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Journal of

MILK and FOOD TECHNOLOGY

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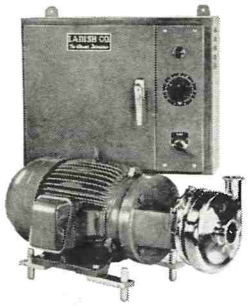

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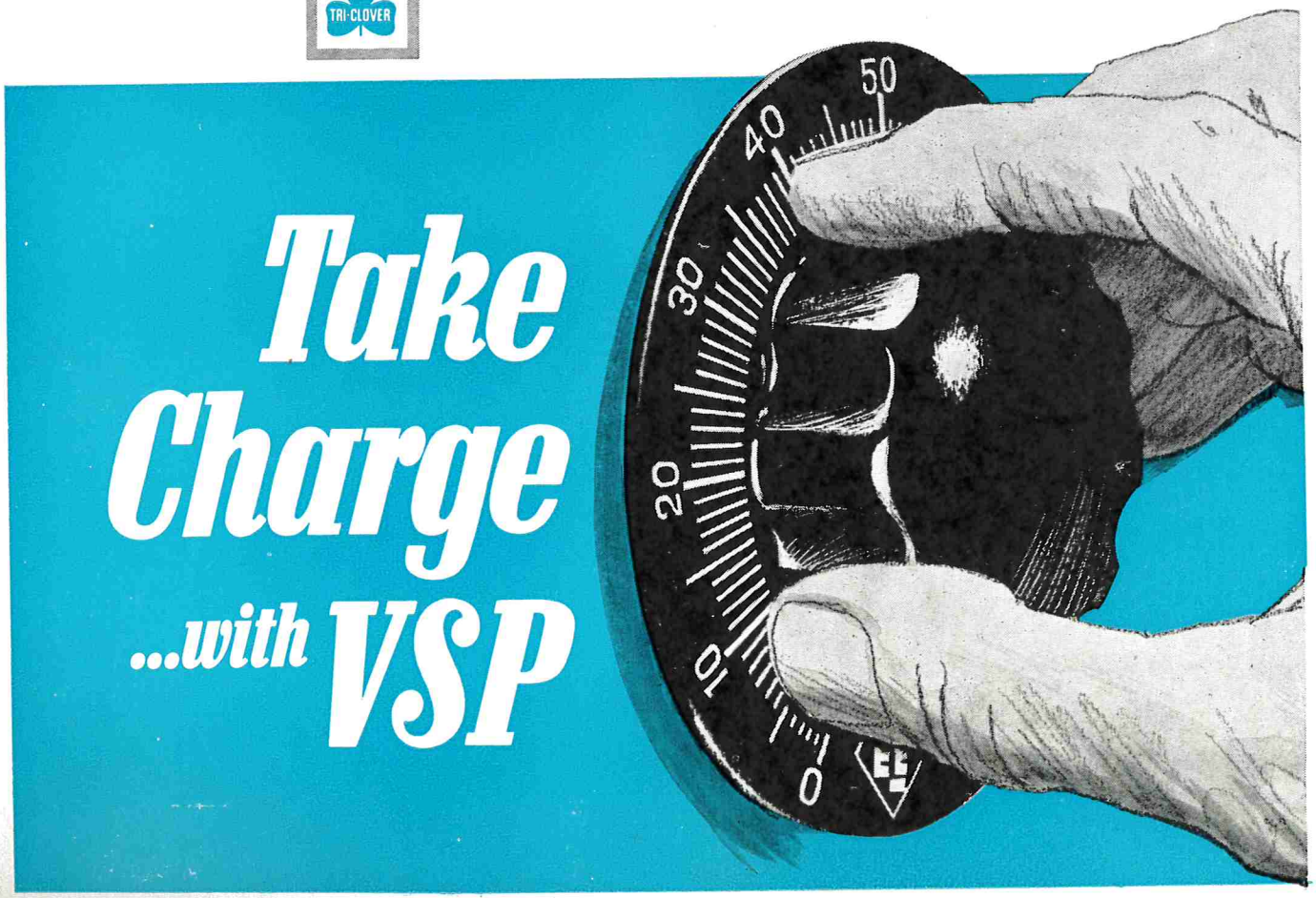

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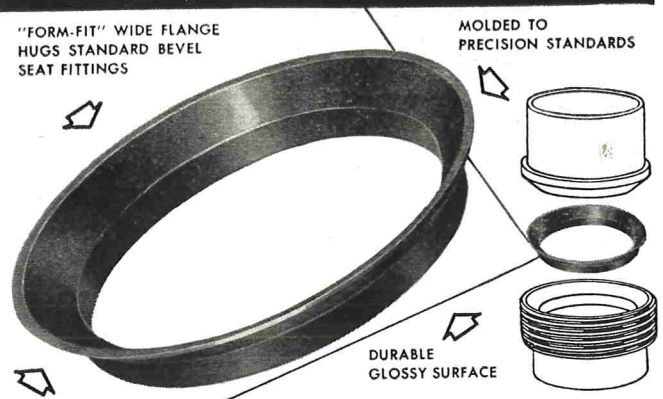

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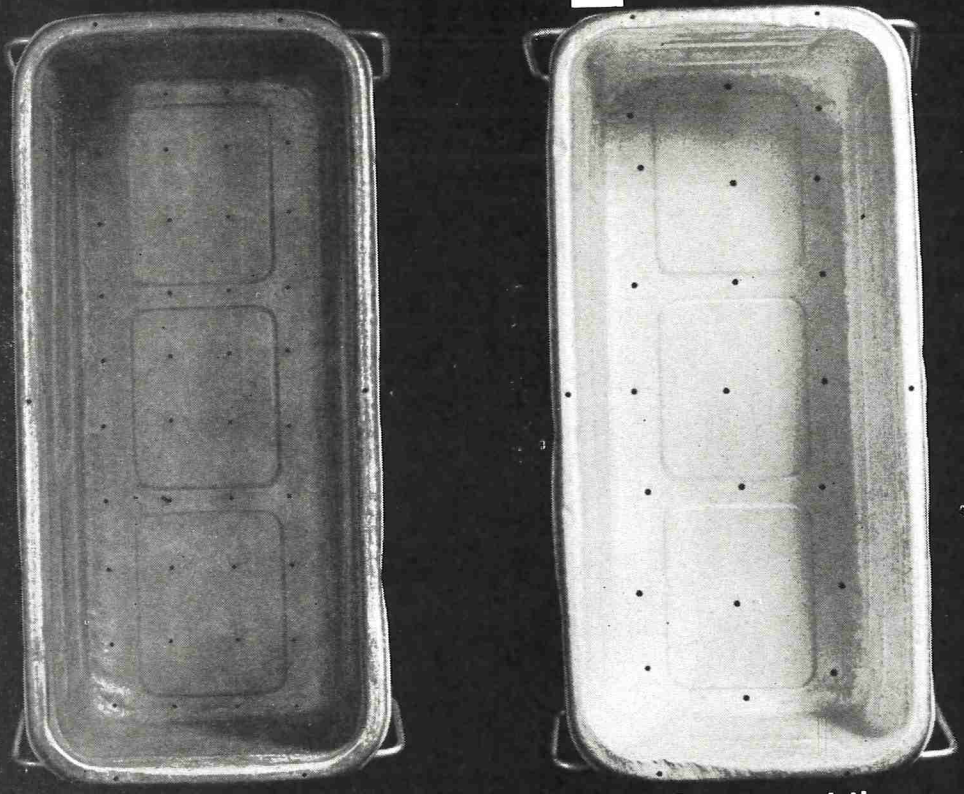

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WHAT THE SANITARIAN SHOULD KNOW ABOUT *CLOSTRIDIUM PERFRINGENS* FOODBORNE ILLNESS

FRANK L. BRYAN

U. S. Department of Health, Education, and Welfare
Public Health Service
Health Services and Mental Health Administration
National Communicable Disease Center
Atlanta, Georgia

(Received for publication August 7, 1969)

ABSTRACT

This paper is a follow-up of two articles, which were previously published in this Journal, concerned with what the sanitarian should know about staphylococci and salmonellae. The nature of *Clostridium perfringens,* including factors that support or limit its growth, is discussed. This organism is widely distributed in the intestinal contents of man and animals, in sewage, and in soil. From these sources foods frequently become contaminated with this organism. Meats and meat products prepared in food-service establishments are frequently involved in outbreaks. To prove that *C. perfringens* is responsible for outbreaks, this organism should be recovered in large numbers from both the patients' stools and the incriminated food, and isolates from both should be correlated serologically. Of the various methods of controlling foodborne diseases—only inhibition of growth is practical for controlling outbreaks of *C. perfringens* foodborne illness. Appropriate control features are delineated.

NATURE OF THE ORGANISM

Clostridium perfringens (*C. welchii*) is a nonmotile, encapsulated, short and thick bacillus with blunt ends. It occurs singly, in pairs, and, less frequently, in short chains. Filamentous cells are sometimes produced. Young cultures are gram-positive, but old cultures may appear gram-negative. Subterminal ovoid spores are produced. Vegetative cells sporulate readily in the intestines, but rarely in cooked meat.

Strains of *C. perfringens* are divided into five toxicological types (A to E) on the basis of four major toxins (*alpha, beta, epsilon, iota*) which may be produced *(58).* Only types A and C have caused human gastroenteritis. Type A strains also cause gas gangrene. Type C (formerly known as type F) has caused outbreaks of necrotic enteritis in Germany *(73)* and in New Guinea *(43).*

The only major toxin produced by *C. perfringens* type A is *alpha* toxin, an enzyme (lecithinase C) which attacks lecithin and similar chemical substance to liberate phosphorylcholine. This reaction is seen as an opaque zone of precipitate around a colony on egg yolk agar. *Clostridium perfringens* type A may or may not be hemolytic (possess *theta* toxin which lyses red blood cells); it does, however, form collagenase (destroys collagen in tissues), hyaluronidase (facilitates the spread of the organism through tissues), and desoxyribonuclease (destroys DNA). A few years ago, *C. perfringens* type A was subdivided into gas gangrene strains and food poisoning strains *(34).* Heat-sensitive organisms that produced large amounts of lecithinase and *theta* toxin were considered as gas gangrene strains. Food poisoning strains were considered those that were heat resistant, produced little lecithinase and no *theta* toxin. Today, however, it is believed that it is neither necessary for a particular strain to possess a specific biochemical characteristic nor for it to be of a certain serotype to produce foodborne illness. Thus, there is no such thing as a "food poisoning *C. perfringens*" per se *(23, 26).*

Some strains of *C. perfringens* produce heat-resistant spores; others produce heat-sensitive spores. Heat-sensitive strains outnumber heat-resistant strains in most habitats. Although spores of most strains are killed in a few minutes at 212 F, the spores of some strains are extremely heat-resistant and survive boiling for 1 to 6 hr *(6, 22, 30, 34, 44, 51, 69).*

Clostridium perfringens is rather demanding in its nutritional needs, requiring 13 to 14 amino acids and 5 to 6 growth factors *(9, 17).* These nutritional needs may play a role in the types of foods associated with foodborne illness. Foods associated with outbreaks caused by this organism are usually high in protein, such as meat or meat dishes.

The optimum temperature for growth of *C. perfringens* is between 109.4 and 116.6 F *(9).* Growth will occur at 122 but not at 131 F. At 122 F a "Phoenix phenomenon" occurs with initial declines in numbers to minimum counts at 4 hr followed by a sudden increase in growth to maximum counts at 6 hr *(13).* In beef (pH 5.7-5.8) growth did not commence until 68 F *(6).* After a long lag period, growth of *C. perfringens* in beef cubes in gravy was observed at 65 F *(21).* No growth of *C. perfringens* was observed in cooked meat after storage for 7 days at 43.7 F *(70).*

Spores of *C. perfringens* in frozen meat were resistant to freezing and holding at 23 F or –4 F *(6)*. Destruction was less rapid during storage at –4 F than at 23 F, and a much slower rate of destruction occurred at temperatures of 33.8 to 59 F. In laboratory media, Canada et al. *(12)* recovered an average of from 16 to 58% of spores after freezing at 0 F for 48 hr; in chicken gravy, 4.3 to 38% survived after 90 days, and 3.7 to 11% after 180 days when held at 0 F *(59)*. A considerable proportion of vegetative cells of *C. perfringens* was destroyed by freezing and frozen storage *(6, 12, 59)*. Storage for 48 hr at refrigeration temperatures (45-50 F) also resulted in substantial decreases of both vegetative cells and spores. Other investigators have observed decreases in numbers during refrigerated storage *(61, 71)*.

Clostridium perfringens is an anaerobe, but stringent anaerobic conditions are not required. The organism requires a low oxidation-reduction potential for initiation of growth (cell division). Oxidation-reduction potential is a measure of a system's reducing tendency (to give up or accept electrons). This potential is usually expressed as the Eh value in millivolts (mv). The major effect of an adverse oxidation-reduction potential is on the lag phase. At an Eh value of –45 mv there is a minimal lag in growth, but the lag increases with increasing redox potentials until a point is reached where growth ceases and cells commence to die (+31 to +231 mv). (For comparison purposes, the potential of venous blood is +180mv.) During the lag period, *C. perfringens* cells reduce the Eh of their immediate surroundings until it becomes sufficiently low to permit logarithmic growth. The optimum Eh of culture media is in the vicinity of –200 mv *(50)*. The pH has a marked effect on limiting Eh for growth. Specific Eh values supporting growth of *C. perfringens* may vary with strain, size of inoculum, metabolic state, pH, and the method of determination *(7, 25, 50)*. The ability of *C. perfringens* to grow appears to be governed, almost entirely, by the oxidation-reduction potential of the medium and not by the presence or absence of gaseous oxygen *(55)*.

No growth of *C. perfringens* was observed at or below pH 5 or at or above pH 9 *(17)*. Fairly rapid growth occurred between pH 5.5 and pH 8 *(54)*. Growth of *C. perfringens* in beef stored at 68 F was variable at pH 5.7-5.8, but growth was rapid at 7.2 *(6)*.

Spores were able to germinate and grow in media containing up to 5% NaCl (a_w 0 97) but not in 10% NaCl *(30)*. Slight growth was observed in 8% NaCl *(19)*. Vegetative cells survivied for 6 days on raw meat covered with brine (22% NaCl and 0.9% $NaNO_3$). The inhibitory effect of salt was greater when 1% $NaNO_3$ was present *(29)*. *Clostridium perfringens* can survive and grow in curing salt solutions that are higher than those used in normal curing operations. Growth also occurred in media containing up to 10,000 ppm $NaNO_3$ or 400 ppm $NaNO_2$. *Clostridium perfringens* survived all steps in curing and smoking hams, up to 113 F internal temperature *(19)*. Growth of *C. perfringens* is inhibited by certain other bacteria, particularly enterococci *(57)*.

EPIDEMIOLOGY

Early reports linking *C. perfringens* with food poisoning were made before the turn of the century *(1, 39)*, but the illness was not reported in the United States until 1945 when McClung described four outbreaks of *C. perfringens* foodborne illness *(41)*. This ailment did not receive much attention, however, until the appearance of the classical paper of Hobbs et al. in 1953 *(34)*. Since then, this illness has become recognized as one of the most common foodborne diseases.

In the United States, during 1966-68, *C. perfringens* foodborne illness accounted for 67 reported, bacteriologically confirmed, outbreaks (13.5% of the outbreaks of known etiology). There were also 26 outbreaks that were clinically and epidemiologically similar to *C. perfringens* foodborne illness. During 1966 and 1968 and some previous years, *C. perfringens* was responsible for more reported cases of foodborne diseases than any other agent *(46)*. In England and Wales during 1966, *C. perfringens* was responsible for 33% of the reported general outbreaks but only 2% of all outbreaks when family outbreaks and sporadic cases were included in the totals. This organism, however, accounted for 30% of the total numbers of reported cases of food poisoning *(68)*.

Foods involved in outbreaks of *C. perfringens* foodborne illness are usually meat or poultry that has been boiled, stewed, or lightly roasted; or meat and poultry stews, sauces, gravies, pies, salads, casseroles, and dressings. The incriminated food invariably is held at room temperature or refrigerated in large masses for several hours, often overnight or longer. Outbreaks frequently follow banquets or meals prepared at hospitals and schools where large amounts of meat or poultry are involved. Thus, *C. perfringens* foodborne illness is a disease intimately associated with the food-service industry.

When sufficient numbers of *C. perfringens* are ingested, diarrhea and abdominal pain accompanied by large volumes of gas in the intestine occur after an incubation period of 4 to 22 hr (12 hr average). Nausea and vomiting are rare. Fever, shivering, headache, and other signs of infection seldom occur *(15, 27, 34, 52)*.

TABLE 1. REPORTS OF *C. perfringens* ISOLATION FROM HUMAN FECES[1]

Group	Number of samples	Number positive	Percent positive	Type[2]	Reference
General population	108	19	18	HR (F)	*(20)*
General population	45	1	2	HR	*(34)*
Hospitalized old people	53	8	15	HR	*(34)*
Healthy hospitalized personnel and families	50	10	20	HR	*(15)*
General population	50	3-4	6-8	HR	*(13)*
Male	59	13	22	HR	*(40)*
Female	131	19	15	HR	*(40)*
Hospital patients	308	96	30	HR	*(40)*
Chinese hospital patients (Hong Kong)	364	229	63	HR	*(67)*
General population (Leeds, England)	57	5	9	HR	*(67)*
General rural population					
Children	461	7	2	HR	*(62)*
Adults	50	3	6	HR	*(62)*
Persons fed in hospitals	48	12	25	HR	*(62)*
Persons fed in boarding schools	53	8	15	HR	*(62)*
Aborigines (Australian)	420	80	19	HR	*(62)*
Normal adults	11	9	82[3]		*(53)*
Normal adults	25	25	100[3]		*(66)*
Normal adults	50	50	100[3]		*(13)*
Food handlers (Louisiana)	219	171	78[3]		*(24)*
		15	7	HR	

[1]Modified from Hobbs *(31)*.
[2]HR = heat resistant; (F) = type F; blank = not differentiated (includes both heat-sensitive and heat-resistant strains).
[3]Percentages are most significant because both heat-sensitive and heat-resistant strains cause foodborne illness.
illness.

In human volunteer studies with heat-resistant strains of *C. perfringens*, doses of 1.9 x 10^8 or larger were required to produce illness. Filtrates from cultures containing a mean of 8.1 x 10^8 viable organisms failed to produce illness in six volunteers *(15)*. A strain of *C. perfringens* that produced heat-sensitive spores caused diarrhea and abdominal cramps in five of six volunteers when cultures containing 4 to 6 x 10^9 vegetative cells were fed to them *(27)*. In children and young adults *C. perfringens* foodborne illness is relatively mild, and symptoms usually subside within 24 hr; but in the elderly, ill, or debilitated, serious consequences, including death, have occurred *(38, 48, 63)*.

The absence of fever, immunity, and secondary spread suggests that the illness is an intoxication; however, culture filtrates or suspensions of dead organisms have failed to produce illness in human volunteers. Nygren *(47)* proposed an interesting hypothesis that lecithin in food was hydrolyzed by the phospholipase C enzyme that is produced by *C. perfringens*, and phosphorylcholine was formed and produced diarrhea. Animals given synthetic phosphorylcholine developed diarrhea. Human volunteer studies, however, have failed to confirm this hypothesis. One volunteer was given 100 mg and 500 mg phosphorylcholine but did not develop diarrhea *(14)*. The specific cause of *C. perfringens* foodborne illness remains an enigma.

Smith and Holdeman *(57)* claim that *C. perfringens* is probably more widespread over the earth than any other pathogenic bacterium. This, of course, is conjecture, but nevertheless its ubiquity is apparent. This organism is widely distributed in the intestinal contents of man and animals, in sewage, and in soil.

Clostridium perfringens is a normal inhabitant of the intestinal tract of man and animals. Percentages for human carriers of *C. perfringens* type A in various population groups are listed in Table 1. Variation in results is dependent in part on the method used for culturing. The investigators chose various media, and only a few used selective or differential media. Most of the studies dealt with the isolation of only heat-resistant strains, and criteria for heat resistance also differed with several investigators. Since it is known that heat-sensitive strains also cause food

TABLE 2. INCIDENCE OF *C. perfringens* TYPE A IN RAW FOODS[1]

Food	Number of samples	Number positive[2]	Percent positive[3]	Reference
RAW RED MEATS				
Beef	50	35	70	[21][4]
	54	13	(24)	[34][4]
frozen boneless	237	32	(14)	[36][4]
carcasses	158	2	(1)	[36][4]
imported-Gr. Brit.	134	28	(21)	[65]
retail	47	17	(36)	[65]
abattoirs	40	2	(5)	[65]
steak and mince	10	6	60	[42][5]
tripe	6	1 H	17	[42][5]
		1 N	17	
Veal				
carcasses	17	14	82	[21][4]
	10	0	(0)	[36][4]
	7	1	(14)	[34][4]
	20	3	(15)	[65]
frozen boneless	163	2	(1)	[36][4]
Pork				
carcasses	41	15	37	[21][4]
	4	0	(0)	[36][4]
	55	11	(20)	[34][4]
retail	55	27	(49)	[65]
abattoirs	14	3	(21)	[65]
sausage	21	10	48	[21][4]
	38	36 H	95	[42][5]
		9 N	24	
		1	(3)	
Lamb and Mutton				
carcasses	27	14	52	[21][4]
	23	1	(4)	[36][4]
	17	0	(0)	[34][4]
imported-Gr. Brit.	76	18	(24)	[65]
abattoir	19	12	(63)	[65]
frozen boneless	163	2	(1)	[36][4]
Liver				
market	100	26	26	[11][5]
abattoir	100	12	12	[11][5]
hospital	2	1	50	[42][5]
Black Pudding	4	2	50	[42][5]
POULTRY	26	15	58	[21][4]
	7	6 H	86	[42][5]
		3 N	44	
		1	(14)	
FISH	18	11 H	61	[42][5]
		3 N	17	
herring	14	8	58	[37][4]
herring	100	1	1	[37][5]
MEAT, POULTRY, FISH no breakdown	122	20	16	[60][5]
MILK	4	1	25	[18][5]
FRUITS AND VEGETABLES	52	2	4	[60][5]
SPICES	60	3	5	[60][5]

[1]Modified from Smith *(54)* and Hobbs *(31)*.
[2]H = hemolytic; N = non-hemolytic.
[3]( ) = heat-resistant strains; percentages are least significant because both heat-sensitive and heat-resistant strains cause foodborne illness.
[4]Enrichment method.
[5]Without enrichment.

poisoning, the true incidence of *C. perfringens* in human feces would be high as indicated in the studies that sought the presence of *C. perfringens* without regard to its heat resistance *(13, 24, 53, 66)*.

Studies by Sutton *(62)* suggested that heat-resistant strains of *C. perfringens* in humans is closely linked with communal feeding and poor hygiene. Of 26 Australian aboriginal families, 24 (92%) had one or more members who were carriers. Families classified as living under conditions of good hygiene had a carrier rate of only 20%. The carrier state of heat-resistant strains appeared to be transient *(62)*.

In an examination of 219 fecal specimens from food handlers in Louisiana, Hall and Hauser *(24)* found that 78% yielded *C. perfringens*. Multiple serotypes were found in 29% of the cultures. Only about 35% of the isolates produced heat-resistant spores (surviving boiling for 30 min or more). There is a definite risk that food workers may contaminate foods with *C. perfringens*.

Since *C. perfringens* is abundant in feces, it is present in sewage, and it has been used as an index of water pollution. In a survey of sewage from about 70 houses, swab samples were positive for heat-resistant strains of *C. perfringens* in 56% of 125 examinations *(34)*.

Animal feces also serve as sources of *C. perfringens (66)*. Heat-resistant strains of *C. perfringens* were recovered from 14 of 76 (18.4%) samples of pig feces obtained from feeding passages, slaughterhouse pens, and pigs; from 2 of 113 (1.7%) of cattle feces obtained from farms and slaughterhouses; and from 6 of 41 (14.6%) samples of rodent pellets *(34)*. *Clostridium perfringens* was found in the feces of cattle, sheep, pigs, and chickens at about the same proportion as in human feces, 10^{2-4}/gm; higher levels, 10^{8-9}, were found in dog and cat feces *(53)*. In poultry feces, Yamamoto et al. *(72)* found *C. perfringens* in 41 of 160 (25.6%) samples. All batches of greenbottle and bluebottle flies, sampled by Hobbs et al. *(34)*, contained *C. perfringens*.

In soil, where it is part of the normal bacterial flora, *C. perfringens* exists in both vegetative and spore forms. Taylor and Gordon *(66)* examined 196 samples of soil; 190 contained *C. perfringens*, mostly type A. Smith and Gardner *(56)* found between 100 and 56,700 *C. perfringens* per gram in various types of soil. Dust obtained from a kitchen environment revealed *C. perfringens* in 81-89.6% of samples *(42)*.

Foods are frequently contaminated with *C. perfringens*. Table 2 lists the incidence of *C. perfringens* type A in raw foods, and Table 3 lists the incidence in processed foods. Care must be exercised when interpreting these data since the investigations differed in technique and media. The results represent

TABLE 3. INCIDENCE OF *C. perfringens* TYPE A IN PROCESSED FOODS[1]

Food	Number of samples	Number positive[2]	Percent positive[3]	Reference
PROCESSED MEATS	101	20	20	[21][4]
Requires full cooking	38	14	37	[21][4]
Requires light cooking	21	4	14	[21][4]
Requires no cooking	42	2	5	[21][4]
Steak and Mince	15	1 H	7	[42][5]
		1 N	7	
Sausage	25	4 H	16	[42][5]
		1 N	4	
Tripe	6	1	17	[42][5]
Black Pudding	2	2	100	[42][5]
Roast (cold)	15	4 H	27	[42][5]
		2 N	13	
Cold Meats	63	4 H	6	[42][5]
		4 N	6	[42][5]
Cooked Chickens	46	13 H	28	[42][5]
		7 N	15	[42][5]
		1	(2)	[42][5]
PROCESSED FISH				
Hospital cooked	6	1	16	[42][5]
Kipper	98	7	7	[37][4]
Kipper	59	13	22	[37][5]
Smoked haddock	16	7	44	[37][4]
	14	0	0	[37][5]
Smoked salmon	9	7	78	[37][4]
	9	0	0	[37][5]
Prawns	2	0	0	[37][4]
Scampi	1	1	100	[37][4]
	1	1	100	[37][5]
PASTEURIZED MILK	10	1	10	[18]
DEHYDRATED SOUPS, GRAVIES, SAUCES AND SPAGHETTI	55	10	18	[45][4]
COMMERCIALLY PREPARED FROZEN FOODS	111	3	3	[60][5]
HOME PREPARED FOODS	165	3	2	[60][5]

[1]Modified from Smith (54).
[2]H = hemolytic; N = non-hemolytic.
[3]( ) = heat-resistant strains; percentages are least significant because both heat-sensitive and heat-resistant strains cause foodborne illness.
[4]Enrichment method.
[5]Without enrichment.

samples that were cultured with and without enrichment. Higher percentages were usually observed when enrichment techniques were used. Data that include heat-sensitive strains are the most meaningful.

Foods of animal origin become contaminated from direct or indirect contact with intestinal contents during processing. During the processing of pork, for instance, *C. perfringens* was isolated from pig carcasses after scalding, scraping, and inspection. Scald-tank water also yielded these organisms (5). Peppers and other spices contained 2 to 12 *C. perfringens* per gram (54). Any food or object directly or indirectly exposed to fecal material or soil may be contaminated with this organism.

DETECTING AND IDENTIFYING THE ORGANISM

In the investigation of cases of foodborne disease with a clinical history similar to that of *C. perfringens*, it is necessary to examine both suspected foods and feces of patients. Stool specimens from food workers and environmental swabs from kitchens or processing plants are also valuable in outbreak investigations or bacterial surveys if serotyping is to be done. Samples of food should be sent to the laboratory refrigerated, but not frozen. Although refrigeration temperatures have an adverse effect on *C. perfringens*, they prevent excessive growth of this organism or overgrowth by other organisms during the period between sampling and laboratory analysis. Fecal specimens can be placed in transport or enrichment broth media.

Quantitative colony counts are generally performed on food samples. In the United States, sulfite-polymixin-sulfadiazine (SPS) agar is usually used (2, 3). Because *C. perfringens* reduces sulfite to sulfide, these organisms appear as black colonies in SPS agar.

The interpretation of *C. perfringens* counts in foods is difficult because the sample selected may not be representative of portions eaten, the degree of contamination may vary in different parts of the sample, and the numbers of organisms may change appreciably during the interim between serving and laboratory analysis. This last change can occur if foods are heated or frozen, thus killing vegetative cells and perhaps some spores, or if foods are stored at temperatures that would allow multiplication of organisms.

Portions of fecal samples are transferred to duplicate tubes of cooked meat or thioglycollate broth. One tube is heated in a 176 F (80 C) water bath for 15 min, the other is left unheated. After anaerobic incubation, each broth culture is streaked onto blood and egg-yolk agar plates (16). Swabs taken from food, carcasses, or environmental surfaces are placed in cooked meat broth and treated in the same manner as described for fecal samples. Feces from patients involved in recent outbreaks usually have large numbers of *C. perfringens*, 10^5 to 10^8/g (35). This is higher than the normal level in feces, 10^{2-4}/g (53).

All isolates should be confirmed morphologically and biochemically. Tests for motility, indol production, and nitrate reduction are useful for screening

cultures *(2)*. *Clostridium perfringens* gives a positive reaction only in the latter test; it reduces nitrates to nitrites. All tubes of liquid media (with caps loosened) should be heated in a boiling water bath for 10 min and cooled before inoculation. This action drives off oxygen.

A number of systems are suitable for cultivation of *C. perfringens* including: Brewer anaerobic jars, Case jars, Torbal jars, Gaspak jars or disposable anaerobic systems, desiccators, plastic pouches *(8)*, roll tubes, deep agar tubes, or anaerobic incubators. A gas mixture containing 10% hydrogen, 10% carbon dioxide, and 80% nitrogen is excellent.

Since *C. perfringens* is an ubiquitous organism, more definitive identification of strains must be used in outbreak investigations than just the isolation of *C. perfringens* from foods and from the feces of patients and workers. This can be accomplished by serotyping. At present there are 91 specific antisera (including the 13 types of Hobbs[1]) available for typing isolates of *C. perfringens*. This test is performed by mixing a drop of formalinized suspension (obtained from sediments of centrifuged pure cultures of isolates) with a drop of pooled or specific antisera on a slide. Clumping occurs when the cells and sera are homologous.

It is rare to find the same serotype of *C. perfringens* in a significant percentage of people selected at random, but after a common-source outbreak, the same serotype can be recovered from the stools of an appreciable number of patients. Isolates from the suspect food and from patients should be correlated serologically. A contaminated food may contain only one serotype, while feces from patients are likely to contain resident and transient strains of *C. perfringens* as well as the serotype from the contaminated food. Therefore, several isolates from each culture must be serotyped *(23)*.

CONTROL

Of the three principles of foodborne disease control—limitation of contamination, inhibition of growth, and destruction of the organism *(10)*—only inhibition of growth is practical for controlling outbreaks of *C. perfringens* foodborne illness.

Foods become contaminated with *C. perfringens* in various ways. Meat and poultry may be contaminated by excrementborne organisms of animal origin during slaughtering and processing operations. Boned or rolled meats are apt to be contaminated. Skewers or thermal pins (heating rods) push surface contamination into internal portions of meat. Raw meat and poultry serve as vehicles for conveying *C. perfringens* into kitchens and can contaminate workers' hands or preparation equipment. From these sources other foods may become contaminated. Heat-resistant strains of *C. perfringens* have been isolated from chopping boards and from other articles of kitchen equipment *(33)*. Hobbs *(29)* stated that in view of the common occurrence of *C. welchii (perfringens)* in raw meat, the human carrier in the kitchen is probably a minor source of contamination. On the other hand, Hall and Angelotti *(21)* reported that the greatest hazard was from contamination after cooking. Any food may be contaminated by dust, contact with contaminated equipment, vectors, and excrement-borne organisms of human origins.

Obviously, sanitation, proper processing techniques, and personal hygiene of food workers reduce, but do not always prevent, the risk of contamination. It is not feasible to prevent carriers from handling food since most people harbor *C. perfringens* in their intestinal tract. Thus, control of human contamination rests in adequate hand washing, care in handling foods (particularly cooked foods), and in knowledge of proper food preparation and storage techniques. Care should be taken to clean and sanitize kitchen equipment such as cutting boards and slicers, and to avoid using the same equipment for both raw and cooked food (unless the implement has been effectively sanitized between usages). Although contamination may be limited by good sanitation and personal hygiene, there does not seem to be any way to assure that *C. perfringens* can be kept out of foods.

Outbreaks of *C. perfringens* foodborne illness would not occur if cooked foods were eaten while still hot, just after initial cooking; or reheated to internal temperatures of 165-212 F immediately before serving. Heat penetration during cooking is more effective for small fowl, small pieces of meat, or small masses of food. Hobbs *(32)* suggested that roasts and joints should be 6 lb or less when cooked in food-service establishments.

Vegetative cells of *C. perfringens* are destroyed by thorough cooking, but heat-resistant spores can survive. Even spores that are not considered heat resistant may survive many cooking processes. For instance, spores of heat-sensitive strains survived in bread and onion stuffing when cooked to doneness (163.4 to 180 F) in ovens set at 201.2 F, 225 F, and 450 F *(71)*. Survival has also been observed in cooked foods: baked hams (138.2 F), turkey rolls (165 F and 185 F), and ground-beef casseroles (160 F and 180 F) *(61)*. Internal temperatures that were obtained are indicated following each food item.

When raw chicken was cooked to obtain a temperature of 185 to 194 F in the breast muscle, a *C.*

[1]Only Hobbs' 1-13 are available commercially. NCDC Anaerobic Bacteriology Laboratory has 78 additional antisera, and Hobbs has 4 additional antisera.

perfringens spore inoculum was reduced from 10,000 spores per gram to 1.5 per gram. Upon incubation of the cooked chickens at 113 F, a lag period was observed for about 4 hr and the growth then became logarithmic. After 14.7 hr, 10 million cells were present. When cooked chicken was inoculated with 1,000 vegetative cells, they multiplied to a total of 10 million cells in 6.3 hr *(49)*. *Clostridium perfringens* survived better during oven roasting at low temperatures (200-210 F) overnight than during roasting at 375 to 425 F for a few hours. During conventional methods of roasting, the internal temperature of the meat reached 185 to 195 F in about 3 hr; during overnight roasting at low temperature, the internal temperature of the meat only reached slightly higher than 150 F. It took 7 to 10 hr before this temperature was reached. Overnight roasting, therefore, is not recommended *(64)*.

Preparation of foods several hours or a day before serving is hazardous and should be avoided. Leftover, cooked meat should never be merely warmed up, but heated to an internal temperature of at least 165 F to destroy vegetative cells of *C. perfringens*, or cut up into small pieces and boiled for a sufficient period so that the interior temperatures become lethal to vegetative cells. Once reheated, the food should be eaten while hot and not allowed to remain at incubating temperatures.

Many competing organisms will succumb to heat treatments that allow *C. perfringens* spores to survive. Heat is a very effective spore germination activator. Spores are heat shocked (activated) by high temperatures, and when conditions become favorable they germinate and multiply rapidly. High percentages (30 to 100%) of spores germinated after meat was heated to 158 to 176 F, but only low percentages (<5%) germinated when raw meat was inoculated *(6)*. Heat also drives off oxygen which results in anaerobiosis in meat and poultry. Thus, cooking may contribute to outbreaks of *C. perfringens* foodborne illness if proper precautions are not taken subsequently to inhibit the multiplication of these organisms in food.

The mere presence of *C. perfringens* in food is not enough to cause illness since millions of viable organisms are required. Contamination alone cannot account for such numbers—multiplication must occur after contamination.

The generation time of *C. perfringens* can be as short as 8.5 min (12 min median for 22 strains) in broth cultures incubated at 114.8 F; it is about 20 min at 98.6 F *(4)*. In various poultry and meat stock soups, the generation time ranged from 24-32 min *(54)*. As temperatures deviate from the optimum, in either direction, the generation time lengthens until such a point is reached at which multiplication ceases. Strains of *C. perfringens* failed to grow at temperatures at or above 131 F or at or below 59 F *(54)*. Based on Arbuckle's data *(4)* for growth of 22 strains of *C. perfringens* in thioglycollate broth, this organism would increase over one thousandfold in 3 hr at the optimum temperature of 114.8 F. To increase the same amount 5.5 hr would be required at 98.6 F; 10 hr at 86 F; and about 30 hr at 68 F. Thus, prevention of *C. perfringens* multiplication can be achieved by the effective use of refrigeration (<45 F) or hot holding (>140 F).

Foods such as barbecued chicken, stews, and gravies that are cooked and held warm, should be held under conditions which provide internal product temperatures of 140 F or above. This temperature prevents the germination of spores of *C. perfringens* as well as the growth of vegetative cells. However, *C. perfringens* survived for over 6 hr when roast turkey slices in broth were held at conditions of steam table storage of 154.4 F *(61)*. When boiled lamb, inoculated with 2×10^5 *C. perfringens* vegetative cells, was stored in gravy on a hot plate at 104-122 F for 3 hr, the count increased to 46 million per slice. Meat stored without gravy, but at the same time and temperature, yielded about half as many organisms. When the meat was stored at room temperature for 3 hr, no increase of organisms was observed *(28)*.

All foods that are not eaten while hot, or that are not held in devices that maintain temperatures of 140 F or above, must be chilled rapidly and refrigerated at 45 F or below. The key to the prevention of *C. perfringens* foodborne outbreaks is to prevent multiplication of these organisms in cooked and cooling meat, poultry, meat broths, and foods containing these items as ingredients.

Foods should never be held at room temperature to cool; they should be refrigerated immediately after removal from warming devices or serving tables. More efficient cooling will usually occur if large walk-in type coolers are used instead of small refrigerators. The walk-in coolers have a greater capacity to dissipate heat and frequently have forced-air circulation. It must be kept in mind that meat, poultry, gravies, and meat casseroles cool slowly by conduction. Every possible effort, practical in an operation, should be made to cool foods rapidly. Techniques for rapid cooling of foods include putting containers of food into freezer compartments, packing containers in ice, immersing containers in running water, and putting stews, dressings, gravies, and meat stocks into shallow containers to induce more rapid heat transfer from the products *(10, 70)*. In each instance, af-

ter the temperature has been sufficiently lowered, food is put into refrigerators.

SUMMARY

Clostridium perfringens is a common, anaerobic, sporeforming organism that is likely to be a contaminant of foods. The foodborne illness that results from the consumption of large numbers of these organisms is associated with food-service operations where large portions of meat are prepared. Control lies in the prevention of spore germination and multiplication of vegetative cells.

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INFLUENCE OF CONDITIONS OF REHYDRATION ON THE ENUMERATION OF BACTERIA FROM FREEZE-DEHYDRATED MODEL FOOD SYSTEMS[1]

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ABSTRACT

Model systems, consisting of solutions of 2% gelatin, 2% gelatin and 6% glucose, and 9% skimmilk were inoculated with cultures of *Pseudomonas, Escherichia coli, Serratia marcescens, Alcaligenes faecalis, Arthrobacter globiformis* and *Microbacterium lacticum*. Viable counts of rehydrated freeze-dehydrated samples usually were highest in skimmilk. Reductions in viable count after rehydration with peptone or tryptone solutions were somewhat smaller than with distilled water. In general, the number of survivors was highest when rehydration was at 25 C with a volume of medium equal to that removed during freeze-dehydration. Rate of rehydration had a marked effect on the number of surviving bacteria. Highest numbers were found when rehydrated at a rate of 10^{-1} mg/sec/mg dry material.

Various studies have shown that many bacterial species present in foods survive freeze-dehydration and rehydration *(2, 9, 17)*. An enumeration of the surviving population by the agar plate method can be useful to evaluate the sanitary quality of these foods. The significance of this bacterial index, however, depends upon the degree of quantitative recovery of the surviving cells. During freeze-dehydration, the microbial population of a food is subjected to freezing, heating, and dehydration. Each of these treatments may cause injury to a proportion of the microbial population *(1, 4, 10, 11, 16, 21)*. Injured survivors are frequently more demanding in their nutritional requirements. Several reports *(4, 10, 11, 12, 21)* indicate that injured cells of several bacterial species could grow on complex media such as trypticase soy agar, nutrient agar, or Plate Count agar but could not develop on a minimal agar medium. Iandolo and Ordal *(5)* reported that sublethal thermal injury to *Staphylococcus aureus* increased sensitivity of the cells to salt and extended the lag phase upon inoculation of a heated suspension in trypticase soy broth. Salt tolerance could be regained in a medium which would not support growth. During thermal injury a marked degradation of ribosomal ribonucleic acid (rRNA) occurred, with subsequent resynthesis in the recovery period *(19)*.

Injured cells then may require somewhat different conditions for resumption of optimal growth than non-treated cells. The quantitative recovery of a microbial population in a freeze-dehydrated food therefore may depend greatly upon the conditions chosen for enumeration. In this connection, the following conditions should be considered, *(a)* rehydration of the food (type of rehydration medium, rate of rehydration, temperature during rehydration, and volume of rehydration medium), *(b)* dilution of the rehydrated food prior to plating (composition and temperature of the diluent and time spent in diluent), and *(c)* conditions related to the composition of the recovery growth medium and time and temperature of plate incubation. This study was conducted to determine the effect of conditions related to rehydration on the recovery of various bacterial species from freeze-dehydrated model food systems.

MATERIALS AND METHODS

Cultures

Pseudomonas fluorescens 13525, *Escherichia coli* 11775, *Alcaligenes faecalis* 8750, *Serratia marcescens* 13880, *Microbacterium lacticum* 8180, and *Arthrobacter globiformis* 8010 were obtained from the American Type Culture Collection. Six other cultures (2, 63, F-01, F-11, FL-E and M-21) from the stock culture collection were *Pseudomonas* sp. isolated from various samples of milk and milk products. The cultures were maintained and carried on Plate Count agar slants (Difco). Prior to each trial, they were grown for two transfers in nutrient broth at 25 C for 24 hr.

Preparation of freeze-dehydrated model systems

Model systems consisted of solutions of 2% gelatin (Difco), 2% gelatin with 6% glucose, and skimmilk. Skimmilk was prepared by reconstitution of 9% low-heat nonfat dry milk solids in distilled water. These solutions were sterilized at 121 C for 15 min prior to being inoculated. Unless stated otherwise, glass-distilled water was used to prepare these systems.

Following inoculation, the model systems (2 ml) were placed into pre-weighed sterile cups made from heavy duty aluminum foil. Cups were 20 mm in height and 15 mm in diameter. Weight of samples was determined by weighing the cup and contents. Aluminum cups then were placed in sterile petri dishes and held at −20 C for 2 hr. Freeze-dehydration was performed in an Industrial Dynamics pilot plant Model CPF-20 freeze-dryer. The platen temperature was kept at 37.7 C for 8 hr. The chamber pressure was

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TABLE 1. EFFECT OF FREEZE-DEHYDRATION ON THE NUMBER OF SURVIVING BACTERIA IN REHYDRATED MODEL SYSTEMS[a]

Species	Model system[b]	Viable count per ml before freezing	Log reduction after rehydration
P. fluorescens	A	6.6 x 10^7	5.44
	B		2.14
	C		1.36
E. coli	A	2.3 x 10^7	1.91
	B		0.44
	C		0.28
S. marcescens	A	7.0 x 10^7	2.81
	B		1.32
	C		0.37
A. faecalis	A	7.6 x 10^7	1.48
	B		1.00
	C		0.88
A. globiformis	A	3.3 x 10^6	>4.52
	B		>4.52
	C		1.10
M. lacticum	A	3.4 x 10^7	2.10
	B		0.38
	C		0.11

[a]Rehydration was with a volume of distilled water equal to that removed during freeze-dehydration; it was added in one portion at 25 C.

[b]A = 2% gelatin, B = 2% gelatin and 6% glucose, C = skimmilk.

0.1 mm Hg. Dehydration rates were established by weighing the samples at various intervals during the dehydration cycle. The cycle was ended when the residual moisture was below 3% of that initially present. Following dehydration the samples were placed in a desiccator.

Rehydration of samples

Unless stated otherwise, freeze-dehydrated samples were rehydrated with sterile distilled water at 25 C. Rehydration temperatures were maintained with water baths. Rates of rehydration were controlled by manual operation of sterile 2-ml syringes. In some experiments solutions of 1% peptone (Difco) and 1% tryptone (Difco) were used as rehydration media. All rehydration media were sterilized by autoclaving.

Viable counts

The number of viable cells before freezing and after freeze-dehydration was determined by pour-plating appropriate dilutions in trypticase soy agar (BBL) supplemented with 0.5% (w/v) yeast extract (Difco). The plates were incubated at 32 C for 48 hr.

RESULTS AND DISCUSSION

The number of bacterial survivors in the rehydrated samples was highest in skimmilk and lowest in 2% gelatin (Table 1). Similar results were obtained with six other cultures of *Pseudomonas*. Within model systems, large variations in number of bacterial survivors were found between cultures of *Pseudomonas*. The reductions in viable count of *Pseudomonas* species after freeze-dehydration and rehydration were larger than with other bacterial species, except for *A. globiformis*. Sinskey et al. *(18)* could not recover survivors of *Pseudomonas fragi* from freeze-dehydrated gelatin and gelatin-glucose systems. The platen temperature during freeze-dehydration in their study, however, was considerably higher (49 to 71 C). In addition, cultural differences may also be involved.

Although the number of model systems is limited, data indicate that composition of the material in which bacteria are freeze-dehydrated influences survival. Sinskey et al. *(18)* made a similar observation. Survival of several bacterial species was higher in egg melange than on beef. They also noted that addition of glucose to a gelatin system increased survival of some species.

Addition of peptone or tryptone to the rehydration medium for the gelatin-glucose systems increased the number of survivors of *P. fluorescens* and *S. marcescens*. This increase in log of viable count after rehydration ranged from 0.15 to 1.75. Little if any effect was noticed with cultures of *A. faecalis* and *M. lacticum*. Various investigators *(6, 7, 15, 20)* have used peptone water as diluent to reduce the destructive effect on bacteria of either distilled water or phosphate buffer. With respect to freeze-dehydrated foods, Pablo et al. *(13)* reported that freeze-dehydrated cooked shrimp rehydrated with trypticase diluent had a higher total count than samples rehydrated with distilled water. This difference in count

TABLE 2. EFFECT OF TEMPERATURE OF REHYDRATION MEDIUM ON THE NUMBER OF SURVIVING BACTERIA IN REHYDRATED FREEZE-DEHYDRATED MODEL SYSTEMS[a]

Species	Rehydration temp (C)	Viable count per ml before freezing	Log reduction after rehydration	
			Model B[b]	Model C[b]
P. fluorescens	3	2.3 x 10^7	3.13	3.50
	25		1.90	1.61
	37		3.39	2.28
S. marcescens	3	3.2 x 10^7	0.79	0.67
	25		0.36	0.25
	37		0.64	0.08
A. faecalis	3	1.1 x 10^7	0.89	0.89
	25		0.34	0.18
	37		1.23	0.11
M. lacticum	3	5.1 x 10^7	0.83	0.81
	25		0.88	0.80
	37		0.85	0.90

[a]The volume of distilled water added was equal to that removed during freeze-dehydration; it was added in one portion.

[b]See Table 1.

TABLE 3. EFFECT OF RATE OF REHYDRATION ON THE NUMBER OF SURVIVING BACTERIA[a]

Species	Rate of rehydration[b]	Viable count per ml before freezing	Log reduction after rehydration	
			Model B[c]	Model C[c]
P. fluorescens	I	3.5 x 10^5	>4.54	4.00
	II		2.76	3.26
	III		>4.54	>4.54
S. marcescens	I	1.2 x 10^8	1.96	1.80
	II		1.09	1.29
	III		1.38	1.90
A. faecalis	I	1.1 x 10^7	1.45	1.96
	II		0.15	0.89
	III		1.29	1.41
M. lacticum	I	1.1 x 10^6	0.13	0.13
	II		−0.07	0.04
	III		0.20	0.06

[a]The samples were rehydrated at 25 C with a volume of distilled water equal to that removed during freeze-dehydration.

[b]I = added in one portion, II = 10^{-1} mg/sec/mg dry material, III = 10^{-3} mg/sec/mg dry material.

[c]See Table 1.

was small initially and increased on storage of the rehydrated product at 4 or 20 C. With freeze-dehydrated chicken (diced, cooked) distilled water was as effective or slightly better than trypticase diluent *(14)*. In contrast, Sinskey et al. *(18)* reported that the addition of trypticase to the rehydration medium did not increase the plate count of freeze-dehydrated raw shrimp. Differences in product and/or processing techniques may have been responsible for the differences in behavior to the rehydration media.

Temperature of the rehydration medium within the limits of these experiments (3 to 37 C) had little if any effect on the number of survivors of *M. lacticum* (Table 2). With the other species, the number of survivors in both model systems was larger with rehydration at 25 C than at 3 C. Rehydration of the gelatin-glucose model systems at 37 C resulted in a smaller number of survivors of *P. fluorescens, S. marcescens,* and *A. faecalis* as compared with rehydration at 25 C. Similar results were found in skimmilk for *P. fluorescens.* However, with *S. marcescens* and *A. faecalis* in skimmilk, rehydration at 37 C resulted in a greater number of survivors than at either 25 or 3 C. Leach and Scott *(8)* also reported differences in the response of various bacterial species to temperature of the rehydration medium. Rehydration of *E. coli, Staphylococcus aureus,* and *S. marcescens* at 0 C gave lower viable counts than at 11, 22, or 37 C. Rehydration of *E. coli* at 37 C resulted in lower counts than at 22 C. With *S. marcescens,* however, higher counts were found with rehydration at 37 than at 22 C. With *Vibrio metschnikovi* rehydration at 37 C resulted in lower counts than at 0, 11, or 22 C.

Large reductions in viability of young cultures of *Pseudomonas aeruginosa* when diluted in cold diluents were reported by Gorrill and McNeil *(3)*. It is possible that in this study, similar factors may have caused the reduction in viable counts observed at 3 C with 3 of the 4 species. On the other hand, the bacterial cells in this study were freeze-dehydrated prior to rehydration which undoubtedly caused some damage to the cells. Pablo et al. *(14)* reported that temperatures of the rehydration medium (20 vs 37 C) did not affect the initial plate count of rehydrated freeze-dehydrated chicken. It is difficult to compare the results obtained with a mixed bacterial flora such as present on rehydrated chicken with those obtained with pure cultures.

The data in Table 3 indicate that the number of surviving bacteria was highest with rehydration at a rate of 10^{-1} mg water/sec/mg dry material. Similar results were reported for *V. metschnikovi (8). M. lacticum* seemed least affected by changes in the rate of rehydration. The present data and those reported by Leach and Scott *(8)* suggest that differences can be expected in the response of various bacterial species to changes in the rate of rehydration.

In general, the number of survivors was highest when the volume of rehydration medium was equal

TABLE 4. EFFECT OF VOLUME OF REHYDRATION MEDIUM ON THE NUMBER OF SURVIVING BACTERIA IN FREEZE-DEHYDRATED MODEL SYSTEMS[a]

Species	Volume of rehydration medium[b]	Viable count per ml before freezing	Log reduction after rehydration	
			Model B[c]	Model C[c]
P. fluorescens	1	1.4 x 10^8	2.67	2.55
	10		3.79	2.61
	50		3.45	—
S. marcescens	1	1.4 x 10^8	1.07	1.37
	10		1.39	1.64
	50		1.39	1.63
A. faecalis	1	9.6 x 10^7	1.37	1.21
	10		1.05	1.25
	50		1.22	1.41
M. lacticum	1	2.0 x 10^7	0.31	0.02
	10		0.79	0.10
	50		1.37	0.15

[a]Rehydration was at 25 C with distilled water, added in one portion.

[b]1, 10, 50 = 1X, 10X, 50X Volume of water removed during freeze-dehydration.

[c]See Table 1.

to that removed during freeze-dehydration (Table 4). Leach and Scott *(8)* reported that increasing the volume for rehydration of *V. metschnikovi* from 1 to about 80 times decreased the number of survivors by factors of 100 to more than 1000. On the other hand, *Pasteurella multocida, Salmonella oranienburg, Salmonella newport, E. coli, P. fluorescens, S. marcescens, Bacillus subtilis, Bacillus stearothermophilus, Lactobacillus casei, Streptococcus lactis* and *S. aureus* were not affected by these differences in the volume of medium used for rehydration.

If optimal recovery of the surviving population of a freeze-dehydrated food is desired, conditions of rehydration must be suitable for retaining structural and functional integrity of cells. The present paper provides data which show that the number of apparent surviving bacteria in rehydrated freeze-dehydrated model systems depends upon composition of the material in which the bacteria are freeze-dehydrated, composition, temperature and volume of the rehydration medium, and rate of rehydration. Although the present data are limited to a few bacterial species, the number of bacterial survivors was generally highest when the model systems were rehydrated at 25 C, with a volume equal to that removed during freeze-dehydration and at a rate of 10^{-1} mg water/sec/mg dry material. In this study, the factors related to rehydration were treated separately. In practice, however, it can be expected that interaction exists between the various factors which influence the number of bacterial survivors in freeze-dehydrated foods. This is illustrated by the differences in response of cells in various model systems to variations in temperature of the rehydration medium. In subsequent work, the effect of various conditions related to the recovery growth medium on the bacterial survivors of freeze-dehydrated foods will be investigated.

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ESTIMATION OF EXTRANEOUS MATTER IN MILK, CREAM, AND BUTTER

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ABSTRACT

Methods for the estimation of amounts of extraneous matter (or sediment) in milk, cream, and butter are discussed and methods suitable for use in routine examinations of these products are suggested. Proposed for estimations in milk, is a mixed sample method in which a 16 fluid oz sample is filtered through an area of 0.40-inch diameter. A guide having four discs of this diameter with 0.05, 0.15, 0.20, and 0.30 mg of standard sediment is preferred for scoring these test discs. For estimations in cream and butter, samples of 100 ml and 45 g, respectively, are dispersed in hot water (or, for cream, in hot sodium citrate solution) and filtered through an area of 0.25-inch diameter. A guide having discs of this diameter with 0.10, 0.15, 0.25, and 0.35 mg of standard sediment is proposed for scoring cream and butter test discs.

The term "extraneous matter" as applied to dairy products might include any substance which is not in milk drawn from a cow and which is not deliberately added, as salt and coloring substances in butter. However, in this paper the term will refer only to material which, collected on a filter paper, is visible to the eye. This normally includes a variety of substances of which some, on standing, tend to settle in milk and cream and some remain suspended indefinitely. Often the shorter term "sediment" is used to refer to all visible extraneous matter and, in this sense, it may be considered a synonym for "extraneous matter".

Methods for the isolation and estimation of amounts of extraneous matter *(1, 2, 6)* depend on the filtration of a sample, diluted when necessary, through a suitable Lintine cotton filter pad. Generally the filtering device is funnel-shaped and terminates in a restricted orifice which rests directly on the filter pad. For some purposes, particularly for off-the-bottom milk tests, a device is used which consists of a cylinder and piston so arranged that on the up-stroke of the piston the desired sample is drawn past the filter pad into the cylinder, and on the down stroke of the piston the sample is forced through the filter pad. All the results reported in this paper were obtained using funnel type equipment with suction. Satisfactory suction can be provided by a Venturi attachment on a water tap or, if this is not available, a filtering unit can be constructed from milking machine parts (Fig. 1). Variations in sampling, sample size, and filter area in a number of published methods are indicated in Table 1.

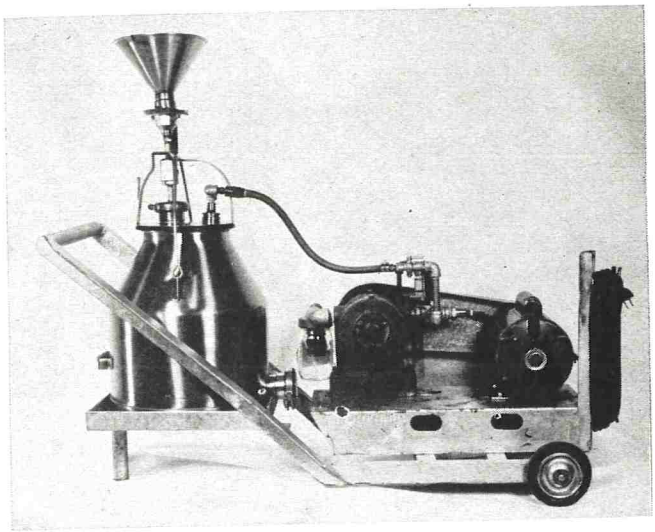

Figure 1. Filtering unit constructed from milking machine parts.

Included in each of the methods is a procedure for the preparation of standard discs to serve as the basis for estimating amounts of sediment on test discs. However, for routine testing it is practical to use photographs of standard discs. Although photograph-

TABLE 1. VARIATIONS IN SEDIMENT TEST METHODS

Product analyzed	Nature of sample	Sample size	Filter diameter (inches)	Method (literature reference)
Milk	Off-bottom	16 fl oz	1.125	*1, 2, 6*
		128 fl oz	1.125	*1, 2, 6*
		32 fl oz	0.64	*6*
			1.125	*6*
	Mixed	16 fl oz	0.44	*6*
			0.40	*1, 2*
Cream		16 fl oz	1.125	*1, 6*
		2 fl oz	1.125	*6*
Butter		1/2 lb	2.75	*1, 6*
			1.125	*6*
		4 oz	2.75	*6*

ic copies of some standards are available it may be preferable for various reasons to prepare and use standard discs that differ from those available. Obviously, discs may be prepared with any amount of sediment and thus the steps in a sediment rating scale can be made exactly as desired. The set of standard discs can then be photographed to provide copies for use where and when they are required.

Either of two methods may be used when scoring test discs. First, the test discs can be scored as equivalent to the nearest matching discs on the guide. This is the method commonly used in Alberta and the results presented in this paper are reported on this basis. Second, test discs can be scored either as "exceeds disc—on the guide" or "less than disc—on the guide". This method has the advantage of increasing the scale divisions, providing, in effect, for plus and minus values about each established point.

Any method for the estimation of amounts of extraneous matter must satisfactorily reflect differences in amounts of sediment in different samples. In addition, amounts having special significance, such as the maximum acceptable amount, or the amount below which a premium is earned, should be readily and clearly indicated. After this, to be suitable for routine use, a method should be economical and convenient. Because the method chosen for milk differed appreciably from that for cream and butter, our consideration of the two will be described separately.

METHODS AND RESULTS

Extraneous matter in milk

Because most milk is now being handled in bulk and because, as several workers have pointed out *(4, 5)*, off-the-bottom methods of sediment testing are not appropriate for bulk milk, in this investigation attention was given to a mixed sample test and to guides suitable for use with this test.

Of the methods described in Table 1, it appeared practical to use samples of 16 fluid oz with a filter area of 0.40-inch diameter. Although it is reported *(5)* that sediment discs of smaller size can satisfactorily be compared with standard discs of 1.125-inch diameter, it was considered preferable that the standard discs should be of the same size as the test discs. At first, test discs were compared with the USDA Sediment Standards for Milk and Milk Products, 7 CFR 58.2731, which offers three standard discs of 0.40-inch diameter: *(a)* 0.0625 mg (0.50 mg equivalent), *(b)* 0.1875 mg (1.50 mg equivalent), and *(c)* 0.3125 mg (2.50 mg equivalent).

However, preliminary tests with this guide indicated that Disc *(b)* represented a particularly important range of values. Scoring to the nearest matching

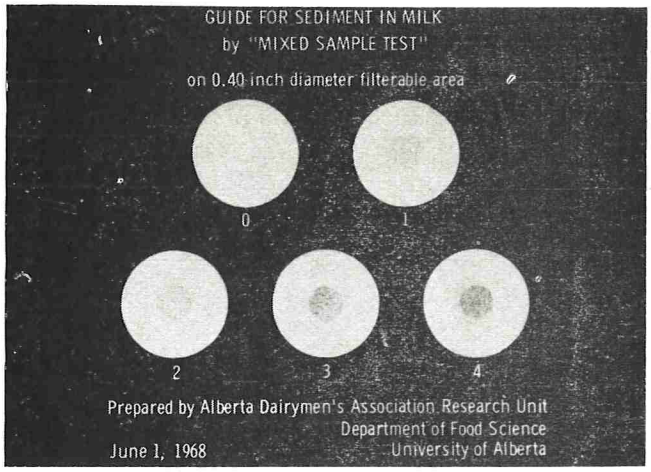

Figure 2. Guide for rating milk sediment discs.

disc, Score 2 would be given to all samples having from 0.12 to 0.25 mg of sediment per 16 fluid oz. Because quality improvement through this range may require special attention, it was considered desirable to provide greater discrimination in this range of the scale. Accordingly, the guide shown in Fig. 2 was prepared with five discs representing the following amounts (mg) of a sediment mixture: 0.00, 0.05, 0.15, 0.20, and 0.30. For simplification of the guide, the discs are identified only by the numbers 0, 1, 2, 3, and 4. The sediment mixture used in making the discs was prepared from Malmo silty clay loam (95%) and manure (5%). The Malmo silty clay loam contains about 6% organic mater and has a (Munsell) color of 10YR 3/1 which is described as very dark gray. Mechanical analysis of the soil shows 46% clay (less than 0.002 mm), 48% silt (0.002-0.05 mm) and 6% sand (greater than 0.05 mm). The manure was the dried and sieved (35 mesh) product obtained from grass-fed animals.

To obtain an indication of the distribution of milk samples when scored with this guide, a number of tests were conducted. Sixteen-ounce samples were obtained from the bulk tanks of 46 milk shippers (22 shipping to a fluid milk plant and 24 to a manufacturing milk plant). The samples, warmed to 90 F, were filtered through a 0.40-inch diameter area. The discs were scored according to the Guide giving the results shown in Table 2.

It will be observed that one-half of the samples received Score 1 representing less than 0.10 mg of sediment per 16 fluid oz, and 83% received Score 1 or 2 representing less than 0.175 mg of sediment per 16 fluid oz. If Score 2 were made the maximum acceptable for a premium price, quality improvement would be demanded for 17% of the milk sampled. If a Score 3 were the maximum acceptable for delivery, 2% of the milk sampled would be unacceptable.

TABLE 2. DISTRIBUTION OF SCORES IN BULK MILK SAMPLES USING THE GUIDE FOR SEDIMENT IN MILK

Score	Mean amount of extraneous matter represented by score (mg/16 fl oz)	Number of samples	Per cent of samples	Accumulative	
				Number	Per cent
1	0.05	23	50	23	50
2	0.15	15	33	38	83
3	0.20	7	15	45	98
4	0.30	1	2	46	100
		46	100		

Extraneous matter in cream and butter

The methods which have been described for cream and butter *(1, 2, 6)* were considered either to require so large a sample as to be excessively costly in routine use, or to distribute the extraneous matter over so large an area as to allow readily for qualitative but not quantitative assessments. Therefore another method was needed for routine quantitative estimates of extraneous matter.

For butter, the method devised by the Dairy Products Division of the Canada Department of Agriculture appeared suitable *(7)*. A 45 g sample of butter was dispersed in 200 ml hot water and filtered through an area of 0.25-inch diameter on discs of 0.5 inch diameter.

For cream, consideration was given to the use of a 0.40-inch diameter filter area. However, samples of approximately 8 fluid oz would be required to give adequate amounts of sediment for satisfactory grading on a disc of this size. Such a sample was considered undersirably large for routine testing. Therefore attention was shifted to the use of a filter area of 0.25-inch diameter, and tests showed that discs of this diameter could be used with 100 ml samples of cream.

Because no standard discs of 0.25-inch diameter were available for scoring test discs of this diameter, it was decided a set should be prepared. In the first guide *(3)*, filter discs were shown representing mixtures of the Malmo silty clay loam and manure in progressively increasing amounts to 0.75 mg. Based on suggestions from the Alberta dairy industry, a second guide (Fig. 3) was prepared with 5 discs representing 0.0, 0.10, 0.15, 0.25, and 0.35 mg of this soil and manure mixture. To simplify the guide for use in routine testing, the discs are numbered simply 0, 1, 2, 3, and 4.

To obtain an indication of the distribution of cream samples when scored with this guide, a number of tests were conducted. Samples of 100 ml each were obtained from 112 cans of cream received at dairy plants. The samples were diluted with an equal volume of hot 4% sodium citrate solution and filtered through a circular area of 0.25-inch diameter. The discs were scored according to the Guide and the results are shown in Table 3.

It will be noticed that approximately one-third of the samples received Score 1 (representing less than 0.125 mg of sediment per 100 ml). Two-thirds of the samples received Score 1 or 2 (representing less than 0.20 mg of sediment per 100 ml). Scores 3 and 4 were assigned to approximately 17 and 15% of the samples respectively. In tests where sediment was measured in cream and in butter made from the cream, it was observed that Score 2 represented the maximum level of extraneous matter in cream that was compatible with the production of butter of the quality (unofficially) rated acceptable by the Canada Department of Agriculture. It appears therefore that, at this time, Score 2, (approximately 0.15 mg extraneous matter per 100 ml), offers a reasonable standard for the maximum acceptable amount of extraneous matter in farm-separated cream.

TABLE 3. DISTRIBUTION OF SCORES IN CREAM SAMPLES USING THE GUIDE FOR SEDIMENT IN CREAM AND BUTTER

Score	Mean amount of ext aneous matter represented by score (mg/100 ml)	Number of samples	Per cent of samples	Accumulative	
				Number	Per cent
1	0.10	38	33.9	38	33.9
2	0.15	38	33.9	76	67.8
3	0.25	19	16.9	95	84.7
4	0.35	17	15.2	112	99.9
		112	100.0		

If such a standard were established, Scores 3 and 4 in the Guide would also be useful. These categories might be expected initially to account for approximately one-third of the samples and, by permitting a system of graded incentives, they could help and encourage a program of cream quality improvement.

A small fraction (5 to 10%) of the cream samples examined have been difficult to filter. In some instances it was found helpful to dilute the sample with a hot 10% sodium citrate solution but even this did not make it possible to filter all samples. For the routine examination of cream samples it would be desirable to have a means of treating each sample to ensure that it could be filtered.

The Guide shown in Fig. 3 also serves satisfactorily for scoring butter sediment discs. Although there exist in Canada no official standards for sediment in butter, the Canada Department of Agriculture has

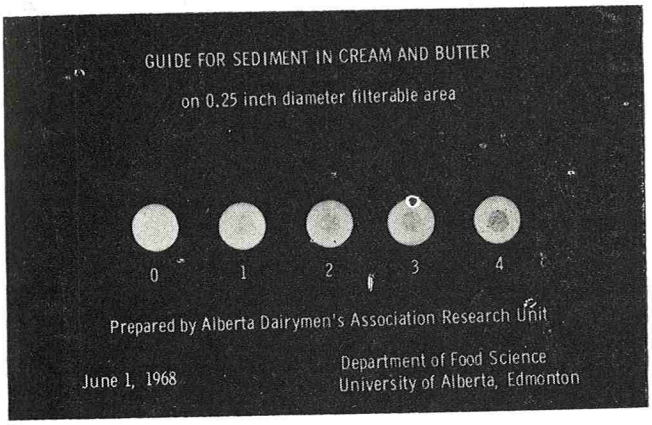

Figure 3. Guide for rating cream and butter sediment discs.

unofficially used a four point scale to indicate what may be considered acceptable and unacceptable amounts of the normal types of sediment from 45 g of butter when it is filtered through an area of 0.25-inch diameter. The two ranges of acceptable amounts of sediment compare with Score 1 on the Guide while two ranges of unacceptable amounts compare with Scores 2, 3, and 4 on the Guide. The Guide may therefore serve suitably as a standard for extraneous matter in butter. Scores 2, 3, and 4, representing various unacceptable amounts of sediment, offer incentives and indicators of improvement in quality.

Satisfactory equipment is available for filtering through an area of 0.40-inch diameter and the same funnels can be fitted with filter plugs of 0.25-inch diameter. It is also possible to provide exchangeable 0.40- and 0.25-inch filter plugs for use with the same funnel.

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A COMPARISON OF DIRECT PLATING AND ENRICHMENT METHODS FOR DETECTION AND ENUMERATION OF COAGULASE-POSITIVE STAPHYLOCOCCI IN FROZEN FEEDS OF ANIMAL ORIGIN[1]

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Abstract

Direct surface plating on five selective and differential agar media and enrichment prior to plating were compared for detection and enumeration of coagulase-positive staphylococci in frozen feeds. The enrichment medium was trypticase soy broth (TSB) fortified with 8, 10, and 12% sodium chloride. In general, direct plating on mannitol salt (MS) and staphylococcus 110 (S110) agars was accompanied by a higher recovery of coagulase-positive staphylococci than was obtained with tellurite polymyxin egg yolk (TPEY), Vogel-Johnson (VJ), and tellurite glycine (TG) agars. Suspected colonies picked from TPEY agar were most likely, and from TG agar were least likely, to be coagulase-positive staphylococci.

Enrichment for 24 hr in TSB containing 8 or 10% sodium chloride yielded more staphylococci than did direct plating on TPEY or TG agars. Numbers obtained by the two procedures usually were more nearly equal when MS or S110 agars were used for direct plating. Enrichment in TSB containing 12% sodium chloride or incubation of broths for 48 instead of 24 hr, even if they contained 8 or 10% sodium chloride, were detrimental to the recovery of coagulase-positive staphylococci. Of the plating media tested, MS, TG, and VJ agars regularly and uniformly recovered coagulase-positive staphylococci from enrichment broths inoculated with highest dilutions of test samples. In contrast to this, erratic results were obtained when S110 and TPEY agars were used to recover these bacteria from the same broth cultures.

Tests on feed-grade frozen meat by-product revealed the presence of 10^3 to 10^4 coagulase-positive staphylococci per gram in two samples and 10^4 to 10^5 per gram in two other samples. Examination of feed-grade frozen liver indicated the presence of 10^3 to 10^4 coagulase-positive staphylococci in one sample and 10^4 to 10^5 per gram in two samples.

Feed-grade frozen meat by-product and liver are incorporated into the diets of mink as sources of protein. Chou and Marth *(6)* examined these feeds and found them to be heavily contaminated with a variety of microorganisms including pathogens such as salmonellae and coagulase-positive staphylococci. Their initial observations on content of coagulase-positive staphylococci in these feeds were based on the use of the surface plating technique and mannitol salt agar *(5)*.

Other media have been suggested for isolation of coagulase-positive staphylococci from foods and other materials. Included are staphylococcus 110 medium *(4)*, tellurite glycine agar *(14)*, Vogel-Johnson agar *(9)*, and tellurite polymyxin egg yolk agar *(8)*. Although still other agar media have been used by different investigators, those just mentioned are readily available from commercial sources.

Use of an enrichment procedure prior to plating also has been suggested for recovery of coagulase-positive staphylococci from naturally contaminated materials *(11)*. Although investigators have published conflicting reports on the efficacy of the enrichment procedure *(3, 10, 13)*, inoculation of trypticase soy broth containing 9.5% added sodium chloride followed by streaking the incubated broth culture on Vogel-Johnson agar has been recommended and accepted as the official method for isolating coagulase-positive staphylococci from foods *(1, 2)*.

The present investigation was conducted to: *(a)* determine numbers of coagulase-positive staphylococci recovered from frozen feeds by five different commercially available agar media intended for growth of these organisms, *(b)* determine the efficiency with which the agar media recover staphylococci from a mixed population as occurs in the frozen feeds, *(c)* compare recovery of staphylococci by trypticase soy broth with 8, 10, and 12% added sodium chloride with that obtained by direct plating, and *(d)* compare the efficiency of the five agar media for recovery of coagulase-positive staphylococci from the enrichment broth.

Materials and Methods

Sampling of products

Samples of feed-grade frozen liver and meat by-product, naturally contaminated with staphylococci, were taken aseptically into sterile jars. They were transported to the labora-

[1]Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

tory immediately and were held frozen until the time of analysis.

Plating media and enrichment broth

Plating media used were commercially available (Difco) dehydrated mannitol salt agar (MS), staphylococcus medium 110 (S110), tellurite glycine agar (TG), Vogel-Johnson agar (VJ), and tellurite polymyxin egg yolk agar (TPEY). They were prepared according to the manufacturer's directions. Plates were poured in the conventional manner and were held partially opened at room temperature for 3 to 4 hr to reduce surface moisture.

The enrichment medium used was commercially available trypticase soy broth fortified with 8, 10, and 12% (w/v) sodium chloride.

Procedure for direct plating and enrichment

An 11 g portion of each sample tested was thawed in a water bath at 30 C for aproximately 10 min and then was transferred to a sterile Waring blendor jar to which was added 99 ml of sterile phosphate-buffered distilled water. This suspension was blended and further decimal dilutions were made in sterile phosphate-buffered distilled water.

Direct plating. Plates poured with media described above were inoculated with 0.1 ml of each decimal dilution of product tested. The inoculum was distributed over the agar surface with a sterile, bent, glass, streaking rod. Inoculated plates of TPEY and TG agars were incubated at 37 C for 24 hr, whereas those of VJ, MS, and S110 agars were incubated at 37 C for 48 hr. Plates were then examined and colonies which were typical of those formed by staphylococci were counted. Some typical colonies were picked from each agar medium and inoculated into brain heart infusion broth which was then incubated at 37 C for 18 to 24 hr before performing the coagulase test. The number of coagulase-positive staphylococci was calculated by multiplying the number of colonies counted by the percentage of colonies which proved to be coagulase-positive. This value was then multiplied by the appropriate dilution factor.

Enrichment. One tube each of trypticase soy broth with 8, 10, and 12% added sodium chloride was inoculated with 1 ml from each decimal dilution of the samples. Tubes were incubated at 37 C for 48 hr. After 24 and 48 hr of incubation, 0.1 ml inoculum from each tube was streaked onto the surfaces of the five plating media described above. After incubation was complete, colonies typical of those produced by staphylococci were picked from each agar medium and inoculated into brain heart infusion broth for later use in the coagulase test.

Coagulase test

Coagulase plasma (Difco) was reconstituted according to the manufacturer's directions. One-half milliliter of the plasma solution was transferred to a Kahn tube and 2 drops of a 16 to 24 hr test culture grown in brain heart infusion broth was added. The plasma was incubated at 37 C for 4 hr and examined periodically for clot formation. Presence of a clot was regarded as a positive reaction.

RESULTS

Direct plating

The number of staphylococci recovered from frozen feeds by direct plating is recorded in Tables 1a, 1b, and 2. Inspection of the data reveals that three of the four samples of meat by-product tested (Tables 1a and 1b) contained less than 7,000 coagulase-positive staphylococci per gram regardless of the plating medium employed, whereas more than 15,000 of these bacteria per gram were found in one sample when tested with four of the five plating media. These results suggest the presence of a somewhat lower level of contamination than was previously observed by Chou and Marth *(6)* who reported that 6 of the 15 samples of meat by-product contained more than 11,000 coagulase-positive staphylococci per gram when tested with MS agar.

Highest numbers of coagulase-positive staphylococci were always recovered from the frozen meat by-product when MS and S110 agars were used. Lowest numbers of these bacteria were most often obtained with VJ or TG agars. In some instances, low numbers also accompanied the use of TPEY agar.

Results obtained from similar tests on feed-grade frozen liver (Table 2) were somewhat different from those just described. The number of coagulase-positive staphylococci in one sample ranged from 20,000 to 110,000 per gram, depending on the medium which was used. Variation in recovery of staphylococci was also rather marked for the other two samples. Data on numbers of coagulase-positive staphylococci in this product are in good agreement with the earlier observations of Chou and Marth *(6)* when they noted that 6 of 15 samples of feed-grade frozen liver contained more than 11,000 of these bacteria per gram. Samples of frozen liver in this study tended to contain somewhat higher numbers of coagulase-positive staphylococci than did the frozen meat by-product.

As was noted in tests on frozen meat by-product, MS and S110 agars again yielded high numbers of staphylococci, but this time VJ agar recovered highest numbers from two of the three samples. Lowest recovery was associated with the use of TPEY and TG agars; the former gave the lowest yield in two tests and the latter in one trial.

Efficiency of plating media

Colonies with an appearance typical of coagulase-positive staphylococci were picked from each agar medium that was inoculated directly with the frozen feeds and were tested for coagulase activity. Results of these tests are summarized in Table 3 and provide a guide to the efficiency with which each medium recovered coagulase-positive staphylococci from the frozen products.

Examination of the data reveals that TPEY agar was least likely to permit growth of organisms other than coagulase-positive staphylococci since 100% of the colonies picked from this medium proved to be that organism, regardless of feed product tested. The second most efficient medium was MS agar when meat by-product was tested (also over-all) and VJ agar when the test product was liver. The least ef-

TABLE 1A. RECOVERY OF COAGULASE-POSITIVE STAPHYLOCOCCI FROM FEED-GRADE FROZEN MEAT BY-PRODUCT BY DIRECT PLATING AND ENRICHMENT PROCEDURES[a]

Sample	Plating medium	Direct plating (No./g)	Enrichment for 24 and 48 hr in trypticase soy broth (TSB) followed by plating: Dilutions of sample in TSB with 8% NaCl, 10⁻³ 24 hr	10⁻³ 48 hr	8% NaCl, 10⁻⁴ 24 hr	8% NaCl, 10⁻⁴ 48 hr	Dilutions of sample in TSB with 10% NaCl, 10⁻³ 24 hr	10⁻³ 48 hr	10% NaCl, 10⁻⁴ 24 hr	10% NaCl, 10⁻⁴ 48 hr	Dilutions of sample in TSB with 12% NaCl, 10⁻³ 24 hr	10⁻³ 48 hr	12% NaCl, 10⁻⁴ 24 hr	10⁻⁴ 48 hr
1	MS[b]	3,800	+[g]	+	+	+	+	+	+	+	+	+	−	−
	S110[c]	2,200	+	+	+	+	+	+	+	+	+	+	−	−
	TG[d]	550	+	+	+	+	+	+	+	+	+	+	−	−
	VJ[e]	1,900	+	+	+	+	+	+	+	+	+	+	−	−
	TPEY[f]	2,100	+	+	+	+	+	+	−	−	+	+	−	−
2	MS	32,000	+	+	+	−	+	+	+	−	+	+	−	−
	S110	30,000	+	+	+	−	+	+	−	−	+	+	−	−
	TG	23,000	+	+	+	−	+	+	+	−	+	+	−	−
	VJ	17,000	+	+	+	−	+	+	+	−	+	+	−	−
	TPEY	6,700	+	−[h]	−	−	+	+	−	−	+	+	−	−

[a]No coagulase-positive staphylococci recovered with any plating medium from any broths inoculated with 1:1000,000 dilution of sample.
[b]Mannitol salt agar
[c]Staphylococcus 110 agar
[d]Tellurite glycine agar
[e]Vogel-Johnson agar
[f]Tellurite polymyxin egg yolk agar
[g]Plus sign signifies coagulase-positive staphylococci were recovered.
[h]Minus sign signifies coagulase-positive staphylococci were not recovered.

TABLE 1B. RECOVERY OF COAGULASE-POSITIVE STAPHYLOCOCCI FROM FEED-GRADE FROZEN MEAT BY-PRODUCT BY DIRECT PLATING AND ENRICHMENT PROCEDURES[a]

Sample	Plating medium	Direct plating (No./g)	Enrichment for 24 and 48 hr in trypticase soy broth (TSB) followed by plating: Dilutions of sample in TSB with 8% NaCl, 10⁻² 24 hr	10⁻² 48 hr	8% NaCl, 10⁻³ 24 hr	8% NaCl, 10⁻³ 48 hr	Dilutions of sample in TSB with 10% NaCl, 10⁻² 24 hr	10⁻² 48 hr	10% NaCl, 10⁻³ 24 hr	10% NaCl, 10⁻³ 48 hr	Dilutions of sample in TSB with 12% NaCl, 10⁻² 24 hr	10⁻² 48 hr	12% NaCl, 10⁻³ 24 hr	10⁻³ 48 hr
3	MS	1,900	+[b]	+	+	+	+	+	+	+	+	+	−	−
	S110	2,000	+	+	+	+	+	+	−	−	+	+	−	−
	TG	580	+	+	+	+	+	+	+	+	+	+	−	−
	VJ	410	+	+	+	+	+	+	+	+	+	+	−	−
	TPEY	1,000	+	−[c]	−	−	−	−	−	−	+	−	−	−
4	MS	4,200	+	+	+	+	+	+	+	+	+	+	+	−
	S110	6,600	+	+	+	+	+	+	+	−	+	+	+	−
	TG	1,800	+	+	+	+	+	+	+	+	+	+	+	−
	VJ	3,300	+	+	+	+	+	+	+	+	+	+	+	−
	TPEY	2,300	+	+	−	−	+	+	−	−	+	+	−	−

[a]No coagulase-positive staphylococci recovered with any plating media from any broths inoculated with 1:10,000 dilution of sample.
[b]Plus sign signified coagulase-positive staphylococci were recovered.
[c]Minus sign signifies coagulase-positive staphylococci were not recovered.

ficient of the agar media tested was TG agar since only 34.4% of the "typical" colonies picked from it were coagulase-positive staphylococci.

Even though TPEY agar was most effective in selecting for staphylococci, it failed to produce highest numbers of these bacteria when inoculated with the frozen feeds (Tables 1a, 1b, and 2). The high degree of selectivity may have made this medium unsuitable for the growth of some cells which were damaged during freezing and thawing, whereas the other media (except for TG agar) allowed some of these bacteria to grow. It is interesting to note that

TG agar is low in both sensitivity (efficiency) and productivity, thus making it the least suitable of the media tested for determining numbers of coagulase-positive staphylococci in frozen feeds of animal origin.

Enrichment and plating

A similar or higher number of coagulase-positive staphylococci was generally recovered from meat by-product with the enrichment rather than the direct plating technique provided that: *(a)* the trypticase soy broth was fortified with either 8 or 10 but not 12% sodium chloride, *(b)* incubation of the enrichment broth was for 24 rather than 48 hr, and *(c)* TPEY and S110 agars were not used to recover staphylococci from the enrichment broths (Tables 1a and 1b.)

Extending incubation of enrichment cultures to 48 hr was accompanied by failure to detect staphylococci at the 10^{-4} dilution of two samples when 24 hr earlier, the bacteria were recovered from this same culture by either three or four of the plating media. An increase from 10 to 12% in sodium chloride content was nearly always accompanied by failure to detect staphylococci at the higher dilution of inoculum. An exception was noted when four of the five agar media recovered staphylococci from broth containing 12% sodium chloride which was inoculated with meat by-product sample number 4 and incubated for 24 hr.

Maximum reliability for detection of staphylococci in enrichment cultures was associated with MS, TG, and VJ agars, whereas TPEY agar proved to be least reliable. Generally, S110 agar recovered staphylococci from enrichment cultures of meat by-product, although noteworthy exceptions occurred with samples 2 and 3.

Use of the enrichment technique on samples 1 and 2 of meat by-product suggests they contained between 10,000 and 100,000 coagulase-positive staphylococci per gram. This value is somewhat higher than that found by direct plating of one sample but is in good agreement with results when the other sample was plated on MS, S110, TG, or VJ agars. Data obtained from samples 3 and 4 indicate that in these instances there was reasonably good agreement in number of staphylococci recovered by the two techniques.

Examination of data obtained from tests on frozen liver (Table 2) reveals that S110 and TPEY agars again were least reliable in recovering staphylococci and that use of 12% sodium chloride inhibited staphylococci so that less were detected than when broth contained either 8 or 10% sodium chloride or, in one instance, when direct plating was done.

TABLE 2. RECOVERY OF COAGULASE-POSITIVE STAPHYLOCOCCI FROM FEED-GRADE FROZEN LIVER BY DIRECT PLATING AND ENRICHMENT PROCEDURES[a]

			Enrichment for 24 and 48 hr in trypticase soy broth (TSB) followed by plating															
			Dilutions of sample in TSB with 8% NaCl						Dilutions of sample in TSB with 10% NaCl						Dilutions of sample in TSB with 12% NaCl			
			10^{-2}		10^{-3}		10^{-4}		10^{-2}		10^{-3}		10^{-4}		10^{-2}		10^{-3}		10^{-4}	
Sample	Plating medium	Direct plating (No./g)	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
1	MS	110,000	+[b]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−
	S110	95,000	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−
	TG	81,000	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−
	VJ	31,000	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−
	TPEY	20,000	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−
2	MS	15,000	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−
	S110	14,000	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	−	−
	TG	3,900	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−
	VJ	35,000	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−
	TPEY	8,200	−[c]	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−
3	MS	3,900	+	+	+	+	−	−	+	+	+	+	−	−	+	+	+	+	−	−
	S110	6,500	−	−	−	−	−	−	−	+	−	−	−	−	+	−	−	−	−	−
	TG	4,300	+	+	+	+	−	−	+	+	+	+	−	−	+	+	+	+	−	−
	VJ	11,000	+	+	+	+	−	−	+	+	+	+	−	−	+	+	+	+	−	−
	TPEY	<300	−	−	−	−	−	−	+	−	−	−	−	−	+	+	−	−	−	−

[a]No coagulase-positive staphylococci recovered with any plating medium from any broths inoculated with 1:100,000 dilution of sample.

[b]Plus sign signifies coagulase-positive staphylococci were recovered.

[c]Minus sign signifies coagulase-positive staphylococci were not recovered.

TABLE 3. EFFICIENCY OF DIFFERENT MEDIA IN RECOVERY OF COAGULASE-POSITIVE STAPHYLOCOCCI BY DIRECT PLATING OF FEED-GRADE FROZEN MEAT BY-PRODUCT AND LIVER

Product	Plating medium	No. colonies picked	Coagulase + staphylococcus colonies	
			Number	Per cent
Meat by-product	MS	34	29	85.3
	S110	33	21	63.6
	TG	31	14	45.1
	VJ	32	21	65.6
	TPEY	28	28	100.0
Liver	MS	30	18	60.0
	S110	30	18	60.0
	TG	30	7	23.3
	VJ	30	22	73.3
	TPEY	20	20	100.0
Both products	MS	64	47	73.4
	S110	63	39	61.9
	TG	61	21	34.4
	VJ	62	43	69.3
	TPEY	48	48	100.0

Data obtained by the enrichment procedure on two samples of frozen liver suggest that they contained between 10,000 and 100,000 coagulase-positive staphylococci per gram. This is in reasonable agreement with values obtained by direct plating of the samples. Similar good agreement was obtained with the third sample although the number of staphylococci was lower.

DISCUSSION

In general, media containing tellurite, such as TPEY, TG, and VJ agars, recovered fewer coagulase-positive staphylococci from frozen feeds than did MS and S110 agars. A similar observation was made by McDivitt and Hussemann *(12)* when they compared MS, S110, and Ludlam's agars for recovery of coagulase-positive staphylococci from ham. Ludlam's agar, which contains potassium tellurite, recovered less than 10% of the staphylococci which were detected by MS and S110 agars.

Crisley et al. *(8)* reported that the type of food being tested influences the efficiency of selective and differential media in detection and enumeration of coagulase-positive staphylococci. Results of these studies, in part, bear out the view of Crisley et al. The frequency with which "typical" colonies proved to be coagulase-positive staphylococci varied markedly between meat by-product and liver when four of the five agar media were used (Table 3).

Although Carter *(3)* reported that direct plating was as efficient as selective enrichment prior to plating, Gilden et al. *(10)* found a great difference in frequency with which coagulase-positive staphylococci were recovered from frozen foods by the two procedures. In one trial on 160 samples of various frozen foods, direct plating on S110 agar served to recover staphylococci from 26.2% of the samples, whereas 93.8% of the samples were found to contain the bacteria when tested with the enrichment procedure (TSB with 9.5% added sodium chloride). In the present studies greater recovery of staphylococci with the enrichment procedure was also observed and especially when TG or TPEY agars served as the direct plating media. When direct plating was done with MS and S110 agars, differences in numbers recovered by the two procedures tended to be minimal.

Several broth media have been suggested for use in the enrichment procedure. Baer et al. *(2)* tested cooked meat and TSB broths each with 9.5% added sodium chloride and a sorbic acid broth. They concluded that the TSB broth with 9.5% added sodium chloride was most satisfactory for regular use. In these experiments only TSB was used but the amount of added salt and the incubation times were varied. Trypticase soy broth with 8 or 10% added sodium chloride appeared to be equally satisfactory, but the addition of 12% sodium chloride created a medium which seemed to be detrimental to some coagulase-positive staphylococci and thus resulted in reduced recovery. The incubation time for enrichment cultures appears to be important when this procedure is used. Although the official method suggests an incubation of 45 to 48 hr at 37 C, results of these tests suggest that greater recovery of coagulase-positive staphylococci may be achieved if the incubation does not exceed 24 hr.

The agar medium used to recover staphylococci from the enrichment broth can also affect the sensitivity of the test. In one test Baer et al. *(2)* found superior recovery with VJ rather than S110 agar. Another trial yielded nearly equal recovery with these two media and markedly inferior recovery with a modified TG agar. Results of the present investigation are essentially in agreement with those of Baer et al. *(2)*. These tests indicated that MS and VJ agars and sometimes S110 agar were superior to TG and TPEY agars for recovery of coagulase-positive staphylococci from TSB enrichment broths.

ACKNOWLEDGMENT

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MASTITIS CAN BE CONTROLLED . . . . IF[1]

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Sanitarians and fieldmen are on the dairy farm to observe milking procedures and techniques more than any other individuals associated with the dairy industry. Poor milking procedures and mastitis cost dairymen approximately $15-30 per cow per year, not including the costly problems resulting from mastitis encountered by processing plants. Everyone wishing to serve the dairy industry properly, must do his part in helping to stamp out this disease.

We do not have the knowledge needed to eliminate mastitis, but we do know enough to control it. Proof of this can be readily obtained by comparing herds relatively free of mastitis with infected herds. Usually, these herds stay in the same category, only a few of them jump back and forth.

Poor management, sloppy milking techniques, or improperly operating machines are usually the culprits. Occasionally we run into a situation that does not fall into these categories, but it is usually the exception and not the rule.

When a herd has a continuous mastitis problem, there is a reason for it. Excessive and prolonged treatment may pull the dairyman out of trouble temporarily, *but if the predisposing cause of his trouble is not corrected,* he will fall right back into trouble again. This is where Sanitarians and Fieldmen come in. It is their responsibility to know enough about mastitis and the predisposing causes of the disease to offer the dairyman a service.

Many derogatory "letters to the editor" (Editor's note: But not to the *Journal of Milk and Food Technology.*) have been written in the past criticizing the national abnormal milk program. But, it has been my experience that these critics were unable to analyze individual screening or bacteriological tests and incapable of performing a complete milking machine analysis to make the proper recommendations that would improve the situation.

Control The Problem

As was stated before, we cannot eliminate all mastitis completely, because it is impossible to eradicate most of the causative organisms. However, if we understand how these organisms are transmitted from cow to cow by improper udder preparation, flooding of milking machines that are operating improperly, general insanitary and hazardous conditions around the barn, we can keep most of our dairymen out of trouble. One man cannot attack the problem

[1]Presented at the 1969 Conference of Fieldmen and Sanitarians, Mammoth Cave National Park, Kentucky, February 25, 1969.

alone. When a herd has a high leucocyte (white blood cell) count, the sanitarian or dairy plant fieldman should work with the dairyman and have a complete milking machine analysis performed and have each individual quarter checked by a veterinarian. Before extensive treatment and culling are carried out, it is necessary to make sure the machines are operating properly and the cow preparation is correct.

Many dairymen balk at the expense incurred, but this approach usually increases incomes. A recent study in Georgia showed that it cost dairymen approximately $1.50 per cow to get out of trouble. The extra milk which resulted meant that the dairymen had their money back in less than 6 months.

SCREENING TESTS

Screening tests on bulk milk samples are a good indication of the mastitis level in a herd. However, it must be remembered that Wisconsin Mastitis Test results vary with the age of milk. Unpublished research results from North Carolina State University indicate that milk which is 45-55 hr old will score approximately 50% lower with the Wisconsin Mastitis Test than when the same milk was 12 hr old. Dairymen who are on every-other-day pick-up should *strive* to produce milk with less than 500,000 leucocytes in the bulk sample, whereas dairymen on every-day pick-up can produce milk with a little higher count and remain out of trouble.

In addition to conducting screening tests on bulk tank samples, all dairymen should use a cow-side test such as the California Mastitis Test (CMT) once a month. Cows scoring 2 or 3 on the CMT are robbing the milk check of added profits. Recent research at Louisiana State University showed that quarters scoring a 2 or a 3 produced 26-46% less milk than corresponding negative quarters. The data is summarized below in Table 1.

CHECK EQUIPMENT

If the job is to be accomplished, sanitarians or fieldmen must be on the dairy farm at milking time. This is a must in order to observe milking procedures and to check the milking equipment. Remember, any milking equipment will perform properly when no cows are being milked. The time to check milking machines is when the cows are being milked with all machines operating.

Space does not permit going into details on vacuum pumps, pipeline sizes, or normal and abnormal leucocyte counts on individual cows, but as a starter there are 18 facts listed below that should be familiar to everyone working in this area.

TABLE 1. INFLUENCE OF SUBCLINICAL MASTITIS ON MILK PRODUCTION AND QUALITY BY COMPARING NEGATIVE CMT QUARTERS WITH CORRESPONDING TRACE, 1, 2, AND 3 SCORING QUARTERS

CMT Score	Decrease in Milk Production (%)	Decrease in Butterfat (%)	Decrease in SNF (%)
Negative	0	0	0
T	3	3	1
1	11	7	3
2	26	10	6
3	46	14	11

1. *Udder infection*: The invasion of the udder cavity by microorganisms that multiply within the gland and cause inflammation.

2. *Subclinical mastitis*: A form of mastitis in which there is no swelling of the gland or gross abnormality of milk, although there are changes in milk that can be detected by special tests.

3. *Clinical mastitis*: A form of mastitis in which the abnormal conditions of the udder and secretion are observable. Mild forms of mastitis may involve changes in the milk such as flakes, clots, and a watery or unusual appearance with signs of swelling, heat, and sensitiveness of the udder being slight or absent. Severe forms of mastitis involve a sudden onset with swelling of the infected quarter, which is hot, hard, and sensitive. The secretion appears abnormal and milk production drops. A systemic reaction may also develop with signs of fever, rapid pulse, depression, weakness, and loss of appetite. The first condition is sometimes referred to as acute local mastitis and the latter as acute systemic mastitis.

4. *Chronic mastitis*: This form is caused by a persistent udder infection that exists most of the time in the nonclinical form but can, occasionally, develop into an active clinical form. Following these flare-ups, there is usually a return to the nonclinical form.

5. *Udder*: Cows with low hanging udders are more susceptible to infection and clinical mastitis. The supporting ligaments of the udder are not in proportion with udder size, thus large udders break down faster.

6. *Teats*: Large teats may become injured easier than smaller teats. Once a teat becomes damaged, that quarter is hard to save. It is not yet clear how mastitis-producing bacteria penetrate the small lactiferous duct at the teat end.

7. *Age*: Some studies indicate that the prevalence of infection with *Streptococcus agalactiae* is likely to increase with increasing age. The same is apparently true of clinical mastitis, but great variation may be encountered.

8. *Milk production*: The evidence has been inconclusive with any possible effects being overshadowed by other factors.

9. *Stage of lactation*: Two-thirds to ¾ of all infection occurs when the cows are in production, the rest occurs during the dry period or before freshening as a heifer.

10. *Hormones*: Estrogenic compounds have been surmised to bring about clinical cases of mastitis. Also, they may affect the bacterial properties of the lining of the teat duct.

11. *Heredity*: At present, there is insufficient evidence that selective breeding for resistance to mastitis could be acomplished to a degree that it would have major significance.

12. *Season*: There is no conclusive evidence to indicate that the season *per se* influences the incidence of udder infections and mastitis. However, the incidence of mastitis is

higher during the first month of lactation and the early dry period than any other time.

13. *Feeds and feeding*: The possible role of feed in mastitis is still a rather confused issue. The feeding of high levels of grain or special components of grain rations have not been demonstrated to exert any marked influence on mastitis.

14. *Housing*: Two factors relating to housing, which are frequently considered as contributing to the problem of mastitis, are udder and teat injuries and improper ventilation. However, little is known about the effect of stress on mastitis resistance.

15. *Milking machine*: In many herds, the improper use of milking machines and the use of faulty equipment has been considered the cause of mastitis problems. Some of the conclusions have been based on presumptive evidence and have not always been substantiated in the laboratory.

16. *Milking act*: The best way to milk a cow is by machine, but overmilking—no matter how—contributes to the mastitis problem.

17. *Pulsator ratio*: There are machines on the market with milk to rest ratios of 40:60 to 70:30 and with pulsator rates of 45-72 pulsation per minute.

18. Any milking machine equipment that does the following, is considered good equipment:

a. Maintains a stable vacuum in the teat cup and at a level adequate for completely milking most udders in 3-5 min.

b. Does not stress the tissues of the teat by excessive stretching and ballooning.

c. Produces massage without harsh action.

d. Is designed so that the entire system can be sanitized efficiently and satisfactorily.

THE DAIRYMAN'S PLIGHT IN MODERN AGRICULTURE[1]

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The American consumers may still be getting milk from contented cows, but it is doubtful if they are getting it from contented dairymen. Dairymen figure they have been getting milked for about all they are worth about as often as their cows are milked.

FOOD COSTS

Americans are eating better than ever and spending only 17.6% of their disposable income for food—the lowest in history. The consumer now spends less than a nickle of every quarter of his disposable income for food. Of this amount, the farmer gets less than two cents. Now let us compare this with food costs in other countries: in England—30%, France—31%, Italy—45% and in Russia—53%. Less than 6% of the American people produce the food and fiber for the entire population of our country. This is the reason why America is a great country and also the reason that we have the highest standard of living any people have ever known.

LOSS OF DAIRY FARMS

In 1964 when I came to North Carolina there were 2,750 Grade "A" dairies, however, in 1954 there were 5,189 and now in 1969 we are down to 2,200 Grade "A" dairies and we are losing between three and four more dairy farms each week.

TABLE 1. SIZE OF HERD AND RETURN TO MANAGEMENT, 110 NORTH CAROLINA DAIRY FARMS, 1968

Cows per farm			
Range	Average	Number of farms	Return to management
Less than 50	40	42	$ 444
50-80	61	43	766
81 or more	118	25	7,425

Now let us look at North Carolina Dairy Farm Business Records and see the average of 110 North Carolina Grade "A" Dairy Farms. The average dairyman has a total investment of $150,659 and 66 milking cows and an investment of $2,407 per cow.

Table 1 illustrates the relationship between size of business and return to management. On the average, as size of herd increased from less than 50 cows to 81 cows or more, return to management increased from $444 to $7,425. The high profit farms averaged $15,171 return to management.

In general, larger businesses make larger net incomes. However, some dairy farms with small herds make larger incomes than some farms with larger herds. Return to management varied within each size-of-herd group and the larger the herd, the greater the variation (Table 2). About 40% of the small farms failed to make a positive return to management, but no losses were larger than minus $7,500. The highest return to management in the group was $10,000 made on a 48-cow farm that sold about

[1]Presented at the Regional Meeting of the National Mastitis Council, Louisville, Kentucky, August 18, 1969.

TABLE 2. VARIATION IN RETURN TO MANAGEMENT, BY SIZE OF HERD, 110 NORTH CAROLINA DAIRY FARMS, 1968

Return to management	Number of cows per farm		
	Less than 50	50-80	81 or more
	— — — Per cent of farms — — —		
Below —$15,000	--	--	4
—$15,000 to —$10,001	--	7	4
—$10,000 to —$5,001	14	14	12
—$5,000 to 0	24	12	8
0 to $4,999	50	44	12
$5,000 to $9,999	10	14	20
$10,000 to $14,999	2	7	12
$15,000 to $19,999	--	--	4
$20,000 or more	--	2	24
Total	100	100	100

12,700 lb of milk per cow. In the 50-80 cow group, 33% of the farms operated at a loss, but 23% made a return to management of $5,000 or more. On farms with 81 or more cows, profits were scattered over a wider range. Losses of $10,000 or more were sustained by about 10% of the farms. In contrast, 60% made profits of at least $5,000. About 25% made a return to management of $20,000 or more.

Size of business has a "multiplier" effect on income. When a profit is realized per hundred weight of milk sold, size increases total profits. Conversely, when a loss per unit of milk sold occurs, large size swells total losses. Therefore, it should be kept in mind that a large size business is no guarantee of success. The addition of more cows, land, labor, and capital puts a premium on management if success is to be obtained. For many North Carolina dairymen, doing "better" with existing farm resources is a more feasible alternative than getting "bigger."

DAIRYMEN IN FINANCIAL TROUBLE

Now after looking at the records we can say that 50% of our dairymen are in financial trouble. The average dairyman has $36,654 debt and there is no way humanly possible for the dairyman to justify 8.5 to 12% interest rates and improve his position. Our best dairyman can barely make 10% profit from his operation and this includes his return for labor, management, and capital investment. President Nixon in announcing Dr. Hardin's appointment said, "the new Secretary, . . . instead of speaking for the President to the farmers, would recognize that it was his responsibility to speak for the farmers to the President." Now it is time to tell the American people that the government and farmers have been subsidizing the consumers with cheap food and it is impossible for the farmers to get increased incomes and not increase the cost of food. We have simply heard too much of this type of reasoning in Washington. The only reason the farmer is in this situation is that he is unorganized and consequently he has no bargaining power. What milk producers have really needed is not simply easier ways to produce milk, as we have had milk pouring out at times like it came out of a broken faucet; what we need is some way to turn the handle open or shut as we please—producing only so much as could be sold for a good fair price. Today's dairyman is seeking bargaining power and he has finally realized that unless he becomes organized he cannot survive.

Our business records show us that our good dairymen are making money, but we apparently don't have enough of them. The dairyman that is in financial trouble is the same dairyman that has a serious mastitis or reproductive problem in his herd.

GOOD DAIRYMEN AND MASTITIS

I can honestly say that our good dairymen have very little trouble with mastitis. They can and always will have to work to keep it under control, but good management and sanitation along with a properly installed milking machine and a properly functioning machine can keep mastitis in check. It is up to the veterinarians, fieldmen, sanitarians, and milking machine service men working together as a team to help our dairyman overcome his problems. We must work together and give the dairyman the right answers or else this mass exodus in dairying will continue. I would like to close with *Lincoln's Ten Guidelines*:

> You cannot bring about prosperity by discouraging thrift.
>
> You cannot help small men by tearing down big men.
>
> You cannot strengthen the weak by weakening the strong.
>
> You cannot lift the wage earner by pulling down the wage payer.
>
> You cannot help the poor man by destroying the rich.
>
> You cannot keep out of trouble by spending more than your income.
>
> You cannot further brotherhood of man by inciting class hatred.
>
> You cannot establish security on borrowed money.
>
> You cannot build character and courage by taking away man's initiative and independence.
>
> You cannot help men permanently by doing for them what they could and should do for themselves.

REPORT OF THE EDITOR JOURNAL OF MILK AND FOOD TECHNOLOGY, 1968-1969

Review of Volume Thirty-One

Publication of the December, 1968 issue of the Journal completed volume 31. This volume contained 540 pages (including covers) and represents an increase of 28 pages over volume 30. The proportion of space in this volume devoted to research papers remained essentially unchanged from the previous volume. There was an increase in the amount of space utilized for publication of general interest papers of a technical nature and concurrent decreases in space devoted to general interest papers of a nontechnical nature and to News and Events. Association Affairs continued to occupy, percentage wise, the same amount of space as in the previous volume. The bulk of the 28 extra pages published in volume 31 was utilized by general interest technical papers with some extra pages devoted to research papers and to Association Affairs. The composition of volume 31 is more fully detailed in Table 1 together with similar information for volume 30.

Research papers in volume 31 again dealt with a wide variety of topics ranging from the quality of restaurant food to the direct microscopic somatic cell count. Technical general interest papers also provided information on many subjects ranging from ultra high-temperature pasteurization to salmonellae and staphylococci in nondairy foods. Subjects covered by nontechnical papers included sanitation problems in Austria, Australia, New Zealand, and India; future developments in the food industry; and other topics.

Table 1. Summary of contents of *Journal of Milk and Food Technology* for 1967 and 1968

Item	Volume 30 (1967)	Volume 31 (1968)
1. Total pages including covers	512	540
2. Research papers		
a. Number	30	32
b. Pages	137	142
c. Per cent of total pages	26.7	26.3
3. General interest papers-technical		
a. Number	11	16
b. Pages	47	74
c. Per cent of total pages	9.2	13.7
4. General interest papers-nontechnical		
a. Number	23	14
b. Pages	72	65
c. Per cent of total pages	14.1	12.0
5. Association affairs		
a. Pages	64	68
b. Per cent of total pages	12.5	12.6
6. News and events		
a. Pages	51	42
b. Per cent of total pages	9.9	7.8
Per cent of pages devoted to technical material	35.9	40.0
Per cent of pages devoted to nontechnical material	36.5	32.4
Per cent of pages devoted to covers, advertising, standards, index, etc.	27.6	27.6

Present Status of Volume Thirty-Two

A complete review of the first 6 issues of volume 32 will not be made at this time. There are a few items about these issues which should be mentioned. At this point 40 extra pages have been published in an attempt to keep the back log of papers to a reasonable number. The first 6 issues of this volume have carried a considerably greater number of research papers than was true of volume 31. Inspite of this, a fairly substantial amount of space has been devoted to both technical and nontechnical general interest papers. In comparison to volume 31, there has been some reduction in the amount of space devoted to Association Affairs and News and Events.

After the June, 1969 issue was published there was a backlog of 12 research papers and 16 technical and nontechnical papers. In addition 10 research papers were in some stage of the review process prior to acceptance for publication. Some of the papers awaiting publication originated at the 1968 IAMFES meeting but most, if not all, of these should be published by September or October of 1969. In view of this backlog, additional extra pages will be required before this volume is completed.

Editorial Board

The present editorial board consists of 18 specialists representing industrial, regulatory, and academic interests. Since last year, two new persons have joined the board. They are Dr. J. C. Olson, Jr., Director of the Microbiology Division of the Food and Drug Administration, Washington, D.C. and Professor Herman Koren, Department of Health and Safety, Indiana State University, Terre Haute.

Many members of the editorial board have been quite busy during the past year since all research papers are reviewed by at least two board members. In addition, the Editor has enlisted help from the following persons in the review of manuscripts: Dr. R. L. Bradley, Jr., Dr. O. R. Fennema, Dr. R. P. Niedermeier, Dr. N. F. Olson, Dr. D. S. Postle, Dr. W. D. Powrie, Dr. L. D. Satter, Dr. G. E. Shook, Dr. H. L. A. Tarr, and Dr. W. C. Winder.

Several members of the Editorial Board have been away from their offices for various periods of time. Dr. G. W. Reinbold was in Norway since August, 1968; Dr. E. A. Zottola was in Venezuela since March, 1969; and Dr. W. E. Sandine went to Canada in May, 1969. All of these scientists will be back in their offices by August or September, 1969. Their absence has meant that some of the other members of the Editorial Board worked harder this past year.

The Editor believes that several persons should be added to the Editorial Board to provide additional expertise. His recommendations have been made to the president.

Instructions To Contributors

In recent years the *Journal* has annually carried a page entitled "Instructions to Contributors." These instructions have been revised and expanded to include changes which have occurred in journal operations and to provide more detailed information to authors for their use in preparation of manuscripts. It is hoped that the revised instructions will appear in the August, 1969 issue of the journal.

E. H. Marth,
Editor
Journal of Milk and Food Technology

RAPID EVALUATION OF VIABLE CELL COUNTS BY USING THE MICROTITER SYSTEM AND MPN TECHNIQUE

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(Received for publication May 26, 1969)

ABSTRACT

A rapid procedure was developed for estimating viable cell counts in bacterial cultures by combining the Microtiter system and Most Probable Number technique. The correlation coefficient of the data between this method and the agar plate method was statistically significant at the 1% level. The advantage of this Microtiter-MPN procedure are savings of time, space, and material. Since the method covers a wide range of dilutions, estimates of cell concentration for proper dilutions are not needed in advance of enumeration procedures. Data are obtainable as early as 6 hr after samples are placed in incubation.

The agar plate method and most probable number (MPN) technique have been extensively used in evaluation of bacterial densities. These methods and their applications are described in detail in *Standard Methods for the Examination of Dairy Products (1).* Recently, a method combining the Microtiter system, developed by Takatsy and modified by Sever *(7)*, and the spot plate technique of McKinney et al. *(6)* was introduced by Fung and Kraft *(3)* for rapid determination of viable cell counts in bacterial cultures and in a turkey meat infusion. This procedure has been successfully used to evaluate spore survival after heat treatment by Baldock et al. *(2)* and viable cell counts in milk samples by Fung and LaGrange *(4)*. Our work describes a related rapid procedure employing the Microtiter system and MPN technique to determine viable cell counts; advantages are that results are obtainable in 6 to 12 hr with substantial savings of time, space, and material in operation.

MATERIALS AND METHODS

Bacterial cultures

Staphylococcus aureus S-6, *S. aureus* 100, *S. aureus* 137, *S. aureus* 196E (from M. S. Bergdoll, University of Wisconsin, Madison), *Salmonella typhimurium*, *S. heidelberg*, *S. anatum* 53, and *S. infantis* (from the Department of Food Technology, Iowa State University, Ames) were grown separately in sterile nutrient broth at 37 C for 8 hr before bacterial densities were evaluated. Also, viable cell counts of stored milk samples were estimated.

Microtiter equipment and serial dilutions

Equipment for the Microtiter system was obtained from the Cooke Engineering Co., Alexandria, Va. Presterilized plates with an arrangement of 8 by 12 wells of 0.35-ml capacity were used as vessels for dilution as well as for growth of bacteria (Fig. 1). Each well was filled with 0.225 ml of sterile nutrient broth dispensed by a sterile automatic pipetting machine (Becton, Dickinson and Company, Rutherford, N. J.) calibrated to deliver 0.225 ml of liquid. Three pretested loops were disinfected by dipping in alcohol and flaming and were used to deliver 0.025 ml each of the same bacterial sample (well-mixed) into the first well of each of three series of 8 wells. The first well then contained a 1:10 dilution of the original sample. The three loops were rotated rapidly 20 times in the first wells before the contents were drain-dried on a piece of blotting paper and again disinfected by alcohol and flaming. Although this step did not appear to influence results when milk samples were tested in earlier work *(4)*, it is a precaution that should be observed in routine analyses. Then the loops were reintroduced to the first wells and rotated for 20 times before being carefully placed into the second series of wells. Serial dilutions to the eighth row of wells then gave a 10^{-8} dilution of the original sample. The alcohol flaming procedure between each dilution is necessary because as little as a single cell contaminant or an accidental carryover from the previous dilution on the loop or the stem will cause erroneous results. Also, in order to minimize airborne contamination, dilutions were made with as little delay as possible. Twelve loops could be operated simultaneously by hand or by a commercially made micro-diluter to dilute four bacterial cultures in triplicate to a dilution of 10^{-8} in about 10 min. The Microtiter plate with diluted cultures was then covered with a dried sterile glass plate and

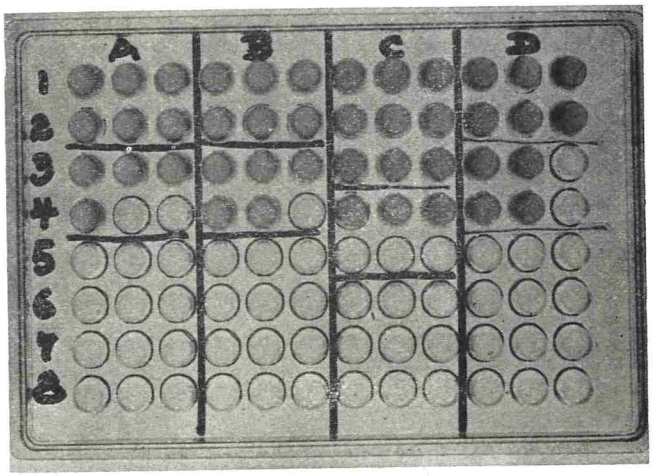

Figure 1. Microtiter plate-MPN evaluation. Turbidity of the wells indicates growth. MPN of sample A is obtained by multiplying 45 (from table 1; 3+/3, 1+/3) x 4 x 10^{4-2} or 1.8 x 10^4 organisms/ml.

[1]Present address: Department of Microbiology, Pennsylvania State University, State College, Pennsylvania 16802.

TABLE 1. MPN PER 0.25 ML OF SAMPLE[1]

Positive wells in 10^{-1} (0.025 ml)	Positive wells in 10^{-2} (0.0025 ml)	MPN/0.25 ml
0	0	0
0	1	3
0	2	6
0	3	10
1	0	4
1	1	7
1	2	12
1	3	16
2	0	9
2	1	15
2	2	20
2	3	30
3	0	25
3	1	45
3	2	110
3	3	250+

[1]Modified from McCrady; instead of 100 ml of sample, 0.25 ml was used.

incubated at 37 C overnight. Presumptive data could be obtained as early as 6 hr after incubation was initiated.

MPN computation

Turbidity of the broth in the wells was indicative of bacterial growth and was designated as positive (Fig. 1). Table 1, modified from McCrady *(5)*, shows the MPN of viable cells from all possible combinations of positive wells of the first 2 dilutions, each in 3 wells of the same sample. Since the original sample, before dilution, was 0.25 ml, the result was reported as MPN per 0.25 ml, or MPN x 4 per ml. When higher dilutions were involved, the MPN was computed from the last 2 dilutions showing positive wells and then adjusted to viable cell counts per ml by multiplying by 4 (to convert 0.25 ml to one ml) and 10^{n-2}, where n is the highest dilution showing a positive result. For example, the MPN of a result such as 3+/3, 3+/3, 1+/3, and 0+/3 of 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7}, respectively, is 45 (from Table 1) x 4 x 10^{6-2}, or 180 x 10^4 cells per ml of the original sample.

Agar plate count

Viable cell counts of the 8 known cultures and 4 milk samples were made by use of the Microtiter-MPN techniques as well as by the agar plate method. For the agar plate method, duplicate plates of each dilution were made by introducing 0.5 ml of a dilution onto the surface of dried nutrient (Difco) agar in a petri dish and spreading evenly with a sterile bent glass rod. The plates were incubated at 37 C for 24 hr before counts were made.

RESULTS AND DISCUSSION

The viable cell counts of the 12 samples obtained from the Microtiter-MPN technique and the conventional agar plate method are presented in Table 2. The results show close correlation between the data of the two methods with a correlation coefficient of 0.801, which was significant at the 1% level

This new procedure provides data comparable to the conventional agar plate method and has the following advantages over both the agar plate method and the conventional MPN method: *(a)* utilizes one Microtiter plate instead of many dilution bottles and test tubes; *(b)* utilizes one set of loops instead of many pipettes; *(c)* occupies a small area of operation and incubation; *(d)* saves time in obtaining data; and *(e)* eliminates dilution estimates for testing since this procedure covers the range from 16 to 4.4 x 10^8 organisms per ml of the original sample.

This procedure provides a potential rapid method to determine the viable cell count in milk, water, food, and in bacterial cultures.

ACKNOWLEDGMENTS

Journal Paper No. J-6266 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa, Project. No. 1749, Center for Agricultural and Economic Development cooperating. This investigation was supported in part by Public Health Service research grant UI 00263-03 from the National Center for Urban and Industrial Health and a Sigma Xi Grant-In-Aid research award to the senior author. We thank Dr. Paul Hartman for suggestions.

TABLE 2. COMPARISONS OF VIABLE CELL COUNTS[1]

Samples	Microtiter-MPN counts/ml 10^7	Agar plate counts/ml 10^7
Milk 1	1.8	4.8
Milk 2	10.0	13.0
Milk 3	0.2	0.6
Milk 4	10.0	52.0
S. *typhimurium*	0.1	0.3
S. *heidelberg*	1.0	0.9
S. *anatum* 53	1.8	2.4
S. *infantis*	1.8	1.1
S. *aureus* S-6	1.0	5.9
S. *aureus* 100	1.0	1.0
S. *aureus* 137	1.0	7.3
S. *aureus* 196 E	1.0	1.4

[1]Correlation coefficient of these methods is 0.801.

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PRESIDENTIAL ADDRESS, FIFTY-SIXTH ANNUAL MEETING, IAMFES

S. O. Noles

Florida Division of Health
Jacksonville, Florida

S. O. Noles giving the presidential address at the opening session.

It is with deep humility that I express to you my appreciation for permitting me to serve as your president during this past year. I also wish to express for all of us a hearty "thank you" to the Kentucky Association of Milk and Food Sanitarians for doing such an excellent job of handling the thousand and one details which are so necessary for a program to operate as smoothly as this one. Always, too, in the background is one beloved and ever-efficient executive secretary, "Red" Thomasson, who is so willing to respond beyond the "call of duty" because of his loyalty and devotion to our organization. To him we are doubly indebted for our continued success and prosperity.

Amalgamation of Sanitarians' Organizations

I know you are interested in the present status of negotiations concerning the joining together of the National Association of Sanitarians (NAS) and the International Association of Milk, Food, and Environmental Sanitarians, Inc., which would yield a new single organization.

You will probably recall from our last meeting that efforts came to somewhat of a standstill as a result of the action of an Executive Committee of NAS changing the proposed constitution and by-laws back to the original document under which that organization was operating. As a result of Nicholas Pohlitt, executive secretary, meeting with our Board and his requesting that IAMFES keep an "open-door" policy toward blending the two into one, with your approval, two things were done. First, an ad hoc committee, consisting of Dick Whitehead, Dr. W. C. Lawton, and Milton Held was appointed to work with a similar Committee of NAS if and when they proposed a constitution and by-laws similar to the one which had been developed by former committees of both organizations . Second, Dr. A. L. Myhr wrote Roger Lewis, then president of NAS, informing him of the action taken by us and asking him to contact us when they were ready. Mr. Lewis did not answer our letter.

In May at the National Conference on Interstate Milk Shipments held in Denver, Dick Whitehead, Dr. W. C. Lawton, and H. L. Thomasson met with Nicholas Pohlitt at his request. During this meeting he was again assured that our "door" is still open for further negotiations. Since the Educational Conference of NAS in June, we have had no further word from anyone in their organization. We are still waiting!

Possibly, it is time for us to reassess our whole thinking concerning such an amalgamation. We should possibly back up a ways, start from some other point and gradually progress toward the ideal through some procedures such as:

(a) Designate certain members of our association to approach the other groups and learn if they would be interested in designating specific members of their associations, all of which could work together, as inter-society committees, to determine common objectives in which we all have mutual interests.

(b) When it is determined that two or more organizations have committees working on similar problems, arrange for methods of communication between such committees so that each may benefit from the efforts and results of the other.

(c) When practically identical activities are being pursued wherein a common goal is sought, efforts of these

individuals working together could well result in a much more far-reaching benefit to all concerned than could be achieved by these individuals working separately.

By using these approaches toward common objectives, tendencies toward professional differences could be eliminated. And through such efforts, resulting in greater cooperation and better understanding of each other by members of all organizations, the natural hesitancy to become associated in one large group could possibly be resolved. Certainly, we should consider carefully any possibility which could lead to greater benefits to members of all organizations in respect to the monumental tasks which face all sanitarians.

OUR OBJECTIVES

In all our work we must pause occasionally and evaluate our efforts. Just what and how much have we accomplished? Life itself is an adventure of experiences. It contains risks, hazards, frustrations, and compensations. The more years that pass, the more clearly do we realize that we make this journey only once. We do not have a round-trip ticket. We see people who are able to remain "young" for many years, while others wear out in early life. Longevity is desirable *only* if it increases our list of worthy accomplishments. The important thing, then, is *not* the number of years that we live, but rather *what* and how much we are able to accomplish in the time we are permitted to live.

You are an important individual. You are living for a *purpose*. The role you play may not always be great. You may not often make the "headlines." The idea to keep in mind is that you *are* an individual, set apart from all others, and only *you* can make your particular contribution.

Time is a precious ingredient. Bernard Berenson, a world-famous art historian and humanist, states that the secret of his achievements—and happiness—is the fact that he has "no time to spare." On his 91st birthday he stated that he was so engrossed in "work in progress" that he wanted to stand on the street corner and beg the idle passers-by for the hours and minutes they were wasting. The happiest people, the ones who accomplish the most, are the ones who have "no time to spare."

OUR PHILOSOPHY OF LIFE

Each of us has formulated a philosophy of life, whether or not we have written it down. This is expressed in your consistent personal attitudes, your beliefs, your principles of conduct. It constitutes the foundation of your plan for daily living. It is expressed in your attitudes and responses toward problems which arise and in your relations with other people. It should often be re-examined and improved upon as you advance through life.

Each of us, to a great extent, must be "on his own" in grappling with the issues which arise in our lives. Often we find ourselves bewildered and discouraged. But such conditions should be looked upon as challenges. Daniel Boone was once asked if he had ever been lost in the woods, to which he replied: "No, I never got lost, but I was bewildered once for three days." Thirty years ago I attempted to express my inner thoughts in the form of a sonnet with the following words:

Oh, Night, what secrets are hidden in thy fold,
Enshrouded with darkness, mysterious gloom—
Thy stillness broken
Only by denizens of indeterminate source?
What kind of spell has thou upon my soul
That drives me restlessly on and on
Seeking for I know not what?
Weary at last, I see the dawn of another day
Approaching to break thy spell—
Thy mysteries are gone with thee.
Oh, Life, what holdest thou for me
In realms beyond today—
Shall I be sad because I lived to see
The morning follow night until the end;
Or shall I be zestful, clinging on
To thee with all my might
Until the time when I shall be no more?
And at last shall I be glad to give thee up,
Realizing that thy mysteries unfold
In Death, as night into the day?

The years which have passed have provided some of the answers.

WORKING WITH OTHERS

We all realize that in order to accomplish desirable objectives in our work we must avail ourselves with the necessary technical knowledge. Your presence at this meeting attests to this fact. In addition, we must *ever* keep in mind that we are *working with people*. In this we are indeed fortunate. To attain the greatest success possible from this venture, we must continuously strive to understand ourselves *and* those with whom we are working. Assimilating a few basic facts to be used as guideposts, such as the following, will be a great help:

(a) We must perform the work in which we are involved to the very best of our ability.
(b) We must realize that we occupy a very responsible position, embodying a trust.
(c) We *must* avoid irresponsible gossip and criticism of our fellow workers.
(d) We must *not* infringe upon the rights of others in order to gain personal objectives.
(e) We must be courteous and considerate at *all* times toward other people.
(f) We must learn to appreciate the other fellow's

point of view.

(g) *Do not use profanity.* It is dirty and does not fit in with good "sanitation!"

(h) We must constantly be aware that each of us is an integral part of a vitally important endeavor, which has as its foundation the fulfillment of many of the great needs of humanity.

Yes, a careful analysis will help in our everyday accomplishments and in our efforts to bring about a closer relationship with those of other organizations who have similar objectives. Virgil A. Mitchell summarized it all very effectively when he said: "Life is filled with broken hopes, frustrated desires and keen disappointments. We make our plans, set our sails, and chart our voyage. We do not always reach the harbor toward which we aim. None of us is immune from such experiences. The important thing is *not* what happens to us, *but* our *attitude toward* what happens. Our responses will determine our happiness, usefulness, character and destiny."

One final thought: We do not live all of life in one day. It is the total sum of all our days. *But* we should live *each* day as completely as if it were the last one we have left on earth. And *one* day we shall be correct! Each day when you arise, make a solemn resolve to make some person happier than he was when he started his day. If you accomplish *this*, *your* rewards will be rich indeed.

FIFTY-SIXTH ANNUAL MEETING OF IAMFES

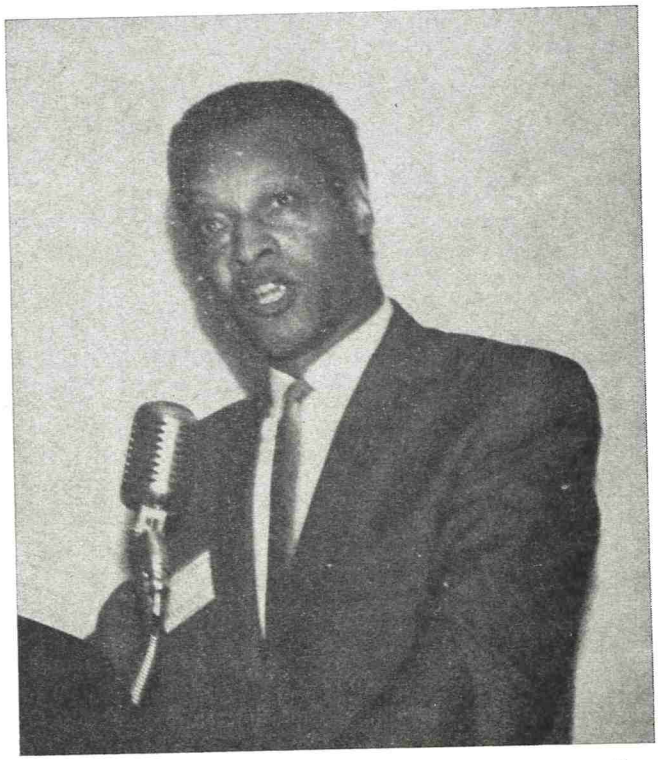
Louisville, Kentucky
August 17-21, 1969

Wendell P. Ford, Lieutenant Governor of Kentucky, welcomes IAMFES members and guests at the opening session.

More than 350 members and guests attended the fifty-sixth annual IAMFES meeting at the Brown Hotel in Louisville, Kentucky. While at the meeting they attended committee meetings, listened to papers on a wide variety of topics, participated in discussion sessions, heard reports at the annual meeting, visited hospitality rooms, and attended the annual awards banquet. The meeting was hosted by the Kentucky Association of Milk, Food, and Environmental Sanitarians.

EXECUTIVE BOARD MEETINGS

Sessions of the IAMFES Executive Board began on Sunday afternoon. The Board heard a report



C. C. Johnson, Jr., Administrator of the Consumer Protection and Environmental Health Services, addresses the opening session.

Robert A. Shaw of the Prudential Life Insurance Company addresses the opening session on working with people to achieve objectives.

from A. E. Parker, the IAMFES representative to the National Mastitis Council and from H. L. Thomasson, Executive-Secretary of IAMFES. Thomasson indicated that IAMFES: *(a)* experienced its largest total and net income ever during the 1968-69 year, *(b)* gained approximately 200 affiliate members and lost approximately 50 direct members, *(c)* participated in development of 3-A Sanitary Standards for the egg industry and *(d)* should move forward in the entire food field.

Dr. C. K. Johns (left), Dr. A. N. Myhr (center), and Mr. Vernon Nickel at the final session.

Reports were also heard from: *(a)* the Editor of the *Journal of Milk and Food Technology* (which will appear in the *Journal*), and *(b)* from Bruce K. Lane, Chairman of the Local Arrangements Committee.

The proposed amalgamation with the National Association of Sanitarians (NAS) was discussed. It was reported that an overture from the Executive-Secretary of NAS was received in May, 1969 to determine if IAMFES was still willing to further consider the prospects of amalgamation. H. L. Thomasson, Dr. W. C. Lawton, and D. B. Whitehead met with Nicholas Pohlitt and reaffirmed the open-door policy of IAMFES. This policy was to be made known to the NAS Executive Board at its Houston meeting in June, 1969. No further communications have been received from NAS.

Refreshments for the ladies in their hospitality room.

Dr. F. W. Barber presented the report of the Journal Management Committee. Barber indicated that institution of the page charge facilitated prompt publication of research papers and made possible the addition of extra pages to the Journal. The committee also recommended the institution of student subscriptions. The Executive Board voted to make journal subscriptions available to full-time students at the rate of $4.00 per year.

Reports on the Sanitarian's Joint Council and on the 3-A Symbol Council were presented by John Fritz and Dr. K. G. Weckel, respectively. Weckel indicated that: *(a)* trustees of the 3-A Symbol Council

authorized a change in the bylaws to prohibit a trustee from serving on a task force, *(b)* Fred Uetz recently has been appointed as a trustee and he will also serve as assistant secretary-treasurer, *(c)* a change has been proposed to prohibit the secretary-treasurer from acting on behalf of the trustees when an application is denied, and *(d)* problems facing the trustees are applications from foreign countries and liability coverage for trustees.

AFFILIATE COUNCIL MEETING

The Affiliate Council meeting was held on Monday evening, August 18, with 14 of 26 affiliate organizations represented. A discussion on the role of the Affiliate Council in the operation of IAMFES was rather extensive but did not lead to any substantial suggestions or recommendations. Questions were raised about the possibility of regional meetings of affiliate representatives, and the composition of the annual meeting program.

Milton Held, president-elect of IAMFES, spoke briefly and outlined an Executive Board suggestion for establishing the Shogren Award to be given annually to the most active affiliate. The Affiliate Council felt that satisfactory criteria for making the award could not be established, and hence voted to turn down the suggestion offered by Held.

Dr. E. H. Marth, Editor of the *Journal of Milk and Food Technology*, reported on institution of student subscriptions for the journal.

Affiliate Council officers chosen to serve for the coming year are: Mr. Ben Luce (Washington), Chairman and Mr. Leon Townsend (Kentucky), Secretary.

TECHNICAL SESSIONS

This year eight papers were given at the General Sessions. These papers dealt with such topics as working with people, consumer protection and environmental health, contamination of food, and imitation foods. Eight papers also were presented at the two sessions devoted to Milk Sanitation. Topics included mastitis control, bulk farm tanks, quality program for manufacturing milk, consolidated cooperatives, the Interstate Milk Shippers Conference, a quality control laboratory, CIP systems, and milk and food combined operations.

Persons attending the two sessions on Food and Environmental Sanitation heard seven papers dealing with such subjects as safe food service, automated food vending, in-flight food service, changes in the Food and Drug Administration, perfringens food poisoning, viruses in foods, and travel trailer safety and sanitation.

One Food Industry Sanitation session offered four papers dealing with good manufacturing practices,

Officers of IAMFES. Left to right: Elmer Kihlstrum, Dick Whitehead, Dr. A. N. Myhr, Milton Held, S. O. Noles, and O. M. Osten. Roy Fairbanks, Sec'y.-Treas. was unable to attend the meeting.

management and sanitation, microbiology of frozen foods, and the egg processing industry. In addition to these formal sessions, three evening discussion groups met to consider food and environmental sanitation, revision of *Standard Methods for the Examination of Dairy Products,* and dairy farm quality control. Most of the papers presented at the annual meeting will appear in subsequent issues of the *Journal of Milk and Food Technology.*

ANNUAL MEETING

The annual business meeting was called to order by President S. O. Noles on Wednesday, August 20, 1969. The membership heard reports from H. L. Thomasson on his activities as executive-secretary and on the financial condition of the association. Representatives from the following association committees also gave reports: Dairy Farm Methods, Sanitary Procedures, Food Protection, Frozen Food Sanitation, Baking Industry Equipment, Communicable Diseases Affecting Man, Food Equipment Sanitary Standards, Professional and Educational Development, and Applied Laboratory Methods. Reports were also heard from the Affiliate Council and the 3-A Symbol Council. Most of these reports will appear in subsequent issues of the *Journal of Milk and Food Technology.*

It was announced that: *(a)* Elmer Kihlstrum was elected as the second vice-president and *(b)* Ivan Parkin has been appointed to serve as secretary-treasurer during the time that Roy Fairbanks is incapacitated.

Future meetings of IAMFES are: *(a)* 1970-the Roosevelt Hotel, Cedar Rapids, Iowa, August 17-21; and *(b)* 1971-San Diego, California.

Resolutions adopted by the membership at the annual meeting are recorded below. The Resolutions Committee consisted of Dr. A. N. Myhr, chairman; Dr. K. G. Weckel; K. K. Jones; and R. A. Belknap.

Resolution No. 1

WHEREAS: The Kentucky Association of Milk, Food, and Environmental Sanitarians has sponsored the 56th Annual Meeting of the International Association of Milk, Food, and Environmental Sanitarians, held at the Brown Hotel, Louisville, Kentucky, August 17 to 21, 1969; and

WHEREAS: The Local Arrangements Committee of the Affiliate provided excellent facilities for the conduct of the meeting as well as exceptionally fine entertainment for the members and their families;

THEREFORE BE IT RESOLVED: that we as members of the IAMFES express our sincere thanks for the cooperation and efforts made by the Kentucky Association of Milk, Food, and Environmental Sanitarians, its officers and friends who have worked so diligently to make this annual meeting a success and that a copy of this resolution be sent to the President of the Kentucky affiliate.

Resolution No. 2

WHEREAS: The current reorganization of the Department of Health, Education, and Welfare has created concern for the fate of the milk sanitation activities of the Public Health Service, and for future participation in the voluntary organizations of the 3-A Sanitary Standards Committees and the National Conference on Interstate Milk Shipments; and

WHEREAS: This concern has been dispelled by both Mr. C. C. Johnson, Administrator of Consumer Protection and Environmental Health Service and Dr. Herbert L. Ley, Commissioner of the Food and Drug Administration, on several occasions in which Mr. Johnson and Dr. Ley have acknowledge the importance of these two voluntary organizations and each have reaffirmed continued support of the 3-A Sanitary Standards Committees and the National Conference on Interstate Milk Shipments;

THEREFORE BE IT RESOLVED: That the International Association of Milk, Food, and Environmental Sanitarians take cognizance of this support and commend Mr. Johnson and Dr. Ley for their assurance of continued support of the 3-A Sanitary Standards Committees and the National Conference on Interstate Milk Shipments and are so notified by the President of the International Association of Milk, Food, and Environmental Sanitarians.

Resolution No. 3

WHEREAS: The Sanitarian's Joint Council's Model Act for Registering Sanitarians which has been in effect for the past ten (10) years; and that through voluntary compliance with legal registration patterned after SJC's Model Act many thousands of professional sanitarians have elected to be registered by their respective State Registration Agencies and,

WHEREAS: Many State and local Health Departments have not understood the value of complying with these Acts or the provisions of the Model Registration Act (in terms of the professional sanitarian's ability to provide better and more efficient service) in the appointment of sanitarian staff members or have elected to ignore the provision therein, thereby frequently appointing personnel who must undergo hardship in many cases to attain the academic training necessary to comply with the Model Registration Act.

THEREFORE BE IT RESOLVED: That the President and Secretary of each affiliate Association encourage their Legislative Committee to develop and arrange for an Act to be adopted at the next session of the State Legislature *requiring* all sanitarians and trainees employed after the effective date of said Act to meet the educational

provisions of the Sanitarians Joint Council's Model Registration Act.

Resolution No. 4

WHEREAS: The International Association of Milk, Food, and Environmental Sanitarians, Inc., represents a group of professional environmental health workers whose membership makes up one of the largest groups of professional public health workers in the Nation's Federal, State, and official Local Health agencies; and that except in a few states this professional competency in Environmental Health is not being utilized in Comprehensive Health Planning Program and

WHEREAS: Many of these Professional Sanitarians have the education and experience to make a significant contribution to the Federal and State Comprehensive Health Planning Program and that one or more should be appointed in each state to serve on the State Health Planning Council to represent the field of Environmental Health,

THEREFORE BE IT RESOLVED: That the President or Secretary of the Association transmit a copy of this resolution to the Secretary of the U. S. Department of Health, Education, and Welfare, and to each Governor and State Health Commissioner requesting the opportunity for more active participation in the State's Comprehensive Planning Council in carrying out its assignment.

Resolution No. 5

WHEREAS: The International Association of Milk, Food, and Environmental Sanitarians is proud of its role in sponsoring and initiating the formation of the National Mastitis Council and,

WHEREAS: The National Mastitis Council has, for the first time this year, held its Regional Meeting in conjunction with the Annual Meeting of IAMFES in the Brown Hotel, Louisville, Kentucky, and

WHEREAS: A common meeting place for the two groups of sanitarians is beneficial to both organizations both from the standpoints of improved attendance and exchange of technical information,

THEREFORE BE IT RESOLVED: That IAMFES extend its appreciation to the National Mastitis Council for selecting this time and place for holding its Regional Meeting and to encourage the Council to regularize the holding of similar future meetings jointly with annual meetings of this association.

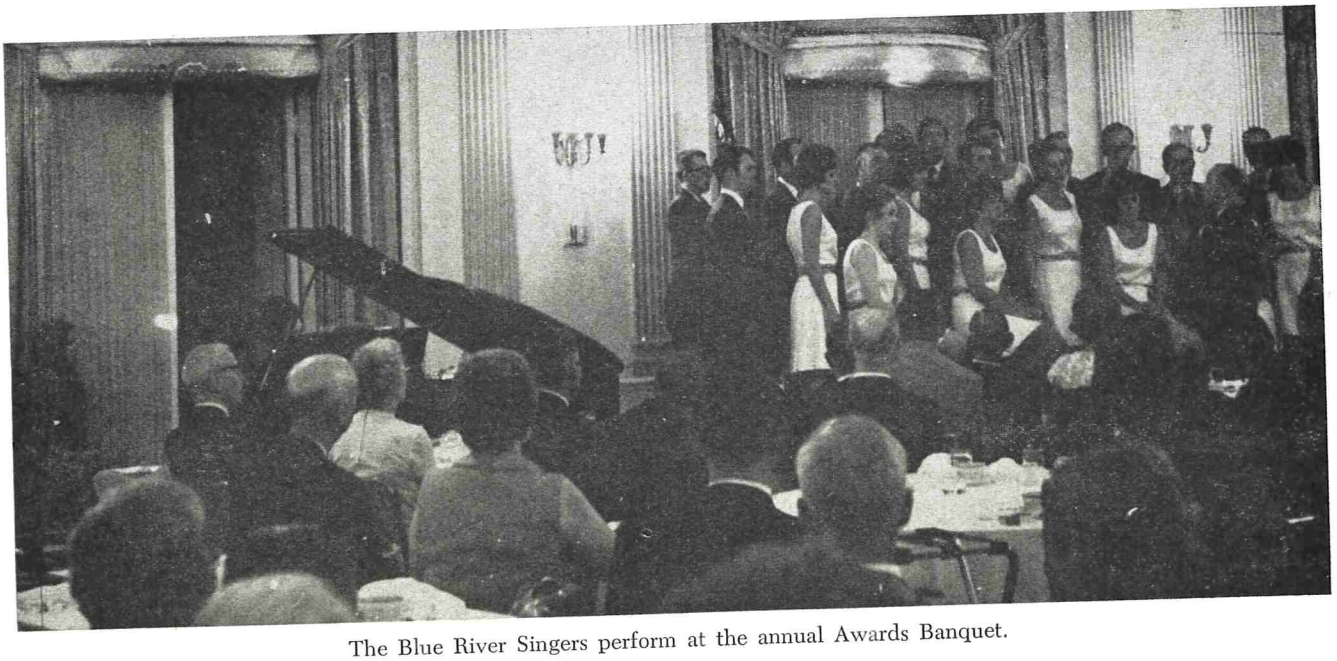
Resolution No. 6

WHEREAS: There is a need for a national conference on food protection to effect a unified and coordinated approach among various agencies and organizations in the establishment of food protection program involving such things as:

1. The establishment of priorities in food protection.
2. Reviewing areas of concern of the various Federal, State and local agencies.
3. The establishment of uniform standards and criteria.

THEREFORE BE IT RESOLVED: That this association recommends to Mr. Charles C. Johnson, Jr., Administrator of the Consumer Protection and Environmental Health Services that a national conference on Food Protection be established at the earliest possible date, and

BE IT FURTHER RESOLVED: That a copy of this resolution be forwarded to the Secretary of Health, Education, and Welfare (Mr. Robert Finch) and the President's Consumer Consultant (Mrs. Virginia Knauer).



The Blue River Singers perform at the annual Awards Banquet.

ANNUAL BANQUET

Members and guests attended a cocktail party hosted by the Local Arrangements Committee prior to participating in the annual banquet on Wednesday evening, August 20, 1969. Mr. Burdette Fisher served as master of ceremonies and entertainment was provided by the Blue River Singers, an excellent choral group from Shelbyville, Indiana.

The Sanitarian's Award and accompanying check for $1,000 went to the W. R. McLean. Karl Jones received the Citation Award and an Honorary Life Membership was given to John Faulkner. A more complete story on the award winners appears elsewhere in the *Journal.*

JONES, FAULKNER, AND MCLEAN HONORED AT FIFTY-SIXTH ANNUAL MEETING OF IAMFES

Karl Jones (left) receives the Citation Award from Ben Luce.

Each year the Committee on Recognition and Awards selects IAMFES members to receive the Citation Award, Honorary Life Membership, and the Sanitarian's Award. Ben Luce, acting on behalf of the Committee, presented the awards at the Annual Awards Banquet which is one of the highlights of the IAMFES Annual Meeting. This year Karl Jones received the Citation Award, John Faulkner joined the ranks of Honorary Life Members, and the Sanitarian's Award went to W. R. McLean. Members of the Committee on Recognition and Awards were: Dr. Paul Elliker, Oregon; Fred Uetz, New Jersey; Harold Barnum, Colorado; Dr. W. C. Lawton, Minnesota; and Paul Corash, New York.

CITATION AWARD—KARL JONES

The Citation Award is presented annually to a member of IAMFES who has given of himself and of his personal time and effort to unselfishly further the aims of IAMFES and to contribute to the professional recognition and status of all Sanitarians. For the year 1969, this award went to Mr. Karl Jones. Jones graduated from Indiana University with a major in milk and food sanitation. During his professional career he has been with the Indiana State Board of Health where, for several years, he served as Chief of the Retail Food Section. Presently Jones is Chief Sanitarian at Purdue University.

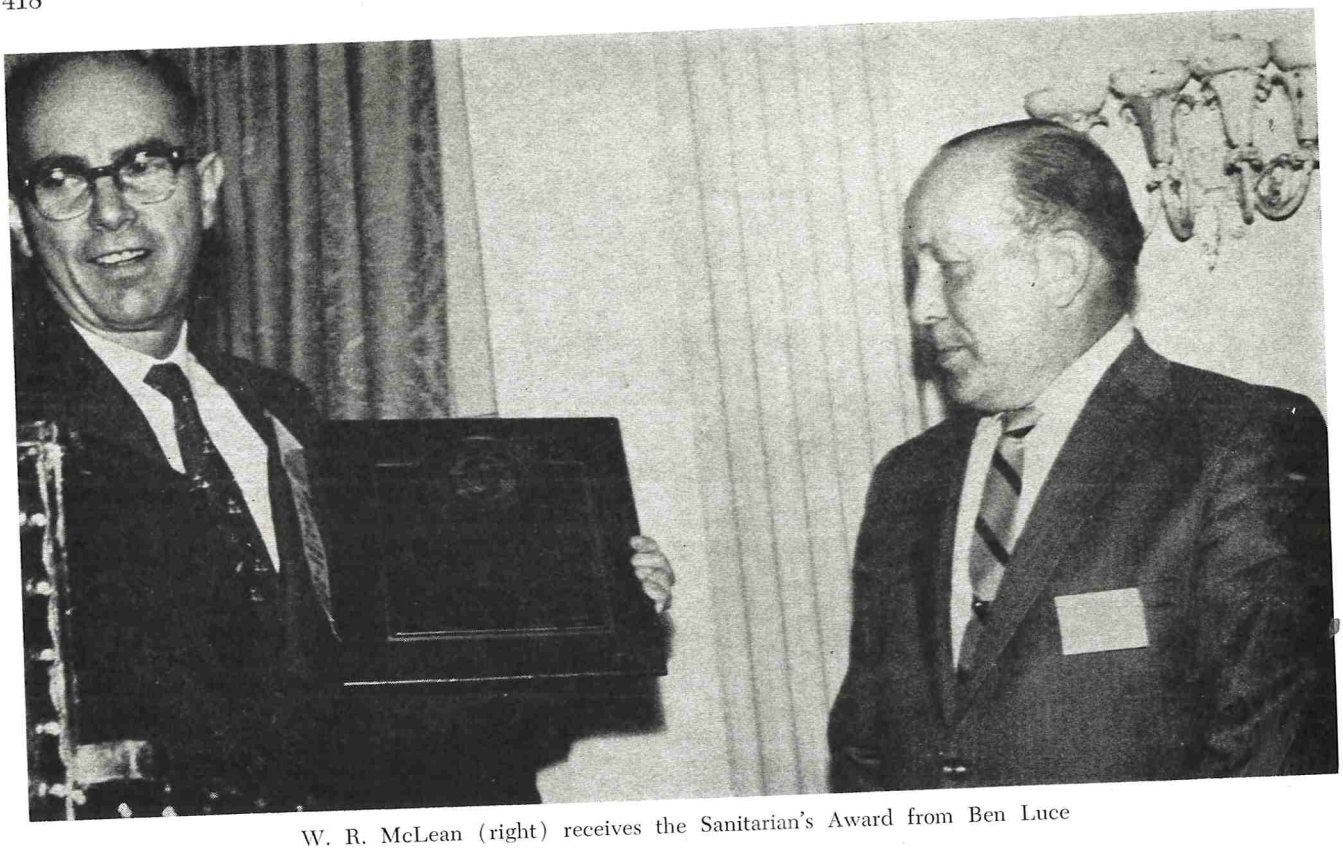
Jones has served IAMFES as secretary for seven years, as chairman of the Food Equipment Committee, and as a representative to the National Sanitation Foundation. Additionally Karl has been secretary-treasurer and president of the Indiana Affiliate and is chairman of the Indiana State Board of Registered Sanitarians.

HONORARY LIFE MEMBER—JOHN FAULKNER

Long and faithful service of IAMFES members is recognized by the awarding of Honorary Life Memberships. This year the honor went to John Faulkner.

Faulkner served as Assistant Chief and as Chief of the Milk and Food Branch of the U. S. Public Health Service. It was during his tenure as Chief

JOHN FAULKNER



W. R. McLean (right) receives the Sanitarian's Award from Ben Luce

that the Public Health Service became more intimately involved in activities such as the 3-A Standards and Interstate Milk Shippers programs.

More recently Faulkner has taken a regional assignment with the Consumer Protection and Environmental Health Service. Ivan E. Parkin accepted the award for John as he was unable to attend.

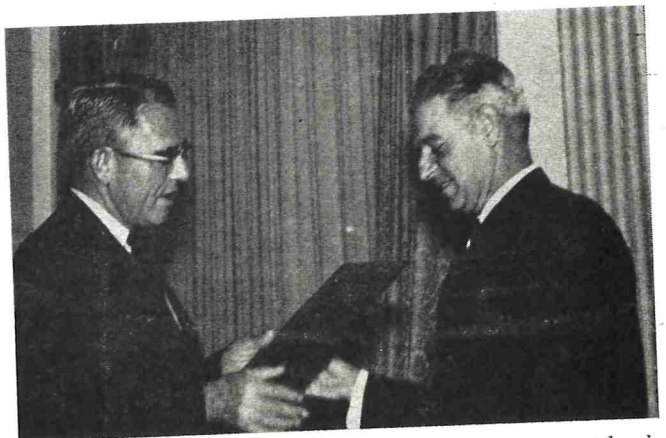
SANITARIAN'S AWARD—W. R. MCLEAN

The Sanitarian's Award is sponsored jointly by the Diversey Company, Klenzade Products, and Pennwalt Corporation and is administered by IAMFES. The award consists of a plaque and $1,000. A policy change was made last year which provided that in odd-numbered years the recipient shall be selected from among State or Federal employees who are members of IAMFES.

The Sanitarian's Award is made each year to the person who in the judgment of the committee, has made the greatest contribution as a Professional Sanitarian. The committee is charged with the responsibility of judging the worthiness of candidates who have been recommended and of selecting the individual who has made the greatest contribution to the field of public health sanitation during the preceding seven years. The recipient of this year's Sanitarian's Award was W. R. McLean.

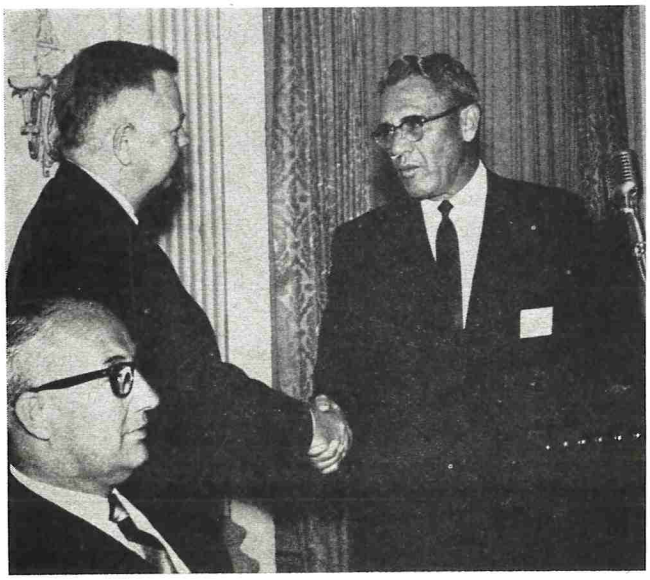
McLean received the B. S. degree from the University of Idaho where he majored in Dairy Science and the M. P. H. degree from the University of Michigan. This year's award winner has demonstrated technical acumen and professional skill in objectively evaluating and reviewing equipment and techniques employed in the milk, food, baking, and vending industries. He has promoted a program of equipment evaluation which has saved the industry millions of dollars through improvements in equipment design and construction.

McLean has assisted with development of: *(a)* 3-A Sanitary Standards for farm milk cooling and holding tanks and for silo-type tanks and *(b)* 3-A Accept-



Dr. A. N. Myhr (right) receives the past-president's plaque from S. O. Noles.

Milton Held (left) receives the president's gavel from William V. Hickey. The gavel received by Mr. Held is the last to bear the name of *Health Officers News Digest* as the donor. This publication is receiving a new format and the name *Environment*.



Milton Held (left), new IAMFES president is congratulated by S. O. Noles, retiring president. Burdette Fisher is in the foreground.

ed Practices for sanitary construction, installation, testing, and operation of HTST pasteurizers and for milk and milk products spray drying systems. He also aided in developing the equipment-related portions of the 1953 Milk Ordinance and Code, the 1965 Grade A pasteurized Milk Ordinance, the 1966 Grade A Powdered Milk Ordinance, Minimum Sanitary Standards for Community Infant Formula Services; the 1968 Ordinance and Code Requesting the Processing of Eggs and Egg Products, the 1967 Sanitary Standards for Smoked Fish Processing, the 1962 Food Service Sanitation Ordinance, the 1956 and 1965 Vending Sanitation Ordinances and the 1965 Manufactured Ice Standard.

During the past year McLean worked closely with Public Health and State Agriculture officials in two of the States within his region on major revisions of State laws pertaining to the regulation of Grade-A pasteurized milk. The legislation was successfully enacted and signed into law in both States. He exhibited great ability and insight in working with conflicting interests of local producers and processors and of State officials and legislators.

In his present position, McLean is responsible for coordinating and organizing activities relating to food protection in cooperation with other regional office activities. He works closely with State officials, officials in Federal agencies, university personnel, and representatives of the food industry. McLean is a Registered Sanitarian in two states, a Diplomat of the American Intersociety Academy for Certification of Sanitarians, a member of the IAMFES committee on Baking Industry Sanitation Standards, and a founder-member of the 3-A Sanitary Standards for Milk Equipment of the National Sanitation Foundation. He has authored papers on vacuum equipment and high heat pasteurizers used for processing milk products, adulteration of Grade-A milk, and infectious hepatitis associated with consumption of raw oysters. In 1967 McLean was cited by the Surgeon General of the U. S. Public Health Service for his outstanding contributions to the development of sanitary standards for milk and food handling equipment.

To Our Contributors

Your participation and generous assistance in our convention made it a huge success for all.

We are most grateful to the following:

American Can Company
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Signed:

BRUCE K. LANE, General Chairman, Local Arrangements

LEON TOWNSEND, Chairman, Finance Committee

M. E. HELD, President, IAMFES, Inc.

NEWS AND EVENTS

DAIRY TECHNOLOGY SHORT COURSES AT UNIVERSITY OF MARYLAND

25th Annual Dairy Technology Conference, November 5, 1969, University of Maryland.

Ice Cream Short Course, January 26 through February 5, 1970, Department of Dairy Science, University of Maryland.

Ice Cream Conference, February 5, 1969, University of Maryland.

Cottage Cheese and Cultured Milk Products Symposium, March 11, 1970, University of Maryland.

For further information, contact Wendell S. Arbuckle, Department of Dairy Science, University of Maryland, College Park, Maryland 20742.

HOUSEKEEPING SEMINARS ANNOUNCED BY SERVICE ENGINEERING ASSOCIATES

Supervisory development and supervisory techniques will receive special emphasis in the Fall Series of three-day seminars in Housekeeping Management and Supervision, to be conducted by Service Engineering Associates, Inc., a consulting firm of registered engineers and sanitarians serving schools, hospitals, industry, buildings, commerce and government.

The announcement was made by the firm's president, Edwin B. Feldmen, P. E., author of numerous books and articles on sanitation. The seminars are schedule as follows: Washington, November 3-5; Toronto, November 12-14; Los Angeles, November 17-19; San Francisco, December 1-3; Seattle, December 8-10.

The speakers will include Keith A. Fitch, Professional Engineer and Registered Sanitarian; Clifford C. Groover, Professional Engineer and labor specialist; as well as Mr. Feldman. Most of the talks are slide-illustrated. The presentations also include demonstrations of techniques for increasing supervisory skills. Audience Research Projects sessions will be devoted to solution of pre-submitted problems. The course is designed for executive housekeepers, physical plant directors, sanitarians, plant engineers, building superintendents and managers, housekeeping foremen and supervisors, methods analysts, maintenance directors, suppliers and contractors. Thousands of such persons have attended S-E-A seminars over the past eight years.

The registration fee is $100, with group rates available. A free brochure provides the complete program, a list of previous participants, biographical data concerning the speakers, and registration form. Write—Service Engineering Associates, Inc., 3954 Peachtree Road, N. E., Atlanta, Georgia 30319, Telephone: 404-261-2050.

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Responsible for developing and administrating a program for Central Soya's Chicago food ingredient processing plant, assuring compliance with the F.D.A., Health Department and company requirements. This individual will give staff direction to production superintendents regarding sanitation. Hiring to $10,600.00. Submit all resumes in confidence to: Richard A. Dent, Central Soya Company, Inc., 300 Fort Wayne Bank Building, Fort Wayne, Indiana 46802—An Equal Opportunity Employer.

SPOKANE, WASH. NEEDS MILK SANITARIAN

Degree in food or dairy science or related. Two years experience in milk processing or inspection. Annual entry salary $8,306—$9,962. Send resume and application to Spokane Civil Service Commission, City Hall, Spokane, Wash. 99201.

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Single Service milk sample tubes. For further information and a catalogue please write, Dairy Technology Inc., P. O. Box 101, Eugene, Oregon 97401.

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Dairy Management Memo

What do dairymen think about dairying?

How do dairymen feel about the future of their business? Do they see imitation milk as a threat? What's the future of price bargaining groups?

These are some of the questions Surge dairy equipment dealers asked dairymen all over the country—over 2,000 farm interviews in all—to find out how they see the dairy business now and in the future.

Answers were tabulated on the basis of herd size and the dairyman's plans for expansion.

Bigger dairymen are generally more satisfied with prospects than smaller ones. They want more action, however, on things like price bargaining—74 percent of the bigger dairymen (compared with 63 percent of smaller dairymen) think dairy farmer price bargaining groups should be larger. And, three out of four of the dairymen interviewed would like to see *all* dairymen support American Dairy Association promotion if a vote showed that two-thirds of dairymen were in favor of this action.

They feel strongly about the need for a compulsory milk check-off for research and promotion—84 percent of bigger dairymen favor it. Of the group milking less than 30 cows, 70 percent favor compulsory milk check-off. And 49 percent of all those asked in this Surge survey are willing to contribute 1 percent or more of milk sales.

The Surge dealers found that five out of six dairymen think imitation milk is a threat, with smaller operators tending to be less afraid of it than bigger operators.

In general, the study indicates that dairymen are optimistic about the state of their business—present and future. But they feel not enough is being done to promote their products or to bolster prices.

The study was made by Surge dealers in connection with Surge's 1968 "Better Cow Milking Week". The special week is held each year during March to help dairymen increase efficiency and reduce milking problems.

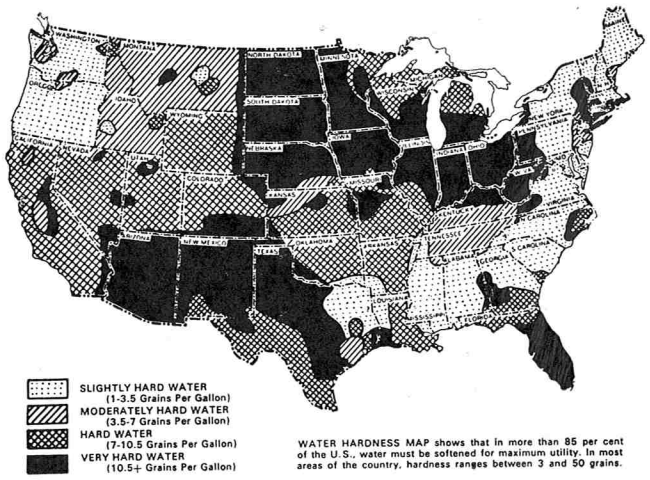
For a full report on this study, see your Surge dealer or write to Babson Bros. Co., 2100 S. York Rd., Oak Brook, Illinois 60521. Ask for the pamphlet "What do dairymen think about dairying?"

What can "CONDITIONED WATER" do?

Good dairy management starts with attention to details. And it's the sum of the small things that adds up to good income from dairying.

Keeping bacteria count low can do more than anything else to assure a dairyman top price for milk. That's why many top managers are taking a hard look at the kind of water they're using to clean with.

In severe hard water areas, milkstone—a hard, calcium-like deposit in milk pipe lines around joints and valves—is a common problem. Water with even moderately high mineral content frequently causes unsightly and unsanitary spotting on milkers, pipe lines, bulk tanks and utensils.



WATER HARDNESS MAP shows that in more than 85 per cent of the U.S., water must be softened for maximum utility. In most areas of the country, hardness ranges between 3 and 50 grains.

Any build-up, however small, can provide a breeding place for bacteria, causing bacteria count to soar.

Other results of hard water are high consumption of cleaning detergents, scum accumulation on surfaces and failure of detergents to provide best cleaning action. More and more dairymen are finding that a water conditioner can solve these problems.

Whether it's calcium-forming minerals, iron bacteria, iron, or the water supply shows bacteria count from contamination, Surge dealers can supply the right equipment to solve the problems. They are trained water treatment specialists. And they're now offering a complete water analysis free of charge.

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