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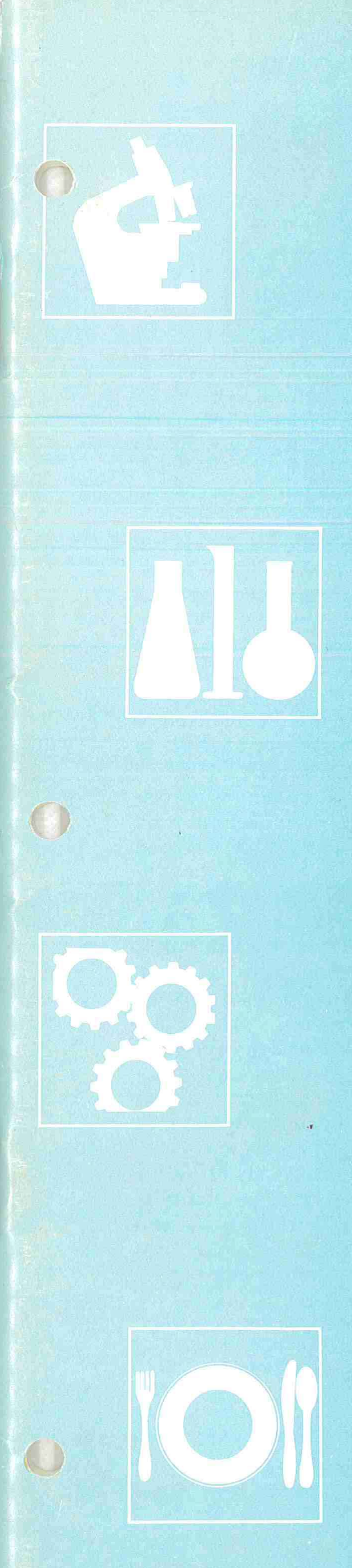

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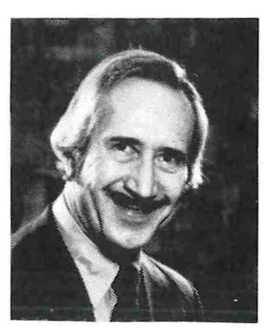
A tribute to America's sanitarians by Robert M. Brown, president of the National Sanitation Foundation. [Reprinted from the new NSF Annual Report.]



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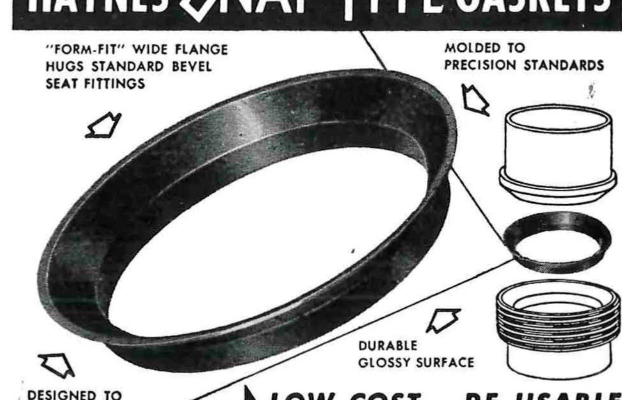

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Effect of Feeding Lactobacilli on the Coliform and Lactobacillus Flora of Intestinal Tissue and Feces from Piglets[1]

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(Received for publication March 24, 1976)

ABSTRACT

Development of fecal *Lactobacillus* and coliform in healthy newborn pigs during the first 48 h after birth was studied. Lactobacilli were detected (10^4 per g) in the feces of newborn pigs as early as 4 h after birth and colifroms by 8 h (10^5 per g). By 24 h the two types were present in near equal numbers (10^8 to 10^9/g). A frozen concentrate of a human isolate of *Lactobacillus lactis* was fed to piglet litters (8 to 10 animals per litter) from the time of their birth. Bottle feeding resulted in reduced fecal coliforms in nursing pigs but lactobacilli were not increased in number. After 54 days of treatment, the *Lactobacillus* to coliform ratio (L/C) was 1280:1; in control pigs not fed lactobacilli, the ratio was 2:1. A continued suppression of coliforms was observed for 30 days after treatment was discontinued. The influence of *Lactobacillus* on the bacterial flora of the gastrointestinal tract was studied. With scouring pigs, enteropathogenic *Escherichia coli* (EEC) were present in larger numbers in tissue homogenates of the tract than in the lumen. The virulence of the EEC found prosent was confirmed by experimental infection in pigs. In control, nonscouring pigs only non-EEC were isolated from tissue sections. In *Lactobacillus*-fed pigs, *E. coli* was reduced to low numbers; also, the few *E. coli* observed were non-enteropathogenic. There were higher numbers of lactobacilli in tissue sections of *Lactobacillus*-fed pigs than in control and scouring pigs. The lactobacilli isolated from tissue homogenates of the treated animals resembled biochemically and serologically (fluorescent antibody staining) the *Lactobacillus* which was fed. Histological studies were done to observe the bacteria in frozen sections of washed intestine obtained from *Lactobacillus*-fed pigs; staining revealed large numbers of gram-positive bacilli. On the other hand, control pigs which died of scouring revealed many coliform types present. Pigs in groups receiving colostrum and lactobacilli did well; no evidence of diarrhea was seen and many lactobacilli were observed in tissue throughout the small intestine. Even after the challenge with EEC serotype 09:K:NM, these two groups of pigs did not show any signs of disease and few coliform types (cocco-bacillary forms) were observed. Pigs not receiving colostrum but only lactobacilli did not scour before challenge and many lactobacilli were present in tissue from the small intestine. However, 72 h after challenge these latter animals revealed symptons of diarrhea and coliforms were seen in the small intestine tissue in addition to lactobacilli.

Since the early studies of Metchnikoff (*18,19,20*) and Stark et al. (*37*), there has been a growing interest in defining the precise role of intestinal bacteria in the health of man and animals. Many reports on this subject were recently reviewed by Sandine et al. (*29*). Preparations of *Lactobacillus acidophilus* are available in pharmacies in various forms and a method for preparing palatable, sweet-tasting acidophilus milk was described in 1959 by Duggan et al. (*8*). An earlier paper by Myers (*21*) also concerned sweet milk containing large numbers of viable *L. acidophilus* cells but details of preparation were not given; Hawley et al. (*10*) have indicated that the effectiveness of lactobacilli in disease therapy requires that large numbers of viable organisms be fed and that a carbohydrate which the organisms can use be available in the intestinal tract. Considering that some lactobacilli are easily killed by freezing and freeze-drying (*12,13,32*), it is important that stability data on preparations available be provided.

For several years, concentrated preparations of different bacteria stored in frozen form have been successfully used in food fermentations to produce sausage, cheese, yogurt, and cultured buttermilk. Such preparations will generally contain 10^{10} cfu/ml, while a fresh conventional culture would contain about 10^8 cfu/ml; thus concentrates provide 100-fold more viable cells than a conventional culture and probably even more cells than are found in dried tablet preparations available in pharmacies.

The commercial availability of frozen concentrates of lactobacilli and the recent renewed interest in the role these organisms play in intestinal health (*29,33,34*) prompted the present study; special emphasis was placed on determining the influence of *Lactobacillus* feeding on numbers of fecal coliforms and lactobacilli present since the adverse economic impact of colibacillosis in the swine industry is of large magnitude and well known (*1*). Possible adherence of lactobacilli to intestinal tissue also was studied by examination of tissue thin sections from sacrificed animals using conventional microscopy and fluorescent antibody staining; the latter approach we used to obtain information on the identity of lactobacilli observed in and recovered from intestinal tissue in relation to those fed.

MATERIALS AND METHODS

Organisms

The *Lactobacillus* concentrate used was obtained from Microlife Technics, Sarasota, Florida in a frozen state. Mr. Stewart M. Farr

[1]*Technical Paper No. 3790, Oregon Agricultural Experiment Station.*

originally isolated the bacterium from the human intestinal tract. The concentrate contained a minimum of 5×10^{10} viable cells/ml when thawed and was stable at this population for at least 48 h after thawing. Because of its source, the organisms was called *L. acidophilus*, though its identity had not actually been determined. Therefore, the concentrate was plated on the medium of Rogosa et al. (*28*) and five single colony isolates were characterized using the tests indicated in the report of the *Taxonmic Sub-Committee on Lactobacilli and Closely Related Organisms* (*2*). The enzymatic method of Mattsson (*17*) was used to determine the type of lactic acid isomer produced. Biochemical properties of the isolates were determined using the API test pack system (Analytab Products Inc., 200 Express St., Plainview, N. Y.). Deoxyribonucleic acid (DNA) extraction and hybridization with one of the isolates were carried out as descibed by Sriranganathan et al. (*36*). Tritium-labeled DNA from *Lactobacillus lactis* ATCC 12315 was used as reference DNA.

Escherichia coli G (serotype 09:K:NM) was provided by Dr. P. J. Glantz, Department of Veterinary Science, Pennsylvania State University. Strain SC of *E. coli* was isolated from fecal liquid excreted by a severely scouring newborn baby pig and strain SC-1 from tissue of the small intestine taken from the same sacrificed animal; both strains were β-hemolytic (Difco blood agar base containing 5% defibrinated human blood), gram-negative rods, formed typical coliform-type colonies on MacConkey's agar (Difco) incubated at 37 C for 48 h and, along with strain G, grew at 45.5 C and produced acid and gas in lactose broth.

Experimental animals

Piglets from the Oregon State University swine herd were used. The herd is operated in confinement on a specific pathogen free-type basis on partially-slotted floors. Animals receive an oral antibiotic (neomycin) at birth; creep feed provided from 10 days to 60-lbs. weight includes ASP 250 (chlortetracycline, sulfamethazine, and penicillin - American Cyanamide Company, New Jersey).

Bottle feeding

Animals were fed *L. lactis* concentrate daily from bottles fitted with rubber nipples as follows, depending on their age: up to 15 days, 10 ml; 16 to 30 days, 15 ml; 31 days to 8 weeks, 30 ml and over 8 weeks 50 ml per pig per day. Animals were fed by litters containing 8 to 10 pigs and control animals came from litters not receiving concentrate.

Sampling

Fecal samples were collected with sterile polyester-fiber-tipped swabs, taking care to prevent contamination from the perineal region. Within 30 min of collection, a 1:10 dilution (wt/vol) was made of the fecal sample in sterile distilled water and mixed well using a vortex mixer to yield a homogeneous suspension. Coliform counts were determined from the number of typical lactose-fermenting colonies on MacConkey's Agar incubated at 37 C for 24 h. *Lactobacillus* counts were based on the number of characteristic colonies found on the agar of Rogosa et al. (*28*) incubated in the presence of 95% nitrogen and 5% carbon dioxide in Torbal anaerobic steel cylinders (Model AJ3, Scientific Products, Seattle, WA) at 37 C for 48 h. Confirmation that colonies appearing on the two media were coliforms and lactobacilli was made on numerous occasions by selecting colonies and examining them microscopically and biochemically.

Bacterial flora of intestinal content and tissue.

To determine how soon after birth lactobacilli and coliforms could be detected in the feces of normal piglets, 24 animals were selected at random and fecal counts were made on samples taken at 4-h intervals during the first 48 h of life. Following this, three replicates of an experiment were conducted to examine the flora of different parts of the intestinal tract of different litters on non-scouring, scouring, and *Lactobacillus*-fed non-scouring pigs. Animals were 3 weeks old and the test group had been fed lactobacilli for 17 days. The parts of the gastrointestinal tract examined encompassed the proximal (first 4 inches) and distal (second 12 inches) parts of the small intestine; fecal samples were also taken for comparison.

Animals (one from each of the three groups) were sacrificed and each intestinal part to be examined was tied to prevent movement of contents from on part to another. Fecal samples from each part were examined for numbers of coliforms and lactobacilli. The remaining fecal material was removed and bacterial adhesion to tissue determined according to Fuller and Turvey (*9*) where the mucosal surface of each of the parts was washed three times with sterile distilled water. The washed tissue was weighed, homogenized and a 1:2 dilution used for enumeration of the same organisms.

Bacteria observed in thin sections of pig intestines

The following groups of animals were studied: (*a*) newborn piglets collected in sterile plastic bags at the time of farrowing to prevent contamination from the surroundings; (*b*) conventionally-raised pigs, 1 week old which were not scouring; (*c*) conventionally-raised pigs, 1 week old which were scouring. Piglets from groups a and b were from the same litter. One animal per group was sacrificed and the small intestines collected. The intestines were divided into nine parts, washed three times with sterile water and frozen sections (3 μ) taken from all parts of the small intestine by means of an Ames microtome cryostat. The sections were gram-stained or stained with toluidine blue using the method of Brown and Brenn (*4*). The experiment was carried out three times.

Influence of feeding Lactobacillus concentrate on bacteria observed in intestinal tissue

A litter of piglets (10 animals) collected in sterile plastic bags at the time of farrowing were grouped as indicated in Table 4. After 72 h animals were sacrificed and the intestines were collected; the unchallenged pig in group I was dead at 36 h while the challenged one was dead at 24 h. The condition of the feces excreted by these pigs (scouring or not) was noted at the feeding intervals. The intestines collected were processed as described above. Tissue and bacteria isolated therefrom were stained by the fluorescent antibody technique; tissue with adhering bacteria was also stained with toluidine blue and gram reagents.

Fluorescent antibody technique

A 3-liter quantity of *L. lactis* was grown in the broth of Rogosa et al. (*28*) and the cells harvested and washed in normal saline six times. The number of organisms per ml was determined by plating serial dilutions on Rogosa's agar. The suspension was mixed (1:1) with complete Freund's adjuvant (Difco) as described in the *Difco Manual Supplement* (*3*). This was stored at 4 C and fresh antigen was prepared every month. Rabbits were initially given 0.5 ml of the antigen intramuscularly and 1.0 ml a week thereafter for each of 5 weeks. On the eighth week, 1.5 ml of antigen were given and the rabbits bled a week thereafter. The agglutination titer of the antisera was determined using a saline suspension of *L. lactis* held at 60 C for 30 min to prevent auto-agglutination. The standard tube agglutination test was used.

Fluorescin isothiocyanate crystalline, and BSA Rhodamine counterstain were obtained from Difco Laboratories, Detroit, Michigan. Antiserum (3.0 ml) was diluted 1:2 with cold (2 to 4 C) saline in the cold room laboratory. A 100% saturated ammonium sulfate solutions was then added dropwise to the serum being stirred with a magnetic stirrer (9 ml of added ammonium sulfate gave a 50% concentration in the serum). After the ammonium sulfate and serum were thoroughly mixed, the mixture was left to stand unstirred in the cold room (4 C). It was then centrifuged at 9000 rpm (5400 × *g*) for 5 min at 0 C and the pellet resuspended in 3.0 ml of 0.85% saline. This suspension was then placed in a previously steamed dialysis tubing (11.2-mm diameter) and dialysed against a liter of water 8 h with a change of water every 4 h. It was then dialysed overnight against a liter of 0.85% saline at 4 C. The protein concentration was determined using the method of Lowry et al. (*15*) with crystalline bovine serum albumin as standard. The antiserum was adjusted to a final protein concentration of 10 mg/ml. Carbonate buffer, pH 9.0, was then added in an amount equal to 10% of volume and the remaining volume (90%) was made up with saline; the mixture was stirred well at 0 C in a closed container. Fluorescin isothioscyanate was then slowly added at the rate of 0.02 mg/mg or protein to the stirring serum mixture, which was then stoppered and stirred overnight. A G-25 coarse Sephadex column was prepared and

the dye-serum mixture was placed in the column. Two layers formed, a layer of densely colored dye on top and the conjugated globulin which migrated. The conjugated protein was collected and filter-sterilized through a Millipore filter (0.45 μ) and was then ready for use.

Staining tissues with the fluorescent antibody (FA) was carried out as follows: Frozen sections of the washed intestines were dried in an incubator at 37 C for 6 to 8 h. They were then fixed in acetone for 20 min and dried again at 37 C for 24 h. They were treated with 0.05 N HCl for 3 to 4 min and fixed in acetone for 20 min and dried at 37 C for 12 to 24 h. The sections were then treated with FA rhodamine counterstain for 30 min at 37 C and washed in FA buffer (Difco) for 20 min with changes of buffer once every 5 min; anti-*L. lactis* serum was applied on the section and incubated at 37 C for 30 min. Slides were washed for 20 min in FA buffer with changes every 5 min and sections were then stained with goat anti-rabbit globulin for 30 min at 37 C. Sections were then washed in FA buffer for 20 min with a change of buffer once every 5 min. Finally, sections were dried and mounted using a No. 1 coverslip and FA mounting fluid (Difco) and examined under the UV microscope.

Challenge by EEC

Pigs were placed in five groups indicated in Table 4. Challenged animals were given 2.5 ml orally of *E. coli* 09:K:NM cells grown 15 h at 37 C in MRS broth and resuspended in the same volume of 0.85% NaCl. The feeding scheldule indicated in Table 4 was then begun. All the pigs were on the test for 72 h; scouring results were recorded. At 72 h after feeding *E. coli*, pigs were sacrificed and intestines were processed in the same manner as described before.

RESULTS

Concentrate organism

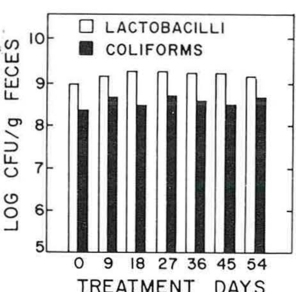
API fermentation tests conducted on the *Lactobacillus* concentrate indicated it was a homofermentative *Thermobacterium*; also, the DNA thermal melting plot revealed a T_m value of 88.5 C and 48.0 moles % guanine plus cytosine (GC) content. Since *L. acidophilus* has a GC content of only 32 to 39% (*23*), it was apparent that the organism had another species identity. Results of the DNA homology studies with known lactobacilli of similar GC content revealed (Table 1) that the concentrate organism had 87 and 72% genetic homology with *L. lactis* and *L. bulgaricus*, respectively. It therefore was called *L. lactis* but shared genes with *L. bulgaricus* also.

TABLE 1. *Hybridization data obtained when tritium-labelled DNA from* Lactobacillus lactis *ATCC 12315 was allowed to reassociate with homologous and heterologous DNA samples*

DNA Source	Cpm		% Homology
	Before hybridization	After hybridization	
L. lactis ATCC 12315 (^{3}H)	2228	—	—
L. lactis ATCC 12315	160	2068	100
Concentrate *Lactobacillus*	429	1799	87
L. salivarius ATCC 11742	2364	0	0
L. bulgaricus ATCC 11842	733	1495	72

Bottle feeding

Preliminary studies conducted with several litters of pigs demonstrated that the bottle feeding of the *L. lactis* concentrate to neonates had a definite suppressing effect on fecal coliform counts. For example, with a litter fed the concentrate for 54 days, mean fecal coliform counts were reduced by 99.9% and the mean *Lactobacillus*: coliform (L/C) ratio changed from near 1:1 to 1290:1 (Fig. 1); after the bottle feeding was discontinued, a continued suppression of the coliform counts was observed during the 30 days until the experiment was terminated. It was observed throughout the study that whenever animals scoured, coliforms increased in number such that the L/C ratio was about 1:1. This is illustrated in Fig. 2 where an 8-pig litter of control animals not fed concentrate was examined for fecal lactobacilli, coliforms, and scouring. At about two weeks of age, when the animals experienced the stress of diet



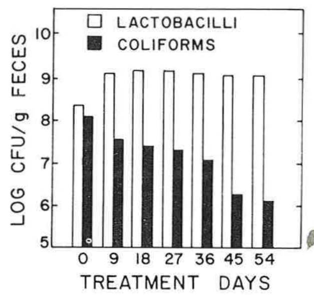


Figure 1. *Geometric means of fecal* Lactobacillus *and coliform counts in 9 control pigs (left) and 8 pigs fed* L. lactis *concentrate (right).*

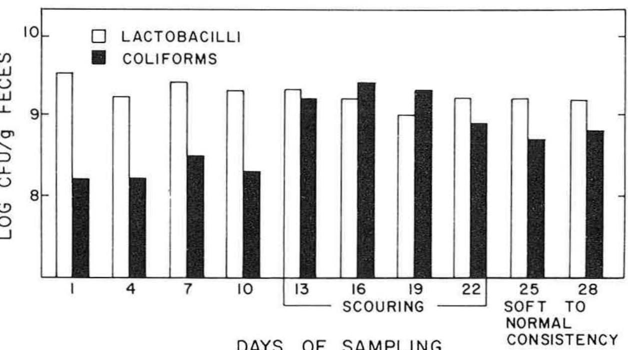


Figure 2. *Geometric means of fecal* Lactobacillus *and coliform counts in 8 suckling pigs from birth to four weeks of age.*

change and were beginning to consume creep feed, scouring was evident and fecal coliforms increased. After the third week as the feces began to assume a more normal consistency, fecal coliforms began to decline in number.

Bacterial flora of intestinal content and tissue

Lactobacilli and coliforms were detected in fecal samples from normal piglet neonates by 4 and 8 h respectively. These first positive samples (geometric means from 24 animals) contained 10^4 cfu/g for lactobacilli and 10^5 cfu/g of feces for coliforms.

Results of the three experiments to examine the intestinal flora of scouring, non-scouring, and lactobacillus-fed pigs were similar in each experiment and typical data appear in Table 2. Ten random colonies taken from MacConkey's agar plates of samples from the scouring animal were found to be *E. coli*; the scouring

pig had 10^{10} coliforms/g of fecal material. Intestinal tissue homogenate from scouring animals had lower numbers of lactobacilli than the *Lactobacillus*-fed animals. Also, homogenates of the intestinal tissue from fed animals had higher numbers of lactobacillli than were found in the scouring or control pigs. This suggested that the lactobacilli were becoming established, thereby reducing the colonization by *E. coli*. The lactobacilli isolated from the tissue homogenate of these pigs were characterized using the API biochemical system as well as by FA staining. The results are presented in Table 3 and Fig. 3 and it can be seen that this *Lactobacillus* was the same biotype as the *L. lactis* strain fed; also, tissue sections revealed flurecing lactobacilli. To ensure that the FA staining was specific, lactobacilli were isolated from normal animals from two differnt herds and used in FA staining employing *L. lactis* fluorescein-conjugated antiserum; no fluorescence reactions were experienced. Also, tissue sections of two animals taken from sows sacrified 48 h after birth revealed no lactobacilli which reacted with the *L. lactis* antiserum.

The *E. coli* strain SC-1 isolated from tissue homogenate of the scouring control animal was further

TABLE 2. *Numbers of coliforms and lactobacilli (cfu/g) found in contents and homogenate of different parts of the small intestine of sacrified three-week old piglets*[a]

	Coliforms				
	Proximal		Distal		
Animal[b]	Contents	Homogenate	Contents	Homogenate	Feces
Normal	1,500,000	140,000	7,000,000	220,000	790,000,000
Scouring	400,000	600,000	2,700,000	30,000	10,000,000,000
Lactobacillus-fed	30,000	300	20,000	1,000	10,000
	Lactobacilli				
	Proximal		Distal		
	Contents	Homogenate	Contents	Homogenate	Feces
Normal	10,000,000	1,600,000	45,000,000	220,000	410,000,000
Scouring	180,000,000	1,500,000	720,000,000	50,000	230,000,000
Lactobacillus-fed	940,000,000	9,300,000	620,000,000	2,700,000	970,000,000

[a]Typical data from one of three experiments with three different litters of at least 8 pigs in each litter.
[b]One animal from three different litters (normal, scouring, Lactobacillus-fed) was sacrified and contents and homogenate examined for lactobacilli and coliforms as described in Methods section.

TABLE 3. *Characterization of* Lactobacillus *organism isolated from intestinal homogenate of* L. lactis-*fed pigs*

Observation	Result	Observation	Result
Origin	Intestinal homogenate of pigs	Scobitol	—
	fed *Lactobacillus* concentrate	Methyl-d-mannoside	+
Optimum growth conditions	Anaerobic environment at 37 C[a]	Methyl-d-glucoside	—
Growth with:		N acetyl-glucosamine	+
2% NaCl	—	Amygdalin	—
2% sodium taurocholate	—	Arbutine iron citrate	—
2% sodium desoxycholate	+	Aesculine iron citrate	+
Catalase	absent	Salicin	+
Pseudocatalase	absent	d (+) cellobiose	—
Type lactic acid produced	DL	Maltose	+
Fermentation:[b]		Lactose	+
Bromcresol purple	—	d (+) melibiose	—
Glycerol	—	Saccharose (sucrose)	+
Erythritol	—	d (+) trehalose	+
d (—) arabinose	—	Inuline	—
1 (+) arabinose	—	d (+) melezitose	—
Ribose	—[c]	d (+) raffinose	+
d (+) xylose	—	Dextrine	—
1 (—) xylose	—	Amylose	—
Adonitol	—	Starch	—
Methyl-xyloside	+	Glycogen	—
Galactose	—	Arginine	—[c]
d (+) glucose	+	Glucose	+, no gas[c]
d (—) levulose fructose	+	Teepol 0.4%	+
d (+) mannose	+	Teepol 0.6%	—
1 (—) sorbose	—	NaCl 4%	+
Rhamnose	+	NaCl 6%	+
Dulcitol	—	NaCl 10%	—
Meso-inositol	—	ONPG	+
Mannitol	—	Potassium nitrate + glucose	—
		Pyruvic acid (V.P.)	+

[a]95% nitrogen, 5% CO_2 atmosphere in evacuated metal cylinder
[b]API test pack system
[c]These characters, when negative, are typical of thermobacteria

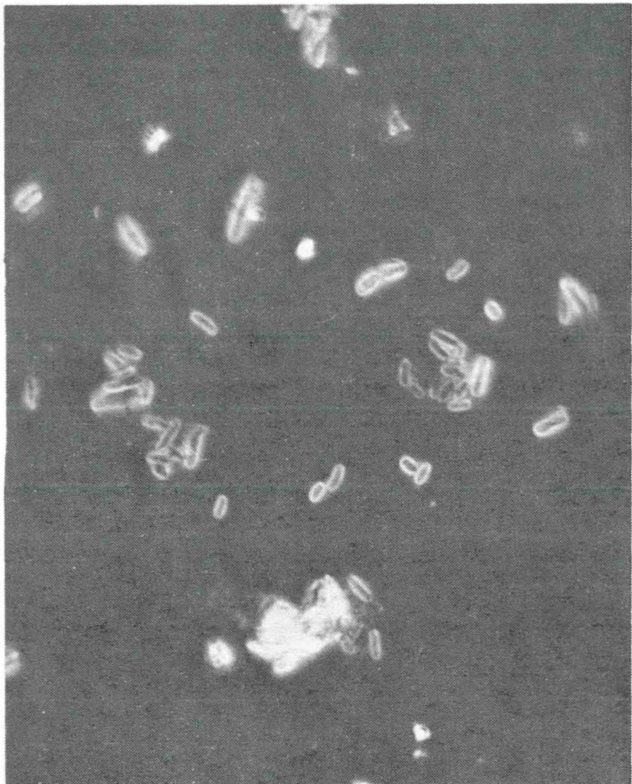


Figure 3. *Fluorescent antibody stained* Lactobacillus *strain isolated from tissue homogenate of* L. lactis*-fed pig.*

examined for its pathogenicity. A broth culture (10 ml) of this hemolytic *E. coli* was fed to a newborn pig and it died within 72 h due to scouring.

Bacteria observed in thin sections of pig intestines

As reported by many workers, the intestines of a newborn pig are sterile; data from the present study also suggested that bacterial colonization was absent in a newborn pig. A typical view can be seen in Fig. 4; no bacteria were observed in thin sections of all parts of the small intestine for animals collected in sterile bags.

Scouring pigs revealed (Fig. 5) large numbers of cocco-bacillary-shaped, gram-negative organisms in all the nine parts of the small intestine. In control non-scouring animals, even though some gram-positive cocci and a few cocco-bacillary organisms were seen, large numbers of gram-positive rod-shaped bacteria (lactobacilli) were found throughout the small intestine (Fig. 6).

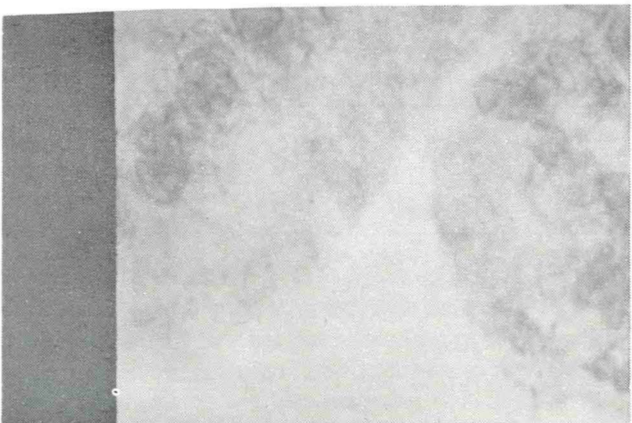


Figure 4. *Photomicrograph of a toluidine blue stained section of the intestine taken from a newborn pig.*

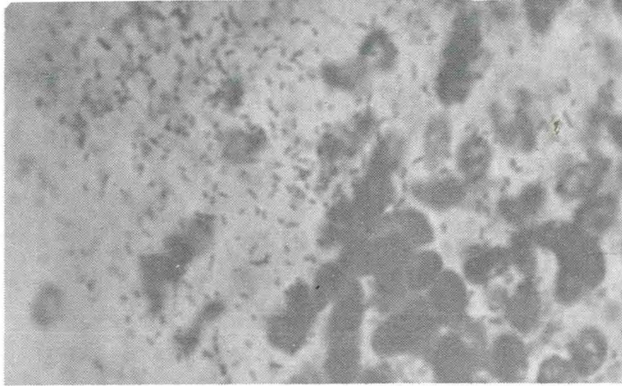


Figure 5. *Cocco-bacillary forms distributed in toluidine blue-stained, frozen sections of the small intestine from a scouring pig.*

Influence of feeding L. lactis *concentrate*

Frozen sections of the intestines from pigs fed concentrates of *L. lactis* revealed (Fig. 7) a large number of gram-positive bacilli throughout. These pigs were kept in a clean place and were fed concentrate: sterile milk (1:1). However, control pigs maintained under similar

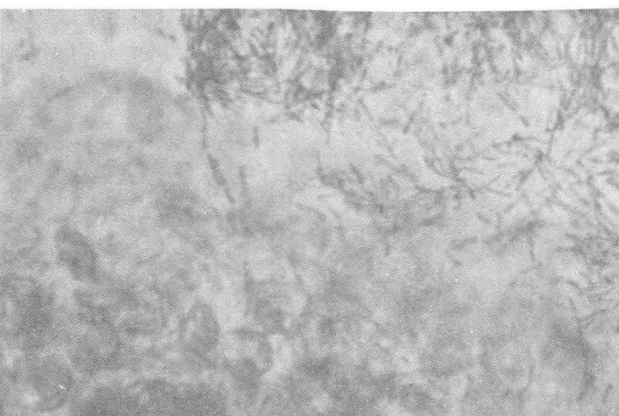


Figure 6. *Photomicrograph of a frozen section of the small intestine from a non-scouring piglet stained with toluidine blue, showing a large number of lactobacilli.*

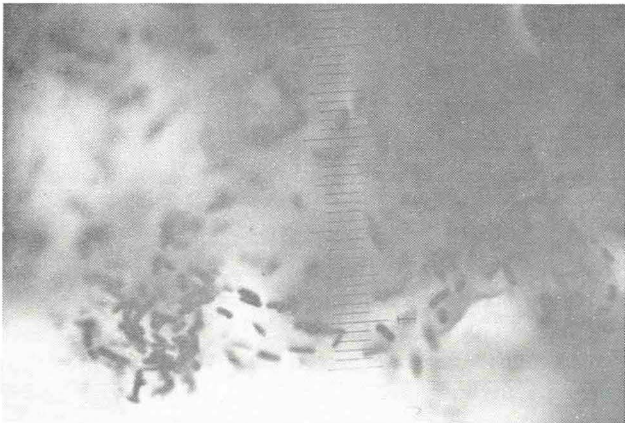


Figure 7. *Gram-stained frozen section of the small intestine from a* Lactobacillus*-fed piglet showing the presence of many lactobacilli.*

conditions (except that they were not fed *Lactobacillus* organisms) revealed a large number of cocco-bacillary organisms throughout the small intestine. In addition, the control pigs died at about 36 h, whereas the test pigs appeared healthy at this time despite never having received any colostrum. Figure 8 shows a typical view observed in control pigs; focusing through the tissue with the microscope revealed many coliform types in all nine segments.

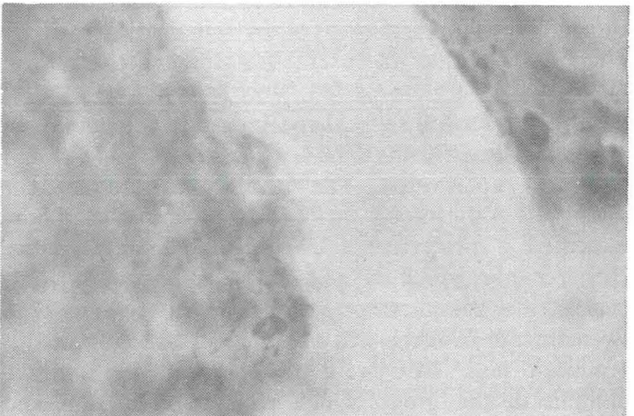


Figure 8. *Photomicrograph of a frozen section of the small intestine from control pigs, showing coccobacillary forms and short rods suggestive of coliforms and lactobacilli.*

TABLE 4. *Scouring and mortality results with ten newborne piglets (2 per group) unchallenged and challenged with EEC 09:K:NM*

Group[a]	Treatment	Scouring		Survival (hours)	
		Un-challenged	Challenged[b]	Un-challenged	Challenged
I	NFM[c]	+	+	36	<24
II	NFM + SOW	—	+	72	72
III	NFM + *L. lactis*	—	+	72	72
IV	NFM + *L. lactis* + sow colostrum	—	—	72	72
V	*L. lactis;* left with sow	—	—	72	72

[a]Pigs in each group were treated as follows:
I-No colostrum was given; animals were fed sterile nonfat milk (20 to 70 ml) every 2 h for the first 24 h and every 4 h for the next 48 h.
II-A 1:1 mixture of sow colostrum and nonfat milk (20 to 30 ml) was fed every 2 h for the first 24 h and every 4 h for the next 38 h.
III-10 ml of *L. lactis* concentrate was given; following this and every 2 h a 1:1 mixture of concentrate and sterile nonfat milk (20 to 30 ml) were fed for the first 24 h and once every 4 h for the next 48 h.
IV-10 ml of *L. lactis* concentrate were given: following this and every 2 h, a 1:1 mixture of concentrate and sow's colostrum (20 ml) was fed for the first 24 h. For the next 48 h, a 1:1 mixture of concentrate and sterile nonfat milk was fed instead of colostrum every 4 h.
V-10 ml of *L. lactis* concentrate were fed and the animals were left with the sow and not weaned.
[b]A 15-h culture was centrifuged and cells suspended in the same volume of 0.85% NaCl. Animals were challenged orally with 2.5 ml of this suspension when feeding began.
[c]NFM = nonfat milk

Scouring and mortality (Table 4) appeared to be related to the number and types of bacteria observed in the thin sections; group I unchallenged animals started scouring and died at abut 36 h, while group II and III animals scoured only after challenge. Colostrum and lactobacilli protected group IV and V animals. Table 5 summarizes the microbial patterns observed in tissue

TABLE 5. *Relative numbers of lactobacilli and coliforms seen in frozen sections of intestine of sacrified animals unchallenged and challenged with EEC as revealed by Gram and toluidine blue staining*

Group	Lactobacilli		Coliforms	
	Unchallenged	Challenged	Unchallenged	Challenged
I	0	0[a]	4+	4+
II	0	1+	4+	2+
III	3+	3+	3+	1+
IV	3+	4+	2+	0
V	4+	4+	1+	0

[a]0 indicates no, 1+ slight, 2+ moderate, 3+ heavy and 4+ very heavy concentrations of organisms seen.

from the five groups of animals before and after EEC challenge. Tissues from *Lactobacillus*-fed animals held many lactobacilli which appeared to prevent appearance of other bacteria. Pigs in group I were given only EEC to check the virulence of the organism and a large number of cocco-bacillary organisms throughout the small intestine was seen; these pigs died at about 24 h. Tissue from group II animals did not contain many lactobacilli when kept away from the sow even though colostrum was fed; coliforms were evident especially after challenge. The third group of pigs which did not receive any colostrum started scouring at about 72 h after the challenge with EEC and frozen sections revealed a significant number of EEC, suggesting that colostrum was required in addition to the concentrates of *Lactobacillus* to prevent establishment by EEC and subsequent scouring. Pigs in group IV and V which received colostrum along with *Lactobacillus* organisms did not scour even 72 h after the challenge and less EEC were observed.

To obtain evidence that the lactobacilli observed in thin sections were the same as those fed, FA-staining was used. Tissue sections were stained and fluorescing *Lactobacillus* organisms (Fig. 9) were seen.

DISCUSSION

In determining the normal bacterial flora of healthy non-scouring pigs, it was noted that the *Lactobacillus* counts were slightly greater (Fig. 1) than the coliform counts. On the other hand, in scouring animals,

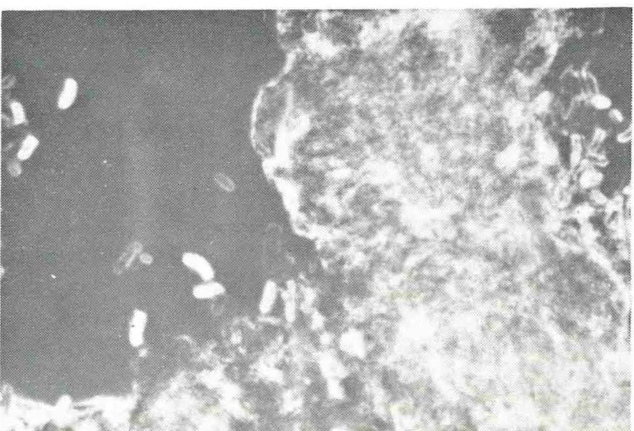


Figure 9. *Fluorescent antibody-stained section of the small intestine from a* Lactobacillus-*fed pig showing the presence of fluorescing lactobacilli.*

invariably the coliform counts were greater than the *Lactobacillus* counts. This is in agreement with work done by Chopra et al. (*6*) who observed an increase in coliform and reduction in lactobacillus counts in scouring pigs. Dubos et at. (*7*) also noted that lactobacilli were predominent in the intestinal tract of mice maintained under unusually clean conditions. This balance between lactobacilli and coliforms is sometimes altered due to unknown stress factors and EEC may increase in numbers and cause colibacillosis under these conditions.

In spite of feeding very large numbers of *L. lactis*, the fecal lactobacilli counts did not increase significantly. This is in agreement with results obtained by Speck et al. (*35*) and Paul and Hoskins (*26*). The reason for this is not known; however, it may be that the lactobacilli fed were colonizing in the small intestine, and only those which were in excess were voided in the feces. Alternately, the lactobacilli might have replaced some indigenous lactobacilli.

Uncertainty exists as to the meaning of reducing fecal coliform counts where animal health is concerned. Intutively, it seems that his should be of benefit. In this regard there have been recent reports indicating the requirements for intestinal pathogenicity by *E. coli* in swine. One of the most important is that the organism becomes localized in the upper part of the pig intestine. Smith and Halls (*30*) found greater numbers of challenge *E. coli* organisms in scrapings of the intestinal wall than in the contents of the small intestine. Thus, they inferred that the strains' ability to proliferate in the anterior small intestine was related to its ability to adhere to the epithelium--a property not possessed by non-pathogenic strains. Also, the diarrhea observed in the present study occurred during the period in which the numbers of *E. coli* in the small intestinal homogenate were maximal. This is compatible with the view that *E. coli* diarrhea results from local action of entertoxin produced by bacteria on the intestinal wall (*31*).

The findings of Kennworthy and Crabb (*14*) that the intestinal tract of healthy pigs is sterile at birth was confirmed in the present study. Later in life pigs come in contact with the surroundings and colonization of the gastrointestinal tract by a variety of micoorganisms results. The bacterial colonization observed in healthy, non-scouring pigs emphasized the importance of a balance between lactobacilli and *E. coli* in maintaining a healthful condition in the intestine. The fecal *Lactobacillus* counts were greater than coliform counts in healthy swine and the reverse in scouring animals. Though not much work has been done on the colonization by indigenous lactobacilli in the pig intestine and their role in the prevention of diarrhea, recent work with infants by Mata et al. (*16*) has given clear evidence that colonization by bifidobacteria in breast-fed children has resulted in a lower incidence of shigellosis and other enteric diseases.

There are many reports describing the possible means by which EEC produce the symptoms of colibacillosis. Porter and Kennworthy (*27*) attributed post-weaning diarrhea in pigs to the increased metabolic activity of *E. coli* in converting proteins to amines. Amines, being irritating and toxic, increase the intestinal peristalsis and thereby produce diarrhea. By feeding *Lactobacillus* organisms Hill et al. (*11*) were able to reduce the fecal amine level and the incidence of diarrhea.

From results obtained ,with the FA technique, it seemed likely that *L. lactis*, when fed to piglets, colonized the small intestine and reduced the colonization by EEC. The FA technique was useful for studying the fate of *Lactobacillus* organisms fed, since it could be used to stain tissues as well as fecal specimens. Results obtained when virulent EEC 09:K:NM was given to *L. lactis*-fed pigs were noteworthy. The inability to produce the symptoms of diarrhea even 72 h after the challenge dose indicated a protective role was played by *L lactis*. However, pigs which did not receive colostrum but only lactobacilli showed symptoms of diarrhea at about 72 h. Even though *L. lactis* was observed in tissue from this group of pigs, EEC were also present. In contrast, animals in the group which received colostrum and lactobacilli showed no signs of diarrhea. This again signified the importance of colostrum in the postnatal health of pigs.

Takeuchi and Savage (*37*) have reported that only certain lactobacilli can attach to the gastric squamous epithelium in the mucosa. This specificity they suggested resided in a particular acid mucopolysaccharide substance produced by the bacteria. Mitsuoka (*24*), reporting on implantation of lactobacilli in the intestine of germ-free chicks, stated that a non-intestinal strain could not become established in the intestine and, even amoung the intestinal strains, species specificity is exhibited. Morishota et al. (*25*) reported that *L acidophilus* ATCC 4356, a human intestinal strain, failed to become established in the intestines of chicks. This again attributed to host specificity. However, the method used by these workers to study the colonization (fecal counts) was indirect and of questionable value.

A strain of *Lactobacillus bulgaricus* has recently been reported (*22*) to neutralize the effect of enterotoxin from *E. coli* pathogenic for pigs; the organism was also beneficial when fed to early weaned pigs. It was noted herein (Table 1) and also by Siringanathan (Ph. D. Thesis, Oregon State University, 1974) that *L. lactis* and *L. bulgaricus* are genetically very similar; therefore certain strains of these organisms may also be beneficial to man where intestinal health is concerned. Since the intestinal tract of pigs is essentially the same as that of man (*5*), further studies with swine may provide data applicable to humans. More studies with human subjects also are needed, however.

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Kinetics of Polychlorinated Biphenyls (Aroclor 1254) in Lactating Bovines and Their Distribution in Dairy Products

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ABSTRACT

Lactating cows were given orally, single or multiple graded doses of polychlorinated biphenyls (PCB) as Aroclor 1254 and the tissue distribution and excretion were measured. Persistence of PCB in milk was determined at all dose levels of PCB administered. A distinct predilection of PCB for tissues with high lipid content was noted. Similarly, PCB appeared in higher concentration in dairy products with high fat content.

It is now common knowledge that distribution of polyschlorinated biphenyls[3] (PCB) in the ecosytem is on a global scale (*14, 27*). These compounds have been detected in various forms of aquatic and terrestrial life (*5, 18 22*) and in human tissues and blood samples (*2, 4, 15, 26*). Contamination of agricultural commodities, including meat-producing animals as well as meat and milk products derived from these animals has been reported (*1, 3, 9, 17, 18, 31*). These biphenyls were used for improvement of chemical and water resistance, flexibility and adhesive properties of plastics, paints, lubricants, hydraulic fluids, etc. (*20*). Their present use is restricted to electrical and electronic industries in capacitors and transformers, especially because of their heat-transfer and dielectric properties.

The very properties of PCB that make them useful industrial applications also prevent them from being degraded once they reach the environment. Since their introduction to industry in 1930, PCB have been accumulating in the environment and have reached the latter from various sources. The largest amounts of PCB reaching the environment are estimated to occur from the industrial and municipal discharges into inland and costal waters (*21*).

Polychlorinated biphenyls possess strong lipophilic properties which, coupled with low biotransformation and excretion rates, result in their accumulation in animal lipids and consequent increase through the trophic levels of the food chain (*22, 27*). The toxicity of PCB in laboratory, wild, and domestic animals has been reviewed (*5, 17, 22*). Pathological lesions attributed to PCB in mammals and man consist mostly of liver lesions (*32*) whereas the most commonly observed lesions in birds are hydropericardium, kidney damage, and reduced spleen size (*32*).

Residues of PCB have been found in the milk of cows. The sources of milk contamination included feeding silage contaminated with PCB from silo sealants containing Aroclor 1254 (*9, 10, 11, 30, 31, 32*), use of discarded transformer oil for defoliant spraying (*8*), drinking water from contaminated streams (*24*), and feeding PCB-containing grain and cereal composites (*3*). The presence of PCB in milk is associated with the unsaponifiable fraction of anhydrous milk fat (*6*). The higher chlorinated isomers of PCB are more eliminated into the milk than are the lower chlorinated biphenyls (*16, 25*).

MATERIALS AND METHODS

Eight actively lactating Jersey cows were given the PCB as Aroclor 1254[4] in a single dose or in 10 consecutive daily doses. The PCB, dissolved in olive oil as a 10% solution, was mixed with the morning dairy concentrate. Animals were treated as follows.

Two cows were given a single dose of 10 mg PCB/kg body weight, the second pair of animals received a single dose of 100 mg/kg, and the third and fourth pairs of animals received 10 daily doses consecutively of 1 and 10 mg/kg, respectively. The first four animals were euthanized 10 days after the single treatment and the other four cows were euthanized 10 days after receiving the last dose.

Total urine, obtained by means of permanently installed catheters, and feces were collected for 2 days before, and 10 and 20 days after the single and first multiple administration of PCB, respectively. Milk was collected twice daily during the same time periods and held for later manufacturing purposes.

The animals were euthanized with sodium pentobarbital and immediately subjected to gross pathological examination. Tissues from selected organs were fixed in formaline and stained with hematoxylin-eosin according to routine procedures. Samples from various organs were removed and stored at − 20 C until analyzed. Half of the brain

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[3]*In North America PCB are manufactured exclusively by Monsanto Co. under the trademark of Aroclor. The Aroclors are complex mixtures of PCB varying from 21 to 70% in chlorine contents and their isomers ranging from mono- to decachlorobiphenyls.*

[4]*Aroclor 1254 is an industrial complex mixture of polychlorinated biphenyls containing 54% chlorine by weight.*

was homogenized before PCB analyses. The PCB were extracted from tissues and various dairy products and the extract cleaned using FDA multipesticide residue method (7) and subsequently detected using a Micro-Tek, Model MT-220 gas chromatograph (Tracor Inc., Augusts, Texas, U.S.A.) equipped with a ^{63}Ni high-temperature electron capture detector. The quantitation of PCB was done by measuring total peak area as the detector response, using Infotronics, Model 208 (Infotronics Ltd., Shannon, Ireland) automatic digital integrator equipped with a baseline tracking and drift corrector.

Dairy products were manufactured from control milk collected before treatment, and from milk of low and high dosed cows. The milk of each treatment was bulked and part of each lot separated for manufacture of spray-dried nonfat dry milk. Skimmilk was given several time-temperature treatments to determine the effect on PCB. These treatments included 1 min exposures at 71, 77, and 82 C, 10 min at 82 C, and 10 min at 82 C with gradual cooling to 55 C over 60 min (the time required to draw the skimmilk into the evaporator). Concentration was in a Rogers laboratory evaporator at 45 C vapor temperature. Concentrate of 32.5% total solids was dried in a Swenson laboratory research spray dryer with inlet air temperature of 188 C and outlet temperature of 88 C. Separated cream which was used to manufacture butter was pasteurized at 71 C for 30 min, cooled, and churned in a 10-liter paddle churn. Separated cream was standardized to approximately 16% milkfat for cultured cream manufacture. It was pasteurized at 71 C for 30 min, homogenized, cooled, inoculated with *Streptococcus cremoris*, and incubated at 21 C. Milk for yogurt manufacture was pasteurized at 71 C for 30 min, cooled to 42 C, inoculated with a mixed culture of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, and incubated for 3 h at 42 C.

RESULTS AND DISCUSSION

Experimental animals did not show any clinical signs of intoxication and upon necropsy, pathological examination failed to reveal any anomalies. Subsequent histological investigation confirmed gross pathology observations.

The concentrations of PCB in tissues of animals are given in Table 1. The highest concentrations of PCB

TABLE 1. *Concentrations*[1] *(in µg/g of fresh weight) of PCB in various tissues of milking cows given single or multiple doses of PCB as Aroclor 1254*

	Single dose		Ten daily doses	
Tissue	10 mg/kg	100 mg/kg	1 mg/kg	10 mg/kg
Brain	0.26	1.47	0.64	0.98
Perirenal fat	9.34	43.69	4.32	63.72
Kidney	0.14	0.49	0.09	0.22
Heart	0.27	1.63	0.76	0.19
Liver	0.48	5.64	0.18	3.30
Diaphragmatic muscle	1.01	7.21	0.04	0.84
Psoas muscle	1.70	8.48	0.25	—
Ovary	0.14	0.07	0.18	0.37
Uterus	0.08	0.09	0.14	<0.01
Adrenal gland	0.40	0.98	0.19	2.32

[1]Mean values of two animals

occurred in the adipose tissues of all animals. In cows given a single dose of PCB, the psoas and diaphragmatic muscles contained the second and third highest level of PCB, respectively. The levels of PCB in the kidney of all animals were low, which indicates a low renal excretion of PCB compounds in milking cows. Brain concentrations, however, were always higher than they were in renal tissues. The relatively high PCB levels in brain are an indication of an important transfer of PCB across the blood-brain barrier. Uterus and ovary were low in PCB but the levels of these pollutants were substantially higher in the adrenal gland.

No attempt was made to detect the presence of metabolites in tissues, milk, feces, or urine. The parent compounds were not, however, detected in urine of all animals analyzed.

The highest concentrations of PCB in milk of cows given a single dose of 10 mg/kg and 100 mg/kg occurred on the second day and were 1.9 ppm and 4.6 ppm, respectively. When the multiple administration of PCB stopped, the average concentrations of PCB in milk of animals given 1 mg/kg and 10 mg/kg were 2.1 ppm. and 5.6 ppm, respectively.

The decline of PCB concentrations in blood and milk is depicted in Fig. 1 and 2. The decline portions of these

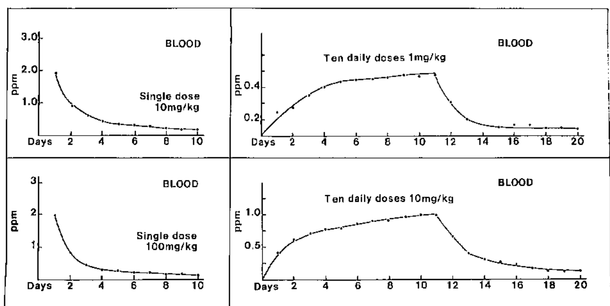

Figure 1. *Concentrations of PCB in the blood of cows as a function of time following the administration of single dose of 10 mg/kg or 100 mg/kg or multiple administration of 1 mg/kg/day or 10 mg/kg/day of Aroclor 1254. Data for each dose level were obtained from two animals.*

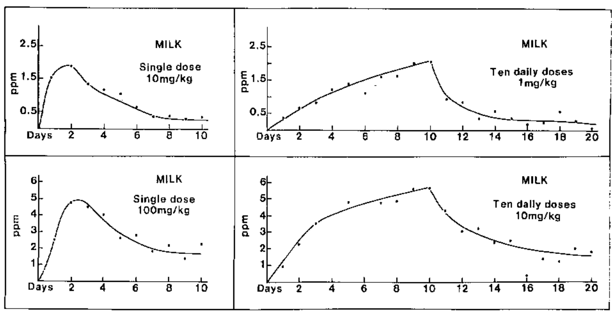

Figure 2. *Concentrations of PCB in the milk of cows as a function of time following the administration of single dose of 10 mg/kg or 100 mg/kg or multiple administration of 1 mg/kg/day or 10 mg/kg/day of Aroclor 1254. Data for each dose level were obtained from two animals.*

curves indicate two compartment systems. The blood half-lives of PCB in animals given a single dose of 10 mg/kg or 100 mg/kg were in the first portion of the curve, 0.8 and 1.1 days, respectively. The corresponding blood half-life following the cessation of PCB administration in animals given miltiple doses of 1 mg/kg was 0.7 day and in those given 10 mg/kg was 1.2 days.

The declines in the milk PCB concentrations were somewhat slower than in the blood. The milk PCB half-lives of the first portion of the elimination curve were 1.4 and 1.8 days in animals given a single dose of 10 mg/kg and 100 mg/kg, respectively, and 1.1 and 1.4 days in cows given multiple doses of 1 mg/kg and 10 mg/kg, respectively.

Cumulative eliminations of PCB into milk and feces of animals given single and multiple daily doses of PCB are depicted in Fig. 3 and 4, respectively. In cows given single

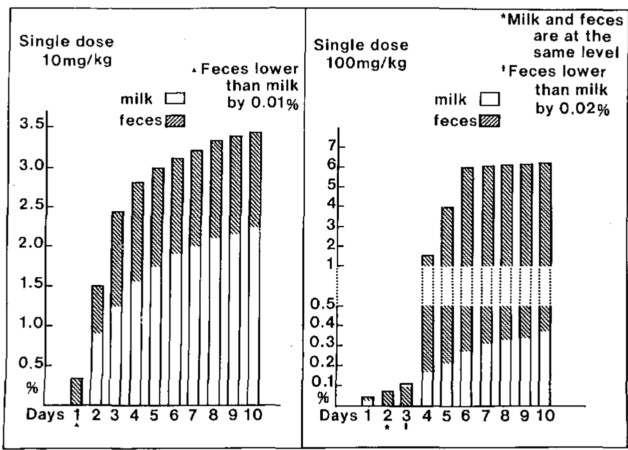

Figure 3. *Percentages of PCB excreted into milk and feces and collected for 10 consecutive days in animals given single dose of 10 mg/kg or 100 mg/kg of PCB. Data are given as cumulative percent of dose administered and obtained from two animals per dose level. Note that both the milk and feces bars begin from the base line.*

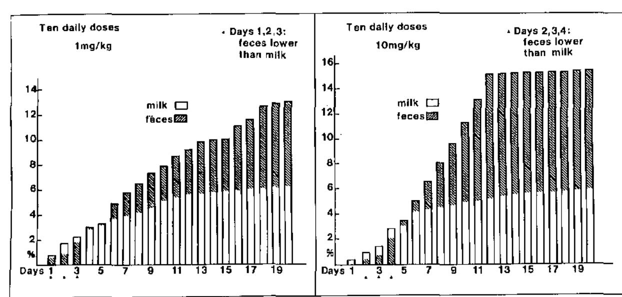

Figure 4. *Percentages of PCB excreted into milk and feces and collected for 20 consecutive days in animals given 10 consecutive daily doses of 1 mg/kg/day or 10 mg/kg/day of PCB. Data are given as cumulative percent of the amount administered and obtained from two animals per dose level. Note that both the milk and feces bars begin from the base line.*

doses of 10 mg/kg or 100 mg/kg, more PCB was eliminated into feces than into milk. Low dosage, however, resulted in proportionally more PCB being eliminated into milk than when cows were given high single doses. Similar results were obtained in cows given multiple doses of PCB. This observation indicates that PCB absorption and consequent elimination in milk is not necessarily proportional to the dose administered to the lactating bovine.

Because of diminishing PCB concentration in the milk of cows given single doses, only the first 4 days' milk from treated cows was used for dairy product manufacture. The bulked milk from cows receiving the higher PCB dosage showed evidence of churning during handling and processing. This sometimes led to difficulties in obtaining representative samples for testing. The same was true to a lesser extent for low dosage milk. The results for PCB and milkfat tests are in Table 2. As expected the PCB followed the fat phase of the milk and dairy products and in all instances the PCB levels were

TABLE 2. *Fat and PCB levels in several dairy products manufactured from milk of control and treated cows*

Product	Control		Low dose		High dose	
	Fat (%)	PCB (μg/g)	Fat (%)	PCB (μg/g)	Fat (%)	PCB (μg/g)
Composite milk	3.83	<0.01	4.38	1.30	3.54	5.90
Skimmilk	0.11	<0.01	0.09	0.04	0.36	0.28
Cream	40.22	<0.01	38.90	12.32	30.60	19.11
Butter	80.14	<0.01	86.14	19.78	85.97	34.10
Buttermilk	0.82	<0.01	0.87	0.61	4.11	5.19
Skimmilk						
71 C-1 min	0.11	<0.01	0.09	0.04	0.36	0.45
77 C-1 min	0.11	<0.01	0.09	0.03	0.36	0.27
82 C-1 min	0.11	<0.01	0.09	0.03	0.36	0.20
82 C-10 min	0.11	<0.01	0.09	0.05	0.36	0.23
82 C-10 min plus 60 min to 55 C	0.11	<0.01	0.09	0.05	0.36	0.16
Nonfat dry milk	1.05	<0.01	1.17	0.15	3.75	0.90
Standardized cream	15.40	<0.01	16.30	6.76	17.52	—
Past'd, homo'd cream + culture	14.47	<0.01	15.34	2.07	16.64	7.02
Cultured cream	14.47	<0.01	15.34	3.97	16.64	8.84
Milk + yogurt culture	3.70	<0.01	3.26	1.58	1.19	1.37
Yogurt	3.70	<0.01	3.26	2.72	1.19	3.20

greater in the higher dose milk. There appeared to be a decline in PCB concentration when skimmilk was heated to 77 C or higher. This supported previous results (*23*). The PCB levels in yogurt and cultured cream appeared to increase in each instance following fermentation. An explanation of this anomaly may be that the conjugated metabolites of PCB and/or the PCB bound to lipoproteins are non-extractable by the method used in the present experiment, but during the fermentation these PCB forms were broken down to their parent compound forms which therefore increased the total PCB present. The low level of fat in the yogurt made from high dose milk is explained by the removal of churned fat before fermentation.

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A Microbial Survey of Various Fresh and Frozen Seafood Products

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ABSTRACT

The microbiological quality of four frozen and seven fresh seafood products (597 units in total) obtained from a local retail store were analyzed. Aerobic plate count means (geometric) ranged from 3.5×10^3/g to 9.3×10^4/g for the frozen products and from 7.8×10^4/g to 2.7×10^8/g for fresh products. Average (geometric) coliform Most Probable Number (MPN) values ranged from 1.0 to 7.7/g for the frozen items and from 7.8/g to 4.8×10^3/g for the fresh seafoods. Employing the MPN method, 4.7% of the 597 units analyzed were positive for *Escherichia coli,* while 7.9% were positive for *Staphylococcus aureus.* Two percent of the samples contained *Clostridium perfringens.* Neither salmonellae nor *Vibrio parahaemolyticus* was isolated in any of the 597 units.

In recent years the subject of microbiological standards for food products has received increasing governmental attention (*5, 6, 7*). The consuming public has demonstrated an increasing interest in being assured that its food supply is safe and wholesome. It is the opinion of many regulatory officials and industry representatives that adoption of microbiological standards for many classes of foods may be inevitable; however, it is felt that these should be based on sound scientific data. This study has focused on the microbiological quality of fresh and frozen seafood products obtained from the retail level. The results of analyses of 597 individual units representing 11 different seafood products are reported.

MATERIALS AND METHODS

Samples were purchased on a weekly basis from a local retail sales establishment and were maintained in the chilled or frozen state, as applicable, before analysis. Products and numbers of units obtained were: frozen catfish filets - 41; frozen flounder filets - 66: frozen salmon steaks - 43; frozen sole filets - 90; fresh clams - 53; fresh cooked crab - 50; fresh salmon steaks - 43; fresh scallops - 51; fresh shrimp - 51; fresh oysters - 59; and fresh prawns - 50. All of the units tested were prepackaged and none were breaded.

Sample preparation

A 25-g portion of each sample was weighed into a sterile 1-liter stainless steel blender cup and 225 ml of sterile buffered water (*8*) was added. The sample was blended at high speed for 3 min, and serial dilutions from 10^{-1} to 10^{-7} were prepared.

Analyses done

Aerobic plate count (APC). Duplicate plates for dilutions 10^{-1} through 10^{-7} were prepared and poured in accordance with *Standard Methods for the Examination of Dairy Products* (*1*). Plates were incubated at 32 C for 72 h.

Total coliform and Escherichia coli *analyses.* Total coliform and *E. coli* Most Probable Number (MPN) determinations were made by the technique described in *Standard Methods for the Examination of Dairy Products* (*1*).

Staphylococcus aureus *analyses. S. aureus* MPN determinations were performed in accordance with the AOAC method (*8*) except that Tellurite Polymyxin Egg Yolk (TPEY) agar was substituted for Vogel and Johnson agar. The tube coagulase test (*8*) was done as needed on isolates from the TPEY agar plates.

Vibrio parahaemolyticus *analyses. V. parahaemolyticus* MPN determinations were performed in accordance with the *Bacteriological Analytical Manual for Foods* (*BAM*) (*2*).

Salmonellae analyses. A 10-ml portion of the 10^{-1} food slurry was transferred in 10 ml double strength lactose broth (DSLB) and incubated at 37 C for 18 h. After incubation 10 ml of the DSLB sample mixture was transferred into 90 ml each of selenite cystine (SC) and tetrathionate (TT) broths and incubated at 37 C. After 18 h of incubation the SC and TT broths were they were streaked onto bismuth brilliant green with sulfadiazine, and *Salmonella-shigella* agars. These sulfite, plates were incubated at 37 C for 18 h; suspect colonies were verified biochemically and serologically following the procedures outlined in *Identification of Enterobacteriaceae* (*10*).

Clostridium perfringens *analyses. C. perfringens* plate count was done in accordance with the procedure outlined in *BAM* (*2*).

Most Probable Number geometric mean calculations. Due to inherent characteristics of the MPN method, the lowest positive number obtainable with a 3-tube series is 3/g, e.g., a 0,0,1 series would give a value of 3 organisms per gram. However, the 3-tube MPN series with the results 0,0,0 is reported as <3/g. Since many of the units analyzed were within the latter group, a value of 1/g was assigned to this series. This technique, used by other investigators (*9*), was necessary to calculate geometric mean values for these MPN analyses.

RESULTS AND DISCUSSION

The aerobic plate count (APC) distributions, ranges, geometric means, and number of samples analyzed for each product are presented in Table 1. The individual APC determinations for frozen products ranged from 2.3×10^2/g in catfish filets to 4.5×10^6/g sole filets. A total of 240 units of frozen fish products were tested. Of

these 70.8% had APCs of less than 1.0×10^5/g, a microbial limit suggested by some researchers (*11*). The International Commission on Microbiological Specifications for Foods (ICMSF) recently published microbial limits for a wide variety of fish products (*14*). This Commission recommended that raw frozen fish products having APCs of less than 1.0×10^6/g should be considered good quality while those having APCs in excess of 1.0×10^7 organisms per gram should be considered unacceptable. Only 2.1% of the frozen products in this study exceeded the ICMSF recommended limit for a good quality product. All frozen products tested were acceptable by the ICMSF recommended limit of 1.0×10^7/g. It must be pointed out that the data presented here were obtained with an APC incubation temperature of 32 C and the ICMSF recommendations are based upon a 25-C incubation temperature. Studies comparing plate counts at different incubation temperatures have indicated that APCs obtained at lower temperatures (7 to 28 C) range from .5 to 1.75 $\log_{10}$ greater than APCs obtained at higher (32 to 35 C) temperatures (*12,13,15*). This variation would negate any comparison of APCs at the two temperatures. In analyzing refrigerated or frozen products, a lower APC value would be expected at the higher incubation temperature (32 C) due to the inhibition of psychrotrophic organisms. However, for purposes of discussion the data obtained in this study will be compared to the recommendations proposed by the ICMSF. The lack of standardized incubation temperatures further justifies the need for careful consideration of various products, their bacterial flora, as well as the need for standardized methodology if realistic guidelines or standards can be established.

Data obtained from the analyses of fresh products are considerably different from those of the frozen products with the exception of fresh clams which compared very closely to the frozen fish products. Fifty-three units of fresh clams were analyzed and the APC mean (geometric) count was 7.8×10^4/g. Again with this product only 3.8% of the samples exceeded the ICMSF recommended limit for fresh products of 1.0×10^6/g to be considered a good quality product. None of the fresh clams tested exceeded the maximum limit for acceptability set forth by the Commission. A possible explanation for this finding is the fact that the products tested were whole unshucked clams which were subsequently shucked under laboratory conditions and weighed into sterile blending vessels. In doing this, the product was not subject to additional handling and processing as are most commercially processed seafood products.

The remaining six fresh products had APCs ranging from 6.8×10^3/g to 3.8×10^9 organisms per gram. Ninety-three percent of these products had APCs of less than 1.0×10^9 while 63.5%, 39.1%, and 31.9% had APCs of less than 10^8/g, 10^7/g, and 10^6/g, respectively. In comparing these data with the ICMSF recommended microbial limit of 1.0×10^7/g for acceptability only 39.1% of the 357 units of the fresh products would comply. The fact that these products have extremely high APCs emphasizes the point that the establishment of microbial guidelines and standards requires an extensive data base and thorough knowledge of the product involved.

The coliform MPN determinations are presented in Table 2 with ranges, geometric means, and the percent of positive samples included. The average (geometric) MPN values for coliforms ranged from <1/g to 7.7/g for the frozen products. The percent of coliform positive samples was 0, 6.9, 40.9, and 48.9 for catfish filets, salmon steaks, flounder filets, and sole filets, respectively. Distribution of the coliform MPN counts for the frozen products

TABLE 1. *Aerobic plate counts for four frozen and seven fresh seafood products*

	Catfish filet frozen		Flunder filet frozen		Salmon steak frozen		Sole filet frozen		Clams fresh		Crab fresh cooked		Salmon steak fresh		Scallops fresh		Shrimp fresh		Oysters fresh		Prawns fresh	
Aerobic plate count range/g	U[a]	CP[b]	U	CP	U	CP	U	CP	U	CP	U	CP	U	CP	U	CP	U	CP	U	CP	U	CP
$<5.0\times10^2$	2	4.9			4	9.3																
5.0×10^2 to 1.0×10^3	6	19.5	1	1.5	2	13.9			1	1.9												
1.1×10^3 to 5.0×10^3	15	56.1	4	7.6	24	69.8	2	2.2	1	3.8												
5.1×10^3 to 1.0×10^4	12	85.4	7	18.2	6	83.7	2	4.4	3	9.4							2	3.9				
1.1×10^4 to 5.0×10^4	5	97.5	21	50.0	6	97.7	17	23.3	17	41.5	1	2.0					4	11.8	1	1.7		
5.1×10^4 to 1.0×10^5	1	100.0	9	63.6			24	50.0	6	52.8	1	4.0	1	2.3			4	19.6				
1.1×10^5 to 5.0×10^5			18	90.9	1	100.0	34	87.8	18	86.8	8	20.0	1	4.7			5	29.4				
5.1×10^5 to 1.0×10^6			4	96.9			8	96.7	5	96.2			1	6.9	3	5.9	1	31.4	3	6.8		
1.1×10^6 to 5.0×10^6			2	100.0			3	100.0	2	100.0	2	24.0	2	11.6	3	11.8	1	33.3	2	10.2	1	2.0
5.1×10^6 to 1.0×10^7											4	32.0	1	13.9	3	17.7	2	37.3	6	20.3	1	4.0
1.1×10^7 to 5.0×10^7											8	48.0	3	20.9	10	37.3	4	45.1	17	49.2	4	12.0
5.1×10^7 to 1.0×10^8											6	60.0	7	37.2	15	66.7	5	54.9	11	67.8	6	24.0
1.1×10^8 to 5.0×10^8											16	92.0	11	62.8	16	98.0	9	72.6	15	93.2	19	62.0
5.1×10^8 to 1.0×10^9											4	100.0	9	83.7	1	100.0	7	86.3	2	96.6	10	82.0
$>1.0\times10^9$													7	100.0			7	100.0	2	100.0	9	100.0
Geometric mean	3.5×10^3		5.4×10^4		3.8×10^3		9.3×10^4		7.8×10^4		2.2×10^7		1.5×10^8		4.6×10^7		1.6×10^7		3.8×10^7		2.7×10^8	
Range	2.3×10^2 to 8.2×10^4		9.8×10^2 to 1.7×10^6		3.0×10^2 to 3.7×10^4		1.7×10^3 to 4.5×10^6		7.9×10^2 to 1.9×10^6		4.8×10^4 to 9.3×10^8		9.4×10^4 to 1.8×10^9		5.4×10^5 to 5.4×10^8		6.8×10^3 to 3.8×10^9		2.7×10^4 to 1.6×10^9		1.2×10^6 to 2.2×10^9	
Total number samples	41		66		43		90		53		50		43		51		51		59		50	

[a]U-Number of items within each count range.

[b]CP-Cumulative percentage of samples within each count range.

TABLE 2. *Coliform MPN determinations for four frozen and seven fresh seafood products*

MPN[a] count range/g	Catfish filet frozen		Flounder filet frozen		Salmon steak frozen		Sole filet frozen		Clams fresh		Crab fresh cooked		Salmon steak fresh		Scallops fresh		Shrimp fresh		Oysters fresh		Prawns fresh	
	U[b]	CP[c]	U	CP	U	CP	U	CP	U	CP	U	CP	U	CP	U	CP	U	CP	U	CP	U	CP
≤3	41	100.0	40	60.6	40	93.0	49	54.4	27	50.9	18	36.0	11	25.6	9	17.7	23	45.1	7	11.9	6	12.0
3.6-19			12	78.8	3	100.0	20	76.7	13	75.5	1	38.0	3	32.6	5	27.5	8	60.8	1	13.6	4	20.0
20-42			6	87.9			12	90.0	4	83.0	4	46.0	4	41.9	1	29.4	1	62.8	1	15.3	5	30.0
43-64			4	93.9			1	91.1	2	86.8	3	52.0			2	33.3	1	64.7			1	32.0
72-150			2	96.9			5	96.7	2	90.6	4	60.0			1	35.3	2	68.6			3	38.0
160-460			2	100.0					5	100.0	6	72.0	4	51.2	12	58.8	5	78.4	3	20.3	13	64.0
530-1,100											2	76.0			4	66.7	3	84.3	1	22.0	2	68.0
1,200-9,500											8	92.0	14	83.7	9	84.3	5	94.1	18	52.5	8	84.0
≥11,000							3	100.0			4	100.0	7	100.0	8	100.0	3	100.0	28	100.0	8	100.0
Geometric mean	1.0		6.4		3.1		7.7		7.8		7.9×10^1		2.5×10^2		1.9×10^2		3.1×10^1		4.8×10^3		2.5×10^2	
Range	—		<3 to 2.4×10^2		<3 to 9.1		<3 to 1.1×10^4		<3 to 4.6×10^2		<3 to 1.1×10^5		<3 to 1.1×10^6		<3 to 2.4×10^4		<3 to 1.1×10^4		<3 to 1.1×10^6		<3 to 1.1×10^4	
% Positive	0%		40.9%		6.9%		48.9%		49.1%		64.0%		74.4%		82.3%		54.9%		88.1%		90%	
Total number samples	41		66		43		90		53		50		43		51		51		59			

[a]MPN-Most Probable Number
[b]U-Number of items within each count range
[c]CP-Cumulative percentage of samples within each count range

TABLE 3. Escherichia coli *values for four frozen and seven fresh seafood products*

MPN[a] count range/g	Catfish filet frozen		Founder filet frozen		Salmon steak frozen		Sole filet frozen		Clams fresh		Crab fresh cooked		Salmon steak fresh		Scallops fresh		Shrimp fresh		Oysters fresh		Prawns fresh	
	U[b]	CP[c]	U	CP	U	CP	U	CP	U	CP	U	CP	U	CP	U	CP	U	CP	U	CP	U	CP
≤3	40	97.6	64	97.0	43	100.0	88	97.8	49	92.5	50	100.0	43	100.0	50	98.0	50	98.0	59	100.0	49	98.0
3.6-19	1	100.0	1	98.5			2	100.0	2	96.3					1	100.0						
20-42			1	100.0																		
43-64																						
72-150									1	98.1												
160-460									1	100.0												
530-1,100																						
1,200-9,500																	1	100.0			1	100.0
≥11,000																						
Geometric mean	1.0		1.1		1.0		1.0		1.3		1.0		1.0		1.0		1.2		1.3		1.1	
Range	—		<3 to 23		—		<3 to 9.1		<3 to 180		—		—		<3 to 3.6		<3 to 1.0×10^3		<3 to 1.1×10^3		<3 to 3.6	
% Positive	0%		3.0%		0%		2.2%		7.5%		0%		0%		1.9%		2.0%		11.9%		4.0%	
Total number samples	41		66		43		90		53		50		43		51		51		59		50	

[a]MPN-Most Probable Number
[b]U-Number of items within each count range
[c]CP-Cumulative percentage of samples within each count range

TABLE 4. Staphylococcus aureus *MPN counts for four frozen and seven fresh seafood products*

MPN[a] count range/g	Catfish filet frozen		Flounder filet frozen		Salmon steak frozen		Sole filet frozen		Clams fresh		Crab fresh cooked		Salmon steak fresh		Scallops fresh		Shrimp fresh		Oysters fresh		Prawns fresh	
	U[b]	CP[c]	U	CP	U	CP	U	CP	U	CP	U	CP	U	CP	U	CP	U	CP	U	CP	U	CP
≤3	41	100.0	58	87.9	42	97.7	74	82.2	48	90.6	47	94.0	42	97.7	48	94.1	49	96.1	53	89.8	48	96.0
3.6-19			8	100.0	1	100.0	15	98.9	2	94.3	1	96.0	1	100.0	3	100.0	1	98.0	5	98.3	2	100.0
20-42							1	100.0	2	98.1	1	98.0										
43-64											1	100.0										
72-150									1	100.0												
160-460																	1	100.0				
530-1,100																			1	100.0		
Geometric mean	1.1		1.3		1.0		1.4		1.3		1.2		1.0		1.1		1.2		1.0		1.1	
Range	<3 to 9.1		<3 to 9.1		<3 to 3.6		<3 to 23		<3 to 93		<3 to 43		<3 to 3.6		<3 to 3.6		<3 to 460		—		<3 to 610	
% Positive	4.8%		15.1%		2.3%		18.9%		9.4%		6.0%		2.3%		5.8%		5.9%		0%		4.0%	
Total number samples	41		66		43		90		53		50		43		51		51		59		50	

[a]MPN-Most Probable Number
[b]U-Number of items within each count range
[c]CP-Cumulative percentage of samples within each count range

corresponds to the findings of other researchers (*9*) for similar products.

Coliform distributions for the fresh products were considerably higher than in the frozen products. Again, fresh clams were the exception in that they compared more closely to the frozen products. The average (geometric) coliform MPN was 4.6/g with the individual sample determinations ranging from <3/g to 4.6×10^2 organisms per gram. Of the 53 units tested, 49.1% were positive for coliform organisms. The remaining fresh products had average (geometric) coliform MPN values

ranging from 18.9/g to 4.2×10^3 organisms per gram. The percentages of coliform positive units for each product were: shrimp -54.9, crab -64.0, salmon steak -74.4, scallops -82.3, oysters -88.1, and prawns -90.0.

The *E. coli* MPN determinations are presented in Table 3 with ranges, geometric means and percentages of positive samples included. The average (geometric) MPN values ranged from <1 to 1.3/g. The percentage of units positive for *E. coli* ranged from 9 to 11.9% for the individual determinations. Of the 597 units tested, 4.7% were *E. coli*- positive by the MPN method. Two units (<1%) had *E. coli* MPN values in excess of 400/g which is the maximum limit for acceptability for fecal coliforms recommended by the ICMSF.

The *S. aureus* MPN determinations with ranges, geometric means, and the percentages of positive samples are presented in Table 4. Average (geometric) *S. aureus* MPN values ranged from <1 to 1.4/g with individual sample determinations ranging from <3 to 610/g. None of the 597 items tested exceeded the recommended limit of 10^3/g suggested by the ICMSF. Ninety-three percent of the products tested had an *S. aureus* MPN value of less than 3/g, a percentage that corresponds to data presented by other investigators (*9*).

C. perfringens was isolated from 12 samples, constituting 2% of all the samples analyzed. Counts ranged from 5 to 20/g. The product and number of samples positive for *C. perfringens* were: frozen flounder filets - 1, fresh clams - 4, and fresh oysters - 7. Due to the ubiquitous nature of this organism these isolations were not unexpected. This does indicate the need for proper handling before and after processing to minimize the possibility of *C. perfringens* food-poisoning.

There were no isolations of *V. parahaemolyticus* or *Salmonella* organisms in the 597 samples tested. Since these organisms have been implicated in food-poisoning outbreaks attributed to seafoods (*3,4*), any guideline or standard pertaining to fresh or frozen seafoods should include a consideration of these organisms.

The data presented clearly indicate that the microflora of seafood products varies considerably. Since many more seafood products are available to the consumer, individual consideration of each product type is necessary before realistic microbial guidelines or standards can be established. In addtion, standardized incubation temperatures and analytical methods should be a primary consideration in developing any microbial guidelines or standards.

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Effect of Neutral Fats and Fatty Acids on Aflatoxin Production [1]

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ABSTRACT

The influence of neutral fats and fatty acids on aflatoxin production by *Aspergillus flavus* (ATCC 15546) was investigated using a chemically defined medium (glucose-salts-amino acids). The fat-fortified medium was inoculated with *A. flavus* spores and incubated at 28 C; samples were solvent-extracted at 3-, 6-, 9-, and 14-day intervals and aflatoxin content quantitated fluorometrically. Increasing concentrations of tricaprylin (15% >10% >5%) repressed aflatoxin G_1 synthesis more than B_1 synthesis as compared to the control. Maximum concentrations of G_1 and B_1 were attained within 3 to 6 days and then declined. Increasing amounts of tricaprylin had little influence on B_1 degradation following 3 days of incubation whereas G_1 degradation was pronounced after 3 days. Tristearin fortification of the medium produced results comparable to those obtained with tricaprylin. Within the 14-day incubation period, G_1 degradation rates exceeded those of B_1 in both the control and fortified samples. As compared to the control, both the 15% linoleic acid and the 15% stearic acid fortification of the medium repressed B_1 and G_1 synthesis; however, the difference became less pronounced with incubation time. The 15% stearic acid fortification facilitated greater B_1 yields than the 15% linoleic acid until the 9th day at which time B_1 accumulation in the linoleic acid fortification surpassed that of the stearic acid. The G_1 level in the 15% stearic acid-fortified medium attained 1300 μg within 3 days and declined to a trace at 14 days. Aflatoxin G_1 synthesis in the 15% linoleic acid-fortified medium was completely repressed throughout the entire incubation period.

Aflatoxins have been produced experimentally on a wide range of substrates including fruit drinks, beans, coconut, peanuts, meats, and cheese (*2, 3, 4, 8, 20, 24*). Because these commodities frequent man's daily diet, extensive research has been directed toward identifying possible factors and conditions enhancing aflatoxin production.

Although much is known about the relative humidity, temperature, gaseous environment, and nutrient requirements for aspergilli growth and toxin production, little information is available regarding the effect of neutral fats and fatty acids on aflatoxin production (*11*).

The objectives of this investigation were threefold: (*a*) to evaluate the effect of a short chain triglyceride (tricaprylin) on aflatoxin production; (*b*) to determine the effect of a long chain triglyceride (tristearin) on aflatoxin production; and (*c*) to investigate the effect of a saturated free fatty acid (stearic) versus a highly unsaturated free fatty acid (linoleic) on aflatoxin production.

MATERIALS AND METHODS

Culture preparation

Aspergillus flavus ATCC 15546, a B_1 and G_1 toxin producing strain, was obtained from the American Type Culture Collection, Rockville, Maryland. Following activation in Czapek Dox broth, the mold was cultured 7 days on Mycological Agar slants at 28 C and harvested by adding 5-10 ml of sterile phosphate buffer containing 0.05% Tween 80 to the slants (*7*). The conidia were decanted, pooled, and the suspension adjusted to an optical density of 0.50 at 550 nm (Spectronic 20, Bausch and Lomb). All adjusted suspensions were washed twice with sterile distilled water (*21*). The standardized suspensions contained 1.8-2.0 × 10^6 spores/ml and were stored at 2 C.

Medium preparation and incubation conditions

Glucose-salts-amino acids medium supplemented with 10 g of Bacto Agar per liter served as the basal medium (*22*). After autoclaving and cooling to 80-85 C, 50 ml of sterile glucose solution (15%) was added to each 100 ml of basal medium. Free fatty acids or triglycerides were uniformly dispersed in the medium by homogenization using a sterilized Club Aluminum Hand Homogenizer. The final concentration of free fatty acids or triglycerides in the culture medium was 0, 5, 10, or 15%. Following homogenization, 20-ml aliquots of the homogenate were placed into petri dishes and rapidly cooled on a table top cooler to uniformly trap the fat in the medium; final pH was 6.0-6.1. Individual plates were inoculated with 1 ml of the standardized spore suspension and incubated at 28 C in the absence of light for 3, 6, 9, or 14 days. All samples were plated in duplicate with the experimental design consisting of two trial determinations.

Tristearin, tricaprylin, stearic acid, and linoleic acid were obtained from ICN Nutritional Biochemicals, Cleveland, Ohio. With the exception of linoleic acid (minimum assay of 75% purity), all fats were pure compounds.

Aflatoxin extraction and purification

Following incubation, aflatoxins were extracted from the agar culture and purified using the method of Shih and Marth (*19*). Aflatoxin extraction entailed blending the entire sample in a Waring blender (1 min) with 190 ml of the monophasic ternary solvent system chloroform: methanol: water (50: 100: 40). Fifty milliliters of chloroform was then added (blended ½ min) followed by the addition of 50 ml of water (blended ½ min). Employing water aspiration, the biphasic system was filtered through a Buchner funnel lined with four layers of cheese cloth to disrupt emulsion formation and remove mycelia. The filtrate was transferred to a 500-ml separatory funnel where the lower chloroform layer containing the aflatoxin(s) was drained into a boiling flask via filtration through a funnel containing .1-.2 g of Celite to remove conidia and other particulate matter. The blender jar and cheese cloth residue were rinsed with an additional 100 ml of chloroform with the rinse being transferred to the original

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methanol: water (100:90) mixture. The chloroform extracts were combined and flash-evaporated until no further reduction in volume occurred.

To prevent interference with thin layer chromatographic (TLC) analysis, a purification step similar to that proposed by Shih and Marth (*19*) was used to remove lipids and/or pigments from the extract. Any emulsions which developed during aflatoxin purification were broken by addition of .3 g of $(NH_4)_2SO_4$ and gentle agitation. All purified chloroform extracts were flash-evaporated to 3-4 ml, transferred to a 10-ml volumetric flask and brought to volume with chloroform. The purified aflatoxin samples were held at room temperature in the absence of light before chromatogram spotting and separation.

Thin layer chromatography procedure

Standard 20 × 20 cm Adsorbosil-1 plates coated with 0.25 mm silica gel (Applied Science Laboratories, Inglewood, California) were used in separating the aflatoxins. The plates were scribed at 1.5 cm intervals to enhance uniform solvent travel and to prevent overlapping and curvilinear arranging of resolved toxins (*18*). All plates were activated 10 min at 100 C and stored in a desiccator 4-5 h before use.

Activated plates were spotted with 5- and 10-μg aliquots of sample extract and 5 μg of aflatoxin standard. The spotted plates were developed in unequilibrated tanks (one plate/tank) containing chloroform: acetone: water (88:12:1.5) as the development solvent (*23*). When the solvent front reached a line scribed 2 cm from the top of the plate (40-50 min), the developed plate was immediately removed, air dried 10 min, and fluorometrically scanned. All aflatoxin analyses were conducted in the presence of reduced illumination to minimize the possibility of toxin degradation.

Primary aflatoxin standards (B_1, B_2, G_1, and G_2) dissolved in benzene: acetonitrile (98:2) were supplied by Dr. L. A. Goldblatt, U.S. Department of Agriculture, New Orleans, Louisiana. The aflatoxin standard was prepared by placing 2 ml of the primary standard into a 10-ml volumetric flask and evaporating to dryness in a vacuum oven before diluting to volume with chloroform. When not in use, the 10-ml aflatoxin standard was wrapped in aluminum foil and stored at -18 C in a chloroform atmosphere.

Aflatoxin quantitation

The silica gel-resolved aflatoxins were quantitated using a Turner Model III Fluorometer. Machine specifications included a 10× sensitivity setting, a narrow pass 7-37 scanning primary filter (366 nm), and a sharp cut 2A secondary filter above 415 nm (*1*). A Model 10 Strip Chart Recorder (Bausch and Lomb) was used to record fluorescent intensity at 10 millivolts. Spots on TLC plates were scanned in order of greatest mobility, $B_1 \rightarrow B_2 \rightarrow G_1 \rightarrow G_2$.

Aflatoxin peak area was obtained by multiplying the peak height times the width at one-half the height, and the average area of duplicate spottings were calculated (*14*). Aflatoxin concentration, μg/20 ml substrate, was determined with the formula employed by Pons et al. (*15*).

Extraction efficiency and purification

Two milliliters of primary standard (10 μg B_1 and 3.0 μg B_2, 10 μg G_1, and 3.0 μg G_2) were added to the basal medium plus various levels of the fatty acids and fat extracted, purified, and fluorometrically quantitated to determine efficiency of recovery (Table 1).

TABLE 1. *Efficiency of aflatoxin extraction from neutral fat-fortified medium*

Sample	Aflatoxin B_1 (10 μg)	Aflatoxin B_2 (3.0 μg)	Aflatoxin G_1 (10 μg)	Aflatoxin G_2 (3.0 μg)
	(% Recovery)			
Control[a]	89.3	105.0	91.5	94.5
Control + linoleic acid	91.0	98.0	93.0	97.0
Control + stearic acid (5%)	86.0	90.0	98.0	93.0
Control + tristearin (5%)	86.0	93.0	87.0	98.0
Control + tricaprylin (5%)	101.0	105.0	94.0	96.0

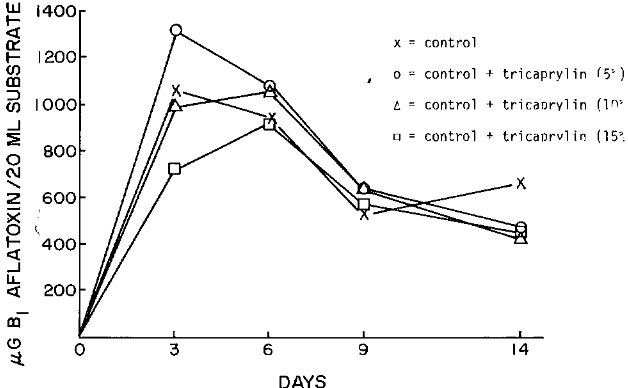
[a]Basal medium

The percentage recovery values are comparable to those reported by Shih and Marth (*19*) when using a similar solvent ratio to extract and purify aflatoxins from spiked cheese samples.

RESULTS AND DISCUSSION

Tricaprylin fortification

Tricaprylin added to the basal medium restricted G_1 synthesis over the entire incubation period (15% > 10% > 5%) (Fig. 1). Compared to the control, the 5% tricaprylin level reduced G_1 formation nearly 500 μg at 3 days of incubation whereas at the 15% level the reduction was nearly 1600 μg. Except at the 3-day incubation time, aflatoxin B_1 synthesis was similar to that of the control (Fig. 1). The 5% tricaprylin level appeared to enhance B_1 production at 3 days of incubation while the 15% level repressed toxin formation. In all instances, B_1 and G_1 concentrations declined following 3 to 6 days of incubation.



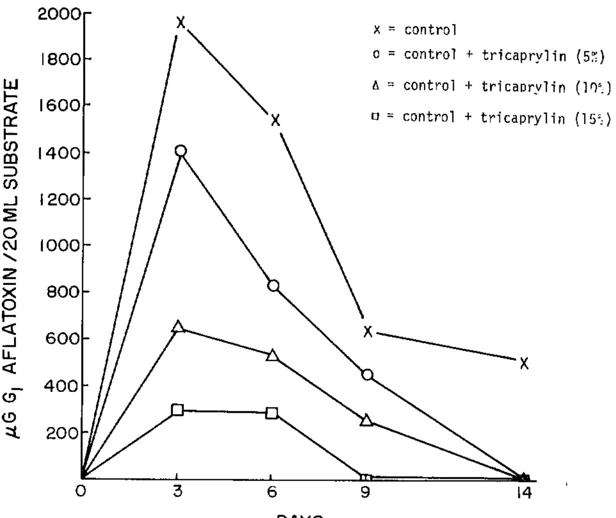


Figure 1. *Effect of tricaprylin on aflatoxin,* G_1 *and* B_1 *production by* Aspergillus flavus *ATCC 15546.*

Based upon the relative proportion of G_1:B_1, G_1 was produced in the control medium at higher quantities than B_1; however, G_1 is also metabolized or degraded more rapidly (Table 2). Increasing levels of tricaprylin not only repressed aflatoxin synthesis, but also on a proportional basis gave a higher yield of aflatoxin B_1 than G_1.

These results are incompatible with the hypothesis advanced by Arseculeratne et al. (*2*) to explain the high

TABLE 2. *Comparison of the ratio of aflatoxin G_1:B_1 on fat-fortified glucose-salts-amino acids medium*

Medium	3 Day	6 Day	9 Day	14 Day
Tricaprylin				
Control	1.86	1.66	1.19	.77
5%	1.07	.77	.71	<.03
10%	.66	.50	.40	<.03
15%	.41	.31	<.02	<.03
Tristearin				
Control	1.61	1.19	.89	.59
5%	1.27	1.20	.68	.54
10%	1.28	.93	.65	.54
15%	1.34	1.02	.63	.53
15% Stearic acid vs. 15% Linoleic acid				
Control	1.57	.63	.76	.60
15% Stearic	1.10	.64	.42	<.05
15% Linoleic acid	.29	<.02	<.02	0

aflatoxin yields on copra. Arseculeratne et al. speculated that *A. flavus* lipolytic enzymes hydrolyzed coconut triglycerides releasing glycerol which is an excellent carbon source for mold growth and aflatoxin production (*6*). Possible explanations for the smaller quantities of B_1 and G_1 in the tricaprylin fortified samples include: (*a*) *A. flavus* lipolytic enzymes hydrolyzed tricaprylin releasing caprylic acid which has fungistatic properties in the 2.0-5.0 pH range (*10*). A subjective rating of mold growth on the inoculated plates (Table 3) lends support to this.

TABLE 3. *Comparison of relative mold growth on fat-fortified glucose-salts-amino acids medium*

Medium	3 Day	6 Day	9 Day	14 Day
Tricaprylin				
Control	++	+++	++++	+++++[b]
5%	+[a]	+	+	+
10%	+	+	+	+
15%	+	+	+	+
Tristearin				
Control	++	+++	++++	+++++
5%	+	++	+++	+++++
10%	+	++	+++	+++++
15%	+	++	+++	+++++
Stearic acid				
Control	++	+++	++++	+++++
15%	+	++	+++	+++++
Linoleic acid				
Control	++	+++	++++	+++++
15%	+	+	+	+

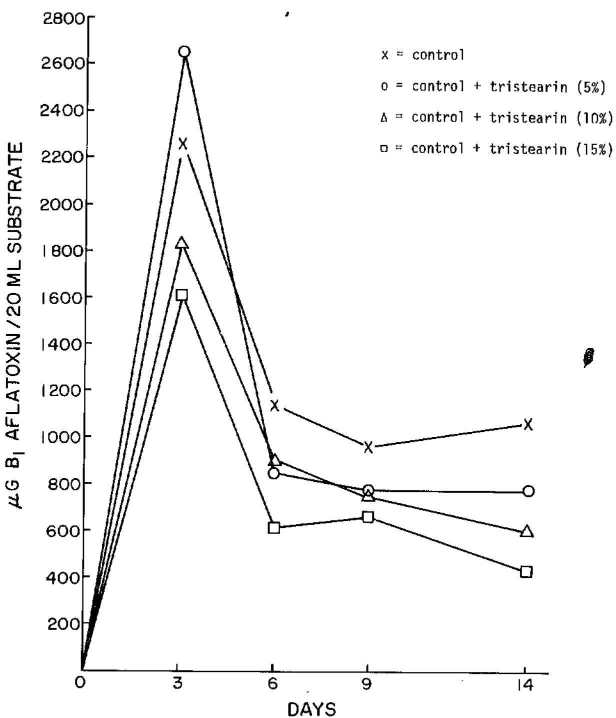
[a]+ = Scanty mold growth
[b]+++++ = Abundant mold growth

Also, within 3 to 6 days of incubation, all samples had attained a 2.2-2.5 pH value; (*b*) the basal medium already was optimal for aflatoxin production and the addition of tricaprylin created a "nutrient imbalance," and/or (*c*) the incubation period was of insufficient duration to permit glycerol utilization. An extended incubation period may have permitted *A. flavus* to utilize glycerol for aflatoxin synthesis, thus resulting in biphasic toxin curves (*1*). However, all of these possibilities fail to explain the ratio differences between G_1 and B_1 as a function of incubation time.

During the 14-day incubation period, small quantities of aflatoxin B_2 and G_2 were also detected by fluorometric analysis. These compounds appeared when the B_1 and G_1 levels declined. Aflatoxins B_2 and G_2 are reported to be derived from the parent compounds by acid-catalyzed conversion or an enzymatic system (*12*).

Tristearin fortification

Fortification of the basal medium with 5%, 10%, and 15% levels of tristearin yielded results comparable to those obtained with tricaprylin, only of greater magnitude (Fig. 2). The 5% tristearin fortification afford-



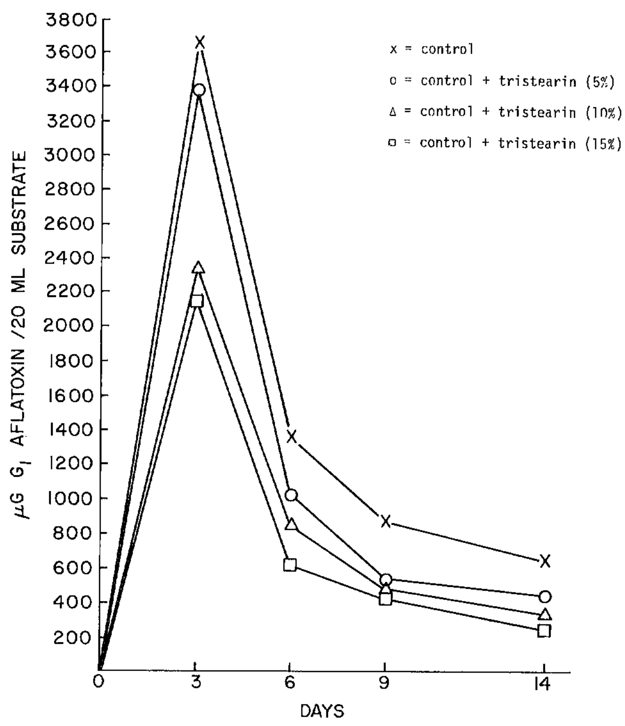


Figure 2. *Effect of tristearin on aflatoxin G_1 and B_1 production by* Aspergillus flavus *ATCC 15546.*

ed B_1 production exceeding that of the control at 3 days (Fig. 2). Increasing tristearin concentrations had a depressing effect on B_1 and G_1 synthesis (15% > 10% > 5%) as compared to the control. Figure 2 also illustrates a rapid decline of B_1 and G_1 production following 3 days of incubation.

Table 2 depicts the G_1:B_1 ratio change with incubation period. Initially, aflatoxin G_1 was synthesized in larger quantities than B_1, but subsequently experienced a more rapid degradation. However, with increasing levels of tristearin, the ratio of G_1:B_1 remained constant.

The tristearin fortification was compared with the tricaprylin fortification on aflatoxin B_1 and G_1 production with the assumption that tristearin would yield proportionately less glycerol than tricaprylin when undergoing lipolytic hydrolysis. Therefore, the tricaprylin system would enhance B_1 and G_1 production due to its greater glycerol content. However, when comparing Fig. 1 and 2, the corresponding controls are radically different, thereby eliminating a valid comparison. The only variable was that the tristearin trial was initiated 24 h later than the tricaprylin trial. In both trials, all plates were inocultaed from the same conidia pool. As shown in Table 3, the tristearin-fortified samples supported mycelial development comparable to the control, and superior to their respective tricaprylin counterparts.

The variability between quantities of aflatoxin production such as described in the above paragraph is not unique to this investigation. Mateles and Adye (*13*) reported that toxin assays of duplicate pairs occasionally differed by 40-50% with most exhibiting less than 20% variability. Likewise, Diener and Davis (*7*) observed "considerable variation between replications and trial runs" using the same *A. flavus* isolate and medium. Explanations accounting for this variability in aflatoxin production are not forthcoming: however, age-of-inoculum studies (*13*) indicated that toxin production was substantially lower with subcultures less than 7 to 11 days old or older than 25 days.

Effect of stearic acid and linoleic acid on aflatoxin production

Although the mechanism(s) or factor(s) associated with aflatoxin disappearance are not well defined, toxin degradation might be more pronounced in a system containing unsaturated fats due to the possible formation of peroxidized radicals. Ciegler et al. (*5*) demonstrated that peroxidized methyl esters of soybean oil partially degraded B_1 in an aqueous system. However, the direct influence of an unsaturated fatty acid on aflatoxin production or subsequent disappearance was not investigated in their study.

Within the 14-day incubation period, the concentration of B_1 and G_1 in the control substrate exceeded that of the 15% linoleic acid and 15% stearic acid fortification (Fig. 3). With the exception of the B_1 concentration with linoleic acid, all samples attained a maximum B_1 concentration at 3 days, followed by a decline. Aflatoxin B_1 concentration in stearic acid exceeded that of linoleic

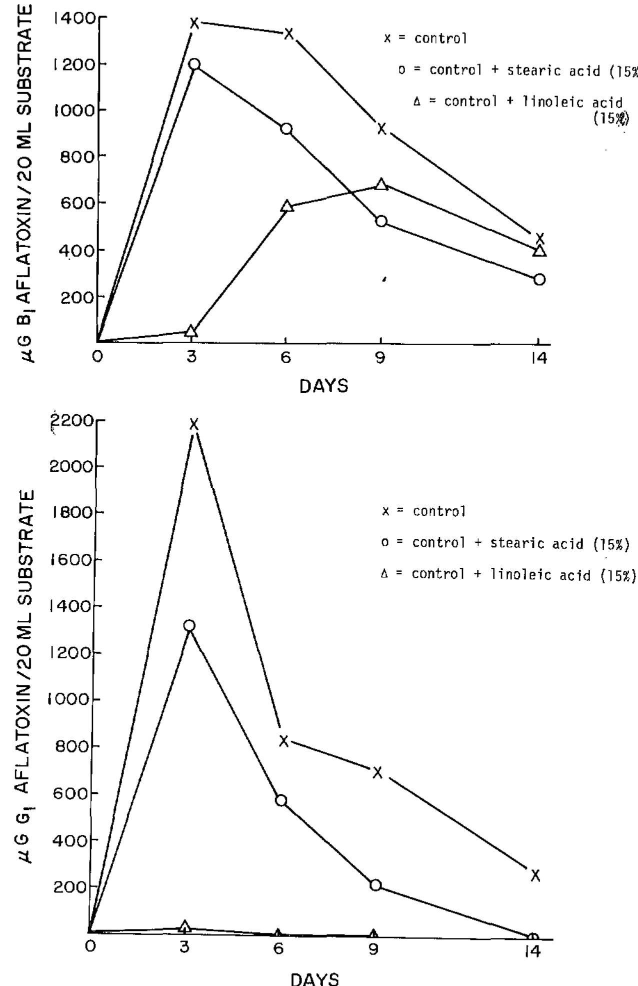


Figure 3. *Effect of stearic acid and linoleic acid on aflatoxin G_1 and B_1 production by* Aspergillus flavus *ATCC 15546.*

acid until 9 days (Fig. 3) at which time the B_1 accumulation with linoleic acid attained a maximum and declined coincident with stearic acid. However, the most pronounced decrease was observed with aflatoxin G_1 (Fig. 3). The aflatoxin G_1 level in stearic acid attained 1,300 μg within 3 days and declined to a trace at 14 days. On the other hand, G_1 synthesis in the 15% linoleic acid system was repressed throughout the entire incubation period.

Based upon a subjective evaluation of mycelial development, the higher initial B_1 and G_1 toxin yields in stearic acid as compared to linoleic acid might be attributed to enhanced mold growth (Table 3). However, the pronounced difference between B_1 and G_1 in linoleic acid is not easily resolved because very little mold growth was observed during the entire incubation period. Although the reason(s) for this inconsistency are not apparent, several possible explanations may exist: (*a*) the formation of peroxidized radicals which have a higher specificity for G_1 than B_1 degradation. Roubal and Tappel (*16*) reported that free radical intermediates of polyunsaturated fatty acids are capable of reacting with and modifying chemical properties of proteins and

enzymes; and/or (*b*) free radical formation may block B_1 conversion to G_1 and dihydroderivatives. Although toxigenic aspergilli vary in their ability to synthesize the different aflatoxins, aflatoxin G_1 in the presence of linoleic acid may be more unstable than aflatoxin B_1 because of the terminal lactone group and consequently degrades to nonfluorescent compound(s).

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Incidence of Toxic and Other Mold Species and Genera in Soybeans

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ABSTRACT

The mold flora of 385 unprocessed soybean samples was determined before and after surface disinfection of the beans with 5% NaOCl. Molds were detected in 99.4% of the 19,250 non-disinfected soybeans examined, and in 52.8% of the 19,250 surface-disinfected soybeans examined indicating that contamination was primarily at the surface, although internal invasion also was substantial. The mold flora, both before and after surface disinfection, was dominated by species of the *Aspergillus glaucus* group, *Asperigillus flavus, Aspergillus candidus, Penicillium cyclopium,* plus species of *Alternaria* and *Cladosporium.* The toxicogenic species *A. flavus, Aspergillus nidulans, Aspergillus ochaeceus, Aspergillus versicolor, Penicillium citrinum, P. cyclopium,* and *Pencillium viridicatum* were encountered regularly in non-surface-disinfected soybeans. Except for *A. flavus* and *P. cyclopium,* the occurrence of toxicogenic species *Aspergillus melleus,* soybeans was sporadic. The toxicogenic species *Aspergillus melleus, Pencillium expansum,* and *Penicillium urticae* were encountered occasionally, but only in non-surface-disinfected soybeans. *Penicillium chrysogenum,* the penicillin-producing organism, was encountered regularly in non-surface-disinfected soybeans, but less frequently in disinfected soybeans.

The amount of soybeans produced yearly in the United States and worldwide is sizeable. In 1973, more than 58 million metric tons were produced worldwide, including more than 1.6 billion bushels from the United States (*1*). Because of their high protein content, soybeans are an important source of livestock food and, usually in processed form, even of human food. Considering these facts, the question arose concerning the presence and the relative prevalence of toxicogenic mold species in and on this agriculture commodity. Reports in the literature indicate that, at present, little is known about the mold flora of soybeans. Dorworth and Christensen (*6*), in a study of soybeans held under controlled temperatures and moistures, reported that species of the *Aspergillus glaucus* group predominated, but that non-speciated isolates of *Penicillium* and *Alternaria* were also encountered regularly. McGee and Christensen (*12*) reported that soybeans held under controlled environmental conditions were dominated by species of the *A. glaucus* group plus *Aspergillus candidus.* Kurata et al. (*11*) isolated 32 fungal cultures from 15 samples of soybena flour but did not itemize the species encountered. Saito et al. (*21*) examined 129 samples of miso (soybean paste) for mycoflora. Although a number of mold species, including certain toxicogenic species, were identified, incidence rates and relative prevalences of any given species were not clearly delineated. Nyiredy et al. (*18*) examined a number of samples of soybean meal for mycoflora. Specifically cited as occurring were *Aspergillus flavus, A. candidus, Aspergillus diversus,* and species of the *A. glaucus* group. Additional molds referred to were *Paecilomyces* spp., *Penicillium* spp., and *Mucor* spp.

Even less is known about actual mycotoxin contamination in soybeans. To date, only aflatoxins have been reported. Bean et al. (*3*) reported the presence of aflatoxin in 14 of 28 soybean samples. Shotwell et at. (*22*) reported that two of 866 samples of soybeans examined contained aflatoxin. Nagarajan et al. (*17*) found that aflatoxin could be produced in the laboratory on autoclaved soybeans.

Considering the above, we conducted a study to determine the mold flora of a number of samples of unprocessed American soybeans to establish: first, whether a potential hazard might exist due to contamination of these soybeans with well-known and extensively studied mycotoxin-producing species; second, whether a potential hazard might exist due to high incidence rates of other fungal genera and species of unknown or poorly understood toxicogenicity.

MATERIALS AND METHODS

Samples

A total of 385 soybean samples, collected in the summer and fall of 1974 by inspectors of the U.S. Food and Drug Administration (FDA) from various soybean outlets, were examined. The exact history of each sample could not be established; however, the samples obtained were either freshly harvested from the 1974 crop or harvested from the 1973 crop and stored less than 1 year. In each instance, sample size received exceeded 1 kg.

Mycoflora determination

Upon receipt, each sample was held at 0 C for 72 h to kill any mites present, since mites are a primary source of cross-contamination in a mycology laboratory. From each sample, 50 randomly selected, intact, and unblemished soybeans were aseptically plated on malt-salt agar containing, per liter, 20 g of malt extract, 75 g of NaCl, 40 mg of chlortetracycline HCl, and 15 g of agar. The antibiotic, added just before the plates were poured, effectively inhibited bacterial growth. The NaCl, added to lower the water activity of the agar substrate, was

utilized for four reasons. First, it adds to bacterial inhibition; second, it retards growth of undersired, fast-growing species of *Mucorales;* third, it slows down, but does not completely inhibit, the growth of most other fungi, thus allowing detection of normally slow-growing species that otherwise might not be detected; fourth, and perhaps most important for the examination of viable seeds, such as soybeans, the xerophytic nature of malt-salt agar prevents bean germination and, therefore, the subsequent rapid seedling development which invariably would result in disorientation of petri dish lids and stacks.

From each sample, 50 addtional intact and unblemished soybeans were surface-disinfected for 1 min in 5% NaOCl, rinsed three times in sterile water, and then plated as above. This was done to provide a basis for determining whether the mold flora subsequently encountered was primarily due to surface contamination or to actual internal seed invasion.

All plates were incubated at 23-26 C for 14-21 days before enumeration and identification of mold flora. Species of *Aspergillus* and *Penicillium* were identified according to Raper and Fennell (*19*) and Raper and Thom (*20*). Other genera were identified according to Barnett (*2*) and Gilman (*8*). Isolates of these other genera rarely were speciated.

RESULTS

In this study, occurrence of molds (genera and species) was defined as the percentage of soybeans examined that contained the respective molds, both before surface disinfection (NSD beans) and after surface disinfection (SD beans). This method of quantitating seed mycoflora is documented in the literature and is used routinely by plant pathologists (*5, 7, 16, 23*).

The total number of soybean samples examined in this study was 385. Molds were evident in virtually all (99.4%) of the 19,250 NSD soybeans examined. Disinfection decidely reduced mold occurrence (52.8% of the 19,250 SD soybeans examined), indicating that developing mold was primarily due to surface contamination. However, the 52.8% occurrence in SD beans indicated that substantial internal invasion had occurred.

Table 1 lists the mold species and non-speciated genera encountered most often in the 385 soybean samples examined (both NSD and SD). The data represent incidence rates on a sample basis, not on a seed percentage basis, and the total number of samples examined versus the number of samples from which the listed organisms were encountered are compared. In the NSD samples, the toxicogenic species, *A. flavus* (aflatoxins), *Aspergillus nidulans* (sterigmatocystin), *Aspergillus ochraceus* (ochratoxins), *Aspergillus versicolor* (sterigmatocystin), *Penicillium cyclopium* (penicillic acid), and *Penicillium viridicatum* (ochratoxin and citrinin), were detected in more than half of all the NSD samples examined. *Penicillium citrinum* (citrinin) (97 samples) and *Penicillium islandicum* (luteoskyrin and islanditoxin) (94 samples) were found regularly. The number of samples containing toxicogenic species was substantially lower in the SD group. Except for the occurrence of *A. flavus* and *P. cyclopium,* the data indicate that the occurrence of the above-listed toxicogenic molds in and on soybeans is mainly due to surface contamination rather than to actual internal invasion. Interestingly, *P. chrysogenum,* the generating organism of penicillin, was encountered regularly in NSD samples but less so in SD samples.

TABLE 1. *Mold species and genera regularly encountered in NSD and SD soybean samples*

Species	No. of samples[a]	
	NSD	SD
Alternaria spp.	208	202
Aspergillus candidus	280	104
A. flavus	316	142
A. glaucus group	369	285
A. nidulans	201	36
A. niger group	188	43
A. ochraceus	267	70
A. restrictus	79	89
A. sydowi	99	23
A. tamarii	91	12
A. versicolor	320	49
A. wentii	136	19
Cladosporium spp.	274	226
Fusarium spp.	124	73
Penicillium chrysogenum	194	45
P. citrinum	97	22
P. cyclopium	335	163
P. islandicum	94	5
P. oxalicum	103	31
P. viridicatum	279	69

[a]385 samples of NSD and SD soybean lots were examined.

TABLE 2. *Occurrence of mold species and genera in NSD and SD soybeans*

Species	Occurrence (%)	
	NSD	SD
Alternaria spp.	6.2	4.5
Aspergillus candidus	16.8	2.1
A. flavus	22.3	2.7
A. glaucus group	61.7	28.0
A. nidulans	10.2	0.4
A. niger group	5.0	0.5
A. ochraceus	18.3	0.8
A. restrictus	1.0	1.5
A. sydowi	1.9	0.2
A. tamarii	2.3	0.1
A. versicolor	17.5	0.4
A. wentii	4.6	0.2
Cladosporium spp.	22.8	10.4
Fusarium spp.	3.0	2.1
Penicillium chrysogenum	6.5	0.4
P. citrinum	2.2	0.2
P. cyclopium	31.2	4.3
P. islandicum	3.2	0.1
P. oxalicum	2.6	0.3
P. viridicatum	12.0	0.8

Table 2 lists the incidence rates of the species and unspeciated genera of molds regularly encountered on all the soybeans examined in this study. The data, based upon the examination of 19,250 NSD and 19,250 SD soybeans, indicate the following: First, the mycoflora, both before and after disinfection, was dominated by species of the *A. glaucus* group. Although not listed separately in Table 3, species of this group most often encountered were *Aspergillus repens, Aspergillus ruber, Aspergillus chevalieri,* and *Aspergillus amstelodami,* in that order. Second, at least 6 toxicogenic species were encountered in NSD beans in more than 10% of all NSD soybeans examined: *A. flavus, A. nidulans, A. ochraceus,*

TABLE 3. *Additional mold species and genera encountered sporadically in the soybean samples*

Absidia spp.	*P. expansum*
Aspergillus caespitosus	*P. frequentans*
A. fumigatus	*P. funiculosum*
A. melleus	*P. martensii*
A. oryzae	*P. multicolor*
A. terreus	*P. olivino-viride*
A. unguis	*P. palitans*
A. ustus	*P. purpurogenum*
Botryosporium spp.	*P. urticae*
Botrytis cinerea	*P. variabile*
Cephalosporium spp.	*Pullularia pullans*
Chaetomium spp.	*Rhizoctonia solani*
Circinella spp.	*Rhizopus* spp.
Mucor spp.	*Scopulariopsis* spp.
Paecilomyces varioti	*Stemphylium* spp.
Penicillium brevi-compactum	*Streptomyces* spp.
P. decumbens	*Trichoderma viride*

A. versicolor, P. cyclopium, and *P. viridicatum.* Incidence rates in SD beans were much lower, with only *A. flavus* and *P. cyclopium* occurring in more than 1% of all soybeans examined. The data in Tables 1 and 2 are consistent in indicating that the presence of these toxicogenic molds is due mainly to surface contamination rather than to internal invasion. Third, unspeciated isolates of the genera *Alternaria* and *Cladosporium* were prominent in both NSD and SD soybeans. Although speciation of all encountered isolates of these two genera was not routinely attempted, the following appeared to be most prominent: *Alternaria alternata* (*A. tenuis*), *Cladosporium herbarum,* and *Cladosporium cladosporioides.*

Table 3 lists additional species and genera encountered sporadically in this study (in less than 1% of all NSD soybeans examined). Although several genera were encountered, sporadically occurring organisms mainly were from the genera *Aspergillus* and *Penicillium.* Not listed in Table 3 are numerous occurrences of unidentifiable non-sporulating cultures. Probably most of these cultures were isolates of the *Moniliaceae* and *Dematiaceae.*

DISCUSSION

The predominance of species of the *A. glaucus* group in soybeans was not unexpected, since members of this group prefer low moisture substrates in equilibrium with a relative humidity of 70-80% or even lower (*4*). Mature, properly harvested, and stored soybeans are relatively low moisture seeds.

Occurrence of the toxicogenic species regularly encountered may also be due to at least in part, their relatively low moistrue requirements. Earlier reports by Mislivec and Tuite (*14*) and Mislivec et al. (*15*) show that the conidia of *A. flavus, A. nidulans, A. ochraceus, A. versicolor, P. citrinum, P. cyclopium,* and *P. viridicatum* have relatively low moisture requirements for germination. Except for *A. ochraceus,* conidia of the seven species germinated at a relative humidity of 81%, while conidia of *A. ochraceus* germinated at a relative humidity of 79%. In addition, except for *P. cyclopium* and *P. viridicatum,* temperatures of 26-30 C (79-86 F) and above favored conidial germination. Soybeans are a relatively high temperature, mid-to-late summer crop.

On the basis of the data compiled in this study, the following points can be made about the existence of animal or human hazard potential due to presence of toxicogenic molds in and on soybeans. First, at least 11 toxicogenic species of *Aspergillus* and *Pencillium* were encountered, including the three sporadically occurring species (Table 3): *A. melleus* (ochratoxin), *P. expansum* (patulin), and *P. urticae* (patulin and griseofulvin). Second, these toxicogenic species occurred primarily in NSD soybeans, indicating surface contamination rather than actual internal invasion. However, the presence of these species, even at the surface, could pose a potential, if not actual, hazard should the beans be subjected to storage conditions which would allow mold growth, i.e., if the beans got wet. Third, and of great interest to us, was the relatively high incidence rates of the genera *Alternaria* and *Cladosporium* in both NSD and SD soybeans. The literature indicates that both genera contain toxicogenic species (*9, 10, 13*). However, although reports of mycotoxins produced by species of the genera *Aspergillus, Penicillium,* and *Fusarium* are readily available in the literature, similar reports concerning mycotoxins produced by the genus *Alternaria* and, even less so, by the genus *Cladosporium* have been sporadic. Perhaps more intense efforts should be made by the scientific community to investigate toxicogenic species of these two genera. Finally, the data collected during this study indicate that the visual examination of any given food or feedstuff for moldiness may not suffice. We examined 19,250 intact, unblemished, and apparently "mold-free" NSD soybeans. When the soybeans were plated upon agar medium, molds were encountered from 99.4% of all of these soybeans examined.

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Comparative Radiation Death Kinetics of *Clostridium botulinum* Spores at Low-Temperature Gamma Irradiation

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ABSTRACT

Spores of *Clostridium botulinum* 33A were irradiated with ^{60}Co gamma rays in 0.067 M Sorensen phosphate buffer (pH 7.0) at -196, -140, -80, -30, or 5 C and incubated in recovery broth for 30 days at 30 C, thus simulating an inoculated pack and eliciting "partial spoilage" data. Resistance of the spores decreased linearly with increasing temperature. A simple empirical equation was derived to predict D values for any desired temperature. An Arrhenius plot of the D value—radiation temperature profile indicated that the death kinetics is not first order. Comparison of the data in this model system with those previously observed in beef indicates a similar radiation death pattern, except that resistance of the spores was somewhat more (1.6-fold) temperature dependent, although significantly more resistant, in the beef. A comparison of the D value-temperature relationship of *Streptoccus faecium* a21, obtained earlier, with strain 33A in the same model system indicated that the spores were considerably less resistant below-20 C and much more resistant above this radiation temperature.

Grecz et al. (*10*) investigated the radiation death kinetics of spores of *Clostridium botulinum* strain 33A in a beef pack. They found that the rate of spore kill (D values) in the radiation temperature range -196 up to about 60 C was represented by the simple linear relationship $D = 379 - 1.06\ t$, where 379 is the D value in Krad at 0 C, 1.06 is the increment of change in D value (Krad/°C) and t is any radiation temperature in the above range. An Arrhenius plot of their D values vs. radiation temperature produced a nonlinear curve, indicating that the kinetics were not first order, but were of a complex nature.

Whether the phenomena observed above for strain 33A are specific for the food substrate used or whether such behavior would reflect a generalized pattern was not known. Hence, studies were conducted with a model system which simulated an inoculated beef pack in which "partial spoilage" (or quantal response) data were elicited.

Results of this investigation provided additional information in a related area. Of 36 nonsporogenic bacteria examined in a model system for comparative radiation resistance, *Streptococcus faecium* strain *a*21 was the most resistant among the organisms of potential public health significance (*3*). Moreover, as the radiation temperature was lowered stepwise from 5 to -196 C, its resistance progressively increased from a D value of 0.09 to 0.38, a relatively high value for vegetative cells. In fact, on the basis of observations with pork irradiated to 5 Mrad at -75 C, Coleby et al. (*8*) cautioned that vegetative organisms such as faecal streptococci may be more of a problem in foods irradiated at cryogenic temperatures than sporeforming bacteria. Conceivably, vegetative microorganisms may experience a relatively higher rate of protection than spore-formers at decreasing radiation temperatures under comparable conditions, so that at some low temperature certain food spoilage non-sporeformers, such as *S. faecium a*21, may equal, if not surpass, the radiation resistance of some bacterial spores. Our present studies with *C. botulinum* 33A, one of the 10 indicator strains we use to establish a microbiologically safe prototype radappertization process (*1, 2, 4-6*), would provide at least a partial basis for comparison of the radiation pattern between these two types of organisms.

MATERIALS AND METHODS

Test organism

Spores of *C. botulinum* strain 33A were produced, harvested and enumerated in TYT agar as cited earlier (*6*). The spores were suspended in 0.067 M Sorenson phosphate buffer ($KH_2PO_4 + Na_2HPO_4$) pH 7.0, and held at 2 ± 1 C until needed.

Radiation resistance

All experiments were done in duplicate using ^{60}Co gamma rays at 5, -30, -80, -140, or -196 C with a variation of ±2 C.

Stock suspension, diluted in buffer to yield 10^7 spores/ml, were dispensed in 1.0-ml quantities into sterile cotton-plugged pyrex tubes (10 × 75 mm) and frozen immediately in a dry ice-acetone bath. Ten tubes were inserted into a polystyrene holder contained in a metal can (size 401 × 411); the holder had two parallel rows of five holes each. The

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cans were sealed under partial pressure (125 mm Hg), equilibrated to the radiation temperature desired, and irradiated, two cans/dose (20 tubes), at 1.0 to 2.0 Mrad in increments of 0.1 Mrad (±3%). The dose rate averaged 5.44×10^4 rad/min and the transient dose was 4.0×10^4 rad.

Recovery of irradiated spores

After irradiation, all samples were refrigerated overnight at 2±1 C. One-ml quantities of steamed (100 C for 10 min) and cooled (20 to 30 C) double strength TYT broth containing 0.15'% $NaHCO_3$ were added aseptically to the irradiated spores, sealed in an oxygen-gas flame and incubated for 4 weeks at 30±2 C. All tubes were then opened in the range where the three highest radiation doses yielded 100% turbidity ("spoilage") through the four lowest consecutive doses which gave no turbidity (no"spoilage"). The tube contents (2.0 ml) were mixed on a Vortex mixer, transferred aseptically to tubes (20 × 150 mm) of single strength TYT broth (20 ml) supplemented with 0.5% glucose and 0.075 M $NaHCO_3$ and incubated at 30±2 C for 14 days. Tubes showing turbidity were centrifuged (2,520 × g for 20 min) and 0.5 ml of supernatant fluid was injected intraperitoneally into two unprotected white male mice (strain CD-1, 15 to 20 g) and into two mice protected with type A botulinal antitoxin. Samples producing death or typical illness in the unprotected mice within 4 days of injection were regarded as positive ("spoiled").

Data processing

The radiation resistance of the organism was estimated as D values (the dose which reduced a population by 90%) from the "spoilage" (quantal response) data (7, equations 3 and 4) at each radiation temperature. The reciprocals of these values were plotted against the reciprocals of the absolute temperatures on semilog coordinates to test the Arrhenius relationship of the spore death kinetics.

RESULTS

The effect of radiation temperature on strain 33A spores is indicated by the D values estimated from the experimental "partial spoilage" data in the buffer (Table 1). The D values increased from a low of 0.180 at 5 C to a high of 0.326 at -196 C. Using these data, linear, exponential and quadratic best fits were computed over the entire temperature range used. The equations

TABLE 1. *Effect of radiation temperature on resistance to gamma rays of* Clostridium botulinum *33A spores in buffer*[a]

Radiation temperature (C)	D values (Mrad) based upon		
	Experimental data[b]	Linear Regression curve[c]	Empirical equation[d]
-196	0.326	0.330	0.333
-140	0.302	0.295	0.297
- 80	0.257	0.255	0.258
- 30	0.253	0.225	0.226
5	0.180	0.205	0.203

[a]Sorensen phosphate buffer, 0.067 M, pH 7.0.
[b]Computed as cited earlier (7, equations 3 and 4).
[c]Obtained by inspection from the curve (see Table 2).
[d]Computed by the equation D = 206-0.65t.

representing these three rates of change in resistance are shown in Table 2.

A comparison of F-values indicated that the quadratic rate of change may be excluded (Table 2). However, both the linear and exponential functions were significant at the 95% confidence level, while the correlation coefficients and standard errors of estimate slightly favored the former. Also, the D values obtained by visual inspection from the linear plot (Fig. 1, curve A)

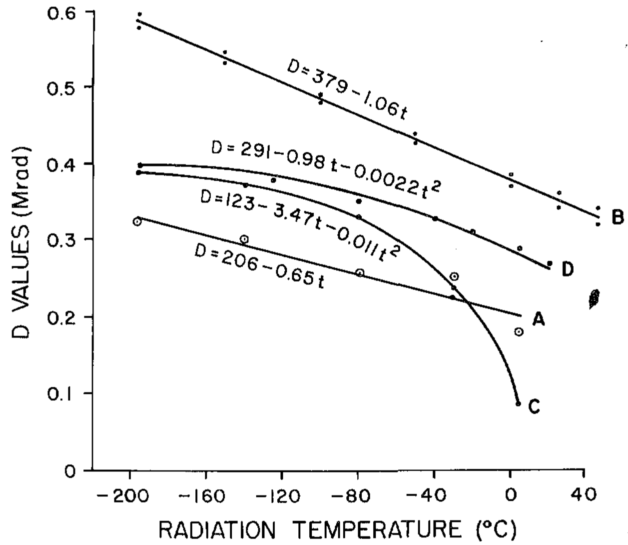

Figure 1. *Comparative effect of radiation temperature on the change in resistance of* Clostridium botulinum *spores and* Streptococcus faecium *a21 to gamma rays. Legend: Curve A, strain 33A spores in buffer; curve B, strain 33A spores in beef; curve C, strain a21 in buffer; curve D, strain 53B spores in beef. Results for curves A and B were obtained from partial spoilage data, and for curves. C and D from survival data.*

compared favorably with the D values estimated from the "partial spoilage" data at each radiation temperature used (Table 1). Hence, the simpler linear correlation between spore resistance and radiation temperature was selected to examine the Arrhenius relationship. Fig. 2 shows that the Arrhenius plot was curvilinear

DISCUSSION

Radiation death of *C. botulinum* 33A spores in our inoculated model "pack" is characterized by the linear regression equation $y = -(6.49 \times 10^{-4})x + 0.206$ (Table 2) which correlates the rate of spore death with radiation

TABLE 2. *Statistical analysis of the change in resistance of* Clostridium botulinum *33A spores in buffer*[a] *with change in radiation temperature from -196 C to 5 C*

Rate of change	Equation	F-value[b]		Corr. coef.	Std. error
		Computed[c]	Critical[d]		
Linear	$y = -(6.49 \times 10^{-4})x + 0.206$	24.72	10.13	0.944	0.021
Exponential	$y = 0.204^{e^{-0.0026}}x$	15.41	10.13	0.914	0.047
Quadratic	$y = -(2.20 \times 10^{-6})x^2 - (1.07 \times 10^{-3})x + 0.20$	0.85	18.51	0.961	0.022

[a]Sorensen phosphate buffer, 0.06 M, pH 7.0.
[b]Significant at the 95% confidence interval.
[c]Based upon experimental data.
[d]Based upon statistical theory.

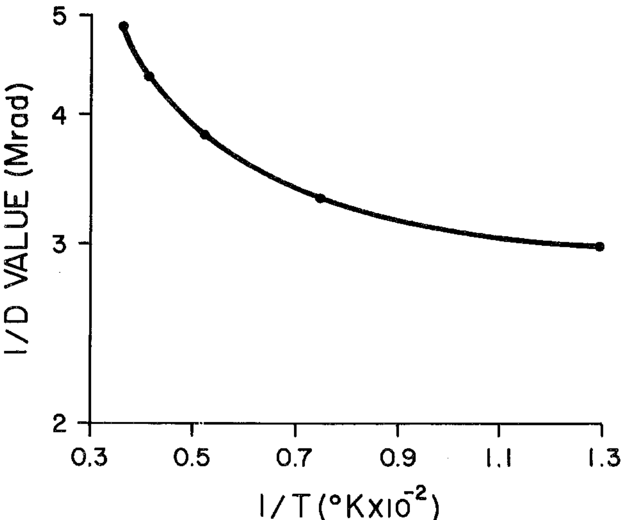

Figure 2. *Arrhenius plot to show kinetic relationship between rate of radiation death and radiation temperature of* Clostridium botulinum *33A spores in buffer. Plot is based on the empirical linear equation D = 206-0.65t.*

temperature and with which an Arrhenius plot was obtained. Hence the linear formula was converted to a simpler, more usable, empirical form, D = 206 - 0.65 t, where 206 is the D value in Krad at 0 C, 0.65 (the slope of the regression curve) is the increment of change in D value (Krad/°C), and t is the radiation temperature (°C) desired. This expression makes it easier (*a*) to obtain the spore radio-resistance in the model system for any radiation temperature from -196 to 5 C, and extrapolate somewhat beyond these limits if desired, and (*b*) to facilitate the graphing of the Arrhenius relationship. Use of this empirical equation produced essentially no change in the D values when compared with both the calculated quantal response, and the graphically obtained data (Table 1). An empirical formula, previously derived for a beef pack inoculated with strain 33A spores, yielded the expression D = 379 - 1.06 t (*10*).

A graphic comparison of the above two equations (Fig. 1) makes it obvious that, although strain 33A was significantly more radioresistant in beef than in buffer, the resistance was 1.6-fold (i.e., a slope ratio of 1.06:0.65 of the two functions) more temperature dependent in the meat than in the simpler model system. The cause(s) for this unexpected observation is unknown at this time, and would be difficult to elucidate, since the phenomenon is probably a reflection of the sum total of a combination of several complex, competitive, and/or synergistic events occurring before, during, and after irradiation of the spores in the two very dissimilar suspending substrates and recovery media. Possible clarification of this observation might be obtained by a series of investigations which would isolate each of the individual variables noted.

Anellis et al. (*3*) had found that a phosphate buffer suspension of *S. faecium a*21, representing the tail portion (0.1% of the initial cell population) of the dose survival curve, was highly radiation resistant at cryogenic temperatures. Although the D value-temperature relationship for this organism was obtained from survival counts instead of from quantal response data, it was still of interest to compare the resulting plot with that derived for strain 33A. Figure 1 indicates that the vegetative cells of *S. faecium a*21 were significantly more radiation resistant than the botulinal spores within much of the cryogenic range studied. Above -80 C, the vegetative cells became increasingly temperature dependent, resulting in a rapid rise in sensitivity until, at about -20 C, the D values of both organisms were similar; above -20 C, strain *a*21 was markedly more radiation sensitive than strain 33A spores. The high radiation temperature dependence of these streptococci on their resistance did not follow a linear regrression curve but was expressed by the quadratic empirical equation $D = 123-3.47t - 0.011\ t^2$ (Fig. 1).

Although the methods used for estimating D values for the two test organisms were not identical, our findings seem to confirm the observation by Coleby et al. (*8*) that faecal streptococci may be more of a problem in foods irradiated at cryogenic temperatures than sporeforming bacteria. Since the radappertization of our foods is based upon radiation temprature of -30±10 C (*11*), fecal streptococcus contaminants might possible survive the botulinal 12D process, assuming that they are not destroyed by the enzyme inactivation treatment (65 - 75 C center temperature) of the product before irradiation. However, in the many years of microbiological sterility testing of radappertized foods in our laboratory, no viable microorganisms have ever been recovered. Irradiation and/or thermal death time inoculated pack studies, using *S. faecium a*21, would determine whether this organism could withstand a radappetization process. This possibility will be investagated at a later date.

Unlike chemical kinetics, the radiation death kinetics did not appear to display an Arrhenius behavior. An Arrhenius plot of the 33A spore D value data obtained from the empirical equation (Table 1) as a function of radiation temperature, produced a smooth non-linear curve (Fig. 2). A similar curvilinear Arrhenius graph was obtained with this organism in beef (*10*). El-Bisi et al. (*9*), using *C. botulinum* 53B spores in beef, determined the D values (from survival counts) as a function of radiation temperature (-196 to 20 C). Although they did not attempt to characterize the Arrhenius nature of their data, we found that their results also followed a nonlinear plot; so did the *S. faecium a*21 radiation resistance data (*3*). Thus there is no relatively simple physiocochemical mechanism discernible which might explain the change in rate of bacterial kill with a change in radiation temperature. Cell death may therefore be due to ionization events which lead to non-repairable DNA strand breaks and , secondarily perhaps, to the cleavage of weak DNA hydrogen bonds.

Our studies with *C. botulinum* 33A spores, using partial spoilage data, indicate that the radiation death kinetics, in both the model system and in beef, follow similar patterns, with the one notable exception that the

TABLE 3. *Lethality rate with radiation temperature and death kinetics of* Clostridium botulinum *spores and* Streptococcus faecium *a21*

Organism	Substate irradiated	Method of acquiring data	Lethality curve[a]	Arrhenius relationship
C. botulinum 33A	Buffer[b]	Quantal response[c]	Linear	Curvilinear[d]
33A	Beef	Quantal response	Linear	Curvilinear
53B	Beef	Survival count	Quadratic	Curvilinear
S. faecium a21	Buffer	Survival count	Quadratic	Curvilinear

[a]Graphed on linear coordinates.
[b]Sorensen phosphate buffer, 0.067M, pH 7.0
[c]Partial spoilage data.
[d]Not first order kinetics.

sensitivity of the organism was somewhat more temperature dependent in beef, although its resistance in the meat was significantly higher.

The mode of radiation death with temperature for *S. faecium a*21 in buffer and for *C. botulinum* 53B spores in beef, was also similar. But unlike strain 33A, these two organisms followed a quadratic (Fig. 1) instead of a linear lethality rate, although strain 53 B, (whose death rate was $D = 291 - 0.98\,t - 0.0022t^2$, Fig. 1) was considerably less temperature dependent that *a*21. Yet both organisms conformed to the same type of non-Arrhenius kinetics as the 3A spores. Table 3 summarizes more clearly the apparent relationship between the experimental variables noted and the lethality rates and death kinetics of the above four organisms. Since the quadratic results were based upon dose survival counts rather than upon quantal response data, it would be of interest to examine more thoroughly the influence of these two procedures and identical substrates on the shape of the death rate curve as a function of radiation temperature.

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Salmonella in Commercially Produced Dried Dog Food: Possible Relationship to a Human Infection Caused by *Salmonella enteritidis* Serotype Havana

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ABSTRACT

Identification of *Salmonella enteritidis* serotype Havana, isolated from a 2½-month old female, engendered efforts to trace the source of infection. The inquiry led to examination of commercially prepared dehydrated dog foods. Twenty-five samples, representing four different manufacturers plus two retail store brands, were examined. Each of 11 samples, produced by one manufacturer, contained one or more *Salmonella* serotypes. Eight of them contained *S. enteritidis* serotype Havana. Isolates of serotype Havana from the index case and her mother had antibiotic susceptibility patterns essentially identical to those of nine of 10 serotype Havana isolates recovered from one sample of dog food. The possibility that the human isolates were related to the dog food isolates could not be eliminated.

Salmonella enteritidis serotype havana had not been encountered by the Milwaukee Health Department laboratory before December, 1975. Therefore, recovery of this serotype from a stool specimen of an infant girl, at a Milwaukee hospital, triggered a series of events which led to presentation of this report.

The child, approximately 2½ months old, had developed a gastrointestinal illness December 6, 1975 and was admitted to a hospital on December 13, 1975. She recovered sufficiently to be returned to her home 2 weeks later. Follow-up cultures, prepared from the child's stool specimens January 13, 1976 and March 8, 1976, were positive for *S. enteritidis* serotype Havana.

Upon interviewing the child's mother on December 29, 1975, it was learned that a canine household pet had become ill November 28, 1975. The dog had refused food for 6 days and was observed to be suffering from "gagging". He was treated at a veterinary clinic for "digestive disruption", a stool specimen was not cultured at that time. However, a specimen collected from the dog January 13, 1976 was found to be positive for *S. enteritidis* serotype Newington.

The child's mother, although asymptomatic, was found to have a stool culture positive for *S. enteritidis* serotype Havan on February 4, 1976; ssecond specimen was negative one month later. A 3-year-old sibling, and the chnild's father, each had stool cultures negative for *Salmonella.*

The occurrence of *Salmonella* infections among dogs may indeed provide a reservoir for transmission of disease to humans (*1,7*). The magnitude of this reservoir may be considerable; a survey conducted by Galton et al. (*4*) revealed 27.6% of 8,157 rectal swabs, collected from dogs, were positive for *Salmonella.* Dogs, on occasion, have been observed to eat carrion and garbage and to practice coprophagy. Therefore, the mechanism for transmission of *Salmonella* to dogs, and re-infection among dogs, may be present continually.

Dried dog foods were incriminated as the source of *Salmonella* infections among colonies of laboratory animals as early as 1952. (*5*). Results of a survey of commercially produced, dehydrated dog meal were published in 1955; Galton et al. (*3*) reported 26.5% of 98 samples contained *Salmonella.* Thus another source for possible transmission of *Salmonella* to dogs became known some 24 years ago.

Bacteriological examination of a portion, approximately 44 g, of commercial dried dog food, obtained January 24, 1976, from a supply at the home of the index case, yielded isolates of *S. enteriditis* serotypes Infantis and Minnesota.

Isolation of four different serotypes of *Salmonella* (from the index case and her mother, canine pet, and dog food) depressed earlier enthusiasm that identification of a relatively rare serotype (Havana) of *Salmonella* (*10*) presented an unique opportunity to trace the source of infection. However, occurrence of *Salmonella* in commercial dried dog food obtained from the home prompted a question as to whether the food became contaminated in the home or provided a vehicle for entry of *Salmonella* into the home. Therefore, various brands of dried dog food, including the brand which had been samples at the home of the index case, were purchased from retail stores in the Milwaukee area. Bacteriological examination of the retail samples was begun March 1, 1976.

MATERIALS AND METHODS

Dehydrated dog foods

Five-pound (2,268 g) bags of dehydrated dog foods, representing four different manufacturers, and two store brands (manufacturer not indentified on bags) were purchased from among 11 supermarkets. Purchases were made between February 26, 1976 and May 21, 1976.

Laboratory analyses

Pre-enrichment, selective enrichment, and isolation procedures were employed essentially as recommended by the International Association of Microbiological Societies (*12*). Biochemical and serological methods were followed as described by Edwards and Ewing (*2*).

Eight samples were subjected to "destructive analysis," i.e., the entire contents of a 5-lb. bag aseptically divided into eight aliquots, approximately 283.5 g, each; each aliquot was transferred to individual 4-liter Erlenmeyer flasks containing 2.5 liters of lactose broth. Flasks were thoroughly shaken to aid in wetting the dry dog food particles. Cultures were incubated at 35 C for approximately 24 h. Incubating flasks were manually shaken periodically during the span of working hours. At completion of pre-enrichment incubation, 1.0-ml inocula from the cultures were transferred into 12.0-ml volumes of selective enrichment media. Both selenite broth and tetrathionate broth were employed. Six tubes, three of each of these media, were prepared for each pre-enrichment culture. Selective enrichment cultures were incubated overnight at 35 C. Selenite broth cultures were subcultured to individual plates of S.S. agar and individual plates of MacConkey agar. Tetrathionate broth cultures were subcultured to individual plates of brilliant green agar. After overnight incubation at 35 C, three suspicious colonies per plate were picked to triple sugar iron agar.

Success in detecting *Salmonella* by the "destructive analysis" procedure led to the question of how many of these organisms might be contained in a 5-lb. sample. A modified most probable (MPN) procedure described by Silliker (appendix C of ref. *8*) was adapted for this purpose. Each of ten 25-g portions, from a 5-lb. (2,268 g) bag, was placed into individual 500-ml Erlenmeyer flasks containing 250 ml of lactose broth. The remaining dog food was divided into seven aliquots, approximately 288 g each, for culture in 4-liter flasks. Fifteen samples were examined by this dual procedure.

In an additional comparative study, a conventional MPN series (*6*), employing five cultures per bank, was set up in conjunction with the Siliiker modified MPN; two 5-lb. bags were cultured to compare the MPN methods. In the first attempt a 60-g portion of dried food was blended with 540 ml of lactose broth. Five 100-ml portions were transferred to individual 16-ounce jars, five 10-ml protions, five 1.0-ml portions and five 0.1-ml portions were transferred to individual tubes containing 10 ml of lactose broth. After overnight incubation at 35 C, 1.0-ml portions of these cultures were transferred to selenite broth and carried on for *Salmonella* detection. Ten portions, 25 g each, of dried dog food were pre-enriched in 500-ml flasks containing 250 ml of lactose broth. In an attempt to determine the effect of abuse, four 60-g portions of dried food were individually combined with 120 ml of sterile distilled water. These portions, which simulated preparation of the food for consumption by pet dogs, were held at room temperature. One portion was sampled after 2 h, one after 4 h, one after 6 h, and the fourth after 24 h. The abuse samples were blended directly with 420 ml of selenite broth. A MPN series was set up including five 100-ml portions for direct culture plus five 10 ml, five 1.0 ml, and five 0.1 ml portions, which were inoculated into tubes containing 10 ml of selenite broth. The remaining 1,718-g portion of the 5-lb. sample was divided into six aliquots for lactose pre-enrichment.

A second 5-lb. bag of dried dog food was also set up for comparison of the conventional MPN with Silliker's modification. In this attempt five 60-g portions were each individually blended with 540 ml of lactose. These five homogenates were combined in a 4-liter flask. Four banks of MPN cultures, five cultures each, were prepared from the composite homogenate as described above. Ten portions, 25 g each, of dried food, were pre-enriched in 500-ml flasks containing 250 ml of lactose broth. Three portions of dried food (300 g each) were distributed into individual beakers of 1 liter capacity. Sterile distilled water, 300 ml, was added to each beaker. These abuse portions were held at room temperature. The first was sampled after 3 h, the second after 6 h, and the third 24 h. A 60-portion of an abuse sample was blended with 540 ml of selenite broth. Four banks of MPN cultures, five cultures each, were prepared in selenite broth. The remaining 818-g portion of dried dog food was divided into three aliquots; each of these was pre-enriched in 2.5 liters of lactose broth contained in 4-liter flasks.

RESULTS

Qualitative detection of salmonellae

Eight packages of dried dog food, representing three different manufacturers, were examined by qualitative destructive analysis. Results are summarized in Table 1.

TABLE 1. Salmonella enteritidis *serotypes isolated from dried dog food by destructive analysis of 5-lb. (2,268 g) samples*[a]

Source	Sample no.	No. flasks pos./ No. cultured	*Salmonella* somatic groups and serotypes isolated	
Mfg. #1[b]	0812	4/8	C_1	Infantis
			E_3	Thomasville
			G	Havana
			K	Siegburg
	0814	1/8	B	Schwarzengrund
			C_1	Infantis
	0810	8/8	B	Schwarzengrund
			C_1	Infantis
			C_1	Livingstone
			G	Havana
			K	Siegburg
	1255	1/8	B	Agona
	1253	7/8	C_1	Infantis
			E_3	Thomasville
			E_4	Senftenberg
			G	Havana
			K	Siegburg
			L	Minnesota
	0813	8/8	B	Schwarzengrund
			C_1	Infantis
			E_1	Lexington
			E_3	Thomasville
			E_4	Senftenberg
			G	Havana
			K	Siegburg
Mfg. #2[c]	1256	1/8	R	Johannesburg
Mfg. #3[c]	1257	0/8		

[a]283.5 g/each of eight 4-liter flasks containing 2,500 ml lactose broth.
[b]Different lot Nos. for each sample except #1255 and #1253 which did not have coded lot numbers. Products produced by alleged manufacturer of product sampled from home of index case.
[c]No lot Nos.

Dog food sampled from the home of the index case was allegedly produced by manufacturer No. 1. It is of interest to note that each of six packages, produced by this manufacturer, contained *Salmonella*. Furthermore, *S. enteritidis* serotype Havana was found in four of these six samples. Isolates of *S. enteritidis* serotype Havana obtained from the index case, her mother, and sample No. 1253, Table 1 were sent to the Enteric Section, Enterobacteriology Branch, Bureau of Laboratories, Center for Disease Control, Atlanta, Georgia. Isolates from the index case, and her mother had antibiotic susceptibility patterns essentially identical to those of nine of 10 isolates recovered from one sample of dried dog food. Although this observation cannot be construed as relating the human isolates to the dog food isolates, the possibility was not ruled out.

Eleven different serotypes were recovered from seven

of eight bags. Ten of the serotypes were recovered from among six bags of dog foods produced by manufacturer No. 1. Although 637 individual isolates were serogrouped, and most of these serotyped, data in Table 1 reflect only the *Salmonella* identified by picking three colonies per plate of selective differential medium. Other serogroups and serotypes may have been present among suspicious colonies remaining on the plates. A 12-ounce packet of dog food, produced by a fourth manufacturer, was received as a sample from a store visited by a laboratory staff member. Destructive analysis of this sample, three aliquots of approximately 57 g per 500 ml of lactose broth, failed to yield detectable *Salmonella*.

Experience with the above samples revealed no additional serotypes of *Salmonella* were detected by the tetrathionate broth-brilliant green agar sequence. Use of triplicate cultures of two selective enrichment media likewise failed to yield additional serotypes. Therefore, only one tube of selenite broth was employed per pre-enrichment culture in subsequent studies. Each of these selenite broth cultures was streaked on individual plates of S.S. agar, MacConkey agar, and brilliant green agar.

Comparison of qualitative detection of Salmonellae *with a modified MPN procedure*

Fifteen bags of dried dog food were examined both by destructive analysis and Silliker's modified MPN technique (*8*). Ten serotypes of *S. enteritidis* were detected among three bags of the dried dog food produced by manufacturer No. 1, Table 2. An additional 12 samples (5-lb. bags) of dried dog food, two retail store brands plus products of three different manufacturers, were examined by this dual procedure; none of these yielded isolates of *Salmonella*.

Comparison of modified MPN and conventional MPN procedures with qualitative detection of Salmonellae *in fresh samples and with quantitative detection of* Salmonellae *in abused samples*

Samples No. 1617, and 1616, Table 2, were coded as being from the same production lot, as samples Nos. 1712 and 1713, Table 3. Each, of the former pair, was found to yield *Salmonella* MPN levels, by a modified technique, which were remarkably consistent with those determined by both modified and conventional techniques for sample No. 1713, Table 3. Although four serotypes of *S. enteritidis* were isolated from sample No. 1712, (Table 3), MPN values of less than 0.4 per 100 g and less than 2 per 100 g were found by modified and conventional techniques, respectively. The 1,718-g mass of food, which remained after distributing the MPN portions, was divided into six aliquots. Five of these aliquots produced cultures from which *Salmonellae* were isolated. Assuming that one *Salmonella* produced a positive culture (modified technique), one can calculate ($5 \div 1718 = .0029/g$) the MPN value to be 1 per 345 g. Therefore, failure to detect multiplication of *Salmonella* in the abused portions of sample No. 1712, Table 3, is not surprising; each abused portion consisted of only 60-g amounts. Portions employed for a second abuse study were increased to 300-g amounts, sample No. 1713, Table 3. An MPN value of 2 *Salmonella* per 100 g in the original sample did not show an increase after 3 or 6 h

TABLE 2. *Detection of* Salmonella *in 5-lb. (2,268 g) samples of dried dog food by culturing of ten 25-g portions per sample and qualitative destructive analysis of the balance*

Source	Sample no.	288-g Portions	25-g Portions		
		No. flasks pos./no. cultured; Somatic groups isolated	No. flasks pos./no. flasks cultured; Somatic groups isolated	Modified M.P.N./ 100 g[a]	*Salmonella* serotypes identified
Mfg. #1 Identical lot nos.	#1617[b]	7/7 B, C_1, E_1, E_3, E_4, G, K	5/10 C_1, G, K	2.0	Agona Schwarzengrund Infantis Livingstone Lexington Thomasville Senftenberg Havana Siegburg
	#1616[b]	7/7 B, C_1, E_1, E_2, E_4, G, K	6/10 C_1, E_4, G, K	2.4	Schwarzengrund Livingstone Lexington Newington Senftenberg Havana Siegburg
Mfg. #1 Different lot no.	#1254	7/7 B, C_1, E_3, G, K	1/10 G	0.4	Agona Infantis Thomasville Havana Siegburg

[a]Silliker's modification. Ref. *8*. No. flasks positive ÷ total mass cultured × 100.
[b]Lot Nos. identical to those of samples #1712 and #1713, Table 3.

TABLE 3. *Detection of* Salmonella enteritidis *in 5-lb. (2,268 g) samples of dried dog food. Comparison of M.P.N. values derived from fresh and abused samples. Balance of sample examined by qualitative destructive analysis.*

		Fresh samples			Abused Samples[a]					
		Destructive analysis No. flasks pos./ No. cultured (Somatic Groups Isolated)	Modified M.P.N./ 100 g[b] No. flasks pos./ No. 25-g portions cultured (Somatic Groups Isolated)	Conventional M.P.N./100 g (Somatic groups isolated	Conventional M.P.N./100 g (Somatic groups isolated) Hours held at room temp.					
Source	Sample no.				2	3	4	6	24	Serotypes identified
Mfg. #1 Identical Lot Nos.[c]	1712	5/6[d] (C_1, E_4, K)	<0.4 0/10	<2	<2		<2	<2	<2	Infantis Livingstone Senftenberg Siegburg
	1713	3/3[e] (E_1, E_3, G, K)	2.4 6/10 (C_1, E_3, G)	2 (K)		<2		2 (C_1)	>24,000 (E_3, K)	Livingstone Lexington Thomasville Havana Siegburg

[a]Food combined with sterile distilled water.
[b]Silliker's modification. Ref. 8, No. flasks positive ÷ total mass cultured × 100.
[c]Lot No. identical to that of samples 1617 and 1616, Table 2.
[d]286 g/culture.
[e]273 g/culture.

of abuse. However, a significant increase, to greater than 24,000 per 100 g, was observed after 24 h.

DISCUSSION

The modified MPN procedure, described by Silliker (*8*), compared very favorably with a conventional technique as employed in this study. Although it requires almost four times the volume of pre-enrichment medium (2500 ml vs 690 ml), it uses one-half the amount of selective enrichment medium and one-half the amount of selective differential media. It also reduces the number of pre-enrichment cultures from 20 to 10 per sample. Most importantly, it obviates the tedium of preparing homogenates and transferring five decimal dilutions per bank into a set of four banks.

Eight aliquots were prepared from a 2,268-g mass of dried dog food for destructive analysis. Three samples were encountered in which only one of the eight produced positive cultures, Table 1. Using the modified MPN premise of 1 *Salmonella* per positive culture (1 ÷ 2,268 = 0.00044/g) one can calculate a MPN of 1 per 2,273 g. However, one of the samples (No. 0814, Table 1) contained two serotypes of *S. enteritidis.* This observation affirms the suspicion that distribution of *Salmonella* within a 5-lb. sample of dried dog food may not be uniform.

Seven of 8 samples, examined by destructive analysis, yielded isolates of *Salmonella.* Three samples were found to have *Salmonella* in one (283.5 g) of eight aliquots, one in four of eight, one in seven of eight, and two in eight of eight, Table 1. With the exception of two of three samples, in which *Salmonella* was detected in only one of eight aliquots, multiple serotypes were isolated from each 283.5-g aliquot. It is reasonable to conclude that quality control and sampling, at the production plant, were inadequate. Sampling and testing plans for *Salmonella*, as employed by the Food and Drug Administration (FDA), have been described (*9*). It would be prudent for the dried dog food industry to consider adopting the FDA sampling recommendation for foods in Category I. That is, random selection of 60 analytical units per production lot. Fifteen analystical units, 25 g each, could then be combined (375 g) for composite cultures. Four composite units (1,500 g) per production lot being negative would provide the manufacturer assurance, at the 95% confidence level, that the lot contained no more that one *Salmonella* per 500 g. The efficacy of compositing multiple analytical units for detection of *Salmonella* in dried foods has been established by Silliker and Gabis (*11*).

Combining of 60 analytical units into four composite units, for product testing, is a less formidable task than the conventional practice of preparing 10 individual cultures of analytical units. Admittedly one does not intend dried dog food to be consumed by infant humans, aged humans, or infirm humans. However, canine pets may also conform with these physiologic states. It is abundantly clear that dogs, infected with *Salmonella*, can provide a link in disease transmission to humans (*7*). Therefore, manufacturers of dried dog food should be interested in adopting more stringent laboratory testing to provide evidence that their products present a low consumer risk.

Eleven 5-lb. bags of dog food, produced by manufacturer No. 1, were examined in the course of this investigation. Multiple serotypes of *S. enteritidis* were recovered from each of 10 bags and a single serotype was recovered from the eleventh. As many as nine serotypes were detected in a single sample. These products were allegedly produced by an expansion extrusion process. The process is briefly described (pg 179, ref. *13*) as one which conditions meal with steam to attain moisture levels of 25 to 30%. Temperatures of 200 to 350 F (93 to 176 C) are reached for 45 to 60 sec. It is reasonable to

consider this a critical control point in production of dried dog food; time, temperature, and moisture parameters lend themselves to continual monitoring for quality control. Methods to eliminate the hazard of post-processing contamination, if it exists, might well be investigated.

Dried dog food products, distributed under labels of four manufacturers and two store brands, were examined in this study. Products of two manufacturers were found to contain *Salmonella*. The possibility that dried dog foods may provide a vehicle to introduce *Salmonella* into the home is not sufficiently recognized by consumers.

This investigation has brought forth the following questions: (*a*) Is it realistic to expect manufacturers to produce *Salmonella*-free dried dog food? (*b*) Should the answer to the first question be negative, what degree of hazard does dried dog food present to pets and to their owners? (*c*) Should pet owners be cautioned about the handling, storage, and potential for abuse of dried dog foods?

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Microbial Flora of Preseasoned Comminuted Turkey Meat

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ABSTRACT

Preseasoned comminuted turkey meat, prepared at the retail level, was examined and revealed the following levels of microbial contamination per gram: mean standard plate count 2.2×10^8, mean coliform plate count 2.0×10^5, *Escherichia coli* count 8.7. Gram-positive and gram-negative microbial flora were isolated and identified.

Comminuted or ground turkey is a product prepared from either manually or mechanically deboned turkey meat. As an alternative to ground beef, comminuted turkey products have appeared at the retail level with increasing frequency. Fresh comminuted turkey meat has been previously characterized as a product having a high degree of bacterial contamination and a short shelf life as well as potential for disseminating salmonellae and other enteric bacilli (*4*).

Recently, preseasoned turkey loaf was added to the growing list of further processed turkey products. Several spices, including garlic and onion, are known to possess antibacterial properties (*6, 7, 8*) as well as having high bacterial counts of their own. Since fresh comminuted turkey meat has been reported to have a high degree of bacterial contamination (*4*), analyses were undertaken to determine the nature and level of contamination in the preseasoned product.

MATERIALS AND METHODS

Product.

The preseasoned turkey loaf was prepared at the retail market by thawing 20-lb lots of frozen comminuted turkey meat and combining it with a prepackaged dehydrated meat loaf mix containing cracker meal, soy flour, salt, onion, celery, bell pepper, leavening, pepper, and garlic.

Samples.

Samples, in approximately 1-lb lots, were purchased by open tray selection at the retail market and transported to the laboratory in a Freez-Safe Styrofoam Case (Glo Brite Foam Plastics Co., Chicago, IL). Blue-Ice cold storage units (Divajex Co. Santa Ana, CA) were used to maintain a temperature of approximately 8 C during transport. Samples were analyzed after holding at 4 C for not longer than 48 h.

Bacteriological analyses.

Standard plate counts (SPC) were made using the pour plate technique and Standard Methods agar (SMA) as recommended in *Standard Methods for the Examination of Dairy Products* (*SMEDP*). (*1*). Plates were incubated at 32 C for 72 h.

Coliform estimates were made using both the Most Probable Number (MPN) technique and the plate count procedure with Violet Red Bile (VRB) agar as described in *SMEDP* (*1*). The MPN and plate count procedures were also used to quantitate the level of *Escherichia*

TABLE 1. *Bacterial analyses of pre-seasoned comminuted turkey meat*

	Count Ranges										
Test	LE[a] 10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90	91-100	GT[b] 100
SPC[c] × 100,000 per gram											
Number samples in range	0	0	0	0	2	2	0	3	0	0	11
Percent in range					11.1	11.1		16.7			61.1
Cumulative percentage					11.1	22.2		38.9			100.0
SPC × 1,000,000 per gram											
Number samples in range	7	1	0	1	2	1	0	0	2	0	4
Percent in range	38.9	5.6		5.6	11.1	5.6			11.1		22.2
Cumulative percentage	38.9	44.4		50.0	61.1	66.7			77.8		100.0
Escherichia coli per gram											
Number samples in range	15	0	1	0	1	0	0	1	0	0	0
Percent in range	83.2		5.6		5.6			5.6			
Cumulative percentage	83.2		88.8		94.4			100.0			
Staphylococcus aureus per gram											
Number samples in range	6	2	5	0	3	0	0	0	0	1	1
Percent in range	33.3	11.1	27.8		16.7					5.6	5.6
Cumulative percentage	33.3	44.4	72.2		88.9					94.4	100.0
Fecal streptococci × 100 per gram											
Number samples in range	6	1	2	0	1	1	1	0	1	0	5
Percent in range	33.3	5.6	11.1		5.6	5.6	5.6		5.6		27.8
Cumulative percentage	33.3	38.9	50.0		55.6	61.1	66.7		72.2		100.0

[a]Less than or equal to
[b]Greater than
[c]Standard Plate Count

coli contained in the product. The IMViC procedures were used for verification of isolates.

Methods outlined by the Association of Official Analytical Chemists (AOAC) were used to determine *Staphylococcus aureus* MPN (3). Tellurite Polymyxin Egg Yolk (TPEY) agar was substituted for Vogel and Johnson Agar in the MPN technique. Colonies exhibiting a positive reaction on TPEY were verified by testing for coagulase production according to AOAC methodology (3).

Fecal streptococci were enumerated using procedures from the *Bacteriological Analytical Manual for Foods* (*BAM*) (2). Ethyl Violet Azide broth was used for confirmation of colonies from KF Streptococcal agar.

Samples were screened for the presence of salmonellae following procedures for raw meat products as outlined in *BAM* (2). The procedures used for isolation and identification of aerobic bacteria as well as the determination of the presence of *Clostridium perfringens* were previously reported by Guthertz et al. (4). In addition, the procedures outlined above were used to ascertain the extent of microbial contamination of the dehydrated meat loaf mix used to prepare the product for market.

RESULTS AND DISCUSSION

The distribution of counts for each of the bacteriological analyses performed on the samples of preseasoned ground turkey can be seen in Table 1. Standard plate counts ranged from 4.7×10^6 to 3.8×10^8 per gram with a mean count 2.2×10^8 per gram. As indicated in Table 1, 61.1% of the samples tested had standard plate counts greater than 1×10^7 per gram with 22.2% of the samples having a standard plate count greater than 1×10^8per gram. When the mean count of these samples is compared with the 8.4×10^7 per gram mean standard plate count reported for fresh comminuted turkey meat by Guthertz et al. (4), the higher microbial density of the preseasoned product can be easily seem. This increase in microbial density may be attributed to increased handling of the product, microbial growth during thawing of frozen ground turkey meat, or addition of bacteria contained in the seasoning mix.

Coliform analysis using the plate count method revealed coliforms present in all samples with counts ranging from 1.7×10^2 to 1.7×10^6 per gram. The mean coliform count obtained using the plating procedure was 2.0×10^5 per gram. The coliform MPN procedure yielded counts ranging from 9.3×10^2 to 1.1×10^6 per gram with a mean count of 2.3×10^5 per gram.

E. coli was detected by the plate count procedure in only one sample. Using the MPN technique, *E. coli* was detected in 28% of the samples tested with a mean count of 8.7 per gram. Comparison with the results of *E. coli* MPN analyses of fresh ground turkey (4) reveals that *E. coli* was recovered less often in the seasoned samples and that the mean of the seasoned samples is half that reported for the fresh product. Thus it appears that addition of spices to the product may have some influence on the numbers of coliform organisms which remain viable.

It is interesting to note that by using the aerobic isolation technique, *E. coli* was recovered from 83% of the samples tested, which is significantly higher than results obtained using either the plate count or MPN techniques. Since the product tested was prepared from frozen meat, this may be a reflection of evidence that freezing injury to coliforms prevents detection by the conventionally used enumeration techniques (9).

Although, in a previous study, *Salmonella* sp. were isolated from 28% of the fresh comminuted turkey meat samples (4), none were isolated during this study. This may reflect the effects of seasonings on enteric organisms. Johnson and Vaughn (6) found both onion and garlic to have lethal effects on growing and resting-cell cutlrues of *Salmonella* typhimurium and *E. coli.*

Fecal streptococci were detected in all samples. Counts ranged from 2.2×10^2 to 2.9×10^5 per gram with a mean count of 3.5×10^4 per gram. *Streptococcus liquefaciens* represented 69% of the fecal streptococci isolated during this study, followed by *Streptococcus faecalis, Streptococcus durans, Streptococcus faecium,* and *Streptococcus bovis*, representing 15.0, 7.7, 3.8, and 3.8% of the fecal streptococci isolated, respectively.

S. aureus was detected in 83% of the samples tested by the MPN technique. Counts ranged from 3.7×10^0 to 2.4×10^3 with a mean of 1.9×10^2 per gram. Using the isolation technique *S. aureus* was isolated from 89% of the samples. This high incidence in isolation of *S. aureus* is indicative of the increased handling that the product receives before sale.

TABLE 2. *Bacterial flora isolated from pre-seasoned comminuted turkey meat*

Organism	Number isolations	% of samples
Gram-positive isolates:		
Bacillus cereus	4	22
Bacillus sp.	5	28
Clostridium perfringens	9	50
Corynebacterium sp.	2	11
Micrococcus sp.	4	22
Staphylococcus aureus	16	89
Staphylococcus epidermidis	10	56
Streptococcus bovis	1	6
Streptococcus durans	2	11
Streptococcus dysgalactiae	1	6
Streptococcus faecalis	4	22
Streptococcus faecium	1	6
Streptococcus lactis	3	17
Streptococcus liquefaciens	18	100
Streptococcus pneumoniae	1	6
Streptococcus sp.	3	17
Gram-negative isolates:		
Acinetobacter calcoaceticus var. *anitratum*	3	17
Citrobacter freundii	14	78
Enterobacter agglomerans	7	39
Enterobacter cloacae	16	89
Enterobacter liquefaciens (*Serratia liquefaciens*)	5	28
Escherichia coli	15	83
Hafnia alvei (*Enterobacter hafniae*)	15	83
Klebsiella pneumoniae	13	72
Klebsiella ozaenae	2	11
Proteus mirabilis	2	11
Proteus morganii	6	33
Proteus vulgaris	2	11
Pseudomonas aeruginosa	1	6
Pseudomonas fluorescens	3	17
Pseudomonas putida	1	6
Pseudomonas sp.	1	6
Serratia marcescens	1	6
Yersinia enterocolitica	1	6

Table 2 lists the identities of organisms isolated during this study. It should be of interest that along with the 13 isolations of *Klebsiella pneumoniae,* there were two isolations of *Klebsiella ozaenae,* an infrequently occurring respiratory pathogen (*5*). In addition, there were several isolations of *Bacillus cereus* and *C. perfringens* both of which are capable of causing foodborne disease (*9*).

A single examination of the dehydrated seasoning mix yielded the following counts per gram: SPC 420,000; psychrotrophic count 560; coliform MPN 0.1. No fecal streptococci, *C. perfringens, S. aureus,* or salmonellae were isolated from this product. The coliforms present were identified as *Enterobacter cloacae.*

From the data presented, it can be seen that preseasoned comminuted turkey meat is a product containing a high degree of bacterial contamination, some species of which are capable of causing foodborne illness as well as opportunistic infections.

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Growth and Enterotoxin Production by Staphylococci in Genoa Salami[1]

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ABSTRACT

Staphylococcus aureus strains 265 and 243 which produce enterotoxins A and B, respectively, were inoculated into meat being made into Genoa salami in the amount of 10^3, 10^5, and 10^7 cells/g. No lactic starter culture was added. Samples were taken at different stages of processing to determine the microbial populations, percentage moisture, total acidity, pH, and enterotoxin content. Staphylococcal populations varying from about 10^7 to 5×10^8/g were detected during tempering of the salami. Enterotoxin A was detected in surface but not in core samples of salami inoculated with 10^5 and 10^7 *S. aureus* 265 cells/g. However, no enterotoxin B was detected in the salami inoculated with *S. aureus* 243, which requires a relatively high a_w for enterotoxin production. Staphylococcal counts were higher in surface samples than in core samples, attributable to the difference in oxygen, but there was no significant difference in microaerophilic lactic acid bacteria in different portions of the salami.

Meat products such as ham, bacon, and fermented sausage have been incriminated in staphylococcal food poisoning, caused by careless manufacturing techniques which render these products vulnerable to staphylococcal development. Use of cultures of lactic acid bacteria has facilitated inhibition of staphylococci during processing. However, "chance inoculation" and "back slopping" are still used to some extent in industry (*4*). In 1971, several outbreaks of gastroenteritis were traced to Genoa salami containing up to 10^6 coagulase positive staphylococci/g and type A enterotoxin was detected in some of these samples (*12, 13*). Niskanen and Nurmi (*6*) found measurable amounts of enterotoxin A in a 200-g sample of dry sausage containing per g 10^6 cells of *Staphylococcus aureus* which produce type A toxin but enterotoxin B was not detected in corresponding samples containing 10^8 cells per g of *S. aureus* which produce type B toxin.

Barber and Deibel (*2*) studied the effect of pH and oxygen tension on staphylococcal growth and enterotoxin formation in fermented sausage. They indicated growth could be controlled with 1.5% glucono-delta-lactone (GDL), whereas a high inoculum of *Pediococcus cerevisiae* failed to suppress aerobic growth. Most staphylococcal strains tested grew and produced enterotoxin aerobically at pH 5.1 in broth media. Control of *S. aureus* in sausage by lactic cultures and chemical acidulation was also reported by Daly et al. (*4*). Partial inhibition of staphylococcal cells was observed by using the starter culture at 10^8 cells/g, or by chemical acidulation with 0.75% GDL and 0.1% citric acid. They also suggested using a combination of chemical acidulation and lactic culture. The effect of water activity (a_w) on enterotoxin production and growth of *S. aureus* was reported by Troller (*10, 11*) who demonstrated the production of enterotoxin B by *S. aureus* C-243 was strongly inhibited by a reduction in a_w from 0.99 to 0.98 in broth despite the attainment of populations of 10^9 cells/ml. However, *S. aureus* 196E produced enterotoxin A at an a_w of 0.90 and final cell counts were 10^8/ml.

The purpose of this investigation was to evaluate growth of staphylococci and production of enterotoxins in Genoa salami.

MATERIALS AND METHODS

Processing of Genoa salami

Frozen pork was thawed, cut into strips and ground through a 1.2-cm plate. Spices and curing agents (Table 1) were mixed in by stirring. The meat was then inoculated with washed cells of *S. aureus* 265 or 243 in the amount of about 10^3, 10^5, and 10^7 cells/g. After inoculation, meat was spread in layers and refrigerated for 2 days at 4 C. It was re-ground and stuffed into pre-soaked, tied collagen casings (9 × 56 cm; Brechteen Co.). The casings of salami were refrigerated at 4 C for 4 days and then put in a tempering room at 20 to 25 C and 80% relative humidity (RH) for 2 days. Following tempering, the casings were heated in air at 38 C for 20 h, 43 C for 2 h, 49 C for 4 h, and 54 C for 3 h at 80 to 90% RH. The salami was then dried at 12 C and 72% RH for about 60 days.

TABLE 1. *Genoa salami ingredients*

Ingredient	Quantity
Pork	9.08kg
Sodium chloride	306.20g
White pepper	11.34 g
Whole pepper	2.84 g
Sodium nitrite	0.71 g
Sodium nitrate	5.68 g
Garlic	1.89 g
Dextrose	68.10 g

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Sampling methods

Samples from the outer 1 cm of surface and samples of the core were taken from salami inoculated with *S. aureus* 265. Samples of the entire cross section were taken from salami inoculated with *S. aureus* 243. The salami were examined at different stages of processing as follows: (*a*) after inoculation, (*b*) before tempering, (*c*) after tempering, (*d*) after heating, and (*e*) at various intervals during drying.

Enumeration of microbial populations

Staphylococcal counts were made using spread plate technique on Mannitol Salt Agar (MSA; Difco). Following incubation at 37 C for 48 h, coagulase tests were made on a representative number of typical *S. aureus* colonies. The aerobic counts were made in Plate Count Agar (PCA; Difco). Lactic acid bacteria were enumerated in plates of Lactobacillus Selective Agar (LBS; BBL).

Lactic acid and pH determination

Twenty grams of salami and 180 ml of de-ionized water were mixed in a Waring belnder for 2 min. The pH of the homogenate was measured on a Beckman pH meter. The homogenate was then filtered through Whatman #1 filter paper and portions of filtrate corresponding to 5 g of sample were titrated with 0.1 N NaOH to pH 8.3. The total titratable acid was calculated as percent lactic acid.

Moisture determination

A 5-g sample of salami was spread in an aluminum moisture dish 5.5 cm in diameter (Sargent and Co.) and dried in a convection oven at 100 C for 16 to 18 h and cooled. The weight loss was expressed as percent moisture.

Determination of water activity

A moisture sensing element (No. 547535, Hygrodynamics, Inc.) was mounted in a rubber stopper on a 170-ml jar containing 20 g of salami and attached to a hygrometer indicator. Water activity measurements were carried out after the samples were equilibrated for 24 h at 22 C.

Extraction and detection of enterotoxin

Enterotoxin was extracted from 100-g samples of salami and determined by the serological procedure described by Casman and Bennett (*3*) with modifications described by Barber and Deibel (*2*).

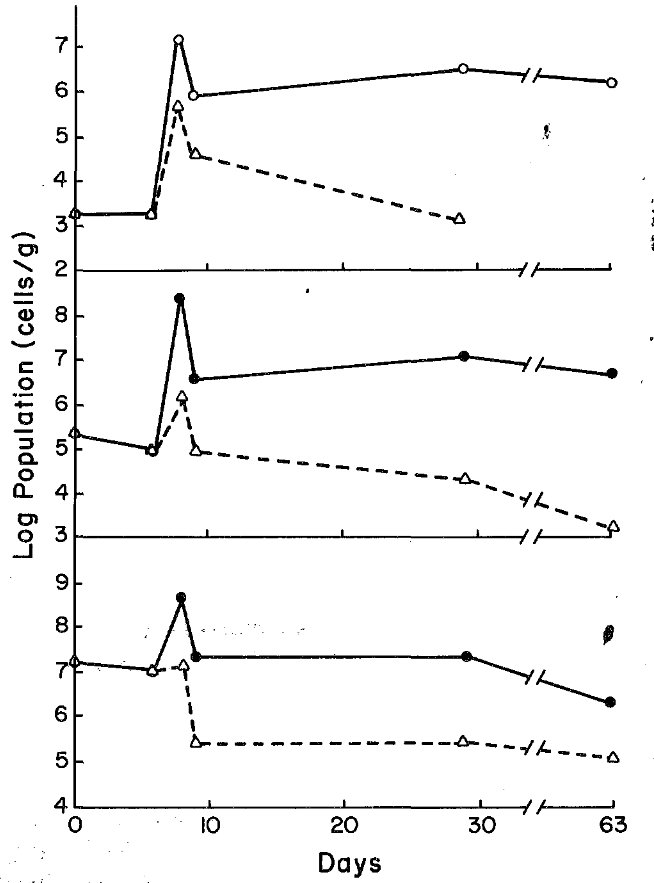

Figure 1. *Populations of* S. aureus *strain 265 determined on MSA plates and enterotoxin A produced in salami inoculated with 10^3 (top), 10^5 (middle), and 10^7 (bottom) cells/g. Legend: -o- surface sample; -△- core sample. Solid symbols indicate enterotoxin A was detected.*

RESULTS AND DISCUSSION

Genoa salami inoculated with S. aureus 265

Samples of salami were obtained for analyses at 0 day (after inoculation), 6 days (after curing in the cooler), 8 days (after tempering), 9 days (after heating), 29 and 63 days (during drying). Data in Fig. 1 illustrate the growth patterns of *S. aureus* 265 in the inoculated salami. The staphylococcal population remained the same or decreased slightly during 6 days of curing in the cooler. After tempering, counts of 1.5×10^7, 2.8×10^8, and 4.9×10^8 cells/g were obtained from the surfaces of the salami inoculated with 10^3, 10^5, and 10^7 staphylococci/g, respectively. In the core samples, however, increases of 300-fold and 15-fold occurred in the salami inoculated with 10^3 and10^5 cells/g, respectively, while only a slight increase occurred in the salami inoculated with 10^7 cells/g. Heating caused a reduction of 10- to 100-fold in populations in both surface and core samples. During the drying period the populations gradually decreased. After drying, counts were more than 10^6 cells/g on the surface of each salami and less than 1.0×10^3, 1.7×10^3 and 1.2×10^5 cells/g in the core of salami inoculated with 10^3, 10^5, and 10^7 cells/g, respectively. The staphylococcal counts were always higher in surface samples than in core samples. These results verify the finding of Barber and Deibel (2) who reported that the uneven distribution of the microbial populations was mainly due to the difference in the oxygen tension. Total population trends for organisms enumerated by aerobic plate counts (Fig.2) were similar to those of the staphylococci, except that during the drying period the total populations decreased less than the staphylococcal populations. Data in Fig. 3 illustrate the population changes of the lactic acid bacteria in salami inoculated with *S. aureus* 265. The original population of these organisms in the pork was less than 150/g of meat, but the count was more than 10^5/g in samples taken after the salami was heated. The anaerobic condition in core samples caused a decrease in the lactic acid bacteria, since they are microaerophilic. Samples taken from different locations of the salami did not show any significant difference in the populations of lactic acid bacteria. Also, there was no significant decrease in the population of lactic organisms during drying.

The pH of salami made in this experiment was relatively high, ranging from 6.06 to 6.33 and probably would have been lower if the meat had been inoculated with lactic organisms. However, low pH values may not inhibit growth and enterotoxin production by staphylococci. Enterotoxin B can be produced in cured meat at an initial pH of 5.0 (7), and an initial pH of 4.5 in

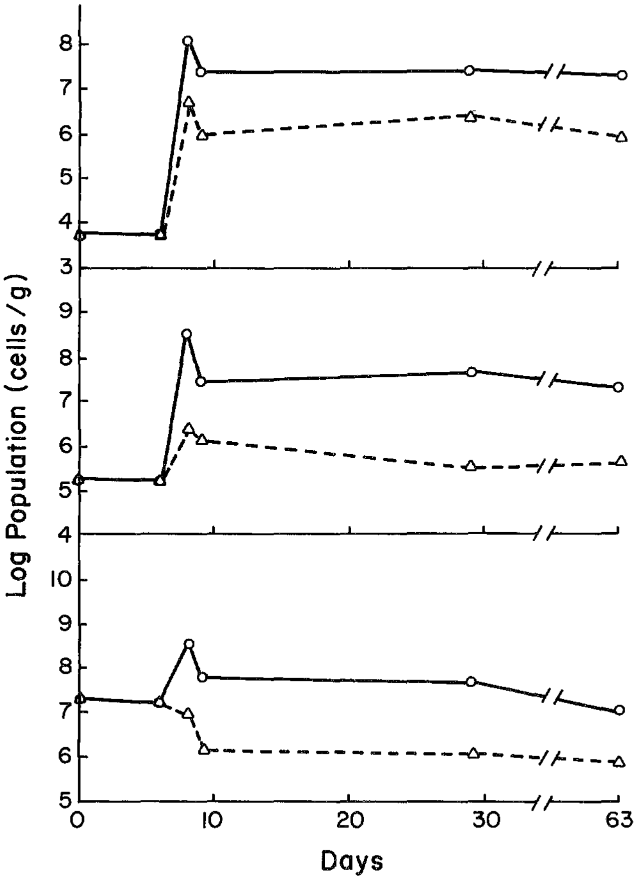

Figure 2. *Aerobic plate counts of salami inoculated with 10^3 (top), 10^5 (middle), and 10^7 (bottom)* S. aureus *strain 265 cells/g. Legend: -o- surface sample; -Δ- core sample.*

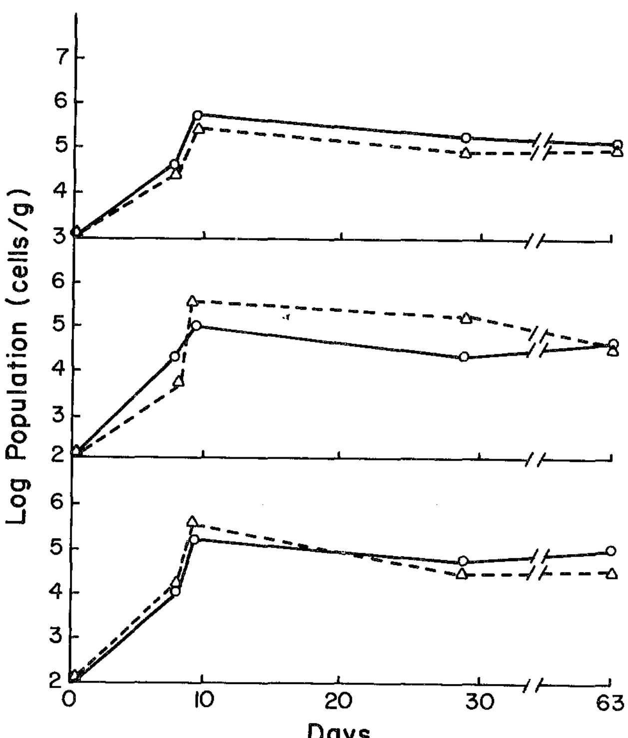

Figure 3. ***Populations of lactic acid bacteria in salami inoculated with 10^3 (top), 10^5 (middle), and 10^7 (bottom)*** **S. aureus** ***strain 265 cells/g.*** *Legend: -o- surface sample; -Δ- core samples.*

reconstituted nonfat milk solids permitted enterotoxin A production according to Tatini et at. (9). The lowest pH value reported to permit formation of staphylococcal enterotoxin was 4.0 in broth medium inoculated with 1×10^8/ml (5). Total acidity developed during the curing period varied from 0.64 to 0.79% expressed as lactic acid. More lactic acid was present in the surface samples of salami inoculated with 10^3 staphylococci than in those inoculated with 10^5 and 10^7/g. The lactic fermentation of the salami was dependent on the indigenous bacterial flora of the meat and the lactic organisms reached a population of only about 10^5/g. Acton et al. (*1*) reported more than 10^8/g of lactic acid bacteria in sausage after 48 h in a controlled fermentation when lactic culture was inoculated at 2×10^6/g.

In the samples taken after 8 or more days, approximately 0.2 μg of enterotoxin A was detected in 100-g portions of surface samples from salami inoculated with 10^5 and 10^7 *S. aureus* 265/g of meat, but no enterotoxin was detected in any of the core samples or in the surface samples of salami inoculated with 10^3/g. Absence of enterotoxin in core samples can be explained by restricted growth of the organisms due to the reduced oxygen tension in the center of the salami.

Genoa salami inoculated with S. aureus 243

Samples were taken from cross sections of this salami instead of from the surface and core as was done with the previous group. Figure 4 illustrates the data obtained on

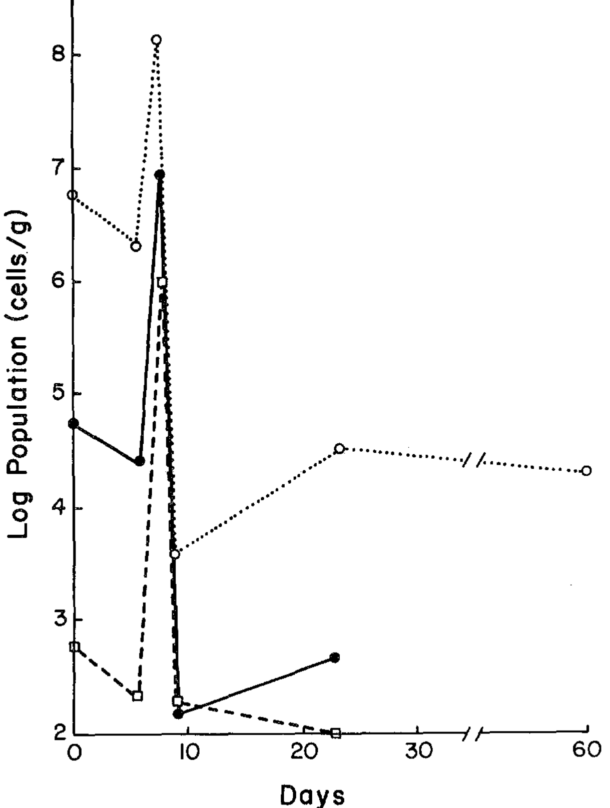

Figure 4. *Populations of* S. aureus *243 as determined on MSA plates from salami inoculated with 10^3 (□), 10^6 (.) and 10^7 (o) cells/g.*

the staphylococcal populations in the inoculated salami. The counts decreased slightly during curing for 6 days in the cooler. Determinations after tempering indicated that the populations increased to 1.0×10^6, 9.0×10^6, and 1.3×10^8/g in the salami inoculated with 10^3, 10^5, and 10^7/g, respectively. Heating on the 9th day reduced the populations 5000-to 59,000-fold in different samples of salami but a slight increase in population at the next sampling time on the 24th day occurred in two of the three samples. Thermally injured cells are inhibited on a highly selective medium and the staphylococcal populations in samples taken immediately after heating probably did not include the heat injured cells. On the 59th day, a population of 2.3×10^4/g remained in the salami inoculated with 10^7/g. The data in Fig. 5 show the

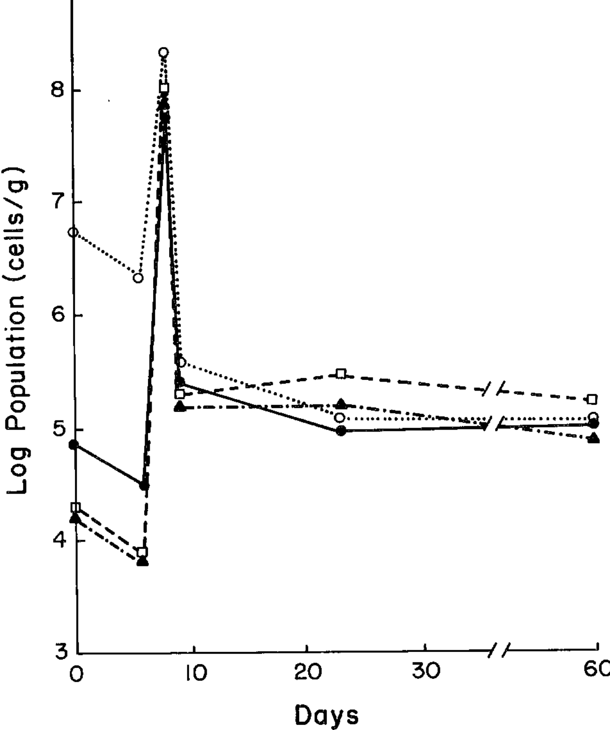

Figure 5. *Aerobic plate counts of non-inoculated salami* (△) *and of salami inoculated with* S. aureus *243 at* 10^3 (□), 10^5 (.), *and* 10^7 (o) *cells/g.*

aerobic plate counts of the salami. Again, the populations increased during tempering and decreased during heating, whereas little change in the population occurred during drying. Data on the population of lactic acid bacteria are summarized in Table 2. No significant

TABLE 2. *Population of lactic acid bacteria in non-inoculated salami and in salami inoculated with* S. aureus *243*

Days	Non-inoculated control	*S. aureus* inocula (cells/g) 10^3	10^5	10^7
		cells/g of lactic acid bacteria		
0	<150	<150	<150	<150
6	<150	<150	<150	<150
8	2.5×10^4	1.7×10^5	1.3×10^4	2.5×10^4
9	9.5×10^3	1.6×10^4	4.0×10^4	3.2×10^4
23	6.0×10^4	5.4×10^4	4.4×10^4	4.4×10^4
59	4.8×10^3	5.5×10^3	3.6×10^3	2.6×10^4

competitive effect was observed between the lactic acid bacteria and the staphylococcal inocula at the various concentrations.

Data showing the moisture content and a_w of the salami are recorded in Table 3. The moisture content

TABLE 3. *Moisture content and* a_w *of a representive blend of salami inoculated with* S. aureus *265 and processed normally*

Days	Moisture (%)	a_w
0	N.D.*	N.D.
6	58.2	0.99
8	58.4	0.98
9	59.9	N.D.
23	55.3	0.97
59	43.4	0.84

* No determination

fluctuated within the range of sampling error during heating and decreased 16.5% during the drying period. Substantial decreases occurred in both moisture and a_w between the 23rd and 59th day. Enterotoxin B was not detected in any of the samples taken from the inoculated salami, although the population of *S. aureus* 243 reached 1.3×10^8/g. The variations in individual staphylococcal strains and environmental conditions have a great influence on the production of enterotoxin. Although the production of enterotoxin is always dependent upon attainment of high cell populations, good growth of staphylococci is not necessarily an indication of the presence of enterotoxin (*2, 7, 8*). In this investigation, the a_w dropped from 0.99 to 0.98 during tempering of the salami and is a limiting factor in the production of enterotoxin A (*11*). Although enterotoxin B was not detected, the high population of the staphylococci in the product could be a potential health hazard since measurable toxin production sometimes occurs in foods containing only a few million coagulase positive staphylococci/g, and some strains of *S. aureus* produce multiple types of toxins detectable only by testing for each toxin individually.

ACKNOWLEDGMENT

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Erratum

Heat-Resistant Psychrotrophic Bacteria Isolated from Pasteurized Milk

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Table 10 was inadvertently omitted from this paper. It should have been provided as follows:

TABLE 10. *Tentative identification of non-spore-producing types*

Type	Tentative identification	Variations from Bergey's
A	*Microbacterium flavum*	Acid from maltose and lactose
B	*Arthrobacter aurescens*	
C	*Microbacterium lacticum*	Starch not hydroloyzed
D	*Corynebacterium equi*	
E	*Arthrobacter* sp.	Did not agree with any specie described in Bergey's Manual
F	*Arthrobacter* sp.	
G	*Streptococcus faecalis* var. *liquefaciens*	
H	*Streptococcus faecalis*	

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Significance of *Clostridium perfringens* in Processed Foods

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ABSTRACT

Clostridium perfringens presents a significant public health hazard to consumers of foods which have undergone improper processing or have been improperly handled at some point before consumption. Factors involved in outbreaks of *C. perfringens* foodborne illness include contamination of food with either spores or vegetative cells of enterotoxigenic strains of *C. perfringens*, suitable growth temperature, pH, media, oxidation reduction potential, and adequate incubation time. With proper handling of food items, the risk of *C. perfringens* foodborne illness outbreaks can be eliminated.

Clostridium perfringens type A has long been recognized as a causative agent in gas gangrene of man and as a potential food spoilage organism. Hobbs and co-workers (*10*), in Great Britain, demonstrated its importance as a foodborne illness agent in 1953. Due to negative results obtained in a human feeding study (*6*), recognition of this organism's role as a foodborne illness entity in the United States was delayed until the late 1950's. The organism is now well established as a cause of foodborne illness throughout the western world. From 1970 through 1974, *C. perfringens* was responsible for 138 outbreaks and 13,268 cases of foodborne illness in the United States. This represented 18.8% of all outbreaks and 25.5% of all cases of foodborne illness confirmed by the Center for Disease Control (*4, 5*).

Over the years, the term 'food processing' has acquired many different meanings to various elements of the food industry. In the context of this paper, food processing pertains to any procedure that modifies a food product in any manner including washing, freezing, waxing, slaughtering, thermosterilizing, dehydrating, fermenting, irradiating, canning, milling, baking, cooking, freezing, packaging, changing or causing to be changed by any one or a combination of procedures before sale for consumption. In modifying or changing a food under this broad definition, a food's ability to support microbial growth may also be modified, not to mention the possibility of altering the natural microbial flora. For example, one would not expect to find viable *C. perfringens* cells in the longissimus dorsi muscle of a healthy beef animal just before slaughter. However, when that muscle is converted, via the various processing steps, into an oven-ready rib roast, the possibility of contamination with *C. perfringens* has been increased considerably.

THE ORGANISM AND THE ILLNESS

C. perfringens is an anaerobic, gram-positive, spore-forming rod. Five types (A through E) have been distinguished on the basis of exotoxins produced. This discussion will be limited to type A, the only type that plays a significant role in foodborne illnesses in the United States. In an excellent review, Smith (*12*) describes the cultural characteristics and requirements of this organism. The clinical symptomology and course of *C. perfringens* foodborne illness are discussed by Walker (*16*).

The typical history of a *C. perfringens* foodborne illness outbreak involves cooked meat items that have been held at either room temperature or under inadequate refrigeration before consumption. In the United States, the most common setting for acquisition of *C. perfringens* foodborne illness is from a food service facility involved in mass feeding. A high percentage of outbreaks have been traced to homes and restaurants, but most cases have usually been associated with mass feeding at banquets, receptions, institutions, or cafeterias where a common meal has been prepared and served. Restaurants may be responsible for a large share of unreported outbreaks because of the diversity of their menu selection and of clientele. In a restaurant feeding situation, a potentially infective menu item may be served to a small percentage of consumers who become ill, but because of minimal or no communication with other victims, the illness is not associated with the restaurant and may be passed off as "intestinal flu" or "something that's going around" (*7*).

C. perfringens foodborne illness requires consumption of between 10^8 and 10^9 vegetative cells. For populations of this size to develop, a suitable growth medium, incubation temperature, and sufficient time are required. The size of

food items can present situations where the growth requirements for *C. perfringens* are more easily met. Foods are cooked to an end point that is determined by a cumulative time-temperature relationship. Thus large items such as beef roasts, and bulk items cooked in large containers are cooked at lower temperatures for longer times than are smaller items. Cooking temperatures are sufficient to drive off gaseous oxygen lowering the oxidation-reduction potential (Eh) to a point more favorable for growth of *C. perfringens*. After cooking, exposure to oxygen is limited to the surface only, leaving subsurface portions free of oxygen and with a reduced Eh. Thus if the food item is large or is being prepared in large bulk containers, oxygen is restricted from the interior of the product for a long period. The resulting reduced Eh is conducive to rapid outgrowth and multiplication of *C. perfringens* when the temperature and other conditions are optimal. Cooking temperatures, in addition to expelling oxygen, also destroy vegetative cells of most microorganisms including *C. perfringens*. However, cooking temperatures are insufficient for the destruction of *C. perfringens* spores but are sufficient to heat shock spores and stimulate their germination. After cooking, as the temperature declines from the cooking range, the freshly germinated cells are able to multiply rapidly. Because of the high optimum growth temperature, *C. perfringens* can grow freely without competition from other organisms.

A CONTAMINANT IN MANY FOODS

C. perfringens is a common contaminant of a wide variety of food items. Its occurrence in a number of common foods has been reported as follows: veal-82%, beef-70%, turkey-62%, chicken-58%, lamb-52%, spaghetti mix-40%, pork-37%, vegetables-5%, fish-2% and luncheon meats-1% (*1, 8, 9, 16*). The organism is also easily isolated from soil (*13*). In human intestinal tracts the carrier rate is 100%. Persons associated with communal feeding have a high carrier rate for heat resistant strains (*7, 14, 15*). Since *C. perfringens* has such universal distribution, precautions must be taken by food processors to prevent foodborne illness outbreaks. This can be accomplished by limiting the dissemination, preventing multiplication, or effecting destruction of the organism.

Meat items, irrespective of species, share a large number of processing procedures. Following slaughter the animal must undergo washing, chilling, cutting, and packaging. Since *C. perfringens* is in the normal flora of the intestinal tract of animals, contamination of the carcass from the intestinal contents, as well as soil, dust, or from workers is virtually unavoidable. Cross-contamination occurs when contaminated and uncontaminated meat is processed in the same facility, using the same equipment and personnel. Once the carcass is removed from the slaughter area, direct contamination from the intestinal contents is minimized, but the danger of cross-contamination and contamination from dust, soil, equipment, and workers persists. When meat is comminuted, any existing contamination may be dispersed throughout the product. Likewise, contamination of an entire lot may occur when products are cured in brine solution. Equipent used to inject curing solutions into hams or bacon slabs is an ideal means of spreading contamination to many pieces of meat.

The slaughter of poultry involves scalding as an aid in removal of feathers and in some processing lines the birds are submerged in a cold water tank to rapidly reduce the carcass temperature. There have been reports of high incidences of *C. perfringens* in both scald and chill water (*11*). Final washing procedures may remove a significant portion of this contamination from the exposed surfaces. Systemic contamination by *C. perfringens* from the scald water has been observed. It was thought that the contamination might have occurred via the jugular vein; however, evidence indicates that contamination occurred via the respiratory system and possibly spread by the circulatory system (*11*).

The most frequently incriminated foods in *C. perfringens* foodborne illness outbreaks are roast beef, turkey, and chicken, along with their associated gravies, juices, and dressings. These foods are convenient to use in mass feeding facilities, and they have a high probability of being contaminated with vegetative cells or spores of *C. perfringens*. Such foods require relatively low cooking temperatures which are normally insufficient to destroy spores. These items can be cooked well in advance of anticipated use and can be served with high acceptance as cold or rewarmed leftovers.

Large food items such as beef roasts or turkeys and products prepared in large bulk containers such as chili require a long time to cool after cooking even when they are placed in a properly functioning conventional refrigerator (*3*). Under ideal conditions, using a refrigerator set at 2 C, 5 h are required to reduce the temperature of a 4-lb roast from 65 to 2 C and 8 h are required to reduce the temperature of a 6.25-lb roast to 2 C. Under these conditions, the temperature of the latter was in the growth range of *C. perfringens* for 2 h, while the former was in the proper growth range for 1.5 h (*2*).

There is a relatively low incidence of *C. perfringens* in the luncheon meat, bologna, and frankfurter type products primarily due to cooking steps followed by continuous cold storage after processing. Likewise, the recovery rate of *C. perfringens* from salami and cured sausages is low due to various conditions that impede the growth of the organism and favor the growth of competitors. Generally, these items have a large surface area thus providing a high Eh. They also have a high salt content, low water activity, and normally are heated sufficiently to destroy vegetative cells. These products are normally stored at or below the minimum growth temperatures for *C. perfringens*. Since these items are

stored for long periods, if significant growth of *C. perfringens* occurred, the product would be rendered unacceptable for consumption.

Fresh and frozen fish and fish products generally are not contaminated with *C. perfringens* with as great a frequency as meats, vegetables, and spices. Given the proper conditions, however, fish and fish products have the potential to be involved in *C. perfringens* foodborne illness outbreaks.

CONTROL

The primary source of *C. perfringens* contamination of vegetables is from soil followed by dust and cross-contamination caused by equipment and people. Although vegetables are not commonly found to be the vehicle of *C. perfringens* foodborne illness, they have the potential to contaminate food service equipment used in the preparation of other foods or to contaminate other foods directly.

C. perfringens can be eliminated from products which are properly canned and thermally sterilized. However, many canned products are only commercially sterile, a term that implies the destruction of all micro-organisms capable of spoiling the food under normal storage conditions. Canned hams are examples of items that are not intended to be commercially sterile. Although these items do receive substantial heat treatment, they require refrigerated storage. In some instances when canned hams are not refrigerated, *C. perfringens* multiplies and causes the can to swell and, on occasion, to rupture the seams. When this happens the product is unacceptable to consumers and thus presents an economic rather than a public health problem. On the other hand, if the product is properly refrigerated and contains relatively low levels of *C. perfringens*, it presents no more of a public health hazard than any other meat item possessing the same degree of contamination.

Many spices are herbs produced in areas of the world where hand labor is plentiful and inexpensive. The possibility of these spices being contaminated by fecal material, soil, and dust is great. Since spices are used extensively to season meats, meat dishes, casseroles and many other food items, they may serve as a source of *C. perfringens* contamination when added to foods.

The possibility of *C. perfringens* causing foodborne illness in processed foods can be controlled by applying good basic food hygiene practices such as limiting contamination, thorough cleaning, proper canning, and maintaining foods at temperatures outside the growth range of *C. perfringens*. Ready to eat food items, particularly meat products, must be held at temperatures either too hot or too cold to permit multiplication of *C. perfringens*. When it is essential that the temperature of a food item pass through the *C. perfringens* growth range, the length of time must be held to a minimum to limit the number of the organisms produced. Foods should be eaten within 3 h after cooking. If the foods are to be held at warm temperatures to be served later, they should be maintained above 55 C. If the temperature falls below 55 C, food should be reheated to above 60 C. Foods that are to be served cold after cooking should be rapidly chilled to 20 C and held at or below 7 C until served.

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Effects of Freezing and Storage on Microorganisms in Frozen Foods: A Review

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ABSTRACT

The fate of bacteria contained in food during freezing, storage, and thawing is usually one that is detrimental. However, many microorganisms considered to have been killed by such treatments actually are only injured. Their viability can be determined by allowing the injury to repair in a non-selective medium before testing for their presence on selective media. Injured cells of pathogens have been found to be as pathogenic as uninjured ones; injured spoilage microorganisms can cause spoilage if permitted to repair and grow. Injured microorganisms and their potential importance in food safety and shelf-life constitute and important problem for the frozed food industry as well as the food sanitarian.

Freezing has become a very important means for food preservation. Frozen foods have two properties that control microbiological activity. One is the limiting a_W; the other is that the temperature of the product is too low to allow microbial growth. Freezing also can maintain a more desirable texture and flavor in foods that can not be accomplished by other preservation procedures. Yet, freezing and frozen storage of foods result in something of a dilemma for the food processor because the attributes of freezing, which permit preservation of the cellular structure of foods, have similar effects on microorganisms that are contained in the foods. Therefore, while some of the micoorganisms may be killed during freezing, many do survive and exist in different states of viability during frozen storage. As a consequence, the microbiologist must now deal with different states of microbial viability in assessing the microbiolobical quality of frozen foods.

FATE OF BACTERIA IN FROZEN FOODS

The successful freezing of food must be done with only minor consideration being given to reducing the bacterial load; yet, depending upon various factors, freezing can be lethal to many bacteria present in foods. As a result, frozen foods contain dead and surviving bacterial cells; many survivors may be in an injured condition. This differentiation is based on the ability of the bacterial cells to form colonies on different types of solid media (*17*). Among gram-negative bacteria, survivors are differentiated from dead cells by their ability to form colonies on a nonselective medium, such as trypticase soy agar (TSA). Among the survivors, injured cells are able to form colonies on TSA, but not on commonly used selective (for "structurally injured cells") or minimal (for "metabolically injured cells") media. The injured cells vary in the extent of their injury; where injury extends beyond the ability of a cell to multiply and form a colony, it is regarded as dead. Differentiation between "structural" and "metabolic" injury probably can be ascribed by the extent of cell damage; all injured cells have damage to cell structure; when injury is more extensive metabolic systems are damaged and such cells are considered metabolically injured.

The numbers of dead, injured, and uninjured bacterial cells in a frozen food are dependent upon many factors. While these factors have been studied primarily with pure cultures (*3, 6, 13, 17*), similar effects can be expected in bacteria present in foods.

Type of bacteria

Bacterial spores are exceedingly resistant to freezing. The vegetative cells of micrococci, staphylococci, and streptococci are very resistant to freezing and frozen storage. However, there is considerable variation in resistance among strains. Gram-negative bacteria generally are more sensitive to freezing than are the gram-positive types. Cells in the stationary phase are more resistant than those in the log phase (*13, 17*).

Composition of food.

Composition of the food in which bacteria are contained can increase or reduce the resistance of bacterial cells to freezing damage. Increased resistance usually is provided by viscous foods and by such food components as proteins, simple and complex carbohydrates, and by triglycerides; reduced resistance is associated with the presence of certain ions, inorganic salts, acids, surface active components, and certain enzymes (e.g., lysozyme, proteases) (*10, 11, 16, 17, 19, 22, 24*).

Treatments of food before, during and after freezing

Freezing can be expected to be more lethal if the food

containing the bacteria is subjected to some other sublethal treatment before freezing. Such treatments could be low heat, irradiation, refrigeration, salting, or acidification.

Within limits, fast freezing is generally less lethal to bacterial cells than slow freezing. The smaller intracellular ice crystals resulting from rapid freezing are less damaging to the cell. Also, rapid thawing is less damaging than slow thawing. Repeated freezing and thawing are highly lethal.

Bacterial cells die rapidly during the initial period of frozen storage and at a reduced rate thereafter. Death rate during frozen storage may increase at higher temperatures of storage, due to fluctuations in temperature and the presence of oxygen (*3, 6, 13, 17*). In frozen foods, pathogenic and indicator bacteria can survive in variable quantities for extended periods (*5, 9*).

IMPORTANCE OF DEAD, INJURED, AND UNINJURED BACTERIA IN FROZEN FOODS

The state in which bacterial cells exist in a frozen food can cause a number of problems with respect to evaluation of its microbiological quality. While freezing and frozen storage can reduce bacterial numbers considerably, this can not be depended upon qualitatively or quantitatively. Therefore, freezing can not replace sanitary production and handling of frozen foods. Consequently, examination of frozen foods for indicator or pathogenic bacteria is important in monitoring frozen food quality. Indicator bacteria, such as the coliform group and *Escherichia coli*, are detected in foods by selective enumeration methods (*1, 4*). Injured cells, which can constitute 90% or more of those surviving, are sensitive to the selective media customarily used to enumerate coliforms and therefore will not be detected (*16, 17, 24*). Consequently, coliform counts on frozen foods can not be depended upon to indicate the quality of sanitary practices used in processing and handling (*2*). It has been shown that the uninjured salmonellae could be equally pathogenic (*21*). Yet injured pathogens are also sensitive to the selective conditions used in their isolation and enumeration (*10, 11, 14*). Therefore, frozen foods contaminated with pathogenic bacteria can be an unsuspected health hazard to consumers.

Effect of selective solid media

Freeze-injured coliforms are extremely sensitive to violet red bile agar (VRBA) and other solid media used for the selective enumeration (*7, 12, 16, 22, 24*). Under some conditions 99% or more of the survivors may be injured and remain undetected. Productivity of VRBA medium varies with the method used in plating; pour plating is more inhibitory than the surface and surface-overlay plating methods (*15, 16, 22, 23, 24*). Increased sensitivity has been shown for freeze-injured *Salmonella* to xylose lysine deoxycholate agar, *Shigella* to Hektoen Enteric agar, *Vibrio parahaemolyticus* to thiosulfate citrate bile salts sucrose agar, and *Staphylococcus aureus* to Vogel-Johnson agar (*10, 11, 14, 17*).

Effect of selective liquid media

Cells of coliforms injured by freezing die rapidly in selective liquid media (*16, 17*). Exposure of such cells for 1 to 2 min in lauryl sulfate tryptose or brilliant green bile broth (LST-BGB) causes them to lose their ability to form colonies on TSA. Similar observations have been made with freeze-injured *Salmonella* in tetrathionate and selenite cysteine broth, with *V. parahaemolyticus* in glucose salt Teepol broth (GSTB), and with *S. aureus* in TSB containing 10% NaCl (Ray, unpublished data).

METHODOLOGY FOR REPAIR OF INJURY AND SELECTIVE ENUMERATION OF COLIFORMS IN FROZEN FOODS

Repair of injured cells cannot occur in selective environments used for their detection. In a non-selective environment injured cells repair their damage and then can proceed with growth and multiplication. Efforts therefore were directed to the development of methods to effect repair of any injured cells before their selective enumeration.

Repair in liquid media

In this method injured cells are allowed to repair in Trypticase Soy Broth (TSB). It has been studied extensively with pure cultures, with sterile foods inoculated with coliforms before freezing, and with commercially processed foods containing coliforms (*16, 17, 22, 24*). Results have indicated that the cells repaired freeze-injury rapidly in TSB; most cells repaired within 1 h at 25 C and were no longer susceptible to VRBA. Resistance of repaired coliforms to liquid selective media has been reported (*16*). Frozen foods, without thawing, can be blended with TSB, incubated 1 h at 25 C to effect repair and then can be enumerated for coliforms with VRBA by plating or with LST by the MPN (most probable number) method.

Repair in liquid media has the advantage of being applicable to foods with small and large coliform populations. For foods having low coliform limits (e.g. $\leq$10/g), a 10-ml portion containing 1 g of a sample could be plated on three plates with VRBA; a smaller amount can be used for foods having high limits. However, this method has several disadvantages. All strains of coliforms do not repair equally well in TSB within 1 h. There is also the possibility that during the repair period uninjured cells may start multiplication; this has been observed with fresh isolates, but not with laboratory cultures or with coliforms in commercial foods. This possibility has made the liquid-repair method of questionable value, especially for regulatory purposes. The other possible disadvantage of the liquid media repair is that certain food components, such as lysozyme, protease, acids, and NaCl, might be detrimental to repair which could limit the recovery of injured coliforms after

incubation in TSB.

Similar repair of freeze-injured *Salmonella, Shigella, V. parahaemolyticus,* and *S. aureus* in TSB has been studied (*10, 11, 14,* Ray, unpublished data).

Repair on solid media

The applicability of this method for enumeration of coliforms in frozen and other semi-preserved foods is being studied currently in our laboratory (*18, 23*). The method consists of either surface or pour plating of blended samples using TSA or PCA (Plate Count Agar); this is followed by a 1 to 2 h of incubation at 25 to 35 C to effect repair. The plates are then overlaid with VRBA in an effort to permit only selective growth of coliform cells during subsequent incubation at 35 C for 24 h. Red to pink colored colonies are enumerated as coliforms. Recent studies have indicated that up to 1 ml of the blended samples could be plated with 5 ml of TSA repair medium. At present, several modifications relating to incubation time and temperature are being studied with the objective to increase coliform colony formation and better recognition of the colonies. In general, for frozen non-dairy products a 50-g sample is blended with 450 ml of 0.1% peptone without prior thawing; coliforms are enumerated by plating (1 ml/plate) by the solid repair procedure (TSA followed by VRBA, to determine the total coliform survivors), by VRBA alone (to determine the uninjured coliforms), and by the standard three tube-three dilution MPN method (*4*) using LST broth followed by confirmation in BGB broth. For frozen dairy products a 50-g sample was thawed by rotating in a waterbath and then plated (1 ml/plate) by the solid repair method and by the standard procedure (*1*). Enumeration of coliforms in 20 commercial ice cream samples by the solid repair procedure resulted in a 4-fold increase in counts. It was of particular interest that 73% of these samples met the customary limit of 10 or less coliforms/g by the standard procedure; by the solid-repair method only 25% were found to meet this standard (*18*). This method has also been found to increase detection of coliforms from different frozen foods, such as seafoods, T.V. dinners, meat products, and vegetable products. Increased counts by use of this procedure also were obtained from other semipreserved foods such as salads, soft ripened cheese, sausage, frankfurters, bacon, sandwiches, refrigerated meat products, and spices (Ray, unpublished data). In most samples coliform counts by the solid-repair method were considerably higher than by the MPN method.

The above results indicate that the solid-repair method could probably be effectively used for enumeration of coliforms from frozen foods as well as from other semi-preserved foods and samples where coliforms may be present in an injured state. This method not only recovered injured coliforms, but also reduced variation in the population enumerated in subsamples, and permitted a reduction of 24 h in the time required for the MPN method. Studies are now in progress to optimize enumeration of coliforms from frozen and other semi-preserved foods by the solid-repair method. Some variation of this method has been reported recently by others (*8, 20*).

CONCLUSIONS

It is becoming increasingly clear that the number of viable microorganisms in frozen foods may markedly exceed that which has been determined by conventional methodology. This presents new problems with respect to assessment of frozen food safety by microbiological analyses. Certainly frozen foods have contained undetected injured microorganisms during the years that conventional analytical methods have been used for monitoring these foods. Whether or not this situation has caused frozen foods to have constituted major dangers to consumers could rightfully be questioned. At the same time, there is no reason for not detecting any pathogen, or enumerating all index bacteria, if they are present in frozen foods and the technique for their detection is available. Furthermore, if other injured cells might affect the shelf-life of the foods during storage or upon thawing, certainly the industry would want to take corrective actions. Conceivably, microbiologal standards for frozen foods, particularly index bacteria, may need some relaxation for a period after regulatory agencies have adopted newer procedures for enumerating injured as well as uninjured bacteria. Similar consideration should be given in the evaluation of microbiological quality and in setting up microbiological standards for different types of semi-preserved products because conditions in such foods may produce sublethal injury to the microbial population present.

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Handling Perishable Foods

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ABSTRACT

Food service industry management has been interested in help to meet sanitary regulations, reduce food spoilage, lengthen keeping quality, and prevent foodborne illness. Materials were developed for 1-day workshops to provide practical suggestions for handling perishable and preserved foods. Products included were meats, poultry and eggs, milk and dairy products, delicatessen, fruits, vegetables and seafood. A 200-page reference book and 12 sets of slides or filmstrips were used to outline spoilage, food poisoning, personal hygiene, equipment sanitation, and proper food handling practices. A program has been conducted at 16 locations in Pennsylvania. Participants have been supervisors from stores, schools, hospitals, nursing homes, restaurants, vending companies, and fast food operations. Educational materials and programs will train food service personnel how to handle perishable foods.

You never know to what two telephone calls may lead. Representatives of two supermarket chains in Pennsylvania wanted educational information presented at meetings of their store managers. The purpose was to present proper handling practices which would help them meet the new retail establishment regulations covering temperature and exposure of dairy products. A suggestion to include handling practices for other perishable foods led to our workshops.

The need for educational programs on handling perishable food is great. More state and local regulations require training programs on food sanitation and licensing of supervisory personnel. No attempt was made to involve regulatory personnel or to cover regulations.

SITUATION

The model ordinance for states covering food service establishments has been revised by the Food and Drug Administration. Last year (1975) they proposed a regulation to require training and licensing of a supervisor in each food service establishment.

A Report to Congress by the Controller General of the United States indicated that present federal support for restaurant sanitation was found to be largely ineffective. On the basis of random sampling and inspection of restaurants during 1974, it was estimated that about 90% of all restaurants were unsanitary. Most of these conditions involved dirty dishes and utensils, and improper protection and storage of perishable food.

State and federal regulatory agencies report an increasing incidence of food spoilage and food poisoning outbreaks. Many of these are traced to food service establishments. The Center for Disease Control reported in 1974 that 151 of 456, or 33% of the incidences of foodborne disease outbreaks were acquired in restaurants or schools.

SCHEDULING OF WORKSHOPS

The Extension Service of the Pennsylvania State University conducts programs for the general public and agricultural producers as requested. Cost to the participants is minimal, usually involving only cost of materials.

Our Perishable Food Handling Workshops have all been organized by regional or county Extension staff. They developed a mailing list, required preregistration, and arranged for meeting facilities and lunch. They determined the interest and promoted the program.

The 16 workshops have been coordinated by 16 different persons. In fact, some workshops have involved two persons and at least two counties. Their mailing lists were prepared with the cooperation of local sanitarians. Notices of the workshops were sent to schools, hospitals, nursing homes, retail stores, fire halls, churches, restaurants, and fast food outlets.

The response has exceeded all expectations in nearly every instance. Evaluations at recent workshops indicated high ratings for the presentations and material. Participants have been especially pleased at how the information was directed toward practical use in their operations.

WORKSHOP PROGRAM AND MATERIALS

Eight separate presentations have been made during the 6-h program. Ample time was allowed after each illustrated talk for questions and a general discussion session was included at the end.

Workshops started with registration and coffee at 8:30 a.m. Background information was provided in the morning to convince participants of the need for good sanitation and proper handling practices for perishable food.

Sets of color slides, which we have prepared, illustrate spoilage, health hazards, personal hygiene, and equipment sanitation. Initially, some commercial film strips and tapes were included.

The highlight of the day has been our specific handling practices provided during the afternoon. Foods covered include dairy, meat, poultry, seafood, delicatiessen, fruit, vegetable, and bakery products in fresh, frozen, and canned forms.

Each person received a certificate of instruction and a reference book of mimeograph material and bulletins. We did not use the reference book during the program, however it included more information than we could cover during the workshops. Copies of the workshop program, reference book index, and evaluation form are included.

TABLE 1. *Perishable food handling workshop*

8:30 AM	— Registration and coffee
9:00	— Introduction
9:05	— *General spoilage of foods*—Mast: bacterial types, growth requirements, and destruction
10:00	— *Public health hazards*—Kuhn: food poisoning, food safety
10:30	— Break
10:45	— *Prevention of hazards and spoilage*—Kuhn
11:15	— *Personal and equipment sanitation*—Barnard: employee hygiene, packaging and handling, cleaning and sanitizing food handling facilities and equipment
12:00	— Lunch
1:00 PM	— *Sanitation and handling practices for perishable products*/meats, seafood, poultry, eggs and delicatessen—Mast
2:00	— *Dairy products*—Barnard
2:45	— Break
3:00	— *Fruits, vegetables, bakery*—Kuhn
3:30	— General discussion
4:00	— Presentation of certificates and adjourn

This workshop is conducted by the following Food Scientists from the Pennsylvania State University in cooperation with County Extension staff:

Dr. Gerald Kuhn - fruits and vegetables
Dr. Morris Mast - poultry, meat, eggs
Mr. Sidney Barnard - dairy products

The registration fee of $15.00 covers the cost of reference materials, visuals, facilities and travel expenses of speakers. For more information or to arrange a workshop, contact:

Sidney E. Barnard
Extension Food Scientist
The Pennsylvania State University
9 Borland Laboratory
University Park, PA 16802

All workshops were set up conference style to provide participants with a table on which to take notes and scan the reference book. Breaks were provided during both morning and afternoon.

We carried with us all of the necessary visual aid equipment which included lecturenette, screen, slide projector and stand, filmstrip and cassette tape projector and speaker, and the necessary extension cords. Registration forms and name tags were also provided.

Each participant paid $15.00 for the days program plus lunch. All comments indicated they received more than their money's worth. The cost of the publications

TABLE 2. *Reference book index, program for workshops*

Section A	— General spoilage of foods Food spoilage and preservation
Section B	— Food safety and wholesomeness
Section C	— Personal and equipment sanitation Sanitation of perishable food handling facilities and equipment in stores Food soils, water hardness, and alkaline cleaner formulations Trouble-shooting films and deposits on equipment Personal hygiene
Section D	— Meats and seafood Sanitation and handling practices for perishable products—Red meats Spoilage of meats Fish and shellfish
Section E	— Eggs, poultry and delicatessen Maintaining shell egg quality Shelf life and spoilage of poultry Recommended procedures for preparation and vending of barbecued meats cooked in rotisseries Additional recommendations for small establishments Sanitary preparation of potentially hazardous foods—salads Selected food poisoning outbreaks and single cases from barbecued food sold at retail outlets
Section F	— Dairy products Shelf life and spoilage of dairy products Store handling and dating fluid milk Milk handling procedures-in envelope Consumer complaints about dairy products
Section G	— Fruits and vegetables Handbook on the storage of fruits and vegetables for farm and commercial use Storage and keeping qualities of canned food
Section H	— Miscellaneous Violations observed in retail food stores Definitions of selected terms Microbiological standards for raw ground beef, cold cuts, and frankfurters

TABLE 3. *Evaluation*

Perishable food handling workshop
Date: ________ Location ____________

Were the meeting room facilities satisfactory?
Yes_____ No_____

Rate presentation of speakers:
Good_____ Fair_____ Poor_____

How were visual aids?
Good_____ Fair_____ Poor_____

Rate reference book for future use:
Good_____ Fair_____ Poor_____

Did you get the information you expected?
Most_____ Some_____ None_____

Do you think this information will help you do a better job?
Yes_____ No_____ Possibly_____

Indicate the category of food service in which you are employed.

Hotel or Restaurant	_____	Church or Fire Hall	_____
School	_____	Vending	_____
Hospital	_____	Retailer or Wholesaler	_____
		Other	_____

Are you interested in similar programs on topics such as?

Waitress training	_____	Nutrition	_____
Kitchen sanitation	_____	Other	_____

Add other comments about today's program and the information presented.

and mimeographed material was nearly $10.00 when assembled.

We tried to conclude each workshop about 4:00 p.m. as some participants traveled for an hour or more.

Although more than half of the participants were female many men did attend.

Attendance at workshops has varied from 18 to 148 persons. We have asked county Extension staff to get at least 25 persons pre-registered at least one week before the date of the workshop.

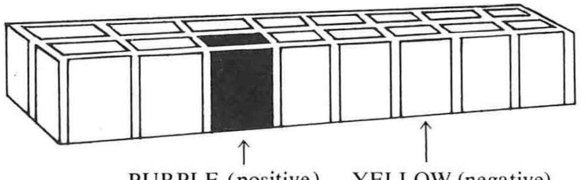
SUMMARY

Sixteen Perishable Food Handling Workshops have been conducted by Extension Food Scientists at the Pennsylvania State University since April, 1975. Total attendance has been 803 persons for an average of about 50 per workshop. Our experience indicates that groups of 25 to 50 persons provide the greatest chance for discussion. We have many requests for the future, but these workshops are only a part of our educational programs. Previous to this our efforts have been primarily with agricultural producers, processors and consumers. There is much interest among food handlers for information about sanitation and handling of perishable foods. We encourage other institutions to conduct similar workshops. We found those in Pennsylvania to be well received and to provide a sense of satisfaction to us.

ACKNOWLEDGMENT

Presented at the 63rd Annual Meeting of the International Association of Milk, Food, and Environmental Sanitarians, Arlington Heights, Illinois, August 8-11, 1976.

New!
For bulk tank screening.



Easy to use!
Economical!

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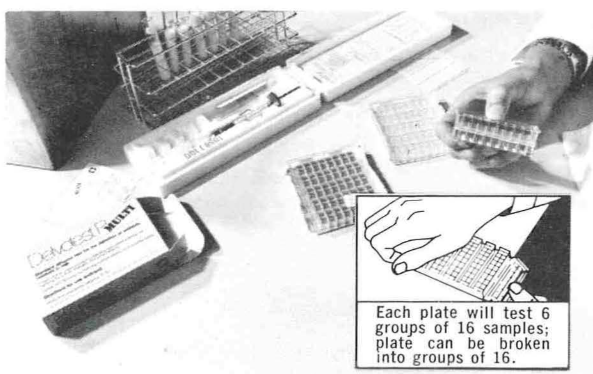

Delvotest® Multi consists of 2 disposable plates, each containing **96** (6x16) cups. One plate contains agar with spores while the other plate contains nutrient tablets.

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It's sensitive. Penicillin concentrations of 0.003 I.U. or less per ml. of milk always yield a negative test result (entirely yellow), while levels of 0.006 I.U. or higher give a positive result (entirely purple). In-between concentrations give yellow-purple colorations.

It's diverse. Also sensitive to all antibiotics used in lactating cattle.

It's economical. Delvotest® Multi is economical enough to allow a complete antibiotic screening program for all incoming bulk tankers to assure antibiotic control also in finished products.

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Journal of Food Protection Vol. 40, No. 5, Pages 340-344 (May, 1977)
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Holders of 3-A Symbol Council Authorizations on February 20, 1977

Questions or statements concerning any of the holders of authorizations listed below, or the equipment fabricated, should be addressed to Earl O. Wright, Sec'y.-Treas., P.O. Box 701, Ames, Iowa 50010.

01-06 Storage Tanks for Milk and Milk Products

28 Cherry-Burrell Corporation (10/ 3/56)
575 E. Mill St.
Little Falls, New York 13365

102 Chester-Jensen Company, Inc. (6/ 6/58)
5th & Tilgham Streets
Chester, Pennsylvania 19013

2 CREPACO, Inc. (5/ 1/56)
100 C.P. Avenue
Lake Mills, Wisconsin 53551

117 Dairy Craft, Inc. (10/28/59)
St. Cloud Industrial Park
St. Cloud, Minnesota 56301

76 Damrow Company (10/31/57)
196 Western Avenue
Fond du Lac, Wisconsin 54935

115 DeLaval Company, Ltd. (9/28 59)
113 Park Street South
Peterborough, Ontario, Canada

109 Girton Manufacturing Company (9/30/58)
State Street
Millville, Pennsylvania 17846

114 C. E. Howard Corporation (9/21/59)
P.O. Box 2507
City of Industry, California 91746

127 Paul Mueller Company (6/29/60)
P.O. Box 828
Springfield, Missouri 65801

31 Walker Stainless Equipment Co. (10/ 4/56)
Elroy, Wisconsin 53929

02-06 Pumps for Milk and Milk Products

214R Ben H. Anderson Manufacturers (5/20/70)
Morrisonville, Wisconsin 53571

212R Babson Bros. Co. (2/20/70)
2100 S. York Rd.
Oak Brook, Illinois 60621

29R Cherry-Burrell Corporation (10/ 3/56)
2400 Sixth St., Southwest
Cedar Rapids, Iowa 52406

63R CREPACO, Inc. (4/29/57)
100 CP Avenue
Lake Mills, Wisconsin 53551

205R Dairy Equipment Company (5/22/69)
1919 South Stoughton Road
Madison, Wisconsin 53716

65R G & H Products, Inc. (5/22/69)
5718 52nd Street
Kenosha, Wisconsin 53140

145R ITT Jabsco, Incorporated (11/20/63)
1485 Dale Way
Costa Mesa, California 92626

26R Ladish Co., Tri-Clover Division (9/29/56)
9201 Wilmot Road
Kenosha, Wisconsin 53140

236 Megator Corporation (5/ 2/72)
125 Gamma Drive
Pittsburgh, Pennsylvania 15238

280 Niro Atomizer (9/10/76)
9165 Rumsey Road
Columbia, Maryland 21045

241 Puriti S. A. (9/12/72)
Alfredo Noble #39, Industrial Pte. de Vigas
Tlalnepantla, Mexico

148 Robbins & Myers, Inc. (4/22/64)
Moyno Pump Division
1345 Lagonda Avenue
Springfield, Ohio 45501

72R L. C. Thomsen & Sons, Inc. (8/15/57)
1303 43rd Street
Kenosha, Wisconsin 53140

219 Tri-Canada Cherry-Burrell Ltd. (2/15/71)
6500 Northwest Drive
Mississauga, Ontario, Canada

175R Universal Milking Machine Div. (10/26/65)
National Cooperatives, Inc.
First Avenue at College
Albert Lea, Minnesota 56007

52R Viking Pump Div. (12/31/56)
Houdaille Industries, Inc.
406 State Street
Cedar Falls, Iowa 50613

5R Waukesha Foundry Company (7/ 6/56)
Waukesha, Wisconsin 53186

282 Knudsen Corporation (11/ 8/76)
715 N. Divisadero Street
Visalia, California 93277

04-03 Homogenizers and High Pressure Pumps of the Plunger Type

247 Bran and Lubbe, Inc. (4/14/73)
2508 Gross Point Road
Evanston, Illinois 60201

87 Cherry-Burrell Company (12/20/57)
2400 Sixth Street, Southwest
Cedar Rapids, Iowa 52404

37 CREPACO, Inc. (10/19/56)
100 CP Avenue
Lake Mills, Wisconsin 53538

75 Gaulin, Inc. (9/26/57)
44 Garden Street
Everett, Massachusetts 02149

237 Graco Inc. (6/ 3/72)
P.O. Box 1441
Minneapolis, Minnesota 55440

256 Hercules, Inc. (1/23/74)
2285 University Avenue
St. Paul, Minnesota 55114

282 Knudsen Corporation (11/ 8/76)
715 N. Divisadero Street
Visalia, California 93277

05-13 Stainless Steel Automotive Milk Transportation Tanks for Bulk Delivery and/or Farm Pick-up Service

131R Almont Welding Works, Inc. (9/ 3/60)
4091 Van Dyke Road
Almont, Michigan 48003

70R Brenner Tank, Inc. (8/ 5/57)
450 Arlington,
Fond du Lac, Wisconsin 54935

40 Butler Manufacturing Co. (10/20/56)
900 Sixth Ave., Southeast
Minneapolis, Minnesota 55114

66 Dairy Equipment Company (5/29/57)
1919 South Stoughton Road
Madison, Wisconsin 53716

45 The Heil Company (10/26/56)
3000 W. Montana Street
Milwaukee, Wisconsin 53235

201 Paul Krohnert Mfg., Ltd. (4/ 1/68)
811 Steeles Avenue
Milton, Ontario, Canada L9T 2Y3

85 Polar Manufacturing Company (12/20/57)
Holdingford, Minnesota 56340

71 Progress Industries, Inc. (8/ 8/57)
400 E. Progress Street
Arthur, Illinois 61911

121 Technova Inc. Gosselin Division (12/ 9/59)
1450 Hebert c.p. 758
Drummondville, Quebec, Canada

189 A. & L. Tougas, Ltee (10/ 3/66)
1 Tougas St.
Iberville, Quebec, Canada

47 Trailmobile, Div. of Pullman, Inc. (11/ 2/56)
701 East 16th Avenue
North Kansas City, Missouri 64116

25 Walker Stainless Equipment Co. (9/28/56)
New Lisbon, Wisconsin 53950

08-17 Fittings Used on Milk and Milk Products Equipment and Used on Sanitary Lines Conducting Milk and Milk Products

79R Alloy Products Corporation (11/23/57)
1045 Perkins Avenue
Waukesha, Wisconsin 53186

138R APV-CREPACO of Canada Limited (12/17/62)
1250 Ormont Dr.
Weston, Ontario, Canada M9L 2V4

245 Babson Brothers Company (2/12/73)
2100 South York Road
Oak Brook, Illinois 60521

284 Bristol Engineering Company (11/18/76)
210 Beaver Street
Yorkville, Illinois 60560

82R Cherry-Burrell Company (12/11/57)
2400 Sixth Street, Southwest
Cedar Rapids, Iowa 52406

266 Condor Manufacturing Company (8/ 1/75)
418 West Magnolia Avenue
Glendale, California 91204

260 CREPACO, Inc. (5/22/74)
100 CP Avenue
Lake Mills, Wisconsin 53551

271 The Foxboro Company (3/ 8/76)
Neponset Street
Foxboro, Massachusetts 02035

67R G & H Products, Inc. (6/10/57)
5718 52nd Street,
Kenosha, Wisconsin 53140

199R Graco, Inc. (12/ 8/67)
P.O. Box 1441
Minneapolis, Minnesota 55440

203R ITT-Grinnell Company (11/ 7/68)
260 W. Exchange St.
Providence, Rhode Island 02901

218 Highland Corporation (2/12/71)
74-10 88th St.
Glendale, New York 11227

34R Ladish Co., Tri-Clover Division (10/15/56)
9201 Wilmot Road
Kenosha, Wisconsin 53140

287 Koltek OY (1/14/77)
Kotinummentieiz
SF-00700 Helsinki 70
Finland

239 LUMACO (6/30/72)
Box 688,
Teaneck, New Jersey 07666

200R Paul Mueller Co. (3/ 5/68)
P.O. Box 828
Springfield, Missouri 65801

242 Puriti, S. A. (9/12/72)
Alfredo Nobel #39 Industrial Pte. de Vigas
Tlalnepantla, Mexico

149R Q Controls (5/18/64)
Occidental, California 95465

73R L. C. Thomsen & Sons, Inc. (8/31/57)
1303 43rd Street
Kenosha, Wisconsin 53140

191R Tri-Canada Cherry-Burrell, Ltd. (11/23/66)
6500 Northwest Drive
Mississauga, Ontario, Canda L4V 1K4

250 Universal Milking Machine Division (6/11/73)
Universal Cooperatives, Inc.
408 First Ave. South
Albert Lea, Minnesota 56007

278 Valex Products (8/30/76)
9421 Winnetka
Chatsworth, California 91311

86R Waukesha Specialty Company, Inc. (12/20/57)
Darien, Wisconsin 53114

08-17 Inlet and Outlet Leak Protector Plug Valves for Batch Pasteurizers

122R Cherry-Burrell Company (12/11/59)
2400 Sixth St., Southwest
Cedar Rapids, Iowa 52406

69 G & H Products Corporation (6/10/57)
5718 52nd Street
Kenosha, Wisconsin 53140

27 Ladish Co., Tri-Clover Division (9/29/56)
9201 Wilmot Rd.
Kenosha, Wisconsin 53140

78 L. C. Thomsen & Sons, Inc. (11/20/57)
1303 43rd Street
Kenosha, Wisconsin 53140

09-07 Instrument Fittings and Connections Used on Milk and Milk Products Equipment

269 Babson Bros. Company (1/23/76)
2100 South York Road
Oak Brook, Illinois 60521

206 The Foxboro Company (8/11/69)
Neponset Avenue
Foxboro, Massachusetts 02035

285 Tank Mate Company (12/ 7/76)
1815 Eleanor
St. Paul, Minnesota 55116

32 Taylor Instrument Process Control (10/ 4/56)
Div. Sybron Corporation
95 Ames Street
Rochester, New York 14601

246 United Electric Controls (3/24/73)
85 School Street
Watertown, Massachusetts 02172

10-00 Milk and Milk Products Filters Using Disposable Filter Media, As Amended

35 Ladish Co., Tri-Clover Division (10/15/56)
9201 Wilmot Road
Kenosha, Wisconsin 53140

11-03 Plate-type Heat Exchangers for Milk and Milk Products

20 A.P.V. Company, Inc. (9/ 4/56)
395 Fillmore Avenue
Tonawanda, New York 14150

30 Cherry-Burrell Corporation (10/ 1/56)
2400 Sixth Street, Southwest
Cedar Rapids, Iowa 52404

14 Chester-Jensen Co., Inc. (8/15/56)
5th & Tilgham Streets
Chester, Pennsylvania 19013

38 CREPACO, Inc. (10/19/56)
100 CP Avenue
Lake Mills, Wisconsin 53551

267 De Danske Mejeriers Maskinfabrik (10/15/75)
The Danish Dairies' Machine Factory
P.O. Box 66, 6000 Kolding, Denmark

120 DeLaval Company, Ltd. (12/ 3/59)
113 Park Street
South Peterborough, Ontario, Canada

17 The DeLaval Separator Company (8/30/56)
350 Dutchess Turnpike
Poughkeepsie, New York 12602

15 Kusel Dairy Equipment Company (8/15/56)
100 W. Milwaukee Street
Watertown, Wisconsin 53094

12-04 Internal Return Tubular Heat Exchangers, for Milk and Milk Products

248 Allegheny Bradford Corporation (4/16/73)
P.O. Box 264
Bradford, Pennsylvania 16701

243 Babson Brothers Company (10/31/72)
2100 S. York Road
Oak Brook, Illinois 60521

103 Chester-Jensen Company, Inc. (6/ 6/58)
5th & Tilgham Street
Chester, Pennsylvania 19013

152 The DeLaval Separator Co. (11/18/69)
350 Dutchess Turnpike
Poughkeepsie, New York 12602

217 Girton Manufacturing Co. (1/23/71)
Millville, Pennsylvania 17846

252 Ernest Laffranchi (12/27/73)
P.O. Box 455
Ferndale, California 95536

238 Paul Mueller Company (6/28/72)
P.O. Box 828
Springfield, Missouri 65801

96 C. E. Rogers Company (3/31/64)
P.O. Box 188
Mora, Minnesota 55051

13-06 Farm Milk Cooling and Holding Tanks

240 Babson Brothers Company (9/ 5/72)
2100 S. York Road
Oak Brook, Illinois 60521

11R CREPACO, Inc. (7/25/56)
100 CP Ave.
Lake Mills, Wisconsin 53551

119R Dairy Craft, Inc. (10/28/59)
St. Cloud Industrial Park
St. Cloud, Minnesota 56301

4R Dairy Equipment Company (6/15/56)
1919 South Stoughton Road
Madison, Wisconsin 53716

92R DeLaval Company, Ltd. (12/27/57)
113 Park Street
South Peterborough, Ontario, Canada

49R The DeLaval Separator Company (12/ 5/56)
Dutchess Turnpike
Poughkeepsie, New York 12602

10R Girton Manufacturing Company (7/25/56)
Millville, Pennsylvania 17846

95R Globe Fabricators, Inc. (3/14/58)
3350 North Gilman Rd.
El Monte, California 91732

179R Heavy Duty Products (Preston), Ltd. (3/ 8/66)
1261 Industrial Road
Preston, Ontario, Canada

12R Paul Mueller Company (7/31/56)
P.O. Box 828
Springfield, Missouri 65801

249 Sunset Equipment Co. (4/16/73)
3765 North Dunlap Street
St. Paul, Minnesota 55112

42R VanVetter, Inc. (10/22/56)
4 South Idaho Street
Seattle, Washington 98134

16R Zero Manufacturing Company (8/27/56)
Washington, Missouri 63090

16-04 Evaporators and Vacuum Pans for Milk and Milk Products

164R Anderson IBEC (4/25/65)
19609 Progress Drive
Strongsville, Ohio 44136

254 Anhydro, Inc. (1/ 7/74)
165 John Dietsch Square
Attleboro Falls, Massachusetts 02763

132R A.P.V. Company, Inc. (10/26/60)
137 Arthur Street
Buffalo, New York 14207

263 C. E. Howard Corporation (12/21/74)
240 N. Orange Avenue
City of Industry, California 91746

107R C. E. Rogers Company (8/ 1/58)
P.O. Box 118
Mora, Minnesota 55051

186R Marriott Walker Corporation (9/ 6/66)
925 East Maple Road
Birmingham, Michigan 48010

273 Niro Atomizer Inc. (5/20/76)
9165 Rumsey Road
Columbia, Maryland 21044

17-04 Fillers and Sealers of Single Service Containers For Milk and Milk Products

192 Cherry-Burrell Corporation (1/ 3/67)
2400 Sixth St., Southwest
Cedar Rapids, Iowa 52404

137 Ex-Cell-O Corporation (10/17/62)
2855 Coolidge,
Troy, Michigan 48084

220 Hercules, Inc., Package Equipment Div. (4/24/71)
2285 University Ave.
St. Paul, Minnesota 55114

281 Purity Packaging Corporation (11/ 8/76)
4190 Fisher Road
Columbus, Ohio 43228

211 Steel & Cohen (2/ 4/70)
745 Fifth Avenue
New York, New York 10022

19-02 Batch and Continuous Freezers, For Ice Cream, Ices and Similarly Frozen Dairy Foods, As Amended

286 Alfa-Hoyer (12/ 8/76)
Soren Nymarksvei 13
DK-8270 Hojbjerb, Denmark

146 Cherry-Burrell Company (12/10/63)
2400 Sixth Street, Southwest
Cedar Rapids, Iowa 52404

141 CREPACO, Inc. (4/15/63)
100 CP Avenue
Lake Mills, Wisconsin 53551

22-04 Silo-Type Storage Tanks for Milk and Milk Products

168 Cherry-Burrell Corporation (6/16/65)
575 E. Mill St.
Little Falls, New York 13365

154 CREPACO, Inc. (2/10/65)
100 CP Avenue
Lake Mills, Wisconsin 53551

160 Dairy Craft, Inc. (4/ 5/65)
St. Cloud Industrial Park
St. Cloud, Minnesota 56301

181 Damrow Company, Division of DEC (5/18/66)
International, Inc., 196 Western Ave.
Fond du Lac, Wisconsin 54935

262 DeLaval Company Limited (11/11/74)
113 Park Street
South, Peterborough, Ontario, Canada

156 C. E. Howard Corporation (3/ 9/65)
9001 Rayo Avenue
South Gate, California 90280

276 Letsch Corporation (8/17/76)
501 N. Belcrest
Springfield, Missouri 65802

155 Paul Mueller Co. (2/10/65)
P.O. Box 828
Springfield, Missouri 65801

165 Walker Stainless Equipment Co. (4/26/65)
Elroy, Wisconsin 53929

23-01 Equipment for Packaging Frozen Desserts, Cottage Cheese and Milk Products Similar to Cottage Cheese in Single Service Containers

174 Anderson Bros. Mfg. Co. (9/28/65)
1303 Samuelson Road
Rockford, Illinois 61109

209 Doboy Packaging Machinery (7/23/69)
Domain Industries, Inc., 869 S. Knowles Ave.
New Richmond, Wisconsin 54017

258 Hercules, Inc. (2/ 8/74)
2285 University Ave.
St. Paul, Minnesota 55114

222 Maryland Cup Corporation (11/15/71)
10100 Reisterstown Road
Owings Mills, Maryland 21117

193 Triangle Package Machinery Co. (1/31/67)
6655 West Diversey Ave.
Chicago, Illinois 60635

24-00 Non-Coil Type Batch Pasteurizers

161 Cherry-Burrell Corporation (4/ 5/65)
575 E. Mill St.
Little Falls, New York 13365

158 CREPACO, Inc. (3/24/65)
100 CP Avenue
Lake Mills, Wisconsin 53551

187 Dairy Craft, Inc. (9/26/66)
St. Cloud Industrial Park
St. Cloud, Minnesota 56301

177 Girton Manufacturing Co. (2/18/66)
Millville, Pennsylvania 17846

166 Paul Mueller Co. (4/26/65)
P.O. Box 828
Springfield, Missouri 65601

25-00 Non-Coil Type Batch Processors for Milk and Milk Products

275 Bepex Corporation (7/12/76)
150 Todd Road
Santa Rosa, California 95402

162 Cherry-Burrell Corporation (4/ 5/65)
575 E. Mill St.
Little Falls, New York 13365

159 CREPACO, Inc. (3/24/65)
100 CP Avenue
Lake Mills, Wisconsin 53551

188 Dairy Craft, Inc. (9/26/66)
St. Cloud Industrial Park
St. Cloud, Minnesota 56301

283 Letsch Corporation (11/10/76)
501 N. Belcrest
Springfield, Missouri 65802

167 Paul Mueller Co. (4/26/65)
Box 828
Springfield, Missouri 65801

202 Walker Stainless Equipment Co. (9/24/68)
New Lisbon, Wisconsin 53950

26-00 Sifters for Dry Milk and Dry Milk Products

228 Day Mixing, Div. LeBlond, Inc. (2/28/72)
4932 Beech Street
Cincinnati, Ohio 45202

229 Russell Finex Inc. (3/15/72)
156 W. Sandford Boulevard
Mt. Vernon, New York 10550

173 B. F. Gump Division (9/20/65)
Blaw-Knox Food & Chem. Equip. Inc.
750 E. Ferry St., P.O. Box 1041
Buffalo, New York 14240

185 Rotex, Inc. (8/10/66)
1230 Knowlton St.
Cincinnati, Ohio 45223

176 Koppers Company, Inc. (1/ 4/66)
Metal Products Division
Sprout-Waldron Operation
Munsy, Pennsylvania 17756

172 SWECO, Inc. (9/ 1/65)
6033 E. Bandini Blvd.
Los Angeles, California 90051

28-00 Flow Meters for Milk and Liquid Milk Products

272 Accurate Metering Systems, Inc. (4/ 2/76)
1731 Carmen Drive
Elk Grove Village, Illinois 60007

253 Badger Meter, Inc. (1/ 2/74)
4545 W. Brown Deer Road
Milwaukee, Wisconsin 53223

223 C-E IN-VAL-CO, Division of Combustion (11/15/71)
Engineering, Inc.
P.O. Box 556, 3102 Charles Page Blvd.
Tulsa, Oklahoma 74101

265 Electronic Flo-Meters, Inc. (3/10/75)
P.O. Box 38269
Dallas, Texas 75238

226 Fischer & Porter Company (12/ 9/71)
County Line Road
Warminster, Pennsylvania 18974

261 Foss America, Inc. (11/ 5/74)
Route 82
Fishkill, New York 12524

224 The Foxboro Company (11/16/71)
Neponset Avenue
Foxboro, Massachusetts 02035

270 Taylor Instrument Process Control (2/ 9/76)
Sybron Corporation, 95 Ames Street
Rochester, New York 14601

29-00 Air Eliminators for Milk and Fluid Milk Products

30-00 Farm Milk Storage Tanks

257 Babson Bros. Co. (2/ 7/74)
2100 S. York Road
Oak Brook, Illinois 60521

31-00 Scraped Surface Heat Exchangers

274 Contherm Corporation (6/25/76)
P.O. Box 352
Newburyport, Massachusetts 01950

32-00 Uninsulated Tanks for Milk and Milk Products

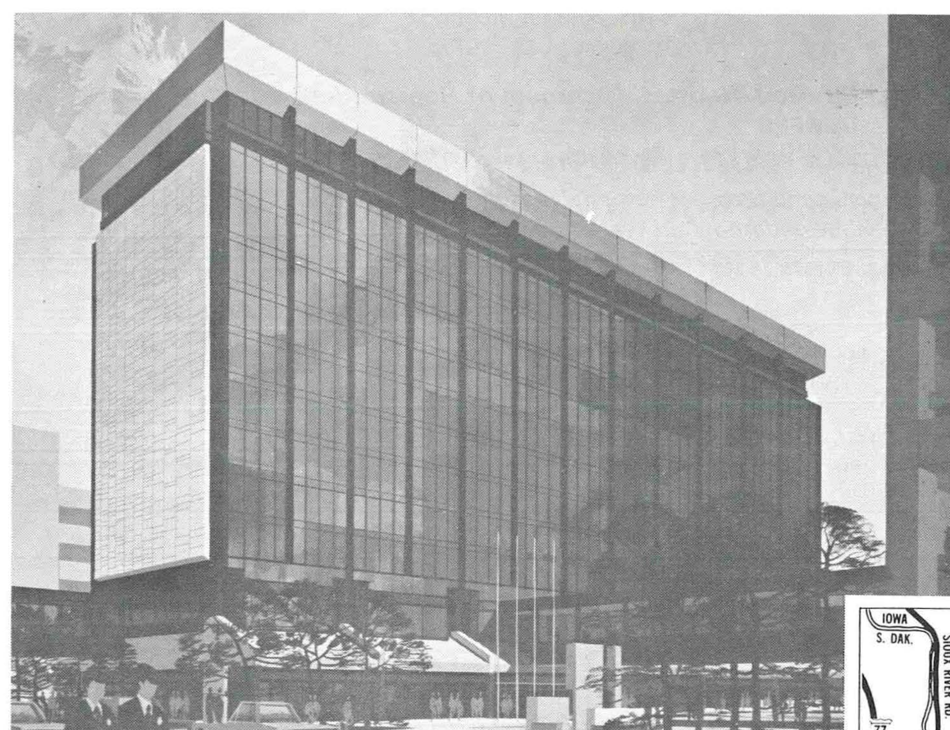
264 Cherry-Burrell Company, Division (1/27/75)
of Paxall, Inc.
575 E. Mill St.
Little Falls, New York 13365

268 Dairy Craft, Inc. (11/21/75)
P.O. Box 1227
St. Cloud, Minnesota 56301

33-00 Polished Metal Tubing for Dairy Products

289 Ladish Co., Tri-Clover Division (1/21/77)
9201 Wilmot Road
Kenosha, Wisconsin 53140

SIOUX CITY IOWA IS THE SPOT



64th Annual Meeting
International Association of Milk, Food
and Environmental Sanitarians, Inc.

AUGUST 14-18, 1977

Hilton Inn on the Plaza, Sioux City, Iowa

Host, Iowa Association of Milk, Food & Environmental Sanitarians

*The National Mastitis Council will hold its summer meeting—August 18, 1977

*KEYNOTE SPEAKERS	*SPOUSES PROGRAM
*BUSINESS MEETING	*COMMITTEE MEETINGS
*RESEARCH PAPERS	*PANEL WORKSHOPS
*EARLY-BIRD PARTY	*AWARDS & PRESENTATIONS
*COCKTAIL PARTY	

BANQUET AND ENTERTAINMENT

Sioux City also offers these attractions

Golf Courses • Race Tracks (Horse and Dog) • War Eagle Monument • SGT Floyd Monument • Tennis Courts • Art Center Museum • Public Museum • Camper Parking • Nearby Shopping • Industry and Agriculture

1977 ANNUAL MEETING I.A.M.F.E.S.

Advance Registration Form
Sioux City, Iowa

64th Annual Meeting
August 14-18, 1977

ATTENTION: **Mr. Carl Webster, Chairman of Registration**
IAMFES
P.O. Box 284 - Waterloo, Iowa 50701

Advance Register and Save — Refundable if you don't attend
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1977 ANNUAL MEETING I.A.M.F.E.S.

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Journal of Food Protection Vol. 40, No. 5, Pages 347-351 (May, 1977)
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PROGRAM

Sixty-Fourth Annual Meeting International Association of Milk, Food and Environmental Sanitarians, Inc.

In cooperation with
Iowa Association of Milk, Food and Environmental Sanitarians, Inc.

August 14-18, 1977

Sioux City Hilton **Sioux City, Iowa**

Henry V. Atherton
President
I.A.M.F.E.S., INC.

REGISTRATION

Sunday, August 14—1:00 p.m.-5:00 p.m.
Monday, August 15—8:00 a.m.-5:00 p.m.
Tuesday, August 16—8:00 a.m.-5:00 p.m.
Wednesday, August 17—8:00 a.m.-12:00 Noon
Thursday, August 18—8:00 a.m.

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Harold Thompson, Jr.	Executive Board

SUNDAY, AUGUST 14, 1977

1:00- 5:00 p.m.—Registration-2nd. Floor Lobby
1:30- 5:30 p.m.—Executive Board-Salon C&D
6:00- 7:00 p.m.—Early Bird Reception-Top of Hilton
8:00-11:00 p.m.—Executive Board Salon C&D

MONDAY, AUGUST 15, 1977

8:00 a.m.-5:00 p.m.—Registration-2nd. Floor Lobby

Special Meetings

9:00 a.m.-12:00 noon—Executive Board-Salon C&D
1. Report on Local Arrangements
2. Report of Executive Secretary
3. Report of Sanitarians Joint Council

9:00 a.m.—Journal Management Committee
1:00 p.m.-3:00 p.m.—Affiliate Council—Ballroom—Plaza South
1. Overview of International Association during the past year
2. Report from the Affiliate who made the largest increase in Membership.
3. Report on Awards Committee
4. Report on Affiliate Imput into Journal
5. Summary of Questionaire sent to Affiliates
6. Election of Affiliate Council Officers
7. Other Pertininent Topics

3:00 p.m.—Sanitarians Joint Council
3:30 p.m.-5:30 p.m.—Executive Board—Salon C&D
1. Regular Agenda
2. Report of Journal Management Committee
3. Committee Chairmen
4. Report of Affiliate Council
5. Meet with Past Presidents

1:30-5:00 p.m.—Individual Committee Meetings
3:00-5:00 p.m.—Farm Methods Committee Ballroom-Plaza South
6:00-7:00 p.m.—Reception—2nd. Floor Lobby

Council of State Sanitarian Registration Agencies
(Place and Time to be Announced)

Individual Committee Meetings are open to all registrants

TUESDAY, AUGUST 16, 1977

8:00-5:00 p.m.—Registration—2nd. Floor Lobby
8:00-9:00 p.m.—Executive Board Salon C&D

Morning-General Session-Ballroom
David D. Fry—Presiding

9:30 a.m.—INVOCATION

9:35 a.m.—ADDRESS OF WELCOME—Paul Berger, Pres. Chamb. of Comm.
9:55 a.m.—PRESIDENTIAL ADDRESS—Henry V. Atherton, Univ. of Vermont
10:25 a.m.—KEYNOTE ADDRESS—Berkley Bedell, U.S. Congressman, 6th Dist., Spirit Lake, Iowa
11:10 a.m.—THE SOCIAL AND ECONOMIC PROBLEMS OF ALCHOLISM IN INDUSTRY. Richard Schick-Kamel Mfg., Middle Village, NY
11:40 a.m.—ANNOUNCEMENTS
Program Highlights
Local Arrangements—Duane Hagedon
New Nominating Committee
Other Announcements

TUESDAY, AUGUST 16, 1977
Afternoon-Milk Sanitation Session
Ballroom-Plaza South
Richard P. March Presiding

1:30 p.m. DOOR PRIZE DRAWING
1:40 p.m. ANTIBIOTIC RESIDUE IN MILK FOLLOWING DRY COW TREATMENTS James H. Martin, S. Dak. State University, Brookings, S. Dak.
2:00 p.m. LABORATORY QUALITY ASSURance, A. Richard Brazis, F.D.A. Cincinnati, Ohio
2:30 p.m. FROZEN YOGURTS, Robert Redford, Pine State Dairy, Raleigh, N.C.
3:00 p.m. INIBITION OF ENTEROPATHOGENIS E. COLI BY LACTIC STARTER CULTURE-J. F. Frank and E. H. Marth, Univ. of Wisc., Madison Wisc.
3:15 p.m. MILK BREAK
3:30 p.m. REPORT FROM NATIONAL CONFERENCE ON INTERSTATE MILK SHIPMENTS-Herb Vaux-State Health Dept. Indianapolis, Ind.
4:00 p.m. RAPID AUTOMATED IMPEDANCE SCREENING OF MILK FOR MICROBIAL CONTENT AND KEEPING QUALITY-P. Cady, D. Hardy, S. W. DuFour and S. J. Kraeger, Bactomatic, Inc. Palo Alto, Calif.

TUESDAY, AUGUST 16th, 1977
Afternoon-Food Sanitation Section
Ballroom Plaza North
Howard E. Hutchings-Presiding

1:30 p.m. DOOR PRIZE DRAWING
1:40 p.m. HEATING PATTERNS OF PRODUCTS IN CROCKERY COOKERS, R. E. Brackett and E. H. Marth, Univ. of Wisc., Madison, Wisc.
2:00 p.m. SANITATION PROGRAM IN RETAIL FOOD STORES-Gale Prince
2:30 p.m. BOTULISM REVISITED-Ralston B. Read
3:00 p.m. A SENSITIVE PROCEDURE FOR DETECTING SALMONALLAE ON WHOLE BROILER CARCASSES WITHOUT PRE-ENRICHMENT. N. A. Cox, A. S. Mercuri, J. E. Thomson, and J. S. Bailey, USDA, ARS, Russell Research Center, Athens, Ga.
3:15 p.m. MILK BREAK
3:30 p.m. POTENTIAL HAZARDS ASSOCIATED WITH HOT LUNCH PROGRAMS, Mitsuru J. Makamura, Univ. of Montana, Missoula, Mt.
3:50 p.m. NATURAL AND ARTIFICIAL SWEETNERS-CURRENT STATUS-Richard A. Daehler, Clinton Corn Processing Co., Clinton, Iowa
4:25 p.m. A STUDY OF COAGULASE-POSITIVE STAPHYLOCOCCI IN SALAMI PRIOR TO FERMENTATION. M. M. Pullen, Univ. of Minn., St. Paul, MN. and C. A. Genigeoris, Univ. of Calif., Davis, CA.

TUESDAY EVENING, AUGUST 16, 1977

7:00-9:00 p.m.—EVENING DISCUSSION GROUPS
7:00-9:00 p.m.—FOOD SANITATION, Salon A and B
7:00-9:00 p.m.—MILK SANITATION, Salon C and D
9:00 p.m. —WINE & CHEESE—Ballroom

WEDNESDAY, AUGUST 17, 1977
General Session
Henry V. Atherton, Presiding-Ballroom

8:30 a.m. DOOR PRIZE DRAWING
8:40 a.m. MANAGEMENT OF SLUDGE USE ON LAND, Charles F. Jelinek, FDA, Wash. D.C.
9:10 a.m. MICROBIOLOGY OF COCOAS, STABILIZERS, FLAVORS AND FOOD ADDITIVES-Robert Morley, Dari-Tech Corp., Atlanta GA.
9:40 a.m. MILK BREAK
9:55 a.m. DOOR PRIZE DRAWING
10:00 a.m. ANNUAL BUSINESS MEETING
1. Report of Executive Secretary
2. Report of Secretary-Treasurer
3. Committee Reports
4. 3-A Symbol Council Reports
5. Report of Resolutions Committee
6. Report of Affiliate Council
7. Old Business
8. New Business
9. Election of Officers
Ivan Parkin-Parliamentarian

WEDNESDAY AFTERNOON, AUGUST 17, 1977
Milk Sanitation Section-Ballroom-Plaza Center
Carl Webster, Presiding

1:30 p.m. DOOR PRIZE DRAWING

1:40 p.m. IMPEDANCE CHANGES IN RAW MILK AS AN ALTERNATIVE METHOD TO THE STANDARD PLATE COUNT. S. O. Gnan and L. O. Luedecke, Wash. State Univ., Pullman, WA.

2:00 p.m. PYRUVATE ANALYSIS-Robert Marshall, Univ. of Missouri, Columbia, MO.

2:30 p.m. DETERMINING VIABLE LACTOBACILLUS ACIDOPHILUS ORGANISM IN DAIRY PRODUCTS-Marvin L. Speck, N. Caro, State Univ. Raleigh, NC

3:00 p.m. MILK BREAK

3:15 p.m. FOULING OF HEAT TRANSFER SURFACES BY FLUIDS CONTAINING SOLUBLE PROTEINS. A. C. Ling and D. B. Lund, Univ. of Wisc., Madison, WIS.

3:35 p.m. AUTOMATED MILK PLANT OPERATIONS-Dale A. Seiberling-Seiberling Associates, South Beloit, Ill.

4:05 p.m. LACTOSE INTOLERANCE-THE PROBLEM AND SOLUTIONS. R. S. Katz, National Dairy Council, Rosemont, Ill.

WEDNESDAY AFTERNOON-MILK PRODUCTION SECTION-BALLROOM-PLAZA NORTH

Sidney Beale, Presiding

1:30 p.m. DOOR PRIZE DRAWING

1:40 p.m. INFLATIONS AND PROBLEMS-Ken Kirby, Hi-Life Rubber Co., Johnson Creek, Wis.

2:20 p.m. FIELDMEN PLANNING THEIR WORK Ed Kalder, Mid-American Dairyman, Inc. St. Paul, Minn.

2:55 p.m. AUTOMATED MILKING SYSTEMS-Bill Bichert, Michigan State Univ., East Lansing, Mich.

3:30 p.m. MILK BREAK

3:45 p.m. APPROACHES TO TROUBLE SHOOTING A MILKING SYSTEM-Sidney Beale, Mich. Milk Producers, Detroit, Mich.

4:00 p.m. BOARD OF DIRECTORS OF NATIONAL MASTITIS COUNCIL

4:20 p.m. BUSINESS MEETING-National Association of Dairy Fieldmen.

WEDNESDAY, AUGUST 17, 1977
FOOD SANITATION SECTION
BALLROOM-PLAZA NORTH

Tom Corothers, Presiding

1:30 p.m. DOOR PRIZE DRAWING

1:40 p.m. A COMPAISON STUDY OF THE ANTIMICROBIAL PROPERTIES OF ISOLATED HUMAN MILK AND EGG WHITE LYSOZYMES. Douglas Peck and K. Ostovar Pennsylvania State Univ., Univ. Park, Penn.

2:00 p.m. PATHOGENIC ANAEROBES IN CONVENIENCE FOODS-Paul R. Middaugh, South Dakota State Univ., Brookings, SD

2:30 p.m. MECHANICAL DEBONED MEAT AND FISH, Robert Rust, Iowa State Univ., Ames, Iowa

3:00 p.m. DEGRADATION OF AFLATOXIN BY POTASSIUM BISULFITE, M. P. Doyle and E. H. Marth, Univ. of Wisc., Madison, WI.

3:15 p.m. MILK BREAK

3:30 p.m. A NATIONAL UNIFORM FOOD SERVICE MANAGER SANITATION TRAINING AND CERTIFICATION PROGRAM, Charles Dee Clingman, National Institute for the Food Service Industry, Chicago, Ill.

3:50 p.m. TRAINING OPPORTUNITIES FOR THE SANITARIAN-Harry Haverland, Chief Training Institute, FDA, Cincinnati, Ohio

4:25 p.m. ASSESSMENT OF FOOD-BORNE DISEASE REPORTING IN CANADA FOR 1973-1975. E. Todd, Health and Welfare Canada, Tunney's Pasture, Ottawa, Ontario.

WEDNESDAY EVENING, AUGUST 17, 1977

6:00-7:00 p.m. RECEPTION-2nd. Floor Lobby-Pool Area-Salons

7:00 p.m. ANNUAL AWARDS BANQUET-Ballroom, Henry V. Atherton, Pres., presiding
INVOCATION-Ivan E. Parking
INTRODUCTIONS
PRESENTATION OF AWARDS, Parnel J. Skulborstad, Chairman

1. Past President Award
2. Citation Awards
3. Honorary Life Membership
4. C. B. Shogren Memorial Award
5. Sanitarians Award-Sponsored By:
   Klenzade Products, Inc.
   Division Economics Laboratories
   Pennwalt Chemical, Inc.
   Diversey Corporation, Inc.
6. Industry and/or Education Award Sponsored By:
   Milking Machine
   Manufacturers Council of the Farm and Industrial Equipment Institute

INSTALLATION OF OFFICERS

9:00 p.m. ENTERTAINMENT

THURSDAY, AUGUST 18, 1977

8:00 a.m. REGISTRATION-NATIONAL MASTITIS COUNCIL-2nd Floor Lobby

7:30 a.m. EXECUTIVE BOARD-Breakfast Meeting-Salon C and D

NATIONAL MASTITIS COUNCIL
1977 SUMMER MEETING
PROGRAM-BALLROOM

John McDonald, Presiding

8:00 a.m.	REGISTRATION-2nd. Floor Lobby
8:30 a.m.	GREETINGS-President James R. Welch, Lenzada Products of St. Paul, Minn.
8:45 a.m.	MOST COMMON CAUSES OF MASTITIS-John Micolai, Jr., Ellicott City, Maryland
9:15 a.m.	THE ROLE OF THE TEAT END IN MASTITIS CONTROL-Claance Johannes D.V.M. Consultant, Ames, Iowa
10:15 a.m.	MILK BREAK
10:45 a.m.	THE PRACTIONERS APPROACH TO AN EFFECTIVE MASTITIS THERAPY PROGRAM, James K. West-D.V.M. Practitionor-Waverly, Iowa
11:30 a.m.	QUESTION AND ANSWER PERIOD
12:00 noon	LUNCH
1:30 p.m.	DAIRY PANEL-My Dairy Management and Mastitis Control-Fred Foreman, ISU, Ames, Iowa Panel Members Arlen Berwald-Toronto, SD Dwayne Stelling-Bloomfield, Nebr. Leroy Meyer-Sac City, Iowa
2:30 p.m.	QUESTION AND ANSWERS
2:45 p.m.	MILK BREAK
3:15 p.m.	IMPLEMENTATION OF A MASTITIS PROGRAM-Allan Bringe, Univ. of Wisc., Madison, Wisc.
3:45 p.m.	MASTITIS CONTROL-PUTTING IT ALL TOGETHER. Nelson Philpot, Louisiana Experiment Station, Homer, LA.
4:30 p.m.	QUESTION AND ANSWERS
5:00 p.m.	Adjournment

ENTERTAINMENT
MEN AND WOMEN

SUNDAY, AUGUST 14, 1977

6:00-7:00 p.m.—RECEPTION-TOP OF HILTON
(Corn Roast-Iowa Style)

MONDAY, AUGUST 15, 1977

6:00-7:00 p.m.—RECEPTION-SECOND FLOOR LOBBY

TUESDAY, AUGUST 16, 1977

9:00 p.m.—WINE & CHEESE PARTY-BALLROOM

WEDNESDAY, AUGUST 17, 1977

6:00-7:00 p.m.—RECEPTION-Second Floor-Pool Area
7:00 p.m. —BANQUET & ENTERTAINMENT

ENTERTAINMENT FOR THE SPOUSES

(Spouses are invite to attend any of the meeting sessions)

Convienent Downtown Shopping—Monday, Aug. 15, 1977

Boat Cruise with a Luncheon—Tuesday, Aug. 16, 1977

K-D Station Tour with a Luncheon—Wednesday, Aug. 17, 1977

Convienent Downtown Shopping—Thursday, Aug. 18, 1977

Letters to the Editor

What about injured coliforms?

DEAR SIR:

We are very concerned that many food microbiologists dealing with the day-to-day monitoring of foods may be seriously misled by a recent paper published in your journal (Hartman, P. A. and P. S. Hartman. 1976. Coliform analyses at 30 C. J. MIlk Food Technol. 39:763-767).

These authors concluded that incubation of violet red bile (VRB) agar should be continued for more than 24 h at 30 C when looking for coliforms in some products. Samples from water or frozen vegetables were said to require 48 h of incubation to detect coliforms that were 'slow in growth initiation.' Although they claimed to have reviewed the literature, they made no suggestion that the apparent slow growth could have been due to sublethal damage. However, tests on pure cultures of many different (undamaged) coliform-aerogenes bacteria have shown that none required more than 24 h of incubation (*6*). On the other hand, there are numerous references in the literature indicating that coliforms and other organisms in foods (particularly frozen, dried, or heated), or in water, may be damaged. Damaged *Enterobacteriaceae* may grow only after an extended lag period, or may be completely unable to grow on VRB agar or other selective media (*2,3,5*). A resuscitation step (incubation for a short time in a non-selective medium) restores the ability to grow in the presence of inhibitors. The most likely explanation for delayed growth observed in the study under discussion (*4*) is that the coliforms present in the frozen vegetables and water were sublethally damaged. Many studies on the recovery of sublethally damaged cells from foods have been published in the last few years; indeed, a review on the occurrence of damaged organisms in foods was published recently in this journal (*1*) and we are preparing a review article on practical and theoretical aspects of damage and resuscitation with respect to organisms in foods.

We should also point out that it is very rash to conclude, on the basis of limited tests on only four commondities in the United States, that only with cottage cheese is there a danger of non-coliforms producing colonies when incubation is continued after 24 h. In our experience this is not so.

In conclusion, not only may counts carried out as suggested in this paper fail to detect all sublethally damaged organisms, but extended incubation may allow non-coliforms to grow. Use of a resuscitation step before plating with VRB agar gives: (*a*) more rapid results, (*b*) allows enumeration of damaged cells which would otherwise be inhibited on VRB agar, and (*c*) prevents problems with the growth of non-coliform types.

JANET E. L. CORRY
Metropolitian Police Forensic Science Laboratory
109, Lambeth Road
London, England SE1 7LP

D. A. A. MOSSEL
Department of Food Microbiology
Faculty of Veterinary Medicine
University of Utrecht
Utrecht, The Netherlands

1. Busta, F. F. 1976. Practical implication of injured microorganisms in foods. J. Milk Food Technol. 39:138-145.
2. Corry, J. E. L., A. G. Kitchell, and T. A. Roberts. 1969. Interactions in the recovery of *Salmonella typhimurium* damaged by heat or gamma radiation. J. App. Bacteriol. 32:415-428.
3. Gunderson, M. F., and K. D. Rose. 1948. Survival of bacteria in a precooked, fresh-frozen food. Food Res. 13:254-263.
4. Hartman, P. A., and P. S. Hartman. 1976. Coliform analyses at 30 C. J. Milk Food Technol. 39:763-767.
5. Hartsell, S. E. 1951. The longevity and behavior of pathogenic bacteria in frozen foods: The influence of plating media. Amer. J. Publ. Health 41:1072-1077.
6. Mossel, D. A. A. 1957. The presumptive enumeration of lactose negative as well as lactose positive *Enterobacteriaceae* in foods. Appl Microbiol. 5:379-381.

Injured coliforms were not forgotten

DEAR SIR:

Corry and Mossel state that some coliforms are 'slow in growth initiation' because they are damaged and that we should have mentioned this fact in our paper (*4*). We did. On page 766 we stated that "The use of violet red bile 2 (VRB-2) agar, which...is satisfactory for the recovery of stressed coliforms (*5*), might be a logical alternative" to the use of regular violet bile (VRB) agar. We might have been remiss, however, in not placing more emphasis on cell injury.

The "claimed to have reviewed the literature" statement in the Corry-Mossel letter is unfair and inappropriate. Many investigators have studied bacterial cell injury, but few have proposed practical solutions to the problem. We have published improvements to both direct plating (*5*) and MPN (*8,9*) methods that permit cell resuscitation without altering normal laboratory routine. More complete discussions of cell injury and means to facilitate repair appear in those publications (*5,8,9*).

I would like to contest other points that Corry and Mossel make in their letter. First, they state that "incubation for a short time in a non-selective medium restores the ability to grow in the presence of inhibitors." This is a naive statement that might be misleading to some readers unless qualified. The time necessary for resuscitation of bacteria in natural materials is not always "short", nor do all cells recover at approximately the same time. We discovered, for example, that resuscitation times of over 6 h are necessary for coliforms under some circumstances (*8*). Recent studies with salmonellae in naturally contaminated products (*9*) also indicated that resuscitation times can be extensive. The only solution is to strike a balance between recovering a reasonable percentage of the injured bacteria present in a sample and the time and effort needed to effect increased recovery. Furthermore, results of resuscitation studies should be interpreted with caution, especially when laboratory cultures are used as the experimental material. Laboratory strains of bacteria are suitable for preliminary studies, but the true efficacy of any procedure can be confirmed only by using naturally contaminated materials.

The second point of their letter that deserves comment regards whether or not there is a danger of non-coliforms producing colonies when incubation is continued after 24 h. We have examined a wide variety of foods (*2,3,4,5*) and other mateials (*1,6,7*) by using VRB agar. Results obtained with a 24-h incubation were reported in our publications, but on all these occassions VRB agar plates were incubated for periods longer that 24 h to discover if further incubation would result in appreciably higher counts. Of all the food samples that we examined, a 24-h limit was necessary only when the food had been subjected to a lactic fermentation.

PAUL A. HARTMAN
Department of Bacteriology
Iowa State University, Ames, Iowa 50011

1. Burmeister, H. R., P. A. Hartman, and R. A. Saul. 1966. Microbiology of ensiled high-moisture corn. Appl. Microbiol. 14:31-34.
2. Hartman, P. A. 1958. The selectivity of autoclave-sterilized violet red bile agar. Food Res. 23:532-535.
3. Hartman, P. A. 1960. Further studies on selecivity of violet red bile agar. J. Milk Food Technol. 23:45-48.
4. Hartman, P. A., and P. S. Hartman. 1976. Coliform analyses at 30 C. J. Milk Food Technol. 39:763-767.
5. Hartman, P. A., P. S. Hartman, and W. W. Lanz. 1975. Violet red bile 2 agar for stressed colifroms. Appl. Microbiol. 29:537-539.
6. Hartman, P. A., R. H. Johnson, L. R. Brown, N. L. Jacobson, R. S. Allen, P. R. Shellenberger, and H. H. Van Horn, Jr. 1962. Relationship of rumen faculative anaerobes to feedlot and pasture bloat. Iowa State J. Sci. 36:217-231.
7. Hartman, P. A., J. L. Morrill, and N. L. Jacobson. 1966. Influence of diet and age on bacterial counts of ileal digesta and feces obtained from young calves. Appl. Microbiol. 14:70-73.

8. Lanz. W. W., and P. A. Hartman. 1976. Timed-release capsule method for coliform enumeration. Appl. Environ. Microbiol. 32:716-722.

9. Sveum, W. H., and P. A. Hartman. 1977. A time-release capsule method for the detection of salmonellae in foods and feeds. Appl. Environ. Microbiol. 33:in press (March issue).

News and Events

Kultures and Kurds Klinic

Smith Dairy Products Company of Orrville, Ohio received the Neil C. Angevine Superior Quality Award at the 1977 Kultures and Kurds Klinic held in Cincinnati, on March 21-23.

The Neil C. Angevine Superior Quality Award is given annually to the dairy plant with the highest cumulative score for all cultured products. Dairy plants are evaluated at the American Cultured Dairy Products Institute training schools.

Vandervoorts Dairy, Fort Worth, Texas placed second in the over-all products category while third place went to Hawthorn Mellody, Inc., Chicago, Illinois.

Also at this year's Kultures and Kurds Klinic, first place certificates of merit for quality products were awarded to the following organizations: Hillside Old Meadow Dairy, Cleveland Heights, Ohio (sour cream); Thompson's Dairy Co., Seymour, Indiana (buttermilk); Smith Dairy Products Co., Orrville, Ohio (cottage cheese and lemon yogurt); Southland Specialty Foods, Sulphur Springs, Texas (plain, strawberry, and all categories yogurt).

Other plants receiving honorable mentions included: Dairylea Cooperative, Inc., Vernon, New York; Borden, Inc., Indianapolis, Indiana; Borden, Inc., Columbus, Ohio; Purity Dairies, Nashville, Tennessee; Pet, Inc., St. Petersburg, Florida; Marigol Foods, Inc., Rochester, Minnesota; LaCorona Foods, Inc., Glendale, Arizona; A & P Coffee/Dairy Div., Kentwood, Louisiana.

This year's Klinic drew 230 delegates from thirty-six states, Mexico, Canada, and West Germany.

(Left to Right), Mr. Norman Berg and David H. Hill (Smith Dairy Products Co., Orrville, Ohio), Dr. C. Bronson Lane (Secretary of the American Cultured Dairy Products Institute) and James Jones (Vandervoorts Dairy, Ft. Worth, Texas) review results of the national cultured products judging contest held in conjunction with the March 21-23, 1977 Kultures and Kurds Klinic, Cincinnati, Ohio.

D.C. Environmental Health Workers Trained in Disease Investigation

Environmental health specialists of the D.C. Environmental Health Administration (EHA) are pursuing a 16-week intensive training course in epidemiological investigation techniques and procedures.

The course is being taught by Dr. Steven Thacker, epidemiological intelligence officer, National Center for Disease Control and one of the investigators of the Legionnaires disease outbreak last year in Philadelphia, along with other health scientists in the Washington, D.C. metropolitan area.

According to Dr. Bailus Walker, Jr., Administrator of EHA, who requested the training, the course is designed to "further our understanding of environmentally-induced diseases and to strengthen our ability to assist in the investigation of these diseases and develop methods of prevention."

"At a time when environmental chemicals and physical stresses are identified as major determinants of disease and disability, regulatory agencies, such as ours, must be prepared to do more than make inspections and issue enforcement citations," Walker added. For further information contact: Dr. Bailus Walker, Jr. (telephone no.) 629-3013 or 3014.

Engineering Orientation to Meat Packing

The revised edition of one of the most complete texts on engineering in the meat packing industry has just been released by the American Meat Institute Center for Continuing Education. "Engineering Orientation to Meat Packing," originally published in 1965, now contains 105 full-size pages with many illustrations and tables covering the engineering aspects of equipment and layout for the abattoir; cutting and boning operations; meat processing; rendering and animal byproducts department; packing and shipping; and plant sanitation, the environment and maintenance. The revised edition also reviews statutory requirements of USDA, OSHA, EPA and local building codes.

The text is the basis for a home-study course on meat plant engineering designed to better prepare qualified engineers for a career in the meat packing industry and to keep meat plant engineers updated on the latest techniques and innovations. The author and instructor for the course is Jackson H. Everds, architect and engineer, Henschien Everds Crombie Inc., Chicago, Illinois.

The registration fee for the course is $60, including the text. Detailed enrollment information is available from the AMI Center for Continuing Education, P.O. Box 3556, Washington, D.C. 20007. The textbook may be purchased separately for $16.95.

"Engineering Orientation of Meat Packing" is just one of the courses offered by the Center, the American Meat Institute's educational division. Currently, eight other courses in meat industry operations are available from the Center. A full course brochure is available on request.

Foodservice Students

The number of students enrolled in foodservice and hospitality courses in the U.S. rose to 328,707 last year, announced Dr. Chester G. Hall, Executive Vice President of the National Institute for the Foodservice Industry (NIFI).

Dr. Hall said enrollments grew by 20,252 over 1975—an increase of 6.5 per cent.

Data for the report is provided annually to NIFI by the Division of Vocational and Technical Education, U.S. Office of Education. Dr. Hall noted that he has obtained such data since 1966. "The 11-year rate of increase in the number of students is 267.1 per cent," he said.

At the same time, Dr. Hall reported that a national survey done by NIFI in cooperation with the National Restaurant Association revealed that there are now 2,280 secondary schools and 438 post-sec-

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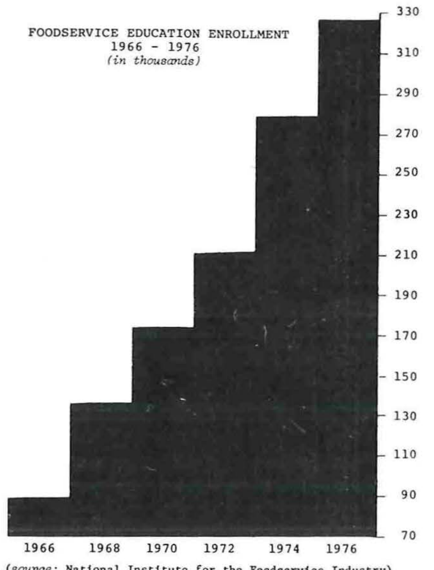

(source: National Institute for the Foodservice Industry)

ondary schools offering foodservice programs. Survey information was obtained from the directors of vocational education of 50 states and four territories.

Enrollments are up at all levels, according to the latest statistics. "It is very significant," Dr. Hall said, "that enrollments in junior and community colleges jumped almost 20 per cent in the past year, from 34,765 to 41,664—an increase of 6,899. There are now almost ten times as many students in industry courses at the post-secondary level as there were in 1966 (4,404)."

More than five times as many high school students (178,625) are studying foodservice hospitality careers now as compared to enrollment in such courses 11 years ago (32,522), Hall pointed out.

The National Institute for the Foodservice Industry is the not-for-profit foundation established by the industry to advance foodservice management standards through education.

Florida Health Department Grants Recognition to NIFI Sanitation Certification

Florida is the latest state to grant official recognition to persons certified in foodservice sanitation by the National Institute for the Foodservice Industry, according to Dr. Chester G. Hall, NIFI Executive Vice-President.

With that action, Dr. Hall noted that all states which have mandatory foodservice sanitation certification now accept completion of the NIFI course as meeting their requirements. Mandatory programs in the state of Illinois, the District of Columbia, and also in the city of Chicago recognize NIFI certification.

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Safety Stressed in Antibiotics in Feeds

All persons who use antibiotics or who cause antibiotics to be used should recognize the potential hazards associated with their use. Farmers and ranchers using such materials in animal feeds should particularly guard against the indiscriminate use of antibiotics, emphasized a keynote speaker at the annual Texas Animal Agriculture Conference in College Station, Texas on April 4.

Dr. J. E. Mosier, head of the Department of Surgery and Medicine in the College of Veterinary Medicine at Kansas State University, stressed that "we must redouble our efforts to learn more about the dangers inherent in the misuse of antibiotics and in their uncontrolled or illegal sale, improper prescription, and unjustified prophylactic use. It is through the combined concern of the physician, the veterinarian, the animal scientist, the microbiologist, the research worker, the maufacturer, the regulatory official and the consumer that we will maximize the benefits and minimize the risks of antibiotics in feeds."

Mosier, speaking to some 1,500 producers and others interested in the livestock industry, noted that of the 20.8 million pounds of antibiotics produced in the United States in 1973, 8.2 million were used primarily in animal feeds while the remainder were destined for medicinal use.

He traced the use of antibiotics in animal feeds and the various committees that have been formed to examine their usage. The most recent of these is the Subcommittee on Antibiotics in Animal Feeds formed last year as a result of discussions in the National Advisory Food and Drug Committee. This subcommittee was asked to consider the risks and benefits involved with the use of a number of antibiotics and sulfonamides and to reach judgments as to whether or not the use of these drugs was worthwhile.

The subcommittee's report as accepted by the National Advisory Food and Drug Committee recommended that Penicillin be discontinued for growth promotion and feed efficiency as well as for disease prevention when effective substitutes are available, that Tetracycline be continued for growth promotion, feed efficiency and disaease prevention, and that Sulfaquinoxyline be continued for approved use in disease prevention.

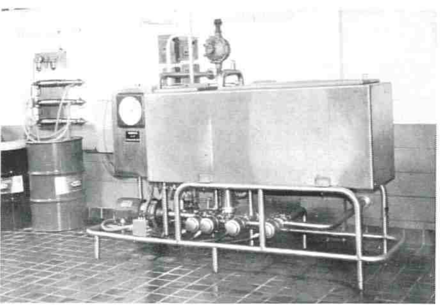
"The major issue of risk in the use of antibiotics in animal feeds rests on the judgment regarding the development of resistant organisms, the transfer of resistance from one bacteria to another, and the existence of multiple resistance," pointed out Mosier. "There is no question but that all three exist. The major question is, do the resistant organisms in animals pose a threat to man?

"Perhaps the most perplexing issue is how to separate the effect of antibiotics in animal feeds from the effect of antibiotics used for medicinal purposes," noted the veterinarian.

Suitable alternates to currently-used antibiotics would resolve some of these major issues, but unfortunately, none have yet been identified, noted Mosier. In accepting alternate drugs it is important that consideration be given to assure that the currently-used products are not replaced with substances endowed with lesser benefits and unexplored hazards.

The Texas Animal Agriculture Conference is sponsored by the Texas Agricultural Extension Service in Cooperation with other parts of the Texas A&M University System.

As and offshoot of DRINC (Dairy Research Inc.), Dairy Research Foundation has been established to secure funding for basic and applied research.



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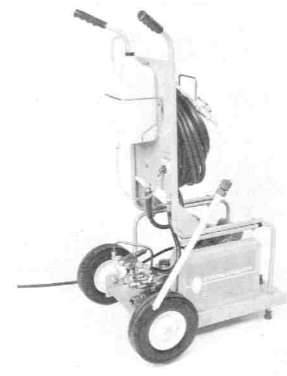

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OVER-TIME 1,130.00
SUPERVISION 1,250.00
MISC SUPPLIES
BRUSHES, BROOMS, EQUIP 750.00
DETERGENTS 500.00
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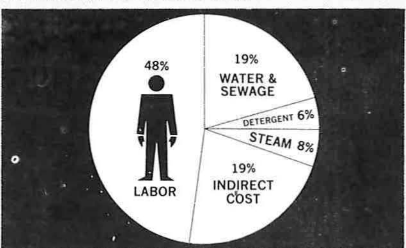
Chuck

Here's how Klenzade can help...

We can show you what happens to your clean-up dollar. And why. Fact is, labor, energy and water normally account for about 75% of clean-up costs. Sanitation chemicals, as little as 6%.

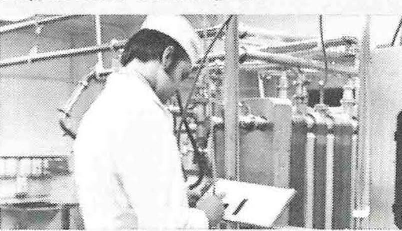


Here's where your Sanitation dollar normally goes.

Therefore, the cost of detergents and sanitizers can vary up or down, usually with minor impact on the total cost picture. But even a small reduction in labor, energy or water costs can quickly add up to bottom-line savings and profit.

That's why we emphasize the KLENZADE SANITATION ENGINEERING STUDY. It provides you with an analysis of cleaning procedures, chemical use, manpower requirements, energy and water consumption. In short, it shows how you can get maximum efficiency from each input of your sanitation program.

For example, many processors have changed from former procedures to a KLENZMATION® CIP System. Fully automated, this system can save man-hours, water and energy. One processor reported saving up to 200% in labor and 75% in water consumption.



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Another way to save labor and water is by switching to low-volume, high-pressure cleaning. Rather than flushing equipment with water at 10 to 15 gpm, available Klenzade Systems can clean and rinse at up to 700 psi and 3 gpm. That's a water saving of 300% or more at each clean-up station.

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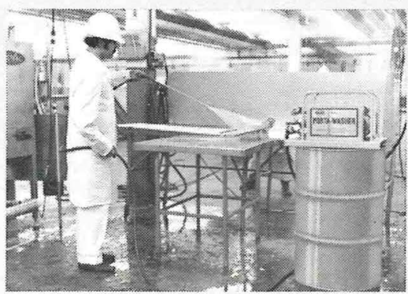

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Suspension of Chemical Safety Data Sheets

The Manufacturing Chemists Association (MCA) has announced the temporary or permanent suspension of nine more Chemical Safety Data Sheets. The suspension of five other data sheets was announced in December of last year.

Four of the nine data sheets have been permanently withdrawn from distribution. These are:

- Arsenic Trioxide (SD-60). This compound has been identified as a possible carcinogen in *Threshold Limit Values for Chemical Substances and Physical Agents in Workroom Environment with Intended Changes for 1976,* published by the American Conference of Governmental Industrial Hygienists. In addition, no MCA member currently manufactures the compound.
- Chlorine (SD-80). This data sheet is a reprint of a bulletin published by the Chlorine Institute and presently is being revised by that organization.
- Boron Hydrides (SD-84). Federal military and space programs, once principal users, no longer use these compounds in large quantity and no other potential users are foreseen.
- Zirconium and Hafnium Powder (SD-92). These are no longer articles of commerce and are not manufactured by any MCA member company.

The remaining five data sheets have been temporarily suspended from circulation until they have been reviewed and revised to reflect current knowledge regarding the properties, characteristics and handling of the materials involved. They are:

- Acrylonitrile (SD-31) and Dimethly Sulfate (SD-19). These have been suspended pending verification or additional knowledge of the possible carcinogenic effects.
- Naphthalene (SD-58). This has been withdrawn pending revision to reflect new recommended threshold limit values and current information about health and fire hazards.
- Perchloroethylene (SD-24) and Trichloroethylene (SD-14). These have been suspended pending clarification and redefinition of health hazards.

Battelle Begins Research on Food Oxidation and Deterioration Using Chemiluminescence

A new research program to study the use of chemiluminescence to determine the degree of food oxidation and resulting deterioration is under way at Battelle's Columbus Laboratories. It is aimed, ultimately, at helping food companies learn more about the shelf life and quality of their products.

The study, currently being supported by three leading food companies, has two objectives, according to Dr. Richard Nathan, who heads the research team.

The first objective is to study the fundamentals of the oxidation of selected components in food products. With this knowledge, the techniques and equipment developed in previous research at Battelle-Columbus can be further refined and applied to practical aspects of food deterioration. The second objective is to use the chemiluminescence technique and equipment to study oxidation in actual food samples furnished by the companies supporting the research and to work with the sponsoring companies to adapt the technique to their in-plant quality-control procedures.

The data from the first portion of the study will be reported to all the member companies, Dr. Nathan said. Data from the second phase will be proprietary to the individual companies since it involves information about their specific products.

"In addition to aiding food companies learn more about product deterioration and quality," said Dr. Nathan, "we believe this research will aid in the development of new antioxidants to help prolong product quality."

Each participating company is investing $9,000 in the 12-month study. Membership in the sponsoring group is still open, Dr. Nathan said, and companies interested in joining may contact him at Battelle's Columbus Laboratories, 505 King Avenue, Columbus, Ohio 43201.

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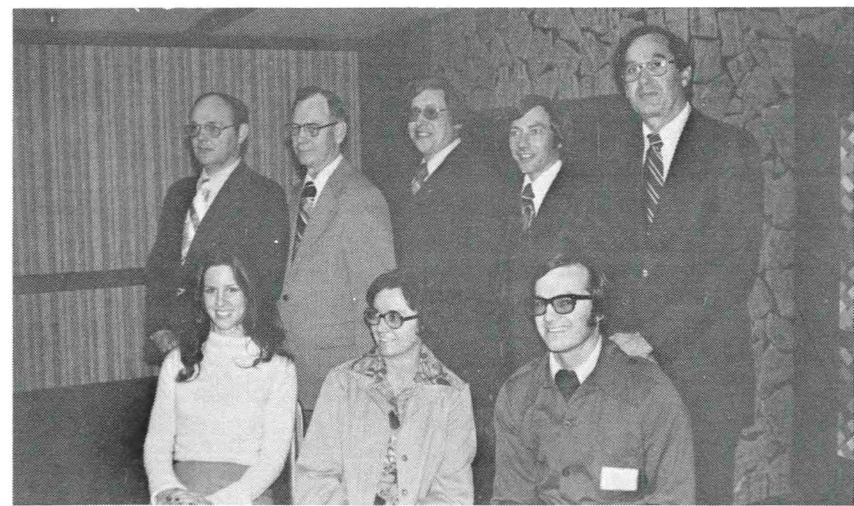
Future IAMFES Annual Meetings

August 14-18, 1977
Sioux City, Iowa

August 6-10, 1978
Kansas City, Missouri

August 12-16, 1979
Orlando, Florida



(L to R) Back Row—Dr. B. E. Langlois, James Spillman, Barry Kinslow, Dudley Conner, Harry Marsh. Front row—Ellen Cook, JoAnn Needham, David Atkinson.

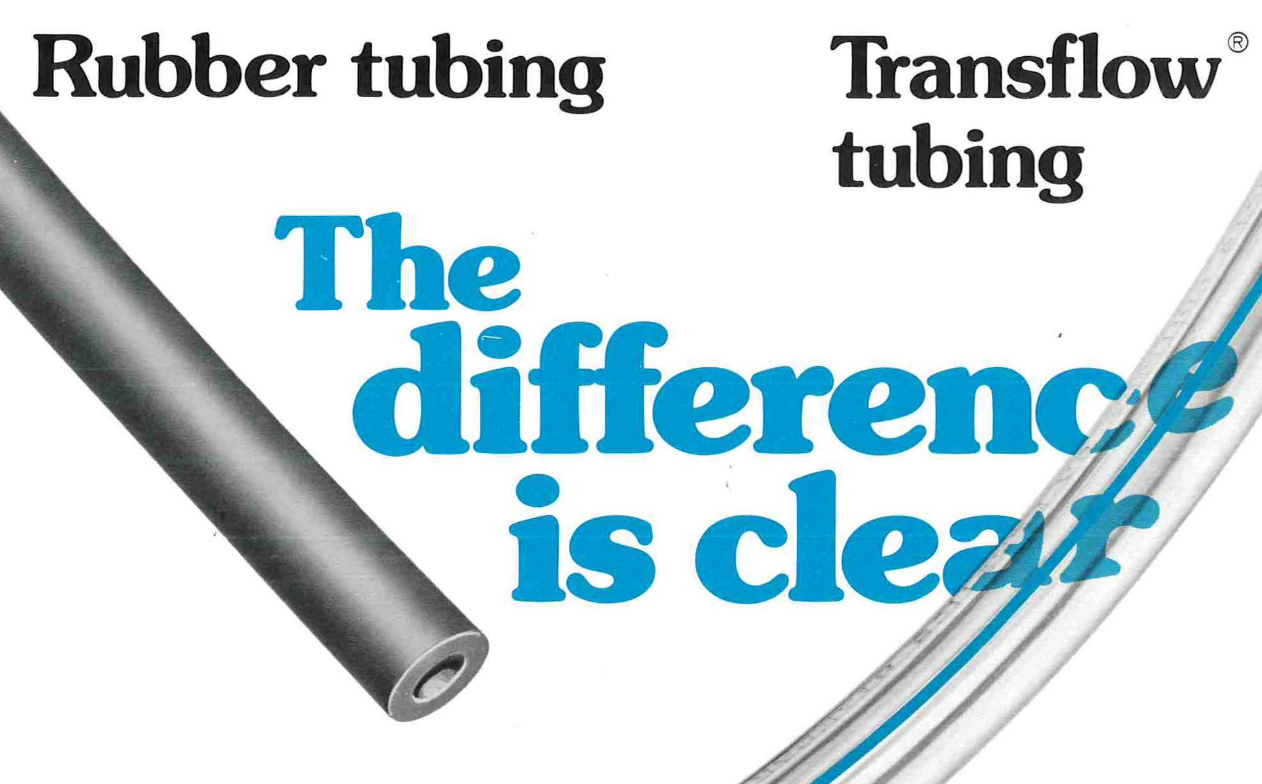
Kentucky Association of Milk, Food and Environmental Sanitarians

The Kentucky Association of Milk, Food and Environmental Sanitarians sponsored the 1977 Educational Conference for Fieldmen and Sanitarians on February 22, 23 at Executive West, Louisville, Kentucky.

Over 300 persons attended the conference, 24 of which were from states other than Kentucky. The 24 included persons from Florida, Georgia, Illinois, Indiana, Iowa, Missouri, New York, Ohio, Tennessee, Texas and Washington D.C.

Officer elections and an awards banquet highlighted the conference. The newly elected officers and directors are: Harry Marsh, Lexington, President; Barry Kinslow, Louisville, President-Elect; Dudley Conner, Frankfort, Vice-President; David E. Atkinson, Frankfort, Secretary-Treasurer; Ellen Cook, Louisville and Dr. B. E. Langlois, Lexington, Directors-North Central Region; Steve McDaniel, Lebanon, Director-South Central Region; JoAnn Needham, Owingsville, Director-Eastern Region.

Kentuckians receiving awards at the awards banquet were: Irving Bell, Frankfort, Outstanding Sanitarian Award; John Bruce Mattingly, Springfield, Outstanding Fieldman Award; Joe Winlock, Glasgow, Outstanding Service Award. Also at the banquet, the KAMFES gave Honorary Membership to Paris Boles, Monticello and Marvin Wesley, Frankfort.



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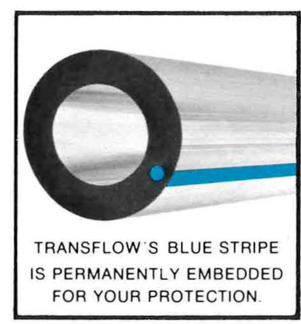

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Total Management Concept: The Team Working For You

Dr. George C. Fisher, Head
Veterinary Services Laboratory,
Ontario Ministry of Agriculture & Food,
Kemptville, Ontario

There is no way the farmer today can operate as a loner. That's why more and more dairymen are looking at their total operations with an eye on Total Management. The Total Management Concept benefits the dairyman because it encourages him to identify and make use of the many people who have been trained to offer professional advice and service. TMC is teamwork with the dairyman as head coach. He knows who his resource people on the bench are and when to call on them. And, like any winning coach, the dairyman knows the special efforts of all team members must be coordinated to succeed. These resource people are the veterinarians, equipment dealers, nutritionists, agricultural engineers, extension service people, D.H.I.A. or R.O.P., and many others who can provide current, specialized information and help with the business of dairying.

Planning to Avoid Emergencies
Through Total Management, you coordinate planning to minimize emergencies, be they in health, equipment, or production. This is done with the help of available experts in six broad areas:

1. Soil Testing
2. Nutrition
3. Cow Performance
4. Equipment Maintenance
5. Herd Health Program
6. Record Keeping

Though some areas may appear more important than others, the exclusion or neglect of any one will result in less profit, or as a sudden problem in production or herd health—a problem easily avoided with proper management and teamwork.

The Team in Action
Total Management provides the farmer with continually updated information, and assures that all areas are working to his benefit. Since the amount of information available is more than any one person can possibly handle, specialists are essential to apply the right facts to your needs. Broadly stated then, the TMC helps you make informed decisions and to put them into practice.

• HERD HEALTH
Use your local veterinarian in a systematic approach to a herd health program (specifically in respect to infertility and mastitis control).

• PRODUCTION GOALS
Set uniform production goals designed to meet the capabilities of the herd and farm unit with the assistance of your County Agent.

• RECORDS
Apply a unified approach to the keeping of records relating to herd health, nutrition, reproduction, and production—(with the help of a milk recording system such as D.H.I.A. or R.O.P.). University extension people are trained to provide guidance in this area.

• EQUIPMENT
Have your dairy equipment dealer perform periodic checks and adjustments of the milking system through a scheduled maintenance program. This will assure proper equipment operation for better production and improved herd health.

• FEEDING PROGRAM
Work with your nutrition specialist to develop an in-depth feeding program using nutrient analysis to determine year-round feeding according to production. Base this program on the production, storage, and utilization of high quality forages.

Keeping pace with the times offers a rewarding challenge for the dairyman who is willing to use progressive management practices. And, if you face the future by working on Total Management with the help of the many professionals available, you will find a bright future. Today, more than ever, your future depends on having a winning team working for you.

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