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THE CHARACTERIZATION OF FECAL STREPTOCOCCI

AN ATTEMPT TO DIFFERENTIATE BETWEEN ANIMAL AND HUMAN SOURCES OF CONTAMINATION[1]

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SUMMARY

Fecal streptococci were isolated from human and animal feces and frozen meat and poultry pies. Organisms from these sources could be classified in three major groups: *Streptococcus faecalis, Streptococcus faecium,* and *Streptococcus bovis.* Attempts to relate the presence of such organisms to their fecal source using the heat-tellurite test were unsuccessful. A positive correlation was noted between the ability of these organisms to reduce tetrazolium and tellurite and their fecal source.

The use of fecal streptococci as an index of fecal contamination in water and food products has received much attention in the past few years. In addition to their use as pollution indicators, it would be advantageous to employ these organisms to trace the sanitary history of a food product so that preventive measures could be initiated in order to minimize or prevent contamination. The studies reported herein describe the cultural similarities between fecal streptococci isolated from fecal sources and from frozen food products. Data are presented that suggest certain weaknesses inherent in existing methods for detecting the source of fecal streptococci.

Various studies have suggested that it may be possible to trace the source of contamination in a food product by determining whether the isolated streptococci are of animal or human origin. Cooper and Ramadan *(4)* and Kjellander *(8)* reported that *Streptococcus faecalis* was more frequently found in human feces and *Streptococcus faecium* was more predominantly found in animal feces. Orla-Jensen *(11, 12)*, Skadhauge *(17)*, Sharpe and Shattuck *(14)*, Shattuck *(15)*, Barnes *(1)*, Kenner et al. *(7)*, Diebel, Lake and Niven *(5)*, Diebel *(6)* and Whittenbury *(18)* have presented evidence for the division of the fecal streptococci into these two major groups.

Ramadan and Sabir *(13)*, confirming previous work *(4)*, suggested the use of the heat tellurite test to distinguish human and animal strains of fecal streptococci. Moreover, they also supported the view of Colobert and Morelis *(3)* that less stress should be placed on *S. faecium* as a separate species.

Contrary to this belief, studies reported herein present evidence for three distinct types of fecal streptococci isolated from frozen pot pies and for their physiological relationship to direct fecal isolates.

METHODS

Fecal samples from 15 humans, 14 sheep, and 12 cattle (from geographically separated flocks and herds) were collected and placed in sterile petri dishes immediately after defecation. Upon arrival in the laboratory, usually within 15 min after collection, a "pea sized" portion was emulsified in 9.0 ml of Butterfield's phosphate buffer (0.15 M, pH 7.2) and serially diluted. One ml of each dilution was added to Azide Dextrose (AD) broth and incubated at 37 C for 48 hrs. Positive AD tubes were confirmed for fecal streptococci by transfer to Ethyl Violet Azide broth (EVA) *(9)*. The positive EVA tubes were streaked on Thallous Acetate Tetrazolium Glucose agar (TLTG) proposed by Barnes *(1)*, and purified by alternate transfers to Brain Heart Infusion (BHI) broth and TLTG agar. Purified isolates were tentatively classified as fecal streptococci by tetrazolium reduction on TLTG agar, growth on 40% bile agar, and microscopic examination.

Frozen pot pies were brought to the laboratory for analysis within an hour after they were purchased at local markets. Eighty-seven samples of 4 national brands and 58 samples of 3 local brands of chicken, turkey, and beef pies were used in this study.

Fecal streptococci from frozen pot pies were presumptively isolated from the cold pie slurry by the methods as outlined for direct fecal samples.

To further classify the isolates, the following tests were performed using a standardized 0.1-ml inoculum of a 24-hr BHI broth culture washed three times and resuspended in 10 ml of Butterfield's phosphate buffer. Unless otherwise noted, all tests were performed in 10 ml of the specified medium and incubated at 37 C.

Growth at 45 C

Seeded tubes of medium A (0.5% NaCl, 1.0% Evans Peptone, 1.0% Lab Lemco Meat Extract, pH 7.2) were incubated at 45 C (± 0.2 C) in a water bath and growth noted daily over a 3-day period.

Growth in 6.5% NaCl broth.

Seeded tubes of medium A containing 6.5% NaCl were incubated and turbidity noted daily for 3 days.

Growth at 10 C.

Precooled tubes of medium A were seeded and incubated

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at 10 C in a refrigerated water bath. Growth was noted daily for 6 days.

Survival at 60 C for 30 minutes.

One-tenth ml of inoculum was carefully added to a preheated tubes of medium A. The tubes were placed in a 60 C water bath for 30 min and then immediately transferred to a 37 C water bath. Growth was noted daily for three days.

Initiation of growth at pH 9.6.

Seeded tubes of medium A buffered at pH 9.6 according to Kenner, Clark, and Kabler (1961), were incubated and growth noted daily for 3 days.

Growth on 40% bile agar.

Medium B (0.5% NaCl, 1.0% Evans Peptone, 1.0% Lab-Lemco Meat Extract, 0.3% Difco Yeast Extract, 1.5% agar, pH 7.2) to which was added 40% bile, was streaked radially and incubated. Growth was noted after 24 hr.

Starch Hydrolysis.

Medium B containing 2% soluble starch was streaked, incubated for 24 hr and overlayed with Gram's iodine. Yellow zones of hydrolysis were noted as positive activity.

Gelatin liquefaction.

Nutrient gelatin was inoculated by a deep stab and incubated at room temperature for 10 days. The gelatin cultures were refrigerated for 30 min prior to recording.

Final pH in 1.0% glucose.

Seeded tubes of Medium A containing 1% glucose were incubated for 96 hr. The final pH was determined on a Beckman Zeromatic pH meter.

Catalase.

A 24-hr tetrazolium glucose agar culture was overlayed with 5% H_2O_2. Bubbles liberated from a colony constituted a positive catalase test.

Carbohydrate fermentation.

Purple Broth Base (Difco) was used for all fermentation determinations. Ten percent solutions of selected carbohydrates were prepared, sterilized by Seitz filtration, and added to the Purple Broth Base to a final concentration of 1%. Acid production was noted daily for 4 days.

Reduction of 0.04% Potassium Tellurite.

Streaked Medium B containing 10% cooked horse blood and 0.04% potassium tellurite was used. Black colonies were recorded as positive reduction (+4), and grey colonies as partial reduction (+2). In instances where negative tellurite reduction was noted, there was no growth of the isolate due to the toxic effect of unreduced tellurite.

Reduction of Litmus Milk.

Seeded tubes of litmus Milk (Difco) were used to determine reduction, acid production, and clotting. Results were noted daily for 3 days.

Reduction of Methylene Blue Milk.

Seeded tubes of Skim Milk (Rifco) containing 0.1% methylene blue were used to determine reduction. Results ranging from +4 (strong reduction) to − (negative reduction) were recorded daily for 3 days.

Reduction of 2, 3, 5 triphenyl tetrazolium chloride.

The reduction of tetrazolium to formazan was observed on tetrazolium glucose agar incubated at 37 C for 48 hr. Reduction was recorded as follows: strong reduction, +4, +3 (red centered colony); moderate reduction, +2 (pink centered colony); weak reduction +1 (pale pink colony); and negative reduction − (white colony).

Hemolysis.

Two methods, streak plates and pour plates, were employed to determine the hemolytic properties of the fecal streptococci. Blood agar base (Difco) to which was added 10% horse blood was inoculated and then incubated for 48 hr followed by refrigeration overnight to enhance the hemolytic reaction.

Heat-tellurite resistance (4).

Cultures were seeded into a 1.0% Evan's Peptone medium and incubated at 37 C for 16 hr. One-half ml of each peptone culture was transferred to a preheated standard size serological tube and incubated in a 63 C water bath for 30 min, followed by incubation in a 37 C water bath for 2 hrs. Each heat-treated culture was streaked on chocolate agar and chocolate tellurite (0.04%) agar using a 4-mm loop. The plates were incubated at 37 C for 24 hr and recorded as follows:

Chocolate agar: growth, greening of the cooked blood under and around the colony.

Chocolate tellurite agar: growth, the extent of tellurite reduction as evidenced by the blackness of the individual colonies.

RESULTS AND DISCUSSION

The data presented in Table 1 indicate that the fecal streptococci isolated from the feces of man, cattle, and sheep can be separated into three distinct groups; the S. *faecalis* group, the S. *faecium* group, and the S. *bovis* group. This separation is based primarily on the reductive capacity and tellurite tolerance of the individual isolates. These data tend to confirm those of Whittenbury *(18)* who reported that, among the established tests used, only tellurite tolerance and reducing activity completely differentiated S. *faecalis* and S. *faecium*, the other commonly used tests being of limited value.

Every fecal sample examined in this study contained fecal streptococci. The majority of isolates from cattle and sheep, 87% and 81%, respectively, were found to be members of the S. *bovis* group. Medrek and Barnes *(10)* also noted that S. *bovis* was the predominant, if not the sole, fecal streptococcus, in the intestines of cattle and sheep. Bartley and Slanetz *(2)*, using themembrane filter technique, reported that S. *bovis* was predominant in the feces of cattle only. This group was differentiated primarily by its hydrolytic activity on starch agar and the inability to grow in 6.5% NaCl broth. Reduction of tetrazolium was sporadic among members of the S. *bovis* group. Colonies growing on TLTG agar varied in color frm brick red to colorless. The S. *bovis* group as isolated from cattle and sheep feces were, otherwise, homogenous.

Two isolates from cattle and three from sheep were classified as atypical S. *faecalis* because of moderately active tetrazolium and methylene-blue

TABLE 1. THE CHARACTERIZATION OF DIRECT ISOLATES OF FECAL STREPTOCOCCI FROM HUMAN, CATTLE AND SHEEP FECES

Source	Species	No. of strains	Tetrazolium reduction	Tellurite reduction	Methylene blue reduction	Growth in 6.5% NaCl broth	Growth at 45 C	Gelatin liquefaction	Starch hydrolysis
Human	S. *faecalis* var. *liquefaciens*	24	+4	+4	+4	+	+	+	−
	S. *faecalis*	8	+4	+4	+4	+	+	−	−
	S. *faecalis* (atypical)	7	+2	+4	+4 (5) +2 (1) − (1)	+	+	−	−
	S. *faecium* (atypical)	8	−	+4	+2	+	+	−	−
	S. *faecium*	3	−	−	+2	+	+	−	−
Cattle	S. *faecalis* (atypical)	2	+2	−	+2	+	+	−	−
	S. *faecium*	2	−	−	+2	+	+	−	−
	S. *bovis*	27	+1[a]	−	+2	−	+	−	−
Sheep	S. *faecalis* (atypical)	3	+2	−	+2	+	+	−	−
	S. *bovis*	13	+1[a]	−	+2	−	+	−	+

[a]pinpoint colonies varying in color from white to pink.

milk reduction and sensitivity to tellurite. Ramadan and Sabir *(13)* have noted that S. *faecium*, characterized by tellurite sensitivity and inability to ferment sorbitol or reduce tetrazolium, could not be distinguished from atypical S. *faecalis*. It is recognized that these five atypical S. *faecalis* isolates were culturally similar to S. *faecium* and could be grouped accordingly. Two isolates from cattle feces were classified as S. *faecium* because of weak reductive capacity and tellurite sensitivity.

Of the 50 human isolates, 24 (48%) were characteristic of S. *faecalis* var. *liquefaciens* due to the active reduction of tetrazolium, tellurite, methylene-blue milk, and gelatinase activity. Eight human isolates were classified as S. *faecalis* and seven as atypical S. *faecalis*. The human atypical S. *faecalis* group was characterized by active tellurite reduction, but weak to moderate tetrazolium reduction resulting in colonies on TLTG agar of various shades of pink. Eleven human isolates (22%) were classified in the S. *faecium* group because of weak reduction of tetrazolium and methylene blue. However, it is significant that of these 11 human isolates, eight reduced tellurite.

The data presented in Table 2 indicate that fecal streptococci isolated from the various brands of frozen pot pies could also be assigned to one of three physiological groups: S. *faecalis*, S. *faecium*, or S. *bovis*. All of the isolates, except those classified as S. *bovis*, were positive for the "Sherman" criteria *(16)* which includes survival at 60 C for 30 min, growth at 45 C and at pH 9.6, growth in 6.5% NaCl and 40% bile, and reduction of 0.1% methlyene-blue milk.

40% bile, and reduction of 0.1% methlyene-blue milk.

Fifty-five isolates were classified in the S. *faecalis* group. Of this total, 17 were determined to be S. *faecalis* based on active reduction of tellurite, tetrazolium, litmus milk, and methylene-blue milk; fermentation of mannitol and sorbitol but not arabinose and raffinose.

Thirty-eight of the 55 isolates also reduced tellurite, tetrazolium, litmus milk, and methylene-blue milk but were classified as S. *faecalis* var. *liquefaciens* due to active gelatinase activity.

All of the isolates classified as S. *faecalis* fermented mannitol and sorbitol while the majority of the group failed to ferment arabinose or raffinose. One isolate was classified as "atypical S. *faecalis*" because of weak reduction of tetrazolium, litmus milk, and methylene-blue milk but active reduction of tellurite.

Twenty-eight isolates were classified as S. *faecium* due to weak reduction of tetrazolium, litmus milk, methylene-blue milk, and sensitivity to 0.04% tellurite. Most of these isolates fermented mannose, sorbitol, arabinose, and raffinose. Five reduced tellurite and did not reduce tetrazolium. However, characteristics similar to other S. *faecium* isolates necessitated that they be grouped as atypical S. *faecium*. Four other isolates resembling S. *bovis*

TABLE 2. THE CHARACTERIZATION OF DIRECT ISOLATES OF FECAL STREPTOCOCCI FROM FROZEN POT PIES

Species	No. of strains	Tetrazolium reduction	Tellurite reduction	Methylene blue reduction	Litmus milk reduction	Growth at 45 C	Growth at 10 C	Growth in 6.5% NaCl broth	Growth at pH 9.6	Growth in 40% bile broth	Survival @ 60 C for 30 min	Starch hydrolysis	Final pH in 1% glucose	Hemolysis	Mannitol	Sorbitol	Arabinose	Raffinose	Lactose	Gelatin liquefaction	Catalase
S. *faecalis* var.	38	+4 (33)	+4	+4 (33)	R (33)	+	+	+	+	+	+	−	3.9	α	+	+	− (33)	− (33)	+	+	−
liquefaciens		+2 (5)	+4	+2 (5)	rA (5)	+	+	+	+	+	+	−	3.9	γ	+	+	+ (5)	+ (5)	+	+	−
S. *faecalis*	17	+4	+4	+4	R	+	+	+	+	+	+	−	3.9	γ	+	+	−	−	+	−	−
S. *faecium*	19	−	−	+2	rAC	+	+	+	+	+	+	−	4.0	γ	+	+	+	+	+	−	−
S. *faecium* (atypical)	5	−	+3	+1	RaC	+	+	+	+	+	+	−	4.0	γ	+	−	−	−	+	−	−
S. *faecium* (atypical)	2	+1[a]	−	+1	rA	+	−	+	+	+	+	−	4.0	γ	+	+	−	−	+	−	−
S. *faecium* (atypical)	1	+1[a]	−	−	−	+	−	−	+	+	+	−	3.9	γ	−	−	−	+	+	−	−
S. *faecium* (atypical)	1	+1[a]	−	+4	RA	+	+	+	+	+	+	−	4.0	γ	−	−	−	+	+	−	−
S. *bovis*	9	+1[a]	−	−	r	+	−	−	−	+	−	+	4.2	γ	−	−	−	+	+	−	−
S. *bovis* (atypical)	2	+1[a]	−	−	−	+	− (1)	− (1)	−	+	− (1)	+	4.2	γ	+	+	−	− (1)	+	−	−
							+ (1)	+ (1)			+ (1)	+						+ (1)			

legend

[a]Tiny pinpoint colonies varying in color from white to pink.

Litmus milk reduction
- R—strong reduction
- r—weak reduction
- A—strong acid
- a—weak acid
- C—clot

colonially on agar media but S. *faecium* physiologically were included as atypical S. *faecium* isolates.

Eleven isolates from frozen pot pies were classified as S. *bovis* due to colonial appearance on TLTG agar, negative results for the "Sherman criteria", and the ability to hydrolyze starch. One was included as S. *bovis* because it hydrolyzed starch and was of a similar colony type to S. *bovis* on solid media although it was positive for the "Sherman criteria."

The results, as presented in Tables 1 and 2, indicate that in both frozen pot pies and fecal samples, the majority of the fecal streptococci isolated fall into three major physiological groups. Moreover, the data from the present study indicate that although diagnostic tests based on tetrazolium reduction, litmus milk, methylene-blue milk, and tellurite reduction differentiated the Group D streptococci; the other commonly employed tests were of limited diagnostic value due to intraspecies variability.

Cooper and Ramadan *(4)* and Ramadan and Sabir *(13)* reported that a series of tests based on Janus green reduction, heat and heat-tellurite tolerance could be used to designate between human and animal fecal streptococci. They concluded that a streptococcus which is capable of surviving the heat-tellurite test, regardless of its action on Janus green, may be regarded of human origin and one which failed to survive the heat-tellurite test and did not reduce Janus green could be considered of animal origin. They designated strains yielding other results of doubtful origin.

To correlate the observed differences in reducing activity of the isolated fecal streptococci and the possibility of determining their source employing the techniques of Cooper and Ramadan *(4)*, all isolates from feces and frozen pot pies were subjected to the heat-tellurite test. The extent of tetrazolium reduction was compared with both the reduction of tellurite and the results of the heat-tellurite test.

The data summarized in Table 3 illustrate that, of the active tetrazolium reducers from the feces of man and animal, approximately eight times as many reduced tellurite as were sensitive to it; of the negative reducers about half reduced tellurite; and of the weak reducers (S. *bovis* type), all were sensitive to tellurite in the media. Results of the heat-tellurite test compared to that of tetrazolium reduction indicate that the majority of the strong tetrazolium reducers (37 human isolates and 3 sheep isolates) were heat resistant but did not reduce tellurite after heat exposure. However, five animal isolates (2 cow, 3 sheep) were both heat and tellurite sensitive. Only two human fecal isolates reduced tellurite after heat exposures. Of the strains that did not reduce tetrazolium, eight human strains were heat resistant but did not reduce tellurite after the heat exposure and five animal strains were heat sensitive. All of the weak tetrazolium reducers were also heat sensitive.

Remembering that 50% of all isolates studied in this phase were of human fecal origin, interpretation of these data, according to Ramadan and Sabir *(13)*, would indicate that only 2% of the isolates were of human origin, 48% (45 human, 3 sheep) were of "doubtful origin" and the remaining 50% were of animal origin.

Isolates of fecal streptococci from frozen pot pies were also subjected to the tetrazolium reduction and

TABLE 3. COMPARISON OF TELLURITE REDUCTION AND THE HEAT-TELLURITE TEST WITH TETRAZOLIUM REDUCTION BY FECAL STREPTOCOCCI FROM HUMAN, CATTLE, AND SHEEP FECES

	Reduction of tetrazolium		
Reduction of tellurite	Positive +4	Negative —	Weak +1[a]
Positive +4	39 (human) 3 (sheep)	8 (human)	0
Negative —	2 (cattle) 3 (sheep)	2 (cattle) 3 (human)	27 (cattle) 13 (sheep)
Heat-tellurite test			
Heat resistant—Tellurite not reduced	37 (human) 3 (sheep)	8 (human)	0
Heat resistant Tellurite reduced	2 (human)	0	0
Heat-sensitive Tellurite not reduced	2 (cattle) 3 (sheep)	2 (cattle) 3 (human)	27 (cattle) 13 (sheep)

[a]Tiny pinpoint colonies varying in color from white to pink.

TABLE 4. COMPARISON OF TELLURITE REDUCTION AND THE HEAT TELLURITE TEST WITH TETRAZOLIUM REDUCTION BY FECAL STREPTOCOCCI ISOLATED FROM FROZEN POT PIES

Reduction of tellurite	Positive (+4)	Negative	Weak (+1[a])
Positive + 4	55	5	0
Negative	0	19	9
Heat-tellurite test			
Heat-resistant-Tellurite not reduced	36	14	0
Heat-resistant-Tellurite reduced	2	0	0
Heat-sensitive-Tellurite not reduced	17	10	9

[a]Tiny pinpoint colonies varying in color from white to pink.

heat-tellurite test, and these results are presented in Table 4. Of the 55 active tetrazolium reducers all reduced tellurite; of the 24 negative tetrazolium reducers only five reduced tellurite and of the nine weak tetrazolium reducers, none reduced tellurite. Of the same 55 active tetrazolium reducers, only two survived the heat test and reduced tellurite following heat exposure. Thirty-six were heat resistant but failed to reduce tellurite after heat exposure, and 17 were heat sensitive. Of the 24 negative tetrazolium reducers, 14 were heat resistant but did not reduce tellurite and ten were heat sensitive. All of the nine weak tetrazolium reducers were heat sensitive. Strict interpretation of the heat-tellurite test suggests that only two of the 94 isolates could be considered of human origin and 50 were of "doubtful origin."

To determine the source of fecal streptococci in frozen pot pies by use of the heat-tellurite test does not, at this time, appear feasible. Results indicate, however, that the majority of fecal streptococci from a human source reduce tetrazolium and tellurite while those from animal sources are usually weak or variable tetrazolium reducers and sensitive to the presence of 0.04% tellurite in the medium. It is therefore suggested that the reducing capabilities of these microorganisms and their relative sensitivity to tellurite may provide a basis for speciation and source determination.

The findings of the present investigation also substantiate the proposal of Medrek and Barnes *(10)* that S. *bovis* should be included as a member of the fecal streptococcus group.

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COMPARISONS OF MASTITIS SCREENING TEST RESULTS FROM QUARTER, BUCKET AND BULK MILK SAMPLES[1, 2]

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Summary

Milk from four dairy farms in southern Wisconsin was examined over a period of one year in a study that was undertaken: *(a)* to determine the agreement between results of mastitis screening tests when applied to bulk, bucket and quarter milk samples; *(b)* to determine the relative efficiencies of five mastitis screening tests using direct microscopic leukocyte counts as a standard, and *(c)* to examine the quality, as determined by leukocyte content and screening test results, of the milk from all quarters contributing to the bulk tank on each farm. Most screening tests examined, when applied to quarter milk samples, gave a higher correlation with direct microscopic leukocyte counts than when applied to either bucket or bulk milk samples. Similarly, efficiency ratings of screening tests applied to quarter samples were higher than those for the same tests applied to bulk samples. Three of the four farms examined maintained bulk tank milk screening test scores that failed to suggest the presence of milk from a substantial number of quarters that were shedding abnormal numbers of leukocytes.

To be of value in a large scale mastitis control program, any bulk milk screening procedure must reflect the health of the quarters contributing to the bulk supply. In order to determine how accurately a single bulk tank milk sample or a series of such samples might reflect the udder health of those cows contributing to the bulk tank, it was necessary to survey and sample milk from several herds to determine conditions contributing to the quality of the farm milk product.

This study was undertaken with three objectives: *(a)* to determine if mastitis screening tests might yield comparable results when applied to quarter, bucket and bulk milk samples; *(b)* to determine the relative efficiencies of the more widely used screening procedures, using the direct microscopic leukocyte count (DMLC) as a standard, and *(c)* to examine the milk from all quarters contributing to farm bulk tanks in an effort to determine the meaning of the results from screening tests applied to bulk tank milk.

Materials and Methods

Samplings.

Permission was obtained to sample all lactating quarters at regular intervals in four dairy herds in southern Wisconsin marketing grade A milk. These herds were selected on the basis of their bulk milk mastitis screening test results from the previous year. One herd was chosen because of frequent isolation of *Streptococcus agalactiae,* along with consistently high scores for bulk milk on mastitis screening tests (Herd S). Two herds were chosen that showed somewhat lower bulk milk tests scores with only occasional isolation of *Str. agalactiae,* (Herds *P* and *R*), and a fourth herd was chosen that produced milk with consistently low screening test scores on bulk samples with very infrequent isolation of *Str. agalactiae* (Herd *H*). In addition to the samples from these herds, quarter samples were collected from two other herds at irregular intervals. The six herds represented 276 cows with 1068 producing quarters.

Quarter samples of not less than three ounces were collected in sterile polyethylere bags[4] using aseptic collecting techniques. Samples were collected after the cows were prepared for morning milking, following milk let-down. Collection followed the strip cup examination by the operator, when applied, and before the application of the milking machine. There was no attempt to change the cow preparation procedures and milking habits of the farm operators as it was desired that the milk samples be truly representative of the market milk production of the various farms. Samples in excess of three ounces were collected as this quantity might more correctly represent the full quarter production than if the samples consisted of exclusively fore-milk or stripping-milk. Care was exercised to collect samples from only those quarters that contributed milk to the bulk tank supply.

A four ounce milk sample was also collected from the farm bulk tank on each visit. Because the bulk milk supply of each of the farms contributing quarter samples was collected every second day, samples of bulk tank milk that were collected concurrently with quarter samples represented the production of either two or four milking periods.

In addition, the collection of bulk samples representing 266 dairy herds, as previously described (9) was continued. Also, bucket milk samples were collected from two herds in which daily milk production of individual cows is weighed. The term bucket sample is used to designate a milk sample representing the entire production from one cow at one milking. Bucket milk samples were collected from the milking machine pail with a dipper immediately following weighing, or drained from the weigh-jars in a milking parlor.

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[3]Presented at the 53rd Annual Meeting of the International Association of Milk, Food, and Environmental Sanitarians, Inc., at Minneapolis, Minnesota, August 15-18, 1966.

[4]Whirl-Pak. NASCO, Inc., Ft. Atkinson, Wisconsin.

TABLE 1. CORRELATION[a] BETWEEN DIRECT MICROSCOPIC LEUKOCYTE COUNTS AND SIX MASTITIS SCREENING TESTS APPLIED TO QUARTER, BUCKET AND BULK MILK SAMPLES

	Quarter milk		Bucket milk		Bulk milk	
Screening tests	Samples (No.)	r	Samples (No.)	r	Samples (No.)	r
Brabant mastitis reaction	2052	0.86	123	0.84	882	0.47
California mastitis test	3972	0.80	96	0.79	1396	0.66
Catalase test	176	0.81	96	0.69	1396	0.47
Feulgen DNA test	3320	0.86	96	0.77	819	0.72
Modified Whiteside reaction	323	0.80	96	0.80	178	0.51
Wisc. mastitis test	869	0.87	131	0.85	641	0.87

[a]Expressed as correlation coefficients.

Milk samples were transported to the laboratory immediately following collection and cooled to 4C. Milk was cultured and screening tests were applied without delay.

At the beginning of the sampling period, a milking time visit was made to the farms contributing quarter milk samples to observe milking practices and mechanical milking equipment. Observations were made from a period before assembly of mechanical milking equipment through the clean-up period following milking. Measurements were taken on vacuum pump capacity, vacuum level at the end or extremity of the vacuum line with all milking units operating, vacuum range at teat ends during milking, and from pulsator tracings made by a pulsation recorder. From these observations, it was determined that the mechanical milking equipment on each of the farms observed was operating within the specifications of the equipment manufacturer.

Bacteriologic Examinations.

Quarter milk samples of 0.01 ml were cultured on both sheep blood agar (5% citrated sheep blood and 95% blood agar base[5]) and a fibrinogen medium *(1)*. The cultures were incubated at 37C for 24 hours. Staphylococcic colonies surrounded by a distinct zone of turbidity on the fibrinogen medium were considered to be coagulase-positive. Streptococcic growth on blood agar was subjected to the CAMP test *(6)* to presumptively identify *Streptococcus agalactiae.*

Screening Tests.

The California mastitis test (CMT) *(11)*, the Brabant mastitis reaction (BMR) *(3)*, the catalase test *(12)*, and the modified Whiteside reaction (MWR) *(5)* were conducted as previously described *(9)* The Wisconsin mastitis test (WMT) *(14)* and the Feulgen DNA test (FDNA *(8)* were also applied. A modification of the technique of Prescott and Breed *(10)* for estimating leukocyte content of milk, as previously described *(9)* produced a coefficient of variability *(13)* of 17%. This value was larger than the coefficients of variability reported for the indirect screening tests examined. In an attempt to improve the reproducibility of the DMLC, a technique was selected in which 0.01 ml milk sample was delivered to a glass slide from a 0.01 ml pipette, spread over a circular 1 cm^2 area and air dried. The slide was suspended in Levowitz-Weber stain *(4)* for two minutes. Cells were counted in eight microscope fields using a 43x objective lens and a 10x occular lens. This combination of lenses and microscope fields afforded a working factor of 10,000. A reproducibility study following this microscope technique was made in which three CMT grades of milk from trace (T) to two plus (+ +) were chosen. Thirty circular 1 cm^2 milk films were made from each milk sample, so that a total of 90 examinations were made for the reproducibility study. The technique described produced a coefficient of variability of 7% for the DMLC.

Duplicate milk films were prepared from each quarter, bucket and bulk sample for microscopic examination. DMLC values were determined following the technique described above, and average values for the duplicate counts were recorded.

RESULTS

The agreement between the results of the various screening tests, when applied to quarter, bucket and bulk milk samples, and the leukocyte numbers estimated by direct microscopic examinations was determined by both a correlation analysis and relative efficiency score. Correlation for each screening procedure, expressed as correlation coefficients *(13)* (Table 1), was generally highest when applied to quarter milk samples and lowest when applied to bulk milk samples.

A method of expressing relative efficiencies of screening procedures was described by Ewbank *(2)* in which screening test results were compared with DMLC values. Screening test results were designated as true or false positive, or true or false negative readings. The 'true' and 'false' ratings were determined by agreement or lack of agreement with DMLC values which were divided into positive or negative classes. Minimum scores considered as positive values for five indirect screening tests and DMLC values for this study were: DMLC, 500,000/ml; catalase test, 30% 0_2 gas; CMT, one plus (+); FDNA test, a score of one on the color chart; WMT, 15mm; and BMR, 10 seconds for flow-through.

[5]Difco Laboratories, Detroit, Michigan.

The efficiency rating, expressed as a percent, was derived by averaging the percent of false positive readings added to the percent of false negative readings and subtracting this value from the average of the percent of true positive readings added to the percent of true negative readings. This can be expressed in equation form as: % efficiency = (*% true pos. +% true neg.*) − (*% false pos. +% false neg.*) Although this procedure produces a comparison that is statistically less sensitive than that produced by correlation analysis, it permits an examination of the areas of disagreement between any screening test and DMLC values (Table 2).

An example demonstrating the procedure for arriving at an efficiency rating may be developed from the data in Table 2. There were 625 bulk milk samples subjected to the BMR. Of these, 504 were positive (samples that required more than five seconds to flow through a capillary funnel) and 121 were negative to the BMR. Of the 504 samples positive to the BMR, 68% also had positive DMLC values (500,000 or more leukocytes/ml) while 32% of the samples were negative to DMLC (less than 500,000 leukocytes/ml).

Those samples (68% of 504) that were positive to both DMLC and BMR were designated as true positives, while those samples positive to BMR and negative to DMLC were designated as false positives. Similarly, the 121 samples negative to the BMR were designated as either true or false negatives.

Generally, there was agreement between efficiency ratings and correlation coefficients, but the data arrayed for developing an efficiency score permit an identification of the area of disagreement. There was close agreement between screening tests applied to quarter milk samples, and DMLC in the "negative results" category, however two screening tests (BMR and FDNA) produced low efficiency ratings because of a relatively high number of "false positive" readings (Table 2).

The proportion of false negative readings from bulk milk samples for all five screening tests increased sharply over false negative readings from quarter milk. Consequently, tests when applied to bulk milk samples produced lower efficiency scores than when applied to quarter milk samples.

During the quarter milk sampling period, mastitis

TABLE 2. RELATIVE EFFICIENCIES OF FIVE SCREENING TESTS APPLIED TO QUARTER AND BULK MILK SAMPLES

Screening tests	Samples (no.)	Number positive	Division of positive results (%) true[a]	false[a]	Number negative	Division of negative results (%) true[a]	false[a]	Efficiency rating[b]
			Quarter milk samples					
BMR	464	108	73	27	356	97	3	70
Catalase	156	112	85	15	44	95	5	80
CMT	1564	733	86	14	831	97	3	83
FDNA	902	566	68	32	336	97	3	65
WMT	598	252	88	12	346	97	3	85
			Bulk milk samples					
BMR	625	504	68	32	121	69	31	37
Catalase	1159	762	79	21	397	69	31	49
CMT	1159	520	93	7	639	63	37	56
FDNA	1159	1067	66	34	992	72	28	37
WMT	1159	751	87	13	408	84	16	70

[a]Indicates agreement or disagreement with direct microscope leucocyte counts. Minimum Scores considered as positive for the tests were: DMLC, 500,000/ml; catalase, 30% 0_2; CMT, one plus (+); FDNA, a score of one on the color chart; WMT, 15; and BMR, 10 seconds.

[b]Efficiency rating determined by equation:

$$\frac{(\%\text{ true positive} + \%\text{ true negative}) - (\%\text{ false positive} + \%\text{false negative})}{2}$$

TABLE 3. ISOLATION OF PATHOGENIC BACTERIA FROM QUARTER SAMPLES FROM FOUR HERDS

Herd	Quarters[a] yielding pathogenic bacteria (%)	
	CAMP-positive streptococci	Coagulase-positive staphylococci
H	10	22
P	31	16
R	37	43
S	83	53

[a]Quarters that contributed at least one sample that yielded CAMP-positive streptococci or coagulase-positive staphylococci, expressed as percent of total number of contributing quarters.

TABLE 4. DATA FROM 1211 QUARTER SAMPLES FROM HERD R DEMONSTRATING THE RELATIONSHIP BETWEEN MILK SAMPLES WITH MEDIUM TO HIGH (>500,000/ML) LEUKOCYTE CONTENT AND THE ISOLATION OF PATHOGENIC BACTERIA[a]

Leukocyte content (cells/ml)	Samples yielding pathogenic bacteria (no.)	Samples not yielding pathogenic bacteria (no.)
<500,000	194	130
>500,000	268	619
	$X^2 = 89$[b]	

[a]CAMP-positive streptococci and coagulase-positive staphylococci.

[b]Chi-square value significant for 0.05 and 0.01 probability level.

treatment within the four herds was limited to either dry cow treatment or treatment of clinical mastitis. The herd (S) that had the greatest number of quarters with high leukocyte content also had the greatest number of quarters that yielded pathogenic bacteria. Herd S had 83% of the contributing quarters that yielded one or more isolations of CAMP-positive streptococci, while 53% of the quarters yielded one or more isolations of coagulase-positive staphylococci. The number of quarters in each herd that yielded CAMP-positive streptococci or coagulase-positive staphylococci was expressed as a percent of the total number of quarters contributing milk (Table 3).

Within each herd there was a significant relationship, demonstrated by Chi-squared test, between the presence of either medium or high leukocyte content and isolation of pathogenic bacteria. An example of this relationship within herd R is presented in Table 4.

For convenience in comparing the proportion of leukocytes contributed to the bulk milk by each of three categories of quarters within a herd, DMLC values for all quarters were designated as low (less than 500,000 leukocytes/ml), medium (500,000 to 1,000,000/ml) or high (greater than 1,000,000/ml). Similarly, the screening test results of all samples were assigned a rating of low, medium or high, so that a determination could be made for each screening test what proportion of low, medium and high score quarter milk was combined to produce the average bulk tank screening test values (Table 5). The data on bulk milk screening test results in Table 5 represent average values derived from several collection periods for each farm.

Herd S had a history of producing milk with high screening test values during the year preceeding this study, and this trend continued during the sampling period. DMLC values for bulk milk from herd S were consistently over one million per ml. Fifty-four percent of the quarters contributing to this bulk milk supply yielded milk with a leukocyte content rating of high, 24% with medium and 22% with a rating of low (Table 5). By way of contrast, the bulk milk from herd R (average leukocyte content of 637,000/ml) was composed primarily of quarter milk with low leukocyte content rating (67% low, 15% medium and 18% of the quarters had a rating of high).

To provide a clearer understanding of the relationship between a bulk tank screening test score and the screening test scores of milk from all constituent quarters, data from two sampling periods for one (Farm R) of the four farms cooperating with the study are illustrated in Figure 1. These data are representative of those from other farms and illustrate quite clearly that a single bulk tank sample did not accurately reflect the proportion of constituent quarters shedding abnormal numbers of leukocytes.

DISCUSSION

Correlation analysis and relative efficiency scores of screening tests applied to fresh quarter milk are consistently high enough for all of the tests examined to suggest that an ideal screening situation would involve only fresh milk. Since this is unrealistic, and since any large scale screening program must use samples of bulk milk for screening purposes to locate problem herds, it would be helpful to determine the factors that reduce the effectiveness of screening procedures applied to stored mixed herd milk.

Other workers investigating the mechanisms of action of some screening tests offer suggestions which may explain some of the discrepancies found between screening test results from quarter and bulk milk. Paape et al. *(8)* suggested that the FDNA test results might reflect the cell content in milk more accurately than the direct microscope method. Nageswararao *(7)* suggested that only living cells entered into the

gel-forming reaction of tests using the CMT principle. The same author found that the proportion of living cells decreased dramatically on storage, while catalase content of milk remained quite stable through six days.

Bulk milk from three of the four herds (herds H, P and R), when screened by DMLC, would be considered acceptable by most grade A milk plants (average DMLC values of less than 700,000/ml). However, with bulk milk DMLC values below 700,000/ml, there were 30, 22, and 18%, respectively, of contributing quarters that yielded more than one million leukocytes/ml.

The data presented in this study suggest that by using existing screening methods applied to two day old or older bulk milk, it would be necessary to establish a screening level much lower than current standards if milk from all quarters shedding abnormal numbers of leukocytes were to be detected in the bulk milk supply. However, the quantity of milk produced by each quarter in the four contributing herds was not available. If the generalization could be assumed that those quarters producing the largest quantity of milk would likely be in a good state of health with a resulting low leukocyte content, and conversely, those quarters shedding grossly abnormal numbers of leukocytes would have the least production, then these data subject a bulk tank screening test score to an unrealistically harsh examination. Further study is necessary to determine the effects to screening test scores when milk with high cell content is diluted in farm bulk tanks with milk from healthy quarters.

Additional areas worthy of investigation that may be helpful in understanding the meaning of bulk milk screening test results include: the problems inherent with mixing and sampling from the farm bulk tanks, and determination of the effects of sample

TABLE 5. SCREENING TEST RESULTS (AVERAGED) FOR BULK MILK FROM FOUR DAIRY HERDS, WITH RATINGS OF CONSTITUENT QUARTER MILK SAMPLES

Screening tests[a]	Average bulk milk screening test results	Quarter samples (No.)	Distribution of ratings of screening tests applied to constituent quarter milk (percent)		
			Low[b]	Medium[b]	High[b]
		Herd H			
DMLC	590,000	232	58	12	30
CMT	+	232	55	38	7
F.DNA	1	232	39	46	15
		Herd P			
DMLC	686,000	803	65	13	22
CMT	+	803	63	31	6
F.DNA	1.2	803	41	45	14
		Herd R			
DMLC	637,000	1,293	67	15	18
CMT	+	1,289	64	33	3
F.DNA	1.16	1,129	57	38	5
BMR	1	159	62	26	12
WMT	15.5	376	68	17	15
MWR	+	167	60	38	2
CATALASE	30	60	52	11	37
		Herd S			
DMLC	1,353,000	530	22	24	54
CMT	+	530	25	64	11
F.DNA	2.2	420	14	65	21
WMT	27	218	23	35	42
CATALASE	52	76	16	13	71

[a]Direct microscopic leukocyte count, California mastitis test, Feulgen DNA test, Brabant mastitis reaction, Wisconsin mastitis test, Modified Whiteside reaction, catalase test.

[b]DMLC: <500,000/ml, 500,000 to 1,000,000/ml, >1,000,000; CMT and MWR: <+, + to ++, >++; F.DNA: <1, 1 to 3, >3 on color chart; BMR: <5 sec, 5 to 20 sec, >20 sec; WMT: <15, 15 to 25, >25; catalase: <30% 0_2, 30% to 40%, >40%.

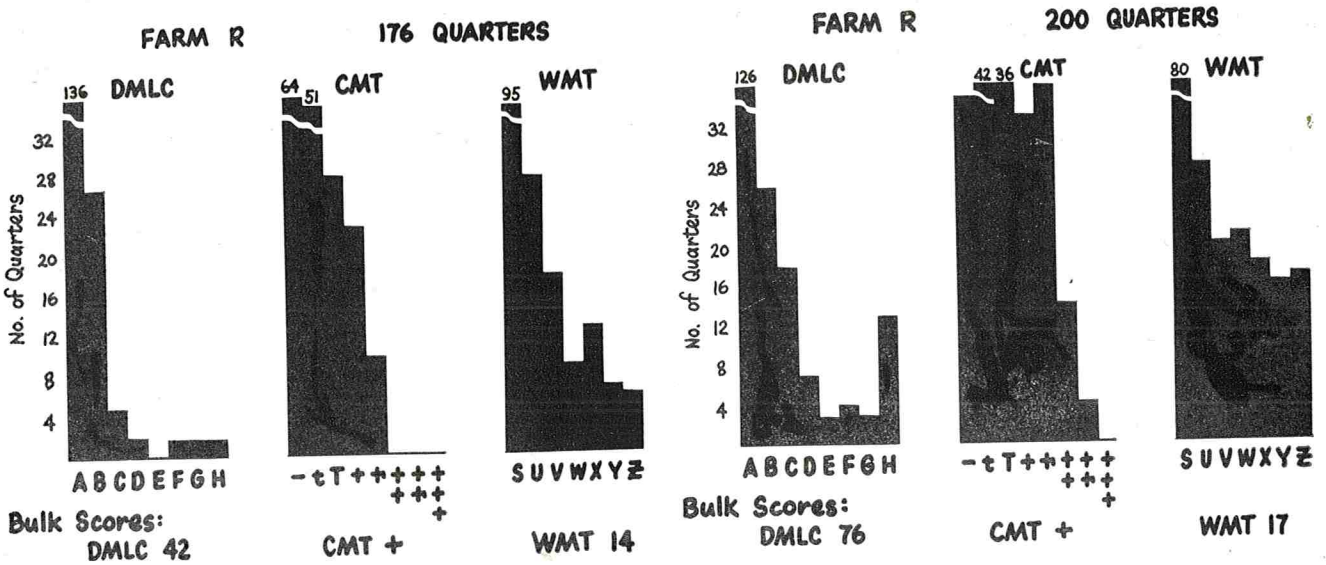

Figure 1. Frequency distributions of screening test scores from quarter milk for two sampling periods for Farm R. Key for DMLC values in cells/ml: A = 0-500,000; B = >500,000-1,000,000; C = >1,000,000-1,500,000; D = >1,-500,000-2,000,000; E = >2,000,000-2,500,000 F = >2,500,000-3,000,000; G = >3,000,000-3,500,000; H = >3,500,000. Key for WMT recordedin mm: S = 3-7; U = 8-12; V = 13-17; W = 18-22; X = 23-27; Y = 28-32; Z = 33-37.

aging as well as sample handling between cow and laboratory.

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SIGNIFICANCE AND CONTROL OF AIR-BORNE CONTAMINATION IN MILK AND FOOD PLANTS[1, 2]

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Summary

The control or elimination of air-borne contamination in the food industry provides many distinct advantages. Obtaining maximum shelf-life of packaged non-sterile food products, gaining full economic advantages of continuous sterilization of fluid food products and maintaining overall high quality levels are among the advantages. By control or elimination of sources such as floor drains, ventilation systems and plant workers, populations of air-borne microorganisms can be reduced significantly. Recent results reveal that 68% of the air-borne bacteria counts in packaging areas of a dairy plant are greater than 6 per ft^3. Nearly 45% of the air-borne bacteria are associated with particles between 2.0 and 5.5 microns. Contamination control can be accomplished by preventing transport of air-borne contaminants from source to point of contamination. Air turbulence is one of the primary mechanisms contributing to transport. Laminar flow of filtered air has provided effective control under experimental situations.

The emphasis on longer shelf-life for food products and trends toward continuous sterilization and aseptic packaging of fluid products has focused increased attention on air-borne contamination. When discussing air-borne contamination in the food industry, reference is most frequently made to the contact of the product with air-borne microorganisms. The extent to which air-borne contamination can occur is then related to two factors: *(a)* the product surface area exposed to air and *(b)* the concentration of air-borne microorganisms present in the air. An additional important factor is the type of microorganisms in the air and numbers of the types which cause product deterioration. The populations and types of air-borne microorganisms present in milk and food processing plants have been reported in several investigations *(1, 2, 3, 7, 8)*.

Two factors which have received insufficient attention are: *(a)* characteristics of microbiological aerosols which exist in the areas of interest and *(b)* the mechanisms which bring about contact between product and microorganisms. Aerosol characteristics such as particle size, size distribution and the actual structure of the microbiological particle will influence the extent of air-borne contamination considerably. The mechanisms which influence contamination probably include air movement and characteristics of product surface along with the characteristics of the microbiological particles.

Significance of Air-borne Contamination

Air-borne contamination is significant in three distinct areas: *(a)* shelf-life of processed non-sterile food products, *(b)* aseptic packaging of sterile products and *(c)* consumer health.

Many food products which are packaged and distributed, are not sterile and therefore have a limited shelf-life. Many situations exist where significant improvements in product shelf-life could be accomplished through effective contamination control procedures. The result would be improved product quality with fewer distribution problems and possibly a new distribution arrangement.

The full significance of contamination control in the food industry should be apparent in the near future. The development of continuous sterilization equipment for fluid food products such as milk has brought about the need for effective aseptic packaging equipment. This implies complete elimination of air-borne and all other microbiological contamination. The accomplishment of the present goals to: *(a)* perfect the sterilization technique to a point where the product will meet consumer acceptance standards and *(b)* develop aseptic packaging equipment which will allow the packaging of sterile product in containers which the consumer accepts, will result in revolutionary changes in distribution policies for milk. These changes are already underway on a small scale. The distribution policies of other food products with unlimited shelf-life provide some idea of how milk may be handled in the future.

A factor which should receive attention is the potential relationship between air-borne contamination and consumer health. Although, there are no known incidents of food poisoning being linked to air-borne contamination of the product, it does not seem beyond reason that such could occur. The many sources of air-borne microorganisms, including the plant worker, certainly have the potential to generate microorganisms which could come in contact with the product through air-borne contamination and in turn be harmful to the consumer's health.

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[2]Presented at the 53rd Annual Meeting of the International Association of Milk, Food and Enviromental Sanitarians, Inc., in Minneapolis, Minnesota, August 15-18, 1966.

TABLE 1. AIR-BORNE BACTERIA COUNTS IN FOOD PACKAGING AREAS[a]

Sampler - year	No. of samples	Mean count	Range of daily mean counts	Percentage distribution of counts/ft³ <3	<6	>6	>10
		(No./ft³)	(No./ft³)	(%)	(%)	(%)	(%)
Casella-1963	315	5	2 - 10	31.4	56.1	43.9	14.3
Casella-1966	161	14	1 - 30	11.4	31.1	68.9	46.4
Andersen-1966	51	9	3 - 21	12.6	31.8	68.2	39.2

[a]Counts based on 35 C - 48 hr. incubation.

Complete control of air-borne contamination eliminates this possibility.

CONTROL OF AIR-BORNE CONTAMINATION

It would appear that control of contamination by air-borne microorganisms in food processing plants can be accomplished in three different ways: *(a)* isolate and eliminate sources of air-borne microorganisms, *(b)* prevent transport of air-borne microorganisms from the source to the point of contamination and *(c)* provide localized control of air-borne contamination. One of the more important factors in any control approach is the population of air-borne microorganisms in the space of interest. Typical populations of air-borne bacteria are illustrated in Table 1 where counts obtained by Heldman et al. *(3)* in food packaging areas of a dairy plant are compared to more recent counts in the same plant and with a different air sampler. A comparison of air-borne counts obtained by collecting air samples on solidified standard plant count medium using a Casella slit sampler and an Andersen air sampler are shown in Table 1. The air samples were collected in the milk, cottage cheese, butter and ice cream packaging areas of a dairy plant. Samples collected in 1966 by both the Casella and Andersen sampler produced counts which are higher than counts obtained with the Casella sampler in 1963. This is attributed to increased activitity due to increased production in the plant, and a construction site adjacent to the plant. Very close agreement in percentage distribution of counts was obtained in the recent counts by the Casella and Andersen samplers with about 68% of counts being greater than 6 per ft³ in both cases.

A second factor of importance for controlling air-borne contamination are the characteristics of the contaminant. One characteristic is the particle size and size distribution of particles with which air-borne microorganisms are associated. Typical results are presented in Figure 1 which shows particle size distributions of air-borne bacteria and mold for air samples collected by an Andersen sampler in four packaging areas of a dairy plant. These results indicate that nearly 45% of the air-borne bacteria were associated with particles sizes of 2.0 to 5.5 μ. Nearly 60% of the air-borne mold collected had particle sizes between 1.0 and 3.3 μ. Particle size characteristics have particular importance when discussing transport of the air-borne microorganisms from source to point of contamination.

SOURCES OF AIR-BORNE MICROORGANISMS

Within the structure of a food processing plant the number and types of sources of air-borne microorganisms may be numerous. Therefore, any attempt to control or eliminate the source may be impractical. However, identification and isolation of the more prevalent sources may lead to significant reductions in overall air-borne populations. Based on available information, there appears to be five prevalent sources of air-borne microorganisms in food packaging areas: *(a)* floor drains, *(b)* ventilation systems, *(c)* plant workers, *(d)* openings between rooms and *(e)* any surface to which air-borne particles will adhere.

The contribution of floor drains, ventilation systems and plant workers has been discussed by Heldman et al. *(3, 4, 5)*. The results *(4)* revealed significant contributions to air-borne populations during flooding of floor drains early in the processing day. Results *(5)* indicated that the contribution of ventilation systems was relatively constant during normal operation. Counts at the system outlet vent increased on starting of the system after an idle period of time.

Although no conclusive evidence is available, the contribution of plant workers cannot be overlooked. Previous results *(3)* indicate a slight relationship between air-borne populations and worker activity. By isolation of a worker in a controlled air space at one hour intervals throughout a typical work day, and sampling the air from the space, the results presented in Figure 2 were obtained. Since the air was

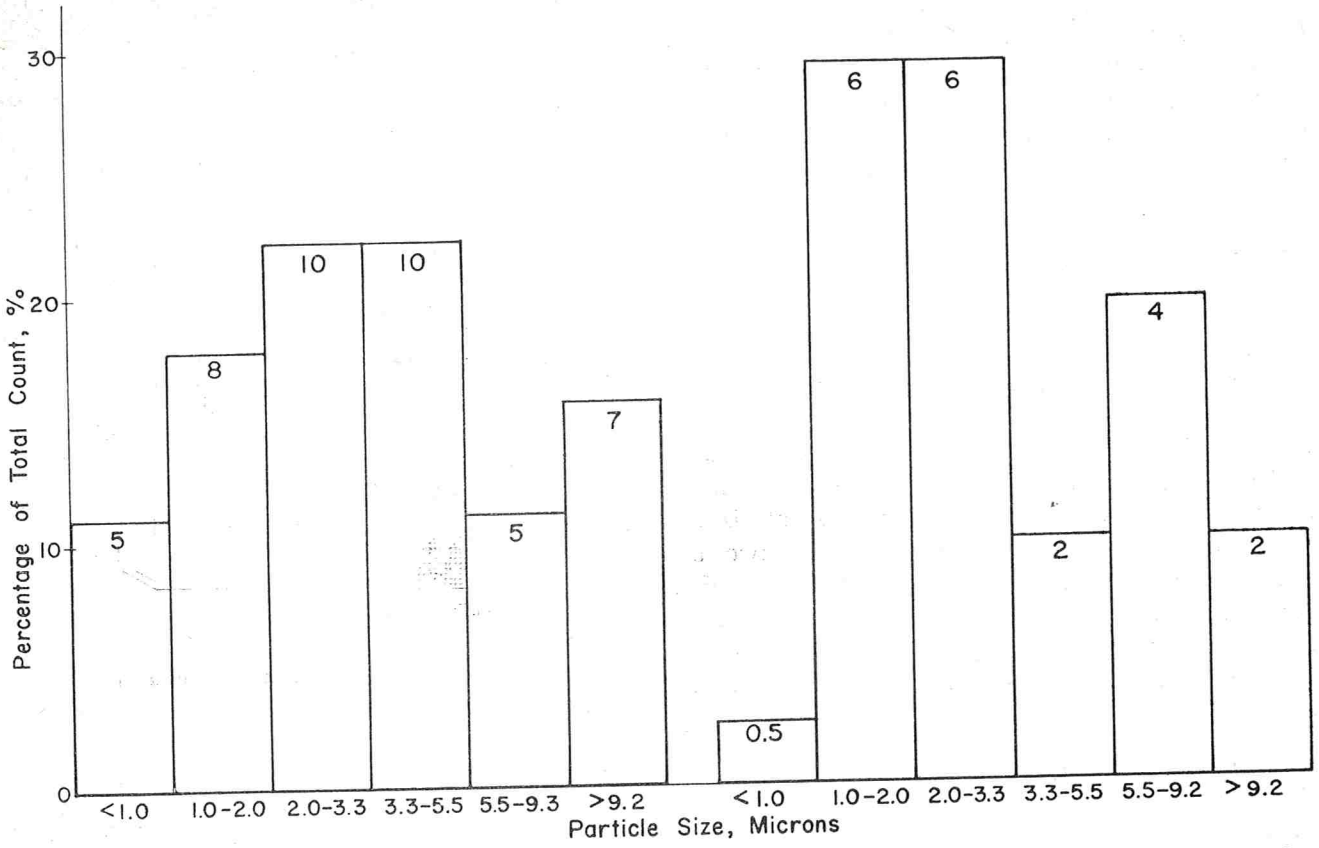

Figure 1. Size distribution of particles with which air-borne bacteria (left) and air-borne mold (right) are associated.

sampled at a rate of one cubic foot per minute, 5 min was required to collect the sample at each time interval and the data in Figure 2 can be expressed as rate of contribution if desired. It is evident from Figure 2 that contributions of air-borne bacteria by workers are relatively high and fluctuate throughout the working day. The results indicate contributions ranging from about 20 to nearly 70 bacteria/minute. These results represent the contributions of one person on a given day working in a given area of the plant. It seems evident that the contribution of the worker will vary with several factors including: *(a)* health, *(b)* condition of clothing, *(c)* hygiene and *(d)* location of work within plant. There is a definite need for more research in this area.

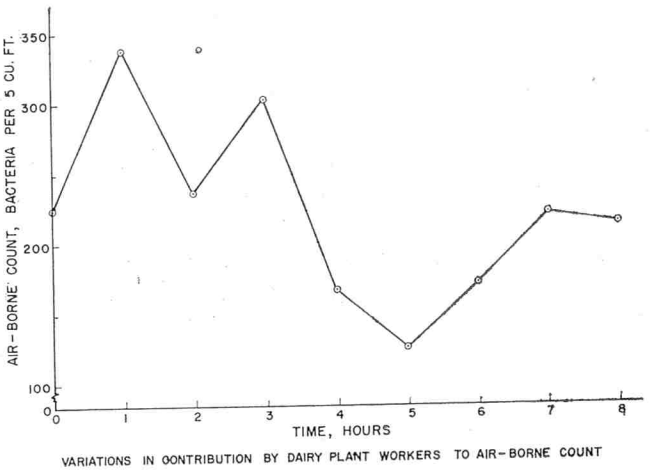

Figure 2. Variations in contribution of dairy plant workers to air-borne count.

Any surface to which a microbiological particle may adhere or settle on will act as a source if conditions are appropriate for the particle to become air-borne. Wilkinson *(19)* has revealed that the survival of bacteria on metal surfaces is dependent on temperature and relative humidity in the surrounding environment. The research illustrates that mircoorganisms can remain viable sufficiently long to become air-borne. The conditions required for reflotation probably include: *(a)* air velocity and turbulence near the surface, *(b)* surface characteristics and *(c)* particle characteristics. Preliminary investigations are needed to evaluate the importance of this particular source.

Openings between rooms, such as doors or windows, may act as a source of air-borne microorganisms if the concentrations in adjoining rooms are higher. Any occurance causing air movement at the

opening will result in transport of air-borne particles from one space to another. These occurances would include movement of the door or any movement of an obstacle through the door. In addition, transport of air-borne contamination, through the opening will occur even without air movement as will be shown in the next section.

TRANSPORT OF AIR-BORNE MICROORGANISMS

Since it is impossible to eliminate all sources of air-borne microorganisms in a food packaging area, an attempt can be made to prevent transport from the source to the point of contamination. The first step in this endeavor is to gain knowledge of mechanisms which cause transport. One of the primary mechanisms, in addition to mass air movement, is air turbulence *(6)*. At an opening between two air spaces with different concentrations, transport of a bacterial aerosol was related directly to turbulent energy as illustrated in Figure 3. Transport is expressed in terms of a turbulent transfer coefficient defined as:

$$k_C = \frac{N_A}{A(C_H - C_L)} \qquad (1)$$

where:

N_A = mass flux of aerosol, No./min.
A = area of opening, ft².
$C_H - C_L$ = concentration gradient. No./ft³.

The turbulent energy is the root mean square of the instantaneous velocity. Fluctuations were measured at the opening by a constant current hot wire anenometer and auxilary equipment. The results indicate that, for the range of paramters measured, there is a second order relationship between the transfer coefficient and turbulent energy.

Investigations are underway to determine the factors which influence dispersion of bacterial aerosol from a point source in a typical food packaging space. Preliminary results indicate that the primary factors of concern are ventilation rate, location of the source and obstacles in the room which may influence air flow characteristics.

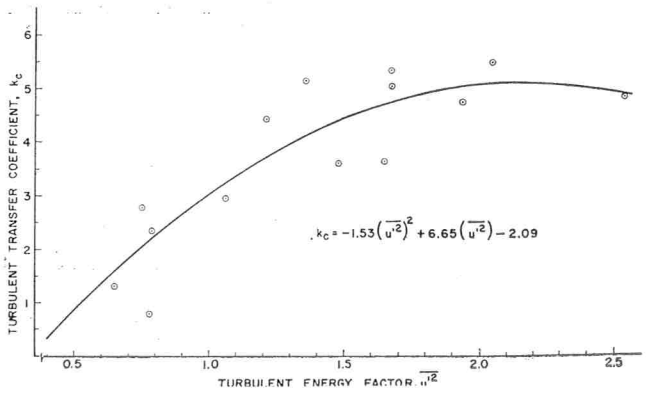

Figure 3. Influence of turbulent energy on transport of a bacterial aerosol.

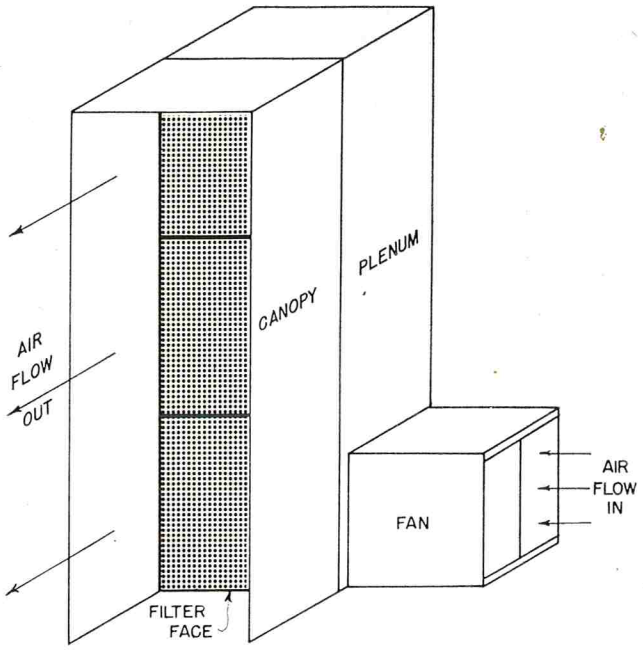

Figure 4. Experimental laminar air flow chamber.

LOCALIZED CONTROL OF AIR-BORNE CONTAMINATION

In some situations, conditions can be created at the potential point of contamination which will prevent contact of product and air-borne contaminant. One such technique-laminar air flow-is commercially available in the form of laminar air flow benches or hoods. The basic concept is to provide a space through which filter air is moving continuously without mixing with unfiltered air. The air in such a space can be maintained in a sterile condition by using ultra-high efficiency filters.

Using the experimental laminar air flow unit shown in Figure 4, experiments were conducted to establish the effectiveness against air-borne contamination by microorganisms. Some 194 colonies were grown from 5068 ft³ of air sampled at 9 different locations in the controlled space. The room in which the unit was operating was maintained at constant concentrations between 8 and 82 per ft³ by generating an aerosol of *Serratia marcescens*. The results indicated the influence of air velocity and location within the controlled space with air turbulence contributing to counts obtained at some locations. The influence of location probably is less prevalent on commercial units due to improved design. Air velocity appeared to be optimum at a mean of 100 ft/min.

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WORLD FOOD SUPPLY PROBLEMS DISCUSSED AT DAIRY SHOW FOOD FORUM

At the Food Technology sessions on October 25—27, 1966, sponsored by the Dairy and Food Industries Association at its Industrial Exposition at Atlantic City there were interesting and enlightening discussions on several subjects dealing with the technology of the world's food supply and management's outlook on current resources.

In reviewing the nutritional aspects of the situation, Dr. E. E. Howe, Director of Nutrition for the Merck Institute for Therapeutic Research, stated that, although food demand must outrun supply at present population growth rates, the deficit can be lessened either by a protein concentrate or by amino acid supplementation or both. Enough protein concentrates exist but it is unlikely that production will keep pace with demand.

Another consideration is that addition of sufficient concentrates makes changes in taste and texture of foods necessitating an education program to alter dietary practices. The ultimate goal for all people should be a reasonable intake of protein comparable in quality to milk proteins, Dr. Howe stated. Cereal grains, most readily available, are in themselves inadequate in protein because of quality and should be upgraded by added concentrates or amino acid supplements. The latter procedure can be accomplished at a central processing plant and the cost is low. Protein concentrates may be introduced in the diet in the form of textured foods of high taste appeal.

Dr. Edward S. Josephson, Director of the Army's Radiation Laboratory at Natick, Mass., discussed the outlook for radiation processing of food. The use of ionizing radiation does much to enhance quality and extend shelf life by destroying pathogens, eliminating insect infestation and slowing down the maturing process. Foods that have been subjected to irradiation are either rendered completely sterile or pasteurized to a state know as "commercially sterile." The latter has application to the widest range of foods.

Application of the process to dairy products changes the flavor and is not practical for use, according to Dr. Josephson. However, with sufficient research into flavor problems, acceptability of the process could well be achieved. At present irradiation of foods is quite costly but Dr. Josephson felt that costs will come down as volumes processed increase.

Speaking on the subject of chemical elements in food. Dr. J. M. Coon, Head of the Department of Pharmacology, Jefferson Medical College of Philadelphia, stated that naturally-occurring elements, whether they be toxicants such as arsenic and fluoride or essential nutrients such as vitamins, amino acids and calcium, can produce harmful effects if consumed out of proportion to other elements in the food supply. Men have long been aware of naturally-occurring toxicants but only recently has formal study of those elements been stimulated by growing concern over man-made chemicals, such as insecticides, in the food supply.

It was emphasized that food processors should be aware of the potentially toxicological effects of naturally-occurring antivitamins, enzyme inhibitors, carcinogens, etc., when consumed in disproportionate amounts. It is important to know the proportion of the potentially harmful level present so that supplementation or enrichment procedures in food precessing did not result in excessive intake or of dietary inbalances.

Dr. Coon concluded that there is as yet no reason for alarm about natural chemical components of foods. Our efforts should be directed toward a more complete knowledge of the natural chemical constitution of our foods and a fuller understanding of long range toxicological significance in the context of the chemical make-up, both natural *and* man-made, of our total diet.

The papers presented at the DFISA sponsored forum as well as comments from the floor will appear in Proceedings of the Forum entitled "The Technology of the Food Supply: Management Looks at Current Resources." Copies will be available from the Dairy and Food Industries Supply Association, 1145 19th St., N.W., Washington, D. C. 20036.

THE OCCURENCE OF CITRATE-FERMENTING BACTERIA IN CHEDDAR CHEESE

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(Received for publication August 8, 1966)

Summary

Forty samples of Cheddar cheese were purchased at retail outlets and examined for citrate-fermenting organisms and scored for flavor. The cheese were grouped into approximate flavor-groupings according to vendor's or manufacturer's label. Assuming that flavor intensity progresses with age, then the aged cheese with the sharp flavor had, on the average, fewer citrate-fermenting organisms. Likewise, the converse was true in that the mild flavored cheese had, on the average, higher counts of citrate-fermenting organisms. The correlation coefficient between the flavor scores and the logarithm of the numbers of citrate-fermenting bacteria was −0.53 which was significant at the 1% level.

The citrate-fermenting organisms contribute to the flavor of cottage cheese and cultured buttermilk through the production of biacetyl; likewise, one could expect a similar contribution to the flavor of Cheddar cheese since these organisms are present in many of the cultures in commercial use. In a survey of lactic cultures in use in a number of dairy establishments, Overcast and Skean *(11)* found that all of the 21 cultures used in making Cheddar cheese contained citrate-fermenting bacteria ranging in number from 2.7×10^6 to 2.0×10^8 per ml. Calbert and Price *(2)* stated that a small quantity of biacetyl seems to be essential in the typical flavor and aroma of Cheddar cheese and in a later study *(3)* found all lots and kinds of cheese examined contained biacetyl. The biacetyl content of the Cheddar cheese ranged from 0.016 mg to 0.335 mg per 100 g of cheese.

Since the establishment by Hammer *(7)* in 1920 that certain volatile acid-producing bacteria were associated with the lactic acid-producing bacteria of dairy cultures, a number of proposals for the enumeration of these "associated" organisms has occurred. Benchetrit *(1)* in 1933 suggested the addition of bromopropionic acid to tomato juice agar as a means of differentiating the *Leuconostoc* species from the lactic organisms. Prouty and Glenn *(12)* developed a limited plate culture method for differentiation and enumeration of *Leuconostoc citrovorum* that utilized brom cresol purple. Lundstedt *(8)* used the iridescent characteristic of these organisms on a citrate whey agar as a means of identification. But due to the various limitations with these procedures none of them has been entirely satisfactory for the enumeration of these organisms. More recently Mayeux et al *(9)* suggested the use of a medium containing 75 ppm of sodium azide, while McDonough et al. *(10)* added 0.15 mg per ml of tetracycline to tomato junice agar to inhibit the streptococci. A medium developed by Galesloot et al. *(4)* and modified by Skean and Overcast *(13)* has been found convenient and effective for enumerating citrate-fermenting bacteria. With this modified medium, it was now possible to examine Cheddar cheese for citrate-fermenting bacteria.

Procedure

Forty samples of natural Cheddar cheese were purchased from retail outlets and grouped in approximate flavor-groups according to vendor's or the manufacturer's label. These groups, presumably from the most aged cheese to the least aged, were as follows: extra sharp, sharp, mellow, medium, and mild with another group as unclassified because there was no identification that would indicate its age or flavor. The cheese packages were opened in the laboratory and with a sterile spatula the outer layers were removed and a one gram sample of the interior portion was weighed into a sterile mortar containing 9 ml of a warm sterile 2% sodium citrate solution. With a sterile pestle the cheese was triturated into a smooth and uniform suspension. From this one to ten dilution the appropriate dilutions for plating were prepared using standard 99-ml water blanks.

Five grams of calcium lactate were added to a liter of tomato juice agar and sterilized in the usual manner. The stabilized calcium citrate suspension was prepared by adding 1.5 g of carboxymethyl cellulose (CMC) to 200 ml of warm distilled water at 45 C and allowing it to stand overnight at this temperature. To this CMC gel was added 20 g powdered calcium citrate and the mixture was homogenized using a Virtis Homogenizer at low speed for approximately two minutes. The calcium citrate suspension was tubed in screw cap tubes and sterilized in the autoclave.

Immediately prior to pouring the plates 6 ml of the warm calcium citrate suspension was added to each 100 ml of the modified tomato juice agar and mixed thoroughly. The plates were poured with 10 to 12 ml of this medium and after solidification they were incubated at 21 C for 5 days. After the incubation period the colonies, encircled by a clear zone or halo, (indicating citrate fermentation) were counted.

This medium contained no inhibitor for gram negative organisms. Colonies of *Aerobacter aerogenes* on this agar were large, thick, white to cream-colored, and raised. Fermentation of citrate and development of clear zones around the colonies progressed slowly at 21 F. Because of these characteristics *A. aerogenes* could easily be distinguished from the citrate-fermenter of lactic culture origin and few, if any,

were present in these cheese at the dilutions used for enumeration.

All the cheese was scored for flavor by one or more experienced judges.

RESULTS AND DISCUSSION

Four of the 40 cheese were criticized on flavor as yeasty. Two of these were in the sharp flavor group and one each in the mellow and mild groups. In each of the yeasty cheese the citrate-fermenting organism population was among the very lowest for its group. The remaining cheese were all in the good to excellent flavor range.

The numbers of citrate-fermenting bacteria from the various cheese in this experiment are shown in Figure 1. On the flavor classification used 13 of the 40 cheese classified as sharp, 7 as mellow, 5 as medium, 6 as mild and 6 as unclassified. In addition, 3 cheese classified as extra sharp which do not appear in the figure because they contained less than one citrate-fermenting organism per gram of cheese. Within each of the groups there was considerable variation in the count. However, the general trend indicates that as the intensity of flavor increases, the citrate-fermenting bacteria decrease in number. Assuming that flavor intensity progresses with age, then the aged cheese with sharp flavor had fewer citrate-fermenting organisms. This is emphasized by the fact that 61% of the cheese with less than one citrate-fermenting organism per gram were classified as sharp or extra sharp in favor.

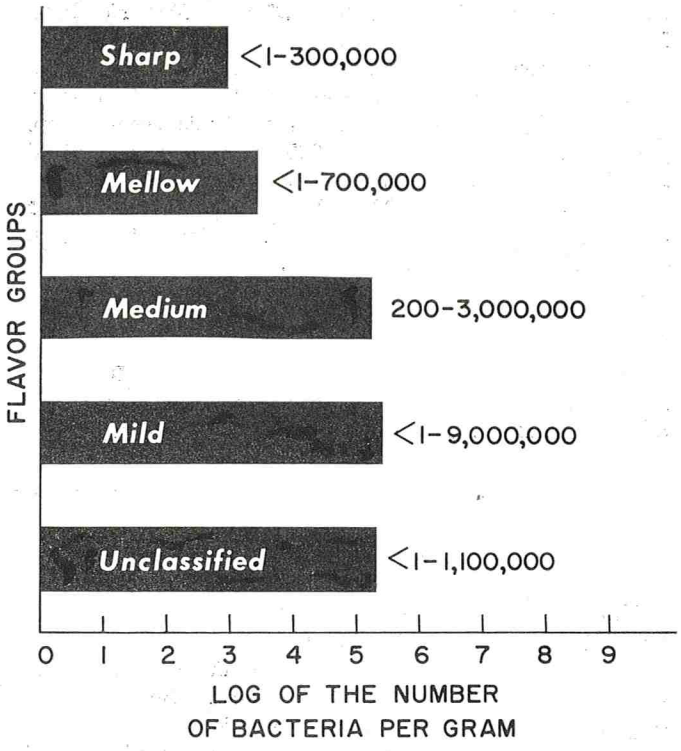

FIGURE 1. The median number of citrate-fermenting bacteria in the five cheese flavor groups and the range in the counts.

Hamamoto et al. *(6)* concluded that *Leuconostoc citrovorum* could not grow in ripening Gouda cheese, probably because of the low pH and/or high salt concentration. This then could be an explanation for less than one organism per gram in the various flavor groups as shown in Figure 1; however, this is doubtful since the citrate-fermenting organism of cheese cultures are capable of growth at the pH found in Cheddar cheese. On the other hand, another explanation could be that these cheese were made with single strain cultures or at least only acid-producing cultures; in which case few, if any, citrate-fermenting organisms would be present. Still a third explanation for few citrate-fermenting organisms in these cheese could be that they die off during the curing of the cheese. This very likely could be the explanation for the aged cheese.

Of the 40 cheese sampled, 13 or 32.5% contained less than one citrate-fermenting organism per gram of cheese. Hamamoto et al. *(5)* found that 49 of 86 ripened cheese contained between 10^2 and 10^7 colonies of *Leuconostoc* species per gram and the other 37 cheese contained less than one per gram of cheese.

Calbert and Price *(3)* and Vedamuthu et al. *(14)* showed that small excesses of biacetyl or high carbonyl content were associated with off-flavors in Cheddar cheese. Since the citrate-fermenting organisms are capable of producing these compounds one might expect a high correlation between their numbers and the flavor scores of cheese. However, with these cheese the correlation coefficient between the flavor scores and the logarithm of the numbers of citrate-fermenting bacteria was only –0.53 but this was significant at the 1% level of probability. Additional research is underway to further elucidate the role of these organisms in the flavor development of Cheddar cheese.

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NMC ANNUAL MEETING

The sixth annual meeting of the National Mastitis Council will be held in Chicago, Ill., February 23-24, 1967.

The first morning session will have three speakers discussing various aspects of mastitis control: Dr. Robert Schroeder, President-elect of the American Veterinary Medical Association, will discuss "The Challenge of a Mastitis Control Program"; Dr. William Merrill of Cornell will present a paper on "An Approach to Mastitis Control"; and Dr. John Mettler, a practicing veterinarian at Copake Falls, New York, will discuss "The Veterinarian's Responsibility in the Control of Mastitis."

Progress with control programs will be featured during the first afternoon session. Dr. K. A. McEwen of the Ontario Department of Agriculture and Food will describe "The Ontario Mastitis Control Program"; and Dr. Donald E. Jasper of the University of California will tell the audience about "The California Mastitis Control Program." Also, Dr. Donald Postle of the University of Wisconsin will be on the afternoon program to discuss "Screening Bulk Milk Samples for *Streptococcus agalactiae*." The session will conclude with a report by Dr. John Herrick, Chairman of the Committee on Abnormal Milk of the National Conference of Interstate Milk Shipments. He will discuss the proposed program being recommended by his committee.

On Thursday evening, February 23, all standing committees of the Council will have meetings. The purpose will be to develop program for Council action. These will be open meetings and everyone in attendance will be invited to attend the meeting of his choice.

Most of the second morning session will be devoted to a review of mastitis research. This session will be handled by the Research Committee of the Council under the chairmanship of Dr. L. W. Slanetz of the University of New Hampshire. Committee reports and the annual business meeting will take up the rest of the morning session.

WISCONSIN SCIENTISTS RECOMMEND ENTEROCOCCI AS WATER POLLUTION INDICATORS

Sewage pollution of water supplies has been traditionally measured by the number of "Coliform" bacteria found in the water, but another bacterial group called "enterococci" may be better indicators of water purity.

Coliform bacteria live in the lower intestine of man and are always found in human sewage. Their presence in ground water indicates contamination by sewage. This could mean that disease producing organisms of intestinal origin are present. Such bacteria can cause typhoid fever, paratyphoid, dysentery, asiatic cholera, and diarrhea.

Coliform bacteria have been a most useful indicator of safe drinking water, but they are not satisfactory for measuring pollution in streams, according to University of Wisconsin scientists G. A. Rohlich and W. B. Sarles. They say that coliform bacteria are everywhere in nature, and they may even multiply under certain conditions in stream water. So, while absence of coliforms mean that water is safe, their presence does not always mean that it is unsafe.

The researchers tested enterococci and coliform bacteria and found enterococci to be better indicators of sewage contamination than coliforms. They found that coliforms often multiplied in warm polluted water while enterococci did not. Populations of coliforms increased four to tenfold in the first two or three days before the bacteria started to die.

Coliforms showed about the same buildup in population in 50, 68, and 86 degree water. In cold water coliform bacteria had a longer "lag phase," the time from the peak population buildup until the bacteria started to die off. Enterococci bacteria died off faster than coliforms in warm polluted water as well as in cool polluted water.

Bacteriologists have known for a long time that both coliforms and enterococci live in the human digestive system. But they did not use enterococci as indicators because they thought their populations were too small to measure. Modern methods of detection show that enterococci do occur in large numbers and therefore are practical to use as indicators of water pollution with human or animal sewage.

APPLICATION OF AN AUTOMATED PROCEDURE TO THE MILK PHOSPHATASE TEST

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(Received for publication October 4, 1966)

Summary

An automated p-nitrophenylphosphate alkaline phosphatase procedure, developed for clinical testing of blood serum, has been adapted with minor modifications for use in the milk phosphatase test. The sensitivity of the automated procedure for the detection of under-pasteurization, and raw milk addition, is considered, and comparisons made with the official Gilcreas-Davis method.

The presence of the enzyme phosphatase in milk has universal acceptance as evidence of faulty pasteurization or contamination with raw milk. The test originally employed by Kay and Graham *(5)* has had many modifications, and new tests have been devised, mostly aimed at simplifying the procedure or shortening the incubation period while retaining or improving upon the sensitivity of the test. Aschaffenburg and Mullen *(3)*, basing their test on the work of Bessey et al. *(4)*, reduced the incubation time to two hours using p-nitrophenyl disodium orthophosphate as the substrate. Following improvements by Tramer and Wright *(9)*, and Aschaffenburg *(2)*, this technique was adopted as the official phosphatase test for pasteurized milk in the United Kingdom *(8)*. In America, Kosikowski *(6)*, utilizing the original disodium phenyl phosphate substrate of Kay and Graham, with an improved buffer system, and separation of unwanted milk constituents by dialysis and extraction with butyl alcohol, reduced the incubation time to one hour and less, and attained excellent sensitivity.

The following adaptation of a technique reported by Morganstern et al. *(7)* for the determination of alkaline phosphatase in blood serum, utilizing the Autoanalyzer[1], combines the use of p-nitrophenylphosphate substrate, a very capable 2-amino-2-methyl-1-propanol buffer, and dialysis of the post incubation stream. Some of the advantages of this combination are: rapid multiple analysis of untreated milk samples with adequate sensitivity; the substrate produces its own chromogen, p-nitrophenol, so does not require separate determination of phenol or phosphate; control analyses are not required.

[1]Technicon Instruments Corporation, Chauncey, N. Y.

Reagents and Apparatus

Buffer, 0.5 M (pH 10.25 ± 0.05, 25 C).

For convenience, a stock 50% w/v solution of 2-amino-2-methyl-1-propanol[2] is prepared. To 95 ml stock buffer solution, in about 700 ml of distilled water, 5N hydrochloric acid is added, with continuous agitation, until the pH of the solution as monitored with a reliable meter is brought to 10.25. (About 22 ml is required.) The solution is diluted to 1 liter and stored under refrigeration.

Magnesium Chloride, 1M.

To 9.25 g of magnesium chloride (dried overnight at 110 C), dissolved in about 80 ml distilled water, 2 drops of concentrated hydrochloric acid are added and the solution diluted to 100 ml.

Buffered Substrate.

Immediately before use, sufficient p-nitrophenyl disodium orthophosphate[3] is weighed at the rate of 2.0 mg/ml of buffer, to prepare enough substrate for the day's run, and dissolved in the required amount of buffer. Sufficient 1M magnesium chloride is added to make the concentration equivalent to 0.001M. The pH is rechecked and readjusted to 10.25 ± 0.05, if required.

p-Nitrophenol Standards.

A stock standard (2 mg/ml) is prepared by dissolving 0.2 g of p-nitrophenol[3] in 100 ml of buffer solution. Working standards containing 10, 20, 30, 40, 50, 100, 150, and 200 μg are prepared by diluting 0.5, 1.0, 1.5, 2.0, 2.5, 5.0, 7.5, and 10.0 ml, respectively, of the stock standard, to 100 ml with buffer solution. Keep under refrigeration.

Manifold and Flow Diagram

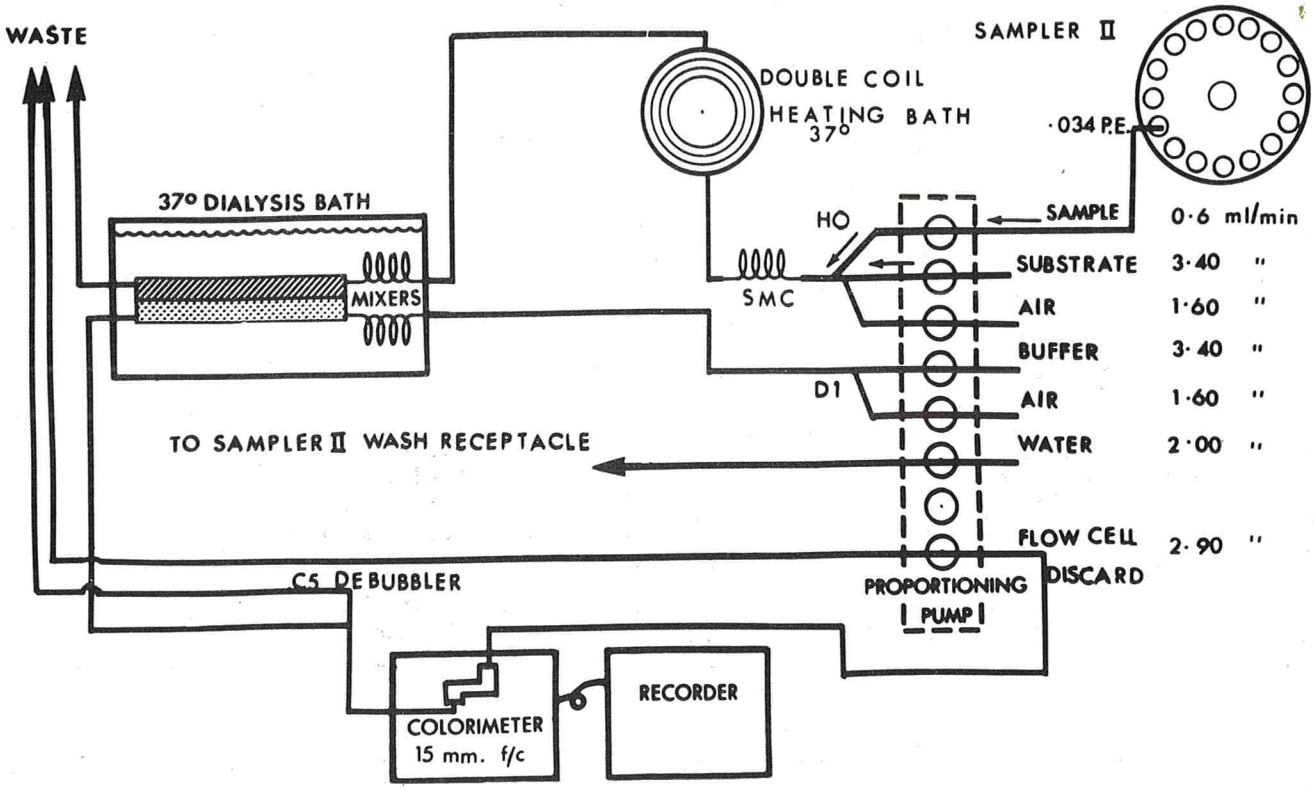
The analytical system here depicted is a continuous flow system in which reagent solutions are added to samples in the required proportions and at appropriate times for the desired chemical reactions to occur within plastic or glass tubing as the solutions pass through. The addition of various modules through which the solutions pass, makes it possible to subject them to incubation, heat, dialysis, digestion and a variety of other conditions which may be required. For further details, the interested reader is referred to the literature of the manufacturer.

The manifold and flow diagram are shown in Figure 1. Sample, substrate and air are pumped through tygon tubing

[2]Distillation Products Industries (Eastman) P-4780 was used in this study.

[3]British Drug Houses, Toronto, Canada. Material from Sigma Chemical Co. is also satisfactory.

Fig. 1 - MANIFOLD AND FLOW DIAGRAM
MILK PHOSPHATASE



by a continuously operating proportioning pump. They are brought together in an HO glass fitting and mixed in a spiral glass mixing coil (S.M.C.). The sample-substrate mixture, segmented by air bubbles, then enters a double coil heating bath where incubation at 37 C begins. Incubation continues as the sample-substrate passes through 80 ft of glass tubing within the heating bath, and then through a mixing coil into the top of a dialyzer unit contained in a 37 C bath. At the same time, the recipient stream, consisting of buffer segmented by air bubbles, is being pumped into the bottom of the dialyzer unit. In the dialyzer, p-nitrophenol produced from the substrate by the action of phosphatase enzyme, dialyzes down from the sample-substrate stream into the recipient stream. The recipient stream passes through a flow cuvette in the colorimeter. The %T of solution in the flow cuvette is constantly recorded. The introduction of p-nitrophenol into the cuvette causes a recorder 'peak', the height of which is directly proportional to the concentration of p-nitrophenol, and hence to the concentration of enzyme in the original sample.

In the work reported here, recordings were made on %T chart paper, chart speed 18 inches/hr, using filters transmitting at 420 mμ. Sampling rate is 40 samples per hour.

Procedure

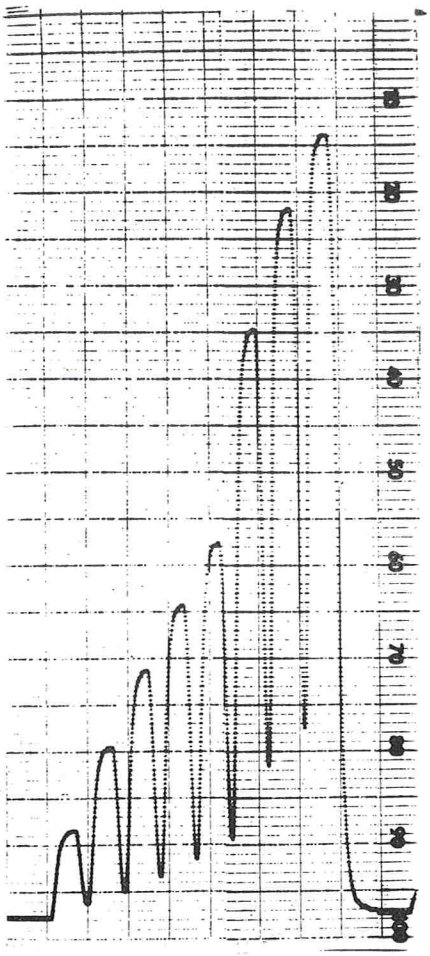
Buffered substrate and buffer are pumped through the manifold. When the system is filled, with the dialyzer recipient stream passing through the colorimeter, the baseline is set at 98%T. Standards and milk samples are then aspirated at the rate of 40 per hour, using 2:1 sample to wash ratio. They are incubated at 37 C, and following dialysis, the recipient stream passes through a 15-mm flow cell cuvette, where the absorbance is measured and recorded.

Calculation of Enzyme Activity

A plot of absorbance of the p-nitrophenol standards (or of %T using semi-log. paper) will produce a calibration curve of activity under the conditions of the test. Enzyme activity of samples can be obtained from the graph by location of the absorbance given by the sample. A typical calibration curve with p-nitrophenol standards, and the strip chart recording from which this was constructed, is illustrated in Figure 2. Excellent linearity is obtained up to 100 μg with the manifold and flow diagram as depicted in Figure 1.

Results

Portions of a mixed supply of fresh raw milk were heated at 143, 142, 141 and 140 F, for 30, 28, and 26 min, respectively, and the phosphatase determined by the automated method and by the Gilcreas-Davis method *(1)*. For further comparison, raw milk was heated to boiling temperature, cooled, and raw milk added to the boiled in the proportions of 0.05%, 0.1%, 0.2%, 0.4% and 1.0%, v/v. Phosphatase determinations were similarly carried out on these samples. The results of these tests are given in Table 1 and Table 2.



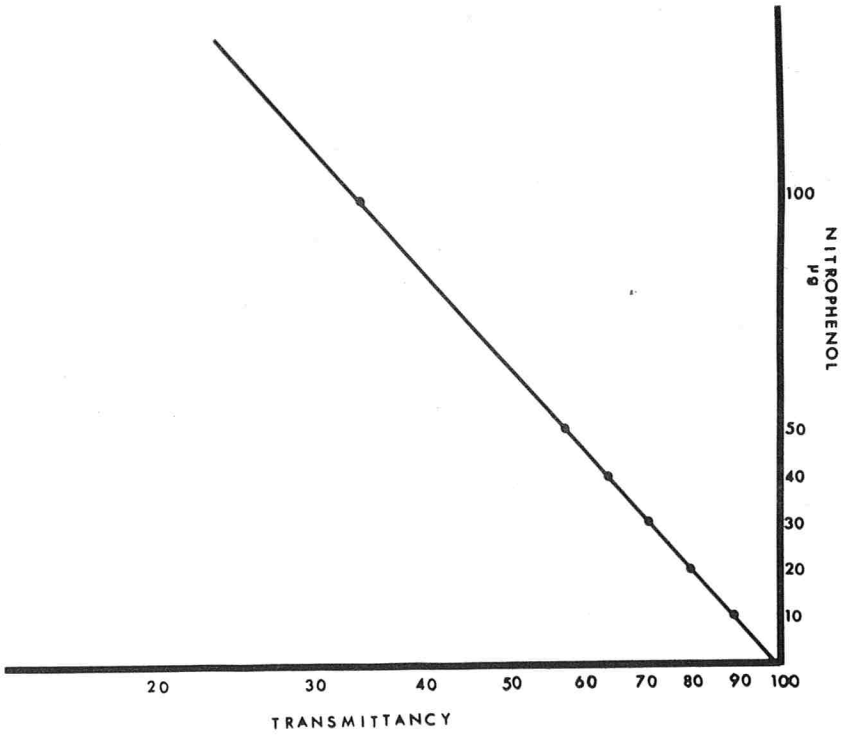


Fig II - Calibration curve, p-nitrophenol standards. Strip chart recording from which it was constructed is at left.

DISCUSSION

Recorded absorbances of p-nitrophenol standards are variable with those factors which can alter the rate of dialysis, irrespective of the incubation time and temperature. If no changes occur in those factors, the recorded absorbances of standards remain very uniform from day to day. On the other hand, the recorded absorbances of samples are a measure of p-nitrophenol production; vary with incubation temperature; and are directly proportional to incubation time. The incubation temperature should be controlled within not more than ± 0.5 C.

In order to calculate enzyme activity in meaningful units which can be compared to standards, and to values obtained by manual techniques and by other laboratories, accurate timing of the incubation period from entry into the heating bath to exit from the dialysis bath is essential. In these laboratories, we have chosen to calculate enzyme activity in terms of micrograms of p-nitrophenol produced per ml of milk in 30 minutes. Our incubation period was timed at 15 minutes, 50 seconds, so that in order to report values in micrograms of p-nitrophenol produced per ml of milk in 30 minutes, readings from the calibration graph were multiplied by a factor of 1.9. The autoanalyzer values in Table 1 and Table 2 have been calculated on this basis.

The system is quite flexible, so that the actual incubation time can be increased as desired, with

TABLE 1. HEAT TREATMENT EXPERIMENT

Milk treatment		Phosphatase	Phosphatase
Temp. °F	Time Min.	autoanalyzer[a]	Gilcrease-Davis[b]
Boiled	—	Nil	Nil
143	30	2.0	64
143	28	3.0	90
143	26	4.0	114
142	30	9.5	232
142	28	15.0	296
142	26	19.0	316
141	30	30.5	516
141	28	34.0	576
141	26	38.0	600+
140	30	74.0	600+
140	28	89.5	600+
140	26	108.5	600+

[a]MICROGRAMS p-nitrophenol produced per ml milk per 30 min.

[b]MICROGRAMS phenol produced per ml milk per 24 hrs.

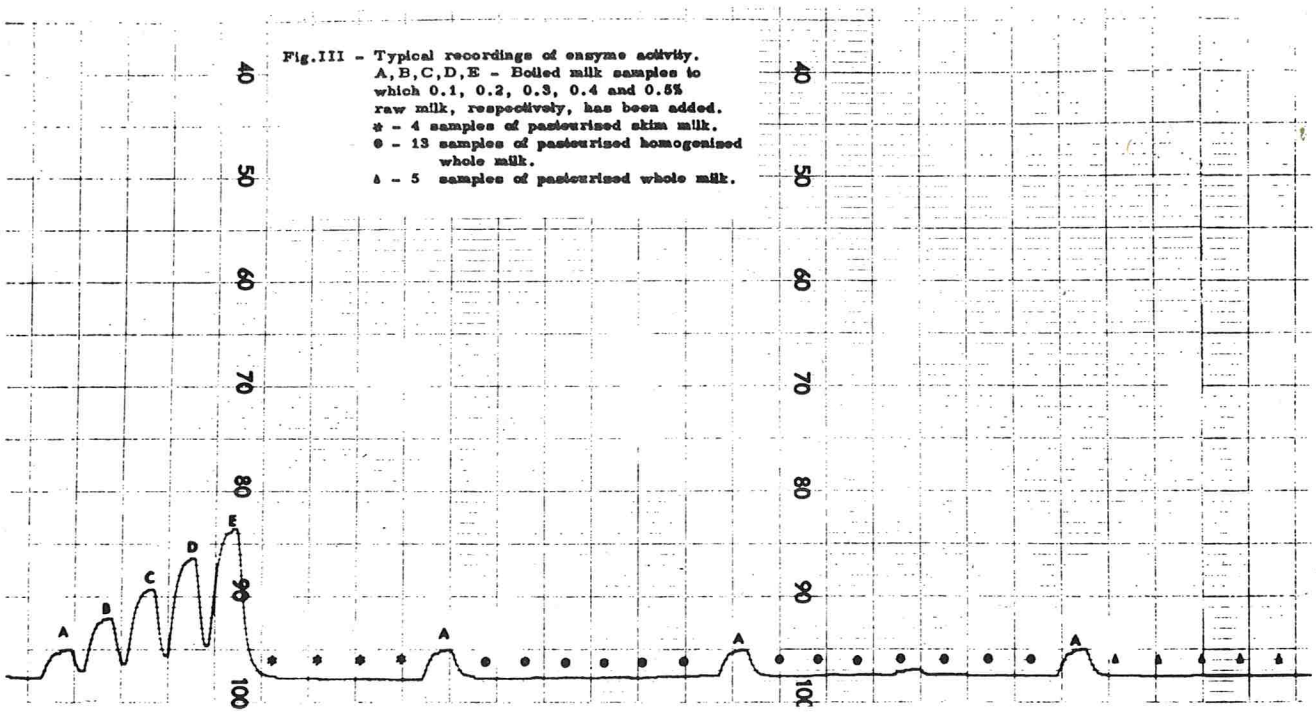


TABLE 2. RAW MILK ADDED TO BOILED MILK

% Raw milk added	Phosphatase autoanalyzer[a]	Phosphatase Gilcrease-Davis[b]
Nil	Nil	Nil
0.05	5.5	128
0.1	7.5	148
0.2	13.0	216
0.3	17.0	260
0.4	21.0	460
1.0	36.0	600+

[a]MICROGRAMS p-nitrophenol produced per ml milk per 30 min.

[b]MICROGRAMS phenol produced per ml milk per 24 hrs.

a corresponding increase in sensitivity, where accurate quantitation in the lower range of phosphatase activity is desired. For example, the addition of a second double coil heating bath to the system would increase the incubation time to approximately 30 minutes. We have found the shorter incubation period to be adequately sensitive for routine screening of pasteurized milks, which is our principal interest in this procedure.

We have set our screening level at 2 μg of p-nitrophenol per ml of milk. Any samples of milk showing a peak beyond that level are selected for confirmation of underpasteurization by a standard manual method. Figure 3 shows a series of 30 determinations, the first 5 being on boiled milk to which 0.1, 0.2, 0.3, 0.4 and 0.5% raw milk was added. These are followed by twenty-two samples of pasteurized milk. Three samples of boiled milk to which 0.1% raw milk has been added were inserted at random among the pasteurized milks to indicate the ability of this technique to pick them out. Of the pasteurized milks, only one shows any appreciable deviation from the base line, and it is well below our screening level.

Because of their high natural color, it was thought that Guernsey and Jersey milk might possibly interfere with the colorimetric procedure. Many successive runs have indicated that this is not the case. Addition of vitamins A, B complex, C and D did not affect values for milks to which they were added.

Six different chocolate milk drinks were analyzed by this procedure, and even when boiled, introduced a color which gave false phosphatase findings. Preliminary experiments indicate that it may be necessary to run controls. No work has as yet been carried out with other dairy products.

ACKNOWLEDGMENT

The authors are indebted to Mr. Murray Nixon of the Sanitary Bacteriology Laboratory, Ontario Department of Health, for the preparation and manual analysis of samples used in the pasteurization and raw milk addition experiments.

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NOTICE TO MEMBERSHIP

In accordance with our Constitution and By-laws which requires our 2nd Vice President and Secretary-Treasurer to be elected by mail ballot you are hereby notified that President Fred E. Uetz, at the annual meeting in Minneapolis, Minnesota appointed A. Bender Luce, State Dept. of Agriculture, Box 120, Olympia, Washington as Chairman of the Nominating Committee.

Nominations for the offices of 2nd Vice-President and Secretary-Treasurer are now open and any member wishing to make a nomination should send a picture and biographical sketch of his nominee to Mr. Luce not later than March 1, 1967.

Karl K. Jones, Secretary-Treasurer, International Association of Milk, Food and Environmental Sanitarians, Inc.

ANNOUNCEMENT CONCERNING THE SANITARIANS AWARD FOR 1967

Announcement is made that nominations will be accepted for the annual Sanitarians Award until June 1, 1967, and the members of the International Association of Milk, Food and Environmental Sanitarians, Inc. are requested to give consideration to the nomination of individuals whose professional work in the field of milk, food, or environmental sanitation in their communities has been outstanding.

The Award consists of a Certificate of Citation and $1,000 in cash, and is sponsored jointly by the Diversey Corporation, Klenzade Products, Inc., and Pennsalt Chemical Corporation. It is administered by the International Association of Milk, Food and Environmental Sanitarians, Inc., and is presented annually. The next presentation of the Sanitarians Award will be made at the 54th annual meeting of the Association which is to be held at Miami Beach, Florida, in August 1967.

The Executive Board of the Association has established the following rules and procedures governing the Sanitarians Award.

Eligibility:

1. *General Criteria*

To be eligible for nomination the Sanitarians Award offered annually by the International Association of Milk, Food and Environmental Sanitarians, candidates must:

a. Have been a member of IAMFES in good standing for a period of five years prior to the date when the Award is to be presented;

b. Be a living citizen of the United States or Canada who, at the time of nomination, is employed as a professional sanitarian in the field of milk, food, and/or environmental sanitation by a county or municipality; provided that any sanitarian employed by a higher political sudivision, up to and including a State, who works in a capacity and is assigned duties comparable in scope and responsibility to those normally expected of county or municipal sanitarians, shall also be deemed eligible to receive this Award.

Members of the Executive Board, members of the Committee on Recognition and Awards of the International Association of Milk, Food, and Environmental Sanitarians, employees of Federal and State agencies (except as provided above), and industry members shall not be eligible for the Award. Race, sex or age shall not enter into the selection of the Award recipient.

c. Have made a meritorious contribution in the field of milk, food or environmental sanitation, to the public health and welfare of a county, counties, district or municipality within the United States or Canada.

d. Have completed the achievements and contributions on which the nomination is based during the seven-year period immediately preceding January 1, of the year in which the Award is to be made.

2. *Additional Criteria*

a. Co-workers are eligible for nominations if both have contributed equally to the work on which the nomination is based and each independently meets the other qualifications for nomination.

b. Where co-workers are selected to receive the Award, each shall receive a certificate and share equally in the cash accompanying the Award.

c. No person who has received, or shared in receipt of the Award, shall be eligible for renomination for this Award.

Nominations

Nominations of candidates for the Sanitarians Award may be submitted by the Affiliate Associations of the IAMFES, or by any member of the Association in good standing except members of the Executive Board, members of the Committee on Recognition and Awards, and employees of the sponsoring companies. Nominations from persons who are not members of the Association cannot be accepted. No member or Affiliate may nominate more than one candidate in any given year.

Each nomination must be accompanied by factual information concerning the candidate, a resume of his work and achievements, evidence supporting his achievements and if available, reprints of publications. A form for the submission of nominations may be obtained upon request from H. L. Thomasson, Executive Secretary, International Association of Milk, Food and Environmental Sanitarians, Inc., P. O. Box 437, Shelbyville. Indiana.

Submission of Nominations

The deadline for submission of nominations is set annually, and all nominations and supporting evidence must be postmarked prior to midnight of that date. The deadline this year is June 1, 1967. Nominations should be submitted to Mr. W. C. Lawton, Sr., Chairman, Committee on Recognition and Awards.

Selection of the Recipient

The Committee on Recognition and Awards of the International Association of Milk, Food and Environmental Sanitarians, Inc., has full responsibility for selecting from among the candidates nominated the recipient of the Sanitarians Award. In judging the contributions of each candidate, the Committee will give special consideration to (*a*) originality of thought, mode of planning, and techniques employed, (*b*) the comprehensive nature of the candidate's achievements, and (*c*) their relative value as they affect the health and welfare of the candidate's community. The Committee will give consideration also to the efforts of the candidate to establish professional recognition in the community in which he serves, as well as to his research, administrative development, program operation and educational achievements. Additional information or verification of submitted information will be requested when considered necessary by the Committee. Testimonial letters in behalf of a candidate are not desired.

If after reviewing the nominations and supporting evidence, the Committee decides that the work and achievements of none of the candidates have been significantly outstanding, the Award shall not be made. In this connection, it is fundamental that if meritorious professional achievement cannot be discerned the Award shall be omitted for a year rather than to lower the standards for selections of a recipient.

W. C. Lawton Sr., Chairman,
Committee on Recognition
and Awards.
2424 Territorial Road
St. Paul, Minn. 55114

INTERIM REPORT OF COMMITTEE ON DAIRY FARM METHODS—1966.

This interim report by A. K. Saunders, Committee Chairman, describes briefly the progress of each of the Task or Subcommittees. Some of the Task Committees have been so busy that a good report could have been submitted at this time. In the final report next year a lot of information beneficial for the dairy industry should be made available.

Below is listed each Task Committee and what progress they have made.

Antibiotics, Pesticides, and other Adulterants

Milton E. Held, *Chairman*
Donald K. Summers, *Co-chairman*

Outline type of questionnaire was circulated throughout the United States and Canada. Responses were received which indicates a philosophy of "permissable residues" as compared to "zero tolerance" is moving ahead. The problem of "added water" may be largely influenced by C.I.P. systems on dairy farms. The Task Committee intends to investigate this as well as the introduction of detergents and sanitizers. It is hoped that agencies, institutions and the Industry, will examine this possibility to aid the Committee.

Cleaning of Farm Bulk Tanks and Transportation Tanks

Albert R. Pernice, *Chairman*

Reports from the different members of this Task Committee indicate many new aids can be reported next year.

C.I.P. Cleaning of Pipeline Milkers

William Pickavance, *Chairman*

This Task Committee at present is gathering information through the medium of a questionnaire to report on next year. Any thoughts pertinent to the above will be welcomed by the Chairman from anyone in the industry.

Education

Vernon D. Nickel, *Chairman*

All the pamphlets from the many sources, gathered in the last two years are being evaluated and being sent to the Journal to be abstracted for publication. In addition, the Committee is seeking out publications on abnormal milk and tests for the same.

Plastics

Bernard Saffian, *Chairman*

This Committee made inquiries of thirty-six people throughout the United States and Canada as to where plastics were being used in the dairy industry. A table has been prepared from the answers to these inquiries and will be published in detail in the final report next year.

Sediment in Fluid Milk

Elmer Kihlstrum, *Chairman*

This Committee is continuing work on testing for sediment in bulk tank milk as well as the relationship of yellow color to mastitis milk.

RELATION OF DAIRY CATTLE HOUSING TO QUALITY MILK PRODUCTION

James Smathers, *Chairman*

In 1965 this Committee submitted a guideline report for "free-stall loose housing" systems. We are instructed to continue our study, placing emphasis on recommendations for handling fluid manure. From all over the country the committee has assembled information on this subject which will give answers to pertinent questions.

For our final report, subject to full committee approval, this committee will attempt to prepare broad guideline recommendations for fluid manure handling, which may be used on a national basis.

COMPATABILITY OF DETERGENTS TO FARM WATER SUPPLIES AND EFFECT OF SOLUTION TEMPERATURE ON C.I.P. CLEANING OF FARM EQUIPMENT

Stephen Spencer, *Chairman*

This committee has now prepared tables and classifications of the tasks assigned to them for publication in the final report. During the next year the committee will attempt to prepare similar tables to aid the industry as to what chemicals can be mixed together in use solutions.

RELATION OF FARM WATER SUPPLIES TO THE QUALITY OF MILK

Henry Atherton, *Chairman*

This committee accepts responsibility in two areas of interest: (1) to serve as a central committee to receive information relating to farm water supplies and to evaluate the findings and (2) to encourage further study of farm water supplies as they affect the cleaning chemicals which are used in dairy farm sanitation programs. To date, the committee has much useful information and will report it all in full in our final report.

DAIRY FARM MILKING MANAGEMENT

T. A. Evans, *Chairman*

This committee's members are gathering data as to the effect of (a) milking equipment – (b) personnel – (c) cow health – (d) milking procedures – and (e) cow cleanliness on quality milk production.

The Farm Methods Committee is made up of the following Members, State Affiliates with Farm Methods Committees, and Consultants:

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Portland, Ore.

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S. H. Beale,
Detroit, Mich.

C. F. Bletch,
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G. D. Coffee,
Washington, D.C.

C. W. Fielder,
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Toronto, Ont.

D. J. Norton,
Poughkeepsie, N. Y.

A. A. Pais,
Baltimore, Md.

Ethan Rasmussen,
Omaha, Neb.

REPORT OF COMMITTEE ON EDUCATION AND PROFESSIONAL DEVELOPMENT—1966

Although the committee has not undertaken large projects this year, it has initiated several small ones which are long overdue. They are listed and discussed below.

READER'S DIGEST. The Sanitarian profession is still not well understood by the public, so a managing editor of the Readers Digest was contacted and requested to run a story on this profession. He expressed an interest in this matter and will keep it in mind for a future issue. The Saturday Evening Post was also contacted and similar interest was expressed by the science editor of that publication.

EXCHANGE OF NEWSLETTERS. During the year affiliates were urged to exchange newsletters with each other. Only in this way can maximum benefit be derived from the

newsletters. Affiliates were also encouraged to send copies to "Red" Thomasson at I.A.M.F.E.S. Headquarters in Shelbyville, Indiana.

COLUMN IN JOURNAL. The committee has discussed with the editor of the Journal of Milk and Food Technology the possibility of assigning a column each month to the committee. This column would be entitled "The Question Box", or other appropriate name, and would present questions and answers on many aspects of environmental health. The primary objective of this column would be to assist the sanitarian in his never ending quest for more knowledge about his field. The Journal editor has reacted favorably to this suggestion and three columns have been prepared for publication.

"ENVIRO-FACTS". Several mimeographed fact sheets entitled "Enviro-Facts" have been prepared for distribution to the affiliates. These sheets are designed not only to aid the new sanitarian in his training program, but also to refresh the memory of experienced personnel in environmental health. Affiliates are urged to reproduce these sheets in future issues of their newsletters. As one of their projects they could also mail copies to training supervisors in various agencies in the state.

BROCHURE ON CAREER OF THE SANITARIAN. Each year I.A.M.F.E.S. receives many requests from schools and individuals for information on the career of the sanitarian. Unfortunately I.A.M.F.E.S. has not had a suitable brochure to send to these people. However, such a brochure has been prepared by this committee and it is hoped that authorization can be obtained to print it this year.

PUBLIC REQUESTS. The committee has assisted two colleges in drawing up plans for environmental science courses. Responses have also been given by the committee to several other public requests for information about the Association, the career of the sanitarian, or other aspects of the field of environmental health.

COLOR SLIDES. There is a shortage of good 35 mm environmental color slides that may be purchased for use in lectures and in training purposes. The committee has made a start on developing a slide series for use in training food service personnel. When the series is complete, it is hoped that Association approval can be obtained to make the series available for purchase by agencies or individuals. Perhaps slide series on other subjects can be prepared by the committee in the future.

J. R. Patillo, *Chairman*
City Health Dept.
Richmond, Va.

1967 ANNUAL PROGRAM PLANNING COMPLETED

Plans for the formal program for the 54th Annual Meeting of IAMFES at the Americana Hotel at Miami Beach, Florida, August 14-17, 1967, were laid out by the Program Committee at a get-together at Chicago early in December. The Program Committee under the chairmanship of A. N. Myhr, President-Elect, consists of the officers and the Executive Board. The Journal editorial staff, meeting concurrently, participated in the planning.

The program, designed to fit the wide variety of interests of the Association, will be organized along the line of previous annual meetings. General sessions will provide for discussions of subjects of national and international concern and topics of more limited yet timely interest will be scheduled for the section meetings on milk, food and environmental sanitation. One evening will be devoted to the informal group discussions which have been so well attended at past meetings. The annual awards banquet will be held the second evening.

Papers to be presented at the general sessions will include a review of food industry developments in the foreseeable future—world food requirements, new products and processing methods, increased utilization of seafoods and of proteins and other food supplements, waste reduction, transportation and new packaging. A top level representative from the Food and Drug Administration will discuss new programs affecting milk and food sanitation. A representative from industry will give his views on the sanitary control of foods at the retail level. The need for graduate level education for sanitarians will be scheduled as an informative topic. A staff member from the Communicable Disease Center at Atlanta will discuss the role of CDC in the local sanitarian's program.

In view of the rapidly changing situation and ever-increasing concern over salmonella and staphylococcus infections, no program today would be complete without an opportunity for review of current information. A panel tentatively entitled "What the Sanitarian Should Know About Salmonella and Staphylococci" will provide for a three-part discussion covering dairy products, non-dairy products, and environmental and non-food items. There have been surprising developments, particularly in the last category, and this entire panel should prove to be of major interest.

Programs for the section meetings will feature such subjects as new standards for frozen desserts manufacture, aseptic dairy products packaging, milking machine standards, sanitation problems in the citrus industry, mass education of food handlers, food salvage problems and control, and recreational sanitation.

A "stand-out" discussion at one section meeting will concern the IAMFES position of extension of the 3A Standards program to food processing equipment. Another session which will no doubt attract much attention will be an evening discussion group on the utilization of visual aids in education and regulatory programs.

Outstanding speakers having special knowledge and experience in their respective fields have been selected to handle the topics assigned. All in all, like a presidential planning program, it looks like the 54th Annual Meeting program will have something for everyone.

NEWS AND EVENTS

FDA TO STUDY ANTIBIOTICS IN THE POULTRY AND LIVESTOCK INDUSTRIES

The Food and Drug Administration has formed an advisory committee to study the safety of veterinary medical and nonmedical uses of antibiotics with respect to animals and humans.

According to the committee's report, these antibiotics are potentially harmful to humans in two ways. First, certain bacteria may develop a resistance to one antibiotic, and a person or animal later infected with these bacteria could fail to respond to the antibiotic. Second, a person allergic to an antibiotic might become ill if he ate food containing it.

Based on this committee report and other information, FDA has asked sponsors of drugs containing any antibiotic intended for use in food-producing animals to submit data for FDA to determine whether such antibiotics leave residues in the meat, milk, or eggs from treated animals. A policy statement regarding these required data was published in the Federal Register on August 23, 1966.

The data must be presented to FDA before February 19, 1967. If these data are not submitted within the specified period, FDA may take action to revoke prior sanctions or suspend approval for continued marketing of these antibiotics for use in food-producing animals.

In addition to their use in animal feed, two of the antibiotics affected by the study (chlortetracycline and oxytetracycline) are used to a limited extent to preserve uncooked poultry and fish. According to the announcement concerning the advisory committee, FDA plans to revoke pesticide regulations so the antibiotics cannot be used for this purpose. When antibiotics are used for food preservation, spoilage may occur due to other organisms such as mold. The drugs might also be used as a substitute for good manufacturing practices.

PHS TO STUDY EUROPEAN METHODS OF SOLID WASTE DISPOSAL

West German and other European techniques for converting and reusing organic solid wastes are being studied for the Public Health Service to obain data for a national solid waste pollution abatement program according to Dr. Richard A. Prindle, Assistant Surgeon General and Chief of the Service's Bureau of State Services, Environmental Health. The studies are being made by Dr. Samuel A. Hart, Associate Professor on leave from the Department of Agricultural Engineering of the University of California at Davis.

The PHS Office of Solid Wastes, which is working with local and state governments on the national solid waste control program, arranged for Dr. Hart's studies under a continuing plan for exchange of U. S. and West German authorities engaged in environmental health science investigations. Dr. Hart was chairman of the National Conference on Solid Wastes Management held at Davis, California, in April, 1966, and has been conducting research with Office of Solid Wastes financial support. He will spend up to eight months observing and evaluating composting and biodegradation technology at the Braunschweig Agricultural Research Station and other European science centers.

"The technique of converting organic wastes into safe materials for soil conditioning and land reclamation, highly developed in Europe, is of great importance to United States needs for sanitary and efficient methods of large-scale solid waste disposal," Dr. Prindle said. "We recognize that the composition of wastes frequently varies from one locality to another and that European methods often may have to be modified for U. S. conditions. The Office of Solid Wastes must examine all meaningful approaches to waste conversion and reuse in moving forward the national program to end health-hazardous and landscape-marring solid waste practices prevailing throughout the country."

Dr. Prindle pointed out that a full-scale study of composting as a means of safely disposing of municipal refuse and raw sewage sludge has been initiated by the Public Health Service under a joint agreement with the Tennessee Valley Authority and Johnson City, Tennessee. (Journ. Milk & Food Tech., April. 1966). Dr. Hart will devote most of his effort abroad, Dr. Prindle said, in becoming familiar with biodegradation techniques being perfected at the Braunschweig station and participating in scientific projects there, but will observe related activities elsewhere in Europe. Dr. Hart will be assisted by a Public Health Service engineer to be named later.

ACCOMPLISHMENTS OF THE SINGLE SERVICE CONTAINER COMMITTEE

During the 1965 National Conference on Interstate Milk Shipment, the Task Force on Responsibilities of the Public Health Service made the following recommendations which were adopted by the conference:

"The Task Force recommends to the Conference that the Public Health Service be requested to publish a list of approved single-service container manufacturing plants and processors.

"Provided that prior to the publication of such a list, the Public Health Service shall establish criteria for listing; and

"Provided that the Conference establish a committee to assist the Public Health Service in this function."

As a result of this charge a committee was appointed with Dr. Richard Parry, Chief Dairy Division, Connecticut Department of Agriculture and Natural Resources as chairman. The committee was composed of representatives of the paper and plastics industry, Paper Cup and Container Institute, Syracuse University Research Corporation, Dairy and Food Industries Supply Association, National Association of Sanitary Milk Bottle Closure Manufacturers and the Public Health Service.

Following a series of meetings over a four months period sanitary guide lines for single-service container manufacturing plants were developed. These guidelines have been published by the Public Health Service in the form of a bulletin entitled "Fabrication of Single Service Containers and Closures for Milk and Milk Products—Guides Sanitation Standards", Public Health Service Publication No. 1465.

This pamphlet is available from the Superintendent of Documents, U. S. Government Printing Office, Washington, D. C. 20402 at a cost of 15 cents. To use in conjunction with the guide is an inspection form PHS-723-3 Revised 5-66. "Manufacturing Plant Inspection Report-Single Service Milk Containers" also available from the Government Printing Office.

CONFERENCE ON BIOLOGICAL ASPECTS OF PESTICIDES

A conference on "Biological Effects of Pesticides" will be held under the auspices of The New York Academy of Sciences at the Waldorf-Astoria in New York City on May 2-5, 1967. It is believed that this will be the first conference directed especially to members of the scientific community who are in a position to contribute to the further sophistication of research on health and related aspects of pesticides. It is planned to invite world authorities on toxicology, metabolism, mode of action, and epidemiological effects of pesticides to participate as speakers.

Interested persons desiring information concerning the conference should address inquiries to the Executive Director, The New York Academy of Sciences, 2 E. 63rd St., New York, N. Y. 10021.

NEW FDA PUBLICATION ANNOUNCED

FDA Papers, official magazine of the Food and Drug Administration, will be launched in February, replacing several other agency publications.

The initial issue will feature articles ranging from a report on medical advertising to an in-depth story, with color illustrations, on salmonella, the microorganisms that are a common cause of food poisoning.

FDA Papers will be distributed by subscription. It will be published ten times a year, including combined July-August and December-January issues. The magazine will be the first Government periodical for domestic distribution featuring a regular four-color section.

James L. Goddard, M.D., Commissioner of Food and Drugs, said the magazine will help clarify the legal and scientific bases of FDA policies and regulatory activities to the food, drug, and cosmetic business world. In this way, he said, the publication will help carry out one of the major recommendations of the Miles Committee, whose proposals for strengthening the organization of the FDA were released last January. The Committee, headed by Rufus Miles, former Assistant Secretary for Administration, said there was an urgent need for a "clear set of policies" to be issued centrally by the FDA to provide guidelines for regulation and compliance.

FDA Papers will be an important tool in achieving this objective since it will contain specific information from official sources on agency policy in scientific and regulatory matters, Dr. Goddard said. The new magazine will replace several of the agency's present periodicals, including the *Monthly Report of Enforcement and Compliance, Food and Drug Review*, and *Notices of Judgment.*

The subscription price for the magazine will be $5.50 a year and subscription requests should be sent to the Government Printing Office, Superintendent of Documents, Washington, D. C. 20402.

OHIO STATE DAIRY CONFERENCE

The Ohio State University 34th Annual Dairy Industry Conference scheduled for February 14-16, 1967 with the theme "Prospecting for Profits," will have a comprehensive program designed to have something for everyone. The Conference program

will be comprised of seven sections: Milk Supplies, Manufactured Products, Management and Operations, Engineering and Processing, Frozen Dairy Desserts, Cultured Dairy Products, and Laboratory Control. The expected attendance is 500.

Approximately 50 educators and industrialists will participate in the program. These will include the following visiting University Staff members: Dr. G. H. Watrous, Jr., Penn. State Univ.; Dr. R. T. Marshall, Univ. of Missouri; Dr. W. C. Winder, Univ. of Wisconsin; and Dr. H. V. Atherton, Univ. of Vermont.

One feature will be a session on "Modified and Imitation Dairy Foods" with presentations to be made by Dr. D. E. Miller, Durkee's Famous Foods; Dr. Andre Bolaffi, General Foods; and Dr. J. E. Long, Nopco Chemical Company.

The Management Section will feature presentations on "Laboratory Requirements for Packages," "Making Home Delivery Profitable," "Practical Application of Computers in Food Processing Plants," "Management Creativity and the Profit Motive," "Operation Customer - A Lesson for Management," "Profit-Planning: The Twelve-Month Valentine" and "Identifying and Using Management Leadership." Featured speakers will be R. M. Weimer, Ohio Department of Agriculture; Dr. E. F. Baumer, Professor, Dairy Marketing, OSU; Hunt Hamill, President, National Sugar Refining Company, New York; R. C. Charbeneau, Director, Public Relations, Ex-Cell-O Corporation; D. P. Gould, Consultant, Trundle and Assoc., Cleveland; and Dr. W. D. Hitt, Chief, Behavioral Science Division, Battelle Institute, Columbus.

The Engineering and Processing Section will include discussions on "Keeping Automation in Perspective," "Equipment Maintenance Programs for Automated and Mechanized Systems," "Instrumentation Innovations" by W. S. Taylor, Honeywell Instruments Company, "Waste Prevention and Control" and a panel discussion on "Packages, Packaging and Aseptic Filling." Among the speakers will be R. Kresge, Associated Dairy Equipment Manufacturers; Dr. G. H. Watrous, Jr., Penn State Univ.; and R. A. Carlson, Cherry-Burrell Corp.

The Milk Supplies Program will include "An Analysis of the Milk Supply Situation" by Judson Mason, Milk Producers Federation, Washington, D. C.; Dr. R. E. Jacobsen, Dairy Marketing Specialist, OSU; discussions on Milk Production Efficiencies, Research to Increase Milk Production, Milk Quality Problems and their Solution including presentations by Dr. H. V. Atherton, Univ. of Vermont; J. O. Mead, Dairy Equipment Company, Madison, Wisc.; Dr. R. T. Marshall, University of Missouri, and OSU Staff members in Dairy Science and Agricultural Engineering; and a report on Ohio's New Milk Sanitation Program by R. B. Watts, Ohio Health Department and R. C. Adams, Columbus Health Department. A major session will deal with "Fieldmen's Responsibilities in Changing Times" with pertinent discussions by R. M. Cook, Maryland Cooperative Producers Assn., and R. F. Rintelmann, Klenzade Products.

The Frozen Desserts Program will feature two major sessions: one on "Selection and Uses of Ingredients" and the second on "Packaging Advances in Materials, Methods, and Equipment." Among the speakers will be Dr. A. J. Leo, President, National Pectin Company, who will discuss stabilizers; and Dr. W. C. Winder, Univ. of Wisconsin, who will deal with physical principles in ice cream manufacturing. Arrangements are made for other leading industrialists to participate.

The Sections on Laboratory Control and Cultured Products will include discussions on effective quality control programs, the Salmonella situation, instrumental methods of analyzing milk, methods for mastitis detection, milk flavor control, stabilizers for cultured products, economic aspects of cultured and acidified cream production, and fortification of cultured products with milk-solids-not-fat. Among the speakers will be P. Klofkorn, Michigan Milk Producers Assn.; Dr. H. V. Atherton, Univ. of Vermont; Dr. R. Marshall, Univ. of Missouri; R. W. Mykleby, Land O'-Lakes Creameries; Dr. G. H. Watrous, Jr., Penn. State Univ.; and Dr. J. E. Long, Nopco Chemical Company.

MSU TO OFFER JOB TRAINING FOR FOOD INDUSTRY PERSONNEL

Michigan State University will offer a training program for jobs in the food processing industry, beginning in the 1967 fall term. The 18-month short course is designed to train workers for jobs in laboratory and quality control work, processing supervision and technical sales and services.

The course is offered by the MSU Department of Food Science in cooperation with the food processing industry and the Michigan Department of Vocational Education. A placement officer will help graduates find employment with firms processing meat, poultry, dairy products, fruits, vegetables, cereals, beverages and related industries.

The program consists of four terms on the MSU campus, plus six months of supervised on-the-job training in the food processing industry. High school graduation or successful work experience, with a recommendation from an employer, is required for admission. The on-the-job training will provide students with an opportunity to help pay for his education.

Applications for the program may be obtained by

writing to the Short Course Department, 120 Agriculture Hall, Michigan State University, East Lansing, 48823.

SANITARIAN ELECTED TO STATE LEGISLATURE[1]

Ben Boyer, chief environmentalist of the Leavenworth City-Co. Health Dept., was elected state representative from the 37th district of Kansas in the recent general election. A Democrat, this was his first campaign for public office.

Ben was a registered pharmacist at 18; he enlisted in the Navy in 1935. During World War II he was a chief pharmacist mate and sanitation officer with a PT boat task group in the southwest Pacific and saw action in the New Guinea and Phillipine Island territories.

When the war was over he accepted an appointment in the United State Public Health Service. Assigned to the federal prison system, Ben was stationed at Lewisburg, Pa., and then in Leavenworth, Kansas where he served until his retirement two and a half years ago. Since his retirement from the public health service he has been in charge of the environmental health section of the Leavenworth City-Co. Health Dept.

[1]From the Kansas Association of Sanitarians Newsletter, December, 1966.

1967 MEETING OF INSTITUTE OF ENVIRONMENTAL SCIENCE

The Institute of Environmental Science 1967 Technical Meeting and Equipment Exposition will be held in Washington, D. C., April 10 through April 12. The Meeting Program will contain a full technical section presenting state of the art papers, and a concurrently running tutorial section in new environments.

The technical program content this year has been expanded to reflect the broader interest of the membership. It will include sessions on Environmental Management, Environmental Equipment covering both new products and maintenance, Simulation Instrumentation, Space Environment, Earth Environment, Marine Environment, and the specialist areas of Dynamics, Electro-magnetic Interference, and Thermo-environment as associated with high speed flight.

The tutorial section this year is being directed to the new interests of Marine and Space Environments, as well as Instrumentation and Simulation techniques. To make this section authoritative, outstanding technical people in the field are being selected as session chairmen. In the Marine Environment, for example, session chairmen are being selected from the Directorate staff of Woods Hole Oceanographic Institute, Scripps Institute of Oceanography, Hudson Laboratories, Texas A & M Department of Oceanography and the Office of Naval Research.

The Institute of Environmental Sciences is a national professional society of engineers and scientists having the responsibility to duplicate the natural and induced environments of the universe in their laboratories. The national office at 940 E. Northwest Highway, Mt. Prospect, Ill. should be addressed for further information on the 1967 meeting.

PUBLIC HEALTH PROGRAMS REVIEWED AT VENDING INDUSTRY HEALTH COUNCIL MEETING

Revision of the Vending Machine Evaluation manual, the addition of ice-maker construction standards, and approval of remanufactured machines from a public health standpoint were among the topics discussed at a recent Automatic Merchandising Health-Industry Council (AMHIC) meeting during the N A M A Convention and Trade Show of Automatic Merchandising in Chicago.

The Council, which consists of 22 representatives of national health organizations, military and vending industry groups, reviewed N A M A's vending machine evaluation manual which was revised in accordance with the 1965 Revised U. S. Public Health Service "Vending Code". AMHIC members also voted to include ice-maker construction standards in the evaluation manual. A proposal to add remanufactured machines to the machine evaluation program was assigned for study by a special committee.

This was the 10th Annual Meeting of AMHIC. The first was held in December, 1956.

RESEARCH AND DEVELOPMENT ASSOCIATES WILL MEET AT RICHMOND

The 21st Annual Meeting of the Research and Development Associates, Inc., will be held April 11-12-13, 1967, at John Marshall Hotel, Richmond, Va. The general theme of the meeting will be "Food Service—Military—Industrial." Registration information may be obtained from Harlan J. Wills, Lt. Col., AUS-Ret'd., Executive Secretary, Research & Development Associates, Inc., U. S. Army Natick Laboratories, Natick, Mass. 01760.

CENTRAL STATES CEREAL CHEMISTS SYMPOSIUM

The Central States Section of the American Association of Cereal Chemists is presenting its Eighth Annual Symposium for Cereal Chemists and Allied Trades in St. Louis, Mo., February 17-18, 1967. The Symposium should be of definite interest to Cereal Chemists, Millers, Bakers, Food Technologists, and others connected with the manufacture or usage of cereal flours.

The ever increasing search for new products and processes has recently created interest in low priced cereal flours other than wheat. The use of corn, oat, soya, sorghum, rice, rye, and potato flours as ingredients in food and non-food processes have brought about changes that are both useful and novel. The program has been designed around a group of expert speakers so as to provide the most up-to-date knowledge on the milling and utilization of these cereal products.

Further information on the program can be obtained by writing Dr. J. D. Commerford, Anheuser-Busch, Inc., 1101 Wyoming St., St. Louis, Missouri 63118.

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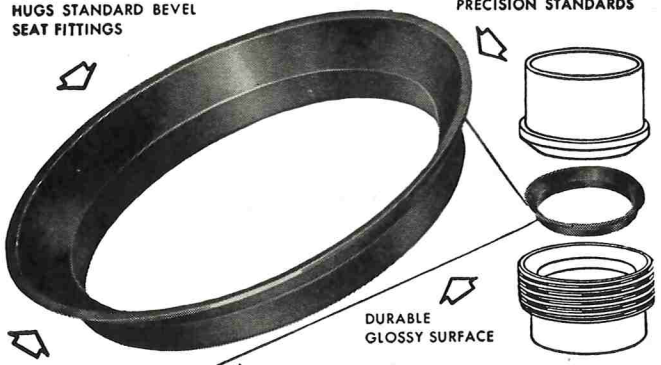
4180 Lorain Avenue • Cleveland 13, Ohio

HAYNES-SPRAY INGREDIENTS CONFORM WITH FDA REGULATIONS AND CAN BE SAFELY USED AS A SANITARY LUBRICANT FOR FOOD PROCESSING EQUIPMENT WHEN USED IN COMPLIANCE WITH A EXISTING FOOD ADDITIVES REGULATION.

HAYNES SNAP-TITE GASKETS

"FORM-FIT" WIDE FLANGE HUGS STANDARD BEVEL SEAT FITTINGS

MOLDED TO PRECISION STANDARDS



DURABLE GLOSSY SURFACE

DESIGNED TO SNAP INTO FITTINGS

LOW COST...RE-USABLE

LEAK-PREVENTING

NEOPRENE GASKET for Sanitary Fittings

Check these SNAP-TITE *Advantages*

Tight joints, no leaks, no shrinkage
Sanitary, unaffected by heat or fats
Non-porous, no seams or crevices
Odorless, polished surfaces, easily cleaned
Withstand sterilization
Time-saving, easy to assemble
Self-centering
No sticking to fittings
Eliminate line blocks
Help overcome line vibrations
Long life, use over and over

Available for 1", 1½", 2", 2½" and 3" fittings.
Packed 100 to the box. Order through your dairy supply house.

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HAYNES
SELF-CENTERING
SNAP-TITE® *Gaskets*

SIZES 1" - 1½"
2" - 2½" - 3"

*MADE FROM
TEFLON®

"The Sophisticated Gasket"

THE IDEAL UNION SEAL FOR BOTH VACUUM AND PRESSURE LINES

Gasket Color . . . slightly off-white

SNAP-TITE self-centering gaskets of TEFLON are designed for all standard bevel seat sanitary fittings. They SNAP into place providing self-alignment and ease of assembly and disassembly.
HAYNES SNAP-TITES of TEFLON are unaffected by cleaning solutions, steam and solvents. They will not embrittle at temperatures as low as minus 200° F. and are impervious to heat up to 500° F.

FOR A FITTING GASKET THAT WILL OUT-PERFORM ALL OTHERS...

Specify . . . HAYNES SNAP-TITES of TEFLON

- *TEFLON ACCEPTED SAFE FOR USE ON FOOD & PROCESSING EQUIPMENT BY U. S. FOOD AND DRUG ADMINISTRATION*

*Gaskets made of DuPont TEFLON® TFE-FLUOROCARBON RESINS

THE HAYNES MANUFACTURING COMPANY
4180 LORAIN AVENUE • CLEVELAND, OHIO 44113

A HEAVY DUTY SANITARY LUBRICANT

Available in both
SPRAY AND TUBE

All Lubri-Film ingredients are approved by F.D.A. and can be safely utilized as a lubricant for food processing equipment when used in compliance with an existing food additive regulation.

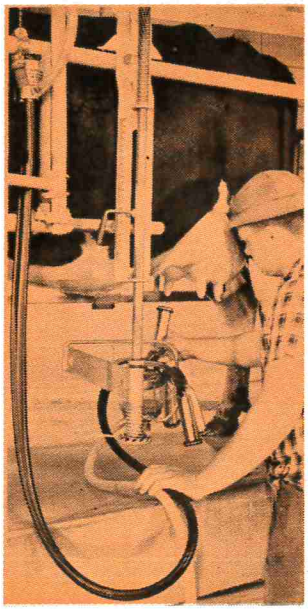
ESPECIALLY DEVELOPED FOR LUBRICATION OF FOOD PROCESSING AND PACKAGING EQUIPMENT

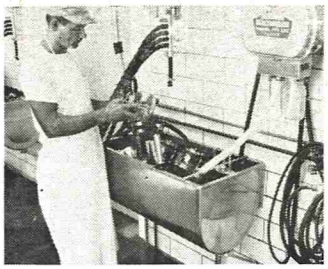
For Use in Dairies — Ice Cream Plants — Breweries — Beverage Plants — Bakeries — Canneries — Packing Plants

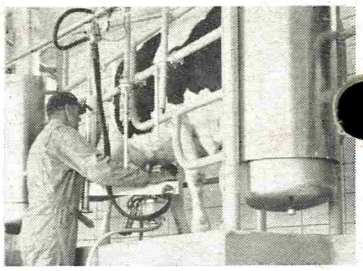
SANITARY • NON TOXIC • ODORLESS • TASTELESS

SPRAY — PACKED 6 — 16 OZ. CANS PER CARTON
TUBES — PACKED 12 — 4 OZ. TUBES PER CARTON

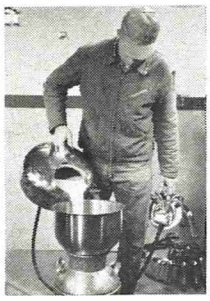
THE HAYNES MANUFACTURING CO.
CLEVELAND 13, OHIO

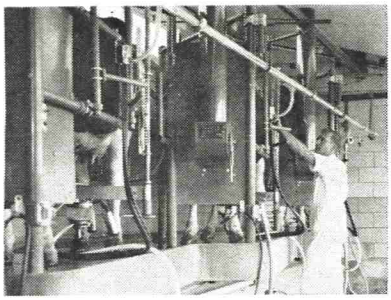


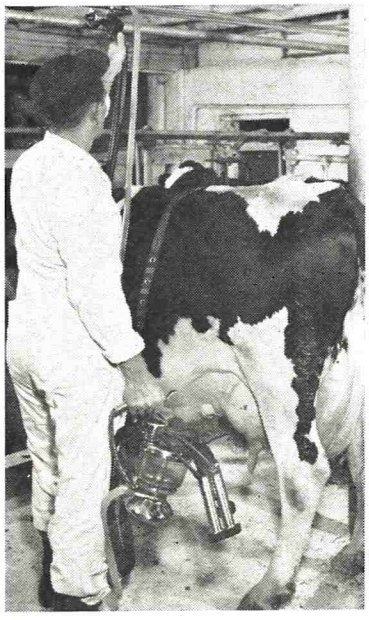


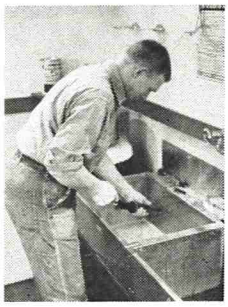


We Are Going To Milk With Thousands of Dairymen During "Surge Better Cow Milking Week" January 23 to 28







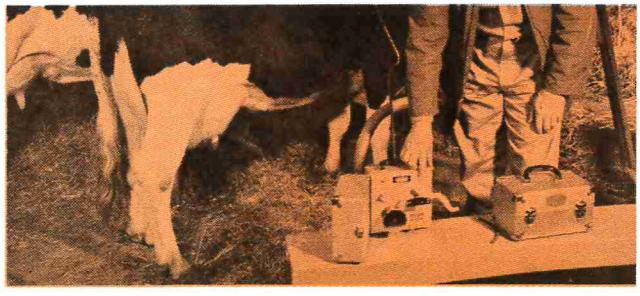


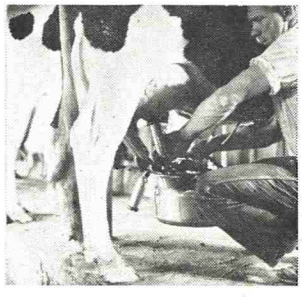
There is no better TIME or PLACE to check the efficiency of your milking equipment than in YOUR barn at milking time.

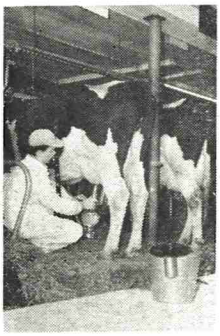
We can't call on all of you . . . everywhere . . . all in one week. But, we are committing every SURGE Dealer, his employees and all qualified BABSON Men to call on FIVE local dairymen at milking time each week night from January 23 through the 28.

These men are going to be there to help YOU by checking both the condition and the performance of your milking equipment . . . to discuss the problem of mastitis with regard to machine operation . . . and to provide helpful pointers on milking techniques.

YOU can also help US by telling us YOUR ideas on dairy management, comment on present equipment and suggestions for future equipment. We want to know how WE can help YOU to make the Future of Dairying more attractive and more profitable.







SURGE

BABSON BROS. CO.
OAK BROOK, ILLINOIS 60521
KANSAS CITY, MEMPHIS, SYRACUSE
MINNEAPOLIS, RENO

BABSON BROS. CO. (Canada) LTD.
REXDALE, ONTARIO