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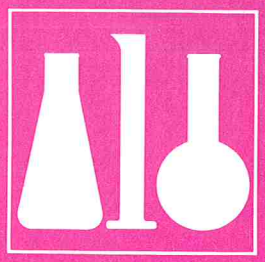

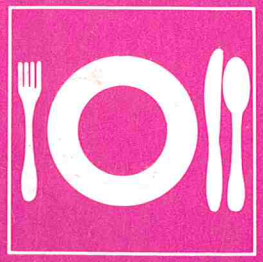

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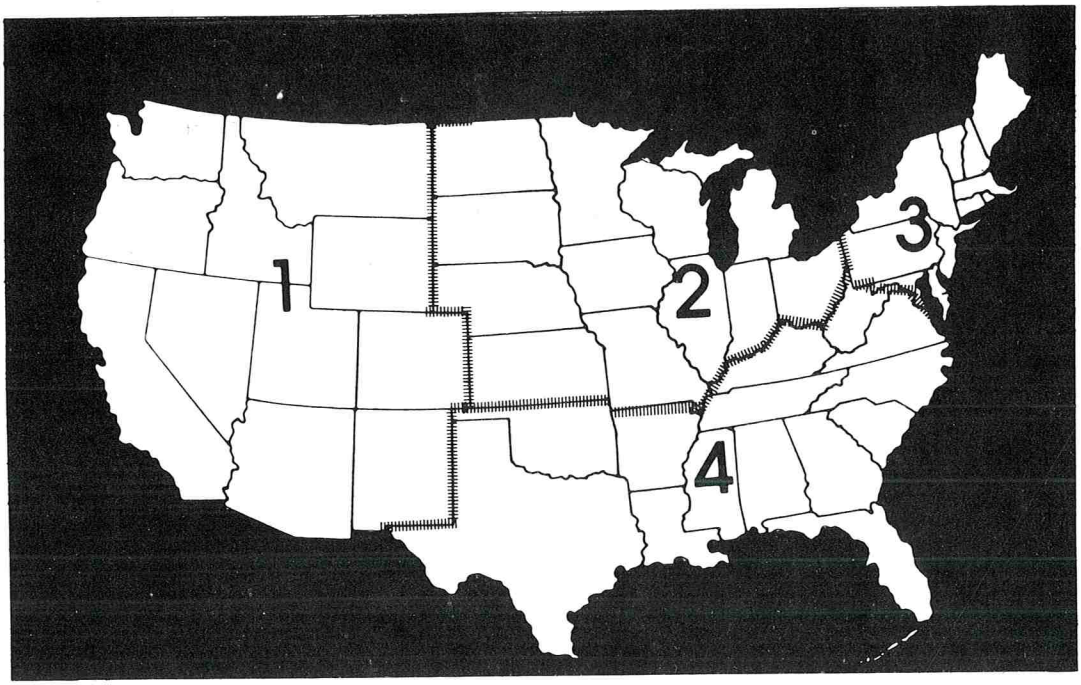

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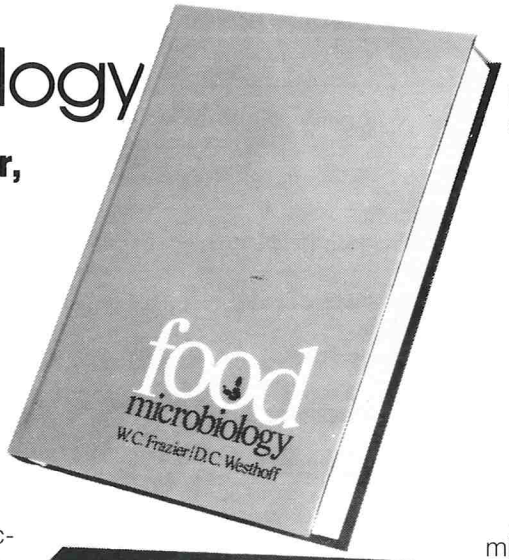

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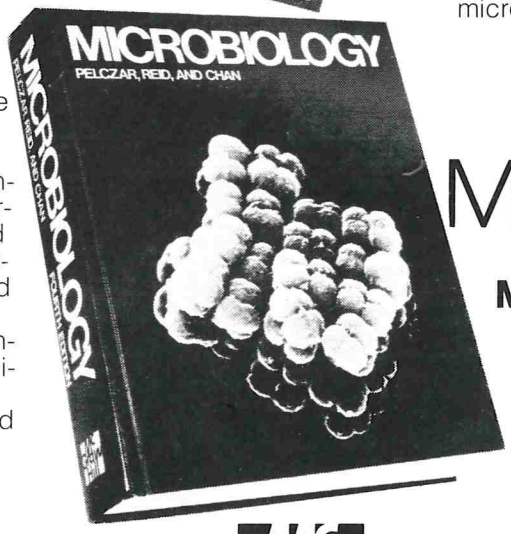

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General Interest

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Time-Temperature Observations of Food and Equipment in Airline Catering Operations[1]

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(Received for publication August 3, 1977)

ABSTRACT

Foods served on aircraft have been implicated as vehicles of foodborne illness. Preparation practices that could contribute to growth or survival of foodborne pathogens were surveyed in several airline catering operations. Although most of the practices would provide bacteriologically safe foods, some would permit pathogenic foodborne bacteria to survive cooking and reheating processes and permit the bacteria to multiply during cold- or hot-storage. Many of the prepared foods were exposed to temperatures within the 45 to 140 F range for several hours. Some of the equipment used could not maintain food temperatures above 140 F or below 45 F because of capacity, insufficient refrigerating medium, or poor condition.

Outbreaks of foodborne illness sporadically affect passengers and crew of aircraft. Several outbreaks appear in public health and disease surveillance literature (*2, 5-14, 16-18*). Others, no doubt, have occurred. But if the disease has an incubation period longer than the plane ride, passengers scatter, and their subsequent illnesses are seldom associated with the meal they had on the airplane.

Staphyloenterotoxicosis, salmonellosis, *Vibrio parahaemolyticus* gastroenteritis, non-cholera vibrio gastroenteritis, and shigellosis have been acquired by eating airline-catered foods. Inadequate refrigeration or inadequate hot-holding of foods, or both, were usually reported as contributing factors that permitted the contaminating pathogenic bacteria to multiply to such an extent that the outbreaks ensued.

Temperatures of various foods and of the units in which they were held during cold storage, preparation, cooking, chilling, hot- and cold-holding, delivery to aircraft, and reheating were evaluated during routine and simulated operations in airline catering kitchens. Data are interpreted by reference to temperatures that are known to kill vegetative bacteria, that allow or enhance growth of common pathogenic foodborne bacteria, and that are within a temperature range not permitted under codes that regulate food service operations (*15*).

METHODS

Six flight kitchens servicing five airlines at three airports were investigated. The operations were selected because they typified operations elsewhere and used similar types of equipment. The following operations were evaluated: (*a*) frozen, precooked meals were reheated in ovens and held in hot-holding transporters in the kitchen, transported in the same modules to the aircraft, and stored hot on the aircraft until served; (*b*) frozen, precooked meals were held frozen in the kitchen, delivered to the aircraft and held there in transporters, and reheated in convection ovens on the aircraft; (*c*) chilled, raw foods were cooked in the kitchen, after which they were assembled into individual-service casseroles and oven inserts and chilled; the following day they were transported to the aircraft and reheated in convection ovens; (*d*) chilled, raw foods were cooked in the kitchen, assembled in meal trays, held in hot-storage transporters in kitchens, and later delivered to the aircraft and held in these modules until they were served; (*e*) precooked, chilled foods or kitchen-prepared foods were chilled, if necessary, put in cold-storage transporters, delivered to the aircraft, and held in these modules until served.

Equipment in the various operations differed somewhat. Hot-storage transporters differed in size; they were electrically heated, usually with a high setting of 175 F and a low setting of 145 F. Several devices were used for storage and delivery of chilled foods. Metal tray carriers were used to hold trays of sandwiches, salads, rolls, and desserts. Low temperatures were maintained by placing dry ice in the doors of the tray carriers. Chilled meals to be reheated on the aircraft were held in oven racks which were put in cardboard boxes. Dry ice was added to the boxes to maintain low temperature during delivery and aircraft storage. Large cold-storage transporters also were used. Interiors were cooled either by putting dry ice into a center holding-well or onto the top shelves of the units, depending on construction. A transporter that had a dry-ice-activated freon-refrigeration system was used on some wide-body aircraft. Transporters for frozen foods had a large, dry-ice holding-well between two compartments. Small convection ovens, with a fan in the rear, were used to reheat foods on narrow-body aircraft. Each oven could hold approximately 30 meals for reheating. A large convection oven with an internal baffle along the side walls to facilitate

[1] *Use of trade names is for identification purposes only and does not constitute endorsement by the U.S. Department of Health, Education, and Welfare.*
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airflow to the front was used on some wide-body aircraft. Other equipment was identical or similar to that employed in other large food service operations.

Cooking, chilling, cold storage, and tray assembly were evaluated during actual routine operations; hot-holding, reheating, and cold-food procedures were evaluated under simulated conditions. All evaluations resulted from tests of random portions of food taken from equipment that is actually used in operations.

Movement of equipment from kitchen to aircraft was simulated by disconnecting the equipment from the current supply for an interval equal to that which would elapse during a trip to the aircraft. Reconnecting the equipment to the power supply simulated arrival at the installation in the aircraft, where in the course of usual operations, the equipment would be connected to a power supply in the aircraft.

Reheating operations in aircraft were simulated in an airline test kitchen, using ovens of the same type and design, supplied by current of the same characteristics, as used in aircraft.

Test food or meals were put into the equipment at locations which would represent the coldest and hottest temperature zones. The remainder of the unit was filled with meal containers with lids so arranged as to represent normal loading. The test procedures simulated actual operations in temperature settings and timing as closely as possible.

Type T thermocouples[6] attached to a recording potentiometer[7] were used to continuously record temperatures of scrambled eggs, omelets, baked eggs, beef dishes, sliced beef sandwiches, chicken, and cream-filled pies during applicable cold storage, cooking, hot- or cold-holding, transportation, and reheating. Thermocouples were attached in the following ways. A drill with a bit the same diameter as the thermocouple wire was used to make a hole to the geometric center of frozen foods. Soldered-end thermocouples were inserted into the holes. Either soldered-end or bayonet (needle) thermocouples were pushed into chilled, hot, or room temperature foods. Soldered end thermocouples were also inserted just beneath the skin of pieces of chicken to take temperatures near the surface. Surface temperatures of other foods were taken with button thermocouples which were attached firmly to the surface by wires wrapped around the thermocouple and woven into the food. Temperatures of the heating, cooling, and storage units were taken with soldered-end thermocouples suspended in the air within a few inches of foods being tested. The procedures followed those previously described (*3, 4*).

RESULTS AND DISCUSSION

The data recorded about egg, poultry, and beef products, and dessert items are summarized in Tables 1 to 4, respectively. These tables give the highest temperature obtained and the minutes above 165, 150, and 140 F during cooking, hot-holding, and reheating. These data are interpreted according to lethality of vegetative pathogenic bacteria based upon the following criteria: a second at or above 165 F, 12 min or more at or above 150 F, and 83 min or more at or above 140 F (*1*). These tables also give the minutes in cold storage, tray assembly, chilling, and hot- and cold-holding during which the foods are held within a rapid multiplication range for mesophilic bacteria (between 70 to 115 F), within the growing range for *Clostridium perfringens* (between 60 to 122 F), and within the temperature range of 45 to 140 F (to comply with food service code requirements, cold foods should be at 45 F or below, and hot foods should be at 140 F or above) (*15*). These data are interpreted according to the potential for growth of mesophilic or pathogenic foodborne bacteria that are characterized by a lag phase of at least 60 min and additional time for one or more generations at the 70 to 115 F range.

Time-temperature relationships of specific foods prepared in flight kitchens and served on aircraft are described for each step of the preparation process to which they were exposed.

Cooking

During the cooking of scrambled eggs or omelets, temperatures near or above 165 F were reached, or the eggs reached or exceeded this temperature during the post-cooking temperature rise (Table 1). An example is illustrated in Fig. 1.

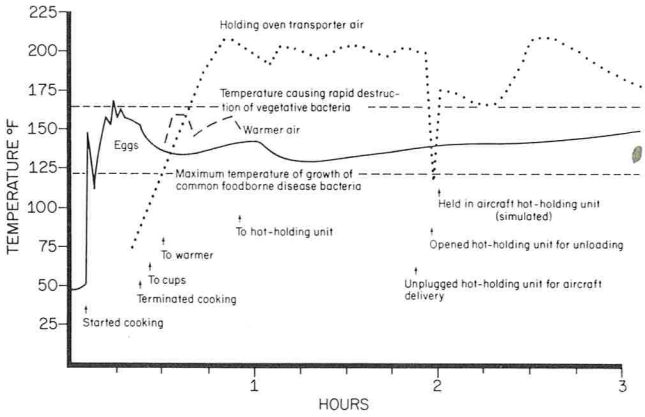

Figure 1. *Temperature of eggs with cream cheese during scrambling-cooking, dispensing in 3.5 ounce (2.5 × 1.5 high) paper cups, holding in warmer, and holding in a holding-oven transporter and simulated holding in an aircraft hot-holding unit.*

During cooking of 1.5 ounce chicken wings (drumettes) in oil at 350 F, surface temperatures exceeded 225 F and internal temperatures exceeded 190 F (Table 1; Fig. 2). During baking or post-oven rise of 4-ounce

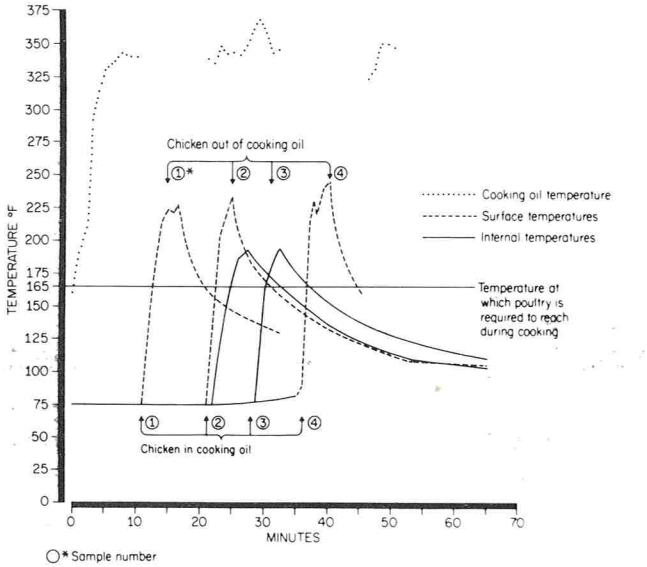

Figure 2. *Internal (geometric center) and surface temperature of chicken wings (drumettes) during cooking in oil and holding at room temperature.*

[6] *Type T refers to copper-constantan junction.*

[7] *Temperatures were recorded on Chart 5270 (type T), 0 to 500F range, in an Electronik 16 Multipoint Recorder, Honeywell, Fort Washington, Pa.*

TABLE 1. *Time-temperature exposure of geometric center of different types of egg preparations during airline catering operations*

Food	Operation	Highest temperature F	Minutes at or above 165 F	Minutes at or above 150 F	Minutes at or above 140 F	Potential for survival (+) of vegetative pathogenic bacteria	Minutes between 70-115 F	Minutes between 60-122 F	Minutes between 45-140 F	Potential for growth (+) of foodborne disease bacteria
Scrambled egg mix, initially frozen (4-pound carton)	Thawed in 38-42 F refrigerator	31					0	0	0	–
Scrambled eggs	Cooked in steam kettle	157 (a)[1]	0	3	7.6	+				
	Post-cooking rise	167 (a)	5	13	16					
	Cooked in steam kettle, including post-cooking rise	200	9	12	n[2]	–				
Scrambled eggs, 3.5 ounces in 3-inch diameter 1.75-inch high, paper cup	Cooled in walk-in refrigerator	145				–	65	105	180	+/–
	Cooled in cart (roll-in type) refrigerator	145				–	90	160	375	+
Scrambled eggs 5 inches deep in 20 × 11 × 6 inch pan	Cooled in cart (roll-in type) refrigerator	164	0	75	120	–	330	505	750+	+++
Scrambled eggs with cream cheese, 3.5-ounce portion in 2.5-inch diameter, 1.5-inch high paper cup	Cooked in tilt fryer (steam-heated pan)	169	2	13	18	–				
	Assembled, stored in warmer, stored in hot-holding transporter, and delivered and stored in aircraft	153	0	1	15	+	0	0	90	–
Omelet	Cooked in pan over gas flame grill	198	1	2	3	–				
	Trays assembled	198	3	4	5.5	–	38	45	52	–
	Cooled on tray in walk-in refrigerator	80					40	67	230+	–
	Total for trays being assembled and cooled	198	3	4	5.5	–	78	112	282+	+/–
Omelet (precooked and frozen)	Reheated in convection oven	50 (b)	0	0	0	+				
	Stored in hot-storage transporter set at 175 F after reheating; delivered and stored in aircraft (190 min)	197 (b)	150	160	165	–	10	15	27	–
	Reheated and stored in a hot-storage transporter set at 145 F; delivered and stored in aircraft (490 min)	154 (d)	0	150	212	–	65	77	153	+/–
Baked eggs (precooked and frozen)	Reheated in convection oven reaching 332 F	28 (c)	0	0	0	+				
	Stored in hot-storage transporter set at 175 F; delivered and stored in aircraft (190 min)	187 (c)	110	130	137	–	19	26	42	–
	Reheated and stored in a hot-storage transporter set at 145 F; delivered and stored in aircraft (490 min)	148 (d)	0	0	90	–	57	80	156	–

[1]Sample identification. In all samples with the same letter, the probe was in the same position during the continuous recording
[2]Not recorded

TABLE 2. *Time-temperature exposure of poultry products during various operations of airline catering*

Food	Operation	Probe position	Highest temperature F	Minutes at or above 165 F	Minutes at or above 150 F	Minutes at or above 140 F	Potential for survival (+) of vegetative pathogenic bacteria	Minutes between 70-115 F	Minutes between 60-122 F	Minutes between 45-140 F	Potential for growth (+) of foodborne disease bacteria
CHICKEN WINGS (Drumettes)											
1.5-ounce, approximately 3.5 long × 1.25 diameter, inches	Stored in walk-in refrigerator	C[2]	42					0	0	0	–
	Cooked in oil approximately 350 F for 3-4 min and stored at room temperature (post-cooking rise) for a maximum of 34 min	S[2](a)	227	8.2	11.6	16	–				
			234	8.5	12.1	15.5	–				
			245	8	n[3]	n	–				
		C (a)	193	8.4	12.6	15.3	–				
			194	7.3	11.2	14.8	–				
	Cooled, piece in middle of pan of other warm wings in a cart (roll-in type) refrigerator (180 min-surface; 105 min-interior)	S (a)	107					82.5+	127+	180+	+
		C (a)	113					105+	105+	105+	+
	Trays assembled in 76 F room for 105 min	C	75					105	105	105	+
	Cooled in plastic dish covered with saran in walk-in refrigerator	C	75					14	38	95	–
	Stored cold, delivered and stored in metal container cooled by dry ice in door in narrow-body aircraft										
	for 120 min	C	68					0	82	120	–
	for 150 min	C	72					15	113	150	–
CHICKEN THIGHS											
4-ounce portion	Baked and stored (post-cooking rise) at room temperature (oven temperature exceeded 500 F)	S (b)	190	11	15	17	–				
		(c)	245	19	22	26	–				
		C (b)	176	8	15	20	–				
		(c)	202	20	27	33	–				
	Cooled in reach-in refrigerator	for 235 min	S (b)	105				155	225	235+	++
		for 40 min	(c)	74				4	12	40+	+
		for 235 min	C (b)	107				145	220	235+	++
		for 40 min	(c)	85				16	29	40+	–
	Trays assembled for 65 min		S (c)	95				33	51	65	–
			C (c)	93				33	54	65	–
	Stored in warmer, delivered and stored in narrow-body aircraft for 190 min		S(c)	212	143	153	159	–	17+	21+	32+	–
			C (c)	212	140	150	155	–	20+	25+	37+	–
	Reheating chilled chicken in warmer at 98-114 F for 115 min		S	88	0	0	0		95	110	115	+
BONELESS CHICKEN	Stored frozen in transporter cooled by dry ice		S/C (d)	26					0	0	0	–
			(e)	28					0	0	0	–
	Reheated in convection oven in wide-body aircraft		S (d)	132	0	0	0	+				
			(e)	168	1	7.5	11.5	–				
			C (d)	72	0	0	0	+				
			(e)	68	0	0	0	+				
	Stored in hot-storage transporter for 20 min		(d)	172	5	12	17	–				
	for 45 min		S (d)	183	35	42	47	–				
	for 20 min		(e)	175	15	28	32	–				
	for 45 min		(e)	183	40	53	57	–				
	for 20 min		(d)	148	0	0	4	+				
	for 45 min		C (d)	180	20	30	34	–				
	for 20 min		(e)	134	0	0	0	+				
	for 45 min		(e)	167	3	16	22.5	–				

TABLE 2. *Continued*

CHICKEN WITH CRAB STUFFING											
Cooked and frozen	Cooked in deep fat (370-374 F)	S (f)	174	1	3	4	–				
		C (f)	30	0	0	0	+				
	Stored, delivered and stored in aircraft for 125 min	C (f)	134	0	0	0	+	47	68	112	–
	for 145 min	(f)	138	0	0	0	+	47	68	142	–
TURKEY											
6 slices, precooked, frozen, thawed	Reheated and stored in hot-storage transporter; delivered and stored in narrow body aircraft for 105 min	B[1]	144	0	0	15	+	38	50	91	–

[1]Sample identification. In all samples with the same letter, the probe was in the same position during the continuous recording.
[2]C refers to probe in geometric center; S refers to probe just beneath skin; B refers to probe between 3rd and 4th slices
[3]Not recorded

TABLE 3. *Time-temperature exposure of beef products during airline catering operations*

Food	Operation	Probe position	Highest temperature F	Minutes at or above 165 F	Minutes at or above 150 F	Minutes at or above 140 F	Potential for survival (+) of vegetative pathogenic bacteria	Minutes between 70-115 F	Minutes between 60-122 F	Minutes between 45-140 F	Potential for survival (+) of vegetative pathogenic bacteria
ROAST BEEF											
1-pound, 9-ounce	Stored in walk-in refrigerator	S[1] (a)[2]	43					0	0	0	–
		C[1] (a)	33					0	0	0	–
1-pound, 14-ounce		C (b)	42					0	0	0	–
1-pound, 9-ounce	Baked in convection oven	S (a)	164	0	22.5	27	–				
		C (a)	147	0	0	2	+				
		S (b)	127	0	0	0	+				
		C (b)	119	0	0	0	+				
		S (c)	177	16	17	18	–				
		C (c)	110	0	0	0	+				
		C (d)	115	0	0	0	+				
1-pound, 13-ounce	Cooled in cart (roll-in type) refrigerator for 46 min	S (a)	164	0	3	6	+	28+	34+	40+	–
		C (a)	127	0	0	0	+	0	6+	46+	–
		S (b)	147	0	0	0.5	+	22+	35+	46+	–
		C (b)	127	0	0	0	+	0	18+	46+	–
	for 41 min	S (c)	170	0.5	2	2.5	–	26+	31+	39+	–
		C (c)	126	0	0	0	+	2+	6+	41+	–
	for 695 min	C (d)	127	0	0	0	–	150	255	675	++
1-ounce slice with three others	Cooled in foil-covered serving casserole (dish) in a walk-in refrigerator	C	82					38	75	165	–
			76					19	60	165	–
1-ounce slice with three others (second from bottom)	Stored cold in metal rack in a cardboard box cooled by dry ice for narrow body aircraft	C (e)[3]	58					0	0	60	–
		(f)[4]	64					0	30	165	–
		(g)[5]	70					0	60	165	–
		(h)[4]	68					0	90	210	–
		(i)[5]	75					60	130	210	+/–
		(j)[3]	74					40	115	210	–
	Cold storage of meals in wide body transporter cooled with dry-ice, heat-exchange activated system	(k)[3]	54					0	0	130	–
		(l)[5]	60					0	0	130	–
		(m)[4]	57					0	0	130	–
1-ounce slice with three others (second from bottom)	Reheating in narrow-body aircraft convection oven (oven setting 350 F for 20 min)	C (e)[3]	110	0	0	0	+				
		(f)[4]	125	0	0	0	+				
		(g)[5]	135	0	0	0	+				
		(h)[4]	175	2	4	6	–				
		(i)[5]	160	0	2	3	+				
		(j)[3]	120	0	0	0	+				
		(k)[3]	85	0	0	0	+				
		(l)[5]	128	0	0	0	+				
		(m)[4]	155	0	1	2	+				
	10-min post-oven rise period	(e)	130	0	0	0	+				
		(f)	165	<1	4	6	–				
		(g)	165	<1	6	8	–				
		(h)	205	12	14	15	–				
		(i)	200	10	12	13	–				

TABLE 3. *Continued*

			(j)	145	0	0	2.5	+			
			(k)	102	0	0	0	+			
			(l)	157	0	6	8	+			
			(m)	180	9	11	12	+			
COOKED BONELESS BEEF											
3-ounce rectangle-shaped, precooked and frozen	Reheated in convection oven	C	(n)	80	0	0	0	+			
			(o)	63	0	0	0	+			
			(p)	55	0	0	0	+			
			(q)	48	0	0	0	+			
			(r)	75	0	0	0	+			
			(s)	78	0	0	0	+			
			(t)	41	0	0	0	+			
			(u)	82	0	0	0	+			
	Held hot, delivered and stored in narrow-body transporter in aircraft										
	for 183 min (60 min after replug into aircraft)		(n)	134	0	0	0	+	20	53	183+ –
	for 223 min (60 min after replug into aircraft)		(n)	134	0	0	0	+	20	53	223+ –
	Held in dry-ice activated freon, heat-exchange system transporter for 170 min (30 min after replug into aircraft)		(o)	148	0	0	55	+	50	66	110+ –
	for 175 min (60 min after replug into aircraft)		(p)	147	0	0	60	+	53	69	115+ –
			(q)	155	0	55	90	–	27	35	83 –
	for 215 min (100 min after replug into aircraft)		(p)	158	0	30	100	–	53	64	115+ –
			(q)	167	10	95	130	–	27	35	83 –
	for 300 min (60 min after replug into aircraft)		(r)	172	25	180	215	+	35	48	85 –
	for 330 min (90 min after replug into aircraft)		(r)	172	55	210	245	+	40	52	90 –
	held in hot-storage transporter										
	for 60 min		(s)	145	0	0	7	+	59	70	79 –
	for 90 min		(s)	160	0	24	38	–	59	70	79 –
	for 120 min		(s)	165	<1	54	68	–	59	70	79 –
	Left at room temperature		(t)	88	0	0	0	+	52	55	64 –
	Held in hot-storage transporter										
	for 175 min (60 min after replug into aircraft)		(u)	160	0	112	132	–	53	60	88 –
	for 205 min (90 min after replug into aircraft)		(u)	160	0	142	162	–	53	60	88 –
COOKED BEEF ROLL											
Frozen and thawed	Stored in walk-in refrigerator	C		42					0	0	0 –
SLICED COOKED ROAST BEEF	Stored in walk-in refrigerator	C		46.5					0	0	290 –
	Awaiting sandwich-making at room temperature (74-78 F) for 70-80 min			72					38+	54+	68+ –
				62					0	10	68+ –
SLICED BEEF SANDWICHES											
1-ounce in bun	Held in sandwich-making room for 25 min; refrigerated for 43 min	C		62					0	66	78+ –
	Stored in 40-60 F walk-in refrigerator for 680 min	C		70					0	8	180 –

TABLE 3. *Continued*

	Stored cold, delivered and stored in narrow-body transporters for 150 min in narrow-body aircraft 1		63.5					0	35	150	–
	for 100 min in narrow-body aircraft 2		62					0	15	95	–
	for 50 min in narrow-body aircraft 3		52					0	0	52	–
COOKED BONELESS BEEF											
3-ounce rectangular shaped, precooked and frozen	Held frozen in transporter cooled with dry ice for 130 min	C (v)	28					0	0	0	–
		(w)	28					0	0	0	–
		(x)	28					0	0	0	–
	for 287 min	(y)	26					0	0	0	–
		(z)	26					0	0	0	–
		(aa)	26					0	0	0	–
	Reheated in wide-body aircraft convection oven	(v)[5]	100	0	0	0	+				
		(w)[3]	120	0	0	0	+				
		(x)[4]	125	0	0	0	+				
		(y)[3]	95	0	0	0	+				
		(z)[5]	105	0	0	0	+				
		(aa)[4]	85	0	0	0	+				
	Stored hot in aircraft cart for 20 min	(v)	172	2.5	4	11	–	7	9	17	–
		(w)	154	0	5	11	+	0	2	17	–
		(x)	175	5	11	15	–	0	0	7	–
		(y)	158	0	0	4	+	5	7	17	–
		(z)	183	<1	8	11	–	2	5	11	–
		(aa)	163	0	4	7	+	5	4	11	–
	Stored hot in aircraft cart for 45 min	(v)	181	27.5	29	36	–	7	9	17	–
		(w)	160	0	30	36	–	0	2	17	–
		(x)	180	30	36	40	–	0	0	7	–
		(y)	158	0	27	33	–	5	7	27	–
		(z)	185	30	38	41	–	2	5	11	–
		(aa)	185	30	35	37	–	2	4	11	–
COOKED SLICED BEEF											
3-ounce portion	Cooled in walk-in refrigerator (37-43 F) for 1020 min	C (ab)	58					0	0	200	–
	Stored cold, delivered and stored in transporter cooled by dry ice in aircraft	C (ab)	50					0	0	85	–
	Reheated in convection oven to simulate aircraft heating	C (ab)	182	15	25	35	–				
	Reheated in steamer (center of 6 slices between 3rd and 4th slice)	C (ac)	81	0	0	0	+				
	Assembled in serving casserole (dish) with lid	C (ac)	104	0	0	0	+	0	0	17	–
	Held hot, delivered and stored in aircraft for 193 min (60 min after replugged into aircraft)	C (ac)	168	22	118	133	–	15	22	50	–
	for 223 min (90 min after replugged into aircraft)	C (ac)	168	22	130	145	–	15	22	50	–
ROLLED ROAST BEEF											
	Reheated and stored, delivered and held in hot transporter in aircraft	C	156	0	6	16	+	28	45	91	–

TABLE 3. *Continued*

STEAK	Grilled	C	77	0	0	0	+				
	Reheated and held hot for 110 min	C	88	0	0	0	+	854	105+	115+	+
	Reheated and held, delivered and stored in aircraft for 119 min (45 min after re-plugged into aircraft)	C	142	0	0	10	+	40	61	120	–
FILET											
Cooked and chilled	Trays assembled, put in refrigerator, and taken out	C	64					0	165	165	–
	Reheated and held hot in transporter	C	136	0	0	0	+	40	75	175	–
	Reheated and held hot, delivered and stored in aircraft for 185 min (60 min after replugged into aircraft)	C/S	131	0	0	0	+	52	79	185	–
	for 215 min (90 min after replugged into aircraft)	C/S	131	0	0	0	+	52	79	215	–
	Reheated and held hot, delivered and stored in aircraft for 175 min (60 min after replugging into aircraft)	C	155	0	30	85	–	33	40	100	–
	for 205 min (60 min after replugging into aircraft)	C	164	10	60	115	–	33	40	100	–

[1]C refers to probe in geometric center; S refers to probe attached to surface
[2]Letters in parentheses (a), (b), etc., refer to probe in same sample at same location during different operations.
[3]Meal located at bottom, front of oven
[4]Meal located at middle, center of oven
[5]Meal located at top, back of oven

chicken thighs, geometric center temperatures exceeded 175 F (Table 2; Fig. 3).

During cooking of cuts of beef which varied in weight from 1.5 to 2 lb., surface temperatures ranged between 147 and 177 F and internal temperatures ranged between 110 and 127 F (Table 3; Fig. 4). The lower temperature of the surface and the temperatures at the interior permit survival of pathogenic foodborne bacteria.

Chilling and cold storage

Raw or precooked convenience foods stored in refrigerators stayed below 45 F (Tables 1, 2,3).

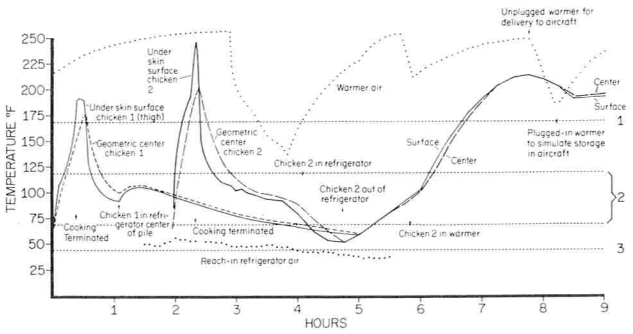

Figure 3. *Temperature of 4-ounce chicken thighs during baking in an oven, room and refrigerator storage, and holding in an electrically-heated holding oven. [1]Temperature which poultry is required to reach during cooking. [2]Temperature range for rapid bacterial growth. [3]Temperature required for refrigerated storage of foods.*

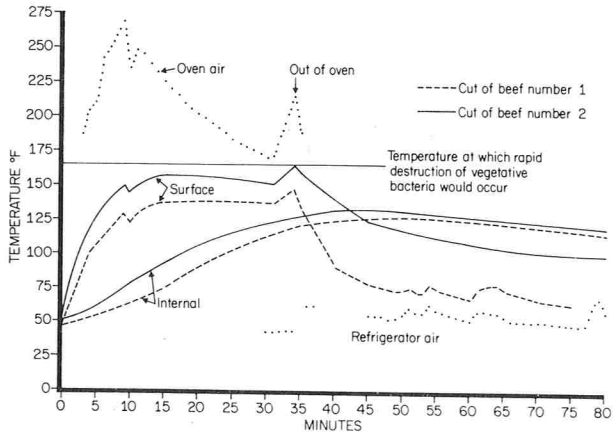

Figure 4. *Internal (geometric center) and surface temperatures of two cuts of beef (1 = 1 pound, 9-ounce; 2 = 1-pound, 14-ounce) during cooking in a malfunctioning convection oven and cooling in a cart refrigerator.*

Foods cool slowly, even in meal-size portions. Cups containing 3.5 ounces of scrambled eggs cooled faster in a walk-in refrigerator than in a cart (roll-in type) refrigerator (Table 1; Fig. 5). The cart refrigerator was opened frequently, and the unit capacity was inadequate to remove added heat.

Large quantities of foods cool slowly. For example, scrambled eggs, 5 inches deep in a 20 × 11 × 6-inch pan, were within a temperature range long enough to permit

TABLE 4. *Time-temperature exposure of geometric center of desserts during assembly, delivery, and storage on aircraft, 84 F ambient temperature*

Food	Aircraft body style	Total storage time (min)	Highest temperature F	Minutes between 70-115°F	Minutes between 60-122°F	Minutes between 45-140°F	Potential for growth (+) of foodborne disease bacteria
Lime pie	Narrow 1	150	58.5	0	0	150	–
	(metal tray carrier[1]		63	0	35	150	–
	cooled by dry		68	0	70	150	–
	ice in door)						
	Narrow 2	45	49	0	0	45	–
	(metal tray carrier[2]						
	cooled by dry						
	ice in door)	120	66	0	45	120	–
	Narrow 3	75	62	0	15	75	–
	(metal tray carrier[3]	135	72	10	90	135	–
	cooled by dry						
	ice in door)	150	72	15	90	150	–
	Narrow 4	110	65	0	40	110	–
	(metal tray carrier[4]		67	0	45	110	–
	cooled by dry		68	0	55	110	–
	ice in door)						
	Insulated wide	150 (a)[7]	42	0	0	0	–
	cart[5] cooled by	(b)	51	0	0	150	–
	dry ice activated	(c)	51	0	0	60	–
	freon heat-exchange	180 (a)	45	0	0	<1	–
	system	(b)	55	0	0	180	–
		(c)	56	0	0	90	–
Cheese cake	Narrow transporter	150 (d)	51.8	0	0	150	–
	compartment[6]	180 (d)	55.1	0	0	180	–
	cooled by	250 (d)	55.9	0	0	250	–
	dry ice on top						
	shelf						

[1]Dimensions of tray carrier not recorded
[2]13 × 18.5 × 19-inch tray carrier
[3]16.5 × 24 × 19.5-inch tray carrier
[4]18.5 × 19.5 × 20-inch tray carrier
[5]71 × 22.5 × 8-inch compartment of tray carrier
[6]27 × 24.5 × 18-inch compartment of transporter
[7]Sample identification

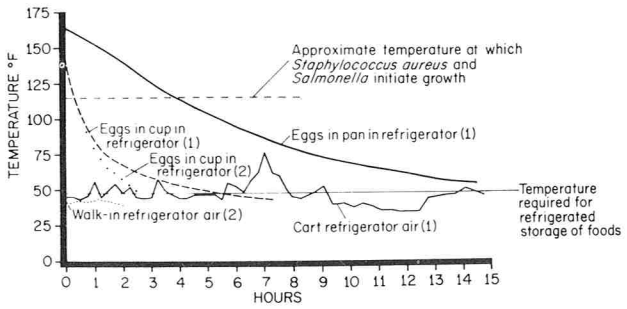

Figure 5. *Temperature of geometric center of 5-inch deep portion of scrambled eggs in a 20 × 11 × 6-inch pan and 3½ oz. portion in 3-inch diameter cup during storage in cart refrigerator and in a walk-in refrigerator.*

considerable growth of bacteria (Table 1; Fig. 5). Both the surface and interior of pieces of chicken in the middle of other warm pieces of chicken in a pan cooled so slowly that bacterial growth could have occurred (Table 2; Fig. 3).

Immediate refrigeration of heated beef reduced the post-oven rise, restricting it to a maximum of 16 F, but usually less, and causing an immediate drop in the surface temperature (Table 3; Fig. 4). When the beef was not removed for slicing and tray assembly, internal portions remained in the rapid multiplication range (70-115 F) for mesophilic bacteria, the growing range (60-122 F) for *C. perfringens,* and between 45 and 140 F for several hours (Table 3; Fig. 6).

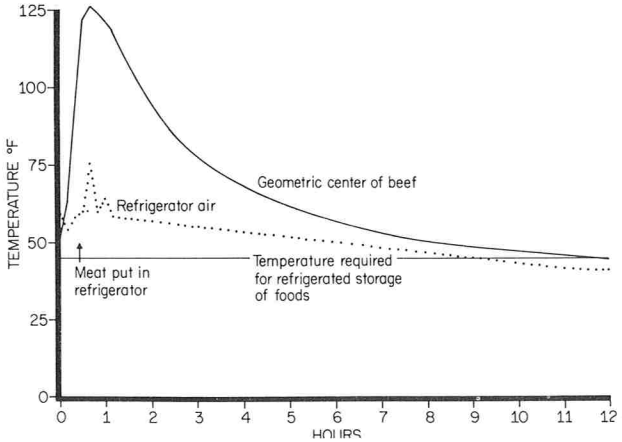

Figure 6. *Internal temperature of 1-pound, 13-ounce cut of beef in a cart refrigerator.*

Meal portions of beef that had cooled to room temperature during slicing and tray assembly, cooled rapidly during subsequent storage in a refrigerator (Table 3; Fig. 7).

The temperature of chilled foods assembled in individual-serving casseroles during kitchen or dock storage, delivery, and aircraft storage usually rose slowly. Foods in metal tray carriers with dry ice in the carriers' doors increased in temperature slowly, but continuously, during the storage period (Tables 2, 3, 4; Fig. 8, 9); so did

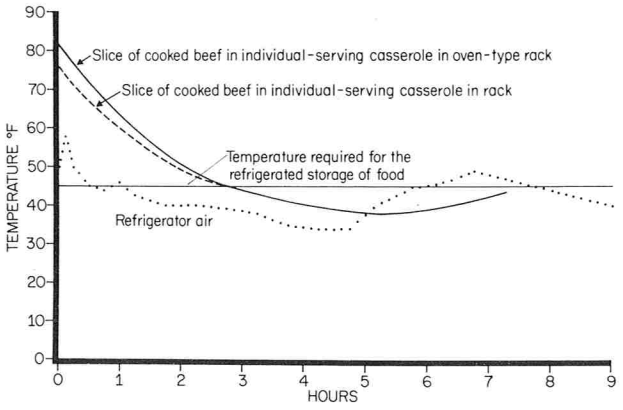

Figure 7. *Internal (geometric center) temperature of 1-ounce slice of beef in foil-covered individual-serving casserole (dish), containing four 1-ounce slices of beef, potatoes, and peas during storage in a walk-in refrigerator.*

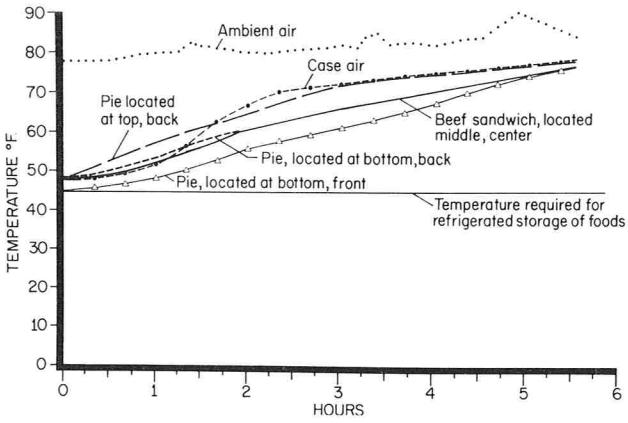

Figure 8. *Internal (geometric center) temperature of pies and roast beef sandwich during storage in narrow-body style metal tray carrier with dry ice in door, during simulated dock storage, delivery, and storage on aircraft.*

meals stored in transporters cooled with dry ice on the top shelves or in dry-ice wells (Tables 3, 4; Fig. 10). During assembly of chilled foods at room temperature, food temperatures rose above 45 F. During subsequent storage in transporters, the temperature did not get back down to 45 F, and the food temperature continued to rise slowly. The quantity of dry ice put on top shelves was insufficient to cool the air of the bottom shelf in the transporter (Fig. 10). The barriers formed by trays interfered with air circulation. The temperature of foods stored in transporters that had dry-ice-activated freon-refrigeration systems remained below 45 F for at least 90 min. (Tables 2, 3, 4; Fig. 11). The temperature of pie at the bottom rear of the cart even decreased.

Frozen storage

Frozen meals did not thaw during storage in transporters that were cooled by dry ice (Tables 2, 3; Fig. 12).

Tray assembly

During tray assembly of recently-heated foods, post-oven temperature rise occurred (Tables 1, 3). Recently-cooked (not reheated) foods sometimes even

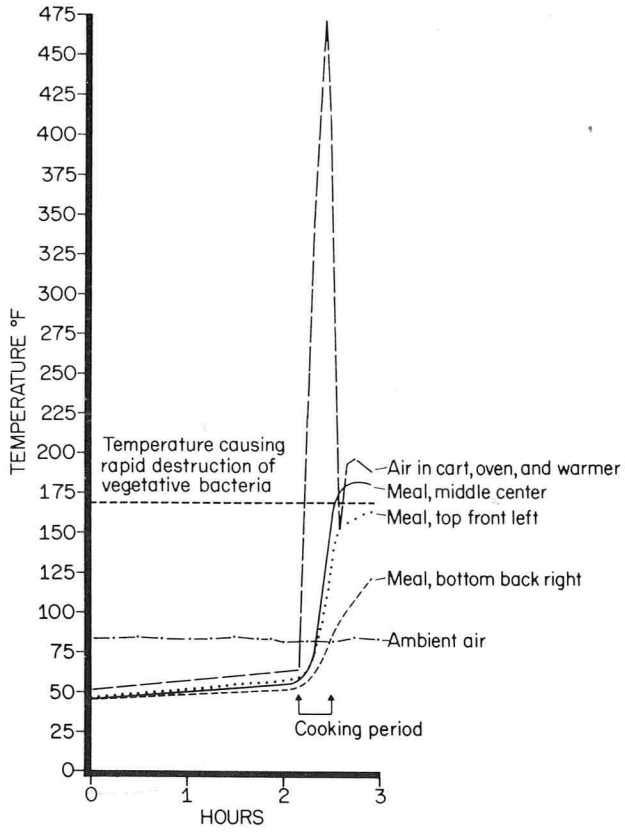

Figure 9. *Internal (geometric center) temperature of three 1-ounce slices of beef (second from bottom of four slices) in plastic dish (casserole) with foil cover in different locations in an insulated compartment of a (multicompartment) wide-body cold-storage cart during simulated dock storage, delivery to planes, storage in planes and warm-holding after cooking. (Heating was done in a narrow-body oven: setting 350 F for 20 minutes.)*

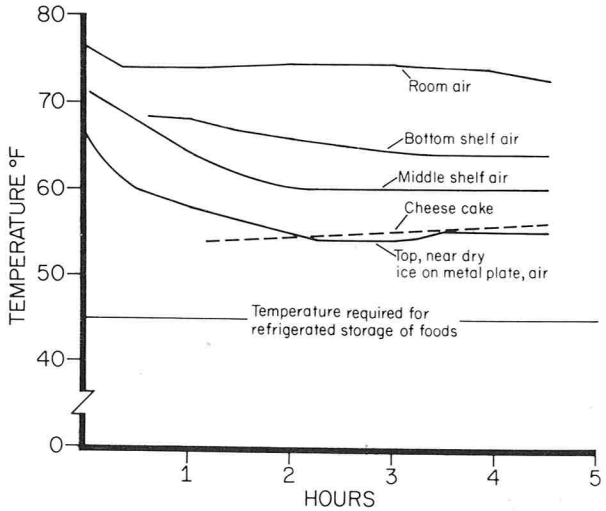

Figure 10. *Temperature of geometric center of a 2-ounce cheese cake during storage in a cold-storage transporter cooled by three 1-pound slabs of dry ice.*

reached temperatures lethal to vegetative pathogenic foodborne bacteria during the post-oven temperature rise that occurred during tray assembly. Temperatures of chilled foods rose slowly during sandwich-making or tray assembly (Fig. 3,10).

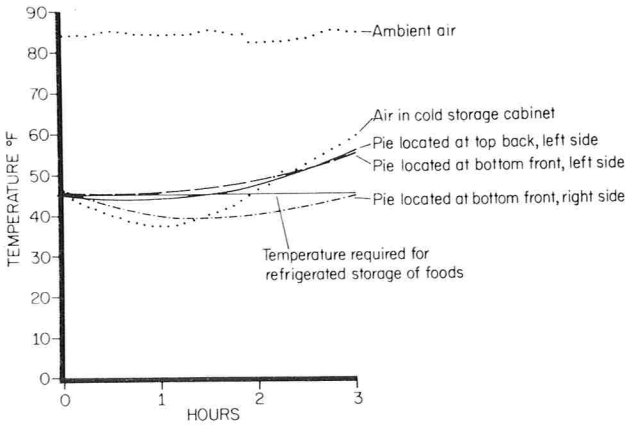

Figure 11. *Internal (geometric center) temperature of lime pies during storage in a wide-body insulated cart (compartment size 17" × 22.5" × 8") with dry ice added to top compartment during simulated dock storage, delivery, and storage on plane.*

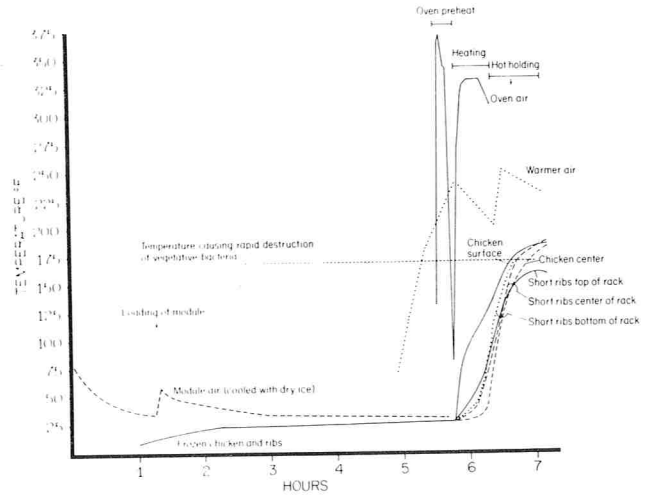

Figure 12. *Temperature of initially frozen short ribs and chicken during holding in a module cooled by dry ice, during heating in wide-body type convection oven, and holding in a warmer cart.*

Hot storage

Considerable temperature variation occurred in hot-storage transporters (Fig. 13). Unit 1, for instance, did not maintain foods at temperatures high enough to prevent growth of bacteria. In fact, it held foods at temperatures that would be optimum for growth of pathogenic foodborne bacteria. Because of the variation of the temperatures in hot-holding transporters, temperatures of the food stored in these modules also varied considerably.

Eggs during hot storage were at temperatures that would either inhibit growth of vegetative pathogenic foodborne bacteria or kill them (Table 1; Fig. 1). Scrambled eggs were at temperatures that inhibited growth of these organisms. When hot-storage transporters, set at 175 F, were used, reheated precooked, frozen omelets and baked eggs reached temperatures lethal to vegetative pathogenic foodborne bacteria. When precooked, frozen omelets and baked eggs were reheated in ovens and then held for 490 min in hot-storage transporters, set at 145 F, temperatures lethal to these organisms were also reached (Table 1; Fig. 14). The latter arrangement is hazardous when foods are thawed and then reheated in a module such as Unit 1 (Table 1; Fig. 1).

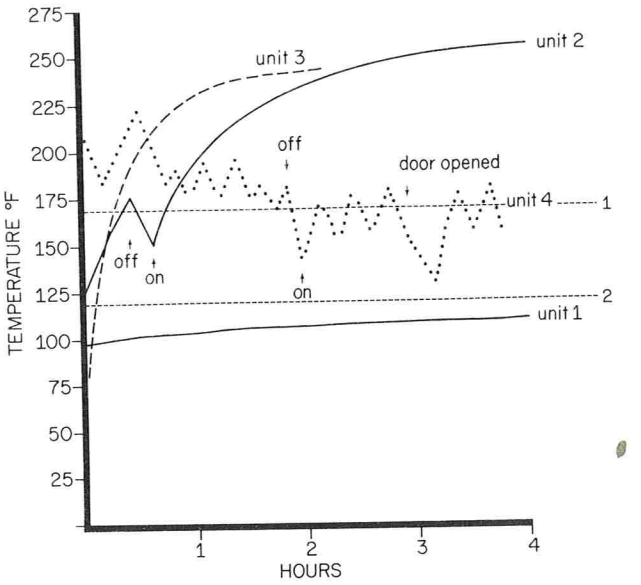

Figure 13. *Variation of air temperature of warmers.* [1]*Temperature causing rapid destruction of vegetative bacteria.* [2]*Temperature at which* Salmonella *and* Staphylococcus aureus *initiate growth.*

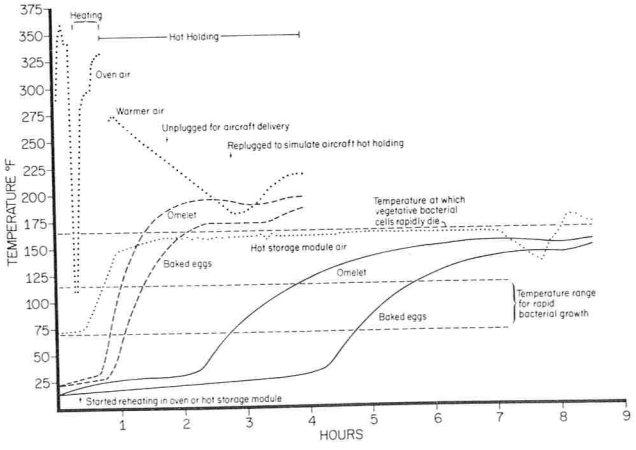

Figure 14. *Temperature of initially-frozen omelets and baked eggs during reheating in a convection oven and then holding in a warmer (set at 175 F) and during reheating in a hot storage module (set at 145 F).*

Chicken thighs reached temperatures lethal to vegetative pathogenic foodborne bacteria after they were assembled in serving casseroles and held in hot-storage transporters (Table 2). Reheated chicken with crab stuffing and sliced turkey, however, did not reach such temperatures. Time-temperature conditions necessary to kill vegetative pathogenic foodborne bacteria were reached after reheating boneless chicken and holding it 45 min; 20 min, however, did not suffice.

Three-ounce pieces of cooked beef in individual-serving casseroles sometimes failed to reach internal temperatures that would be lethal to vegetative pathogenic foodborne bacteria. Occasionally they were in

the optimal temperature ranges for multiplication for almost an hour. They were within the temperature range 45 to 140 F from 64 to 223 min (Table 3).

Reheating

Reheating frozen, precooked meals or entrees did not bring foods to temperatures lethal to vegetative pathogenic foodborne bacteria (Tables 1, 2, 3; Fig. 15). Temperatures of chicken stuffed with crab rose to only 30 F, egg products reached 50 F, and 3-ounce pieces of beef rose to 82 F at the final point of reheating. Subsequent storage of the food in hot-storage transporters increased food temperatures to a level that would be lethal to vegetative pathogenic foodborne bacteria in eggs and in some beef meals (Tables 1,3; Fig. 15).

Reheating chilled, sliced beef in a steamer raised its temperature to 81 F. During individual-serving casserole assembly, the temperature increased to 104 F (Table 3; Fig. 16). Subsequent storage of the beef in hot-storage transporters (with air temperatures fluctuating between 140 and 185 F) raised the beef temperature to 168 F.

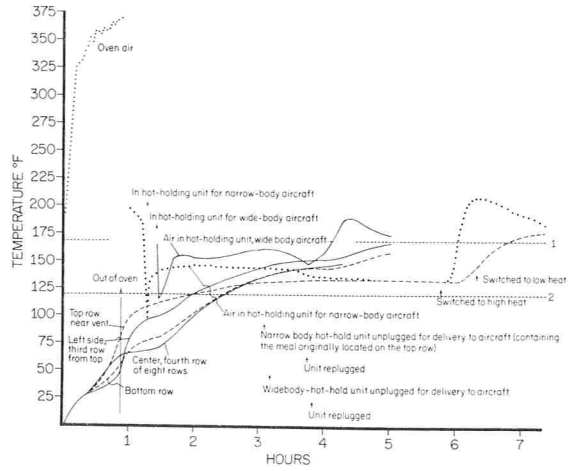

Figure 15. *Temperature of geometric center of 3-ounce piece of initially-frozen beef in plastic dish with hard-foil cover during cooking in convection oven and hot-holding in air-carrier hot-holding transporters.* [1]*Temperature causing rapid destruction of vegetative bacteria.* [2]*Temperature at which* Salmonella *and* Staphylococcus aureus *initiate growth.*

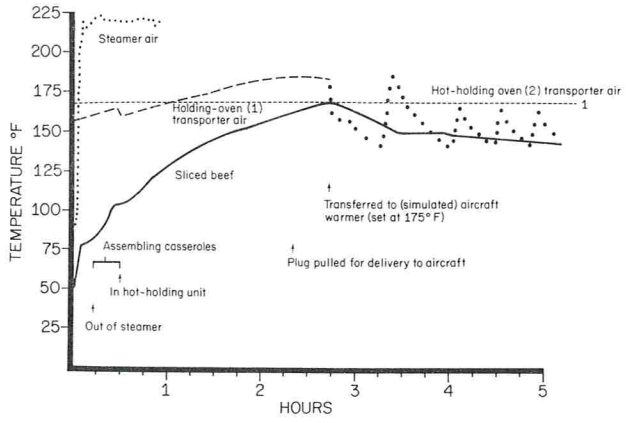

Figure 16. *Temperature in geometric center of six slices (between third and fourth slice) of cooked, chilled beef (3 ounces) during reheating in a steamer, serving casserole (dish), holding in a holding-oven transporter, and holding in a second hot-holding unit to simulate aircraft conditions.* [1]*Temperature causing rapid destruction of vegetative bacteria.*

Food was sometimes reheated in hot-storage modules. The results varied with the temperature and condition of the hot-storage transporter. Turkey rose to 144 F only after 105 min of storage (Table 2). Beef filets rose to temperatures between 131 and 164 F only. Rolled roast beef reached 156 F after 105 min; steaks reached temperatures between 88 and 142 F (Table 3).

The convection ovens on narrow-body aircraft produced food temperatures that varied with the location of the food in the ovens. Meals stored at the top rear of the oven usually rose to the highest temperature and those located at the bottom front rose to the lowest temperature—not high enough to kill vegetative pathogenic foodborne bacteria even after a post-oven temperature rise for 10 min (Table 3; Fig. 9). The fan possibly forced the air over the top of the bottom layer of meals and up away from them.

The convection ovens on wide-body aircraft gave more consistent results. Temperatures of 3-ounce pieces of frozen, precooked beef reached a temperature range of 85 to 125 F. After 20 min in a hot-storage transporter, the beef temperatures ranged from 145 to 175 F. After 45 min. of storage in a hot-storage transporter, beef temperatures increased to the 158 to 185 F range (Table 3; Fig. 12). Internal temperatures of boneless chicken ranged from 72 to 88 F after reheating, increased to the 134 to 148 F range after 20 min, and to the 167 to 180 F range after 45 min of storage in a hot-storage transporter (Table 2; Fig. 12). The meals served immediately to up to 45 min after reheating would not reach a temperature of 165 F.

CONCLUSIONS

Although most of the data show that the surveyed airline caterers produced safe meals, there were a few indications that had some of the foods been contaminated with pathogenic foodborne bacteria, the pathogens would have survived cooking and reheating and multiplied excessively during cold- or hot-storage. Scrambled eggs stored in pans to a depth of 5 inches, 1-pound, 13-ounce cuts of roast beef cooled in a cart refrigerator, and cooked chicken thighs stored in a reach-in refrigerator are examples. Periods during which many of the prepared foods were exposed to temperatures within the 45 to 140 F range were also excessively long.

Some equipment was inadequate to maintain temperatures of foods above 140 and below 45 F. Hot-storage transporters were occasionally either of a capacity producing inadequate heat or were in poor condition. Cold-storage transporters, other than those with the dry-ice-activated freon-refrigeration systems, did not maintain a temperature of 45 F or lower during kitchen and dock storage, delivery, and aircraft storage. The amount of dry ice used for cooling was inadequate to maintain cold air temperatures.

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Heat Resistant Psychrotrophic Bacteria in Raw Milk and Their Growth at 7 C

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ABSTRACT

Microbiological data are presented for 109 raw milk samples, and for the same samples after heat treatment at 80 C for 12 min, and upon subsequent storage at 7 C for 7, 14, and 28 days. The Standard Plate Count of the raw milk averaged 110,000/ml with 65% of these organisms being penicillin-resistant. Immediately after heat treatment, 87% of the samples had psychrotrophic spore counts < 10/ml. After 14 and 28 days of storage, 50 and 83% of the samples had psychrotrophic counts ≥ 100,000/ml. It was concluded that growth of heat-resistant psychrotrophic organisms may cause spoilage of heated milk. No relationship could be demonstrated between gram-negative counts of raw milk, or initial mesophilic spore counts of heated milk, and bacterial numbers in heated, stored milk.

Martin et al. (*13*) encountered sporeforming microorganisms in 285 of 287 Ohio milk samples which had been heat-treated at 80 C for 10 min. Seventy-five percent of the samples had mesophilic spore counts in excess of 1,000/ml. Approximately 80% of the sporeformers were *Bacillus licheniformis* or *Bacillus cereus.*

Maxcy (*14*) examined fresh pasteurized packaged milk and found the distribution of microorganisms as follows: 40% gram-positive nonsporeforming rods, 32% micrococci, 22% bacilli, 5% streptococci, and 1% coliforms. Credit et al. (*4*) identified 84% of the bacteria in commercially pasteurized milk stored at 4.5 C for 30 days as members of the genus *Bacillus.*

The sweet curdling defect has been associated with high heat-treated milk and milk products (*5*). The defect is attributed to a rennin-like enzyme produced by sporeforming organisms, particularly *B. cereus* (*8,9,10*). Overcast and Atmaram (*15*) showed that 28% of commercially pasteurized milk samples stored for up to 10 days at 7 C exhibited sweet curdling due to *B. cereus.*

In 1969, Grosskopf and Harper (*6*) reported the presence of psychrophilic sporeformers in pasteurized and "sterilized" milks. In a subsequent paper (*7*), they demonstrated the psychrotrophic nature of these organisms and their apparent loss of ability to grow at 4 C after maintenance in Tryptic Soy broth at 21 C for several weeks.

Other workers (*3,16,17,18*) have also reported on the presence and selected growth characteristics of psychrotrophic sporeformers in pasteurized milk. The percentages of raw milk samples containing psychrotrophic sporeformers ranged from 25 (*6*) to 25 to 35 (*16*) to 83 (*3*). Excessive growth of gram-negative bacteria in milk before processing may have a stimulatory effect on subsequent growth of psychrotrophic sporeformers (*15*).

This study was undertaken to (*a*) ascertain the extent of sporeforming microorganisms in raw milk supplies and their ability to grow out at different incubation temperatures initially and following storage of heated (80 C-12 min) milk samples at 7 C for extended periods and (*b*) determine whether any relationship exists between gram-negative counts of the raw milk and counts of the heated milk initially and upon subsequent storage.

PROCEDURE

Raw milk samples

Over an 18-month period, Grade A raw milk samples (100-150 ml) from individual producers or bulk tank truck were collected in sterile plastic sample bags, iced, and shipped to the laboratory. Samples not analyzed immediately were stored at 2-4 C for a period not exceeding 48 h.

Just before analysis, approximately 100 ml of each sample were transferred from the plastic bag into a previously sterilized screw-cap milk dilution bottle (Kimax 14925). Each raw milk sample was subjected to the following microbiological analyses: (*a*) Standard Plate Count (*2*) and (*b*) gram-negative counts as suggested by Lightbody (*11,12*) using Standard Methods agar to which was added at 45 C sufficient filter-sterilized potassium penicillin G to yield a final concentration of 5 units of penicillin/ml of agar. Plating procedures here followed Standard Methods (*2*) excepting plates were incubated at 21 C for 72 h.

Heated milk samples

The raw milk samples contained in the milk dilution bottles were placed in a thermostatically controlled ethylene glycol bath. The level of the ethylene glycol was at least 5 cm above the level of the milk. The milk, with intermittent shaking, was heated to 80 C and held for 12 min after attaining the desired temperature. The temperature was monitored by use of a milk dilution bottle containing an equivalent amount of water and an immersed mercury thermometer. Immediately following the heat treatment, milk samples were cooled rapidly in ice

water. Spore counts were done on the samples following Standard Methods (*2*) but using Standard Methods agar containing 0.1% soluble starch. Plates were incubated at three different temperatures: (*a*) mesophilic counts at 32 C for 48 h (*b*) intermediate counts at 21 C for 72 h, and (*c*) psychrotrophic counts at 7 C for 10 days.

Stored heated milk samples

After the initial platings were completed, heated milk samples were stored at 7 C in a Forma Model 2095 ethylene glycol bath for 7, 14, and 28 days. The heated stored milk samples did not receive additional heat treatments. At the end of the 7-day storage period, analyses were made for mesophilic, intermediate, and psychrotrophic counts. For the 14- and 28-day storage periods, only psychrotrophic counts were made. Every effort was made to prevent recontamination of the samples.

RESULTS AND DISCUSSION

The Standard Plate (mesophilic) Counts of the raw milk ranged from 2,500 to 2,300,000/ml, with an average of 110,000/ml (Table 1). Seventy-nine percent of the raw milk samples had mesophilic counts of <100,000/ml with 66% of the samples having counts of <50,000/ml (Table 2). Thus, most of the raw samples met Ohio Standards for Grade A raw milk for pasteurization which specify that bacterial limits for individual producer milk shall not exceed 100,000/ml.

TABLE 1. *Microbiological quality of raw milk and of the same milk upon heat treatment at 80 C for 12 minutes and subsequent storage at 7 C.*

Sample and plating procedure	No. samples	Microbial numbers	
		Range	Average[c]
		—(CFU/ml)—	
Raw milk			
Standard Plate Count	109	2.5 T-2.3 M	110 T
Gram-negative count	109	80-1.0 M	72 T
Heated milk			
Initial			
Mesophilic	109	<1-1.5 T	170
Intermediate	109	<1-830	76
Psychrotrophic	109	<1-140	7
7-day storage			
Mesophilic	109	1-140 T	1.8 T
Intermediate	109	<1- 30 T	700
Psychrotrophic	109	<1- 10 T	340
14-day storage[a]			
Psychrotrophic	80	<10-330 M	10 M
28-day storage[b]			
Psychrotrophic	93	<10-180 M	20 M

[a]Counts were not done on the first 29 samples
[b]Counts were not done on 16 of the last 44 samples because the 14 day counts were ⩾1 M/ml
[c]Arithmetic averages were calculated

TABLE 2. *Distribution of mesophilic and gram-negative counts of raw milk samples*[a]

Range of counts	Mesophilic counts	Gram-negative counts
—(CFU/ml)—	—(% of samples)—	
<1 T	0	17
⩾1 T-<5 T	6	25
⩾5 T-<10 T	11	14
⩾10 T-<25 T	33	16
⩾25 T-<50 T	16	5
⩾50 T-<100 T	13	8
⩾100 T-<250 T	11	7
⩾250 T-<500 T	5	3
⩾500 T-<1 M	3	4
⩾1 M	2	1

[a]Data are for 109 raw milk samples

The gram-negative counts as determined by the Penicillin agar plate method (*11,12*) ranged from 80 to >1,000,000/ml with an average of 72,000/ml (Table 1). Thus, a high percentage (65%) of the organisms counted by Standard Methods procedure was resistant to penicillin. It should be noted that average counts may be misleading because 77% of the raw milk samples had gram-negative counts of <50,000/ml and 56% had counts of <10,000/ml (Table 2).

Upon heat treatment of the raw milk at 80 C for 12 min, approximately 88% of the samples contained 10 or more sporeformers/ml which were capable of outgrowth at mesophilic (32 C) or intermediate (21 C) incubation temperatures (Table 3). The average mesophilic spore count was 170/ml and the average intermediate spore count was 76/ml.

TABLE 3. *Distribution of initial mesophilic, intermediate, and psychrotrophic spore counts of heated milk*[a]

Range of counts	Mesophilic	Intermediate	Psychrotrophic
—(CFU/ml)—		—(% of samples)—	
<1	1	2	39
⩾1-<2	3	3	19
⩾2-<10	9	7	29
⩾10-<100	60	68	11
⩾100-<1 T	24	20	2
⩾1 T	3	0	0

[a]Data are for 109 samples

Only 13% of the heated samples had psychrotrophic spore counts of 10 or more/ml (Table 3). Thirty-nine percent of the samples had counts of less than 1/ml. The highest psychrotrophic spore count found was 140/ml. The presence of sporeforming bacilli was confirmed by microscopic examination of growth on Brain Heart Infusion agar slants prepared by streaking colonies picked from psychrotrophic plates.

Following storage of the heated milk for 7 days at 7 C (Table 4), 92, 85, and 58% of the samples had mesophilic, intermediate, and psychrotrophic counts, respectively, of 10 or more/ml. The percentages of milk samples having mesophilic and intermediate counts ⩾ 10/ml were about the same as that observed initially, but the percentage of psychrotrophic samples in the ⩾ 10/ml count range had increased from 13 to 58, and in the ⩾ 100/ml range, the increase was from 2 to 28%. The average mesophilic, intermediate, and psychrotrophic plate counts of the 7-day stored milk samples were 1,800, 700, and 340/ml respectively (Table 1). The mesophilic and intermediate counts had increased 10-fold, whereas the psychrotrophic counts had increased 50-fold.

Only psychrotrophic counts were made of the heated milk following storage for 14 and 28 days (Table 5). At 14 days storage, 50% of the samples had psychrotrophic counts ⩾ 100,000/ml and 34% contained ⩾ 1,000,000/ml. At 28 days, 83% of the samples had counts ⩾ 100,000/ml and 71% ⩾ 1,000,000/ml. These data reveal that heat treatment of milk at 80 C for 12 min with storage at 7 C will not ensure a product of unlimited keeping quality.

TABLE 4. *Distribution of mesophilic, intermediate and psychrotrophic counts of heated milk stored for 7 days at 7 C.*[a]

Range of counts	Mesophilic	Intermediate	Psychrotrophic
—(CFU/ml)—		—(% of samples)—	
<1	0	6	26
⩾1-<2	1	3	3
⩾2-<10	7	6	13
⩾10-<100	41	46	30
⩾100-<1 T	38	32	19
⩾1 T	13	7	9

[a]Data are for 109 samples

TABLE 5. *Distribution of psychrotrophic counts of heated milk stored for 14 and 28 days at 7 C*

Range of counts	Stored for 14 Days[a]	Stored for 28 Days[b]
—(CFU/ml)—	—(% of samples)—	
⩽10	19	3
⩾10-<50	2	3
⩾50-<100	9	0
⩾100-<1 T	5	3
⩾1 T-<10 T	4	4
⩾10 T-<100 T	11	4
⩾100 T-<1 M	16	12
⩾1 M-<10 M	19	31
⩾10 M	15	40

[a]Data are for 80 samples. No 14 days counts were made of the first 29 samples

[b]Data here are for 93 samples; counts were not done on 16 of the last 44 samples because the 14 day counts were ⩾1 M/ml.

Statistical analysis revealed no significant relationship between the gram-negative counts of the raw milk and mesophilic, intermediate, or psychrotrophic plate counts of the same milk upon heat treatment and storage (Table 6). Most (72%) of the samples had gram-negative counts of <25,000/ml. Therefore, it is unlikely that the number of gram-negative organisms would have produced sufficient stimulatory agents for spore outgrowth as noted by Overcast and Atmaram (*15*). However, Adams and coworkers (*1*) have shown that numbers as low as 10,000/ml could produce measurable amounts of heat-resistant proteases in milk. Normally, gram-negative organisms would be destroyed by proper pasteurization (*19*).

TABLE 6. *Relationship between the gram negative counts of raw milk and the mesophilic, intermediate and psychrotrophic plate counts of heated milk stored at 0, 7, and 14 days at 7 C*

Days - heated milk stored	Plate count incubation conditions	Correlation coefficient[a]
0	Mesophilic	0.0760
	Intermediate	0.0312
	Psychrotrophic	0.1232
7	Mesophilic	-0.0260
	Intermediate	-0.0346
	Psychrotrophic	-0.0214
14	Psychrotrophic	-0.0712

[a]Linear correlation coefficients were determined by regression analysis

Also, no significant relationship could be demonstrated between the initial mesophilic spore count of the raw milk and the number of organisms present in the milk upon storage (Table 7). Thus mesophilic spore counts are not good indicators of the keeping quality of heated milk.

TABLE 7. *Relationship between the initial mesophilic spore count of heated milk and the number of organisms present in the same milk after 7 and 14 days storage at 7 C*

Days - heated milk stored	Plate count incubation conditions	Correlation coefficient[a]
7	Mesophilic	0.0174
	Intermediate	0.0542
	Psychrotrophic	0.3832
14	Psychrotrophic	-0.0278

[a]Linear correlation coefficients were determined by regression analysis

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Effect of Several Factors on Total and Differential Counting of Somatic Cells in Farm Bulk Tank Milk

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ABSTRACT

Samples from approximately 1000 bulk tanks from widely separated areas of Ontario were examined over a period of 1 year. Total and differential somatic cell content was estimated electronically. A high degree of reproducibility was shown both in total cell count, and percent of cell volume in specific size ranges (channels) determined with a Coulter Model TA electronic counter. Use of a single count per dilution made little practical difference as compared to using the average of two counts per dilution. There were no significant differences between cell count means and cell volume percentages in channel 8 (cells from 89.2 to 178.3 μm^3) from 24- and 48-h-old samples from either fluid or industrial shippers. There was a small significant difference in both measurements in duplicate samples shipped 275 miles before and after formalin fixation, and the cell volume in channel 8 was markedly reduced by shipping samples the same distance. Data from a small group of herds which were quarter sampled and submitted for mastitis laboratory diagnosis indicated the possibility that the percentage of cell volume in channel 8 found in bulk tank milk may be a better indicator of infection incidence in a herd than the total cell count alone.

Estimation of numbers of somatic cells in farm bulk tanks by the method of Pearson et al. (*3*), modified by use of buffered formalin fixative, was found to be accurate and reproducible (*1*). An extension of this method to differentiate the cells counted by size based on volume has been described (*2*). This was made possible by using a Model TA Coulter Counter (Coulter Electronics, Hialeah, Fla.) which sizes counted particles into 16 groups or channels, and instantly produces a histogram visible on an oscilloscope, and, if desired, plotted on paper. The minimum size of individual cells in each channel is one half of that in the next higher channel and so on. Using 10 of the 16 possible channels, i.e. numbers 7 to 16, typical histograms or patterns for normal milk, colostrum, and milk from infected quarters were described. In addition bulk tank milks with similar patterns to all three types of milk were found; some tanks of milk with mean somatic cell counts of less than 500,000/ml giving patterns identical to those from infected quarters. Quarter sampling of such herds confirmed high incidences of infected cows and quarters (*2*).

The height of each channel of the histogram indicates the percentage in that channel of the total volume of the cells counted by the machine in the sample passing through the orifice. With milk from infected quarters, it was found that the greatest change occurred in the percentage of cell volume in channel 8, which includes those cells with individual volumes from 89.2 to 178.3 μm^3, equivalent to spheres with diameters between 5.54 and 6.98 μm. These sizes are rather lower than one would expect for neutrophils, but result from shrinkage following formalin fixation.

In addition some limited data are presented from a small group of herds from which cow quarter samples were taken, and mean bulk tank total somatic cell counts and percentage volumes in channel 8 over a number of months were available.

Reported here are results of the application of the method to approximately 1000 bulk tanks from three areas of Ontario over a period of approximately one year, and some effects of source and age of the milk and of shipping samples under different conditions.

MATERIALS AND METHODS

Samples

To demonstrate reproducibility of volume percentages in channels, 16 separate sample were taken from each of two farm tanks by a fieldman for the Milk Industry Branch of the Ontario Ministry of Agriculture and Food (OMAF). All other samples were taken by milk transport drivers routinely picking up milk from farm bulk tanks containing four milkings at the time of pick-up. They were from three areas of Ontario: A, farms approximately 50-75 miles west of Guelph; B, farms supplying milk to plants in the city of Guelph; and C, farms approximately 275 miles east of Guelph. Samples from area A were collected at one point and brought to the Guelph Laboratory in refrigerated closed vans by OMAF personnel. At the time of delivery to the laboratory, half of these samples were 24 h old and half 48 h old, i.e. after they were taken. Samples from area B were obtained directly from the local plants and were no more than 24 h old. All samples from Areas A and B were fixed and counted at the Guelph laboratory. Samples from Area C were routinely fixed at the regional OMAF Veterinary Services Branch (VSB) Laboratory and then shipped by commercial courier un-iced in insulated containers to Guelph for counting. For comparative purposes, as indicated later, some samples were shipped unfixed, and iced in insulated containers.

Milk in Ontario is bought by the Ontario Milk Marketing Board either for fluid consumption (Pool 1) or manufacturing purposes (Pool 2). The samples examined in these experiments came from both pools.

Electronic counting

All samples were fixed, diluted, and counted with a Coulter Model TA Counter as described previously (*1*). Percentages of cell volume in channels were read from the digital display of the counter.

Statistical analysis

Analyses of variance, significance of differences between means as determined by the Scheffe procedure, and paired t-tests were carried out by standard programs at the University Insititute of Computer Science.

Description of esperiments

(*a*) Single total somatic cell counts and percentages of cell volume in channels 7 to 12 were determined in each of 16 samples taken from two farm bulk tanks, and also in 16 replicates from one sample of the original 16 from each tank.

(*b*) It has been our practice to make two machine counts on each diluted sample, originally to overcome slight differences in count with a switch in polarity of the orifice current, and/or reduce the inherent variation produced by the Poisson distribution of cells in suspension. These were then averaged for estimating the total cell count per ml of milk. With the possibility of automation, for which it would be more convenient to use a single count fed directly to a computer, a two-tailed t-test was done on the first and second machine counts of 8648 samples.

(*c*) As described above, some samples from Area A were 24 h old, while others were 48 h old when delivered to the laboratory. Resulting from the pick-up schedule, there were approximately equal numbers of 24- and 48-h samples from any one farm during the year. While this did not provide a direct comparison of the cell counts and volume percentages from duplicate 24- and 48-h samples, it was possible to compare the means and their variances from two large groups of samples of the two ages from both Pool 1 and Pool 2 milks.

(*d*) (i) Milk samples coming from a distance into a laboratory for counting could be shipped either fixed or unfixed. A group of samples from Area C were utilized in a comparison of counts and volume percentages obtained from duplicate samples of which one was shipped after fixation and the other shipped unfixed and iced.

(ii) In a further experiment, samples were split at the regional laboratory in Area C; one subsample was fixed and counted there, while the other was fixed and then shipped to the Guelph laboratory for counting. This provided a comparison between counts obtained from samples close to their origin and those shipped a long distance.

(*e*) Individual quarter samples were taken by an OMAF fieldman from cows in 46 herds in Area A for which mean bulk tank data were available for periods of 10-12 months. The quarter samples were subdivided into two subsamples, one of which was submitted to the VSB Guelph laboratory for mastitis diagnosis. Quarters were classified as negative, i.e. in the milk no increased California Mastitis Test (CMT) reaction was demonstrable; or as positive, i.e. in the milk a CMT 1+ or more was found and pathogens were isolated. The other subsample was used to determine somatic cell counts and percentages of volume in channel 8, from which were calculated the means for all those samples classified as negative and all those positive.

RESULTS

Reproducibility of total cell counts and percentages of cell volume in 6 channels in replicate samples from two bulk tanks

From the data in Table 1, it can be seen that not only were the total somatic cell counts highly reproducible, but that the percentages of cell volume in the recorded channels were also reproducible. It should be noted that in tank sample 1, the standard error among the 16 counts on a single sample was much lower than that among the 16 tank samples, which suggests that at least some of the latter variance resulted from the original sampling of the tank.

Mean difference between first and second counts on single diluted samples

As indicated above, two counts were made on each diluted and prepared sample placed in the cuvette of the counter. The means of these counts from the three areas are shown in Table 2. The second count in samples from all areas was, on the average, slightly higher than the first, the difference being significant in two of the three areas. However, since the total somatic cell count/ml of milk has been calculated by multiplying by 100 the sum of count 1 and count 2, the mean difference in cell count/ml, if only the first count were used, would have been -1060, -3680 and -4610 for Areas A, B and C respectively.

TABLE 2. *Mean differences between first and second instrument counts on single dilutions of 8648 samples from three areas of Ontario*

Area	A	B	C
No. of samples	2222	4199	2227
Count 1 mean	3167.7	3195.9	3514.1
Count 2 mean	3178.3	3232.7	3560.2
Difference between means	10.6	36.8	46.1
S.E. of difference	±4.30	±2.03	±1.34

[1] Figures given indicate the means of the actual numbers of cells counted in 0.5 ml of a 1:100 dilution of original milk sample.

Comparison of 24- and 48-h-old samples

The results are shown in Table 3. The mean cell counts

TABLE 1. *Variation in total cell counts and percent of cell volume in 6 channels in duplicate samples from 2 bulk tanks*

No. and type of sample	Mean count ± S.E. (× 10^3)	% of Cell Volume in Channels ± S.E.					
		7	8	9	10	11	12
Bulk tank 1							
16 separate samples from tank. 1 count each	1185 ±8.03	11.7 ±0.28	36.2 ±0.98	12.1 ±0.38	8.1 ±0.2	8.1 ±0.25	7.2 ±0.28
16 counts from 1 of above	1149 ±2.23	12.2 ±0.18	36.3 ±0.38	11.8 ±0.2	7.6 ±0.13	7.1 ±0.2	7.3 ±0.2
Bulk tank 2							
16 separate samples from tank. 1 count each	502 ±3.23	7.5 ±0.18	23.3 ±0.28	9.5 ±0.2	9.8 ±0.28	13.1 ±0.33	13.7 ±0.25
16 counts from 1 of above	499 ±2.15	7.4 ±0.18	23.1 ±0.25	9.0 ±0.15	9.4 ±0.28	12.6 ±0.25	13.7 ±0.13

TABLE 3. *Comparison of mean somatic cell counts in 24- and 48-hour-old samples from Pool 1 and Pool 2 bulk tanks*

Sample source	No. of samples	Mean Count ± S.E. ($\times 10^{-3}$)	Mean % in chan. 8 ± S.E.
Pool 1 Milks			
24-h-old	1666	641,951 ± 8537	32.66 ± 0.27
48-h-old	1614	631,508 ± 7250	31.04 ± 0.29
Pool 2 Milks			
24-h-old	645	610,440 ± 12,776	32.66 ± 0.43
48-h-old	640	681,735 ± 33,667	32-93 ± 0.49

obtained from 24- and 48-h-old milks were not significantly different (p 0.05), nor were the mean cell counts of Pool 1 and Pool 2 milks. However, the standard error of the mean of 48-h-old Pool 2 milks was somewhat higher than those of the other three groups. This might be related to a previous observation that in a small group of 48-h Pool 2 milks there was a comparatively low correlation between the Electronic Count and DMSCC, although the mean counts for the two methods were almost identical. There were no differences in the mean percentage volumes in channel 8 among the four groups of samples, although the standard errors were somewhat higher for both Pool 2 means.

Comparison of total somatic cell counts and percent of cell volume in channel 8 from duplicate subsamples shipped from a distant laboratory before and after fixing

The comparative results obtained are shown in Table 4. While the mean differences in both parameters were

TABLE 4. *Comparison of total somatic cell counts and percent of cell volume in channel 8 in 283 pairs of subsamples shipped before and after formalin fixing*

	Fixed not iced	Unfixed iced	Mean difference ± S.E.
Mean count ($\times 10^{-3}$)	721.64	776.92	55.3 ± 14.97*
Mean % in channel 8	34.62	30.82	3.53 ± 0.58*

* $p > 0.01$

statistically significant, it is doubtful if either has practical significance.

Comparison of mean total somatic cell counts and percent of cell volume in channel 8 obtained in replicate subsamples at a regional laboratory and after shipment to a distant central laboratory

One hundred and twenty-one bulk tank samples were split into duplicate subsamples at the regional laboratory. Both were fixed and one subsample was counted there, while the second subsample was shipped to Guelph for counting. The Coulter Counter used at the regional laboratory was carefully calibrated before being taken there, to agree with the instrument in use at Guelph. Counts at both locations were made by the same technician. The results are in Table 5. The slightly lower

TABLE 5. *Comparison of mean total somatic cell counts and percent of cell volume in channel 8 in 121 duplicate subsamples of bulk tank milk, determined at two laboratories*

	Regional laboratory	Guelph laboratory	Mean difference ± S.E.
Mean count ($\times 10^{-3}$)	681.56	636.90	44.65 ± 9.74*
Mean % in channel 8	39.64	28.73	10.90 ± 0.91*

* $p > 0.01$

count obtained at the Guelph laboratory, while statistically significant, had small practical importance. Of considerable importance was the decrease in mean percent volume in channel 8 in those samples counted at Guelph, after shipment by commercial courier approximately 275 miles. This would have to be taken into account in any program involving such sample handling.

Mean somatic cell counts and percentage volumes in channel 8 in milks from negative and positive quarters

Results obtained are shown in Table 6. Values for

TABLE 6. *Mean somatic cell counts and percentages of cell volume in channel 8 in milk from positive and negative quarters on 46 Ontario farms*

Quarter infection status	No. of quarters	Mean cell count ± S.E. ($\times 10^{-3}$)	Mean %vol. ± S.E. in channel 8
Negative	3026	283.01 ± 6.37	15.65 ± 0.19
Positive all infections	2328	1117.76 ±31.11	32.48 ± 0.31
Positive *S. agalactiae*[1]	560	1676.99 ± 81.01	39.58 ± 0.55
Positive *S. aureus*	1104	945.47 ± 36.09	31.72 ± 0.45
Positive other streptococci[2]	559	955.84 ± 60.61	28.52 ± 0.62

[1] CAMP positive, Esculin negative streptococci
[2] Esculin positive streptococci

negative quarters are probably affected by inclusion of early and late lactation cows, since no allowance was made for stage of lactation in the diagnostic classification. In positive quarter milks there was a higher coefficient of variation in total cell count than in percentage of volume in channel 8, the figures being 134.28 and 46.2%, respectively.

The similarity of the mean percentage volume in

TABLE 7. *Comparison of percent of months in which cell count and percent volume in channel 8 exceeded arbitrary limits in herds with and without* S. agalactiae infections

Infections present	No. farms	Mean % Quarters Infected[1] ± S.E.	Mean % of months bulk tank exceeded[2] ± S.E.		Students' "t"
			750,000 cells/ml	30% in ch. 8	
S agalactiae[3]	24	16.1 ± 2.31[4]	31.3 ± 5.59	58.6 ± 5.7	2.93[6]
All other infections[5]	16	28.4 ± 5.0	5.9 ± 1.73	24.7 ± 6.28	2.88[6]

[1] Positive quarters found on a single quarter sampling
[2] Mean number of months tanks sampled: 11 for *S. agalactiae* herds, 10 for other herds
[3] Herds with some *S. agalactiae* infections; other infections may or may not have been found
[4] *S. agalactiae* quarters only
[5] Herds with no *S. agalactiae* found
[6] $p = 0.01$

channel 8 in the positive samples and those in bulk tank milk (Table 3) is striking, although the mean total somatic cell counts were higher in milk from infected quarters. This suggests that percent of volume in channel 8 in bulk tanks may be a better indicator of infection incidence than cell count alone. This hypothesis, which needs positive data from a large group of farms for confirmation, is supported by the data in Table 7, which show that an arbitrarily selected limit of 30% of cell volume in channel 8 was exceeded significantly more often in bulk tank milk from farms both with and without *Streptococcus agalactiae*-infected quarters, than was a limit of 750×10^3 cells per ml in the same tanks. In a group of 60 tanks, selected from Area A, from which over a period of 10 to 12 months the milk averaged 528.6×10^3 (S.E. 32.24) cells/ml the mean percent in channel 8 was 27.6 (S.E. 2.27), which suggests that not a little bulk tank milk with somatic cell counts below 500×10^3/ml is essentially similar to milk from infected quarters.

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Thermal Inactivation of *Clostridium perfringens* Enterotoxin

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ABSTRACT

Thermal inactivation studies of *Clostridium perfringens* enterotoxin revealed that biological activity was destroyed within 5 min at 60 C, whereas about 10% or less residual serological activity could be detected even after 80 min of exposure at 60 C in saline or in phosphate buffer, pH 7.0 or 8.0. Loss of serological activity was more rapid at 60 C at pH 5.4 or 6.0 than at pH 7.0 or 8.0. Flocculation of enterotoxin was visible in phosphate buffer after 20 min of exposure at 60 C, pH 5.4, 70 min at pH 6.0 but not at all at pH 7.0 or 8.0. Rapid loss of serological activity also occurred at 60 C in cooked turkey, chicken gravy, beef gravy as well as in 5, 10, and 20% bovine serum albumin and gelatin. Up to about 12% of the heat-inactivated serological activity could be recovered by treating toxin in the food samples with urea for 1 h at room temperature. However, serological activity of toxin heated in phosphate buffer could not be reactivated by urea treatment.

The enterotoxin of *Clostridium perfringens* is produced during sporulation (*2, 3, 5*) and has a molecular weight of approximately 35,000 with an isoelectric point of 4.3. Although this toxin is not normally thought to be preformed in foods, recent evidence indicates that in some outbreaks preformed toxin in foods may be responsible for symptoms that occur earlier than 5-6 h after ingestion of contaminated food (*9, 10, 11*). It has been well-documented that cell-free enterotoxin experimentally administered to human subjects and monkeys can induce food poisoning symptoms (*4, 13, 15*). Recently we have demonstrated preformed *C. perfringens* enterotoxin in various foods inoculated with enterotoxigenic strains (*8*), as well as in food from a documented *C. perfringens* outbreak. Because of the potential involvement of preformed toxin in a food poisoning outbreak, the present investigation was made to determine the thermal stability of *C. perfringens* enterotoxin in different environmental conditions.

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MATERIALS AND METHODS

Toxin production

C. perfringens type A strain, National Collection of Type Cultures (NCTC) 8239 was grown in Duncan and Strong (DS) sporulation medium (*1*) for 6-8 h. Cells were harvested and sonicated to produce crude cell extract (CCE) (*1, 12*). Enterotoxin in the CCE was quantified by counterimmunoelectrophoresis as described by Naik and Duncan (*7*).

Heat inactivation of enterotoxin in foods

Ten μg of enterotoxin/g was added to moist cooked turkey (*8*), pH 6.4 and macerated with a mortar and pestle. Fifty grams of this mixture were distributed into half-pint blending jars which were then heated in a water bath at 60 C. One-ml quantities of commercially canned chicken and beef gravy, pH 6.46 and 6.07, respectively, containing 10 μg of enterotoxin/ml were placed in 13 × 100-mm test tubes and also heated at 60 C. Come-up time was determined using a thermocouple. Samples were removed from the water bath at 0, 5, 10, 20, 30, 40, 50, 60, 70 and 80-min intervals. Samples were immediately diluted 1:2 with ice-cold distilled water to stop further inactivation. Samples were then centrifuged at 16,300 × *g* for 30 min and the supernatant fluids were analyzed for serologically active toxin by CIEP (*7*).

Heat inactivation of enterotoxin in cooked turkey dialysate

Two hundred grams of ground cooked turkey were dialysed against 800 ml of distilled water for 72 h at 4 C. The dialysate was concentrated to 200 ml using carbowax 20,000. Enterotoxin was added to the dialysate at a concentration of 10 μg/ml and 1-ml quantities were placed in 13 × 100 mm test tubes. These tubes were then heated at 60 C for intervals similar to those used for cooked turkey. Toxin heated in 0.85% saline served as a control. Residual serological as well as biological activity was measured using CIEP (*7*) and the guinea pig skin test for erythemal activity (*12*), respectively.

Heat inactivation of enterotoxin in saline

Enterotoxin, 10 μg/ml in saline, was distributed in 1.0-ml quantities in 13 × 100 mm test tubes. Thermal inactivation was carried out at 45, 50, 55, 60, 65, and 70 C as described above.

Effect of different concentrations of proteins on thermal inactivation of enterotoxin

The effect of 5.0, 10.0 and 20.0% bovine serum albumin (BSA) and gelatin at pH 6.8 on thermal inactivation of enterotoxin at 60 C was determined. Heating conditions were similar to those described above. The effect of 0.1 M sodium phosphate buffer at pH 5.4, 6.0, 7.0 and 8.0 on thermal inactivation of enterotoxin was also measured in a similar fashion.

Recovery of heat-inactivated enterotoxin by treatment with urea

Heat-inactivated toxin in various menstrua was mixed 1:1 with 8 M urea and held for 1 h at room temperature. Urea was removed by dialysis at 4 C for 72 h before assaying for serological and biological activity.

RESULTS AND DISCUSSION

Heat inactivation of enterotoxin in foods

After 10 min of heating enterotoxin at 60 C in cooked turkey (Fig. 1), there was a 75.0% loss of serological activity and a 95% loss after 20 min. A subsequent gradual decrease in serological activity occurred after 20 min with no detectable toxin being present after 80 min. The come-up time to 60 C in the 50-g quantity of turkey used per sample was 32 min. Even though 95% of the serological activity was lost at this time, the residual activity detected up until 70 min would indicate the presence of either a more heat resistant form of the toxin or that perhaps conformational changes occurred in the toxin during heating which would make it more heat stable. Similar results were obtained with toxin heated in chicken or beef gravy.

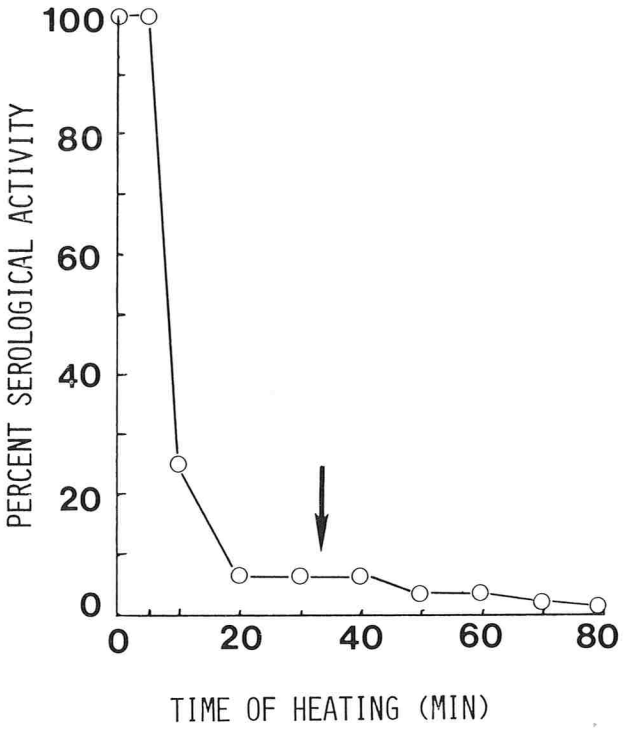

Figure 1. *Heat inactivation of* C. perfringens *enterotoxin in cooked turkey at 60 C. Arrow indicates time at which internal temperature of turkey meat reached 60 C.*

Heat-inactivation kinetics of enterotoxin in cooked turkey dialysate were also similar to those in cooked turkey meat except that serologically active toxin was not detected after 60 min (Fig. 2). Although the kinetics of toxin in saline were also similar to those in turkey meat or dialysate, the residual level of toxin was higher after 20-70 min of heating in saline and serologically active toxin was still detectable after 80 min of heating. In contrast to serological activity, biological activity of enterotoxin as measured in guinea pig skin was completely destroyed within 5 min of exposure at 60 C in saline and within 10 min of exposure in turkey dialysate. Thus, *C. perfringens* enterotoxin may have two or more different specific sites in the protein for biological and serological activity. Biological activity seems to be easily destroyed by heat, whereas serological activity, although also heat labile, seems to be more stable under similar heating conditions.

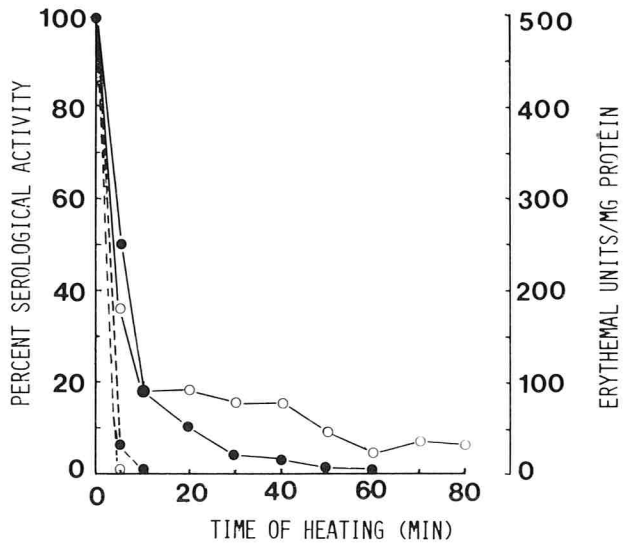

Figure 2. *Heat inactivation of* C. perfringens *enterotoxin in saline and cooked turkey dialysate at 60 C.* O—O *Serologically active enterotoxin in saline,* O-----O *erythemal activity in saline,* •—• *serologically active enterotoxin in carbowax concentrated turkey dialysate,* •-----• *erythemal activity in turkey dialysate.*

Recently, Granum and Skjelkvale (*6*) also reported that *C. perfringens* enterotoxin was very heat labile with 90% of its biological activity being destroyed after only 1 min of exposure at 60 C. They also observed that enterotoxin heated for 1 min at 55 C (1 mg/ml in 0.02 M phosphate buffer) rapidly recovered its biological activity from 30% initially to an apparently stable level of about 50% after 4 days at 20 C. Enterotoxin heat-treated for 5 min recovered its biological activity slowly, i.e., from 15% initially to only 20% after 6 days of storage at 20 C. With enterotoxin heated for 15 min at 55 C there was no recovery of biological activity from an initial 10% residual activity. We were unable to reactivate serologically active enterotoxin heated in saline at 60 C for 80 min by storage at 20 or 4 C.

Effect of different proteins on thermal inactivation of enterotoxin

Biologically active enterotoxin was completely inactivated after 5 min at 60 C in 5.0, 10.0 and 20.0% BSA or gelatin at pH 6.8. Serological activity was destroyed after 10 min of heating under similar conditions.

Effect of pH on thermal inactivation of enterotoxin

Heat-inactivation of enterotoxin suspended in 0.1 M phosphate buffer at pH 5.4, 6.0, 7.0 or 8.0 is shown in

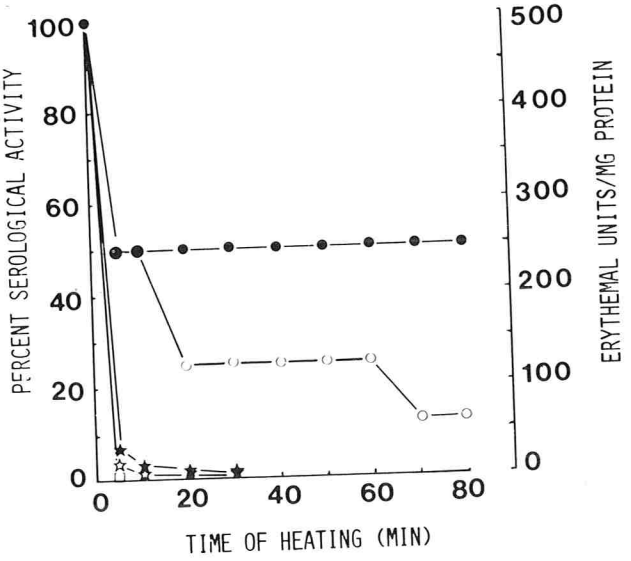

Figure 3. *Heat inactivation of* C. perfringens *enterotoxin in phosphate (0.1 M) at pH 5.4, 6.0, 7.0 and 8.0 at 60 C.* •—• *pH 8.0,* O—O *pH 7.0,* ★ ★ *pH 6.0,* ☆—☆ *pH 5.4,* □ *indicates that biological activity was destroyed within 5 min at all pH values.*

Fig. 3. At pH 5.4 and 6.0 there was a loss of more than 90% of serological activity within 5 min of exposure at 60 C. Less than 5% of the initial serological activity was detected after 20 min and none after 30 min of exposure. The enterotoxin flocculated after 20 min of exposure to 60 C at pH 5.4 and after 70 min of exposure at pH 6.0.

At pH 7.0 the loss of serological activity with heating time was very slow as compared to that at pH 5.4 and 6.0 with only 50% of the serological activity remaining after 10 min, 25% after 20-60 min and about 12% being present after 80 min. Thus, a neutral or alkaline pH protected the serological activity of enterotoxin at 60 C, whereas at an acidic pH in conjunction with heat, the loss of serological activity was very rapid. However, biological activity was not observed after 5 min of exposure of *C. perfringens* enterotoxin at 60 C, no matter whether toxin was suspended at pH 5.4, 6.0, 7.0 or 8.0 in phosphate buffer. This further indicates that biological activity of *C. perfringens* enterotoxin is more heat-labile than its serological activity and depending upon environmental factors serological activity may be heat-stable for a reasonable period at 60 C.

Effect of urea treatment on the recovery of enterotoxin serological activity

Enterotoxin in 5% gelatin or BSA (pH 7.0) lost about 90% of its serological activity within 5 min of exposure at 60 C and all activity after 20 min. However, in samples treated with urea after heating in BSA a recovery of 50% of the serological activity at 5 min, 6% up to 50 min and 3% even after 80 min of exposure at 60 C was observed in the case of 5% BSA (Fig. 4). Urea-induced recovery was also observed with toxin heated in gelatin, chicken gravy, beef gravy or moist cooked turkey, though to a lesser extent (results not shown). Biological activity could not be recovered by urea treatment regardless of the heating menstruum. The urea treatment may have exposed

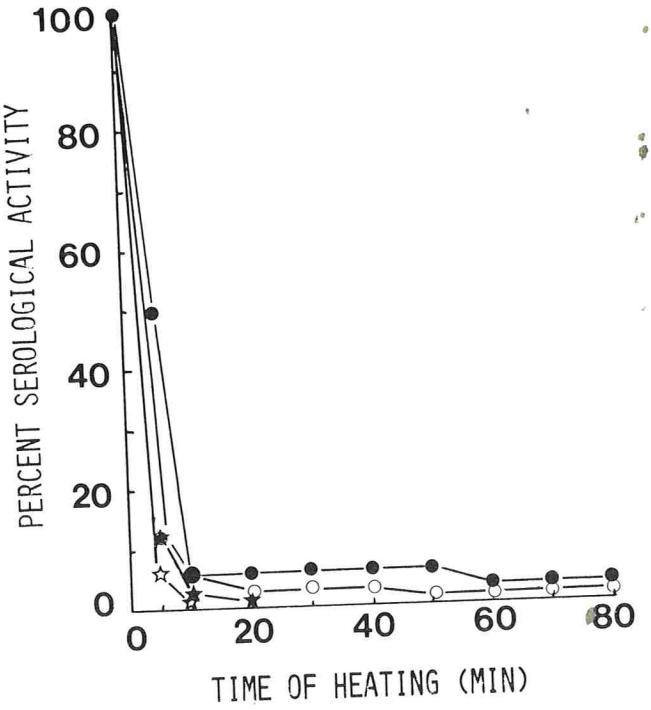

Figure 4. *Heat-inactivation of* C. perfringens *enterotoxin at 60 C in 5% bovine serum albumin and gelatin and recovery of heat-inactivated serological activity by urea treatment. See materials and methods for urea treatment.* ★—★ *5% BSA pH 7.0,* •—• *5% BSA pH 7.0 with subsequent urea treatment,* ☆—☆ *5% gelatin pH 7.0,* O—O *5% gelatin pH 7.0 with subsequent urea treatment.*

serologically active sites for reaction with antienterotoxin which were not available as a result of heating the toxin in the presence of secondary proteins such as gelatin or BSA. It was interesting to note that urea treatment did not affect the detection level of serologically active enterotoxin heated at 60 C in phosphate buffer pH 6.0 or 7.0.

Tatini (*14*) has also reported some enhancement in serological detectability of heat-inactivated staphylococcal enterotoxin A and D after urea treatment. He also reported increased biological activity of urea-treated enterotoxin A. Our results suggest that *C. perfringens* enterotoxin is heat labile in foods as well as in laboratory media. Under normal food cooking conditions the toxin would most likely be inactivated. In testing for preformed enterotoxin in suspect foods from food poisoning outbreaks, it appears desirable to treat the toxin with urea for potential recovery of serologically active toxin that may have been heat-inactivated.

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Microbiological Evaluation of Retail Ground Beef in Izmir, Turkey

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ABSTRACT

Observations of hygiene standards in the abattoir and retail situation in Izmir, Turkey, prompted a survey in which 100 samples of retail ground beef were analyzed for total aerobic plate count, coliforms, staphylococci, and salmonellae. The hygienic quality of ground beef was found to be similar to that established by a number of recent surveys in Canada and U.S.A

Several surveys have been published on the hygienic quality of ground beef (*1-7*). These surveys, carried out in the U.S.A. and Canada, monitored processing and retailing hygiene, in situations which are closely supervised by regulatory bodies at the national, state/provincial, and local levels.

Observations of the processing and retailing of meat products in Turkey revealed less regulation. Additionally, processing techniques and hygiene standards both in abattoirs and in retail shops were very primitive, similar to techniques and standards used in developed countries (e.g. North America, United Kingdom, Australasia) one or two decades previous, since which time the meat industries of all these countries have invested huge capital sums in upgrading of premises and in enlargement of inspectional responsibility. Therefore it was of interest to monitor the hygienic quality of ground beef in a developing country, and a survey was carried out in Izmir, Turkey, a city of more than 600,000 inhabitants. Samples were purchased from small butcher shops and from a large supermarket. Since some Turkish housewives refrigerate ground beef before consumption, samples were analyzed both immediately after purchase and following periods of storage at 5 C.

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MATERIALS AND METHODS

Samples

A total of 100 samples was purchased during April 1977, air ambient temperatures on the days of purchase ranging from 18-28 C. Eighty samples were purchased from small retail butcher shops where, by custom, the gound beef was prepared to order, the butcher cutting the appropriate quantity of meat from the carcass and then grinding it. Of 80 samples, 16 were analyzed within 1 h of purchase, 52 samples stored at 5 C for 48 h before analysis. Samples of ground beef stored at 5 C for 72 h were judged to be organoleptically unacceptable and were not analyzed.

An additional 20 samples were purchased from a large supermarket in which bulk quantities of ground beef were displayed for purchase at ambient (20 C) temperature; all samples were stored at 5 C for 24 h before analysis.

Test Methods

A decimal dilution of each sample with peptone water (0.1% wt/vol) homogenized in a Colworth Stomacher for 60 sec was used to prepare serial dilutions with peptone water which were plated on culture media. The total (aerobic) count was done by surface plating appropriate dilutions in duplicate petri plates containing Nutrient Agar and incubating at 25 C for 48 h. Duplicate plates containing 30-300 colonies were counted, results were averaged and reported as the total count/g.

Coliforms were counted on duplicate plates of Violet Red Bile Agar incubated at 37 C for 24 h, typical colonies being dark red, 0.5-2.0 mm in diameter with entire margins, and surrounded by a zone of precipitation accompanied by a purple discoloration of the agar.

Following surface plating on Baird-Parker Agar and incubation at 37 C for 18-24 h, colonies presumed to be staphylococci were counted when colonies were 1.0-1.5 mm in diameter, black, convex, shiny, and surrounded by a clear zone in the opaque medium.

Two 25-g subsamples were used for *Salmonella* analysis. Samples were enriched in Tetrathionate Broth at 37 C for 24 and 48 h and in Selenite Cystine Broth at 43 C for 24 and 48 h after which incubation periods a loop was used to transfer broth to Xylose Lysine Desoxycholate and Brilliant Green Agars which were incubated at 37 C for 24 h. All suspect colonies were transferred to tubes of Lysine Iron Agar, Triple Sugar Iron Agar, Urea Agar, and ONPG (orthonitrophenyl-β-D-galactopyranoside) broth and incubated at 37 C for 24 h.

RESULTS AND DISCUSSION

Ground beef purchased from small retail shops initially had total counts in the range 10^5-10^7/g rising after storage at 5 C so that after 24 h almost 50% of samples had counts in excess of 10^7/g and after 48 h almost all samples were 10^7-10^8/g (Table 1). Ground beef purchased from supermarkets was of inferior quality — samples stored at 5 C for 24 h all had counts of 10^7-10^8/g (Table 1).

TABLE 1. *Total aerobic count, coliform and staphylococcal levels in 100 samples of raw ground beef*

	Number of samples in each category			
	Small butcher shop			Supermarket
	Storage Time (h) at 5 C			
Test and count	<1	24	48	24
Total Count				
5[a]	6 (37)[b]	5 (10)	0	0
6	8 (50)	24 (46)	1 (8)	0
7	2 (13)	22 (42)	4 (33)	20 (100)
8	0	1 (2)	7 (59)	0
Coliforms				
2	4 (25)	4 (8)	0	0
3	6 (37)	24 (46)	4 (33)	0
4	5 (31)	19 (36)	4 (33)	3 (15)
5	1 (7)	5 (10)	4 (33)	6 (30)
6	0	0	0	11 (55)
Staphylococci				
2	5 (31)	3 (6)	0	0
3	1 (7)	7 (14)	0	0
4	7(43)	22(42)	3(33)	6(30)
5	3 (19)	10 (38)	9 (67)	7 (35)
6	0	0	0	7 (35)

[a]Log. count of sample e.g. category "5" refers to log. counts >5.0 and <6.0

[b]Percentage of samples in each category

Coliforms and staphylococci in ground beef from small butcher shops both ranged over 10^2-10^6/g with a larger percentage of samples having counts at the upper end of this range following periods of refrigerated storage. Higher counts of coliforms and staphylococci were found in ground beef purchased at a supermarket, the range being 10^4-10^7/g. Salmonellae were not detected in any of the samples analyzed.

Recent surveys of the hygienic quality of ground beef (*1-7*) considered a maximum desirable level to be 10^7/g; however, in each survey a large percentage (30-64%) of samples tested was found to exceed 10^7/g (Table 2).

The present survey in Izmir established that, of ground beef prepared to order in retail shops, only 13% of samples tested exceeded 10^7/g; even after storage at 5 C for 24 h only 43% of samples exceeded 10^7/g. Levels of coliforms and staphylococci were found to be similar to those established in Canadian and American surveys (*1,3,4,6*).

TABLE 2. *Summary of hygiene status of ground beef established in recent surveys*

Survey	Aerobic plate count (Percentage of samples)
Al-delaimy and Stiles (*1*)	41% > 1×10^7
Chambers et al. (*2*)	30% > 1×10^7
Duitschaever et al. (*3*)	64% > 1×10^7
Goepfert (*4*)	39% > 5×10^6
Pivnick et al. (*5*)	44% > 10^7
Shoup and Oblinger (*6*)	50% > 10^7
Westhoff and Feldstein (*7*)	average count 7.9×10^6

Considering the circumstances surrounding the slaughter, dressing, transport and retailing of meat products in Izmir (Table 3), it is surprising that the hygienic quality of ground beef should so closely approximate that established in North America. In the Turkish situation, hygienic quality of ground beef revolves around the custom of preparing to order; firstly the bacterial loading, because it is confined to the surface of the carcass, probably increases only slowly because of surface drying. Secondly, the period during which the beef is in the ground state is very much reduced compared with beef in the bulk. Ground beef produced in bulk by the large supermarket was of inferior hygienic quality, due to unspecified time/temperature storage before purchase.

TABLE 3. *Hygiene during slaughter and transport of beef and during retailing of ground beef in Izmir, Turkey*

Abattoir:	Absence of impervious working surfaces.
	Knife sterilizers and handwashing facilities absent.
	Unlimited access of outside personnel.
	Animals await slaughter in full view of carcasses being processed.
	No physical separation of clean and dirty areas.
Dressing:	Begun on floor; gut and hide removed and carcass butchered in hanging position.
Aging:	Carcasses hung 0.5 C for 24 h.
Personnel:	No protective clothing provided—very old, fat and blood-soaked clothing worn.
	Smoking while working is normal.
	Toilet facilities inadequate.
Transport:	Non-refrigerated transport.
	Carcasses stacked in a pile on the floor.
Retail:	Meat stored overnight in chiller.
	Displayed by day in window—unrefrigerated.
	Most butchers smoke while working.
	Many shops have pets.
	Mincer not cleaned during working day.

The meat industries of many developed countries have invested huge capital sums in the upgrading of abattoirs and in the intensification of inspectional activities. For example, to the 30-40 export abattoirs in New Zealand, the hygiene cost of upgrading premises to meet U.S.A. hygiene requirements exceeded NZ$100,000,000 while more recent hygiene requirements to ensure access to the European Economic Community required expenditure of approximately NZ$200,000,000 - 500,000,000.

Yet the present study indicates that ground beef can be produced in a developing country under primitive hygiene conditions to at least the same standard as established by Canadian and American surveys.

This study underscores the view that end-product hygiene is an amalgam of the hygiene status of each facet of the process, transport and retail chain, and, further, that financial inputs commensurate with those invested in the process sector are necessary for the upgrading of premises and the education of personnel in the retail sector.

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Comparison of Two Minikits (API and R-B) for Identification of *Enterobacteriaceae* Isolated from Poultry and Meat Products

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ABSTRACT

Two miniaturized commercial kits (API and R-B) were evaluated for identification of 373 strains of *Enterobacteriaceae* isolated from selected poultry and meat and meat products, such as frozen chicken pot pie, frozen comminuted chicken, processed chicken, ground beef, and pork sausage. The taxonomic classification by these two systems was identical for 59% (221/373) of the isolates. Whenever the two systems disagreed, cultures were identified by conventional methods. The API correctly classified 82% (306/373) of the isolates, while R-B correctly classified 72% (267/373). Most of the disagreements involved organisms of the *Klebsiella-Enterobacter-Serratia* group of the *Enterobacteriaceae* family.

Knowledge of the identity of *Enterobacteriaceae* in different foods is essential for assessing the value of such quality or safety indices as "the coliform count," "fecal coliforms," and the "*Enterobacteriaceae* count" (*10*). This information also may be of importance in selection of methods for isolation and differentiation of salmonellae and other enteric pathogens from foods. The number and nature of biochemical and other tests often required to speciate members of this family have frequently discouraged food microbiologists from obtaining these data. Recently developed commercial "test kits" for identification of *Enterobacteriaceae,* although designed primarily for use in clinical laboratories, may provide suitable alternatives to the conventional tests. The API-20E and the R-B Enteric Differential System are two examples of these biochemical micromethods. A number of investigators (*1,9,13,14,15,18*) have shown that API and R-B have an accuracy of 90% or greater for identification of *Enterobacteriaceae* isolated from clinical specimens and have recommended their routine use in the clinical bacteriology laboratory. Nord and co-workers (*11*) compared five test kits with conventional biochemical tests by using 329 strains of *Enterobacteriaceae;* API correctly identified 94% and R-B 91% of the strains.

We evaluated the efficiency of two of these systems for identification of enteric bacteria isolated from selected foods. If these systems could identify food isolates with an acceptable degree of accuracy when compared to conventional tests, they have the following advantages: (*a*) many biochemical tests are available, (*b*) they require minimal storage and incubator space, (*c*) have a long shelf life, (*d*) require less inoculation time, and (*e*) would reduce laboratory-to-laboratory errors.

MATERIALS AND METHODS

Frozen chicken pot pie, frozen comminuted chicken meat, processed broiler carcasses, ground beef, and pork sausage were procured from a local supermarket. The samples (50 g) were weighed in a Waring Blendor and blended for 1 min with 450 ml of 0.1% peptone. For broiler carcasses, 50 g of breast skin were used. Serial dilutions were made, and double-poured plates of violet red bile agar (Difco) with 1% glucose (*10*) were prepared and incubated 24 h at 37 C. After incubation, a numerical grid and a table of random numbers (*16*) were used to select isolates that would be studied further. A total of 373 colonies was picked and streaked on plates of MacConkey agar (Difco) for purification. After 24 h of incubation, we identified each isolate by

TABLE 1. *Biochemical tests of the API-20E and the R-B systems and the conventional tests used in this study*

API	R-B	Conventional
Amygdalin	Arabinose	Arabinose
Arabinose	Citrate	Citrate
Arginine	DNase[c]	DNase
Citrate	Glucose	Hydrogen Sulfide
Gelatin	Hydrogen Sulfide	Indole
Glucose	Indole	Inositol
Hydrogen Sulfide	Lactose	Lysine
Indole	Lysine	Malonate
Inositol	Motility	ONPG
Lysine	Ornithine	Ornithine
Mannitol	PAD[d]	PAD
Melibiose	Raffinose	Rhamnose
ONPG[a]	Rhamnose	Urease
Ornithine	Sorbitol	Voges-Proskauer
Rhamnose		Others as needed
Sorbitol		
Sucrose		
TDA[b]		
Urease		
Voges-Proskauer		

[a]Ortho-nitrophenyl-β-D-galactopyranoside
[b]Tryptophane deamination
[c]Deoxyribonuclease
[d]Phenylalanine deamination

using two miniaturized systems, the API-20E strip and the R-B Enteric Differential System. The API-20E system, manufactured by Analytab Products, Inc., Plainview, New York, includes a series of 20 plastic cupules affixed to a plastic strip; each cupule contains a dehydrated substrate for a different biochemical test. A colony of the culture to be identified was dispersed in 5.0 ml of sterile distilled water (pH 7.0) and each cupule was filled by using a sterile Pasteur pipette. The strip was then incubated for 18-24 h at 37 C inside a small plastic container supplied by the manufacturer. A small amount of water was added to the bottom of the container to retard dehydration.

The R-B Enteric Differential System, manufactured by Diagnostics Research, Roslyn, New York, incorporates 14 standard biochemical tests into a four-tube system. A colony was picked from the plate with a specially prepared needle and then all four tubes were consecutively inoculated without retouching the colony. The tubes were incubated for 18-24 h at 37 C.

By using the computerized data systems of API (profile register) and R-B (enteric analyzer) and the biochemical data obtained with each isolate, the cultures were identified with each miniaturized system. When the two systems did not agree on the identity of a culture, the organism was identified by conventional methods (*6*).

Table 1 shows the biochemical tests that comprise the API, the R-B, and conventional methods.

RESULTS AND DISCUSSION

Table 2 is a list of the organisms isolated and identified in this study. The predominant *Enterobacteriaceae* from each food type samples were, respectively: frozen chicken pot pie (*Enterobacter agglomerans*), frozen chicken comminuted meat (*Escherichia coli*), processed broiler carcasses (*E. coli*), ground beef (*Serratia liquefaciens*), and pork sausage (*E. agglomerans*). Table 3 shows the number of isolates from each type of food and the number of times that the API and R-B systems agreed or disagreed with each other and with conventional methods for the taxonomic classification of these isolates. If an incorrect identification is defined as one in which the classification of an isolate by a commercial system did not agree with the classification of the other commercial system or the classification by the conventional tests, then the API and R-B systems correctly identified 82% and 72% of the food isolates, respectively. The two systems were in complete agreement for identification of 59% (221/373) of the isolates. If the KES (*Klebsiella-Enterobacter- Serratia*) group of organisms, which are very difficult to speciate accurately with any schema or system are excluded, then the API was 89% accurate and R-B, 82%. Goldin (*7*), working with clinical isolates, reported 84% agreement between R-B and conventional tests. He encountered difficulty in interpreting the lysine decarboxylase reaction and in differentiating *Serratia* from *Enterobacter*. Working with 245 clinical isolates, Hayek and Willis (*8*) found that two commercial methods (API and Enterotube) agreed in the identification of 85% of the cultures.

TABLE 2. Enterobacteriaceae *isolated in this study*

Genus and species	Number of isolates
Arizona sp.	1
Citrobacter freundii	9
Enterobacter aerogenes	1
E. agglomerans	142
E. cloacae	3
E. hafniae	13
Escherichia coli	139
Klebsiella ozaenae	10
K. pneumoniae	5
Pectobacterium sp.	1
Proteus mirabilis	7
P. morganii	2
P. rettgeri	1
Serratia liquefaciens	46
S. marcescens	1
S. rubideae	1
Total	373

Part of the problem of classifying the KES group is caused by lack of universal agreement concerning the nomenclature of these organisms. Some of the confusion results from the different data upon which each system bases its identification procedure. For example, whereas these various minikits are based on the data of Edwards and Ewing (*5*) and Ewing (*6*), some conventional schemes in certain laboratories may be based on the data and nomenclature found in *Bergey's Manual of Determinative Bacteriology* (*2*). In addition to this, many medical microbiologists in Europe use the data of Cowan and Steel (*4*) or Cowan (*3*) for identification of *Enterobacteriaceae* and this further complicates the comparison of results among different laboratories. Thus, the same species may have different names, depending on the classification system used for identification. For example, *Hafnia alvei, Serratia marcescens,* and *Salmonella arizonae,* as named by Bergey's (*2*) are called *Enterobacter hafniae, Serratia rubideae,* and *Arizona hinshawii*, respectively, by Ewing (*6*).

In our study, several isolates identified by both R-B and conventional methods as *E. coli* were identified as *Shigella* by the API method. Those disagreements

TABLE 3. *Agreement between API and R-B in the identification of isolates from selected foods*

Type of food	Number of isolates	Number of agreements between API and R-B	Number of disagreements between API and R-B	Number of disagreements between API and R-B in which conventional agreed with		
				API	R-B	Neither
Raw Chicken	73	45 (62)[a]	28 (38)	12	12	4
Hamburger	79	32 (41)	47 (59)	28	10	9
Pork Sausage	73	38 (52)	35 (48)	21	11	3
Chicken Pot Pie	68	50 (74)	18 (26)	13	5	0
Raw Comminuted Chicken	80	56 (70)	24 (30)	11	8	5
Total	373	221 (59)	152 (41)	85 (56)	46 (30)	21 (14)

[a]Percentages are shown in parentheses.

apparently were caused by negative lysine decarboxylase tests on the API strip and positive lysine tests by R-B and conventional methods. In addition, the API system did not include the motility test which would have helped differentiate *Shigella* and *Escherichia.* For other cultures identified as *Shigella* by API, but as *E. agglomerans* or *S. liquefaciens* by R-B and conventional tests, lysine decarboxylase results were consistently similar for all systems, but false-positive reactions with the Voges-Proskauer test (i.e., the result was negative with R-B and conventional tests) and an occasional false-positive citrate test apparently contributed to the misclassification. However, the API system does not recommend the classification of an isolate as *Shigella* until serological confirmation has been completed. Another group of cultures was identified as *E. coli* with API but as *E. agglomerans* by both R-B and conventional. A false-positive reaction for the arginine dihydrolase test on the API strip also may have been responsible for this disagreement. A false-positive reaction with the Voges-Proskauer test and the absence of a motility test probably accounted for the misidentification of several *E. coli* cultures as *Klebsiella pneumoniae* by the API system.

Numerous cultures identified as *E. hafniae* by API and conventional were classified as *S. liquefaciens* by R-B. A false-positive reaction on sorbitol was observed with R-B for all these cultures. Also, the gelatin liquefaction test was useful for differentiating these organisms and the R-B system did not include this test. The R-B system repeatedly assigned the epithet *S. liquefaciens* to cultures that were identified as indole-negative strains of *E. coli* by the API and conventional tests; these isolates were arginine-positive by both API and conventional tests. The arginine test, which would have minimized the chances of misclassification, was not included in the R-B system.

The R-B system incorrectly classified 13 strains of *E. agglomerans* as *S. liquefaciens* because of false-positive reactions with the lysine and ornithine decarboyxlase tests.

According to Stevens (*17*), inoculum size, incubation time and interpretation of the result by different laboratory workers are only a few of the many factors that can affect biochemical test results with miniaturized systems. Some of the above reactions referred to as false-positives by both API and R-B could have been due to incorrect reading of the test result.

Poelma et al. (*12*), working with 124 strains of *Enterobacteriaceae* isolated from various foods, recently reported an overall correlation between conventional biochemical tests and the API and R-B systems to be 99% and 96%, respectively. However, most of their cultures were *Salmonella,* while only five were *Enterobacter* and none were *Klebsiella* or *Serratia.* As previously stated in our paper, members of this KES group seem to be the most troublesome to correctly classify and in some foods (as shown in this study), these organisms may be frequently encountered.

The results of our study suggest that these two systems may be less efficient for separation and identification of food isolates than previous studies have shown them to be with clinical isolates. For example, clinical studies involving the API-20E reported 93% correct speciation (*18*), 96% accuracy (*15*), 98% agreement with conventional (*1*) and 99% correct identification (*19*). Studies involving the R-B system reported 90% (*13*), 91% (*11*), and 96% (*14*) correct identification. Both systems were designed primarily for separation and identification of *Enterobacteriaceae* commonly encountered in clinical laboratories. Their efficiency with food isolates might be increased if additional tests were included to more easily differentiate those *Enterobacteriaceae* frequently encountered in various foods, e.g. *E. agglomerans, S. liquefaciens.*

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Mention of specific brand names does not imply endorsement of the authors or institutions at which they are employed to the exclusion of others not mentioned.

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Behavior of Enteropathogenic *Escherichia coli* During Manufacture and Ripening of Brick Cheese

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ABSTRACT

The ability of enteropathogenic *Escherichia coli* (EEC) to grow and survive during the manufacture and ripening of brick cheese was determined. Pasteurized milk artificially contaminated with EEC was used to make cheese by the washed-curd method. EEC was enumerated by surface plating samples on Trypticase Soy Agar (TSA) with an overlay of Violet Red Bile Agar (VRB), and by using VRB Agar pour plates. EEC increased 1000-fold during the manufacturing process and numbers decreased slowly during ripening while the smear developed and during refrigerated storage of cheese. Seven weeks after manufacture, numbers of EEC in cheese ranged from 700 to 20,000/g with initial contamination of milk at about 500/ml. Limited growth of EEC on the surface of brick cheese occurred during ripening and these bacteria were inactivated slowly during storage. Counts of EEC obtained with VRB Agar pour plates were 6-59% as large as counts obtained using TSA surface plating with VRB Agar overlay.

Foodborne illness associated with *Escherichia coli* has recently become a public health concern (*12,13*), although enteropathogenic *E. coli* (EEC) has been most often associated with outbreaks of infantile diarrhea in nurseries (*20*) and with outbreaks of travelers' diarrhea (*3*). There are two types of enteropathogenic *E. coli* capable of causing foodborne disease. Enterotoxin producing strains cause a cholera-like illness, and invasive strains cause a *Shigella*-like illness (dysentery). The incidence of EEC foodborne illness is difficult to estimate because of problems in diagnosis of the disease (*21*). Also, presence of EEC in our food supply has not been adequately determined because of problems in isolating the organisms (*14*) and in determining pathogenicity of isolates (*13*).

Presence of EEC in some dairy products can be of public health significance as evidenced by an outbreak in 1971 of gastroenteritis associated with Camembert cheese that contained EEC (*12*). The ability of EEC to grow during initial stages of Camembert cheese manufacture, and subsequent survival of the bacteria in the ripening cheese have been reported by Fantasia et al. (*5*), Frank et al. (*8*), and Park et al. (*16*). Growth of EEC on the surface of ripening Camembert cheese has also been observed (*8*). Behavior of *E. coli* in skim milk fermented by lactic acid bacteria has been described by Frank and Marth (*6,7*). The combination of high starter concentration and low incubation temperature was most effective of the variables tested in controlling growth of *E. coli*.

Although there are no documented outbreaks of foodborne illness caused by EEC and associated with surface-ripened semisoft cheese, Olson (*15*) has noted that these varieties of cheese have some characteristics which are cause for concern. Included are slow acid development during manufacturing, initial low salt concentrations at the interior of the cheese, and relatively high ripening temperatures. "Sweet curd" brick cheese was chosen for this study as representative of semisoft cheeses because it has all the characteristics just described. By manufacturing brick cheese with pasteurized milk artifically contaminated with EEC, we hoped to help evaluate the safety of the brick cheese manufacturing process.

MATERIALS AND METHODS

Cultures

EEC cultures used in this study were an enterotoxigenic strain, B2C, and two invasive strains, 1624 and 4608. Lactose is fermented slowly by strain 1624. These strains were obtained from the FDA, Washington, D.C. Cultures of *E. coli* were grown in nutrient broth at 37 C for 24 h before use as an inoculum. Enough culture was added to coliform-free pasteurized milk to provide about 500 CFU/ml. The starter culture used was *Streptococcus lactis* 4175 from Marschall Div., Miles Lab., Madison, Wisconsin. The starter culture was incubated at 21 C for 20-22 h before cheesemaking; the skimmilk was coagulated under these conditions. A 0.25% inoculum of starter was used.

Manufacture of cheese

Brick cheese was manufactured by the washed-curd method described in detail by Price and Buyens (*17*) and Olson (*15*). Starter, rennet, and coliform were added simultaneously to 21 kg of pasteurized whole milk tempered at 30 C. Approximately 30 min later, coagulated milk was cut using 6-mm knives. Ten minutes later, curd was slowly heated to 36.5 C (this took 25 min). After 15 min at 36.5 C, whey was drained to 50% of the original volume of milk, and the same amount of water was mixed with the curd. This mixture was stirred for 30 min at 36.5 C. Then the whey-water mixture was drained and curd was put into one hoop. The hoop was turned perodically for 6 h with a 2.3-kg weight placed on top for a 2-h period. Eight hours after the start of manufacture, cheese was placed in a salt (22%) brine at 15.5 C for 24 h. Cheese was removed from the brine and ripened quiescently at 15.5 C for 2 weeks in an incubator with high humidity. The block was turned and the surface was rubbed with 0.5% salt solution each day of

ripening. After ripening, the surface smear was gently washed from the block. Cheese was allowed to dry and then was cut into six pieces. Each piece was wrapped in Saran, heat sealed, and stored at 7 C for up to 5 weeks. Duplicate trials were made with each strain of *E. coli*. Results are reported as average values.

Surface inoculations

E. coli was inoculated on the surface of blocks of coliform-free cheese ripened for 1 week. The surface of each block was marked into areas of 20 cm^2 each and each area was inoculated with 0.1 ml of water containing approximately 5000 CFU. Excess water on the surface of the block from the inoculum was allowed to dry before cheese was returned to the ripening chamber. Thereafter, cheese was treated normally. After one additional week of ripening the surface smear was washed from cheese and before packaging, inoculated areas were reinoculated with the same strain and amount of *E. coli* as used initially. At each sampling time, analyses were done on two 20-cm^2 areas. The average of the two is reported. To enumerate *E. coli*, each 20-cm^2 area was cut from the block deep enough to give 20 g of cheese. This was blended with 180 ml of sterile 2.0% sodium citrate solution. Appropriate serial dilutions were made in 2.0% sodium citrate.

Enumeration of E. coli

Two methods were used to enumerate *E. coli*. The first was that suggested by Speck et al. (*22*) to enumerate sublethally injured coliforms. It involves surface plating the sample on Trypticase Soy Agar (TSA, Difco) and a 1-h incubation at room temperature followed by adding an overlay of Violet Red Bile Agar (VRB, Difco). Incubation was then at 37 C for 24 h. This method is referred to as the TSA + VRB surface plating method, and was used to enumerate *E. coli* in all experiments. The second method used VRB Agar pour plates with incubation at 37 C for 24 h. This method was used only with trials that involved inoculated milk.

Cheese samples for enumeration of *E. coli* were diluted by adding 20 g of cheese to 180 ml of sterile 2.0% sodium citrate solution and blending with a Waring Blendor operating at low speed for 3 min. Serial dilutions were made using 9.0 ml of sterile sodium citrate solution. Cheese was sampled by taking a rectangular shaped cross-section of the cheese block to include portions both at the surface and the interior of the block.

Measurement of pH and moisture

These analysis were done with the same methods and equipment as described in a previous paper (*8*). Cheese contained 41.7-43.8% moisture and developed an apparently normal surface smear during ripening.

RESULTS AND DISCUSSION

Growth of EEC during manufacture of cheese

Both strains 1624 and 4608 increased approximately 1000-fold in numbers during the first 7 h of cheese manufacture (Fig. 1). The day after manufacture numbers of these strains began a slow decline. Strain B2C had a different pattern of growth, increasing 100-fold in the first 7 h, but then increasing further after 24 h before numbers began to decline. The pH value for cheese manufactured during one of the trials with strain B2C was higher than normal after 7 h (5.9). The increase in numbers occurring in this trial was greater than when the pH of the cheese was normal. This accounts for some of the growth between 7 and 24 h which did not occur with the other strains.

The 2- to 3-log increase in numbers of *E. coli* during the initial hours of cheese manufacture is partially the result of concentration by entrapment of cells in curd. However, entrapment would account for only a 10-fold

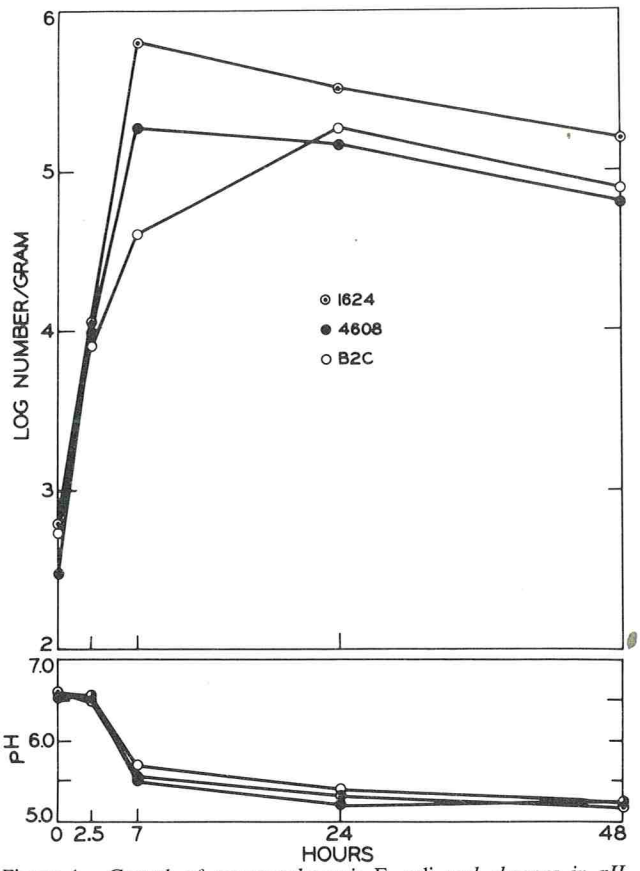

Figure 1. *Growth of enteropathogenic* E. coli *and changes in pH during manufacture of brick cheese.*

increase in numbers. The pH of cheese after 48 h in nearly all instances are approximately 5.2. Behavior of these strains of EEC during fermentation of skim milk at 32 and 21 C, and during manufacture of Camembert cheese has been previously reported (*6,8*). The amount of growth occurring during the initial hours of brick cheese manufacture was about 10 times greater with strains 4608 and 1624 than occurred during the initial hours of Camembert cheese manufacture. This additional growth may have occurred because of the higher temperature and less rapid decline in pH during brick cheese manufacture than during manufacture of Camambert cheese. Data in Fig. 1 show that inhibition of EEC occurred at pH 5.2-5.5, whereas during Camembert cheese manufacture these same strains were inhibited at pH values of 5.0-5.2. It has been previously demonstrated that inhibition of pathogens by lactic acid bacteria is not solely dependent on acid production and pH (*1,18*).

Survival of EEC during ripening and storage of brick cheese

Numbers of EEC strains 1624 and 4608 decreased by 90-95% during the 2-week ripening period (Fig. 2). Strain B2C decreased by only 50% during the same time. This decrease may not be significant since the coefficient of variation of these data is 45%. Upon refrigeration of the cheese after ripening (data from 2-7 weeks, Fig. 2), each strain decreased only slightly in numbers. These strains

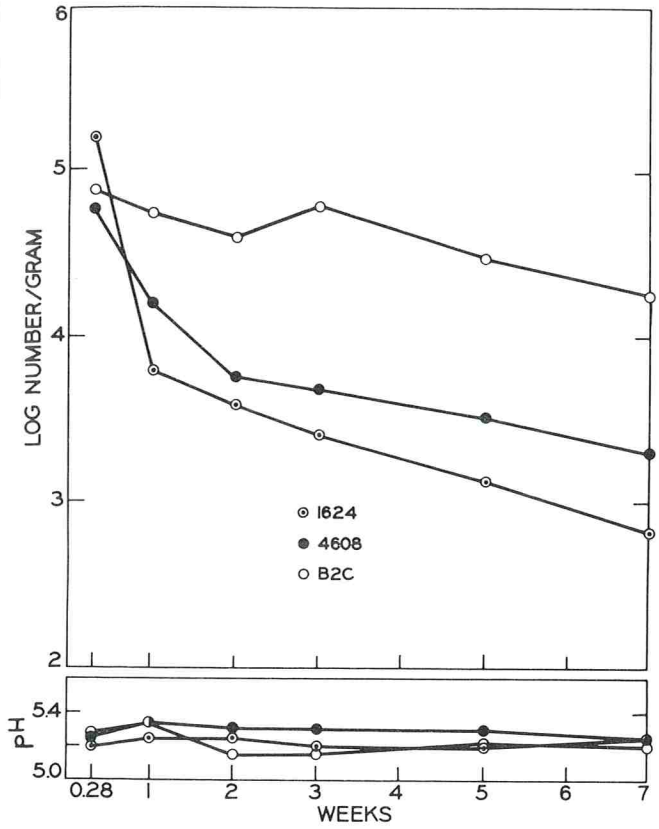

Figure 2. *Survival of enteropathogenic* E. coli *during ripening and refrigerated storage of brick cheese.*

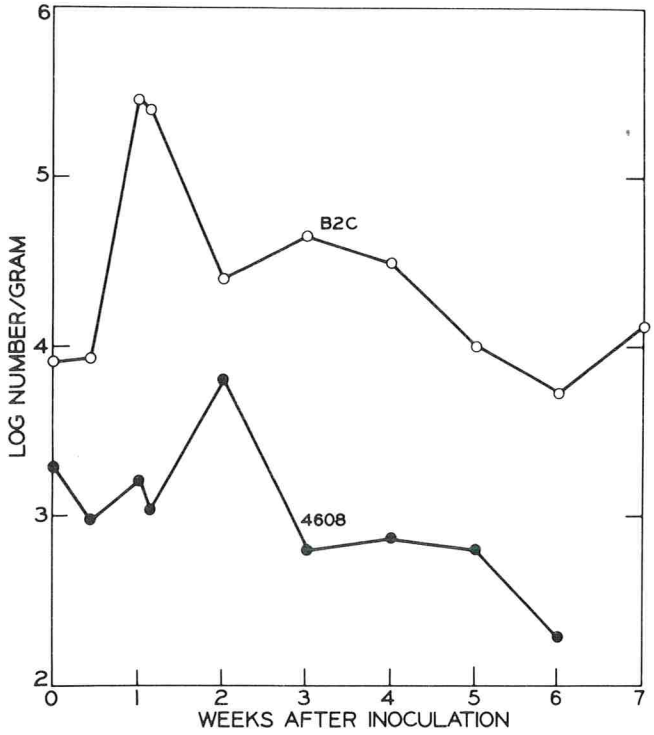

Figure 3. *Behavior of enteropathogenic* E. coli *inoculated onto the surface of brick cheese.*

of EEC were inactivated more rapidly in Camembert than in brick cheese (*8*). This probably is related to the lower pH of unripened Camembert cheese. When brick cheese samples ranged in pH from 5.15 to 5.3, little difference was found in survival of the coliform. Most brick cheese is at a pH of 5.3 after 2 weeks of ripening (*15*).

Populations of EEC in brick cheese after 7 weeks were 20,000, 2,000, and 700/g for strains B2C, 4608, and 1624, respectively. The number of bacteria necessary to cause illness, as estimated through feeding studies, is 10^6 to 10^8 for invasive EEC and 10^8 to 10^9 for enterotoxigenic EEC (*13*). If one assumes ingestion of 100 g of cheese, brick cheese made from milk contaminated with 10^3 to 10^4 CFU/ml of strains B2C or 4608 could cause illness. There is also the possibility of toxigenic strains of EEC producing enterotoxins during growth, then declining in numbers and leaving the enterotoxin behind to cause illness. This possibility is yet to be investigated.

Survival of EEC on the surface of brick cheese

Strain B2C was able to grow during the second week of ripening when inoculated onto the surface of cheese (the first week after inoculation, Fig. 3). Subsequently, there was a general decrease in numbers and after 7 weeks the population of strain B2C was about the same as the initial inoculum. Growth of strain 4608 was more limited than that of strain B2C, and after 6 weeks strain 4608 was at about 10% of the initial inoculum. The behavior of these strains of *E. coli* on the surface of brick cheese contrasts with their ability to grown by 3 to 4 log cycles on the surface of Camembert cheese (*8*). The major difference in the environment at the surface of these cheese varieties is the presence of *Penicillium camembertii* on Camembert cheese. Yeasts and micrococci predominate on the surface of brick cheese during the early stage of ripening, causing the pH at the surface to increase to 5.4-5.5 (*11*). The pH at the surface of Camembert cheese is much higher, thus probably providing a more favorable environment for growth of the coliform. Also, the microflora of the brick cheese surface may inhibit growth of *E. coli* through competitive inhibition (*23*) or by production of inhibitory substances (*9*).

Recovery of EEC from brick cheese using VRB pour plates

Recovery of EEC on VRB Agar pour plates relative to VRB + TSA surface plating decreased during the first hours of cheese manufacture and varied from 6 to 59% of the VRB + TSA count throughout the ripening and aging of the cheese (Table 1). Results from the VRB + TSA surface plating method have been previously shown to compare favorably with those obtained with TSA pour plates and MPN enumeration when these strains of EEC were in Camembert cheese or fermented skimmilk (*7,8*). The poor recovery on VRB Agar of the strains listed in Table 1 is similar in degree to that reported for refrigerated skimmilk which had been inoculated with these strains of *E. coli* and fermented with lactic starter cultures for 15 h at 21 C (*7*). A similar

TABLE 1. *Recovery of enteropathogenic* E. coli *on VRB agar pour plates during manufacture, ripening, and refrigerated storage of brick cheese.*

Strain	Time									
	Hours					Weeks				
	0	2.5	7	24	48	1	2	3	5	7
	(% of TSA + VRB counts[a])									
4608	77	44	44	59	50	23	39	25	26	19
1624	83	25	13	17	14	52	40	26	12	7
B2C	33	16	—	30	7	42	6	9	40	13

[a]VRB counts as a % of TSA + VRB surface plating counts.

magnitude of decreased recovery of these strains on VRB Agar was also observed during ripening of Camembert cheese (*8*). These comparisons are interesting because both fermented skimmilk and Camembert cheese exposed *E. coli* to a pH of 4.6-4.7, considerably lower than the pH of brick cheese. Yet there was no increased recovery on VRB Agar, as would be expected if there was less sublethal acid injury to the cells. It has been demonstrated that lactic acid can cause sublethal injury to *E. coli* cells (*11*). Exposure of the cells to the heat of molten agar could also be a factor in the decreased recovery of these strains with VRB Agar pour plates (*22*).

These results do not imply that the VRB Agar pour plate method for estimating coliforms in cheese, as recommended in *Standard Methods* (*10*), is inaccurate to the extent shown with these strains of *E. coli*. The strains used in this study are not typical of coliforms that naturally contaminate dairy products. Elliott and Millard (*4*) have shown that the VRB pour plate procedure is reliable to estimate the amount of coliform contamination in commercial cheese.

The amount of growth and subsequent survival of EEC during brick cheese manufacture and ripening indicate the need for the manufacturer to strictly control the amount of coliform contamination in this product. This can best be done through strict sanitation measures. Results of this study show that relatively high levels of coliform contamination would be necessary to produce cheese likely to cause EEC foodborne illness. Collins-Thompson et al. (*2*) found in a survey of Canadian cheeses that 13.6% of semisoft and 18.1% of soft cheeses contained more than 1600 coliforms/g. Also, the numbers of fecal coliforms exceeded 1600/g in 0.8% of semisoft and 2.1% of soft cheese. Although this does not necessarily indicate a level of contamination capable of causing illness if EEC were present, it indicates the occurrence of unnecessary coliform contamination during the manufacture of these cheese varieties. The disease-causing potential of large numbers of *E. coli* in dairy products should not be ignored.

ACKNOWLEDGMENTS

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A Research Note

Enhancement of Synthesis of *Bacillus cereus* Enterotoxin Using a Sac-Culture Technique

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ABSTRACT

Synthesis of *Bacillus cereus* enterotoxin was enchanced when the organism was grown by the sac-culture method. Of the three other major extracellular proteins examined, only lecithinase synthesis was enhanced. Advantages of the sac-culture method are that it helps simplify purification of *B. cereus* enterotoxin, and that while being simpler and more reduced in scale than that of a fermenter culture, it still yields the same enterotoxin levels on a per ml basis.

Bacillus cereus has been incriminated in outbreaks of food poisoning throughout the world (*2,6*). Currently, it is thought that this illness is mediated by a toxin(s). A diarrheagenic factor, which evidence indicates is an enterotoxin (*3,7*), is normally produced when *B. cereus* is grown in shake flasks containing complex media. Recently, optimum conditions for synthesis of this enterotoxin in a fermenter have been described by Glatz and Goepfert (*3*). This study was undertaken to devise a method in which large amounts of enterotoxin could be produced in the absence of the larger molecular weight proteins present in complex media and at a scale smaller than that of a fermenter. The sac-culture technique described by Donnelly et al. (*1*) was examined for this purpose.

MATERIALS AND METHODS

Set up of sac-cultures and shake flasks

The sac-culture assembly consisted of 100 ml of double strength medium in a 3.3-cm wide 24Å pore-size Visking dialysis membrane (Union Carbide Corp., Chicago, Ill.). Two media were employed, brain heart infusion broth (BHI), and casamino acids plus 1% glucose (CAD) (*3*). The sac was placed in a 250-ml Erlenmeyer flask which was then sterilized at 121 C for 15 min. For the CAD sacs, the sterile 1% glucose was placed outside of the sterilized sac. Twenty ml of sterile 0.04 M PBS, pH 8.0 and prewarmed to 32 C, were added to the flask, outside the sac of medium. Shake flasks (250-ml Erlenmeyer) containing 20 ml of medium were prepared for comparison with the sac-culture method. Each sac-culture and shake flask was prewarmed to 32 C before inoculation.

Inoculum preparation and culture growth

The inoculum, *B. cereus* B-4ac-L, was grown for 17 h at 32 C on a reciprocal shaker, centrifuged at 1,400 × *g*, washed twice with 0.04 M PBS, pH 8.0, and resuspended to its original volume in PBS. Inoculation of the sac-culture assembly and shake flasks was achieved by adding 0.2 ml of the washed culture. All cultures were grown for 8 h at 32 C on a reciprocal shaker at 84 cycles/minute. Two-ml samples of the dialysate and the normal shake flask cultures were taken hourly, centrifuged at 1,400 × *g*, and the supernatant fluid filtered through 0.45-μm membrane filters. Samples were stored at 4 C for approximately 18 h before being assayed for enzyme activities.

Enzyme assays

Assays for lecithinase, heat labile hemolysin, heat stable hemolysin, and enterotoxin as measured by skin capillary permeability factor were done as described previously (*4, 5*) except that the hemolysin assay was modified by mixing equal volumes of culture filtrate (CF) and a washed 2% erythrocyte suspension. The heat stable hemolysin assay differs from the heat labile hemolysin assay in that the sample is heated at 45 C for 30 min before addition of the washed erythrocyte suspension. Toxin activity is expressed as capillary permeability factor units administered in 0.05 ml (CPFUA) (*5*). Values were standardized with a positive control (CPFUA =15.20) which was injected into all rabbits. Surface plating on nutrient agar was used to determine cell numbers.

RESULTS AND DISCUSSION

In preliminary trials single and double strength media and PBS were used for the sac-culture assemblies. Greatest enterotoxin production occurred when double strength medium was used within the sac and with 0.04 M PBS rather than 0.02 M PBS surrounding the sac. Results in Table 1 are based on sac-cultures possessing these concentrations of media and buffer. More enterotoxin was also produced in shake flasks when double strength medium was used.

TABLE 1. *Comparison of production of enterotoxin by* B. cereus *B-4ac-L in shake flasks and sac-cultures*[a]

Medium and cultivation method[b]	Maximum CPFUA obtained[c]	Time (h) to maximum toxin production	Cells/ml at the time of maximum toxin production
BHI/sac-culture	101.57	8.0	3.7×10^9
1 × BHI/shake flask	9.13	8.0	1.7×10^8
2 × BHI/shake flask	11.68	8.0	2.7×10^8
CAD/sac-culture	63.92	5.0	1.8×10^9
1 × CAD/shake flask	0.60	5.0	—
2 × CAD/shake flask	1.18	5.0	1.9×10^9

[a]Values are representative of data obtained
[b]1 × is single strength medium. 2 × is double strength medium
[c]Values are for a 0.05-ml sample

Higher total yields of enterotoxin were obtained in each instance when the sac-culture method was employed. Several explanations are available for this. First, the difference in aeration due to volume differences

in the flasks could be responsible for the greater amount of enterotoxin in the sac cultures. Greater aeration would be experienced in the normal shake flasks with a small volume to flask volume ratio as compared to the sac-cultures. Increased aeration leads to increased oxygen transfer rates and perhaps excessive foaming which would cause lowered enterotoxin recovery (*3*). Second, use of double strength BHI in shake flasks appreciably reduced cell yield but did not affect enterotoxin synthesis. This indicates that among the large molecular weight constituents in BHI there could be an inhibitor which affects growth but not toxin production. Larger amounts of enterotoxin were produced by cells growing in the dialyzable fraction of BHI than in CAD. The toxin produced was apparently more stable in this fraction as well since the large amounts persisted longer than in CAD. There was no corresponding enhancement in synthesis of either heat labile or heat stable hemolysin (Table 2). However, synthesis of lecithinase was enhanced.

TABLE 2. *Comparison of production of lecithinase, heat labile and heat stable hemolysins in BHI shake flasks and sac-cultures*[a]

Medium and cultivation method[b]	Lecithinase titer	Heat labile hemolysin titer	Heat stable hemolysin titer
BHI/sac culture	128	2	8
1 × BHI/shake flask	32	16	2
2 × BHI/shake flask	16	4	4

[a]Results are representative of data obtained and are based on the time of maximum enterotoxin production

[b]1 × is single strength medium. 2 × is double strength medium

The sac-culture technique is desirable because *B. cereus* enterotoxin cannot pass through the dialysis membrane and thus is contained within a small volume of PBS. During this time cells, which also are confined within the small volume of PBS, are nurtured by a large volume of medium which allows for a large yield of enterotoxin. The proteins of large molecular weight which apparently do not inhibit toxin synthesis but which may affect growth are retained within the dialysis tubing. Thus, the sac-culture method simplifies purification of *B. cereus* enterotoxin by permitting use of nutritionally desirable complex media for enterotoxin production by the cells without the medium components of large molecular weight hindering purification efforts. An additional advantage is that synthesis of at least two of the proteins which complicate purification efforts is apparently not enhanced by this approach. Lastly, the sac-culture technique is more simple than and much reduced in scale from a fermenter, yet yields the same amount of toxin obtainable with the latter piece of equipment.

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A Research Note

Relationship of Chlorine Treatment Levels and Development of Off-Flavors in Canned Sweet Corn

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ABSTRACT

Various amounts of chlorine were added to raw, cut, whole-kernel sweet corn before heat processing. Sensory evaluation of canned sweet corn samples showed that experienced panelists could detect off-flavors in corn treated with 25 ppm of chlorine. A laboratory sensory evaluation panel was less critical, and found off-flavors noticeable between 35 and 45 ppm of chlorine.

Chlorine is used extensively for germicidal treatment of water used in food processing plants and in water sprays applied to food processing and handling equipment. Mechanical harvesting of fruit and vegetable products has led to their being handled in bulk bin, tank, and cargo quantities for transport between field and plant, and between plants. There also has been application of field-site preparation of fruits and vegetables, such as washing, trimming, cutting size grading and dicing. In these instances discrete solids and other effluents can better be retained and disposed of, and the prepared product can be economically transported to plants for further processing. Use of chlorine in solution has been experimentally, if not practically, considered for such operations to beneficially inhibit microbial activity during handling and transport. Whole tomatoes submerged in chlorine solutions containing up to 1000 ppm chlorine have been held for intervals up to 72 h; husked sweet corn and cut whole kernel corn have been sprayed with chlorine solution containing up to 500 ppm chlorine and held 2½ h.

In the light of these applications of chlorine in food handling, it was considered advisable to determine the maximum amount of chlorine which could be used without significant detrimental effect on flavor of canned corn. Such information is needed to establish parameters of procedures for handling corn. Somers (*5*), Mercer and Somers (*3*), and National Canners' Association (*4*) have reported on the lowest concentrations of added chlorine which, when added via water or brines and sirups, produced off-flavors in certain canned foods. No flavor effect has been indicated in canned corn containing a total concentration of 15 ppm added chlorine and no maximum limit was cited.

[1]*Deceased, July 9, 1977.*

PROCEDURE

Five oz (142 g) of solutions at 88 C containing different concentrations of monodichloroisocyanate as the chlorine source were measured into 303 × 406 C-enameled cans containing 11 oz (312 g) cut, whole kernel sweet corn (variety Jubilee) which had been previously obtained from a usual processing line in a cannery. The concentration of chlorine in the total contents of the cans ranged from 4 to 442 ppm (Table 1). The cans were closed promptly, and were thermally processed in a continuous retort. The corn was examined after 3 months of storage at 21 C.

Four experienced judges first evaluated the flavor of 14 different lots of corn in comparison with a control lot to which no chlorine had been added. The evaluation questionnaire provided for a profile description of flavor, and for notations whether or not the flavor was objectionable (*1*). The experienced judges selected lots 5 through 9 to have flavors demonstrating threshold levels of effect (Table 1).

Lots 5 through 9 were then evaluated for flavor by 19 members of a laboratory sensory evaluation panel in comparison with the control (lot 1) using a difference intensity procedure (*2*). The samples were prepared by draining the liquid from each can of corn, and portioning 1-oz (28 g) servings into 2-oz (56 g) paper cups coded with random 3-digit numbers. Two cups of the control lot, one identified as a reference sample, the other with a numerical code were submitted along with two or three other experimental samples to the panelists. The absolute scores for the internal reference samples were calculated and used for computing relative intensity scores. An intensity difference ballot required panelists to compare each numerically coded sample with the identified reference, and to note any intensity of flavor difference on a nine point scale (1 =no difference to 9 =extreme difference).

Coded values from ballots were punched into computer cards, and the data were analyzed by the University of Wisconsin 1110 computer for analysis of variance (ANOVA) appropriate for a randomized complete block design (*6*). Mean degree of difference scores for each sample, F-values for the whole comparison of samples, and least significant differences (LSD) for each pair of samples were obtained when a significant F-value was found for the whole comparison.

RESULTS

The evaluations of the four experienced judges are given in Table 1. A chlorine off-flavor in the canned corn was apparent to all judges at a level of 44 ppm. Three of the four judges could detect the specific chlorine off-flavor at 35 and 26 ppm levels. All 4 judges stated

corn with 35 ppm chlorine was not acceptable due to off-flavors; only two stated corn with chlorine at the 26 ppm level was not acceptable. The actual chlorine off-flavor was indicated to be slight at the threshold levels indicated, but the corn had a distinctive "flat" flavor. None of the four judges could detect any chlorine

TABLE 1. *Experienced judge sensory evaluating of canned corn with added chlorine*

Lot no.	Equilibration chlorine content (ppm)	Acceptable flavor[a]		Chlorine flavor detectable[a]		Judge comments
		Yes	No	Yes	No	
		(—No. of Responses—)				
1	Control (0)	4	0	0	4	Chlorine not noticeable
2	4	4	0	0	4	Chlorine not noticeable
3	8	4	0	0	4	Chlorine not noticeable
4	12	4	0	0	4	Chlorine not noticeable
5	17	4	0	0	4	Chlorine not noticeable
6	26	2	2	3	1	Flat flavor, very slight chlorine flavor detectable
7	35	0	4	3	1	
8	44	0	4	4	0	Sl. off-flavor
9	66	0	4	4	0	Off-flavor
10	88	0	4	4	0	Strong off-flavor
11	176	0	4	4	0	Strong off-flavor
12	264	0	4	4	0	Strong off-flavor
13	353	0	4	4	0	Strong off-flavor, dull appearance
14	442	0	4	4	0	Strong off-flavor, dull appearance

[a]Four experienced judges evaluated each sample.

off-flavor at the 17 ppm level and all considered the sample acceptable.

The results of the evaluations by the laboratory sensory evaluation panel are shown in Table 2. Samples containing 12, 17, and 26 ppm chlorine were evaluated in Test 1; samples containing 35 and 44 ppm were evaluated in Test 2. Those containing 12, 17, and 26 ppm chlorine were scored not significantly different from the control. The samples containing 35 ppm were found not significantly different from the control, but the sample with 44 ppm chlorine was statistically different from the control and the sample containing 35 ppm chlorine.

CONCLUSION

The critical concentration of chlorine in canned whole kernel corn appears to be about 25 ppm at which level a flatness in flavor and a slight chlorine off-flavor was evident to experienced judges. The critical range of concentration for chlorine in canned corn as measured by the laboratory sensory evaluation panel was between

TABLE 2. *Small sensory panel evaluation of canned corn with added chlorine*

Lot no.	Equilibration chlorine content (ppm)	Mean of difference intensity scoring[a,b]
Evaluation 1		
1	0	2.4[a]
4	12	2.8[a]
5	17	2.3[a]
6	26	3.1[a]
F-Value		Not sig
Evaluation 2		
1	0	2.1[a]
7	35	2.6[a]
8	44	3.9[b]
F-Value		Sig
LSD 5%		1.1

[1]No. of Judges = 19.
[2]Difference Intensity Scale: 1 = No difference from control, 9 = Extreme difference from control.
[a,b]Mean scores with similar superscripts are not significantly different at the 5% level.

35 and 45 ppm. The sample containing 35 ppm of chlorine was found not significantly different from the control, but the sample with 44 ppm was found significantly different from the control. These data indicate that the four experienced flavor judges were each somewhat more sensitive to chlorine-caused off-flavors in canned corn than the average individual encountered in the larger laboratory panel.

Levels of chlorine which may be retained in canned corn whether via spray treatment or submersion in chlorine treated water for subsequent hold intervals should be determined to be below the critical levels.

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A Research Note

Evaluation of the Botulism Hazard in Fresh Mushrooms Wrapped in Commercial Polyvinylchloride Film

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ABSTRACT

The botulism hazard in fresh mushrooms wrapped in commercial polyvinylchloride (PVC) film appears to be minimal. At the end of their normal shelf-life, 1,078 packages of PVC-wrapped mushrooms were all free of botulinum toxin. Since inoculated mushrooms were occasionally found to be toxic (14 in 250 packages) when only one 1/8-inch hole was punched in the wrapper, and none became toxic when two holes were present, it seems prudent to recommend that PVC-wrapped tills of mushrooms have two holes in the wrapper.

Mushrooms are often regarded as a delicacy, as the major portion, or as the garnish in a variety of dishes. However, since fresh mushrooms are readily perishable, it was desirable to find some means of prolonging their marketable condition. Producers found that mushrooms packaged in papier maché tills and wrapped and sealed in a polyvinylchloride (PVC) film had a longer shelf-life than bulk mushrooms. The till absorbs moisture and the wrapper permits very little gas exchange between the contents and the outside atmosphere. Respiration of the mushrooms, therefore, could be expected to use up the available O_2 and produce CO_2, which would result in an anaerobic atmosphere, favorable to the growth of *Clostridium botulinum*.

Analysis of headspace gas in these packages by Sugiyama and Yang (*2*) showed that the O_2 in the container was quickly reduced to less than 2%. Mushrooms inoculated with large numbers of *C. botulinum* spores of type A or type B and sealed in tills with a PVC wrap developed botulinum toxin while still appearing to be acceptable. Both types of spores grew and produced toxin, but type A grew more readily than type B. Later, Sugiyama (Private Communication, 1977) showed that if one or two holes of 1/8-inch diameter were punched in the PVC wrap without being blocked by either a mushroom or the till, mushrooms inoculated with *C. botulinum* would not become toxic.

In 1976, Craig (Private Communication) found *C. botulinum* type A spores in all samples of mushroom compost and topping which he tested, and Hauschild et al. (*1*) reported low numbers of *C. botulinum* spores in all examined samples of uninoculated mushrooms. The purpose of this study, therefore, was to examine packaged mushrooms wrapped in PVC as offered to the public, for the presence of botulinum toxin after holding them at room temperature for their approximate shelf-life.

MATERIALS AND METHODS

By special arrangement with a local supermarket, mushrooms were obtained as soon as they were received from the producers. The mushrooms were kept in the tills in which they were received but were rewrapped with fresh PVC film without holes. The PVC used was that used commercially for wrapping mushrooms, either Borden's Resinite VC-71 60-gauge polyvinylchloride film or commercial plasticized PVC (0.65 mil thick).

After being held for 7 days at room temperature, mushrooms were tested for toxicity in mice. In preparation for toxicity testing, mushrooms were macerated in a Waring Blendor without addition of liquid and were centrifuged to sediment the pulp. The supernatant fluid was diluted 1:5 and 1:10 in gel-phosphate buffer (pH 6.2), and 0.5 ml of both dilutions was injected intraperitoneally into each of two mice. The mice were observed for 72 h for symptoms of botulism.

RESULTS AND DISCUSSION

The possibility of botulism resulting from mushrooms wrapped in PVC appears to be minimal. Of the 1,078 packages of fresh mushrooms examined in this study, all remained free of botulinum toxin as shown by mouse toxicity testing. The results of Hauschild et al. (*1*) indicate that the number of viable spores on the mushrooms which they examined was very low, and the results of Sugiyama and Yang (*2*) indicate that detectable toxin is not produced unless large numbers of spores are inoculated into the mushrooms. Experiments in our own laboratories have produced results similar to those of Sugiyama with one difference: Some of our inoculated mushrooms wrapped in PVC with only one hole became toxic, whereas those with two holes did not (Unpublished data). Although these experiments do not determine the extent of the problem, they do show the possible hazard

and how it can be prevented.

It seems unlikely that the number of spores required for toxin production would normally be present in fresh mushrooms. However, since our experiments showed that inoculated mushrooms occasionally became toxic (14 in 250 packages) when only one 1/8-inch hole was made in the wrapper, it seems prudent to recommend that PVC-wrapped tills of mushrooms have two unblocked holes in the wrapper.

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Heating Milk for Microbial Destruction: A Historical Outline and Update[1]

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ABSTRACT

Attitudes, developments, and progress related to heating of milk for microbial destruction are outlined. Milk was first heated to increase its shelf-life. Eventually heat was applied to satisfy a requirement for prevention of milkborne diseases. Selection of proper time-temperature combinations of thermal processing can result in sterilized milk. Interest in sterilized milk for non-refrigerated distribution is at an all time high because of potential saving in energy and transportation costs.

Commmercial sterilization, aseptic packaging, and non-refrigerated distribution of fluid milk appear imminent in the United States. Thousands of references on the microbiological quality of milk following heating have appeared in the literature. Therefore, a comprehensive review of this subject was not attempted here — instead the progression of the major developments related specifically to thermal destruction of microorganisms in milk is outlined.

When did it all begin? "We may suppose that the custom of preserving milk by heat is as old as the cow and the use of fire" (*83*).

BEFORE 1900

The beneficial effects derived from the heat treatment of milk were probably not first realized by any recognized scientists, but rather as Hall and Trout (*52*) state in their book, "Undoubtedly, housewives had resorted to cooking or boiling milk for ages, for such practices still exist where pasteurized milk is not available."

Crediting Louis Pasteur for the original concept we must: (*a*) assume that he applied his "par-boiling or under-boiling" heating principle to milk; and (*b*) overlook two rather significant heat treatments for milk which were recommended before Pasteur's work. For example, William Dewees recommended heating of milk as early as 1824 (*52*), close to 40 years before Pasteur's experiments. Dewees' suggestions included the heat treatment of milk in the home before infant feeding. He recognized the advantages of boiling milk to increase its shelf life, as is evident from his statement, "the tendency to decompose is diminished in the milk." He suggested that boiling per se was not required, by just heating the milk to the boiling point and cooling it quickly, the "formation of that strong pellicle (protein denaturation), which is always observed on the top of boiled milk," could be avoided. Also, before Pasteur's work was the major contribution of Gail Borden, who obtained a patent in 1853 for heating and condensing milk under vacuum followed by addition of sugar for preservation. However, heating milk for microbial destruction was not appreciated until Pasteur's work. He experimented and suggested the use of fairly low temperatures (50-60 C) which were sufficient to destroy spoilage organisms (in wine and later beer), but low enough so as not to alter the original characteristics of the liquid.

Application of this low-temperature heat treatment (pasteurization) to milk may have been first suggested and applied by Soxhlet (*89*). He accomplished pasteurization of bottled milk to be fed to infants (*34*). Gerber and Wieske were pasteurizing milk in bottles by the method of Gerber (1 h at 65 C) in 1888 (*51*).

The first commercial milk pasteurizer was made by Albert Fesca (Germany) in 1882, who termed it "a continuously working apparatus for the preservation of milk by heat" (*83*). Heating in this apparatus was at 74 to 77 C for an unspecified time. The apparatus and process were later adopted in Denmark (*63*). In 1884, a Danish dairy scientist, N. J. Fjord, constructed and received credit for a similar apparatus for heating milk (*70*). This heater was designed to heat milk to 70 C with the resulting product being stable for 24 h. Apparently, Fjord had been applying pasteurizatin techniques to milk as early as 1870. Pasteurization of milk on a commercial scale was a common practice in Denmark and in Sweden during the mid-1880's. Heaters of greater efficiency were developed quickly, since it was required

[1]*Scientific Article No. A2352, Contribution No. 5362 of Maryland Agricultural Experiment Station, Department of Dairy Science.*

in Denmark that milk be heated to 85 C to prevent the spread of tuberculosis (*70*). For example, a continuous heater which momentarily heated milk to 85 C was developed in Denmark in the early 1890's. This apparatus, known at the time as the Danish pasteurizer, was first introduced into the United States by Reid in 1895 (*63*). A few years before this, in 1893, L. B. Halsey, Sheffield Farms Company, installed a German pasteurizer in its Bloomville, New York plant, which is considered to be the first commercially operated milk pasteurizer in the United States (*52*).

Most of the credit for dissemination of knowledge and benefits from the heat treatment of milk must be given to two men, namely Henry Koplix, a pediatrician, and Nathan Straus, a philanthropist (*92*). Koplix was a strong supporter of the concept of heating milk before feeding it to infants as had been suggested earlier by others, including Dewees (1824), Jacobi (1873), Soxhlet (1884), and Caille (1888) (*52*). Koplix, however, actually set up a dispensary for heated milk in New York City around 1889. Nathan Straus was so impressed with Koplix's work that he financed and established (1893) numerous milk depots in New York City where heat-treated milk could be purchased and, in fact, sold at cost or given away to the poor (*47*). Straus, "carried the message of pasteurization to not only every large city of the United States, but to Europe as well" (*52*). The Straus milk depots used an in-bottle system of pasteurization which had been described by Freeman (*46*). Glass bottles were first sterilized by dry heat at 150 C for 1 h, cooled, and then filled with milk. The final heat treatment was at 75 C for 20 min (10-min come-up time), and then the bottles were cooled rapidly in running water (*47,92*). Other systems in operation before 1900 are summarized in Table 1.

Despite the lack of compulsory pasteurization requirements in the United States, heat treatment of milk on a large scale was becoming increasingly popular in the late 1890's, since it was quickly recognized by dairy microbiologists and dairy processors as a way to increase the shelf life of milk. The debate over benefits and objections of the heat treatment applied to milk was just beginning and would not be settled until the early 1900's. For this reason early commercial pasteurization of milk was not generally accepted, but many milk companies had adopted it secretly, or at least were not informing their patrons of the fact.

Several milkborne diseases were recognized before 1900, including typhoid fever, diphtheria, scarlet fever, tuberculosis, anthrax, and foot and mouth disease (*29,48,49*). Information before 1900 on heat destruction of pathogens in milk was very limited and variable. For example, it was reported that *Mycobacterium tuberculosis* was killed in milk heated to 60 C in 15 min; however, if a pellicle developed, it would survive for 60 min at 60 C (*88*). Russell and Hastings (*82*) found that if milk was heated in a tightly closed commercial pasteurizer, *M. tuberculosis* was killed at 60 C in 10 min. These workers recommended, in 1900, heating at 60 C for 20 min to insure thorough pasteurization based on their data (*82*).

Pasteur's outstanding work exposed the role of microorganisms in causing undesirable changes in foods. Many of the workers following Pasteur applied this heat treatment to milk to kill microorganisms. Some recognized it as a way to prevent milkborne diseases, and others as a way to increase the shelf life or at least make the milk stable for short periods. Soxhlet and many who followed believed that this "par-boiling" or pasteurization of milk rendered the milk sterile, although this was questioned by Flügge (1894) and Weber (1900) as noted by Savage (*84*). The systems referred to as "sterilizing processes" were in actuality not sterilizing the milk. It is noted, however, that many of these processes were considerably above what was, at the time, considered adequate for destruction of milkborne disease causing agents.

1900 - 1925

Jordan (*61*) suggested that about 5% of New York City's milk supply was receiving a heat treatment in 1902. By 1914 this figure had jumped to 88% (*5*).

TABLE 1. *Examples of heat treatments applied to milk on a commercial scale before 1900*[1]

System	Description
"Pasteurization techniques"	
Dr. Koplix's Good Samaritan Dispensary in New York City	Glass bottles (2, 4 and 5 ounces) heated at 160-170 C for 40 min, then filled with milk and heated at 85 C for 30 min and corked with sterile stoppers
Nathan Straus Milk Depots New York City	As described in text
Fjord's "Danish pasteurizer"	A continuous system, milk heated to 160-180 F (71 to 82 C), no time specified, and then filled into bottles
Posen, Germany	Milk heated in metal casks by steam (104 C), no time specified
Dresden, Germany	Milk preheated to 65 C, filled into one-third liter flasks and then heated under steam for 105 min
Pauling, Dutchess County, N.Y., Appleberg Hygienic Milk Company	Milk vat pasteurized in the patented Appleberg sterilizer 160-180 F (71 to 82 C) for 20 to 30 min, with agitation, then filled into sterile bottles
"Sterilizing techniques"	
Neuhauss-Gronwald-Oehlman System	Milk heated in bottles at 85-90 C for 30 min and then reheated (same day) at 102-103 C for 30 min
Paul Ritter von Hamm System	Milk heated in bottles at 110 C for 30 min (come up time 1 h)

[1]Condensed from: de Schweinitz, E. A. 1894 (*36*).

At the turn of the century low-temperature heat treatments (71 C or less) were accepted by many investigators as being effective for destruction of pathogenic microorganisms. Medical authorities, however, were much opposed to milk dealers heating milk. They felt that this process should be done in the home to avoid further post heat-treatment contamination (*72*). The early 1900's produced an abundance of studies detailing the effects of heat on milk which began to mold the opinions on the merits of pasteruization. For example, in 1903, Park and Holt (*72*) compared mortality and infant diarrhea rates in children fed raw and pasteurized milk. Children consuming pasteurized milk experienced a demonstrated reduction in both mortality and incidence of infant diarrhea. This provided an undisputed benefit which was difficult for the medical and public health profession to ignore. Many, however, were still skeptical of some of the methods of pasteurization. Criticism was leveled particularly at the "flash" methods of pasteurization. These methods usually employed a temperature of 71 C for 30 sec to 1 min (see Table 2). Inadequately pasteurized milk could result from these processes, since there was great variation in flow rate, temperature, and holding time. The work of Rosenau in 1908 (*81*) on thermal death points of pathogenic bacteria, stands out as one of the factors that instilled considerable confidence in the low-temperature holding method for adequate pasteurization. His recommendation of 60 C for 20 min was much more acceptable to the medical and public health profession than the variable "flash" methods. A large commercial pasteurizer was installed in New York in 1907 (*72*) which employed temperatures of 60 to 66 C and holding times of 30 to 45 min. The great success of this technique on a commercial scale marked the beginning of what would become the "holding method" of pasteurization. The idea spread rapidly in the dairy industry, and most of the "flash" heating systems were replaced, or converted to preliminary heaters before pumping into the "delay" or "holding tanks". There was, of course, still a significant amount of milk being pasteurized by an in-bottle technique. This procedure normally would incorporate temperatures of about 63 C for 20 to 30 min. A series of papers (*6,7,80*) did appear on the advantages and disadvantages of in-bottle milk pasteurization. In general, these authors were supporting the in-bottle procedure because it eliminated post-pasteurization contamination problems. This technique, however, was beginning to lose favor on a commercial scale.

TABLE 2. *Examples of commercial methods used for the heat treatment of milk between 1900 and 1925*

Method	Description
Holding method	Milk heated in vat or heated then pumped into vats—140 to 150 F (60-65.6 C) for 30 min
Flash	Continuous flow of milk heated to about 160 F (71.1 C) for 30 sec to 1 min
In-bottle	Bottles filled with milk and heated at 145 F (62.8 C) for 20 to 30 min
Bottling hot	Milk heated at 145 F (62-8 C) for 30 min then filled hot into steamed bottles (2 min)

Consider, for a moment, the dilemma of public health officials and regulatory agencies in the early 1900's. Emotional objections to pasteurization were numerous. The number and variety of available commercial milk heating systems were perplexing. Data for thermal destruction of milk-borne pathogenic bacteria were confusing. Between 1883 and 1906 no fewer than 26 reports appeared in the literature on the thermal death time of *M. tuberculosis* (*72*). The temperatures and times selected for these studies were arbitrary, and ranged from 50 to 100 C and 1 min to 6 h, respectively. It is indeed understandable, then, that between 1890 and 1927 there appeared at least 31 different reommendations for time-temperature combinations to be used for adequate pasteurization of milk (*72*).

Several New York philanthropists who recognized the merits of pasteurization of milk formed the New York Milk Committee. This body, in turn, appointed the National Committee on Milk Standards (NCMS) which was composed of seven bacteriologists and eight public health officials who were all recognized at the time as leaders in their fields. In 1911, they (NCMS) unanimously recommended a time-temperature combination of 145 F (62.8 C) for 30 min (*72*). The prestige and the expertise composing this Committee made their recommendation indeed credible, but it was not uniformly adopted.

Many legal definitions of milk pasteurization (time-temperature combination) appeared in the milk regulations of cities and towns in the United States, since local health officials exercised independent judgement, for the most part, on what should be required. In 1922 the lowest time-temperature combination which appeared in local regulations was 60 C (140 F) for 20 min. The most popular choices, however, were 61.1 C (142 F) or 62.8 C (145 F) for 30 min. Pasteurization at these temperatures is, therefore, slightly above what was at the time considered by many to be adequate exposure for destruction of *M. tuberculosis* (about 60 C for 20 min). Higher temperatures for longer times would, of course, have increased the margin of safety. This would have had a significant commercial disadvantage since it had been shown that higher temperatures destroyed the cream line or the creaming ability of milk and additionally might have imparted a cooked flavor. The effect of time-temperature combinations on various milk properties had been summarized by North in 1911 (*71*).

Several pertinent observations were made in a report of the Committee on Milk Supply, American Public Health Association, in 1920. The committee reported that of the approximately 4,200 pasteurization plants in operation in the United States, *none* of them afforded full protection against ineffective pasteurization. Furthermore, they criticized most of the existing pasteurization equipment but did state that the holding

method was superior. In their report they recommended a temperature of 145 F (62.8 C) for 30 min (*3*).

In 1921, Harry Cronk (Borden's Farm Products Company) suggested to Dr. Charles E. North (North Public Health Bureau) that his organization carry out research on commercial milk factory operations to establish a standard for pasteurization (time-temperature combination) and standards for pasteurizing machinery. Dr. North agreed to supervise this research and invited many other experts to cooperate with him. This was the beginning of what would be referred to as the "Endicott" experiments. Endicott, New York was selected as the site for an extensive series of experiments which were to last several years, and during which time up to 25 experts were involved. Several commercially available pasteurizers were tested, and extensive engineering studies done. Bacteriologists on the project composed the single largest group of investigators and they represented six different testing laboratories. Large volumes of milk (4,000 quarts) were inoculated with bovine tubercule bacillus, human tubercule bacillus, typhoid bacillus, paratyphoid bacillus, diphtheria bacillus, and hemolytic streptococci. The most extensive studies were done on *M. tuberculosis* (1,958 guinea pig inoculations) and with the typhoid bacillus. There was some criticism of the initial series of experiments, these were reported and the questions would be resolved in the next several years (*73*). The "Endicot experiments dramatically pointed out defects in then available pasteurizing machinery. Many irregularities such as uneven temperature of heated milk, defects in vats, lack of proper insulation of tanks, leaky valves, dead ends in piping, excessive formation of foam, excessive splashing, and imprecise measurements of holding times were indicated. The "Endicott" experiments as reported (*69,72,74,75*) concluded that there was a great necessity for improvements in construction of equipment, and indicated the importance of better engineering and controls over such equipment to provide the proper safeguards for pasteurization from a public health viewpoint.

The commercial success of the "holding method" and the acceptance of it as an adequate method of pasteurization resulted in the first pasteurized milk ordinance being published in 1924. In the November, 1924 issue of *Public Health Reports*, pasteurization was defined as a heating process of not less than 142 F (61.1 C) for 30 min in approved equipment.

1925 - 1950

The numerous and quite frequently variable reports on thermal destruction of *M. tuberculosis* in milk were finally clarified by the work of North and Park (*73*). This paper contained an excellent review of the major papers that had been published on the thermal destruction of *M. tuberculosis*. Their data confirmed the work of several earlier investigators (*81,82,88*), and supported the recommendation of 61.1 C (142 F) for 30 min as providing an ample safety margin for destruction of *M. tuberculosis* in milk.

Although the "holding method" of pasteurization was the most widely used during the late 1920's and early 1930's, there was already considerable work underway on the effects of higher temperatures and shorter times on thermal destruction of *M. tuberculosis*. Plate heat exchangers were being developed and used for high temperature-short time methods which were evolved into the current method of milk pasteurization, that is, high temperature-short time (HTST) pasteurization. As one would expect, there was considerable reluctance on the part of public health and regulatory officials to accept the HTST equipment and procedure. Their reluctance was based on difficulties in assuring that products would be held for the specified period. Technology of this equipment, however, was developing rapidly and the availability of adequate control mechanisms finally permitted approval of HTST equipment for pasteurization of milk. HTST standards were included in the 1933 U.S. Public Health Service Milk Ordinance and Code.

It was difficult to predict the adequacy of pasteurization at high temperatures for short times from the data of North and Park (*73*), since minimum times at temperatures above 65.6 C were not reported in their paper. Additional data on thermal destruction of *M. tuberculosis* at higher temperatures and shorter minimum times were reported by several investigators (*31,54,67,98*). Workman (*98*), for example, enumerated the requirements for proper pasteurization by studying the effects of time and temperature on 17 strains of human and bovine tubercle bacilli, 74 stains of *Brucella abortus*, and on 218 strains of human and 186 strains of bovine streptococci. He concluded that laboratory experiments at 160 F (71.1 C) for 15 sec resulted in complete destruction of all these organisms, since in no instance did any of the above strains survive these temperatures.

The HTST standard of 161 F (71.7 C) for 15 sec resulted from a consideration of the effect of HTST treatments on the creaming ability of milk, practical experience, and the result of numerous investigations on the thermal destruction of microorganisms in milk at elevated temperatures.

HTST techniques were rapidly being accepted on a commercial scale during the late 1930's and early 1940's. The feasibility of pasteurization at even higher temperatures was also being examined. Some of these newer techniques employed temperatures above 76.7 C and were referred to as "Quick-Time" pasteurization (*33*).

In 1927, Grindrod received a patent for a steam injection system which was capable of heating milk to 110 C. The milk was first heated by injection of steam at 2 atmospheres of pressure, and subsequently cooled in an expansion chamber which removed the condensed steam. Holding time of 1 to 2 min were recommended. The steam injection process was improved considerably during this period, and temperatures approaching 150 C

with holding periods as short as 0.5 sec were being reported (*55*). The process of the direct heating of milk by injection of steam and the coming development of the steam infusion process—that is, milk being injected into an atmosphere of steam—along with improvements of several indirect heating methods would eventually evolve into time-temperature combinations that are now commonly referred to as ultra-high temperature (UHT) pasteurization.

The rickettsia responsible for Q-fever, *Coxiella burnetii,* was first described by Derrick (*35*). Research that followed detailed the wide distribution of the organisms in nature, and most significantly, its presence in raw milk. Early investigations of Q-fever in California provided evidence that the organism was more heat resistant than *M. tuberculosis*, and that it could be isolated from pasteurized milk which had been processed according to then recommended minimum standards (*57*).

1950 - 1975

In 1950, Bell and his associates (*9*) suggested that use of raw milk infected with *C. burnetii* might be responsible for the appreciable number of human cases of Q-fever observed in southern California. The effect of pasteurization on survival of *C. burnetii* was investigated by Lennett (*65*), and it was concluded that further work on the thermal death point of this organims was needed. The United States Public Health Service, in cooperation with the University of California, established a research project to study the thermal resistance of *C. burnetii*. The report of this work (*40*) showed that if large numbers of *C. burnetii* were present in raw milk some might survive pasteurization at 143 F (61.7 C) for 30 min. This study resulted in the recommendation by the Public Health Service to increase the time-temperature combination of pasteurization to 145 F (62.8 C) for 30 min. Furthermore, it was suggested that at least an additional 5 F be employed for pasteurization of milk products containing more fat than in whole milk or added sugar. Interestingly, a more recent study on *C. burnetii* has noted that the clinical disease in human volunteers was not demonstrated following ingestion of raw milk naturally infected with the organism (*64*).

In 1959, Kells and Lear (*62*) updated the thermal death time curve of *M. tuberculosis* in milk. Their results indicated that the z-values of three strains ranged from 4.8 C (8.6 F) to 5.2 C (9.4 F), which were considerably lower than the previously used z-value of about 12 F as calculated by Ball (*8*) from the existing data at that time. It was concluded that the present pasteurization standard provides a margin of safety of approximately 28.5 min at 61.7 C, and approximately 14 sec at 71.7 C. Figure 1 depicts a comparison of thermal death time curves for *M. tuberculosis* and the pasteurization curve.

Many aspects of the use of higher temperatures for pasteurization and even sterilization of milk were developing simutaneously, and therefore it is appropriate to clarify some terms. A description of many of the systems used to process milk at temperatures above pasteurization can be found elsewhere (*12,16-22,56, 86,95*). Conventional HTST systems (plate heat exchangers — indirect heaters) were being used at temperatures above the minimum time-temperature combinations established for pasteurization to further reduce bacterial loads and thereby increase the shelf-life. Indirect heaters were also being used to sterilize milk. Additionally, direct heating systems, in the form of either steam-injection (steam being injected into milk) or steam infusion (milk entering a steam atmosphere), were being developed and tested for use at high temperatures. All of the temperatures used were above pasteurization or ultra-high temperature (UHT) processes, and hence begins the confusion. Any temperature above pasteurization temperatures could be referred to as a UHT process. Selection of the time-temperature combination obviously would dictate whether it was a UHT *pasteurization* technique or a UHT *sterilization* technique. According to the International Dairy Federation, UHT processes refer to pasteurization techniques with temperatures of at least 130 C in a continuous flow and holding times of approximately 1 sec or more. In most European nations, UHT refers to a time-temperature combination rendering the product

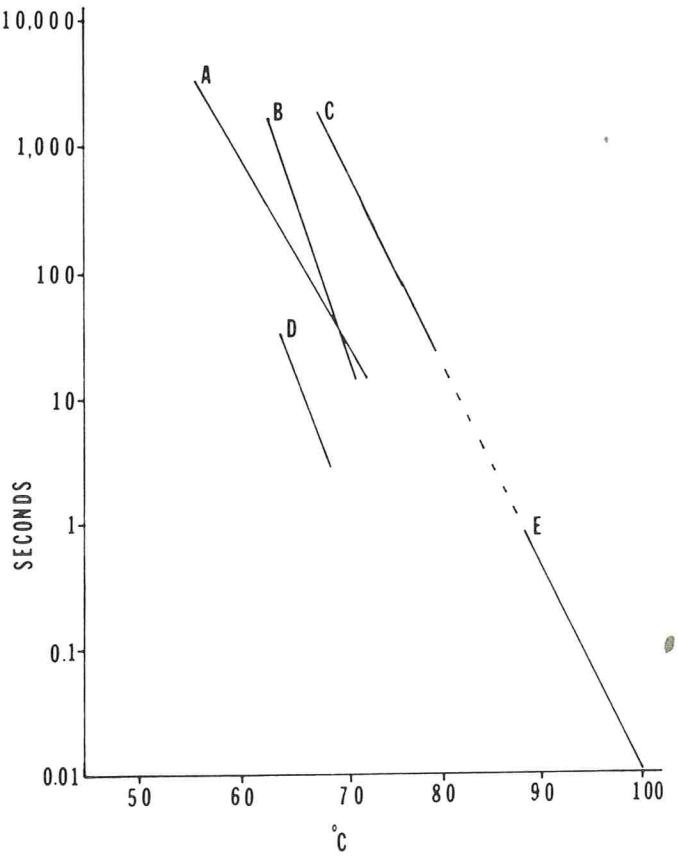

Figure 1. *Thermal death time curves for* Mycobacterium tuberculosis: *line A is from data of North and Park, 1927; line D is from data of Kells and Lear, 1970; and pasteurization curves for: milk, line B; ice cream mix, line C; and the UHT pasteurization curve, line E.*

commercially sterile. Until some new terms are introduced, or at least redefined, attention should be given to the fact that an UHT system may be an UHT pasteurization or an UHT sterilization procedure. Suggested references for a broad view of UHT processes and their implications would include the following (*1,14, 15,60,56*).

UHT PASTEURIZATION

The interest in and development of UHT pasteurization time-temperature combinations for milk have culminated in legal standards (Table 3). In the absence of data on thermal destruction of pathogenic organisms at UHT temperatures, these standards were developed by extrapolating existing data with the addition of a wide

TABLE 3. *Current minimum pasteurization standards*

Methods and product	Time-temperature
Vat or batch pasteurization	
milk	30 min - 145 F (62.8 C)
Cream	30 min - 150 F (65.6 C)
Ice cream Mix	30 min - 155 F (68.3 C)
HTST - pasteurization	
Milk	15 sec - 161 F (71.7 C)
Cream	15 sec - 166 F (74.4 C)
Ice cream Mix	25 sec - 175 F (79.4 C)
UHT - Pasteurization	
All products	1.0 sec - 191 F (88.3 C)
	0.5 sec - 194 F (90.0 C)
	0.1 sec - 201 F (93-9 C)
	0.05 sec - 204 F (95.6 C)
	0.01 sec - 212 F (100 C)
Ultra-pasteurized	
All products	2 sec - 280 F (137.8 C)

margin of safety. The pasteurization curve for milk (Fig. 1, 62.8 C, 30 min; 71.7 C, 15 sec) was not extrapolated from because the slope (z value) of this line is lower than the z values for some vegetative cells. However, extrapolation of the existing pasteurization curve for ice cream mix (68.3 C, 30 min; 79.4 C, 25 sec) was considered acceptable (*76*) (See Fig. 1). The time-temperature standards for UHT pasteurization of milk and milk products by plate heat exchangers (*77*) were later suggested as acceptable minimum combinations for UHT pasteurization by steam injection (*37,38*). The heat resistance of certain pathogenic bacteria in milk was studied in 1971 (*41*) and it was concluded that 82.2 C would adequately pasteurize milk.

UHT STERILIZATION

During the late 1950's extensive investigations were being conducted on an UHT milk sterilizing plant at the National Institute for Research in Dairying, England. Description of the system and procedures used for calculation and determination of the bactericidal and sporicidal effectiveness of this system were discussed in a series of papers (*11,12,25,44,45,97*). Burton (*13*) summarized the reported sporicidal efficiencies of several UHT milk sterlizing plants (both direct and indirect heating systems) operating at 130 to 135 C and concluded that they were capable of producing milk with the sterility that would likely be required for commercial operation.

Interest in UHT sterilization of milk was increasing, since it now appeared feasible to produce a commercially sterile milk with acceptable organoleptic and nutritional properties. The aseptic packaging of this milk could result in a product with an unrefrigerated shelf-life of several months. Both direct and indirect heating systems for use at temperatures of 135 to 150 C for short holding times of approximately 2 sec or more were rapidly being developed and tested.

Use of higher temperatures for processing of milk stimulated research on thermal inactivation of bacteria and spores at UHT temperatures (*10,28,39,41,53,59, 60,66,68,78,79,85,90,93*). Many of the earlier papers on thermal resistance of spores in milk suggested that there was an inhibitor in UHT milk that prevented growth of *Bacillus stearothermophilus*. This complicated determination of sporicidal efficiencies and recovery of surviving *B. stearothermophilus* spores from milk. The inhibitory components of UHT milk were later characterized (*4,27,30*).

During the early 1970's researchers at the National Institute for Research in Dairying published a series of papers comparing an indirect heater (plate heat exchanger) and a direct heater (steam injection system). The operating characteristics of each of these systems were described by Burton and Perkin (*24*). A comparison of the sporicidal efficiencies of each system suggested that a direct heating system had to be operated at 3 to 4 C higher than the indirect system to produce equal sporicidal effects (*43*). The differences in vitamin and nutritional value of the milk processed by a direct or indirect system appeared negligible (*24*).

UPDATE

Selection of the appropriate time-temperature combination for thermal destruction of microorganisms in milk would insure commercial sterility. Temperatures in excess of 138 C for several seconds are usually considered adequate and commercially feasible. Microbiological considerations would therefore include destruction of pathogens and heat-resistant spores. Additionally, processors considering extended non-refrigerated shelf-life would have to consider the presence of residual microbial heat-resistant enzymes (*1*).

Many UHT systems can produce a commercially sterile milk which, if aseptically packaged, could be marketed without refrigeration. This is evident from the large volume of milk so produced in many European countries (Table 4). The reluctance to go this route in the United States is somewhat interesting. Questions are raised about consumer acceptance of a possible "UHT flavor" and currently available aseptic carton configurations. The fact that we have such an excellent pasteurized milk (keeping quality) supply could also be considered as a

TABLE 4. *Approximate percentages of the fluid milk supply sold as UHT aseptically packaged milk*[1]

Country	Percent
Belgium	8
France	18
Germany	36
Italy	42
Netherlands	3
Spain	8
Switzerland	35
Yugoslavia	18

[1]From: Slater, L. E., 1976 (*86*)

reason for lack of development of a UHT market.

What about legal restrictions? All UHT systems would readily meet the heat requirements for *pasteurization* of fluid milk (Table 3), since holding times would exceed the required 0.01 sec. What is restrictive, however, is the fact that all of these milks must be cooled immediately following the process to 7.2 C, and maintained thereat. Milk could be processed and labeled "Ultra-pasteurized" (as was defined by the FDA in the *Federal Register* of October 10, 1973, milk processed at or above 138 C (280 F) for at least 2 sec), however, this product must be refrigerated. Consideration and a regulatory interpretation, therefore, must be given for a commercially sterile *non-refrigerated* milk. Various legal definitions of UHT milk are listed in Table 5. If provision could not be made in milk regulations, and alternative might be to consider the product covered by the Code of Federal Regulations

TABLE 5. *Legal definitions of UHT milk*[1]

Country	Definition
United Kingdom	—Milk which has been heated treated to not less than 270 F (132.2 C) and held for not less than 1 sec.
West Germany	—Milk must be heated to 275-300 F (135-148.9 C) in approved equipment. Holding time determined for each system.
Sweden	—Milk which has undergone such a heat treatment as to render the product free from living bacteria.
Denmark	—If aseptically packaged in cartons, it is treated as pasteurized milk for legal purposes.
United States	—No official definition for sterilizing milk aseptically packaged in flexible containers for non-refrigerated distributions.

[1]Condensed from: Aggarwal, 1974 (*2*).

21 CFR 10.1, Part 128B — Thermally Processed Low-Acid Foods Packaged in Hermetically Sealed Containers. The potential problem here is with the term "hermetically sealed." FDA defines "hermetically sealed" as "a container which has been designed and intended to be secure against the entry of microorganisms, and to maintain the commercial sterility of its contents after processing." This definition differs somewhat from a dictionary definition referring to airtight containers. The appropriate question, therefore, is "do conventional paper milk cartons satisfy the definition of a hermetically sealed container?" All legal restrictions could be resolved by presentation of data or appropriate facts. What remains is the need for an *acceptable* aseptic packaging system, acceptable to regulatory agencies, processors and the consumer.

Is it simply a matter of time before a major dairy company in the United States takes the initial step and markets UHT non-refrigerated milk? Perhaps we are experiencing a phenomenon that occurred at the time of the introduction of HTST systems. The following is from a paper by Dahlberg (*32*) in 1946, and if we just substitute a few words as is shown in parentheses, the paragraph is still very appropriate.

"I think that there is a natural hesitation for milk companies to avoid new processes that may not have the whole-hearted support of public health officials. These officials and dairy executives remember the unfortunate experience with flash pastuerizers (now HTST systems). It is expensive to buy equipment which will not function properly, and then have to discard it for good equipment. The natural tendency is to blame the trouble on the newer equipment so it must be good to endure. There is a real advantage to hold to low-temperature pasteurization (now it is to hold to HTST) until the time comes when the newer process has gained universal approval through satisfactory performance. That time is here."

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Education and Training to Prevent Problems in Food Protection: Experience in the Nation's Capital

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ABSTRACT

Public health scientists are increasingly recognizing that the foundation of food sanitation practice in foodservice establishments is, to a large extent, based on the knowledge, attitude and behavior of the foodservice worker. Accordingly, regulatory agencies throughout the United States are renewing their interests in training and certification of foodservice workers. This article outlines the fundamental strategy of the education and training program in the nation's capital.

Training of foodservice personnel is not a new activity for state and local food control agencies. As early as 1938, the Flint, Michigan Department of Public Health started foodservice training classes, and a year later amended the food ordinance to require mandatory attendance at semi-annual instruction seminars. In the same year the Texas State Health Department and the Texas Department of Vocational Education developed training courses incorporating all of the essentials of sanitary foodservice.

At the national level, interest in foodservice training was clearly expressed in the 1943 edition of the Ordinance and Code Regulating Eating and Drinking Establishments, recommended by the United States Public Health Service. The Code urged and encouraged regulatory agencies to institute training for foodservice workers.

Adams (*1*) suggests that these earlier interests in foodservice training were the result of a change in policy regarding the medical examination of foodservice personnel which occurred in 1937 when Dr. William Best of the New York City Health Department presented a paper on routine physical examinations to the American Public Health Association.

In his very comprehensive paper, Dr. Best questioned the efficacy of routine physical examinations, pointing out that, generally, such examinations were of a cursory nature, they did not reveal conditions that might be conducive to the transmission of diseases through food, they gave no assurance that persons examined would remain free of communicable diseases during the tenure of the certificate and they were not commensurate with the public health benefits obtained.

NEED FOR TRAINING

While some state and municipal health agencies still require "food handlers' examinations," it is increasingly recognized that the foundation of food sanitation practice is not inspection and enforcement, but the knowledge, attitude, and behavior of the foodservice worker. At least three reinforcing factors constitute the basis for this conclusion (*2*).

In the first place foodservice involves personal contact by humans, a contact which in so many instances occurs at a stage in processing after which destruction of pathogens is no longer possible without altering the flavor of the food product. A second difficulty arises from the fact that contamination at the preparation and service levels is, in general, related more to habits and practices of foodservice personnel than to equipment and the physical plant. A third problem is that very often food is not served by professional foodservice personnel, but by volunteers who are working under physical conditions not primarily designed for dispensing food. This is illustrated by the large number of outbreaks of food intoxications and food infections traced to church suppers, picnics and improvised banquets.

Within this context, food scientists and regulatory agencies are concerned about the continuing prevalence of gastroenteritis or gastroenteritis-like illnesses, many of which are traceable to substandard foodservice practices, the increasing gap between changes in the food industry and the resources available to evaluate and control food-associated hazards.

Since there is ample scientific evidence of the need to establish more effective multiple barriers to the transmission of pathogenic organisms through environmental vehicles such as contaminated food, we have renewed our interest in a system—training and certifica-

tion—through which foodservice workers can be informed and motivated to take an active part in preventing food contamination and, at the same time, improve the esthetic aspects of foodservice hygiene and sanitation.

Moreover, efforts have been made by several governmental agencies, educational institutions and technical trade associations to inform consumers, foodservice workers and the food industry about the scientific and epidemiological basis for preventing foodborne illnesses.

An extensive list of short-term, specialized training courses is offered by several agencies. Curricula for training at the college level have also been recommended. Numerous technical and nontechnical articles and manuals have been published, and the National Center for Disease Control has done much to stimulate interest in food protection through publication of surveillance reports.

Seizure of contaminated food products by enforcement agencies has also gained the attention of the food industry and consumers. This action has helped to sensitize and motivate them. There remains, however, much to be done in crystallizing this interest into a comprehensive national training program, thus sustaining the effort on a long-term basis.

PLANNING AND IMPLEMENTATION

Against that broad background, allow me to outline what we in the District of Columbia have attempted to do and what we have learned in an effort to bring about more effective participation of the food industry in protecting consumers from food infection and food intoxication.

First, a responsive legislative body, the District of Columbia City Council, after maximum support and cooperation from the foodservice industry and a full spectrum of consumer groups, amended the D.C. General Food Regulations to require training and certification of foodservice managers. It also required a refresher training course every 3 years following the initial course. The regulations further stipulate that the training program may be provided by the food industry or any other person or group of persons qualified to conduct a training program which is prescribed by the District of Columbia government.

Here one may logically ask why is it necessary to make training and certification mandatory? Hanlon (*3*) provides a partial answer. Referring to the mass of mandatory requirements in the field of milk and food control, he states:

> "Undesirable as this situation undoubtedly is, all will agree that certain types of legal controls are necessary. It is obviously important for a community to exercise some control of those who produce and handle its food and milk supplies. This has repeatedly been upheld by the courts."

Therefore, compulsory regulations, such as mandatory foodservice training and immunization requirements, are often important in protecting community health. They also encourage desirable and long-lasting changes in behavior. This judgement further recognizes that all educational efforts are designed to promote individual dignity and responsibility, as well as community solidarity. Education and training for foodservice workers are designed, on the same basis.

In addition, our experience and that of other state and local governments indicate that voluntary programs have not been successful in attracting a significant number of foodservice workers. The dubious assumption was made that individuals who voluntarily attended training courses would in turn pass this information along to others. But this expectation was not brought to fruition.

Before we could proceed to implement a training program, it was necessary to obtain more information on our target population and to collect certain base-line data required for future evaluation and program revision. This data-gathering phase required a careful analysis of the Washington, D.C. foodservice industry, including a study of in-house environmental management practices, hours of operation, staff size, distribution per work-shift and the characteristics, communication, exposure and behavior of the foodservice workers. All of this information had to be known before we could intelligently plan, implement and evaluate a foodservice training program. Without such knowledge it is extremely difficult to judge the applicability of different training strategies to the target foodservice population.

For example, some adults may effectively respond to the often-used pure lecture strategy. Others may be more motivated by self-instructional materials, single games or exercises, computer-assisted problems or simulations. As a result those who benefit from education services—the target population—should be involved *very early* in identification of need and in determination of what actions should be undertaken in the training process.

We should emphasize that educational aspects of food protection are too important to be allowed to occur by chance or haphazardly; planned educational intervention should transpire as the result of carefully conceived goals and objectives, realizing the maximal gains received from potential educational situations. Not only should basic hygiene and sanitation facts be the focus of training, but also an understanding of human motivation and behavior, as well as information concerning the cultural background of the foodservice worker, must all be incorporated in this process for successful communication.

While much careful planning was needed to determine the technical points to be emphasized in our foodservice training program, the more difficult problems related to (*a*) devising acceptable effective means for educating audiences having diverse backgrounds and interests, and (*b*) generating a desire to use the information on a day-to-day basis. With the necessary background

information, we assembled the applicable subject-matter and designed a minimum course which could be offered by qualified organizations.

The initial training courses were conducted by an accredited academic institution. Later, the foodservice industry and several other accredited institutions implemented training programs. To date some 700 separate classes have been conducted as part of their continuing education services.

For the convenience of the trainees, classes are held at several readily accessible locations and at different time periods, ranging from early morning to late evening. Obviously all foodservice workers cannot attend the same course but generally there is a lull in business between meals when some workers can be relieved to attend classes.

Using our role as a catalyst in solving food protection problems and our knowledge of community resources, we felt uniquely able to give impetus to the foodservice industry, to academic institutions and others in implementing appropriate, acceptable and effective training programs. We assist in registration, maintain records of attendance, monitor examinations, award certificates of recognition to those successfully completing the course, publicize the courses to arouse interest and encourage participation by the foodservice workers, as well as create a public awareness of the food protection program. These activities have been of real value to the process of the program and we have utilized them to the fullest extent.

Involvement of non-governmental forces in the program was based on the premise that training and education should be left to those agencies and institutions that are most suitable to do the job. It is also consistent with the general view that non-governmental institutions should be used to the maximum extent to achieve governmentally determined objectives.

As a consequence, rather than attempt to provide training, we concentrate upon our important roles as community health analysts, counselors and catalysts, to insure involvement of consumer groups in planning, implementation and evaluation.

In addition to the classroom instruction, provisions are made for "self-teaching" of individual employees on a schedule compatible with their other responsibilities. This programmed instruction is particularly useful where large numbers of workers need training in specific skills and procedures such as are required for sanitary preparation, service and holding of food.

Development of the instructional material requires great skill and much effort, but once done, the "self-teaching" course can be given with minimal supervision. Our thrust from the outset was not to teach the "dos" and "don'ts" of foodservice sanitation, but to *sensitize, educate* and *motivate* food workers. The mere act of presenting "dos" and "don'ts" or "hows" accomplishes very little.

A foodservice worker must fully understand the microbiological and chemical aspects of food poisoning and the principles of multiple barriers in preventing the transmission of bacteria and toxic chemicals from source or reservoir to host by way of the food chain. Accordingly, he or she must know why hot water of 185 F is necessary in the final rinse cycle of the mechanical dishwasher and must appreciate the fact that a quick rinse of contaminated hands is not adequate to prevent transmission of the contaminants to foods and beverages.

We believe this approach is paying some measurable dividends. Our field inspectors tell us, and we have adequate evidence, that communication with restaurant operators is much better than it was before mandatory training and we are receiving positive feedback from the members of the Washington Restaurant Association.

In addition to our primary training in foodservice sanitation we are finalizing plans for an advanced training program—the refresher course. This course will focus on a *mutual participation model* in which foodservice managers will be trained to carry out their own evaluation of foodservice practices with periodic consultations or direct contact with the regulatory agency.

To assist in this effort we have developed a special workbook as a guide to good internal management and self-evaluation. We are field testing this publication and it will prove to be a useful and practical tool for the daily management of foodservice facilities.

EVALUATION

Finally, in this era of accountability and evaluation there are demands from all sides for some measure of outcome and cost of training and certification. The design of a sound system of evaluation may be one of our most complex tasks. How do we measure results when we are concerned about change in human attitudes and behavior?

We have learned that it is possible to measure these changes through accumulation of good base-line data. Gathering the data, as tedious as it may be, must be a major concern of the training institutions and the regulatory agency. We must avoid, as much as possible, the meretricious use of the "numbers game"—the frustrating, but usually necessary attempt to express a series of complicated behavior changes, i.e. foodservice practices and attitudes, in numbers alone.

Did the foodservice worker change his or her attitude and behavior toward food preparation and service? Does he or she understand the value of refrigerating prepared foods such as salads, hash or leftovers in shallow pans? Did the foodservice manager develop a self-inspection program? Does he or she maintain an effective cleaning and preventive maintenance program? The numbers in answer to these questions may be small, but meaningful. However, along with numbers, individual episodes of major improvements in foodservice practices or case histories of problem-solving techniques by the food worker should be sought.

The introduction of other variables, such as inspections, publicity, change in management and incentive awards, which we offer in the District, may account for improvements or changes in behavior, and also make evaluation difficult.

An elementary yet frequently overlooked point is that foodservice managers differ in the value they place on health and food sanitation. The difference sharpens upon reaching specific objectives that compete or even conflict with other objectives such as realizing maximum profit from the noon meal, when most customers are pressed to eat their lunch within an hour and return to work. Thus, large volumes of food may be prepared well in advance of the meal regardless of the lack of adequate storage space (i.e. refrigeration or steam tables) to maintain necessary temperatures.

In this connection a waitress/waiter may be advised to take short cuts, which are inconsistent with good foodservice practices, to accommodate the profit motives.

CONCLUSION

These, then, are some of the dimensions of our experience in planning and implementing a training and certification program for foodservice personnel. Our concerns encompassed improvements in foodservice practice, reforms in consumer health protection and improved relationships among the consumer, the industry and the regulatory agency.

In this setting sound educational planning required a strategy based on an analysis of the target population and the complexities of foodservice management in a highly compacted urban center. Moreover, societal decisions regarding food protection and foodservice sanitation extend far beyond the regulatory agency or the health service system per se. The decision, as to what resources can be allocated, often depends on social opinion as to what is desirable and on professional judgement as to what is effective.

But as concern about food protection deepens and the time for more intensive public and private actions draws near, it would be reassuring to know that there exists in the foodservice system a cadre of workers who possess a fairly clear and coherent definition of food sanitation problems and a general theory of action for managing these problems.

ACKNOWLEDGMENT

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Enumeration of Viable *Lactobacillus acidophilus* Organisms in Dairy Products

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ABSTRACT

Pasteurized low fat milk to which is added *Lactobacillus acidophilus* has now become a product of major volume on the U.S. market. The product contains millions of *L. acidophilus* per ml and is considered to be in the general category of cultured milk products for application of regulatory monitoring. There is a growing interest in developing standards for allowable minimum numbers of *L. acidophilus* in such products at point of sale. This can be accomplished best by use of a medium selective for lactobacilli such as Lactobacillus Selection Agar (LBS); non-selective media, such as APT agar, can also be used to enumerate *L. acidophilus*. The latter would provide less assurance that the counts were due to lactobacilli. The non-selectivity of APT, however, usually results in higher counts of *L. acidophilus*. There is need for application of accurate monitoring of such products to ensure the numbers as well as the identity of the culture used as the milk supplement.

The microbiological monitoring of noncultured pasteurized dairy products is accomplished primarily by performing the Standard Plate Count and the coliform count, although tests to detect other groups of microorganisms that may affect the shelf-life of the products also may be applied. In cultured milk products, the Standard Plate Count is not used because the starter bacteria are so numerous that they would obscure the much lower numbers of nonstarter bacteria present. In such products, only the coliform count is done along with the phosphatase test. It is generally agreed that regulatory procedures currently in use satisfactorily evaluate the sanitary quality of milk and cultured milk products.

A little over two years ago, a noncultured pasteurized milk product to which *Lactobacillus acidophilus* is added appeared on the American market. Understandably, this presented some immediate problems to regulatory officials who were charged with evaluating the sanitary quality of this product, and yet were faced with the possibility of having to condemn the product because the *L. acidophilus* bacteria often would form pin point colonies on media used for doing the Standard Plate Count. On October 28, 1975, a notice was sent from the Chief of the Dairy and Lipid Products Branch, FDA, to all regional food and drug directors for attention of the regional milk specialist. This memorandum in part states:

> "In order for the requirements of the Grade A Pasteurized Milk Ordinance to apply to this fluid milk product the words 'safe and suitable bacterial culture' need to be inserted in Definition Q, Optional Ingredients, following the words 'similar ingredients.' Definition Q would now read, 'Optional ingredients shall mean and include Grade A dry milk products, concentrated milk, concentrated milk products, flavors, sweeteners, stabilizers, emulsifiers, acidifiers, vitamins, minerals and similar ingredients and safe and suitable bacterial cultures.' We believe this is the most expeditious way to handle this product as the addition of a pure *Lactobacillus acidophilus* culture to a milk product is analogous to the addition of other optional ingredients.
>
> We recognize that certain other changes in the administrative and sanitation requirements of the Ordinance need to be made and we have these under consideration.
>
> With respect to labeling, the name of the product is considered to be 'Lowfat milk with *Lactobacillus acidophilus* culture added' and the label should contain the necessary ingredient labeling. The words 'Sweet acidophilus' is considered simply a fanciful term and there would be no objection to the use of these words immediately preceding the name of the product."

Subsequently, it was decided that this product should be microbiologically monitored in the same manner as cultured milk products, viz., by the use of coliform count and phosphatase test.

WHY ADD THE LACTOBACILLUS?

At this point, it should be understood that *L. acidophilus* is added to milk because of evidence in the literature that the microorganism can be of benefit to many people (for comprehensive reviews of the literature

on the roles of the intestinal microflora, see references 2, 3, 4, 7, 8). Information is still being obtained on how the lactobacilli may benefit humans. The role of the intestinal lactobacilli is now considered to be somewhat different from that when regular cultured acidophilus milk was being studied. Formerly, the thrust was to consume sufficient quantities of *L. acidophilus* whereby the predominant flora in the intestinal tract would consist of this microorganism and basically eliminate most of the others. Later, however, it was realized that the intestinal microflora in healthy individuals contains a balance that is favorable to the host. Efforts to have the intestinal microflora dominated by *L. acidophilus* have essentially be abandoned. In maintaining the balance among the groups present, it has been observed that the lactobacilli appear to be particularly vulnerable and often are lowered tremendously or are absent. In such instances, it is not unusual to find intestinal upsets occurring which are characterized by diarrhea, flatulence or a combination of the two. To re-establish the lactobacilli, consumption of moderate numbers of *L. acidophilus* seems to be sufficient. For example, the daily consumption of 1×10^8 to 1×10^9 viable lactobacilli was the most satisfactory level of intake (*5*). More recent studies have shown that the consumption of $1\text{-}2 \times 10^9$ *L. acidophilus* per day was adequate for increasing the number of intestinal lactobacilli appearing in the feces (*6*). Consumption of these numbers of *L. acidophilus* has made it possible to prepare a milk product that is not altered in flavor or taste, but which contains approximately 1 billion cells of *L. acidophilus* per half pint. Furthermore, this can be accomplished with a very modest increase in cost of the product to the consumer. This type of product was developed in our laboratories and introduced on the market in 1975; subsequently, similar products have appeared.

REGULATORY CONTROL

Since the lactobacilli were being added to milk to improve its nutritional qualities for the consumer, it was reasonable for regulatory agencies to be concerned that adequate numbers of the microorganism were present in the product. However, methodology for enumerating *L. acidophilus* is not contained in *Standard Methods for the Examination of Dairy Products* (*1*). Enumeration of *L. acidophilus* presents some problems not encountered in doing the Standard Plate Count. These are caused by *L. acidophilus* being more fastidious in its nutritional requirements than are the microorganisms normally present in milk that are enumerated by the Standard Plate Count procedure. To enumerate *L. acidophilus,* the medium, temperature of incubation, and, in certain instances, the gaseous atmoshpere, must be specifically adjusted for obtaining accurate counts. These impose new procedures and equipment requirements on laboratories charged with performing such counts. It is important that accurate methods of enumeration be available since a number of states are now adopting a standard of not less than 2 million *L. acidophilus* per ml of milk for products in which the culture has been added to pasteurized low fat milk.

The procedure which we have used consists of the following:

1. Prepare and plate dilutions of the milk through the one to one hundred thousand dilution (1:100,000).
2. Pour the plates with Lactobacillus Selection Agar (BBL).
3. Place the plates in an atmosphere of CO_2. This can be obtained by the Gaspak jar using the CO_2 generator, or by placing plates in a plastic container which is flushed with CO_2.
4. Incubate the plates at 37 C for 3 days.
5. Count all of the colonies and report as number of *L. acidophilus* per ml of the milk.
6. Incubation at 35 C can be substituted for 37 C if necessary.
7. It is possible that the Rogosa SL medium could be used as well as the Lactobacillus Selection medium, but we have collected no data on this.

Microbiologists will quickly detect the differences in this procedure from that used for the Standard Plate Count (*1*).

Two other media have been used extensively for enumerating *L. acidophilus* and these are Lactobacilli MRS broth (Difco) plus 1.5% agar and APT agar. These two media are not selective for lactobacilli and their non-selectivity often allows higher counts to be obtained in enumerating *L. acidophilus* in a given milk sample. Also, when these media are used, it is not necessary to use a CO_2 atmosphere for *L. acidophilus* colonies to develop.

There may be an understandable reluctance to use the more selective medium and costly procedure for routine monitoring of the population of *L. acidophilus* in milk to which this culture is added. However, it must be realized that use of the Lactobacillus Selection (LBS) medium does permit more confidence in the count obtained as being caused by lactobacilli, rather than by other microorganisms that may have been present because of some undesirable practice(s) in handling of the milk.

It should be noted that the *L. acidophilus* which is used in preparing uncultured acidophilus milk has to be made with extreme care and a high level of sanitation. The product developed at our University and which is sold as Sweet Acidophilus™ milk must not contain any of the following: psychrotrophs, coliforms, staphylococci, salmonellae, or *C. perfringens.* Futhermore, through a monitoring program required of each licensed dairy, results must be submitted by the dairy routinely for the phosphatase test and for counts of *L. acidophilus* and coliforms. In addition, an approved independent laboratory must, on a quarterly basis, perform and submit results on the foregoing tests and test the milk for the presence of salmonellae and the number of staphylococci. It is believed that such a program benefits the dairy, the consumer, and also assists regulatory agencies in their monitoring responsibilities.

BILE-RESISTANT LACTOBACILLI

Certainly it must be agreed that if the *L. acidophilus* is to have any benefit in the intestinal tract it must be viable when consumed. However, there are additional considerations that should be given to the quality of the product. *L. acidophilus* and certain other lactobacilli found in the intestinal tract are able to grow in the presence of bile, and in our laboratories we have developed a procedure for enumerating these bile-resistant bacteria. The medium we have used is the LBS agar to which is added 0.15% oxgall, and is designated as LBSO agar. The plating procedure is done in the same manner as for the LBS agar cited earlier in this discussion. Cultures of *L. acidophilus* produce equal numbers of colonies on the LBS and LBSO media. However, we have found that when the culture has been subjected to certain stresses the LBSO count will frequently be markedly less than the LBS count. When the cells are placed in a favorable environment in the absence of bile, they regain their ability to grow in its presence. This is a typical manifestation of microbial injury. Some of the factors which promote injury are: storage of frozen concentrates at insufficiently low temperatures (for example, storage at –20 C will promote much more injury than storage at –86 C or –196 C); growth of the cultures in media that are inadequate nutritionally; and improper preparation and storage of the milk to which *L. acidophilus* has been added. It would seem that maintenance of a maximum level of bile-resistant cells in the milk would be in the best interests of those consuming milk containing *L. acidophilus.*

In marketing a milk to which many *L. acidophilus* cells are added, it is obvious that much care must be taken to maintain the viability and bile resistance of the culture; but, it is essential that the identity and purity of the culture must be maintained. Fortunately, culture supply companies have this capability and are accustomed to preparing large quantities of cultures in a pure form, which can maintain the desired qualities of the culture. Nevertheless, surveillance of the acidophilus milk products does involve certain procedures which are not currently used routinely. Possibly this is an area wherein industry monitoring programs can be shared with regulatory agencies to accomplish goals of mutual interest.

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Basic Techniques in Studying Mycotoxins: Isolation of Mycotoxins

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ABSTRACT

The basic techniques used and the potential problems encountered in isolation of mycotoxins from naturally contaminated commodities and fungal cultures are discussed. Specifically presented are the factors to be considered in selecting a bioassay organism, methods of preparing formulations for mycotoxin administration, modes of administration, methods available for purification, and problems encountered during purification.

Acute clinical syndromes and deaths caused by mycotoxins may be diagnosed or suspected by veterinary diagnosticians. However, it is believed that in many instances the chronic or nonlethal effects of mycotoxins are not recognized. These latter effects, although frustrating to the veterinary diagnostician, may be economically devastating to the producer through losses incurred from lower productivity, reduced weight gains, and impaired resistance to infection (*5-10*).

The techniques employed to detect and isolate mycotoxins depend largely on the chemical and physical nature of the toxin involved, the nature and number of contaminants, and the titre of the toxin being produced by the particular fungus.

Two general approaches to detection and isolation of mycotoxins have been used. The first is the consequence of a natural outbreak of suspected mycotoxicosis similar to that which occurred in England with the aflatoxins (*4*). The clinical signs in this case were acute and resulted from a heavily contaminated feed source.

In the other approach to detection and isolation of mycotoxins, the fungi that commonly invade an agricultural commodity are evaluated for toxin-producing potential (*2*). This latter approach is indirect and it attempts to identify or predict mycotoxin problems that might be occurring on a chronic level. Once the potential for toxin production and the incidence of particular toxin producers have been determined for a commodity, analytical techniques can be developed to determine whether or not those toxins occur naturally in that commodity.

ISOLATION OF MYCOTOXINS FROM COMMODITIES SUSPECTED OF CAUSING NATURAL MYCOTOXICOSES

Before initiating in-depth analyses for mycotoxins in a suspect commodity, it is best to ascertain that mycotoxins are the probable cause of illness. This can be determined by elimination of other causes at necropsy, and/or on-site inspection. Mycotoxicoses are best confirmed when identical clinical signs are produced when the commodity is fed to an experimental animal, preferably of the same species involved in the initial outbreak. Since one of the first inclinations is to destroy suspect feed, a problem frequently encountered is the non-availability of adequate amounts of the suspect feed for feeding trials and analyses.

It is best, if sufficient contaminated material is available, to isolate and identify the toxin causing the problem before determining which fungus is responsible for toxin production. It is not difficult to isolate several toxigenic fungi invading a particular lot of commodity; therefore, to prevent confusion, it is better to first isolate the toxin involved, then determine which fungus has produced the toxin. Again, this was true with the aflatoxins which were isolated from contaminated groundnut before determining which fungus produced the toxins (*4*).

ISOLATION OF MYCOTOXINS FROM CULTURES OF FUNGI CONTAMINATING A PARTICULAR COMMODITY

This approach involves screening a commodity for toxigenic fungal isolates, followed by isolation and identification of the toxic metabolites (*1*). The relative frequency of invasion by individual toxin-producers is determined in samples of the commodity from various sources. If the commodity proves to be an adequate substrate for toxin production by the fungi found most often invading the commodity, analytical techniques can

then be developed to determine if the toxin occurs as a natural contaminant of the commodity.

SELECTION OF BIOASSAY ORGANISMS

Several factors must be considered in selecting a bioassay organism for isolation of mycotoxins. These include availability, economics, and validity of the bioassay. An effective and economical small animal bioassay organism can be obtained from chick hatcheries producing the Leghorn breed which are used exclusively for egg laying. The Leghorn cockerels are of no economic value and, therefore, can be purchased at a fraction of the cost of other bioassay animals. These are readily available in large numbers at frequent intervals and provide a sensitive bioassay if used before 2 days of age, preferably at 1-day-old and before being put on feed. Other small animals such as mice, rats, hamsters, etc. may be used if they are available; however, these are more expensive than day-old-cockerels. Use of non-vertebrate bioassays (bacteria, brine shrimp, etc.) are not valid in isolation of vertebrate toxins since there is little correlation between vertebrate toxicity and toxicity in microorganisms and invertebrate animals. In addition, results from administration of metabolites via routes other than oral are often confusing since a metabolite must be toxic orally to a vertebrate animal to be considered a mycotoxin. Use of chick embryo and other injection-type bioassays for the initial isolation of mycotoxins are, therefore, not recommended.

FORMULATIONS FOR ADMINISTRATION OF MYCOTOXINS

The suspect commodity or the fungal culture being tested may be dried, ground, and fed ad libitum to test animals. However, the grinding, mixing and feeding of dried fungal-invaded substrate may present a problem concerning worker safety. Without elaborate precautions, airborne spores and microparticles are unavoidable when samples of dried, molded substrate are prepared. Since fungal spores may contain toxins and be allergenic, dangers from exposure to this material must be a primary consideration. Also, these fungi are mainly saprophytic but some species (e.g. *Aspergillus fumigatus*) can be pathogenic to man (*3*).

Health hazards can be alleviated by autoclaving and/or extracting the fungal cultures or moldy substrate with an appropriate solvent such as chloroform. Addition of solvents such as chloroform to the culture flasks or molded substrate and subsequent heating to reflux results in complete wetting and precipitation of airborne spores within the container and destruction of the viability of the spores. The investigator can then complete the remaining steps without risk from airborne fungal spores and microparticles. This procedure should be given serious consideration because extraction is the first step in isolation of the toxin.

Inert carriers that can be used in preparing mycotoxin formulations are vegetable oil, Tween 20, and propylene glycol. Although most mycotoxins are poorly soluble in these carriers, a suitable preparation can be made by first dissolving the mycotoxin in an appropriate solvent, adding the solution to the carrier and removing the solvent under vacuum (*2*).

At dosage levels up to 15 mg/ml, the mycotoxin generally will remain in solution and provide an excellent surface area for absorption in the experimental animal. In some instances, combinations of the above carriers will give better solubility than any used alone.

The relative toxicities of these carriers to day-old-cockerels dosed orally via crop intubation at 1 ml/chick in order of increasing toxicity are vegetable oil, Tween 20, and propylene glycol. One-day-old cockerels show no adverse effects when dosed orally with 1 ml of vegetable oil, minor effects with 1 ml of Tween, and 1 ml of propylene glycol is acutely toxic to day-old cockerels.

PURIFICATION OF MYCOTOXINS

Techniques used in purification of a particular mycotoxin monitored by bioassay depend largely on the nature of the mycotoxin involved. An example of a technique that can be used is as follows: The toxin is extracted with an appropriate solvent and fractionated with any number of column adsorbents (e.g. silica gel, florisil, etc.). The typical elution series for a crude chloroform extract on a silica gel column could be as follows: hexane, benzene, ethyl ether, chloroform, ethyl acetate, acetone, and methanol (three column volumes of each eluent). A second column with a linear gradient elution series and a fraction collector could then be used. The linear gradient elution selected depends on which gross fraction contains the toxin. If the toxin was in the ethyl acetate fraction, a linear gradient from chloroform to ethyl acetate, benzene to ethyl acetate, or from ethyl ether to ethyl acetate may be appropriate. If purification is inadequate, additional adsorbents such as florisil, alumina, ion-exchange resin, or molecular sieve chromatography may be used. Preparative thin-layer chromatography and high pressure liquid chromatography are excellent techniques, if available. Mycotoxins that are polar in nature (i.e. soluble in methanol and/or water) are not amenable to all types of adsorbents. Aqueous adsorbents available are molecular sieve, ion-exchange and DEAE cellulose for conventional column chromatography.

POTENTIAL PROBLEMS ENCOUNTERED DURING TOXIN PURIFICATION

1. Decreased solubility of the toxin as a function of purity and, therefore, decreased absorption and loss of toxicity. The metabolite may be toxic in crude preparations but relatively non-toxic in pure form due to decreased solubility. A recent example of this in our laboratory was encountered during the isolation of chaetoglobosin C (Unpublished) and, to some extent, with flavutoxin (*1*).

2. Instability of the toxin as function of purity. The toxin may be quite stable in crude preparations but highly unstable in pure form.

3. Loss of toxicity during purification due to removal of synergistic metabolites and/or due to removal of companion toxins acting in concert.

4. Loss of toxin-producing potential by the mold as a result of subculture or "domestication."

Those problems could occur individually or simultaneously to various degrees.

Isolation of each mycotoxin is a unique challenge. The isolation may require crystallization from crude extracts or the use of several combinations of sophisticated techniques which may be frustrated by failure due to the presence of stubborn impurities or to instability of the toxin in pure form.

ACKNOWLEDGMENT

Mention of firm names or trade products does not imply endorsement or recommendation by the U.S. Department of Agriculture over other firms or similar products not mentioned.

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Proceedings of the Sixteenth National Conference on Interstate Milk Shipments

Cincinnati, Ohio, May 22-26, 1977

J. C. McCAFFREY

National Conference on Interstate Milk Shipments
3306 Glouster Street-Kensington Park, Sarasota, Florida 33580

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EXECUTIVE BOARD MEETING, May 22, 1977

Chairman Vaux convened the meeting at 4:10 p.m., in the Commodore Room, Stouffer's Inn, Cincinnati, Ohio. Voting members present: Arledge, Boosinger, Causey, Gay, McGarrahan, Race, Rich, Rowley, Rubis, Russell, Schilling, Van Patten, Vaux; absent: Harvey, Seaman, Weckel. Non-voting members present: Huskey, Speer; absent: Conner.

Minutes of the February 23, 1977, meeting were accepted as mailed. The Treasurer's report, showing a balance of $6,186.08, as of May 16, 1977, was accepted as read.

The following proposed Council changes were accepted: (*a*) Council II - Judy Heady for Wm. Ullman; W. S. Phillips for Russell Wright. (*b*) Council III - Konsoer for Kirschbaum for this meeting; Rundle for Evans for this meeting; Morrow for Webb on a permanent basis. Chairman Vaux was authorized to make any necessary changes in council membership between Board meetings at this conference. Council chairmen were instructed to see that all submitted problems are considered; otherwise the chairman is to operate the council as he sees fit.

The Board voted to discuss proposed changes in the constitution and new by-laws on Wednesday, following the Council reports. Race proposed that all amendments to the revised constitution and/or by-laws be submitted in writing, and presented on Tuesday; also that the final voting by delegates be on Wednesday afternoon. Motion passed. The Executive Secretary was directed to take an official roll call at that time, and to have delegates seated separately.

The Board voted to allow non-voting members of the Conference to address the session on both Wednesday afternoon and Thursday morning, provided they have been requested to do so by a voting delegate.

Chairman Vaux appointed a nominating committee of of Clarence Luchterhand as chairman, Osten Baghott, Hurst, Ruppert, and George; a credentials committee of Kennedy as chairman, Dennler and Noles; a resolutions committee of Atherton as chairman, Tipton and Blevins. The Board approved all proposed appointments.

Chairman Vaux reported that he had been asked to appear at the NASDA meeting in San Diego, July 18-20, to report on progress being made by the Conference. The Board will be polled by mail on the advisability of the Chairman accepting this invitation. The meeting was adjourned at 5:55 p.m.

FIRST GENERAL SESSION

The first general session of the Conference was called to order at 10:10 a.m. on Monday, May 23, 1977, by Chairman Vaux. The invocation was given by Sam Noles, Florida Department of Agriculture. The address of welcome was presented by Dr. John H. Ackerman, Director, Ohio Department of Health.

Chairman Vaux named the members of the Credentials, Nominating and Resolutions committees and gave the respective charges. Secretary McCaffrey presented the Treasurer's report and moved acceptance of both the report and the minutes of the 1975 Conference as mailed to participants. Motion was approved. Chairman Vaux called for reports of the standing committees: (*a*) Abnormal Milk, (*b*) Laboratory, (*c*) Single Service Containers and Closures, and (*d*) Constitutional Revision. The Conference accepted the reports and referred them to the appropriate councils.

Chairman Vaux discussed procedures under which the Conference would operate. He then assigned the various meeting rooms for the respective Councils, and instructed chairmen to make their preliminary reports to the membership on Tuesday afternoon.

SECOND GENERAL SESSION

The second general session was convened by Chairman Vaux at 1:40 p.m. on Tuesday, May 24, 1977. The first roll call of states and delegates authorized to vote on Conference agreements was taken by Secretary

McCaffrey. The Chairman then called on each Council chairman to present the preliminary report of their deliberations. Dudley Conner reported for Council I, Jay Boosinger for Council II, and Glen Huskey for Council III.

THIRD GENERAL SESSION

Chairman Vaux convened the third general session at 1:30 p.m. on Wednesday, May 25, 1977. Each Council Chairman presented his complete council report, listing all problems and proposed solutions to be voted on in the final session. Each report included both changes in and additions to existing "Procedures." Complete council reports were given to each participant as he/she entered the meeting room for this session.

After completion of the council reports Chairman Vaux called on Clarence Luchterhand, chairman of the nominating committee, to give the first report of this committee. The selections were: John Baghott of Colorado to complete the unexpired term of Keith Harvey of Idaho, John Schilling representing local health, H. H. Vaux representing state health, Kenneth VanPatten representing state agriculture, Wm. Arledge representing industry, Berry Gay representing laboratory, and Henry Atherton representing education.

Chairman Vaux then called for a discussion of the proposed revised constitution and by-laws. Rowley moved, seconded by McAvoy, that the complete reading of the proposed revision be dispensed with. Motion carried. Boosinger moved, seconded by Dally, that the provisions in the proposed constitution dealing with not allowing personnel from states identified as not practicing reciprocity will not take effect until the conclusion of the 1979 Conference. Motion carried.

Motion was then made that the revised constitution and by-laws be accepted. Ruppert moved, seconded by Griffin, to amend Article IV, section 1 by deleting the words "and from a state practicing reciprocity", and to amend Article VIII, section 4, subdivision 2, by deleting the words "practicing reciprocity". Motion carried. Ruppert moved, seconded by Kennedy, to amend Article IV, section 4, by substituting for the word "municipal", wherever used in this section, the word "local." Motion carried. Conner moved, seconded by Van Devender, that the last sentence in Article IV, section 3 be deleted and that the sentence "Elected full term Board members may not succeed themselves" be substituted for the deleted sentence. Motion carried. Helmreich moved, seconded by Kennedy, that Article V, section 10 be amended by deleting the words "Conference participation, voting privileges" and adding, at the end of the sentence, the words "as outlined in Article IV, section 1". Motion carried. Rowley moved, seconded by Ruppert, that the amended constitution and by-laws be voted on separately. Motion carried. The amended constitution was voted on and unanimously accepted.

Dally moved, seconded by Kimball, that the proposed by-laws be adopted. Conner moved, seconded by Amick, that Article II, section 1, of the by-laws be amended by deleting section 1 in its entirety, and that section 2 become section 1. Motion carried. Dally moved, seconded by Kimball that the amended by-laws be accepted. Motion carried.

FINAL GENERAL SESSION

The final general session was called to order by Chairman Vaux at 8:40 a.m. on Thursday, May 26, 1977. Chairman Vaux introduced Mr. Glen Kilpatrick, Director of Federal-State Relations, Food and Drug Administration. The final roll call of states and delegates was taken by Secretary McCaffrey. The roll call showed that 46 states were officially represented, 12 by both agriculture and health, 16 by agriculture only, and 18 by health only. The District of Columbia was also represented.

Chairman Vaux called on Orlowe Osten to read the report of the nominating committee. Recommendations were: John Baghott to complete the term of Keith Harvey who has retired (Region III); from Region II - John Schilling, local health; H.H. Vaux, state health; Kenneth VanPatten, state agriculture; William Arledge, industry; at-large representing laboratory, Berry E. Gay, Jr.; at-large representing education, Doctor Henry Atherton. The slate was accepted as read. Dr. Henry Atherton presented six resolutions as the committee report.

Resolution No. 1

Whereas; the success of this conference which is now drawing to a close is dependent upon the untiring efforts of dedicated individuals who have given freely of their time in making arrangements, providing facilities, conducting the business of this conference, and making every possible effort which adds to the comfort of all those in attendance.

Therefore be it resolved; that the conference go on record as giving a special vote of thanks and appreciation to those named below:

First, to conference Chairman H.H. "Herb" Vaux for his dedication and leadership during his term on the Executive Board, and especially for his calm and effective guidance of conference affairs during this recent critical period in conference history.

Second, to the Council Chairmen Dudley Conner, Jay Boosinger and Glen Huskey whose leadership of council sessions gave all the opportunity to carefully address problems identified as important in conference relations and responsibilities.

Third, to John Speer whose interest in all affairs of the IMS concept continually serves our needs.

Fourth, to Robert Farst and members of the Local Arrangements Committee (T. A. Batsche, R. A. Belknap, D. J. Conner, Harold Cope, E. M. Helmreich, D. R. Kimball, R. L. Moran, Robert Quade and E. K. Vickrey) who handled so well the many on-site arrangements necessary for the smooth operation of the sixteenth Interstate Milk Shippers Conference.

Finally, be it resolved; that all registrants of this Conference focus their attention and appreciation to those contributors and hostesses at the Dairy Bar. Those we express our appreciation to include:

Mid-East United Dairy Industry Association; Dairy Councils of Cincinnati, Columbus and Dayton; Central Ohio Cooperation Milk Producers, Inc., Columbus; Miami Valley Milk Producers Association, Dayton; Milk, Inc., Cleveland; Huntington Interstate Milk Producers Association, Huntington, W. Virginia; Cincinnati Coop. Milk Sales. Inc; and Milk Dealers of Greater Cincinnati.

Resolution No. 2

Whereas; Council II discussed and recognized the importance of surveillance, supervision, enforcement and handling of milk products

from the source to the ultimate consumer,

Be it therefore resolved; that the delegate body at the sixteenth National Conference on Interstate Milk Shipments express a sense of urgency to the FDA to propose at the earliest possible date adequate sanitary standards for storage and handling of milk and milk products while in storage and at the point of sale, and the associated program for supervision and enforcement.

Resolution No. 3

It is the opinion of Council II that the proposed "methods of making sanitation ratings of milk supplies" is in need of further study.

Therefore; we hereby recommend that an expert committee consisting of rating agencies, enforcement agencies and the dairy industry be formed to assist FDA in an effort to rewrite the "methods of making sanitation ratings of milk supplies" for resubmission to the states for their comments before adoption by the NCIMS.

Resolution No. 4

Whereas; the National Conference on Interstate Milk Shipments has gone on record in favor of a memorandum of understanding between the National Conference and the Food and Drug Administration specifying the responsibilities of the Food and Drug Administration and the National Conference on Interstate Milk Shipments relative to the State-Federal Cooperative Interstate Milk Shippers Program.

Therefore; be it resolved that the National Conference on Interstate Milk Shipments recommends to the Food and Drug Administration that the National Conference, in cooperation with FDA, proceed to sponsor appropriate regional meetings involving FDA personnel from the Dairy and Lipid Products Branch, regional FDA milk consultants, appropriate state enforcement and survey personnel and industry representatives; the purpose of such regional meetings to bring about a greater awareness and understanding of the most recent interpretations of the Grade "A" Pasteurized Milk Ordinance and related documents, as well as the Procedures of the National Conference on Interstate Milk Shipments.

Be it further resolved; that NCIMS recommend to the Food and Drug Administration that the Memorandum of Interpretation of the Grade "A" PMO and IMS Procedures issued by the Dairy and Lipid Products Branch, FDA, to regional FDA personnel and state regulatory and survey personnel be published on a regular and continuing basis in the FDA Quarterly Listing of Sanitation Compliance and Enforcement Ratings of Interstate Milk Shippers.

Resolution No. 5

Whereas; natural constituents of milk and dairy foods have unique nutritional benefits for humans everywhere; and

Whereas; the complexity of the size and sanitation requirements of modern dairy equipment render it impossible to recover all milk nutrients in the several stages as milk moves from the dairy cow to the ultimate consumer; and

Whereas; there is such widespread concern over the environmental impact of waste disposal of lost product in milk handling; and

Whereas; many of our major cooperatives and dairy processors are now restricted in the location of milk handling and processing facilities due to lack of approved recovery equipment and methods for both large and small operations to recover milk solids and thus reduce the effect of milk solid wastes on existing as well as new facilities of public waste disposal systems; and

Whereas; regulatory programs should provide for maximum recovery and utilization of these valuable nutrients; and

Whereas; there now exists considerable confusion as to the best and proper procedures to economically and safely recover these lost nutrients which are so important to the economic health of the dairy industry and the physical well-being of the consuming public; and

Whereas; regulatory standards should be developed which are consistent with these needs.

Therefore be it resolved; that the delegates to this conference initiate cooperative activity and committee response by members of the dairy industry and those responsible for safeguarding the public to recommend suitable procedures for maximum recovery and utilization of all available milk nutrients.

Resolution No. 6

Whereas; delegates and members attending the 1973, 1975 and 1977 Interstate Milk Shippers Conferences have expressed the need for official recognition of bacteriological methods which have merit as alternative procedure to the Standard Plate Count; and

Whereas; the report of the Laboratory Committee to the 1975 IMS Conference was returned by the Executive Board for more detailed study following the 1975 Conference, and as a result of this the Laboratory Committee has carefully studied pertinent literature during several years of review; and

Whereas; these studies have shown ample evidence of the merit of the procedure known as Preliminary Incubation in providing better information concerning the degree of sanitary care of milk handling equipment on dairy farms.

Therefore be it resolved; that the Executive Board of the 1977 IMS Conference request the Intersociety Council now preparing the 14th Edition of *Standard Methods for the Examination of Dairy Products* take action that the procedure commonly known as "Preliminary Incubation" be incorporated among the Supplemental Microbiological Control Methods of Appendix A, with a reference to Acceptable use of the PI procedure shown in Chapter 5.

Further, said Executive Board should consider immediately the recommendations of the 1977 report of the IMS Laboratory Committee and proceed to implement as soon as possible those recommendations considered acceptable by members of this 1977 Executive Board.

REPORTS OF COUNCILS

The next order of business is the regular reports of Councils. In the summation of these deliberations your Secretary will discuss only those proposed changes that were accepted or rejected by the delegates. If accepted, no comment will be made; if rejected, the action will be so noted.

Council I

Chairman Vaux called on Dudley Conner to give the report of Council I.

The Council was given 42 problems, 21 of which were reported as "No action". Problem 15 was withdrawn. Problems 2 and 3 were referred to Council II. Problem 7, dealing with temperature requirements for Grade "A" raw milk for pasteurization was accepted as amended to read "raw milk for pasteurization shall be cooled to 45d.F or less within two hours after each milking. The temperature of the milk in the farm tank must not exceed 50d.F. at any time during the addition of subsequent milkings." Problems 10 and 11, dealing with Administrative Procedures, Item 2R, were combined. Council recommends to amend line 105, page 73 by striking the word "enclosed" and page 46 by striking the sentence beginning with the word "however" on line 971½. Council accepted problem 12 and recommended on page 47, line 986, after the word "uncleanliness" add the words "and increase the potential for the spread of disease." Problem 19, dealing with Item 19r, cooling, was recommended as "No action" by the Council. The delegates rejected and amended to add the word "begins" after the word "milking" on line 329. Problem 22, page 55, item 9, proposed P.M.O. dealing with vents and lighting fixtures. Council accepted a proposed amendment reading "vents, if installed, and lighting fixtures are installed in a manner to preclude the contamination of bulk milk tanks or clean utensil storage

areas."

Problem 24 - page 86, iten 21r - Insect and Rodent Control - Council recommended that, on line 403, the word "infect" be replaced with the word "contaminate." Problem 28, dealing with item 6p, 17p, 21p, involving what appears to be contradictory statements, was accepted by Council. Problem 31, page 180- lines 1611 and 1647 - Capping - was accepted for fluid milk and fluid milk products only. Problem 32, page 117 - lines 257-261, was commented upon. Council accepted the following comment as written: "The proposed requirement for 7-day recording thermometers should not apply to storage tanks and silo tanks which currently are not equipped with them. It is economically unjustifiable to require the installation of new thermometers on existing equipment when adequate verification can now be obtained by other means - e.g., use of more than one 24-h chart. As new storage facilities are utilized and brought on stream, 7-day temperature recording devices can then be efficiently incorporated. Problem 33, Item IV, under Continuous Water Disinfection, Council recommended that, on line 1482, page 272, after the word "supplies" insert the following sentence: "This does not preclude the use of other chemicals or procedures demonstrated to be safe and effective." Problem 34 - Council recommemded that, on page 120, line 317, the deletion of the word "immediately."

Problem 36 deals with the Dry Milk, Condensed Milk Ordinance. Council recommended the deletion of "condensed whey" from page 78, line 833; page 79, lines 840-842. Problem 38 (problem 48 from Council II) dealt with changes in the *Official Methods of Analysis* of the AOAC and was recommended by Council. Problem 39 (problem 26 from Council II) deals with laboratory problems and was recommended by Council. Problem 40 (problem 37 from Council II) deals with a proposed change of wording on page 31, lines 694-697 and was recommended by Council. Problem 42 (Laboratory Committee problem 10) dealing with WMT and DMSCC results was accepted by Council. Council recommended that the report of the committee on Fabrication of Single-Service Containers and Closures for Milk and Milk Products listing page, line and word changes be accepted. Council also recommended that the report of the committee on Abnormal Milk Control be accepted. The Council recommended that the Laboratory Committee report on Antibiotic Residues, Addition of vitamins A and D, Pesticide Residues, be accepted. The report on added water in milk was accepted as amended by deleting recommendation #2 which stated "that the freezing point for milk as specified by the A.O.A.C., currently -0.525d.C., be accepted as the freezing point standard." Ken Whaley, chairman of the Laboratory Committee, moved that the following statement be included in the Evaluation Manual: "The IMS Laboratory Committee recommends that the evaluation of milk laboratories portion of the proposed revised PMO should be expanded to include selected portions of the current "Blue Book" document, namely, *Guide Lines for Officials Conducting Laboratory Surveys, Equipment and Apparatus of Aid to the Survey Officer,* and *Summary Statements of Laboratory Capability.* The additions, however, should exclude the keyed Recommendations for correction of Deviations except for pertinent examples. The listings of keyed recommendations should be provided separately by the Food and Drug Administration as a guideline for the Evaluation officers. The delegates approved.

Council recommended that in Section 7, proposed PMO, item 13, top of page 56, there be added after "the port is in use" the statement "an impervious easily cleanable, raised surface shall be constructed under the hose port adjacent to the outside wall, sufficiently large to protect milk hose from contamination."The statement was amended to delete the words "impervious" and "raised." Council recommended to add item 12p (or appropriate place) the following: "Whenever a bulk tank has been cleaned and sanitized, as required by the inspection authority, it shall bear a tag showing the date, place, time and signature of the employee or contract operator doing the work, unless the truck delivers to only one receiving unit where responsibility for cleaning and sanitizing can be definitely established without tagging. The tag is to be removed at the first stop on route and kept on file for the inspection authority." The statement was amended to add after the word "tag" and before the word "showing" the phrase "or a record shall be kept." Council recommended - item 15p, page 127, line 479 to delete sentence beginning "milk or milk products" and ending with "milk products" on line 481. Item 20p - Personnel - cleanliness - add "requirement for head covering in processing plant only." In Appendix D, Part II, Construction, page 252, item 4, delete sentence starting with "casing", line 1014½ and ending with "directions" ending on line 1016½. Council recommended, item 16p, Pasteurization. administrative procedure, that FDA work out proper wording to permit crystallization of whey at over 45d.F., cooled to 45d.F. for transporting. Council recommended - Appendix D, Standards for water sources, page 242, add, at the beginning of the first paragraph the following: "Where a state has an Administrative Code or regulation equal to or better than EPA or FDA regulation, such state administrative code or regulation shall be accepted."

Council II

Chairman Vaux called on Jay Boosinger, chairman, to give the report of Council II.

Council was given 66 problems, 29 of which were reported as "No action." In addition, parts 1, 2, and 3 of both problem 3 and 5 were reported as "No action". Problem 5, dealing with definitions, was considered first. Council recommended in part 4, that definitions should be included for nonstandardizing dietary foods, and that, in part 5, page 11, line 278, the word "producer" be deleted. In problem 3, part 4, council recommended that on page 37, line 831, the words "raw cream" and

"cream" be deleted and the words "raw products" be substituted in both locations. In part 5, page 197, line 1980, be amended to delete the words "each shipment of." Problem 7, PMO Definitions, amend Section I, beginning on line 32, page 2, as follows: "The following definitions shall apply and shall continue to apply notwithstanding any pending or subsequent amendment of 21C.F.R., Part 18 or 21C.F.R., Part 19 unless and until amendment is approved by NCIMS." Problem 12, definitions - description of the word "person," page 11, line 280, should include the word "institution." Problem 15, dealing with permits, was amended as follows and recommended by Council. The following changes on page 15, line 340: delete the words "written application of" and substitute the words "notification acceptable to the regulatory agency by." Delegates rejected the recommendation by roll call vote. Council opposed problem 20, dealing with labeling, but recommended the adoption of the following substitute wording for line 426, page 19 of Section 4 - "3. Grade 'A'."

Problem 21, dealing with the addition of the reference wording to Appendix L, dealing with labeling, lines 410 and 421 should have Appendix referenced. Problem 22, dealing with seals, was recommmended as amended, by removing the word "number" and substituting the word "identification." Problem 23, page 20, delete lines 472 to 475 and insert wording according to page 5, Item 3, 4 and 5 of IMS Procedures. Council recommended. Problem 24, labeling, page 21, recommend deletion of lines 479-482. Problem 30, Pesticide Tests. While Council recommended 'No action," Harold Thompson stated that the frequency of testing for pesticide residues would be left to the discretion of the health authority and that all references to that frequency will be uniform throughout the entire document. Problem 31, page 30, line 676, remove the semicolon (;). Problem 32 - recommended on page 30, line 659, the deletion of the words "antibiotic tests." Rejected by delegates. Problem 34, page 31, line 681, recommend the insertion of the word "raw" before "milk" (and no *raw* milk shall...) Rejected by delegates.

Problem 36, page 30, line 668 and page 32, line 704, recommend to change 14 to 21 on line 668 and change Appendix E, page 298 to reflect 21 days. Problem 42, page 34, line 769 and 771. Recommend on line 771 change the word "shall" to "should." Problem 43, Deviation from acceptable practices by the milk hauler, recommends that, beginning on line 779½ on page 35, strike the words "producer and/or the milk processing plant being debited on official inspections and ratings." Insert, after the word "the" beginning on line 779½ the words "suspension and/or revocation of his permit," so that the sentence beginning on line 799 shall read as follows: "Any deviation from acceptable practices by the milk hauler may result in the suspension and/or revocation of his permit." Problem 49, Certification of Industry Personnel, recommends that line 625 on page 28, line 462 on page 226, and line 469 on page 227 be amended to provide certification of industry approved personnel *every three years* instead of annually.

Problem 52, District of Columbia, council voted "No action" but the minority report was accepted. The minority report: It is recommended that (a) Section VI, paragraph G on page 10 of the Procedures Manual be deleted. (b) There shall be inserted in the Procedures Manual a new paragraph 5 in Section I-A to read: "For the purpose of these procedures and NCIMS in total, the District of Columbia shall be considered as a State, with all the rights, duties, responsibilities and privileges of a state." Problem 55, amendment to MMSR, recommends to amend line 174½ on page 8 by striking the words "no credit is given" and by striking the words "for compliance" on line 175. Insert the phrase "the regulatory agency only shall be debited" before the word "when" on line 175. Add a new item (4) on page 8, line 180½, following item (3), to read as follows: "Producers and plants shall not be debited for failure of the regulatory agency to perform." (Similar changes are recommended to be applied to amend line 273, 274, 274½ on page 12 of MMSR). Problem 56 dealing with making sanitation ratings of milk, recommend corrections where needed. Line 405, page 19 - Receiving station. The wording "except items 4, 5, 6" should be added after Part III since these items do not apply to receiving stations. Line 408 and 410, page 19 - number 75 should be changed to 70. Items 5, 8 and 9 each carry a weight of 10. Line 475, page 23 - raw milk from without the jurisdiction will be evaluated in Item 4, Part III. Item 4 does not apply to raw milk. Line 479, page 23, gives examples of partial credit but lines 175 and 273 state that "no credit is given for compliance when less than the required number of samples has been examined during the preceding 6 months."

Problem 58, dealing with Methods of Making Sanitation Ratings of Milk Supplies, on pages 7 and 8, lines 172-174 of the Document states that compliance with bacterial, somatic cell and cooling temperature requirements is based on a dairyman's compliance with standards of Section 7 of the PMO on 2 out of 4 samples. However, Section 7, pages 37-39 does not refer in any way to 2 out of 4 counts. Section 6, pages 29-36, lines 634-816, covers action to be taken when 2 out of 4 counts and 3 out of 5 counts are high but Section 7 does not contain information as to when a producer is debited on a survey. Council recommends adoption. Problem 60 - amendment to MMSR. Recommend to amend line 174½ on page 8 to insert, after the word "samples," the following phrase: "unless the last official test exceeded the standard." The delegates rejected the recommendation. Problem 61, dealing with MMSR, recommends "amend paragraph 3, page 30, by deleting the word "sources" on line 657 and substitute the word "imported." On page 31 delete line 658 and substitute "imported milk supplies from a listed source, the raw milk sanitation compliance rating shall be pro-rated on a weighted average basis." Following this, delete line 664 through line 670, on page

31 of MMSR. Further, delete line 679½ beginning with "If raw etc." through line 717 on page 33. Problem 63 deals with communication. Recommend that it should be spelled out in the Procedures that where agencies are separated, regional personnel should take all matters pertaining to survey and certification through the certification agency which is competent to deal with the enforcement agency. Direct service by regional personnel should be kept at a minimum.

Problem 64, recommends: "To eliminate the number of visits for recertification, FDA should be asked to make a continuing evaluation of survey officers throughout the year and compile this information for certification. There is no reason why a determination for recertification could not be done on this basis." Problem 65 requests that the FDA *Quarterly Publication of Interstate Milk Shippers* include a product code for condensed milk and milk products. Recommends adoption. Problem 66, recommends, in Section 5, page 24, line 543, insert the following after the word "months": "except for those states that have a program which is equal to this requirement and complies with at least the specifications outlined in Appendix B." Council recommends inclusion of the requirement that the bulk milk tanker and its appurtenances be inspected at least once a year. Council recommended inclusion of the following before line 636 and changing the first sentence in lines 636 through 640 to read: "It shall be the responsibility of the milk hauler to collect a representative sample of milk from each farm bulk tank before transferring milk from a farm bulk tank, truck or other container. All samples shall be collected and held in substantial compliance with the most recent edition of *Standard Methods for the Examination of Dairy Products* and delivered to a milk plant, receiving station, transfer station, or other locations approved by the regulatory agency. During any consecutive 6 months, at least four samples of raw milk for pasteurization taken and delivered in accordance with this section from each producer shall be obtained under the direction of the regulatory agency or shall be taken from each producer under the direction of the regulatory agency. During any consecutive 6 months, at least four samples of raw milk for pasteurization shall be taken under the direction of the regulatory agency from each milk plant after receipt of the milk by the milk plant and prior to pasteurization." Council recommends adoption of a standard for bulk shipments of pasteurized products for repasteurization with a coliform standard not to exceed 100 per ml. Council recommended that the word "shall" on line 703, page 32, be changed to "should."

The various sections were reviewed with the following recommendations. Section 1 - Recommend that reference be made to Appendix L in the definition section of the PMO. Section 4 - Recommend the addition of the words "or ultrapasteurized" where applicable. Section 5 - Recommend that "shall" on line 550½ on page 24 be changed to "may" and also on line 590, page 26. Appendix G - Recommend that the word "equivalent" on line 445½ be defined.

Council III

Chairman Vaux called on Glen Huskey, chairman, to give report of Council III.

Council III received 10 problems, six of which were voted "No action." Problems 1 and 2 were combined as one problem dealing with the Procedures Manual. Section III, parts F and G, and adding part J. Council recommended (a) Section III-F, 1. add words "or paragraph J of this section" after the words "Section VII" of the present sentence. (b) Section III, G - add words "or paragraph J of this section" after the words "Section VII" of the present sentence. (c) Add paragraph J, dealing with the opportunity for re-rating. Delegates rejected all three recommendations. Problem 3 was combined with a similar problem referred to Council III from Council II, here-in-after called Problem 11 dealing with Section VII of the Procedures Manual. Recommended, in Section VII, C. 1, add 85 in place of the present 90 at bottom of page 11 and insert 80 in place of present 85 on page 12, line five. Delegates rejected the recommendation. Delegate Gadd moved, as a substitute for the rejected Part J, providing for a re-rating within 60 days, that Section III-F-1 and Section III-G of the Procedures Manual be amended by changing the figure "90" to "15" in both cases. Delegates approved. Council voted no separate action be taken on Sections 9-18 of the PMO, Apendixes C, F, and K to the PMO, Sections 8-15 of the Grade "A" condensed and Dry Milk Products and Condensed and Dry Whey and Appendix B to the same document.

Chairman Huskey announced that Wm. Fouse has been appointed as the chairman of the Subcommittee on Reciprocity and that the subcommittee was given an expanded definition of reciprocity and procedures for removal of the asterisk (*) for consideration to be reported back to Council at the next N.C.I.M.S. Conference.

Chairman Vaux asked for a motion authorizing the Executive Board to act on behalf of the Conference in attempting to resolve any still unresolved issues with FDA between now and the 1979 Conference. A delegate so moved and the delegates approved.

NEW BOARD MEMBERS

Chairman Vaux named the slate of Board members proposed for Region II by the nominating committee, and asked for any nominations from the floor. Mr. Kennedy nominated Harold Bengsch, Springfield, Missouri Health Depratment, to represent local health from Region II. Motion was made for a unanimous vote for all candidates, with the exception of the local health department representative. Delegates approved. The secretary was instructed to take a written ballot on the two candidates to represent local health, namely, John Schilling and Harold Bengsch. Harold Bengsch was declared the winner to represent local health from Region II.

The final business session of the sixteenth National Conference on Interstante Milk Shipments was adjourned at 2:30 p.m. on Thursday, May 26, 1977.

EXECUTIVE BOARD MEETING, May 26, 1977

Chairman Vaux convened the meeting at 2:45 p.m. in the Commodore Room. Voting members present: Arledge, Baghott, Bengsch, Causey, Gay, McGarrahan, Race, Rich, Rowley, Rubis, Russell, Schilling, Van Patten, Vaux; absent: Seaman. Non-voting members present: Huskey, Smathers. Dudley Conner asked that Noles be replaced on Council I, due to his retirement, and that Holliday replace Jefferson. Board approved.

Chairman Vaux called for election of officers, turning the meeting over to McCaffrey while he and Boosinger left the room. Vaux and Boosinger were unanimously reelected to their respective positions as chairman and vice-chairman. McCaffrey was reelected Executive Secretary-Treasurer. The Memorandum of Understanding was discussed by McGarrahan, as the result of comments in writing presented by Adams of NMPF and Lofthouse of Montana. McGarrahan stated that FDA would check with their legal counsel regarding possible changes.

Don Race, as incoming chairman of council I, asked to be relieved of those duties. The Board accepted his request with regret. Three new vice-chairmen of councils will be appointed at the Board meeting in August, as well as a replacement of Don Race. Chairman Vaux will contact John Speer regarding his again being program chairman.

Huskey reported on the activities of the sub-committee on reciprocity, and recommended that Alabama, S. Carolina and Texas have the asterisks removed from their names as now appearing in the *Quarterly Publication of Interstate Milk Shippers.* Council III members Chris Sykes, Massachusetts; Ed. Ruppert, North Carolina; and Clinton VanDevender, Mississippi request the records indicate they abstained from the above recommended action. Chris Sykes requested the records show that the Chairman of Council III did appoint a Reciprocity sub-committee at the non-terminally adjourned reconvened meeting in St. Louis, in January 1976, and that his sub-committee did not take any action since that time, since there are currently no guidelines to define reciprocity and there have been no reports submitted to the Executive Board of the NCIMS. Mr. Sykes and Dr. Wildasin have requested the records to indicate they have abstained from all voting restricting the free flow of milk to or from any state. Huskey stated further that the Louisiana-Florida problem has not been solved at this time. The Board voted to accept Huskey's report, as moved by Huskey and seconded by Arledge.

Huskey then presented the report of the sub-committee on procedures to be used in reviewing problems of reciprocity. The Board accepted the report and voted (Russell moved, seconded by Schilling) to have the Executive Secretary incorporate the report into the minutes of this Board meeting. The report is attached hereto. The Board voted to hold the 1981 meeting at the Arlington Hotel, Hot Springs, Arkansas, the dates to be arranged by the Executive Secretary.

The meeting was adjourned at 3:15 p.m., with the understanding that the Board will meet in Sioux City, Iowa, at 8:30 a.m. on Monday, August 15, the room to be arranged by the Executive Secretary, who will so notify the members.

REPORT OF SUB-COMMITTEE ON RECIPROCITY

The following recommendations and definition regarding reciprocity were recommended by Council III to the Reciprocity Sub-committee to use in problems regarding reciprocity.

Definition: Section I,A,4.

Reciprocity for the purpose of NCIMS procedures shall mean that a regulatory agency will follow the requirements of the Grade "A" Pasteurized Milk Ordinance and the NCIMS procedures, in accepting milk from out of state, in accordance with the provisions of Section 11 of the Grade "A" Pasteurized Milk Ordinance.

(A) Procedure for placing an asterisk (star)

1. A grievance, with rationale, must be filed with the Conference Chairman in writing, by a concerned person, with copies to the respective state delegates.
2. The Conference Chairman shall transmit the grievances to the Chairman of Council III.
3. Council Chairman must contact affected state(s) for specific information concerning complaint.
4. A sub-committee within this Council III would review the information and make a recommendation to the Council. The sub-committee should consist of two industry and two regulatory members from Council III, plus the Council Chairman.
5. The affected state should have an opportunity for presenting information in writing or orally before the sub-committee.
6. Council III recommendations go to the Executive Board of the Conference for final decision.

(B) Procedure for removal of asterisk (star).

1. State involved must submit a formal request to the Conference Chairman for removal of asterisk.
2. Referral would be made by Conference Chairman to Council III Chairman.
3. Council III Chaiman would inform parties making initial complaint concerning request for removal and ask for current status showing cause for not honoring request.
4. Request should then have sub-committee review with recommendations to Council III.
5. Council III would make recommendations to the Conference Executive Board for final action.

STATISTICS ON THE 1977 MEETING

Forty-six states and the District of Columbia had official delegates present. Hawaii, Maine, Rhode Island and Washington did not have official delegates in attendance.

Two hundred seventy (270) persons registered for the meeting. All states except Hawaii, Maine and Rhode Island were represented. Ohio led with 38 persons registered; Illinois second with 19; New York third with 14; Texas and District of Columbia with 12 each; Michigan, Missouri and Wisconsin 10 each; all other states had less than 10 persons in attendance.

Broken down discipline-wise, local health had 21 representatives; state agriculture 46; state health 57; industry 106; universities 6; F.D.A. 26, U.S.D.A. 2; and miscellaneous 6.

Long-Time IAMFES Leader H. L. "Red" Thomasson Dies

H. L. "Red" Thomasson, 74, died in the emergency room at Major Hospital in Shelbyville, Indiana on December 31, 1977. Red became ill at his home and was taken to the hospital by the local Emergency Unit.

A former executive secretary of IAMFES and managing editor of the *Journal of Milk and Food Technology,* Red will be deeply missed by all who worked and served with him.

Red at the 1977 Annual IAMFES Awards Banquet.

Red was very active in the milk industry. A graduate of Franklin College in Indiana, he was instrumental in establishing grade A milk standards in Indiana. In addition, he was a member of the board that established triple A dairy standards for the United States. Also, he was a member of both the state board for registration of sanitarians and the World Congress for Utilization of Milk.

As a member of the Committee on Sanitary Procedures from 1949 on, Red devoted much time to improving the knowledge of sanitarians and others so they could more effectively improve the Nation's public health. He first joined IAMFES in 1939. He was elected vice-president in 1949, thus served as president in 1951-52. Further, he was IAMFES Executive secretary and managing editor of the Journal for 22 years beginning in 1952.

Red was married to Margaret Davis on April 28, 1929. She survives him along with four children: Mrs. Warren (Jackie) Weiss, Cincinnati; Dan King Thomasson, Washington, D.C.; Mrs. Clark (Peggy) Brown, Country Club Heights, Indiana; and Michael Thomasson, Columbus. Red was born Sept. 14, 1903.

Dr. J. H. Martin Moves to Clemson

Dr. James H. Martin, Head of the Dairy Science Department at South Dakota State University for the past 5 years, has been named Professor and Head of the Dairy Science Department at Clemson University, Clemson, South Carolina, effective February 1, 1978.

Martin is a native of Tennessee, and holds the B.S. degree in Dairy Manufacturing from the University of Tennessee, Knoxville. He also earned the M.S. degree in Dairy Technology and the Ph.D. degree in Dairy Microbiology, both from the Ohio State University. He served as a faculty member at Mississippi State University, Ohio State University, and the University of Georgia before assuming the position as Department Head at South Dakota State University.

Dr. Martin is a member of the American Dairy Science Association, Institute of Food Technologists, and the American Cultured Dairy Products Institute, for which he serves as Editor of the *Cultured Dairy Products Journal.* Martin has presented papers at annual meetings of IAMFES and results of some of his research have appeared in the *Journal of Food Protection.* He is married to the former Syble Hunter, and they have two children, a son, Dana Keith, who is an engineer with Texaco in Houston, TX, and a daughter, Pamela Dawn, who is a college sophomore majoring in music.

The IAMFES and Iowa Association gratefully acknowledge the following organizations for their generous support of the 64th Annual Meeting of IAMFES in Sioux City, Iowa on August 14-18, 1977.

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United States Chemical Co.
Universal Milking Machine
Upstate Milk Co-op
Valley of Virginia Co-operative Milk Producers Assn.
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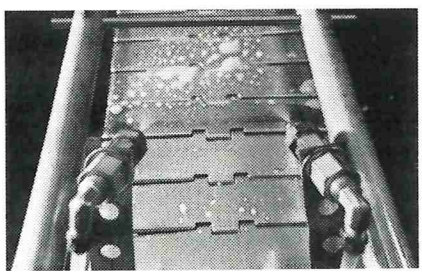

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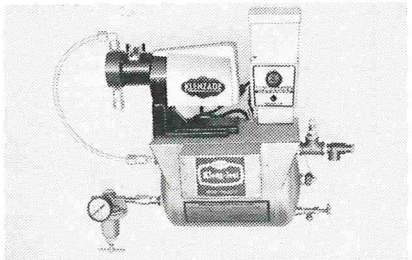


Super KLENZ-JECT Model 26 is the heart of the Central Conveyor Lubrication Program.

FDA Approves Uniform Sanitation Plan; Awards Reciprocity Contract to NIFI

Important progress in sanitation training and certification was signaled by the U.S. Food and Drug Administration (FDA) as it informed the National Institute for the Foodservice Industry (NIFI) of two significant actions:

1. FDA has approved for implementation NIFI's report on a uniform national plan for sanitation training of foodservice managers.

2. NIFI has been selected by FDA to develop a national reciprocity plan for foodservice manager training and certification programs and to undertake a model project to test and refine the plan.

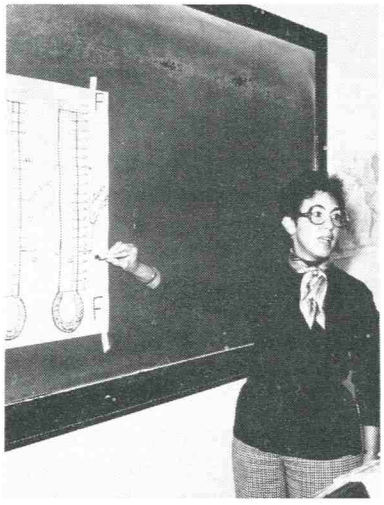
In notifying NIFI of the FDA actions, A. S. Davis, FDA Director, Division of Foodservice, said: "FDA has accepted the NIFI report and recommends its use for establishment of statewide or local programs and as guidance for development of a uniform national plan."

"We believe," Davis said, "that interest in sanitary aspects of foodservice operations is at an all-time high. We believe that regulatory agency involvement in industry training complements enforcement activities and enhances common goals of compliance with regulations and protection of the consumer. To this end, FDA will continue to support industry sanitation training to the extent that resources will permit."

Davis told regulatory agencies and foodservice industry organizations: "We hope that you will fully support the concept of foodservice manager training and the recommendations submitted by NIFI and supported by FDA. We further hope that you find the material contained in the report useful in promoting or implementing sanitation training for industry management personnel."

NIFI, which is the foodservice industry's not-for-profit educational foundation established by the industry to advance foodservice management standards through education, recently submitted a 74-page report to FDA, recommending that all foodservice manager sanitation training and certification programs be designed to facilitate reciprocity among and between industry and government.

Several states will participate initially in NIFI's model project to test and refine the reciprocity plan and other states will be added later. The plan will incorporate emphasis on minimum standards of training content and of uniformly applied certification administration.



Foodservice Training Now Bilingual

Listening intently to their instructor is a class of 28 Spanish-speaking foodservice managers at the Loop branch of Chicago City College, which for the first time is using the new Spanish-language edition of *Applied Foodservice Sanitation,* a course of the National Institute for the Foodservice Industry, the foodservice industry's not-for-profit educational foundation. Entitled *Manejo Higienico De Viveres,* the new course was published and translated in Mexico City.

The instructor, Mrs. Zulma Samos, is shown describing the temperature "danger zone" (45°-140° F) for storing and holding foods. This is the temperature region in which bacteria multiply rapidly.

In addition to Spanish, examinations for the course are available in Korean, Chinese, Italian and Greek for foodservice managers and supervisors preparing for mandatory sanitation certification which is law in the Windy City, beginning Jan. 1, 1978. More than 4,000 Chicagoans have already completed the course on a voluntary basis.

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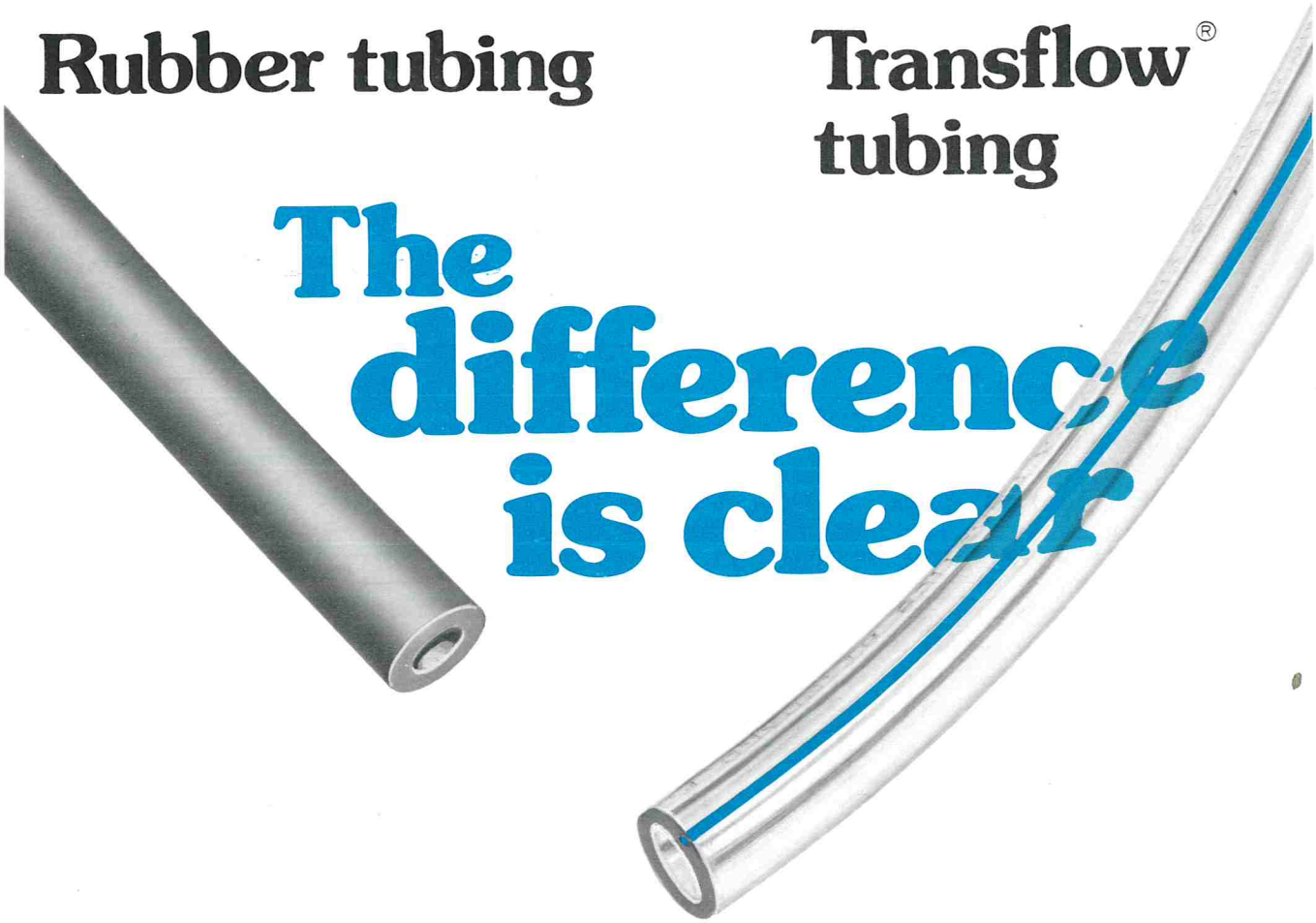

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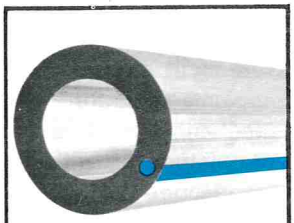

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University of Maryland Assesses Energy Use in Fluid Milk Processing

Under a contract with the Department of Energy (DOE), a university team is investigating a process for sterilizing milk at very high temperatures, without giving the milk a "cooked" flavor. DOE officials believe that the process has potential to save energy in the production, distribution, and storage of milk.

Sterilized fluid milk is used widely in Europe, but its flavor has been a major obstacle to consumer acceptance in the U.S. Most sterilization methods produce a flavor similar to that of evaporated milk.

The process being investigated by the University of Maryland for DOE produces a fresh-tasting product because the milk is heated briefly at a very high temperature, without touching any metal surfaces hotter than the milk itself. The process was developed and patented by DASI Industries, Inc. of Chevy Chase, Maryland.

In the new process, milk is preheated to 150 degrees F., and then is fed into tubes at the top of the sterilizing chamber. Each tube has a very thin slit. Milk falls through each slit in a very thin film.

As a thin film of milk falls through the sterilizing chamber, it comes into contact with very hot steam. The milk absorbs the steam's heat and much of its moisture. Before it falls to the bottom of the chamber, the milk is heated to 280 degrees F.

The University of Maryland's one-year $420,000 contract with the Department of Energy will involve assessing energy use in conventional milk processing, and investigating the energy-saving possibilities of using sterilized fluid milk. The Maryland group will develop ideas or "models" of systems to maximize energy savings using an acceptable sterile fluid milk product.

"We have several things in mind," Harris said. "One idea may be to supply an institution with our milk. It could be stored on the shelf and refrigerated only during the day it's being used. Another idea would be to supply the milk to a ship that is out to sea for several months."

Harris said that his group will also identify the possible constraints to commercializing the new process, including the cost of new capital equipment needed by dairy operations to use the process.

"Basically it boils down to one question." Harris said. "Would you buy a carton of milk that's just sitting on an unrefrigerated grocery store shelf?"

Tomorrow's consumer might answer that question differently than the consumer of today, Harris noted. "If the product looks good, tastes good, and if the price is right," Harris said, "there's a good chance that consumers will buy it, and we'll all benefit from the energy savings."

KAB, Inc. Issues Transcripts of Clean Community Systems Seminar

Keep America Beautiful, Inc. has issued complete transcripts of the first National Seminar on the Clean Community System (CCS). More than 350 representatives of federal, state and local governments, professional and civic organizations and a broad cross-section of industries attended the Sept. 28 Seminar in Washington, D.C.

CCS is the only program using behavioral science to change attitudes toward waste handling at the local level, through cooperative efforts by the public and private sectors. By drying up the generation of loose trash at seven major sources in a given community, litter reductions as high as 70 percent have been achieved by the 81 cities now implementing the CCS. These cities, from Massachusetts to California, range in size from Houston (pop. 1,500,000) to Bardstown, Ky. (pop. 5,800).

Seminar highlights included major addresses by Senator Jennings Randolph of West Virginia, Chairman, Senate Committee on Environment and Public Works, and Mayors William H. Hudnut, III, of Indianapolis, and Buckner F. Melton of Macon, Ga., both certified CCS cities.

Seminar panels discussed the:

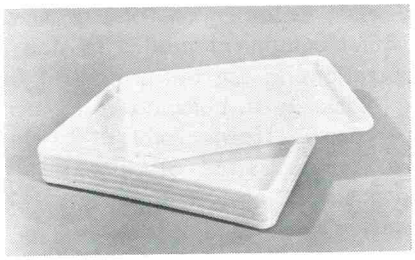
—role of public works directors in the CCS. Panel moderator was Robert S. Hopson, Director of Public Works, Charlotte, N.C. (the principal CCS test site) and Past President, American Public Works Association.

—involvement of businesses, including representatives of the banking, beverage, utility and solid waste management industries.

—participation of grassroots civic group leaders, who have found their membership on local CCS committees to be a logical extension of their involvement in other citizen action and environmental organizations.

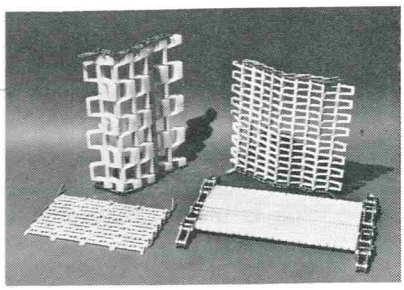
Transcripts of the entire Seminar are available for $12 from KAB, 99 Park Avenue, New York, N.Y. 10016. Information on transcripts of individuals panels and speeches is also available.

Founded in 1953, KAB is a national, nonprofit, public service organization dedicated to promoting individual action to improve the environment. It is recognized nationally by the advertising campaign featuring the "crying Indian."



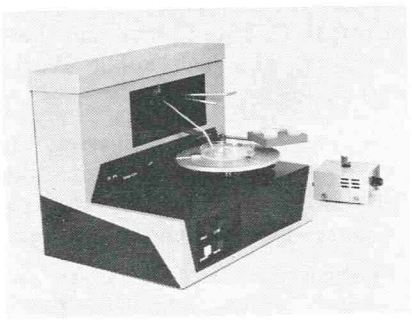
•*Polypropylene trays* that remain stable over a wide temperature range are available from WRH Industries, LTD. The non-staining, chip resistant polypropylene material approved by FDA, USDA and NSF can be frozen without getting brittle and steam cleaned without deforming. For more information contact: WRH Industries, LTD., 5 Industrial Way, East Providence, RI 02915.

•*Jaco announed* a new line of acetal copolymer plastic fittings that were cleared by USDA and FDA for use with coffee, milk and antibiotics. High tensile strength and good impact resistance over a broad temperature range makes these non-toxic fittings ideal for many plumbing and tubing applications. The fittings are corrosion resistant to most inorganics, except for sulfuric, nitric and hydrochloric acids. Tested to 2,000 p.s.i., these fittings stop galvanic corrosion, deaden noise, resist vibration, and are less expensive than metal fittings, according to the manufacturer. Write Acetal Copolymer Bulletin, c/o Irving W. Davies, Jaco Manufacturing Co., 468 Geiger St., Berea, OH 44017.



•*Modular plastic belting* from Plastic Components Corp. (PCC), Windsor, CT, is offered in four configurations for food processing lines. The lightweight plastic belting runs quietly and creates less system drag and lower sprocket and shaft loads than steel belts, Less power is required with plastic belting. For information contact Plastics Components Corp., 611 Palisado Ave., Windsor, CT 06095.

Product Potpourri



•*Spiral System Microbial Assays* claim drastically reduced cost because (1) serial dilution is eliminated, (2) replicate plating is not required and (3) plating is done automatically. As a result, analyst time (and skill requirements) are reduced, there is no waste of petri dishes and agar, there is no need for dilution blanks and pipets, and laboratory equipment and space are more efficiently utilized. The heart of the Spiral System is a precision built dispenser (see photo) which distributes the sample on the surface of a rotating agar plate. Counting can be done manually using a specially designed viewing grid, or automatically. Laser colony counter will count spiral plates and standard pour plates. Spiral plating is recognized as an official method of analysis by the Association of Official Analytical Chemists. Spiral plating and counting instruments are available from Spiral Systems Marketing, 1200 Quince Orchard Blvd., Gaithersburg, MD. 20760.

•*Aflatoxin M_1 Standard* is now available from the Eureka Laboratories, Inc. Aflatoxin M_1 level in milk and meat can be identified by thin layer chromatography using the M_1 standard for comparison. For a data sheet on purity and price contact Eureka Laboratories, Inc., 401 North 16th St., Sacramento, CA 95814.

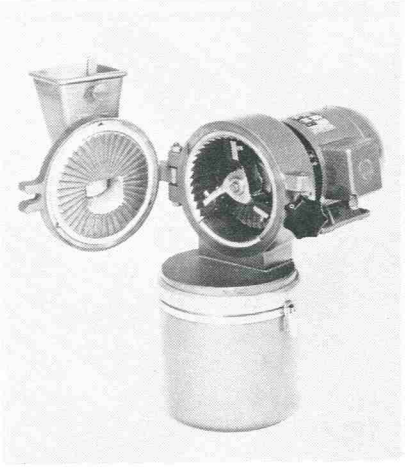
•*3M Company's Packaging Systems Division* announces an easy-to-attach packaging platform for the 3M-Matic 5A Box Sealer. The platform provides an easy convenient means of forming and filling boxes before sealing. The 3M-Matic 5A Box Sealer simultaneously applies tape to the top and bottom seams of regular-slotted containers and will seal up to 26 uniform-size boxes per minutes. For information contact 3M Company, Department PS7-16A, P.O. Box 33600, St. Paul, MN 55133.

•*A four-page brochure* describing a new line of wheeled containers, in 12, 24, and 40 cubic foot sizes, is available from Plastics Omnium Inc. The containers, tradenamed IRONMAN, are ideal for storage, protection, and transport of a wide variety of materials, parts, and products, Easy to clean injection molded containers have rugged, thick walls of high density polyethylene for strength and resistance to heat and cold. For a brochure write Plastics Omnium Inc., 70 Shawmut Rd., Canton, MA 02021.

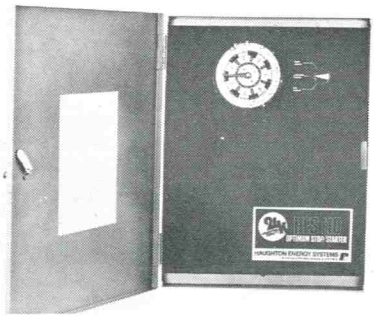
•*Stainless Steel Hand Homogenizer* emulsifies small batch solutions containing either acids or alkalis reducing particles or globules to one micron or less. It features a durable yet lightweight base and handle of hammer-toned aluminum on rubber-cushioned legs; 12 oz. capacity bowl, piston, cylinder and adjustable nozzle—all of stainless steel. Simple disassembly/reassembly for easy cleaning and sterilization. Overall height is 10"; base length 9". For information write Chase-Logeman Co., 59 Tec St. P.O. Box 814, Hicksville, N.Y. 11801.

•*A New Concept in fine particulate removal* is described in a brochure now available from Bird Machine Company, Inc. The Bird Vortex Clarifier is covered in the four-page bulletin (BVK101). Using a forced vortex principle, the Bird Vortex Clarifier is designed to remove fine particulate matter from streams generally containing less than 1000 PPM producing, in many cases, effluents in the range of 10 to 40 PPM. Advantages apply in (1) clarification of waste water prior to discharge, (2) recovery of solid valuables from discharge water, (3) and polishing of liquid valuables in industrial processes. For information write Bird Machine Co., Inc., South Walpole, MA 02071.

• *Laboratory Steam Jacketed Kettles* now feature the same top quality, heavy duty performance capabilities as production sized equipment. The Groen TDC/2/RA-10 quart steam jacketed kettle, with scraper type mixer, processes products under pressure or vacuum. The special tilting unit readily achieves high temperatures at top speeds for fast, efficient cooking and cooling. Gentle handling of heat sensitive products is ensured. Cookers are available in 10 and 20 quart, as well as 5, 10 and 20 gallon capacities. For information contact: Groen Division, Dover Corporation, 1900 Pratt Blvd., Elk Grove, Village, IL 60007.



•*Microjet 2,* an advanced cross beater mill ideal for batch or continuous coarse and fine crushing of both organic and inorganic substances, was introduced by Micro Materials Co. Microjet 2 was designed for crushing and disintegrating materials up to a hardness of 6 on MOH's scale. The maximum material size to be ground is 30mm. The achievable final fineness is 0.07—8mm, depending on sieve size and material to be crushed. Ideal for laboratory or plant sample preparation the Microjet 2 is capable of crushing and shearing material on a continuous basis. For information write Micro Materials Co., 100 Grand St. Westbury, NY 11590.



•*Haughton Energy Systems* offers optimum stop/starter for energy cost reduction. Haughton Model HES-110 controls comfort, lighting and other building systems by automatically compensating for changes in wind, temperature, daylight level and day of work week. Plug-in computer modules are programmed to match building comfort systems to local climate and integrate other building systems. Local override controls compensate for unusual conditions. For information contact Haughton Energy Systems, 671 Spencer St., Toledo, OH 43609.

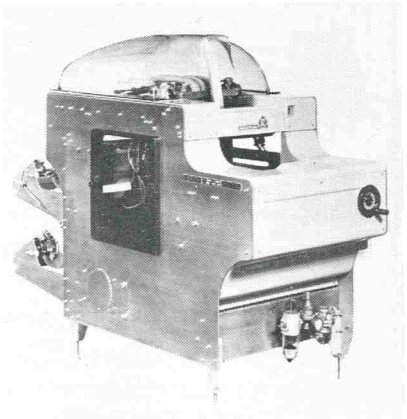
•*Pope Scientific* announced a new 24-page catalog of stainless steel products specially selected for utility in laboratories where durability, corrosion resistance and non-contaminating characteristics of stainless steel are important. Examples of items listed are adhesive backed stainless steel tape and stainless steel spray coatings. For a free copy write Pope Scientific, Inc., P.O. Box 495, Menomonee Falls, Wisconsin 53051.

•*A unique wastewater treatment system* removing more than 98 percent of the BOD in a sugar refinery is outlined in a four-page color brochure available from Activox Inc. Case history study covers the operation and features a patented, advance oxidation ditch treatment system designed by Activox and installed at the Amstar "Domino Sugar" refinery in Chalmette, Louisiana. For information write Activox Inc. 468 Park Ave. South, New York, NY 10016.

•*Typical properties* and suggested uses for a sodium 2-ethylhexyl sulfate designated Witcolate^{TM} D-510, which acts as an effective wetting aid and penetrant are discussed in a new bulletin offered by the Organics Division of Witco Chemical Co. The surfactant exhibits stability, compatability and effectiveness in strong acidic or alkaline solutions and in concentrated salt solutions. Uses include produce washing and lye-peeling, bleaching, mercerizing, metal cleaning and paper wetting. For copies of bulleting No. 228 write Witco Chemical Corporation, Organics Div., Dept. 1458, 3230 Brookfield St., Houston, TX 77045.

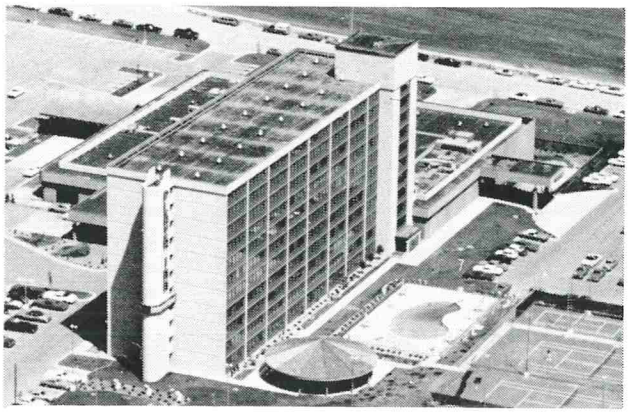
•*New Bulletin on high-volume reverse osmosis* systems describes two central water purification systems designed to produce 125 to 250 liters per hour (800 and 1600 gallons per day). Designed and engineered for central building distribution, boiler beeder or pyrogen-free pharmaceutical production, these systems claim lower capital and operating costs than comparable distillation. The Millipore Milli-RO systems use disposable cartridges for less maintenance. Unique features include automatic operation, solid-state control, built-in conductivity monitoring and a water-saver to reduce water consumption. Fittings are all stainless steel. Millipore bulletin PB843 on "Milli-RO High Volume Reverse Osmosis Systems" is available free, on request, from Millipore Corporation, Water Systems Division, Bedford, MA, 01730.

•*An illustrated bulletin* on the new Metalwash Model RWI insulated Rack and Cart Washer is now available. Design and construction features of the housing that is fabricated from modern materials, are fully described and the advantages over stainless steel construction in application are presented. Specifications given for the Rack Washer cover; tank heating and capacity, pump horsepower and gallonage, wash and rinse spray systems, service and utility requirements, instrumentation and controls, optional equipment and accessories, and crating and shipping information. For a free copy of Metalwash Bulletin No. B-10, contact: Metalwash Machinery Corporation, 901 North Ave., Elizabeth, NJ 07207.



•*Weldotron Corporation* introduces an improved version of A12ST automatic, stainless steel design, stretch wrapping system. Compact and simple to use the A12ST operates on a patented technique which grips and stretches wrapping film on all sides. Weldotron says the method offers up to 75% energy savings over other methods. For information contact Weldotron Co., 1532 So. Washington Ave., Piscataway, NJ 08854.

If you care about professional improvement



Plan to attend the 65th Annual Meeting of IAMFES on August 13-17, 1978 at the

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For years IAMFES Annual Meetings have offered informative group sessions and discussions in a relaxed and enjoyable atmosphere.

This year promises that much and more. A wide variety of general and technical issues will be presented in a format that lets you plunge right in and tackle current problem issues in your field.

And while your pondering events of the day the evenings offer nothing but relaxation. As in years past, there's the Annual Awards banquet. But this year the Missouri and Kansas Associations have teamed up to present special evening events you, and even your family, won't want to miss.

Also, you'll have a chance to see Kansas City and all it has to offer, from the Kansas City Royals to the finest restaurants in the Midwest.

So make plans now to meet in Kansas City for a professional program and an enjoyable meeting.

With so much to offer, you can't afford to miss it.

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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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SANITARY METHOD OF APPLYING
A U. S. P. LUBRICANT
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Haynes Spray

U. S. P. LIQUID PETROLATUM SPRAY

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CONTAINS NO ANIMAL OR VEGETABLE FATS. ABSOLUTELY NEUTRAL. WILL NOT TURN RANCID — CONTAMINATE OR TAINT WHEN IN CONTACT WITH FOOD PRODUCTS.

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This Fine Mist-like
HAYNES-SPRAY
should be used to lubricate:

SANITARY VALVES
HOMOGENIZER PISTONS — RINGS
SANITARY SEALS & PARTS
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GLASS & PAPER FILLING MACHINE PARTS
and for ALL OTHER SANITARY MACHINE PARTS which are cleaned daily.

The Modern HAYNES-SPRAY Method of Lubrication Conforms with the Milk Ordinance and Code Recommended by the U. S. Public Health Service

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PACKED 6-12 oz. CANS PER CARTON
SHIPPING WEIGHT—7 LBS.

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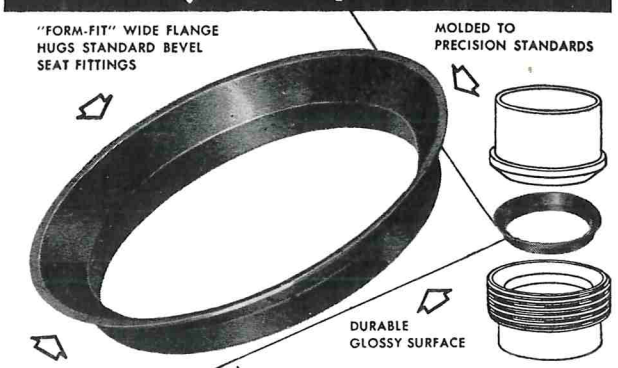
HAYNES-SPRAY INGREDIENTS ARE APPROVED ADDITIVES AND CAN BE SAFELY USED AS A LUBRICANT FOR FOOD PROCESSING EQUIPMENT WHEN USED IN COMPLIANCE WITH EXISTING FOOD ADDITIVES REGULATIONS.

HAYNES SNAP-TITE GASKETS



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LEAK-PREVENTING
NEOPRENE GASKET for Sanitary Fittings

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Available for 1", 1½", 2", 2½" and 3" fittings.
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SELF-CENTERING
SNAP-TITE® *Gaskets*

SIZES 1" - 1½"
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*MADE FROM
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"The Sophisticated Gasket"

THE IDEAL UNION SEAL FOR BOTH VACUUM AND PRESSURE LINES

Gasket Color . . . slightly off-white

SNAP-TITE self-centering gaskets of TEFLON are designed for all standard bevel seat sanitary fittings. They SNAP into place providing self-alignment and ease of assembly and disassembly.
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FOR A FITTING GASKET THAT WILL OUT-PERFORM ALL OTHERS...

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

* Gaskets made of DuPont TEFLON ® TFE-FLUOROCARBON RESINS

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All Lubri-Film ingredients are approved additives and can be safely utilized as a lubricant for food processing equipment when used in compliance with existing food additive regulations.

ESPECIALLY DEVELOPED FOR LUBRICATION OF FOOD PROCESSING AND PACKAGING EQUIPMENT

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SANITARY • NON TOXIC • ODORLESS • TASTELESS

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

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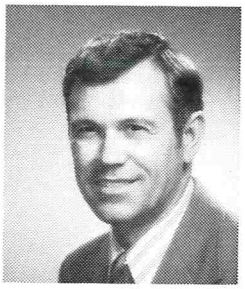
A Better Milk Harvest Through Good Milking Practices

By Dr. John R. Campbell
Professor of Dairy Husbandry
University of Missouri—Columbia



For the corn producer, the most important harvest he makes occurs only once a year—when he goes into the fields with his corn picker. But, for the dairyman, the most important harvest takes place two, and in some cases, three times a day, every day of the year. And, the use of good milking practices helps dairymen to have a good harvest every time they milk their cows. Additionally, a complete milk harvest today will help the cow produce more milk tomorrow.

Milk-making Cells Work Harder With Use

The milk-making (epithelial) cells work the hardest immediately following milking because that is when intramammary pressure is the lowest. At each milking a hormone called prolactin or lactogen is released and its effect is to cause the milk-making cells to go back to work. But, if through poor milking practices, some of the milk is left in the udder, intramammary pressure mounts faster and this, in turn, slows down milk secretion. Research indicates that milk secretion each hour following milking is approximately 90 to 95 percent of that of the preceding hour. But as the udder fills, this percentage decreases. Naturally, milk left in the udder following milking will shorten the period of time that the milk-making cells work at maximal capacity.

Repeated failure to remove milk from mammary glands causes the milk-making cells to become inactive. Thus, for maximal milk production, the milk secreting cells must be challenged... and that means removing all of the milk possible at each and every milking. Although incomplete milking will not have a big detrimental effect in one or two milkings, it sure will over a period of several days. Not only will the milk left in the udder not be harvested and, therefore, not be sold, it will, in addition, accelerate the cow's decline in level of production and, thereby contribute to unprofitable dairying.

Persistency: A Slower Decline Means More Profit

A cow reaches her peak production about two months into the lactation. After this, a natural, gradual decline in milk production occurs. The relationship between milk given one month compared to that produced the next is known as persistency. Persistency can be improved if good milking practices are used to assure a full harvest of the milk crop at each milking.

Eight Steps Toward Getting a Full Milk Harvest

Good cow milking practices include eight steps which, when done properly, will achieve the fullest possible harvest of your valuable milk crop.

1. Environment: Provide a comfortable, stress-free environment.
2. Proper Stimulation: A vigorous massage of the mammary glands will help insure complete letdown of milk.
3. Strip Foremilk: Stripping acts to further stimulate the cow and, at the same time, eliminates much of the bacteria-laden first milk.
4. Timely Application of Milking Machine: This should be done one minute after starting stimulation to take advantage of maximal letdown.
5. Adjust Machine: Proper forward/downward adjustment is important for complete milking.
6. Remove Teat Cups as Quarters Milk Out: Avoid over-milking which can lead to tissue irritation and mastitis.
7. Dip Teats: Teat dipping gives the teat end the protection it needs against mastitis-causing bacteria while the streak canal regains its full constriction.
8. Maintain Equipment Properly: Properly adjusted and maintained equipment is an essential step toward better milking.

The amount of milk a cow produces in a lactation from the time she freshens until she stops lactating is determined by a number of factors; some can be controlled—others cannot. However, good milking practices will go a long way toward helping you achieve more nearly the full potential of your cows' production. In other words, good milking will give a more complete harvest of your most important crop—and that means more profit.