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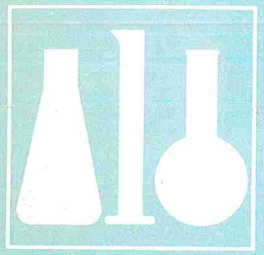

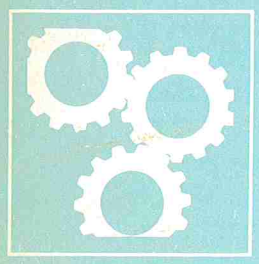

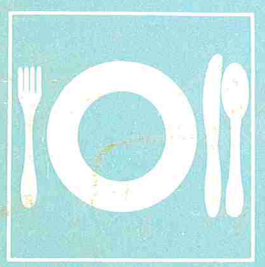

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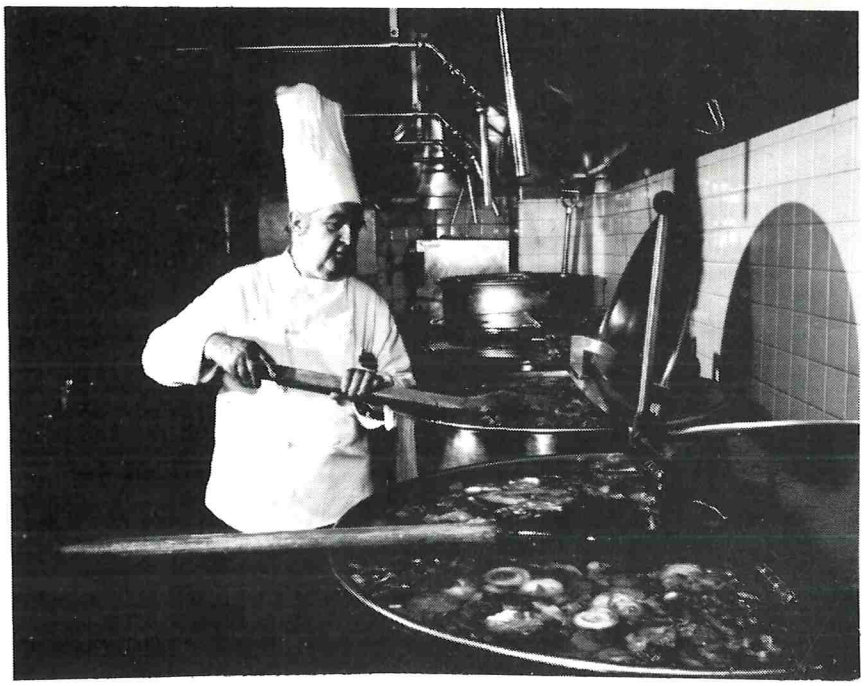

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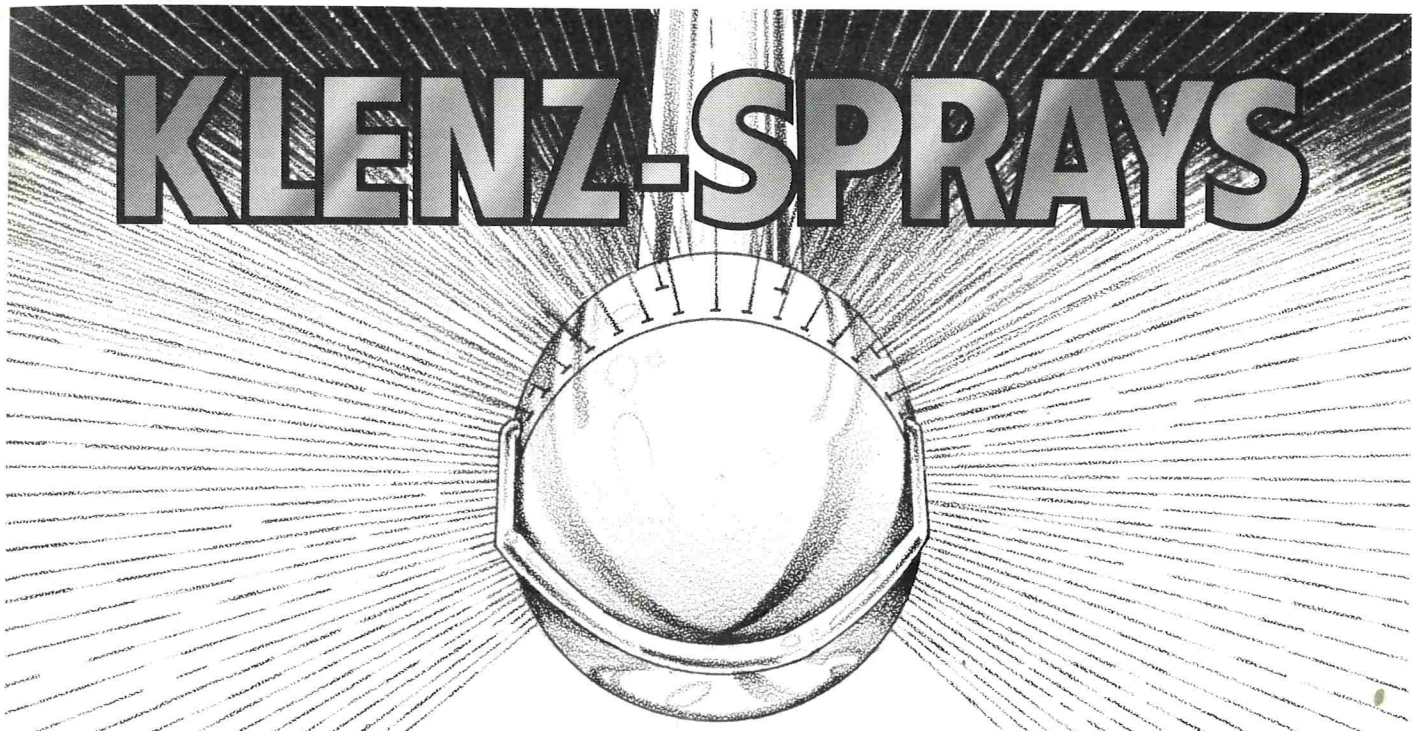

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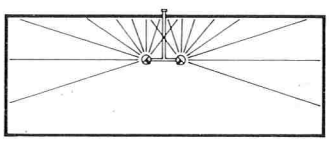

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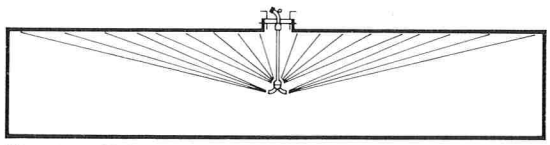

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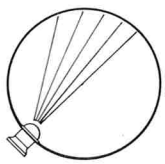

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Manuscripts: Correspondence regarding manuscripts and other reading material should be addressed to Dr. Elmer H. Marth, Dept. of Food Science, University of Wisconsin, Madison, Wis. 53706.

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Anisakine Nematodes in Fresh Commercial Fish From Waters Along the Washington, Oregon and California Coasts

BETTY JUNE MYERS

Division of Research Grants, National Institutes of Health, Westwood Building Room 319, Bethesda, Maryland 20014

(Received for publication February 27, 1978)

ABSTRACT

Two thousand and seventy four fresh fish representing 24 genera and 43 species were purchased from commercial marine fisheries in Washington, Oregon and California. They were examined for anisakine nematodes by dissection, candling or digestion of the muscle and elution of the viscera. Thirty thousand and thirty-two anisakine nematodes were recovered from the fish examined; however, no anisakine nematodes were recovered from the shellfish examined. Both *Anisakis* sp. and *Phocanema* sp., which are considered pathogens for human consumers of raw or semiraw fish, were encountered. Of the anisakines, *Anisakis* sp. larvae were found in a greater number of the fish hosts examined than *Phocanema* sp. larvae, which were found in fewer fish hosts and at lower incidence. The higher incidence of *Anisakis* sp. larvae was probably due to the large population of whales, the host in which this species reaches maturity. The lower incidence of *Phocanema* sp. larvae is a reflection of the small population of seals, the definitive host for this species.

The anisakine nematodes attracted attention during the early 1950s because their presence reduced the commercial value of fish (*8*). Large anisakines, such as *Phocanema* sp. larvae, were easy to detect in both the edible and nonedible muscle of a large variety of fish, especially the cod. Extensive studies were conducted in Canada on this so-called "codworm" but the parasite was thought to be merely unappetizing. Work was directed toward removing it or reducing the number of nematodes visible in fish fillets (*8*). That this anisakine could be a human pathogen was not considered. During the past 10 years, however, there have been an increasing number of reports that these nematodes can infect humans (*6*).

Human infection, i.e. anisakiasis cases, have been reported from Europe (particularly the Netherlands), Japan, the United States and Chile (*14*). All these cases have been linked with consumption of raw or semiraw fish (*6*). The first confirmed case in the United States occurred in 1967, but was not published until another case had been diagnosed in 1970 (*4*). Since then, seven additional cases have been reported and there are known to be more (*1,3*). Six of the cases were attributed to *Phocanema* sp. larva; two cases were attributed to *Anisakis* sp. larva, the so-called "herring worm" which is the principal cause of anisakiasis in Europe.

With the apparently increasing incidence (or recognition) of human anisakiasis, the presence of the causative nematode in edible fish is no longer a problem of esthetics but one of human health. Studies, including the U.S. Pacific Coast survey reported here, of the incidence and geographic distribution of anisakine nematodes in commercially marketed fish from the United States waters were begun under the auspices of the U.S. Food and Drug Administration.

Systematic surveys of fish for anisakine nematodes have been done in Canada's Atlantic and Pacific fisheries, but previous U.S. reports were confined to incidental findings or local surveys. Often during routine surveys, the fish musculature was not examined and the resulting parasite data pertained only to the external surfaces, body cavity, discrete organs and the gastrointestinal surfaces.

MATERIALS AND METHODS

During a 2-year survey, 1974-1976, selected fresh fish and shellfish from the U.S. Pacific Coast waters were examined for anisakine nematodes. Fish to be examined were chosen for their commercial importance and were collected from the landings at Seattle, Washington and Astoria, Oregon, as well as from San Francisco, California shipments. Pertinent information on catch statistics were recorded after purchase.

The fish to be examined were iced but not frozen. After purchase, the fish were taken to the School of Fisheries at the University of Washington in Seattle, Washington and examined as quickly as possible for nematodes or stored in a refrigerator (ca. 5 C) until examined.

Before examination the fish were identified as to species using the morphological characteristics of Hart (*5*). Individual fish were measured and weighed. As a first step in examining fish for parasitic nematodes, the fish were eviscerated. The viscera and the eviscerated portion (muscle) of each fish were examined separately.

The muscle was divided into two portions for examination - the edible and the nonedible portion (the flap muscle). While the nonedible portion is usually discarded, in the salmon the entire muscle including the flap muscle is marketed. The muscle portion was examined by a candling process or by artificial digestion. In candling light is passed through the muscle, and a parasite appears as an opacity (*12*). Artificial digestion of the muscle was done for 4 h at 35-37 C in a 1% pepsin solution adjusted to a pH of 3 with 1 N hydrochloric acid after the addition of 200 g of fish muscle per liter (*7*). Shellfish were also examined by gross dissection and artificial digestion techniques. For recovery of nematodes from the viscera, each set of viscera was first examined microscopically; any visible nematode was collected manually. The contents of the stomach and the small and large intestine were examined by repeated washing of each organ; nematodes were collected manually from the wash fluid. Nematodes collected were washed in saline and fixed in 70% alcohol at ca. 58 C (hot but not boiling); they were stored in 5% glycerine alcohol.

To identify the nematodes by light microscopy, they were cleared in either glycerine or lactophenol and placed in temporary mounts. Upon

completion of identification, lactophenol specimens were washed in 70% alcohol (to remove traces of lactophenol which has a deleterious effect on nematodes) and stored in 10% glycerine alcohol. Identification of the specimens was based on the morphology of the digestive tract and the position of the excretory pore (*9*).

RESULTS

Over two thousand fish were examined, mostly from the U.S. coastal waters off Washington and Oregon shores, and to a lesser extent from off the California shore. Twenty-four genera of fish, encompassing 43 species, were represented in this survey. *Anisakis* sp. was the most abundant of the larval anisakine nematodes occurring in the fish; these nematodes occurred in the edible and nonedible muscle, in the viscera and were encysted on the viscera of fish from all geographic areas sampled during the 2 years of this survey (Table 1). Although the anisakine nematodes encountered in the mesenteries and those encysted on the viscera were identified only by random sampling, most appeared to be *Anisakis* sp. larvae and *Thynnascaris* sp. adults. The latter probably migrated from the lumen of the intestinal tract upon death of the fish, since adult *Thynnascaris* are normally parasitic in the digestive tract (*11*). For *Phocanema* sp., the percentage of fish infected and the average number of nematodes found in the muscle was the same for both Oregon and Washington during the 1976 survey (Table 2).

No anisakine nematodes were encountered in examination of over 2,000 shellfish constituting six genera and 10 species: 160 specimens of *Crassostrea gigas* (Pacific oyster), 135 specimens of *Siliqua patula* (razor clam), 60 specimens of *Venorupis japonicus* (Manila clam), 155 specimens of *Pandalus borealis* (pink shrimp), 152 specimens of *Pandalus danae* (coonstriped shrimp), 164 specimens of *Pandalus hypsinotus* (humpy shrimp), 164 specimens of *Panalopsis dispar* (side striped shrimp), 1090 specimens of *Pandalus jordani* (pink shrimp) and 158 specimens of *Pandalus platyceros.*

DISCUSSION

The anisakine nematodes are members of the order Ascarida and suborder Ascaridae (*2*). The suborder Ascaridae contains three families: (*a*) Ascarididae, of which the most familiar is the large nematode of primates - *Ascaris lumbricoides* Linnaeus, 1758; (*b*) Toxocaridae, containing the well-known dog ascarid *Toxocara canis* Werner, 1782; which is responsible for visceral larval migrans in humans; and (*c*) Anisakidae, of which the anisakine nematodes responsible for human anisakiasis are members. There are 30 genera in the family Anisakidae (*8*). While fish are usually an intermediate or transport host for larval anisakines, fish may also serve as definitive host for some adult anisakidae. Amphibians, reptiles, birds or mammals (*6,9*) serve as definitive host for other anisakidae.

The larval stages of anisakines in fish hosts were reported as early as the 13th century (*10*). While there was some evidence concerning possible human infection with the fishborne stages of these anisakines that mature in mammals, proven cases have been reported only in recent decades (*6*). Only four genera of anisakine nematodes from fish have been implicated in human anisakiasis: *Anisakis, Phocanema* (*-Terranova* of the Japanese), *Porrocaecum* and *Contracaecum* (*6*). These nematodes may be identified microscopically by features of their digestive tract and the position of the excretory pore (*9*).

This survey has demonstrated the presence of anisakine nematodes in the commercial fish of the U.S. Pacific Coast, including the occurrence of potential human pathogens (*Anisakis* and *Phocanema* spp.) in the edible muscle of fish intented for raw or semiraw consumption.

In the commercial fish surveyed, the fish that feed predominantly on other fish showed a higher incidence (e.g. *Sebastes paucispinis* begins feeding on fish during

TABLE 1. *Survey of anisakine nematodes in commercial fish of the U.S. Pacific Coast 1975-1976.*

Fish host	Number of fish examined	No. nematodes/No. fish						
		Anisakis sp. in muscle		*Phocanema* sp. in Muscle		Anisakine in viscera	Av. no. nemas	% Fish infected
		Edible	Nonedible	Edible	Nonedible			
ANOPLOPOMATIDAE								
Anoplopoma fimbria (Sablefish)	4	1/3	1/3	0/0	0/0	3/4	1.0	100%
BOTHIDAE								
Citharichthys sordidus (Pacific Sanddab)	23	5/1	4/3	0/0	0/0	44/21	2.0	91%
CLUPEIDAE								
Clupea harengus pallasi (Pacific Herring)	127	0/0	0/0	0/0	0/0	1094/119	9.0	88%
COTTIDAE								
Scorpaenichthys marmoratus (Cabezon)	3	3/3	0/0	0/0	0/0	3/3	1.0	33%
EMBIOTOCIDAE								
Embiotoca lateralis (Striped Seaperch)	1	0/0	0/0	0/0	0/0	0/0	0.0	0%
GADIDAE								
Gadus macrocephalus (Pacific Cod)	509	103/49	437/140	83/52	19/5	5836/430	9.5	84%
Theragra chalcogrammus (Walleye Pollack)	10	0/0	0/0	0/0	0/0	4/4	2.5	40%
HEXAGRAMMIDAE								
Hexagrammos decagrammus	1	0/0	0/0	0/0	0/0	0/0	0.0	0%

(Kelp Greenling)								
Ophiodon elongatus (Lingcod)	27	3/3	5/5	0/0	0/0	30/15	2.5	56%
PLEURONECTIDAE								
Atherethes stomias (Arrowtooth Flounder)	5	0/0	0/0	0/0	0/0	4/4	0.8	80%
Eopsetta jordani (Petrale Sole)	207	110/29	234/54	0/0	0/0	2054/151	72.0	15%
Glytocephalus zachirus (Rex Sole)	120	1/1	0/0	0/0	0/0	25/19	0.2	16%
Hippoglossoides elassodon (Flatheaded Sole)	1	0/0	0/0	0/0	0/0	0/0	0.0	0%
Isopsetta isolepis (Butter sole)	24	0/0	0/0	0/0	0/0	11/7	0.5	29%
Lyopsetta exilis (Slender Sole)	3	0/0	2/3	0/0	0/0	14/3	5.0	100%
Microstomus pacificus (Dover Sole)	9	0/0	0/0	0/0	0/0	?/4	—	67%
Parophrys vetulus (English Sole)	117	0/0	0/0	0/0	0/0	32/16	2.0	13%
Platichthys stellatus (Starry Flounder)	25	0/0	0/0	0/0	0/0	3/2	1.5	5%
Psettichthys melanostictus (Sand Sole)	4	0/0	2/2	0/0	0/0	6/2	2.6	75%
Reinhardtius hippoglossoides (Greenland Halibut)	5	0/0	4/1	0/0	0/0	22/4	5.0	100%
SALMONIDAE								
Oncorhynchus keta (Chum Salmon)	1	1/1	—	—	—	0/0	—	100%
Oncorhynchus kisutch (Coho Salmon)	55	20/20	—	—	—	0/0	—	36%
Oncorhynchus nerka (Sockeye Salmon)	10	?/4	—	—	—	0/0	—	40%
SCOMBERIDAE								
Thunnus alalunga (Albacore)	10	0/0	0/0	0/0	0/0	2/2	0.2	2%
SCORPAENIDAE								
Sebastes alutus (Pacific Ocean Perch)	220	8/3	0/0	0/0	0/0	121/129	1.3	45%
Sebastes auriculatus (Brown Rockfish)	10	0/0	2/1	0/0	0/0	15/4	0.5	40%
Sebastes babcocki (Redbanded Rockfish)	32	0/0	8/3	0/0	0/0	17/4	6.2	12%
Sebastes brevispinis (Silvergray Rockfish)	47	27/15	623/26	12/3	0/0	2963/26	51.8	55%
Sebastes caurinus (Copper Rockfish)	10	0/0	0/0	2/1	0/0	3/2	2.5	20%
Sebastes chlorostitctus (Greenspotted Rockfish)	4	0/0	0/0	0/0	0/0	5/1	1.2	25%
Sebastes crameri (Darkblotched Rockfish)	5	0/0	0/0	0/0	0/0	35/5	7.0	100%
Sebastes dipoloroa (Splitnosed Rockfish)	1	0/0	0/0	0/0	0/0	2/1	2.0	100%
Sebastes elongatus (Greenstripped Rockfish)	12	0/0	32/8	0/0	0/0	25/5	4.4	58%
Sebastes entomelas (Widow Rockfish)	22	30/10	8/8	0/0	0/0	1120/20	52.6	100%
Sebastes flavidus (Yellowtail Rockfish)	102	14/11	76/28	7/4	0/0	454/69	5.2	77%
Sebastes goodei (Chilipepper Rockfish)	19	0/0	0/0	0/0	0/0	29/17	1.5	89%
Sebastes maliger (Quillback Rockfish)	26	0/0	1/1	0/0	0/0	2/1	0.7	7%
Sebastes paucispinis (Bocaccio)	68	636/53	1865/58	19/19	0/0	9794/59	65.1	95%
Sebastes pinnger (Canary Rockfish)	179	7/4	154/30	1/1	0/0	1651/132	10.9	93%
Sebastes ruberrimus (Yellow Rockfish)	1	0/0	0/0	0/0	0/0	0/0	0.0	0%
Sebastes rufis (Bank Rockfish)	4	0/0	1/1	0/0	0/0	39/4	10.0	100%
Sebastes saxicola (Stripedtail Rockfish)	11	0/0	1/1	0/0	0/0	0/0	0.09	9%
TOTALS	2074	969/210	3460/376	124/80	19/5	25462/1289		

Total No. Nemas: 30032
No. Fish Species: 43

TABLE 2. *Anisakine nematode infection rates from the 1976 U.S. Pacific Coast fish survey.*

State	No exam	Total No. anisakines	*Anisakis* sp. in fish muscle				*Phocanema* sp. in fish muscle				Anisakine in fish viscera			
			% Fish edible	Av. no. nemas	% Fish nonedible	Av. No. nemas	% Fish edible	Av. no. nemas	% Fish nonedible	Av. no. nemas	% Fish viscera	Av. no. nemas	No. fish species
CALIFORNIA	114	601	16%	0.3	22%	0.7	5%	0.03	0	0	4%	0.6	12
OREGON	614	3030	5%	0.04	23%	0.4	4%	0.04	1%	0.06	67%	4.4	11
WASHINGTON	890	15051	13%	0.4	30%	2.0	4%	0.08	1%	0.05	75%	14.3	10

Total number of nematodes 18682
Number of fish examined 1617

the first year of life). In contrast, those species which feed primarily on benthos have a lower incidence of anisakine nematodes (e.g. *Parophrys vetulus*).

Most of the anisakine nematodes (predominantly *Anisakis* sp.) occur in or on the viscera; the next highest incidence is encountered in the edible and nonedible muscle (Table 1).

With the high incidence of anisakine nematodes encysted in or on the viscera and mesenteries a very important factor concerning the processing of fish should not be overlooked. In the commercial fisheries of the U.S. Pacific Coast, the fish are not gutted at sea but placed on ice until the fish reach the market. The vessels may not return to port for 5 days and the fish will remain on ice until they are processed. Smith and Wotten (*15*) have shown experimentally that *Anisakis* sp. larvae migrate from the viscera into the muscle of dead herrings; the author has likewise observed a migration of the *Anisakis* sp. larvae from the viscera into the muscle of fish which were placed on ice. Ruitenberg (*13*) observed that the time elapsing between catching and preparation of the herring could be an important factor in the chance of human infections and as a result certain regulatory measures were established in the Netherlands. It appears that the length of time between the catch of the fish and actual gutting is an important factor in governing migration of the anisakine nematodes, particularly *Anisakis* sp., into the muscles of commercial fish on the West Coast. The incidence could probably be reduced if the catch was processed more quickly or the fish were gutted at sea.

In the Pacific Coast waters of the United States the higher incidence of *Anisakis* sp. larvae than of *Phocanema* sp. larvae is attributed to the large population of whales, the host in which *Anisakis* sp. reaches maturity (*7*). Seals, the host in which *Phocanema* sp. larvae reaches maturity, are not as populous along the Pacific coast (*10*). The opposite conditions exist in the Eastern Canadian regions of the Gulf of Saint Lawrence (*8*).

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by a method described by Boland and Garner (*4*). Five g of methoxyamine hydrochloride (Eastman Kodak Co.) were weighed into a flask containing the dried purified extract. One ml of naphthalene (J. T. Baker Chemical Co.) internal standard (5 mg/ml in dried pyridine) was added. Although the flask was shaken several times with a swirling motion, most of the residue did not dissolve. The mixture was allowed to stand for 8 min at room temperature. At the end of 8 min, 1 ml N,O-Bis-(Trimethylsilyl)-Acetamide, "BSA" (Pierce Chemical Co.) was added, and the contents of the flask were mixed for at least 30 sec. At this point all the residue dissolved, and a clear yellow solution was obtained. The solution was then heated in a stoppered flask for 30 min at 70 C in an oil bath. When derivatization was completed, the solution was allowed to cool for 3 min and analyzed by gas-liquid chromatography.

Gas-liquid chromatography

The separation and identification of the non-volatile organic acids as their trimethylsilyl derivatives was carried out in a Bendix Model 2600 (Process Instrument Div., Bendix Corp.) equipped with a U-shaped stainless steel column (1.8 m × 0.64 cm o.d.). The column packing consisted of 3% SE-52 (Applied Science Laboratories) coated on high performance, acid washed DMCS treated Chromosorb W (Hewlett-Packard). Three microliters of the derivatized solution were injected into the column with a micro syringe (Hamilton Co.). The column was programmed isothermally from 90 to 190 C at the rate of 2 C/min with the injection port and the flame ionization detector maintained at 200 and 270 C, respectively. The flow rates were 55 ml/min for the nitrogen carrier gas, 35 ml/min for the hydrogen and 500 ml/min for the air.

The peaks were recorded by Honeywell Electronic 194 two-pen 10-in. laboratory recorder (Honeywell Co.). The areas of the peaks were integrated by a computing integrator (Infotronics Model CRS-309, International Technical Instruments Inc.). Relative retention times were calculated according to the following formula:

$$\text{Relative retention time} = \frac{t_R \text{ unknown}}{t_R \text{ naphthalene}}$$

GLC calibration curves

One milliliter of a standard solution of organic acid containing 0.5, 1.0, 3.0 or 5.0 mg/ml each of lactic (J. T. Baker Co.), citric, tartaric (Fisher Scientific Co.), fumaric, malic (Eastman Kodak Co.), glutaric, malonic, succinic (United States Biochemicals Co.) and oxalic (Sargent-Welch Scientific Co.) acids and 5 mg of naphthalene in pyridine were silylated and chromatographed as described for the purified extract. The relative area ratios of the TMS derivatives of the organic acids and the naphthalene peak were calculated and plotted against concentration. A typical chromatogram of the standard solution of the organic acids is presented in Fig. 1.

Statistical analysis

Six replications were conducted, and the data were subjected to analysis of variance (*16*) to determine the effects of cooking on non-volatile organic acids. When significant differences occurred, Duncan's (*6*) new multiple range test was applied to locate the significant ($P < 0.05$) differences among means. Results for sensory scores for flavor of peas and carrots in the study of Mabesa and Baldwin (*9*) were correlated (*16*) with the organic acid content found in this study. Also, retention of chlorophyll components of peas reported in the former study was correlated with organic acid content.

RESULTS AND DISCUSSION

Peas

Based upon comparisons of relative retention times of the non-volatile organic acid standards and the peaks of the purified extracts from peas, lactic, succinic, malic and citric acids were identified (Fig. 1 and 2, Table 1). Johnston and Hammill (*7*) also reported the presence of succinic, malic and citric acids in peas, but not lactic.

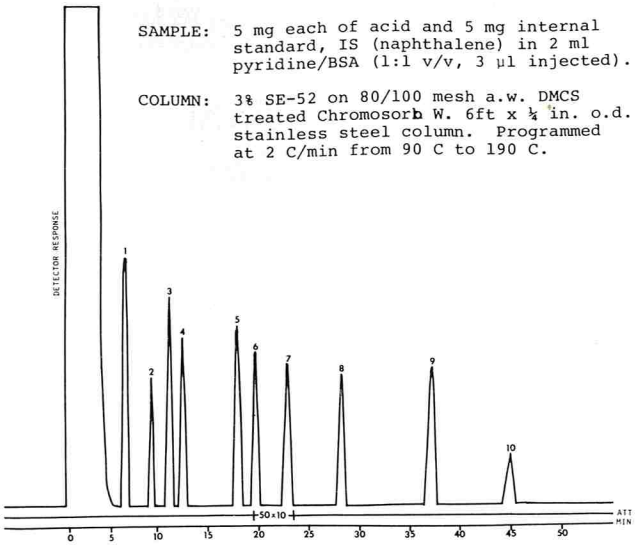

Figure 1. *GLC chromatogram of a standard mixture of organic acids as TMS derivatives. Peaks: 1) lactic 2) oxalic 3) naphthalene, IS 4) malonic 5) succinic 6) fumaric 7) glutaric 8) malic 9) tartartic 10) citric.*

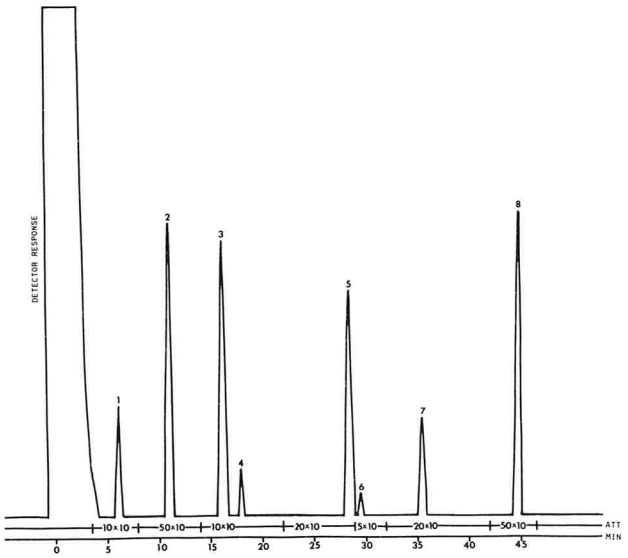

Figure 2. *GLC chromatogram of non-volatile organic acids isolated from peas. Peaks: 1) lactic 2) naphthalene, IS 3) unknown 4) succinic 5) malic 6) unknown 7) unknown 8) citric.*

Among the various cooking methods, the MW-115V procedure appeared to have the most pronounced effect in increasing the amounts of measurable organic acids in peas (Table 2). For lactic acid, the amount was significantly greater than in peas cooked by any other method and also greater than in raw peas. The succinic acid content of peas cooked by the Conv method was significantly larger than in peas cooked by microwaves regardless of the addition of water. Malic acid content of peas cooked by the MW-115V procedure was significantly higher than in the raw and in the other cooked samples. The malic acid content of peas cooked in the consumer microwave oven (MW-115V, MW-115V +) was significantly greater than that of the peas cooked in the institutional microwave oven, regardless of the addition of water. The citric acid content of peas cooked

TABLE 1. *Relative retention times of organic acid standards and organic acids extracted from peas and carrots.*

Organic acid	Standard mixture	Peas	Carrots
Lactic	0.59	0.50	
Oxalic	0.80		
Malonic	1.16		
Unknown		1.55	1.55
Succinic	1.72	1.76	
Fumaric	1.90		
Glutaric	2.22		
Malic	2.77	2.78	2.77
Unknown		2.99	2.96
Unknown		3.52	
Tartaric	3.66		
Citric	4.56	4.59	
Unknown			4.77

TABLE 2. *Mean[a] non-volatile organic acid content (wet weight) of peas cooked by microwaves and conventional methods.*

Cooking method	Lactic (mg/100g)	Succinic (mg/100 g)	Malic (mg/100g)	Citric (mg/100g)
Raw	6.98^{b}	1.73^{c}	15.56^{bc}	67.29^{c}
Conv	4.56^{d}	5.31^{a}	14.05^{bc}	78.60^{b}
MW-115V	8.77^{a}	2.77^{b}	24.63^{a}	104.93^{a}
MW-115V +	4.96^{cd}	1.06^{c}	16.37^{b}	90.32^{ab}
MW-220V	6.09^{bc}	1.95^{bc}	11.25^{c}	73.50^{b}
MW-220V +	4.45^{d}	1.65^{c}	11.79^{c}	68.76^{b}

[a]N = 6. Where exponent letters differ within a column, means differ significantly (P < 0.05) from each other (*6*).

by the MW-115V method was significantly larger than that of the raw and of all the cooked samples except for peas cooked by the MW-115V + method. Also, the citric acid content of the cooked samples was significantly higher than the raw.

Although cooking vegetables does not require the high temperature that commercial processing does, heating may be responsible for changes in organic acid content in both cases. Several researchers (*1,2,8*) observed an increase in organic acid contents after processing vegetables. No postulations concerning this increase were presented by any of these researchers. It appears that, in both cooking and processing, losses of constituents other than organic acids may have occurred also. This then resulted in an increase in concentration of organic acids in the vegetables.

Carrots

Several unidentified peaks were present in the chromatograms of carrot extract. The only peak from the injected derivatized extract that coincided with the relative retention time of the standard acid was malic acid (Fig. 1 and 3, Table 1). Since this was also the largest peak on the chromatogram, malic acid was the dominant acid in carrots. In the study of Bibeau and Clydesdale (*1*), citric acid was identified in fresh carrots in lesser amounts than malic acid, but a peak with a retention time equal to citric acid was not observed in our research. These researchers found a reduction in the amounts of citric acid in carrots subjected to heat processing. Therefore, preparation of the carrots for freezing in the present study may have caused a loss of citric acid.

Malic acid content of carrots on wet basis was not

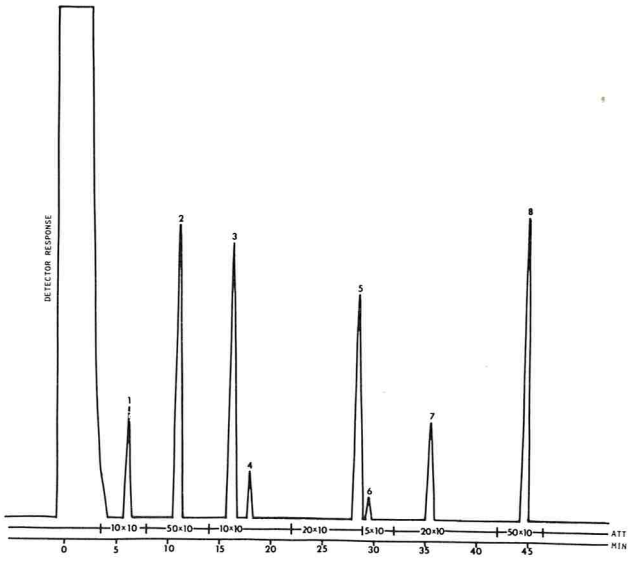

Figure 3. *GLC chromatogram of organic acids isolated from carrots. Peaks: 1) naphthalene, IS 2) unknown 3) malic 4) unknown 5) unknown.*

affected by the different methods of cooking. No significant differences were found between malic acid content of the raw and cooked samples (Table 3).

Correlations

A significant negative correlation (r = - 0.931) between total chlorophyll and total non-volatile organic acids in peas cooked by the MW-115V method was found. Although no other significant correlations were observed, it was noteworthy that all the correlations were negative. This trend was expected since greater amounts of acid would be responsible for greater destruction of chlorophyll components. As pointed out by McKinney and Weast (*10*), destruction of chlorophyll is dependent on the amount of acid in the system and this is aggravated in the aqueous media.

There were no significant correlations observed between different non-volatile organic acid contents (mg/100 g, wet basis) of peas and the sensory scores for flavor (*9*) for any of the cooked peas. However, a significant positive correlation between sensory scores for flavor of cooked carrots and malic acid content on a wet basis was found for carrots cooked by the MW-115V (r = 0.813) and by the MW-115V + (r = 0.831) procedures. These two significant correlations could not be attributed solely to the malic acid content since malic acid content of the cooked carrots did not differ

TABLE 3. *Mean[a] malic acid content (wet weight) of carrots cooked by microwaves and conventional methods.*

Cooking method	Content (mg/100g)
Raw	93.38^{a}
Conv	94.31^{a}
MW-115V	83.81^{a}
MW-115V +	87.39^{a}
MW-220V	95.01^{a}
MW-220V +	86.20^{a}

[a]N = 6. Where exponent letters differ, means differ significantly (P < 0.05) from each other (*6*).

significantly from either the raw or the other cooked samples.

Further investigations might reveal a closer relationship between flavor and non-volatile organic acids. Bibeau et al. (3) found that off-flavors in processed pureed carrots were due to pyrrolidone-carboxylic acid (PCA), which was derived from glutamine. Their investigation revealed that carrot puree processed at temperatures of 116 C contained PCA in sufficient quantities to elicit a taste response.

CONCLUSIONS

Based on the above results, the following conclusions were drawn.

1. Effects of microwave cooking on non-volatile organic acids of vegetables are dependent on the particular vegetable.
2. Cooking vegetables in a consumer microwave oven (550 watts of cooking power) either with or without water favors retention of non-volatile organic acids.
3. The extent of destruction of chlorophyll is related to the amount of acids present in the system, regardless of method of cooking.
4. Non-volatile organic acids play a minor role, if any, in the flavor of cooked peas and carrots.

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Microbial Growth on Plate Beef During Extended Storage After Washing and Sanitizing

M. E. ANDERSON[1], R. T. MARSHALL[2]*, W. C. STRINGER[2], and H. D. NAUMANN[2]

University of Missouri, Columbia, Missouri 65211

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ABSTRACT

Plate beef was washed and/or sanitized with cold water, hot water, steam, sodium hypochlorite, or acetic acid before being stored for up to 28 days at 3.3 C and 90% relative humidity. Microbial counts initially and at regular intervals thereafter disclosed that, compared with untreated controls, time to reach counts of 10^8 bacteria per cm^2 were (*a*) 1 day less with steam- or water-treated samples, (*b*) 2 to 3 days more with hypochlorite-treated samples, (*c*) 5 days more with hot-water-treated samples, and (*d*) 16 to 17 days more with acetic acid-treated samples. Re-sanitization with acetic acid extended time to reach equivalent counts by 7 additional days.

Numerous reports describing the short-term effects of cleaning and sanitizing meat on microbial counts have appeared in recent literature (*1-4,6,7,9-11*). However, long-term effects of these treatments have not been reported. Objective of the present study was to assess the effects of several washing and sanitizing treatments on aerobic plate counts of meat held under refrigeration up to 28 days.

MATERIALS AND EQUIPMENT

Materials

Strips of fresh beef plate meat that were about 25 × 30 × 2 cm were obtained from a commercial slaughterhouse and frozen (- 20 C) until used in the study. Chemical sanitizers were industrial sodium hypochlorite (200-250 μg/ml, pH adjusted to 6.0 with acetic acid) and glacial acetic acid (3.0%). Solutions were prepared with tap water and sprayed at temperatures of 12.8 to 15.6 C.

Equipment

Meat samples were washed and sanitized in a previously described cleaning unit (*3*). Water and sanitizers were sprayed onto the meat with nozzles nos. 5015 and 5008 (Spraying Systems, Wheaton, Illinois), respectively. Nozzles were located 40 cm above the meat. Measurements of pH at the surface of the meat were made with a Corning pH meter Model 12 equipped with a combination electrode.

PROCEDURE

Sixteen strips of plate beef were thawed for each of six replications of the washing and sanitizing experiment. For a uniform distribution of bacteria, all surfaces to be treated were rubbed against each other. The strips of beef were then randomly paired and placed on holding frames. Two samples (2.54 cm in diameter and 3 mm deep) were removed (Fig. 1a) from each strip of beef of each pair. These four samples (two from each strip) were blended in 99 ml of sterile dilution water for 60 sec. Petri dishes were prepared and aerobic bacterial colonies were counted according to *Standard Methods for the Examination of Dairy Products* (*7*), except that incubation was at 28 C for 72 h.

After removal of initial samples, each pair of beef strips was subjected to one of the following treatments: (*a*) no washing or sanitizing--control, (*b*) cold water--15.6 C, (*c*) hot water--76 to 80 C at meat surface, (*d*) steam--95 C at meat surface, (*e*) sodium hypochlorite, (*f*) acetic acid, (*g*) cold water then sodium hypochlorite, and (*h*) cold water then acetic acid. Conditions of application of wash water (both hot and cold) were 14 kg/cm^2 pressure, 12.8 l/min volume, and 10 cm/sec speed of meat travel under the spray. Application conditions for sanitizing solutions were 14 kg/cm^2 pressure, 6.8 l/min volume, and 2 cm/sec speed of meat travel under the spray. Steam was directed onto the meat from a distance of 10 cm with a locally fabricated nozzle. Line pressure was 17 kg/cm^2.

Immediately after treatment, each strip of meat was held with the long axis vertical and allowed to drain for 1 min. Two samples were then removed from each strip of each pair of beef strips (Fig. 1a).

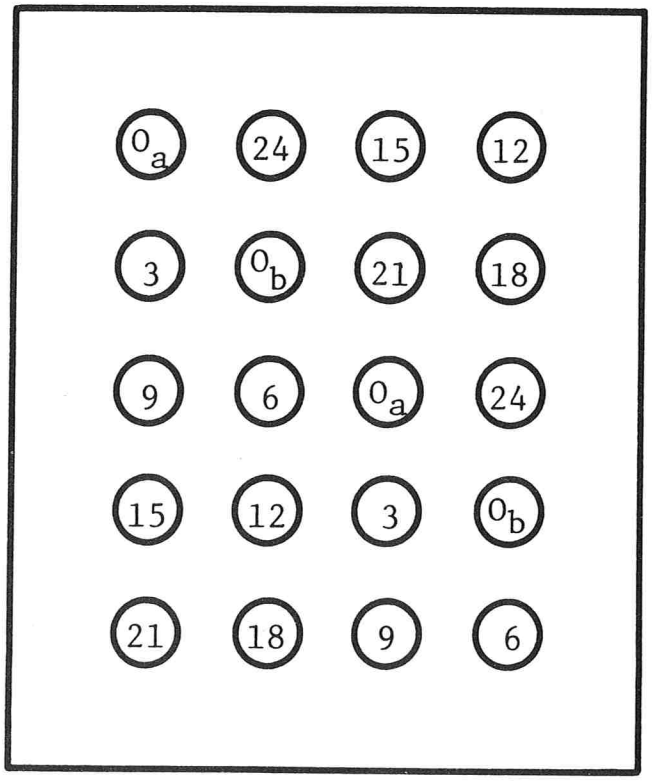

Figure 1a. *Sampling pattern for washing and sanitizing experiment and pH experiment: immediately before (O_a) and after (O_b) treatment and then after the following numbers of days; (3), (6), (9), (12), (15), (18), (21) and (24).*

[1]*Federal Research, Science and Education Administration, United States Department of Agriculture.*
[2]*Department of Food Science and Nutrition.*
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Samples were blended and plated as described above. The strips of beef were then stored at 3.3 C and 90% relative humidity (RH). Samples were taken and plated every 72 h for 12 to 24 days.

Four strips of plate beef were used in each of five replications of experiments to determine the pH of meat sanitized with acetic acid. Measurements of pH were made as follows: The area of the meat to be sampled was located by use of the sampling pattern (Fig. 1a). A drop of distilled water was placed on the area to be sampled, and this area was touched with the combination electrode. The pH was also measured immediately after sanitizing and at 3-day intervals thereafter.

In the experiment on resanitization of meat there were 18 randomly paired strips of beef or nine pairs per replicate in each of four replications. Three paired strips of beef were untreated controls, and six were sanitized initially with 3% acetic acid. After 7 days of storage three of the six paired strips of beef were resanitized with 3% acetic acid, then placed back in storage (3.3 C and 90% RH). Bacterial counts were made at the following times: before and immediately after sanitizing and then every 7 days for 28 days (Fig. 1b), except that unsanitized controls were sampled every 7 days for only 14 days.

The average numbers of colonies on duplicate plates were transformed to logarithms. Logarithmic means and significant differences between means (Duncan's) were determined by the Statistical Analysis System (SAS) in an IBM 370 Computer (5).

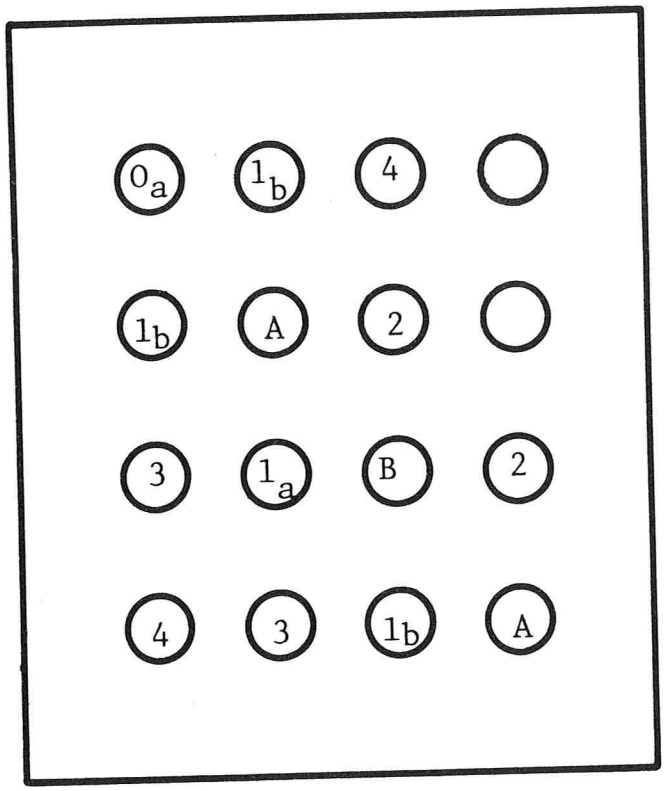

Figure 1b. *Sampling pattern for resanitizing experiment: immediately before (O_a) and after (O_b) the first treatment; before (1_b) and after (1_a) resanitizing 1 week into the storage period; and after 2 (2), 3 (3) and 4 (4) weeks of storage.*

RESULTS AND DISCUSSION

The geometric mean initial aerobic plate count (APC) of beef used in the first experiment was 5.29/cm², and the standard error of the mean was 0.17.

Figures 2 and 3 show changes in APC (log of the number of colony-forming units/cm², CFU/cm²) during refrigerated storage of sanitized and control samples. Means in both figures with different letters (a-d) for each storage time differed significantly ($P < 0.05$). Treatment with sodium hypochlorite, cold water plus sodium hypochlorite, and cold or hot water alone reduced counts (before vs. after) by about 1 log, whereas steam reduced the count only 0.06 log (Fig. 2). Initial reductions in counts by hot water alone and acetic acid alone were 2.0 and 1.5 log, respectively (Fig. 3). Counts of samples treated with cold water plus acetic acid differed little from counts of samples treated with only acetic acid.

Counts of bacteria on control samples increased at a logarithmic rate that approximated 1.4 generations per day throughout the 12 days of storage. The rates of growth on samples treated with water (cold and hot), steam, and hypochlorite were higher than on controls, probably because of the drier surfaces of the controls. Rate of growth was fastest, 2.1 generations per day, on

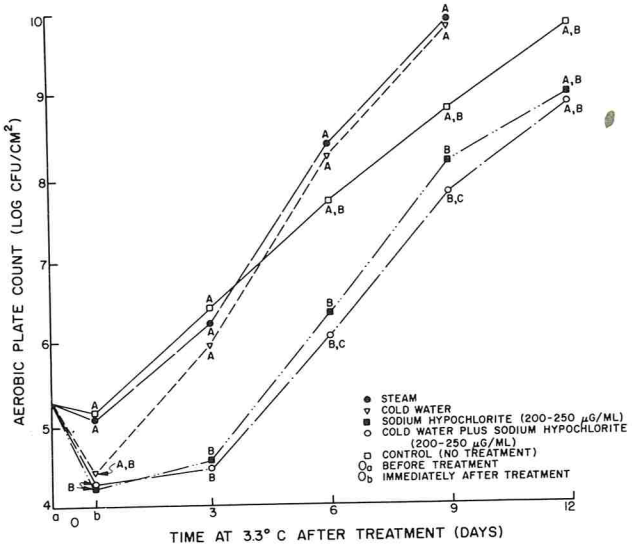

Figure 2. *Changes in aerobic plate counts with time in storage (days) at 3.3 C. Means in Fig. 2 and 3 with different letters (a-d) for each time in storage differed significantly ($P \leq 0.05$). Each data point represents an average of six values except for data points which have exceeded APC of 10^8 or 18 days of storage.*

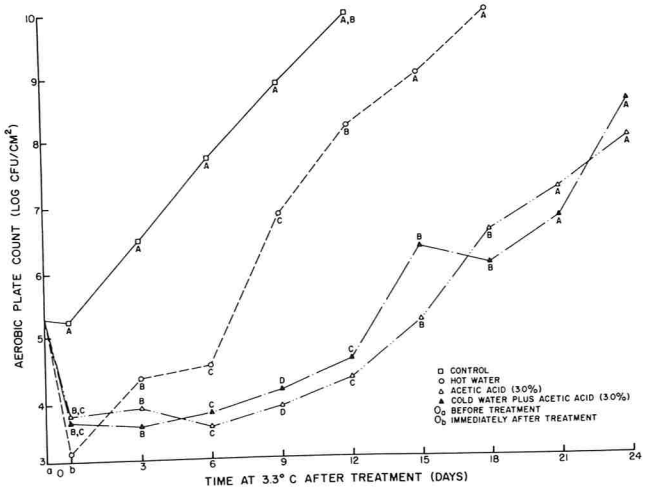

Figure 3. *Changes in aerobic plate counts with time in storage (days) at 3.3 C. Means in Fig. 2 and 3 with different letters (a-d) for each time in storage differed significantly ($P \leq 0.05$). Each data point represents an average of six values except for data points which have exceeded APC of 10^8 or 18 days storage.*

samples treated with cold water. Assuming that an APC of $10^8/cm^2$ indicated spoilage, steam- and cold water-treated samples kept 1 day less (6 vs. 7) than the controls, and hypochlorite-treated samples kept 2 to 3 (9 to 10 vs. 7) days longer than the controls.

The rate of bacterial growth on samples treated with hot water was similar to that on controls, but the initial 2-log difference in counts caused by hot water remained through 12 days of storage. The time required to reach an APC of $10^8/cm^2$ was nearly 5 days longer with the hot-water-treated samples.

The most effective treatment was acetic acid, and prior washing with cold water was of no consequence. With these samples 23 to 24 days passed before APC's reached $10^8/cm^2$. In fact, 14 to 16 days were required for counts to return to the initial level.

Treatment of the beef with acetic acid dropped the pH from about 5.9 to 3.9 (Fig. 4). The mean pH rose rapidly until about the ninth day of storage and stabilized at about 5.5. During the first 3 days, a substantial proportion of acetic acid (pKa = 4.76) was in the inhibitory unionized form.

Since in the first experiments it appeared that the rate of growth on acetic acid-treated samples began to increase after about 14 days of storage, another experiment was done in which beef was re-sanitized after 7 days of storage (Fig. 5). This meat had a lower initial count than that used in the first experiments (APC = 3.79 logs; SE = 0.1 log). Sanitization immediately dropped the count to 2.43 logs, and no growth was observed after 7 days of storage at 3.3 C. At that time the APC of the controls averaged more than $10^7/cm^2$. Re-sanitization dropped the mean APC to 1.46 logs/cm^2. After 14 days of storage, counts of the twice-sanitized samples were 2 logs lower than those of once-sanitized

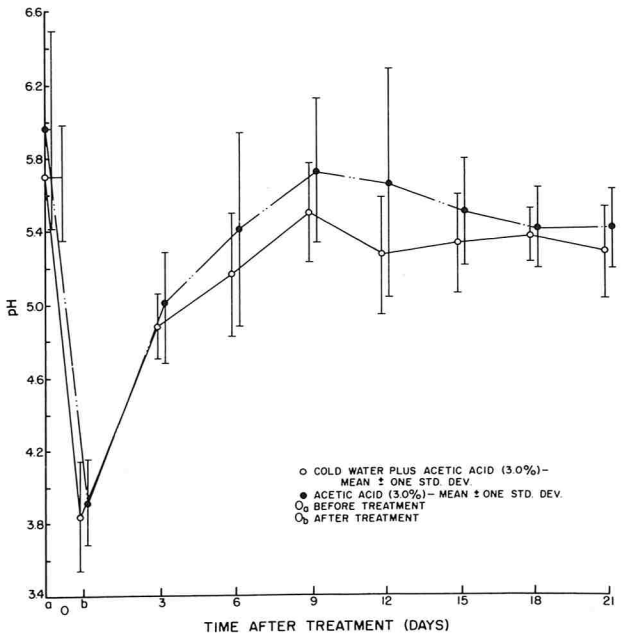

Figure 4. *pH at surfaces during storage at 3.3 C after treatment with acetic acid (3.0%) or cold water plus acetic acid (3.0%).*

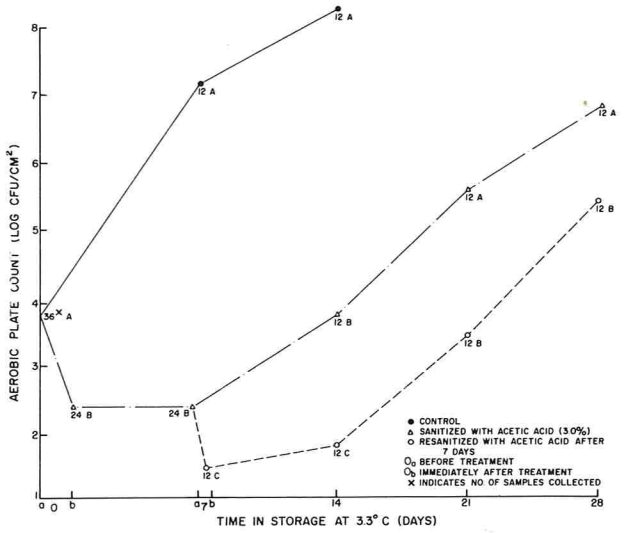

Figure 5. *Changes in aerobic plate counts with time of storage (days) at 3.3 C. Means with different letters (a-c) for each time in storage differed significantly ($P \leq 0.05$).*

samples, and they remained that way for the remaining 14 days of storage. Counts of controls exceeded 10^8 after 2 weeks of storage.

We concluded from these experiments, based on aerobic plate counts as the indicator of shelf life, that application of 3% acetic acid (as described) increased shelf life of refrigerated meat by 18 to 21 days. However, when microbial spoilage of meat is delayed, other defects may become evident. We did not test the meat for other defects. Re-sanitization provided about 7 days additional storage time.

No effort was made to evaluate decoloration or the possibility of odors arising from the use of acetic acid.

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Salmonellosis, *Salmonella* Gastroenteritis Oubreaks Associated with Dry Milk, Milk

(excerpts from the Center for Disease Control's "Morbidity and Mortality Weekly." March 16 and March 23, 1979 issues)

A brand of nonfat powdered milk was implicated in one symptomatic and two asymptomatic cases of *Salmonella* infections in Oregon early this year. The implicated lots of milk were recalled voluntarily.

In the symptomatic case, a 14-month-old child developed acute illness involving diarrhea and fever on January 15, 1979. Stool cultures from the child showed *S. agona* and *S. typhimurium*. Cultures of an already open box of nonfat powdered milk from the patient's home showed the same organisms. Stool cultures of the child's family yielded *S. typhimurium* and *S. agona* infections in a asymptomatic 3-year-old sibling.

A laboratory worker, aware of the first case, who had consumed the same brand of nonfat powdered milk in his home, submitted stool cultures from himself and his wife. His wife had not consumed the nonfat powdered milk. His culture was positive for *S. typhimurium* and *S. agona* while his wife's was negative. *S. agona* and *S. typhimurium* organisms were isolated from the box of powdered milk from their home.

The Oregon Department of Human Resources and local health departments then conducted a phone survey of persons in Oregon who had had salmonellosis caused by *S. typhimurium* or *S. agona* after June, 1978. None of these 55 patients had consumed the implicated dry milk.

Investigation by the FDA showed that the lot of milk consumed by the three infected persons was packaged on Oct. 26, 1978 and shipped to Oregon. The state health division released information on the problem Feb. 14, 1979 and lots of the implicated brand packaged between May 1 and Nov. 1, 1978 were voluntarily recalled.

Cultures taken by the FDA of the only six available unopened packages of nonfat milk from the same lot as that consumed by the infected persons were negative for *Salmonella* organisms.

A different milk-related outbreak occurred in Arizona last October as *Salmonella typhimurium* var *copenhagen* gastroenteritis was linked to a commercial milk supplier. The outbreak occurred Oct. 2-16, 1978 in two northern Arizona cities 60 miles apart.

The increase in cases related to the organism was detected in mid-October. Following public announcement and contact with physicians and labs, 66 primary cases of diarrhea were identified. Fifteen patients were hospitalized. Cultures of stools from 23 patients resulted in *S. typhimurium* var *copenhagen*, one yielded *S. anatum*, one *S. oranienburg*, one salmonellae that were not typed, 20 were negative, and 20 were not cultured. Only one additional isolate of *S. typhimurium* var *copenhagen* was identified from other parts of Arizona in the same month.

Demographic data and food histories by means of two questionnaire surveys conducted Oct. 17-26 to persons known to have had diarrhea produced 23 confirmed cases and 23 controls. In addition to diarrhea, the patients reported the following: fever (91%), abdominal pain (87%), nausea (57%) and vomiting (52%).

One questionnaire produced data that showed 83% of the patients and 30% of the controls had consumed one brand of milk.

A second questionnaire asked about the use of food items the month before the illness. The same brand of milk implicated in the first questionnaire was significantly associated with the illness as 96% of the patients but only 48% of the controls had drunk the milk. None of the other food items, including seven other brands of milk, showed significant differences between cases and controls.

A review of routine samples submitted to the state laboratory on October 3 from the dairy whose milk was implicated showed a sample of pasteurized whole milk with a coliform count of 230 colonies per ml. The accepted coliform count in Grade A pasteurized milk is 10 colonies per ml. The absence of phosphatase in the sample indicated adequate pasteurization. (Phosphatase, an enzyme normally present in raw milk, is inactivated by high temperatures used in the process). All pasteurized samples taken on Oct. 16 were free of coliforms. One sample of raw milk yielded *S. typhimurium* var *copenhagen*. Stool specimens from all dairy employees were negative and there were no coliform organisms found in the water from the well which supplied the plant.

Control measures included the recall of all milk produced in the plant before Oct. 16 and biweekly culturing of samples of the dairy's pasteurized milk products.

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Technique for In-vitro Evaluation of Release of Antibiotics into Milk from Carrier Vehicle[1]

I. A. SCHIPPER,[2]* R. N. REOPELLE[3] and L. J. SCHERMEISTER[3]

Department of Veterinary Medicine and College of Pharmacy, North Dakota State University, Fargo, North Dakota 58105

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ABSTRACT

An apparatus to determine the rate of release of chemotherapeutics from vehicles was developed. It consists of a semi-permeable chamber within a larger glass chamber. The semi-permeable chamber has direct contact with milk within the glass chamber. Preliminary evaluation for rapidity of release of chemotherapeutics by vehicles, in decreasing amount of antibiotic released, showed them to be water, sesame oil, sweet cream butter, peanut oil with aluminum monostearate, lanolin and petrolatum.

The efficaciousness of bovine intramammary mastitis therapy depends on the rapidity and completeness of release of the chemotherapeutic agent from the vehicle (*2,6,9,15,19,29*). Factors such as chemotherapeutic dosage and frequency of administration, route of administration, stage of lactation, degree of inflammation, frequency of milking or nursing and type of vehicle or carrier used may all contribute to the degree of dispersion and longevity of chemotherapeutic materials in the mammary secretions (*3-5,7,8,10,11,13-18,21-30, 32,33,35,37-39*). Intramammary administration of chemotherapeutic materials is one of the primary sources of chemotherapeutic residues in milk. The degree of alveolar tissue penetration is also dependent upon the vehicular binding of chemotherapeutic agent (*7,8,15,34, 35*). Methods of evaluation of chemotherapeutic release from vehicles have been reported (*9*). Use of animal sacrifice and mammary gland analysis have been demonstrated. Though very applicable, this method is also costly (*31,36*).

This investigation was initiated primarily to attempt to establish a practical applicable technique for preliminary determination of release of chemotherapeutics from vehicles used in intramammary medicants and to use several basic vehicular materials as test compounds.

MATERIALS AND METHODS

Testing apparatus

An in-vitro method of using a semi-permeable (6000-8000 MWCO cellulose) membrane within a glass tube was employed for determining chemotherapeutic agent release (Fig. 1). Vehicles to be tested were placed in the dialysing chamber which had previously been placed within a sealed glass tube. The dialysing chamber was then filled with 50 ml of sterile whole milk and sealed on both ends with glass plugs.

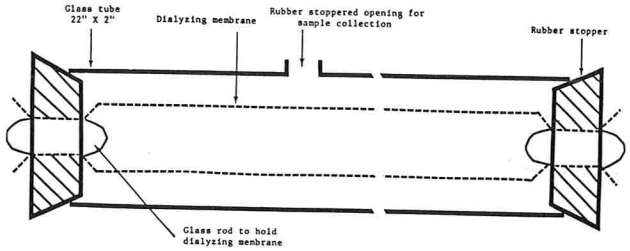

Figure 1. *An outline of a glass tube - semipermeable membrane apparatus for determining the rate of release of antibiotics from various vehicles.*

The glass chamber was filled with 450 ml of sterile milk. The apparatus was placed in an incubator at 37.5 C and on a tilting apparatus which changed position by 45° every 5 min. Samples for antibiotic assay were periodically removed from the glass chamber through a sample collection outlet. A quantity of sterile milk equal to that removed was replaced in a glass chamber to insure complete immersion of the dialysing chamber.

Assay procedure

The samples collected were placed in 15-mm screw-capped vials and frozen until the antibiotic content could be determined. Frozen storage for up to 12 weeks did not affect the accuracy of the antibiotic assay (*12*). For this reason it was convenient to keep the samples frozen for 1 or 2 days before analysis.

All samples were assayed by the cylinder plate bioassay method recommended by the Food and Drug Administration, using petri dishes and the test organism *Sarcina lutea* (ATCC 9341) (*1*). The minimal detectable quantity of antibiotics by the method was 0.066 unit per milliliter.

Vehicles evaluated

The compounds used in this investigation were the basic materials in many commercial vehicles for mastitis chemotherapeutic preparations plus sweet cream butter. They included sterile 0.15 M saline solution (aqueous), lanolin (U.S.P.), peanut oil (U.S.P.) with 3% aluminum monostearate, sesame oil (U.S.P.), sweet cream butter and petrolatum (U.S.P.).

Crystalline oxytetracycline hydrochloride was added to each vehicle at a concentration of 2.5 mg/g of vehicle. Ten grams of vehicle were placed within the semi-permeable sac for each assay. Oxytetracycline was added to the vehicle by homogenization or mixing in a mortar and pestle.

RESULTS

Release of oxytetracycline from all the vehicles studies was determined by an in-vitro method and is represented by Fig. 1. The method employed a cellulose membrane that simulated the cell membranes through which the medication after release from the vehicle must diffuse to penetrate intra- and interalveolar areas of mammary glands.

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[2]*Department of Veterinary Medicine.*
[3]*College of Pharmacy.*

The aqueous vehicle released oxytetracycline in increasing amounts, reaching a maximum at 4 h (Fig. 2). Thereafter, 37 to 43 μg/ml were present in the milk. This amount of oxytetracycline was four times the concentration generally lethal for mastitic organisms (*19*). The amount of free diffusable antibiotic did not increase after a 4-h period.

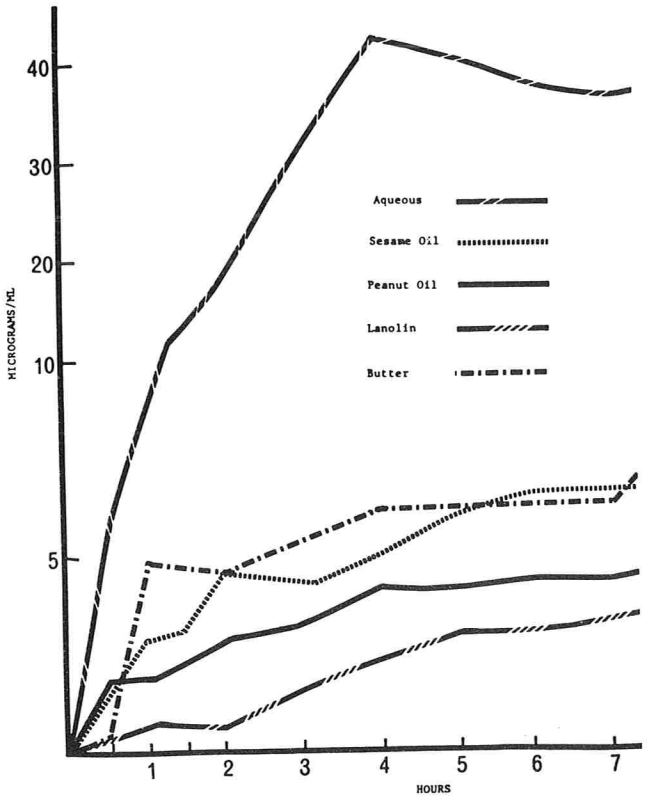

Figure 2. *The rate of release of tetracycline hydrochloride from various vehicles over a seven-hour period as determined by the glass tube - semipermeable membrane apparatus.*

The sesame oil vehicle released the antibiotic at a relatively low constant rate, when compared with the aqueous vehicle, reaching a maximum of 6.7 μg/ml at 8 h. However, the rate of release, when compared to most other vehicles, was comparatively rapid. A slightly different type of release was exhibited by peanut oil, which, after an initial rapid rise within a 30-min period, released the medication at a slowly increasing rate until 4 h had elapsed. No further marked change was detected in the milk up to 9 h. Although peanut oil consistently released the medication at constant rate, the sesame oil released higher therapeutic levels over similar periods. This difference in release could possibly be a result of a gel-like consistency of the peanut oil which was produced by the presence of aluminum monostearate in this oil.

As indicated in Fig. 2, lanolin did not release therapeutic amounts of 3 to 5 μg of antibiotic/ml for 5 h (*19,20*). Thereafter, rapid release occurred up to 9 h after contact with milk.

The possible reason for the initially higher rate of diffusion from sesame and peanut oil when compared to the lanolin ointment is the presence of a greater surface area available for diffusion due to droplet formation of the dispersed sesame or peanut oil in milk.

In addition to the three standard carriers, butter and white petrolatum were tested as possible vehicles. Butter was chosen because it is a naturally occurring fraction of milk and white petrolatum because it is anhydrous. Commercial butter rapidly released the antibiotic within a 1-h period and thereafter the release rate was comparable to that of the sesame oil vehicle. Under the conditions of the experiment, white petrolatum did not release the antibiotic.

DISCUSSION

Based on this investigation, it would appear that the apparatus described provides an inexpensive means of making preliminary evaluations of the rapidity of chemotherapeutic agent release from vehicles.

Evaluation of the common vehicles demonstrated that if rapidity of chemotherapeutic agent release is desired, that the aqueous vehicle would be the one of choice. Previous investigations have demonstrated that the aqueous vehicle provides a more rapid and complete distribution of chemotherapeutic agents under in-vivo evaluation (*31*). Butter offered the advantage of a comparatively rapid release. It also is a natural lacteal secretion and less likely to be toxic to the mammary tissue. Both lanolin and peanut oil with aluminum monostearate provided nearly comparable rates of release. The investigation indicates that petrolatum would not be an acceptable vehicle for intramammary mastitis chemotherapeutic agents, based on antibiotic release.

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Optimum Cultural Conditions for Induction of Temperate Bacteriophages in Lactic Streptococci

K. A. MEISTER and R. A. LEDFORD*

Department of Food Science, Cornell University, Ithaca, New York 14853

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ABSTRACT

Nineteen strains of lactic-group streptococci were examined for lysogeny by treatment with ultraviolet light; eight were inducible. The effectiveness of lysogenic induction with ultraviolet light or mitomycin C depended on the dose of inducing agent, temperature of incubation, and age of the culture. Strain-specific differences in responses to all of these factors were observed; however, in general induction was most effective at temperatures of 30 C or less and when cultures were in mid-exponential growth. No single ultraviolet or mitomycin C dose would induce all eight strains. Cultures which had been frozen and thawed were generally not inducible immediately after thawing. This was attributed to inhibition of bacterial growth by freezing. Cultures which had been chilled without freezing remained inducible. Lysogenic induction was not affected by either calcium or phosphate.

Lysogeny in lactic streptococci has been demonstrated by several workers (*4,6,8,10,14*) and commercial dairy starter cultures used in the United States have been shown to contain lysogenic bacterial strains (*12*). Although the incidence of lysogeny in lactic streptococci is believed to be widespread (*4*), its significance in starter culture technology is unclear (*7*). Lysogenic starter strains may act as a reservoir of phages (*2*) and a means by which phage are introduced into cheese plants. It has therefore been suggested that lysogenic starter strains should be avoided (*10*). However, the high frequency of such strains may make their avoidance impractical.

Since bacteriophages released by lysogenic bacteria may be able to infect other lactic streptococcal strains, screening for lysogenic strains and for their indicator strains would be useful in selection of cultures to be used in starter rotations. However, little is known about conditions that favor or prevent detection of lysogeny in lactic streptococci. A variety of doses of inducing agents, media, incubation temperatures, and other experimental conditions have been used in tests for lysogenic induction in lactic streptococci, and widely varying frequencies of lysogeny, from 8% (*6*) to 60% (*4*), have been reported in studies in which large numbers of strains were screened. Some of this variation may have been due to differences in inducibility of temperate phages under the different conditions used.

Park and McKay (*12*) observed that the optimum dose of ultraviolet light or mitomycin C for prophage induction varied among strains of lactic streptococci. In other bacterial genera, factors such as temperature (*17,18,19*), stage of growth (*3,9,19*), and cell damage (*1*) have been shown to affect the inducibility of lysogenic phages. In this study, the effects of several factors, including dose of inducing agent, temperature, culture age, injury by chilling or freezing, and addition of calcium or phosphate, on prophage induction in lactic streptococci were examined.

MATERIALS AND METHODS

Cultures

Streptococcus lactis strains N12, N17, N27, N58, T12, and 1-3 and *Streptococcus cremoris* strains 1-4, 1-10, 1-14, 1-22, 2-16, 2-38, 1A-2, and 29W were obtained from a cheese manufacturer. *S. lactis* strains C2, C10, M18, and ML3 were obtained from Dr. L. L. McKay, University of Minnesota. *S. cremoris* strain 1-S was obtained from Dr. W. E. Sandine, Oregon State University. Species identifications were confirmed by the method of Reddy et al. (*13*). Cultures were maintained by weekly transfer of a 2% inoculum into 10% litmus milk (BBL); after incubation for 48 h at 30 C, they were stored at 4 C. Reserve stocks were maintained in frozen storage at - 12 C. Cultures were transferred twice in M17 broth (*15*) before use in phage induction experiments.

Phage induction

Ultraviolet (UV) induction was performed by a method adapted from that of Huggins and Sandine (*4*). Active overnight M17 broth cultures were transferred into 10 ml of M17 broth at the 2% level and preincubated at 30 C for 1 to 2.5 h. The cultures were then transferred to sterile polypropylene centrifuge tubes, centrifuged at $5000 \times g$ for 10 min at room temperature, and resuspended in 5 ml of sterile 0.1 M $MgSO_4$. They were then transferred to sterile petri dishes (glass) and irradiated for a timed period with constant swirling (120 rpm). UV exposures of 5, 10, 15, 20, 30, and 40 sec were used. The UV source was a pair of General Electric G15T8 15-watt lamps at a distance of 16 cm from the plates. After irradiation, cultures were transferred to sterile unscratched 15 × 100-mm screw-capped tubes containing 5 ml of double-strength M17 broth, and incubated at 30 C. Turbidity readings were taken every 30 min for 5 h at 600 nm using a Bausch and Lomb Spectronic 710 spectrophotometer. Absorbance was read directly against a tube of uninoculated M17 broth as a blank.

Induction with mitomycin C (MC) (Sigma) was carried out by a similar procedure. Cultures were preincubated and centrifuged as described above, and then resuspended in 5 ml of a solution of twice the desired concentration of MC in 0.1 M $MgSO_4$ and immediately transferred to a tube containing 5 ml of double strength M17 broth. MC doses of 0.4, 1.0, 1.4, 2.0, 3.0, 4.0, 5.0, and 6.0 μg/ml were used. Incubation was continued as described above for UV induction.

Confirmation of lysogeny

All of the UV- or MC-inducible bacterial strains in this study were shown to be resistant to superinfection by their own temperate phages. Neither lysis of broth cultures to which phages were added nor plaque production on the original host strain could be obtained. All inducible strains were shown to remain inducible after repeated purification by streaking on M17 agar (*15*) plates and subculturing of isolated colonies in M17 broth. Since phage-carrying cultures of lactic streptococci can be freed of their phages by streaking and subculturing (*5*), while

lysogenic cultures can not, it was concluded that the inducible strains were true lysogens.

RESULTS

Two of the stock strains (*S. lactis* C2 and C10) were known to be lysogenic (*4*). Six other strains, *S. lactis* M18, N27, T12, and 1-3, and *S. cremoris* 1-4 and 1-10, were also shown to lyse after UV treatment.

Cultures of each of the eight UV-inducible strains were exposed to UV for six different time periods, and the effects on turbidity during subsequent incubation at 30 C were observed. The dose of UV was critical for phage induction (Table 1). Different bacterial strains showed different optimum UV doses for induction and no single UV dose would induce all eight strains. Other characteristic differences in responses of different strains to UV were observed. Both the length of the latent period between UV treatment and lysis and the extent of lysis after UV treatment varied among strains. Since there is a linear relationship between absorbance at 600 nm and cell counts of lactic streptococcal cultures within the absorbance range 0.1 to 1.0, the proportion of cells lysed by the various inducing treatments could be calculated. In some strains (*S. lactis* C2, T12 and 1-3) the proportion of cells lysed characteristically exceeded 90%, while in others (*S. lactis* C10 and *S. cremoris* 1-4 and 1-10) no UV dose would lyse more than 25% of the cells.

The effectiveness of lysogenic induction by MC was also dose-dependent, and the optimum dose varied among strains (Table 1). One strain, *S. lactis* M18, was not MC-inducible under the conditions studied. The strain-specific differences in UV and MC induction were highly reproducible; very similar results were obtained in two sets of experiments done 7 months apart.

Lysogenic induction was strongly affected by temperature (Table 2). At 40 C, no strain could be induced, although the *S. lactis* strains grew well at this temperature. At 37 C, several strains failed to lyse. In general, lysogenic induction was more effective at relatively low temperatures; however, a few strains lysed more effectively at 30 than at 22 C. *S. lactis* M18, the strain mentioned above which was not MC-inducible at 30 C, was MC-inducible at 22 C; it was UV-inducible at both temperatures. This was one of several strains for which the effects of temperature on UV induction and MC induction differed. The temperature of incubation before treatment with the inducing agent did not affect induction in these experiments. The experiments summarized in Table 2 were performed on cultures which had been incubated at 30 C until treated with the inducing agent. When similar experiments were done with cultures incubated at 22 or 37 C before treatment, very similar results were obtained.

The age of the culture at the time of treatment with the inducing agent also affected inducibility. Normally, cultures were induced during mid-exponential phase,

TABLE 1. *Responses of eight strains of lactic streptococci to UV- and MC-induction at 30 C.*

Strain	Minimum length of latent period (min)[a]	Extent of lysis by UV[b]	Optimum UV exposure (sec)	Range of effective UV exposures (sec)	Extent of lysis by MC[b]	Optimum MC dose (μg/ml)	Range of effective MC doses (μg/ml)
S. lactis T12	120	+++	15	5-30	+++	1.0	0.4-3.0
S. lactis 1-3	90	+++	20	15-30	+++	4.0	3.0-6.0
S. lactis C2	90	+++	15	10-40	+++	1.0	0.4-3.0
S. lactis M18	120	++	10	5-10	–		
S. lactis N27	60	++	5	5-10	++	2.0	1.4-3.0
S. lactis C10	120	+	15	15-20	+	5.0	4.0-5.0
S. cremoris 1-4	120	+	15	10-15	+++	1.4	1.4-2.0
S. cremoris 1-10	120	+	10	5-15	++	1.4	1.0-2.0

[a]Latent period = time between UV or MC treatment and the start of the decrease in culture turbidity.
[b]Extent of lysis: +++ = > 70%, ++ = 40-70%, and + = < 40%.

TABLE 2. *Extent of lysis of eight lactic streptococcal cultures after UV or MC treatment and incubation at four temperatures.*

Strain	Inducing agent	Temperature (C) 22	30	37	40
S. lactis T12	UV	++[a]	+++	–	–
	MC	+	+++	+++	–
S. lactis C2	UV	++	+++	++	–
	MC	+++	+++	+++	–
S. lactis 1-3	UV	+++	+++	+	–
	MC	+++	+++	–	–
S. lactis C10	UV	++	+	–	–
	MC	+	+	–	–
S. lactis M18	UV	+++	++	+	–
	MC	++	–	–	–
S. lactis N27	UV	++	++	–	–
	MC	++	++	–	–
S. cremoris 1-4	UV	++	+	–	–
	MC	++	+++	–	–
S. cremoris 1-10	UV	+	+	+	–
	MC	+	++	+	–

[a] +++ = > 70%, ++ = 40-70%, and + = < 40%.

after 1 to 2.5 h of growth. When cultures were grown for longer periods, particularly when they had reached stationary phase, (4 h or later) they became less inducible. The maximum age at which cultures could be induced varied among strains and with the inducing agent used (Table 3). Cultures generally remained MC-inducible for longer than they were UV-inducible. This may have been due to cultures growing into exponential phase after addition of the inducing agent, and then being induced by MC still present in the medium. In some strains induction was more effective in mid-exponential phase than in the earliest stages of growth. One strain, *S. cremoris* 1-10, was not inducible during the first hour of growth; in several other strains, the fraction of cells lysed by UV or MC in 1-h cultures was less than that in 2- or 3-h cultures.

TABLE 3. *Maximum culture ages for UV- and MC-induction of lysis at 30 C.*

Strain	Oldest UV-inducible culture (h)	Oldest MC-inducible culture (h)
S. lactis T12	11	11
S. lactis C2	8	48
S. lactis N27	4	18
S. lactis 1-3	4	24
S. lactis C10	3	5
S. lactis M18	3	4 (at 22 C)
S. cremoris 1-10	5	5
S. cremoris 1-4	not examined	4

Since dairy starter cultures are usually stored and transported in the frozen state, the effects of chilling and freezing on lactic streptococcal temperate phages may be of practical importance. Cultures which were chilled to 4 C and held at that temperature for 2 h before treatment with an inducing agent could be induced successfully. However, with one exception, cultures which had been frozen either in a -12 C freezer or by immersion in liquid nitrogen, held for 2 h at -12 C, and then thawed by immersion in water at 25 C could not be induced immediately after thawing. The exception, *S. lactis* T12, which was both UV- and MC-inducible after freezing and thawing, also appeared to be unusually resistant to cell injury by freezing; uninduced cultures of this strain grew rapidly and with little delay after thawing, while cultures of the other strains grew slowly and showed long lag periods after thawing (Fig. 1).

Addition of calcium (0.1 ml of 1.0 M $CaCl_2$ in 10 ml of M17 broth) did not alter the effect of UV on the lactic streptococcal strains studied. Although Ca^{++} is known to be required for virulent phage infection in lactic streptococci (*7*), it did not appear to be required for phage induction. Induction of all eight inducible strains was indifferent to $CaCl_2$. Also, none of the other 11 strains became inducible when $CaCl_2$ was included in the medium. Similarly, the presence of phosphate, which binds Ca^{++}, did not affect phage induction. Phage induction was equally effective in M17 broth and in a variant medium in which a pH 6.8 phosphate buffer (0.2 M) had been substituted for β-glycerophosphate.

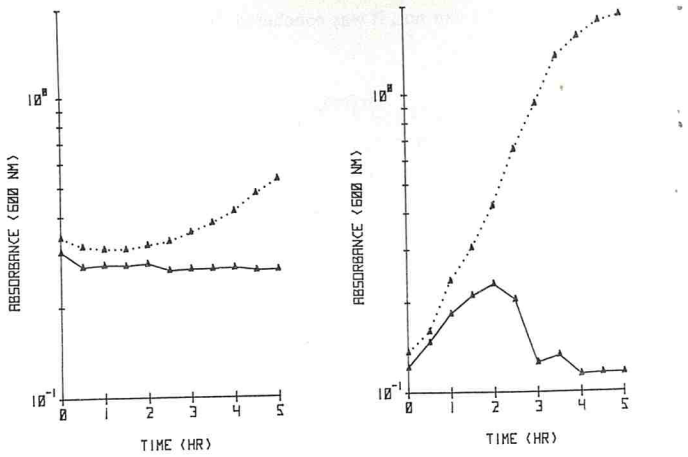

Figure 1. *Effects of freezing/thawing and UV irradiation on culture turbidity.*
Left - S. lactis *1-3: — Frozen, thawed, irradiated. Culture did not induce. ..... Frozen, thawed, not irradiated. Control culture showed delayed growth, indicating injury.*
Right - S. lactis *N27: — Frozen, thawed, irradiated. Culture showed induced lysis. ..... Frozen, thawed, not irradiated. Control culture showed prompt, rapid growth, indicating no injury.*

DISCUSSION

The need to screen starter culture strains for lysogeny has been pointed out in a recent review (*7*). The data presented in this study indicate that, for most effective detection of inducible strains, it is necessary to use a range of doses of the inducing agent, since no one UV- or MC-dose could induce all strains. Also, screening should be carried out at a temperature no greater than 30 C and cultures should be induced in the mid-exponential phase of growth.

In a recent extensive study of lactic streptococcal temperate phages, Huggins and Sandine (*4*) showed that there was considerable morphological diversity among the phages released by different strains. Our data show that lysogenic host/phage systems in lactic streptococci also differ physiologically, and that the responses that they exhibit are stable for a particular host/phage combination over time. The strain-specific differences that we have observed could be attributable to genetic differences among the temperate phage strains, to genetic or adaptive differences among the bacterial strains, or to some feature of the phage-host interaction. Further research will be needed to identify the specific components of the lysogenic system that are responsible for these differences. It is possible that such factors as temperature-sensitivity and dose-responses may be of use in the eventual development of a classification scheme for lactic streptococcal temperate phages and in the identification of related phages carried by different bacterial strains.

Failure of lysogenic induction at 40 C, a temperature which supports growth of *S. lactis*, and failure of induction at 37 C in some strains suggest that some step in the phage-induction process may be inactivated at high temperatures. However, this would not explain why some strains were induced more effectively at 30 than at

22 C. Once phage induction has started, multiplication of the phage is directed by phage genes, and occurs in the same way regardless of the nature of the inducing agent. Therefore, the observation that temperature affects UV- and MC-induction differently suggests that one of the temperature-sensitive steps in induction must be a very early one, that occurs when the inducing agent is initiating the induction process. Evidently, the physiological bases for the effects of temperature on prophage induction in the lactic streptococci are complex, and multiple effects may be present in some strains.

In several bacterial genera, including *Bacillus* (*9*), *Micrococcus* (*3*), and *Lactobacillus* (*19*), it has been shown that exponentially growing cultures are more effectively induced by UV or MC than stationary-phase cultures. Our observations show that the situation is the same in the lactic streptococci. In general, it is believed that induction is most effective when cultures are growing rapidly in a medium that is well supplied with nutrients and are not deprived of nutrients or otherwise injured (*1*). Cultures which are exposed to conditions that injure the cells and delay their growth are less inducible. The inhibition of induction that we observed after freezing and thawing appeared to fit this pattern; the one strain which could be induced after freezing and thawing was also capable of rapid growth; it did not appear to have been injured by freezing.

In *Lactobacillus*, it has been shown that Ca^{++} is required for penetration of phage DNA into the host cells, but not for the multiplication of phage within the cells (*16*). The basis for the Ca^{++} requirement for lactic streptococcal virulent phage growth is believed to be similar (*7*). Since temperate phages are already inside the cell when they are induced, it would be expected that induction would not require Ca^{++}. Our data, which show that induction is indifferent to both Ca^{++} and phosphate, support this hypothesis. Similarly, Huggins and Sandine (*4*) have shown that lysogenic induction in lactic streptococci can occur in commercial phage-inhibitory media, in which Ca^{++} availability has been reduced by the addition of phosphate.

It is not yet known whether dairy starter cultures are exposed to conditions that induce prophages. However, it is known that the prophages carried by some lactic streptococcal strains are spontaneously inducible (*4,6,8*) and that spontaneous induction can occur when such strains are grown in milk (*4*). Since the study of spontaneous induction requires indicator strains, which are rare in lactic streptococci (*7*), relatively little is known about spontaneous induction or about the effects of environmental conditions on it. In our laboratory, we have identified an indicator strain for the phage released by *S. lactis* N27, and, using the indicator, we have found that this phage is spontaneously inducible at a high rate, yielding titers of 10^6 plaque-forming units/ml in uninduced cultures (*11*). Preliminary data suggest that the cultural requirements for spontaneous induction in this strain may not be the same as those required for UV- or MC-induction. For instance, this phage is spontaneously inducible, but not UV- or MC-inducible, at 37 C. Since spontaneous induction decreases the number of active cells in a starter culture, further investigations to determine what conditions permit or prevent spontaneous induction could be of practical value in starter technology.

Since indicator strains are known for only two of our eight temperate phages [those related by *S. lactis* N27 (*11*) and C2 (*4*)] and since phage particles have been observed in lysates of only two of our eight inducible bacterial strains [*S. lactis* C2 and C10 (*4*)], it is possible that the inducible particles released by some of the other strains might be defective bacteriophages or bacteriocins rather than intact, infectious bacteriophages. However, our data do not show any clear differences between the induction responses of the three bacterial strains known to release complete phage particles and the other five inducible strains.

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Attachment of Microorganisms to Pork Skin and Surfaces of Beef and Lamb Carcasses

J. L. BUTLER, J. C. STEWART, C. VANDERZANT*, Z. L. CARPENTER and G. C. SMITH

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

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ABSTRACT

A model system was developed to study attachment to and possibly detachment of bacteria from pork skin and thin-surface slices of beef and lamb carcasses. The technique involves embedding pork skin and beef and lamb surfaces in solidified wax with the skin surface exposed. After exposure of the skin or carcass surface to bacterial suspensions and subsequent manipulations, the sample is removed aseptically from the wax and subjected to agar plate counting methods. A direct relationship existed between bacterial counts of the skin or carcass surface and concentration of bacterial cells in the attachment medium. Much of the bacterial attachment occurred during the first minute of immersion in the attachment medium, although in some instances continued attachment occurred over a 30-min period. Gram-negative motile bacteria showed greater attachment than did gram-positive non-motile species. Temperature and pH of the attachment medium had little effect on the extent of bacterial attachment.

Determinations of the numbers and types of microorganisms on the surface of meat carcasses are important from the standpoint of public health, for judging effectiveness of sanitary handling during processing and for estimating quality characteristics including shelf-life. Numerous procedures are available for determining microbial populations on the surface of meat carcasses. For specific information on these procedures as applied to meat and poultry, reviews by Favero et al. (*6*), Patterson (*17*), Barnes et al. (*3*), Kitchell et al. (*8*) and Baldock (*2*) should be consulted. Before selecting a particular test procedure, the advantages and limitations of the test should be carefully evaluated. This applies particularly to the nature of the sample and the objectives of the procedure. In most instances, little information is available regarding the effectiveness of the method in removing microorganisms from the meat surface. Some techniques, such as certain swabbing procedures, remove only about 10% of the microbial population, as compared with skin or surface maceration techniques (*11*).

Different test procedures exert different forces upon the surface of meats and thus most likely remove microorganisms at different rates. In addition, removal and subsequent recovery of microorganisms from meat and poultry surfaces by any of the test procedures will depend upon the forces by which the microorganisms are held in or on the meat. An understanding of this variable requires information about the mechanism of bacterial attachment on and detachment of bacteria from meat surfaces.

The sorption of microorganisms to surfaces is a widespread phenomenon in nature but information about the mechanisms involved is limited. In the marine environment, for example, microorganisms often form a primary film on surfaces. This film is frequently a prerequisite for attachment of an invertebrate fouling population. Marshall et al. (*10*) reported that the "reversible sorption" (weakly held near the surface) of *Achromobacter* on glass cover slips depended upon the electrolyte concentration in artificial seawater. They suggested that this sorption is associated with the London-van der Waals attractive energies between two surfaces (surface and bacterium) and the electrical repulsive energies resulting from overlapping ionic atmospheres around the surfaces. "Irreversible sorption" (firmer adhesion to surface) through formation of viscous polymers (polymeric bridging) by bacteria may overcome the repulsion barrier between such surfaces. Fletcher and Floodgate (*7*) demonstrated an acidic polysaccharide layer on a marine bacterium which was involved in its adhesion to surfaces. Bacteria which had attached to a surface produced a secondary fibrous acidic polysaccharide. Deinema and Zevenhuizen (*5*) reported exocellular cellulose fibrils on many gram-negative bacteria. In addition, chemotaxis (*1*) may also be involved in attachment and detachment of bacteria. Chet et al. (*4*) reported that organic compounds such as acrylamide, benzoic acid and tannic acid, in concentrations not toxic to bacteria, were capable of repelling motile bacteria from marine surfaces. Detailed information on the behavior of bacteria in solid-liquid interfaces has been presented by Marshall (*9*).

A better understanding of the forces involved in attachment on and detachment of microorganisms from the surface of meat carcasses would aid in selection of an analytical procedure for estimating numbers and types of bacteria on meat surfaces. In addition, this information may be useful in designing techniques to reduce numbers of bacteria on the meat surface, to maintain them at reduced levels which could improve shelf-life and possibly reduce public health hazards. This study reports on the development of a model system to examine bacterial attachment to and detachment from meat surfaces and on the effects of various environmental conditions on attachment.

MATERIALS AND METHODS

Bacterial cultures

Escherichia coli, Pseudomonas putrefaciens, Erwinia herbicola, a *Lactobacillus* sp. and a *Staphylococcus* sp. were used. These cultures were isolated from raw beef in previous experiments. They were maintained on trypticase soy agar (TSA, BBL) slants at 25 C. Inocula for the individual studies were obtained as follows: A loopful of *E. coli* from a 24-h-old TSA slant was placed in each of two tubes with brain heart infusion (BHI, BBL). The BHI tubes were incubated for 24 h at 25 C. After centrifugation (20 min, $1000 \times g$, at 10 C) the pellets were resuspended in 20 ml of sterile attachment medium (*12*) consisting of 8.7 g of NaCl/liter, 0.0062 M Na_2HPO_4, 0.0021 M NaH_2PO_4 and 0.001 M EDTA, adjusted to pH 7.0. This yielded a concentration of about 10^8 cells per ml. A similar procedure was used for *P. putrefaciens* and *E. herbicola*. To prepare the inoculum for the *Lactobacillus* sp., the growth of two 24-h-old TSA slants was removed by washing with 10 ml of lactobacilli MRS broth (MRS, Difco). The MRS tubes were incubated for 24 h at 25 C. Following centrifugation (20 min, $1000 \times g$, at 10 C) the pellets were resuspended in 20 ml of attachment medium. This yielded a cell concentration of about 10^9 cells per ml. Preparation of the inocula with the *Staphylococcus* sp. was as described for the *Lactobacillus* sp. except for the use of BHI broth in place of MRS broth.

Preparation of skin samples

Skin samples were obtained from 100- to 110-kg (live weight) Yorkshire barrows processed according to normal slaughter procedures in the Texas A&M University Meats Laboratory. Animals were electrically stunned, exsanguinated, scalded for 4-8 min in 60-63-C water, mechanically dehaired, further dehaired with bell scrapers and shaved to remove residual hair, singed and washed with cool (25 C) water. Before evisceration and splitting of carcasses, a strip of skin (20-cm wide and 50-cm long) was removed from the midline of the back of the carcass approximating an anatomical location from a point adjacent to the third thoracic vertebra to the last lumbar vertebra. The skin was allowed to cool to room temperature (20-25 C). The skin was cut aseptically into pieces ($3.2 \times 3.2 \times 0.5$ cm), placed in small plastic dishes (50-mm diameter) and embedded in liquid wax (Gulf wax) except for the skin surface. A piece of sterile string was partially embedded in the wax. After hardening of the wax, the skin sample held by the string was immersed for 3 min in 180 ml of sterile attachment medium in a 400-ml beaker. After drainage for 10 sec, the skin samples were dipped in attachment medium (200 ml) containing *E. coli, P. putrefaciens, E. herbicola, Lactobacillus* sp. or *Staphylococcus* sp. Concentration of the bacterial cells in the attachment medium ranged from about 10^2-10^8 cells per ml. Appropriate dilutions of the concentrated inoculum were made with sterile attachment medium. Attachment times ranged from 1 to 30 min, temperature of the attachment medium ranged from 2.5 to 37 C, and pH of the attachment medium ranged from 4 to 8.8. After drainage for 10 sec, the skin samples were rinsed by immersion for 1 min in 180 ml of sterile 0.1% peptone.

Preparation of beef and lamb samples

Samples of cutaneous trunci muscle were taken from 450- to 510-kg (liveweight) Brahman and Brahman crossbred steers. After exsanguination, hide removal, evisceration and splitting, the carcasses were washed with water (25 C). A strip of muscle (approximately 20-cm wide and 60-cm long) then was removed from one side of the carcass beginning at a point approximately 5 cm anterior of the posterior end of the cutaneous trunci in the region of the flank and continuing forward across the flank and wholesale rib. Samples of cutaneous trunci muscle were also obtained from 45- to 50-kg (liveweight) crossbred wethers. After exsanguination, pelt removal and evisceration, the carcasses were washed with water (25 C). A strip of muscle (approximately 15-cm wide and 40-cm long) then was removed from each side of the carcass beginning at a point approximately 2.5 cm from the posterior end of the cutaneous trunci in the region of the flank and continuing to a point just posterior to the shoulder. Beef and lamb samples were handled (cut, embedded in wax, inoculated, etc.) as described for pork skin.

Determination of bacterial counts

The skin or muscle sample was removed from the wax with sterile forceps and placed in a plastic bag with 100 ml of sterile 0.1% peptone. Agitation was carried out for 1 min in a Stomacher - 400. Counts of appropriate dilutions of inoculated and non-inoculated samples were determined as follows: *E. coli* on violet red bile agar (VRB, Difco) with plate incubation for 24 h at 35 C; *P. putrefaciens* on peptone iron agar (PIA, Difco) with plate incubation for 48-72 h at 25 C; *E. herbicola* on eosin methylene blue agar (EMB, Difco) with plate incubation for 48-72 h at 25 C; *Lactobacillus* sp. on MRS broth plus 1.5% agar with plate incubation for 48-72 h at 25 C; and *Staphylococcus* sp. on Staphylococcus 110 agar (S110, Difco) with plate incubation for 72-96 h at 35 C.

RESULTS AND DISCUSSION

Considerable time and effort was devoted to development of a model system to study attachment of bacteria to skin and meat surfaces. After extensive experimentation, the method described in the Materials and Methods section was chosen because results were reproducible. In the experiments with pork skin, five test organisms (*E. coli, P. putrefaciens, E. herbicola,* a *Lactobacillus* sp. and a *Staphylococcus* sp.) were used. With beef and lamb samples only *P. putrefaciens* and the *Lactobacillus* sp. were used. An examination of the effects of bacterial concentration, time of immersion in inoculated attachment medium, temperature and pH of inoculated attachment medium on attachment revealed only minor differences between the three types of samples (pork skin, beef and lamb muscle). For this reason, only results pertaining to attachment on pork skin will be presented here.

Effect of concentration of bacteria in attachment medium on attachment

In the first series of experiments pork skin was immersed in attachment medium containing *E. coli, P. putrefaciens, E. herbicola, Lactobacillus* or *Staphylococcus* sp. Attachment was carried out for 10 min at 25 C with the attachment medium adjusted to pH 7.0. The concentration of the test organisms in the attachment medium ranged from about 10^2 to 10^8 viable cells per ml. Data for *P. putrefaciens* and the *Lactobacillus* sp. are presented in Fig. 1 and 2. The results indicate increased attachment of bacteria onto pork skin as the concentration of these test organisms in the attachment medium was increased. With concentration of the test organisms ranging from 10^7 to 10^8 cells per ml, attachment ranged from about 10^4 to 10^5 cells per cm^2 of skin. At lower concentration of the test organisms (10^3-10^4 per ml) attachment ranged from 10^1 to 10^2 per cm^2 of skin. Differences in attachment between test organisms were noted at the higher cell concentrations. At cell concentrations ranging from about 10^5 to 10^8 per ml, attachment of *P. putrefaciens* appeared slightly greater than that of *E. coli* and *E. herbicola* but definitely greater than that of the *Lactobacillus* or *Staphylococcus* sp. The *Lactobacillus* or *Staphylococcus* sp. exhibited the least attachment among the test

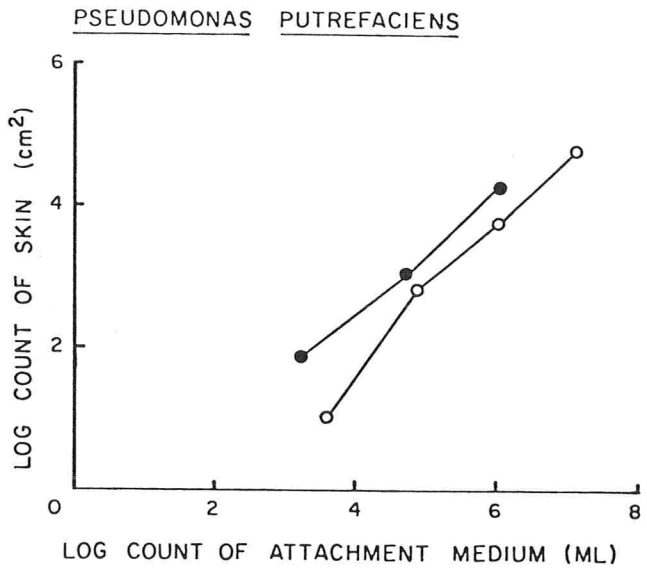

Figure 1. *Effect of the concentration of* Pseudomonas putrefaciens *on attachment to pork skin. Attachment was carried out for 10 min at 25 C with the attachment medium adjusted to pH 7.0 (two trials).*

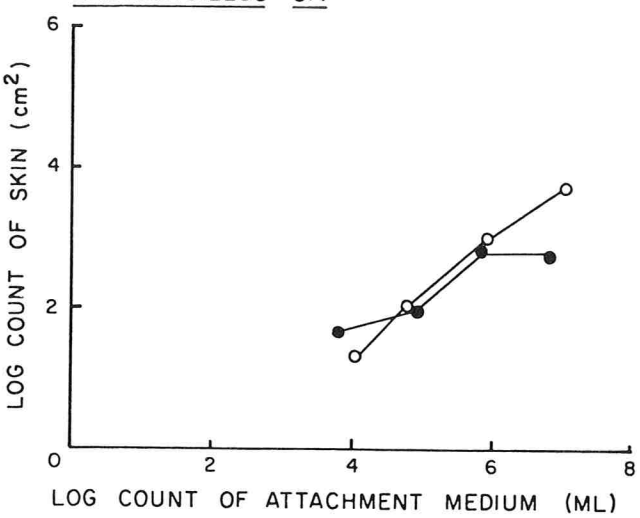

Figure 2. *Effect of the concentration of* Lactobacillus *sp. on attachment to pork skin. Attachment was carried out for 10 min at 25 C with the attachment medium adjusted to pH 7.0 (two trials).*

organisms. At the lower cell concentrations (10^2-10^5 per ml) in the attachment medium, differences in attachment between test organisms were not conclusive.

Effect of immersion time in attachment medium on attachment

In this series of experiments, attachment of five bacterial cultures on pork skin was carried out by immersion in inoculated attachment medium adjusted to 25 C and pH 7.0 for 1-30 min. Cell concentrations of the attachment medium were 10^3 and 10^6-10^7 per ml. Data for *P. putrefaciens* and the *Lactobacillus* sp. are presented in Fig. 3 and 4. With the *Lactobacillus* and *Staphylococcus* spp., little increase in attachment occurred after 1 min of immersion in the attachment medium. With *E. coli, P. putrefaciens* and *E. herbicola* increases in attachment were noted between 1-30 min of immersion in the

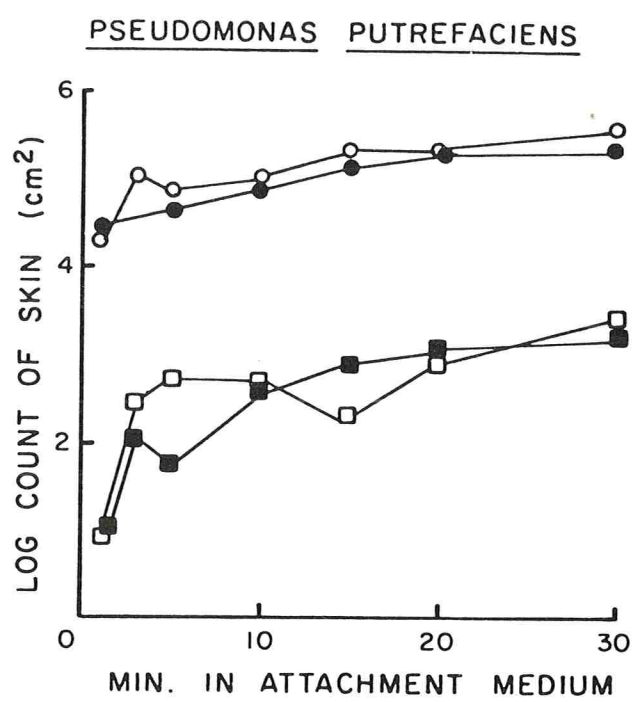

Figure 3. *Effect of immersion time in attachment medium on attachment of* Pseudomonas putrefaciens *to pork skin. Attachment was carried out at 25 C with a cell concentration of approximately 10^7 per ml (○, ●) and 10^3 per ml (□, ■) with the attachment medium adjusted to pH 7.0 (two trials).*

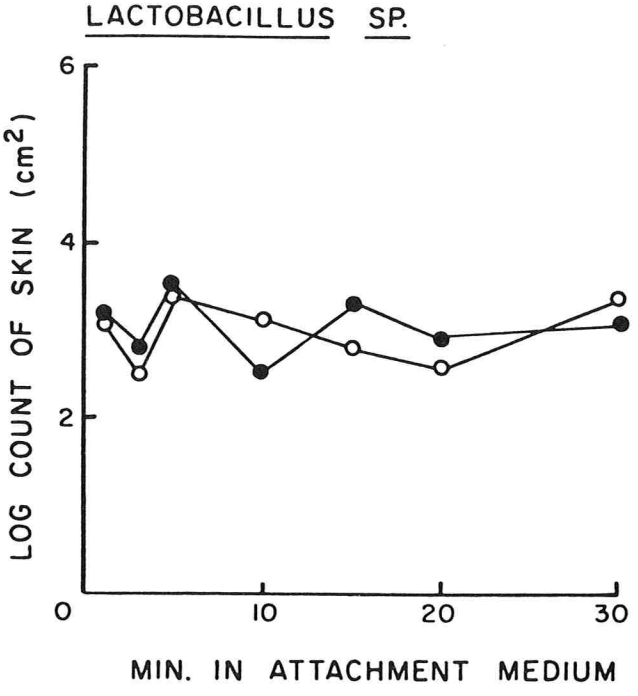

Figure 4. *Effect of immersion time in attachment medium on attachment of* Lactobacillus *sp. to pork skin. Attachment was carried out at 25 C with a cell concentration of approximately 10^6 per ml with the attachment medium adjusted to pH 7.0 (two trials).*

attachment medium. In most instances, little additional attachment occurred after 20 min of immersion in the attachment medium. At both (high and low) cell concentrations, attachment of *P. putrefaciens* was somewhat greater than that of *E. coli* or *E. herbicola*. Attachment of *E. herbicola* was similar to that of *E. coli*

at the higher cell concentrations. Attachment of the *Lactobacillus* and *Staphylococcus* spp. after 20 min in the attachment medium with 10^6 cells per ml was not much different than that of *P. putrefaciens* at a cell concentration of 10^3 cells per ml.

Effect of temperature of the attachment medium on attachment of bacteria onto pork skin

Attachment of five bacterial cultures to pork skin was carried out for 20 min in inoculated attachment medium adjusted to pH 7.0 with a cell concentration of about 10^5 cells per ml at temperatures ranging from 2.5 to 37 C. Data for *P. putrefaciens* and *E. herbicola* are presented in Fig. 5 and 6. With *E. coli, P. putrefaciens,* and the *Lactobacillus* and *Staphylococcus* spp. little difference in attachment occurred over a wide range of temperature

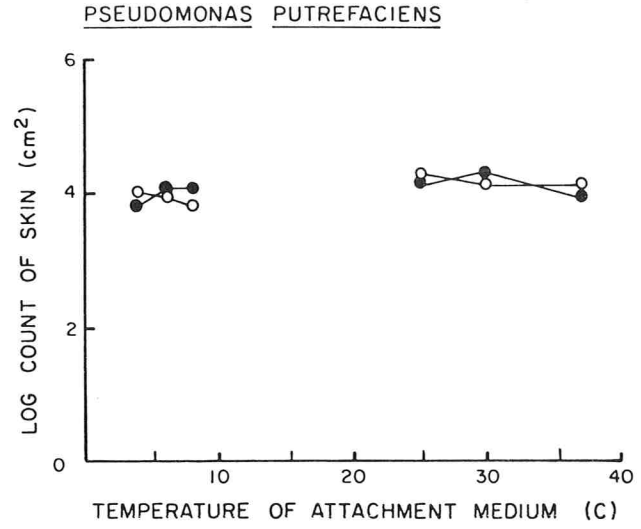

Figure 5. *Effect of temperature on attachment of* Pseudomonas putrefaciens *to pork skin. The concentration of cells in the attachment medium was approximately 10^5 per ml. Attachment was carried out for 20 min with the medium adjusted to pH 7.0 (two trials).*

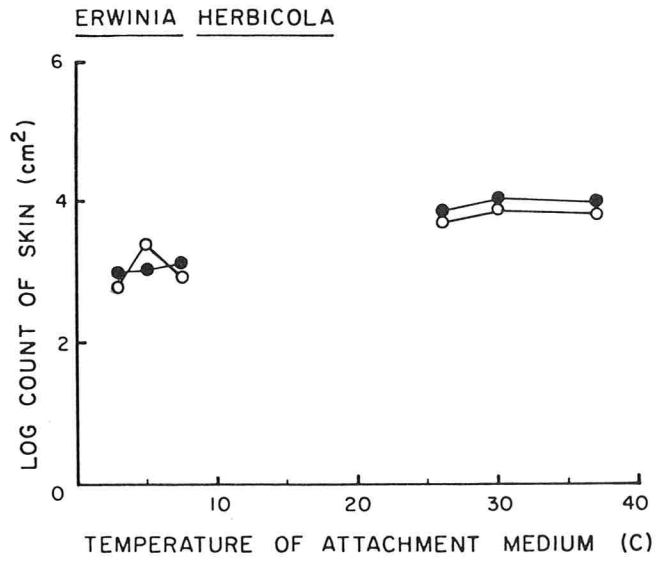

Figure 6. *Effect of temperature on attachment of* Erwinia herbicola *to pork skin. The concentration of cells in the attachment medium was approximately 10^5 per ml. Attachment was carried out for 20 min with the medium adjusted to pH 7.0 (two trials).*

(2.5-37 C) of the attachment medium. With *E. herbicola,* attachment was somewhat greater at temperatures ranging from 26 to 37 C than at the lower temperatures (3-7.5 C). Attachment of *P. putrefaciens* was higher than that of *E. coli,* the *Lactobacillus* sp. or *Staphylococcus* sp. Attachment of *P. putrefaciens* was similar to that of *E. herbicola* at the higher temperatures (25-37 C), but greater than that of *E. herbicola* at the lower temperatures (4-8 C) of the attachment medium.

Effect of pH of the attachment medium on the attachment of bacteria onto pork skin

Attachment of five test organisms was carried out for 20 min at 25 C in attachment medium with approximately 10^5 cells per ml adjusted to pH levels ranging from 4.0 to 8.8. Data for *P. putrefaciens* are presented in Fig. 7. Differences in attachment in media adjusted to different pH levels were only minor. No particular pH value seemed to favor attachment of all five organisms tested. Attachment of *P. putrefaciens, E. coli* and *E. herbicola* was much greater than that of the *Lactobacillus* sp.

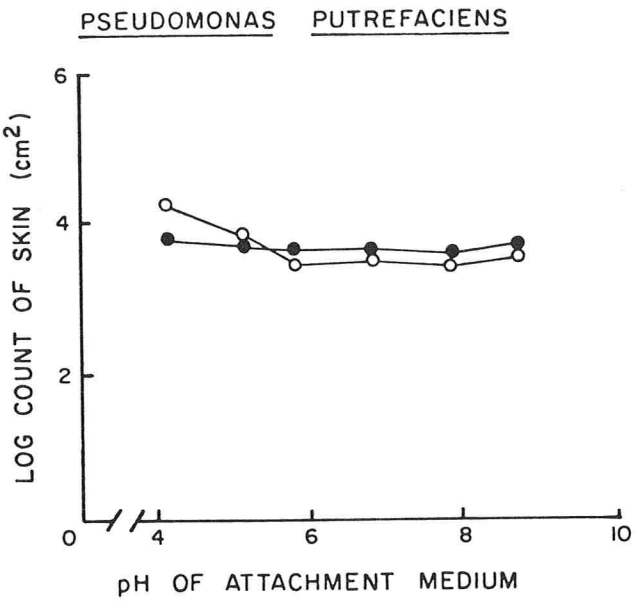

Figure 7. *Effect of pH of the attachment medium on attachment of* Pseudomonas putrefaciens *to pork skin. The cell concentration of the medium was approximately 10^5 per ml. Attachment was carried out for 20 min at 25 C (two trials).*

The results of the present study indicate a direct relationship between bacterial increase on pork skin and concentration of the test organisms in the attachment medium. In general, attachment of motile gram-negative species (*E. coli, P. putrefaciens, E. herbicola*) was greater than that of non-motile, gram-positive organisms (*Lactobacillus, Staphylococcus*). These results are similar to those reported by Notermans and Kampelmacher (*12*) for the skin of broilers; in their experiments broiler carcasses (1-kg each) were dipped in a bath containing 25 l of inoculated attachment medium. Bacterial counts were made on a 5-g sample taken from the lateral

surface of the carcass. They reported that non-flagellated bacteria did not attach or only attached in low numbers, whereas flagellated bacteria showed a very marked attachment. In their studies, the polarly flagellated *Pseudomonas* sp. attached at 21 C faster than the peritrichously flagellated bacteria. The attachment rate of a non-flagellated mutant of *E. coli* k12 was about 20% of that of the flagellated strain. In the present study, the polarly flagellated *P. putrefaciens* showed superior attachment. In other aspects, the results of the present studies with pork skin and beef and lamb muscle differed from those of Notermans and Kampelmacher (*12*) with broiler skin. Most of the attachment of the *Lactobacillus* and *Staphylococcus* spp. had occurred after 1 min of immersion in the inoculated attachment medium; with *P. putrefaciens, E. herbicola* and *E. coli* some increases in attachment frequently occurred after 1 min of immersion. With broiler skin considerable increases in bacterial attachment were noted between 1-25 min of immersion. In addition, in the present study with pork, beef and lamb samples the effects of temperature and pH of the inoculated attachment medium on attachment were small. However, with broiler skin, attachment was optimum at 21 C and at pH 8.0. According to Notermans and Kampelmacher (*12*) the effect of lowering the pH of the attachment medium on the rate of attachment is related to a decrease in bacterial mobility. Increased attachment to broiler skin with increases in temperature from 0 to 21 C were explained (*12*) by an increased activity of the bacteria combined with the flagellar activity. The results of the present study with pork, beef and lamb surfaces show that the effects of immersion time in inoculated attachment medium and temperature and pH of the attachment medium on bacterial attachment are different than for broiler skin. This is not surprising since marked differences exist in skin characteristics of the samples. Notermans and Kampelmacher (*14*) also showed that a part of the bacterial flora of the broiler skin is present in the surrounding water film and can be removed easily by rinsing for 3 min in running water. The rate of attachment with or without removal of the water film was about the same; however, removal of the portion "more firmly attached" to the skin was more restricted. In the present study, samples after immersion in inoculated attachment medium were allowed to drain and then were rinsed by immersion in sterile 0.1% peptone water. Under these conditions attachment to the samples probably was restricted to the "more firmly attached" bacteria. This view is supported by the results of trials in which rinsing of samples in peptone water was replaced by rinsing for 3 min in running distilled water. Differences in the rinsing procedures caused little difference in attachment. Notermans and Kampelmacher (*13,14*) also showed that the distribution of the microflora between the skin proper and the water film affected bacterial counts obtained with different procedures. The microflora "more firmly" attached to the skin could be evaluated more effectively by a skin maceration procedure than by a skin-rinse method. In addition, differences in the position of bacteria on the skin or surface (in the water film versus the skin or surface proper) may affect their survival during heating, freezing or thawing. The reports by Notermans and Kampelmacher (*12,13,14*), van Schothorst et al. (*18*) and Notermans et al. (*15,16*) provide a better understanding of the discrepancies in bacteriological counts and types when different sampling and testing procedures are used. Some bacteria may be more or less firmly trapped mechanically in skin or meat surface crevices or in hair follicles such as those on hogs which are scalded and dehaired. Others may be more firmly associated with the skin or meat surfaces, for example through polymer bridging with the aid of polysaccharides. The degree of association may be influenced by "attractant substances" which would direct the bacteria toward the skin or meat surface (positive chemotaxis). In addition, there are bacteria associated with the moisture film on the skin or meat surfaces. These bacteria probably are less firmly associated with the surface. Changes in the electrolyte concentration and other physical-chemical properties of this layer may be responsible for changes in the degree of attachment to or detachment from surfaces. In view of this, it is surprising that differences in pH of the inoculated attachment medium had little effect on the degree of attachment. Subsequent studies will be focused on attachment and detachment experiments which may reveal more precise information on the mechanisms of attachment and detachment.

ACKNOWLEDGMENT

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Journal of Food Protection Vol. 42, No. 5, Pages 407-409 (May, 1979)

Effect of Delayed Heading on Some Quality Attributes of *Penaeus* Shrimp[1]

R. J. ALVAREZ and J. A. KOBURGER*

Food Science and Human Nutrition Department, University of Florida, Gainesville, Florida 32611

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ABSTRACT

To determine the effect of delayed heading on shrimp quality, shrimp were stored on ice with and without heads for 10 days. Some shrimp were delay-headed after 5 days and returned to ice for the remainder of the storage period. Microbiological studies were conducted at 0, 5 and 10 days of storage. Total aerobic plate counts were done using Standard Plate Count agar with an added 0.5% NaCl. Incubation was at 20 C for 5 days. Analyses indicated similar counts on shrimp tails stored with or without heads and those delayed-headed. Counts ranged from 2.4×10^6 bacteria/gram at 0 day to 1.6×10^9 bacteria/gram on the 10th day. Identification of the flora present revealed that the same major groups of organisms predominated on shrimp tails subjected to the different storage treatments and the head did not alter development of the usual flora. *Flavobacterium, Pseudomonas, Planococcus, Moraxella* and the *Vibrio/Aeromonas* group were the major genera encountered. A shift in bacterial populations was observed during storage. *Flavobacterium* species predominated during the first 5 days of storage; however, after the fifth day *Pseudomonas* species predominated. Sensory panel data revealed no differences in acceptability between shrimp tails stored with or without heads and those delay-headed.

The accepted practice in handling *Penaeus* shrimp is to remove the heads before ice storage, based on the observation that this procedure results in a significant reduction in microbial load (*10*). Numerous reports state that shrimp with heads off keep longer and maintain lower bacterial counts than shrimp stored with heads on (*4,5,6,10,15*). Recently Bieler et al. (*2*) and Koburger et al. (*13*) reported that this practice may not be necessary under all conditions of storage. They showed that the edible portion of shrimp stored with heads on maintained similar bacterial counts and sensory quality as those shrimp stored with heads off.

The effects on quality due to holding shrimp on ice for various periods before heading are poorly understood. The increasingly greater amounts of shrimp landed with heads on stresses the need for answers to the effect of this practice on overall shrimp quality. The objective of this study was to determine the effect of heading, whether immediate or delayed, on the microbial and sensory characteristics of shrimp.

EXPERIMENTAL

Samples

Five studies were conducted, three with shrimp from Mayport Beach, Florida (East Coast) and two from Crystal River, Florida (West Coast). The shrimp were transported on ice to the laboratory and processed within 4 h of landing. Approximately one third of each shipment of shrimp was headed before storage. The tails and whole shrimp were rinsed in tap water, iced and stored in separate STYROFOAM® containers. Ice was replaced as needed. Half of the shrimp stored with heads on were delay-headed on the fifth day of storage and returned to a separate container. Samples were drawn for analysis at 0, 5 and 10 days.

Bacteriological analyses

Shrimp stored with heads on and delay-headed. Immediately before analysis a sample of 10 shrimp with heads on was headed. Depending on the weight, appropriate dilutions using Butterfield's phosphate dilution water were prepared. All subsequent dilutions were prepared on a volume-to-volume ratio following methods in the *Compendium of Methods for the Microbiological Examination of Foods* (*1*). Serial dilutions of these buffered homogenates were used with pour plates of Standard Plate Count (SPC) agar with 0.5% added NaCl. Delay-headed shrimp were analyzed using the same procedure.

Tails stored separately. Three 50-g samples of tails were drawn at specified intervals and each homogenized in 450 ml of Butterfield's phosphate dilution water. Appropriate dilutions were made following the same procedure as stated above.

Isolation and identification of microbial flora. A total of 516 random colonies were picked throughout the study from SPC plates that were used to determine aerobic plate counts. Organisms were identified according to descriptions presented in the 8th edition of *Bergey's Manual of Determinative Bacteriology* (*3*) and *A Guide to the Identification of the Genera of Bacteria* (*19*). Standard microbiological methods were used throughout (*20*) except that 0.5% NaCl was added to all media.

Sensory evaluation

Organoleptic evaluation of the shrimp was accomplished using a 20-member panel. Samples for use in the sensory panels were prepared by heading those shrimp stored with heads on and rinsing them in tap water. Shrimp were then boiled for 5 min, allowed to cool, peeled, deveined and presented to the panelists. A 9-point hedonic scale was used, with 9 being assigned "like extremely" and 1 "dislike extremely". Panelists analyzed the shrimp for odor, color, texture, flavor and general acceptability on the first, sixth and eleventh day of storage.

Statistical methods

Hedonic ratings were converted to numerical scores and subsequently analyzed by means, standard deviation (*21*) and analysis of variance using the Statistical Analysis System (SAS) program package.

RESULTS AND DISCUSSION

Figure 1 shows data from the five studies describing the average bacterial counts of shrimp tails stored with and without heads and those delay-headed from both coasts of Florida. The fresh shrimp tails had bacterial counts ranging from 7.5×10^5 to 2.2×10^6 bacteria/gram.

[1]*Florida Agricultural Experiment Stations Journal Series No. 1413.*

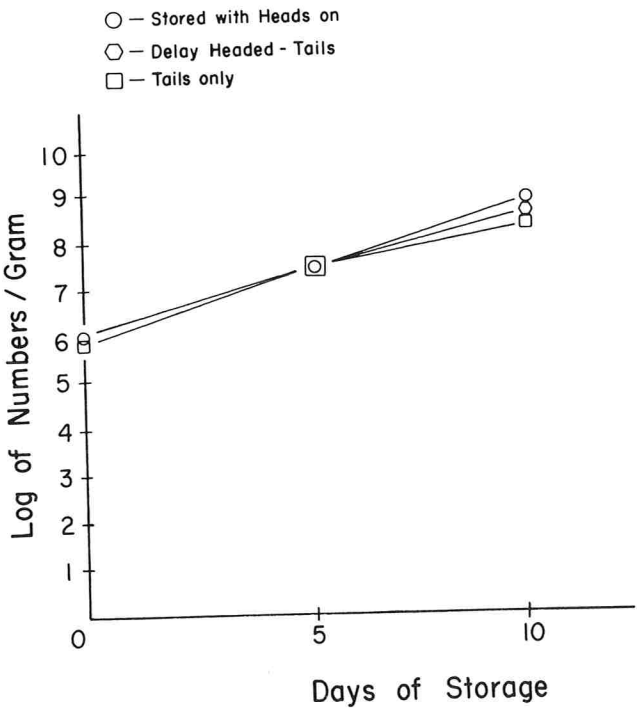

Figure 1. *Aerobic plate counts of iced stored shrimp.*

Although these initial counts seem high, they are within the ranges reported by other investigators (*4,5,7,8,10,22*). Bacterial growth proceeded at the same rate on those shrimp immediately headed and those stored with heads on at the fifth day of ice storage (Fig. 1), indicating that the head while containing 60% of the organisms (*10*), did not influence bacterial growth in the tail. Bacterial growth in delay-headed tails proceeded at the same rate as in tails stored separately. At the 10th day of storage, shrimp stored without heads had an average bacterial count of 9.0×10^8 bacteria/gram while delay-headed shrimp tails contained 1.2×10^9 bacteria/gram, indicating little difference in counts due to the different storage treatments.

The same five major bacterial groups were found in shrimp tails subjected to ice storage with or without heads and those delay-headed (Table 1). *Planococcus, Moraxella, Vibrio-Aeromonas, Flavobacterium* and *Pseudomonas* were the predominant groups isolated. Table 1 shows the percentage of organisms isolated at the different storage periods. The same organisms were present at each sampling time and their numbers were similar regardless of treatment. Therefore, time of heading does not appear to selectively affect proliferation of bacterial flora in shrimp tails.

Other organisms isolated from shrimp subjected to the various treatments were *Bacillus, Micrococcus, Acinetobacter, Azotobacter, Alcaligenes, Proteus* and *Cytophaga* in order of decreasing occurrence.

The numbers of *Planococcus, Moraxella* and *Vibrio/Aeromonas* isolated remained relatively constant throughout the storage period (Table 1). However, the *Flavobacterium* isolates increased until the fifth day, then decreased rapidly. *Pseudomonas* species showed the opposite effect. Other workers have observed the presence of *Flavobacterium* in raw shrimp (*4,11,12,14, 22,23*) and they have noted this decrease in numbers during ice-storage with a subsequent increase in *Pseudomonas* species. Cook (*7*) was unable to produce typical spoilage when shrimp were inoculated with *Flavobacterium* species, indicating that they are probably an inert group of organisms found in shrimp. In contrast, *Pseudomonas* species have been implicated by numerous workers (*17,18*) as the organisms primarily responsible for spoilage of marine products stored on ice.

Figure 2 shows the shift in bacterial flora from *Flavobacterium* to *Pseudomonas* during ice-storage of shrimp stored with heads off and those shrimp delay-headed at the fifth day. This change was also

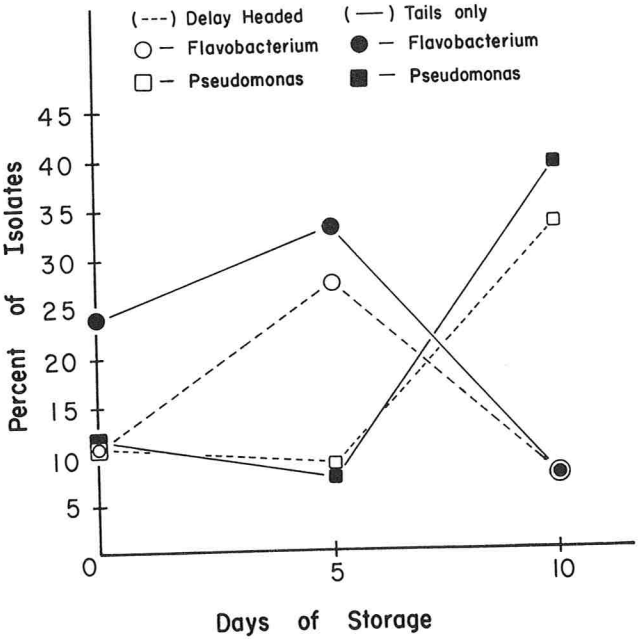

Figure 2. *Changes in bacterial flora of shrimp stored with heads off and those delay-headed.*

TABLE 1. *Distribution of the major bacterial groups present in shrimp tails stored on ice at various periods.*

Bacteria	Days of storage						
	0		5		10		
	Heads off	Heads on	Heads off	Heads on	Heads off	Heads on	Delayed
	(Percent of isolates)						
Planococcus	24	17	23	17	20	17	13
Moraxella	18	23	16	23	13	14	13
Vibrio/Aeromonas	17	22	21	23	15	11	15
Flavobacterium	24	11	33	27	8	13	8
Pseudomonas	12	11	8	9	39	44	33

TABLE 2. *Sensory preference ratings of shrimp stored with and without heads and those delay-headed. (Average of 2 studies)*

	Odor	Color	Texture	Flavor	General acceptance
1 day					
Heads off	$7.1^{a} \pm 1.0$*	$8.1^{a} \pm 0.6$	$7.5^{a} \pm 0.9$	$6.9^{a} \pm 1.2$	$7.2^{a} \pm 1.0$
Heads on	$7.4^{a} \pm 0.8$	$8.1^{a} \pm 0.6$	$7.2^{a} \pm 0.9$	$7.2^{a} \pm 1.2$	$7.4^{a} \pm 1.1$
6 days					
Heads off	$7.1^{a} \pm 1.1$	$7.4^{b} \pm 0.8$	$7.1^{a} \pm 0.8$	$7.2^{a} \pm 1.3$	$7.2^{a} \pm 1.1$
Heads on	$6.9^{a} \pm 1.4$	$7.3^{b} \pm 1.4$	$7.3^{a} \pm 1.2$	$6.9^{a} \pm 1.5$	$6.9^{a} \pm 1.4$
11 days					
Heads off	$6.4^{b} \pm 1.4$	$7.4^{c} \pm 1.1$	$5.7^{b} \pm 1.5$	$6.0^{b} \pm 2.2$	$6.2^{b} \pm 1.8$
Heads on	$6.4^{b} \pm 1.4$	$7.4^{c} \pm 1.0$	$5.6^{b} \pm 1.7$	$6.0^{b} \pm 2.2$	$5.8^{b} \pm 2.0$
Delayed-headed	$6.4^{b} \pm 1.3$	$7.2^{c} \pm 0.9$	$5.8^{b} \pm 1.3$	$6.3^{b} \pm 1.9$	$6.0^{b} \pm 1.6$

*Means of duplicate trials ± standard deviation. Those quality attributes followed by the same letter do not differ significantly at $P < .05$. Scale: 9 = like extremely, to 1 = dislike extremely.

observed in shrimp stored with heads on. Consequently, heading did not alter development of the normal spoilage pattern on shrimp; however, it might add additional surface area for microbial proliferation (*16*).

Table 2 lists the results of the sensory evaluation of the shrimp. The data show no statistical differences between shrimp tails stored with heads on or off or those delay-headed. No significant differences in odor, color, texture, flavor, or general acceptability were observed by the panelists for the shrimp subjected to the different storage treatments. However, after the fifth day, the flesh started to soften and a "fishy" aroma became evident in all shrimp. By the eleventh day of storage, these changes were even more noticeable as shown by the fact that the flavor and texture scores dropped dramatically. These changes in flavor and acceptability coincide with those reported by other researchers (*7,9*) working with shrimp headed immediately upon landing. Consequently, it appears that shrimp headed immediately upon landing or delay-headed will be equally acceptable to the consumer.

As evidenced by the data in this paper, the microbial quality of shrimp and organoleptic acceptability are not markedly affected by delaying the heading of freshly harvested shrimp for a reasonable period. Bacterial counts, bacterial flora and organoleptic acceptability were similar regardless of treatment.

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Inactivation of Salmonellae in Autoclaved Ground Beef Exposed to Constantly Rising Temperatures[1]

W. S. THOMPSON, F. F. BUSTA*, D. R. THOMPSON and C. E. ALLEN

Department of Food Science and Nutrition, University of Minnesota, 1334 Eckles Avenue, St. Paul, Minnesota 55108

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ABSTRACT

Inactivation of a composite of five serotypes of salmonellae was monitored in autoclaved ground beef exposed to constantly rising temperatures increased at rates similar to those used in beef cookery. Rising temperature rates of 6.0 C/h, 8.5 C/h and 12.5 C/h and constant temperatures of 55, 57, 61 and 63 C were examined. Survival of *Salmonella typhimurium* TM-1 was compared to survival of the composite. D and z values were compared for constant and rising temperature rates. The $D_{50\ C}$ for constant temperature data was 30.2 min, and the $D_{50\ C}$ for changing temperature data was 78.6 min (6.0 C/h), 82.4 min (8.5 C/h), and 49.8 min (12.5 C/h). Neither serotype nor heat treatment of ground beef had a major influence on apparent heat resistance of salmonellae. A comparison of these results to previous rising temperature work with *Clostridium perfringens* suggested that controlling *C. perfringens* will result in control of salmonellae. On the basis of these results, the July 18, 1978, USDA processing ruling appears adequate to control salmonellae in precooked beef roasts.

The increased incidence of human salmonellosis contracted from precooked roasts of beef in the last three years has been of major public health concern (*6*). In the 1975 outbreak caused by *Salmonella saint paul* (*11*), the beef had been injected with a spice mix in preparation for cooking. Subsequent cases have involved intact roasts where no such procedures were used, yet salmonellae were isolated from external and internal surfaces of the roasts (*13*). Indications were that the roasts were not being cooked to internal temperatures greater than 54.4 C (130 F) (*13*). For example, in one outbreak the roasts were removed from the oven and the internal temperature was allowed to rise to only 51.7 C (125 F) (*12*). Ten outbreaks with 110 cases of salmonellosis were attributed to roast beef in 1976 and 1977 (*1*). Several of the *Salmonella* serotypes involved in these outbreaks, including *Salmonella newport, Salmonella chester, Salmonella typhimurium, Salmonella waycross,* and *Salmonella bovis-morbificans*, were isolated from unopened precooked roast beef (*14*).

In an effort to eliminate this public health hazard, the USDA passed a ruling on September 2, 1977, requiring processed roasts to be cooked to a minimum internal temperature of 63 C (145 F) in all parts of the roast (*1*). This ruling was intended to control potential salmonellosis due to roast beef. However, many consumers prefer rare roast beef which is difficult to produce at this temperature. A new ruling was proposed on May 2, 1978 (*2*), to provide alternative times and temperatures to the first ruling, and the final version of this ruling was published on July 18, 1978 (*3*).

Much of the work which was the basis for these USDA proposals dealt with inactivation of salmonellae during timed exposures to constant temperatures (*7,16*). However, these constant temperatures may not represent the actual heat treatment incurred in precooked roasts of beef.

The objectives of this research were to study inactivation of representative serotypes of salmonellae while increasing the temperature at rates which are representative of beef cookery over a range of cooking temperatures, to compare the results with previous work on *Clostridium perfringens* by Willardsen et al. (*22*), and to use the data to evaluate the recent USDA proposals.

MATERIALS AND METHODS

Test organisms

Salmonella typhimurium Tm-1 was obtained from J. A. Garibaldi, USDA, Western Regional Research Laboratories, Albany, CA. *Salmonella saint paul, Salmonella newport,* and *Salmonella waycross* were obtained from the Center for Disease Control, Atlanta, GA. *Salmonella heidelberg* was obtained from the food microbiology culture collection, Department of Food Science and Nutrition, University of Minnesota, St. Paul, MN. The five serotypes selected for this study were intended to be representative of strains implicated in salmonellosis. The first four serotypes have been implicated in outbreaks from roast beef.

Culture preparation

The stock culture was prepared by transferring one loopful of inoculum into 10 ml of brain heart infusion (BHI; BBL). This was incubated at 37 C for 24 h. From this culture, 1% inoculum was transferred into 10 ml of BHI and stored at - 20 C.

Test cultures were prepared according to the method of Bayne et al. (*9*). The stock culture was thawed as needed and incubated 24 h at 37 C. From this culture, 1% inoculum was placed in 100 ml of Trypticase Soy Broth (BBL) with 2% yeast extract (BBL). This was incubated with shaking at 150 rpm and 37 C (New Brunswick Scientific, Gyrotory Water Bath Shaker, Model G76) for 72 h. After 72 h, the cells were centrifuged at 3000 × *g* for 10 min and were then washed twice with sterile 1% NaCl. After washing and resuspension in 1% NaCl, a composite of the five strains was prepared by combining 2 ml of each culture into a 25 × 150-mm screw-capped test tube and mixing for 10 sec with a Maxi-Mix. (Maxi-Mix, Model No. M16715, Thermodyne, Dubuque, IA).

Media

The ground beef was prepared in the laboratory according to the

[1]*Paper No. 10,505, Scientific Journal Series, Minnesota Agricultural Experiment Station, St. Paul, MN 55108.*

method of Willardsen et al. (*22*) from beef chucks using 0.5-mm and 3.2-mm grinder plates in succession and was frozen at - 30 C for a maximum of 8 months. Three batches (fat content 23.9, 17.0 and 20.5%) were used during this research. A 15-g portion of ground beef was rolled into a cylindrical shape, tamped with a glass rod (8-mm diameter) into 25 × 150-mm screw-capped test tubes (Pyrex No. 9825) and autoclaved at 121 C for 15 min. After cooling overnight, the tubes were inoculated with 0.1 ml of inoculum using a 15-gauge needle 13 cm in length.

Xylose lysine agar (XL) and xylose lysine deoxycholate agar (XLD) (Difco) were used as enumeration media. Plates were poured 2 days before use and held at room temperature to ensure dryness.

Apparatus

A constant-temperature water bath (Blue M Model MW-1140E-1) was supplemented with a variable wattage heater-circulator (Haake Model E-52) to allow control of rising temperature rates. Water bath temperatures were monitored on a strip chart temperature recorder (Honeywell Model 153X64P16-X-40) and an electronic probe thermometer (Fluke 2100A Digital Thermometer) was used to measure all temperatures of the samples reported in this study.

Temperature treatments and determination of constant

Rising temperature rates of 6.0, 8.5, and 12.5 C/h were selected to simulate cooking rates used in the home or in the meat processing and foodservice industries. Samplings were taken frequently between 47 C and 60 C where the most inactivation effects were expected to occur. Because *S. typhimurium* Tm-1 has been reported to be one of the most heat resistant serotypes (personal communication, J. A. Garibaldi, Western Regional Research Laboratory, Albany, CA 94710), it was studied separately at the same rising temperature rates to determine its contribution to the composite's response.

Constant temperature inactivation of *S. typhimurium* Tm-1 at 55, 57, 61 and 63 C also was measured. D and z values were compared between constant and changing temperature data. Data were calculated and plotted by computer to present curves of best fit (*19*).

Enumeration procedure

Constant-temperature samples were placed in an ice bath immediately after removal from the heating bath. Changing-temperature samples were not chilled but immediately transferred as follows. A sterile 23-cm chrome letter opener was used to transfer the sample into a 18 × 30-cm, 3 ½-mil polyethylene bag. The sample was diluted to 100 g with sterile distilled water (85 ml added), and macerated for 30 sec in a Colworth 400 Stomacher (*21*). Serial dilutions were made into 9.9 ml of 0.1% peptone (Difco), spread plated on XL or XLD agar and incubated for 48 h at 37 C.

RESULTS AND DISCUSSION

Previous work has indicated that a time-temperature relationship exists for inactivation of salmonellae (*4,5*). Much of the work that was the basis for the USDA proposals was based on inactivation of salmonellae exposed to various constant temperatures for several periods (*7,16*). However, as discussed by Willardsen et al. (*22*), food does not remain at any single constant temperature in most home, industrial, and institutional food handling, preparation, processing and storage procedures. Pre-cooked beef roasts are an excellent example of a changing temperature process.

Although studies have indicated that surface contamination of salmonellae can result in survival more frequently than that observed with internal contamination (*8,10*), the effect of surface contamination was not considered in this study.

A representative temperature - based inactivation curve for salmonellae exposed to constantly-rising temperatures is presented in Fig. 1. The data show inactivation of a composite of five serotypes of salmonellae in autoclaved ground beef at temperatures rising at a rate of 6.0 C/h. The N_0, or initial population, was developed using an average of colony forming units (CFU) in samples from 25 to 46 C. This temperature range was used because no inactivation effects were observed in this interval. N was the CFU at each sampling time and N/N_0 is plotted vs. temperature (C). No inactivation occurred until a temperature of approximately 51 C was attained and at 60 C less than 0.0001% survival was observed. This was the most inactivation observable within the sensitivity of this experiment.

Figure 2 shows salmonellae inactivation at 8.5 C/h and is similar to the curve in Fig. 1. Inactivation appears to begin at 51 C and is below the measurement threshold at 60 C. The solid line was computer-drawn based on kinetic parameter values determined by the least squares method. The square of the vertical distance between the line and the data points has been minimized in fitting the curve. This method, first order kinetics and least squares, results in some variation between the computer

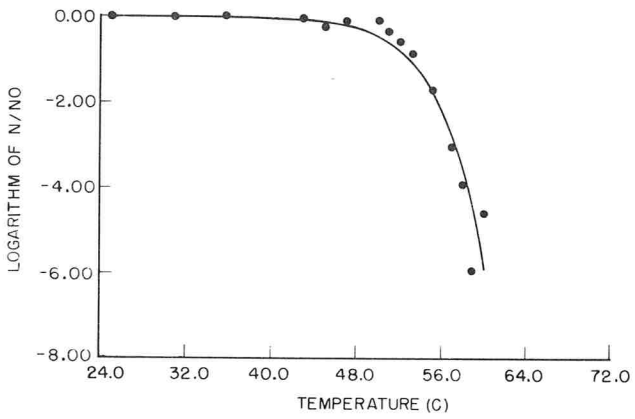

Figure 1. *Survival of five-strain composite of salmonellae exposed to constantly rising temperatures at 6.0 C/h in autoclaved ground beef. N/N_0 determined from CFU/g data. Curve plotted with computer assistance.*

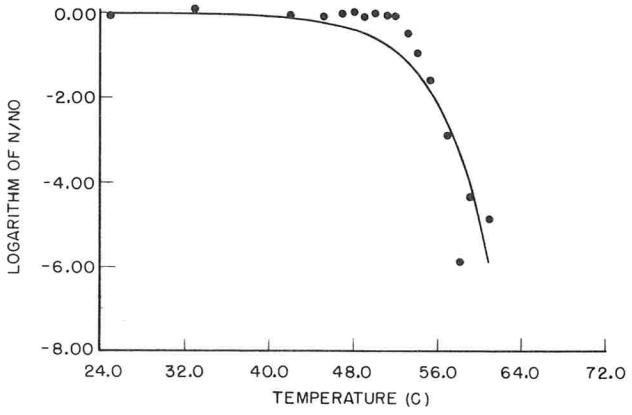

Figure 2. *Survival of five-strain composite of salmonellae exposed to constantly rising temperatures at 8.5 C/h in autoclaved ground beef. N/N_0 determined from CFU/g data. Curve plotted with computer assistance.*

line and actual data points.

Figure 3 demonstrates inactivation at 12.5 C/h. This curve also compares well with the previous curves, with inactivation beginning at 51 C and being beyond minimal levels at 60 C.

All trials were done in duplicate. Figure 4 shows trial reproducibility with duplicate curves from the 8.5 C/h temperature rate trials. The variation between curves was not unexpected for the small number of trials.

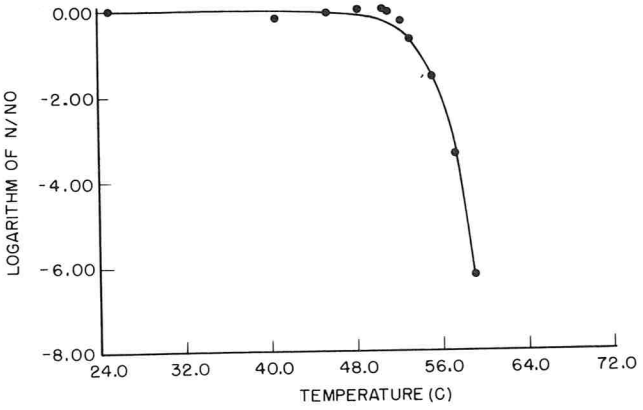

Figure 3. *Survival of five-strain composite of salmonellae exposed to constantly rising temperatures at 12.5 C/h in autoclaved ground beef. N/N_0 determined from CFU/g data. Curve plotted with computer assistance.*

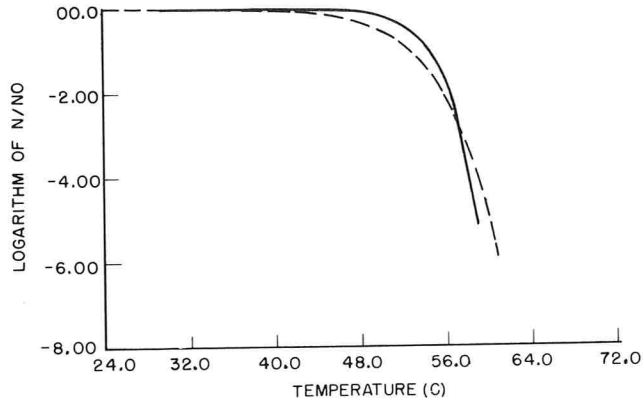

Figure 4. *Survival pattern in duplicate trials of five-strain composite of salmonellae exposed to constantly rising temperatures at 8.5 C/h in autoclaved ground beef. Curves generated with computer assistance from CFU/g data.*

S. typhimurium Tm-1 has been the subject of many thermal inactivation studies (*18*). Its heat resistance when grown under the appropriate conditions is greater than the other strains used in this study (personal communication, J. A. Garibaldi, Western Regional Research Laboratory, Albany, CA 94710). It was studied separately at all three rising temperature rates to determine its contribution to the composite's response. A comparison (Fig. 5) reveals little difference between the two curves, since inactivation began at approximately the same temperature and continued at similar rates. This seems to indicate that all five of the composite organisms had similar heat resistance, which could be due to growing the organisms to the late stationary phase through the 72-h incubation. Late stationary cells are the most heat resistant cells (*17,20*).

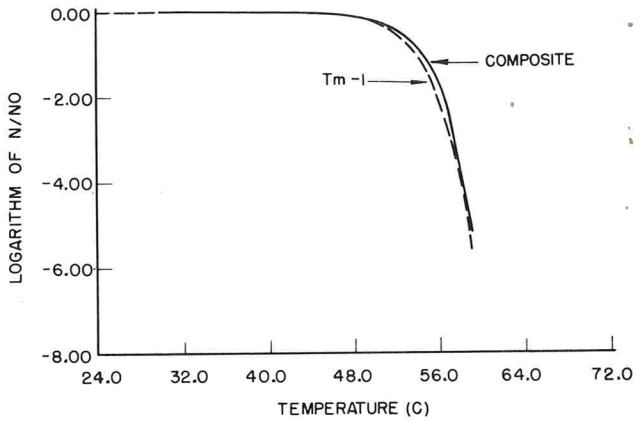

Figure 5. *Survival of* Salmonella typhimurium *Tm-1 compared to survival of five-strain composite of salmonellae exposed to constantly rising temperatures at 8.5 C/h in autoclaved ground beef. Curves generated with computer assistance from CFU/g data.*

To determine the influence of autoclaving, raw ground beef was compared to autoclaved ground beef. In both constant and changing data, heat resistance of *S. typhimurium* Tm-1 in raw ground beef, was consistently equal to or less than that in autoclaved ground beef (Fig. 6). This indicates that the D-values found in autoclaved beef should be an adequate prediction for raw beef products.

Constant-temperature inactivation was studied in duplicate at 55, 57, 61 and 63 C with examples of trials at 57 and 63 C shown in Fig. 7. The time axis for the 57-C curve is 10 times the scale of the time axis for the 63-C curve. All constant temperature survivor curves showed this definite tail. A culture produced from a survivor isolated at the end of one of these tails was used in a special separate study. No mutation or heat resistant substrain was observed because data from this survivor isolate were identical to those obtained originally,

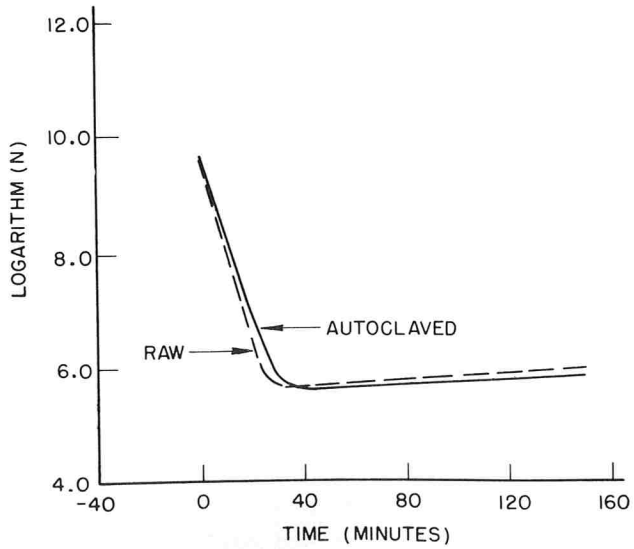

Figure 6. *Survival of* Salmonella typhimurium *Tm-1 exposed to 55 C in raw ground beef (broken line) or autoclaved ground beef (solid line). Curves generated with computer assistance from CFU/g data; natural logarithm of N represents conversion of CFU/g data.*

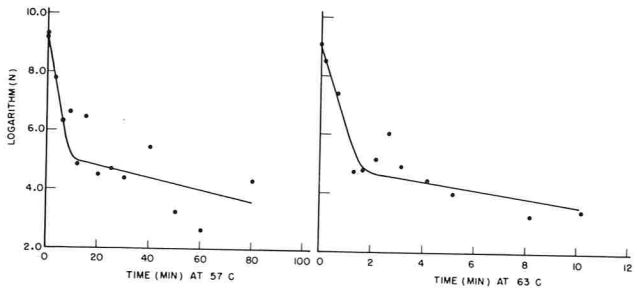

Figure 7. *Comparison of survival of* Salmonella typhimurium *Tm-1 exposed to 57 C (left curve) vs. 63 C (right curve). Curves generated with computer assistance from CFU/g data; natural logarithm of N represents conversion of CFU/g data.*

including the tail. This tail could be due to heterogeneity of heat resistance resulting from growing the cells over 72 h (*15*).

Table 1 presents D, k and R^2 values for both temperatures (57 and 63 C). D is the time of heating at a specific temperature to result in 10% survival, k is the rate constant for inactivation in min^{-1}. D values of 2.130 and 2.667 min were observed at 57 C and these corresponded to rate constants (k) of 1.081 and 0.863 min^{-1}. For 63 C the D was 0.359 min and the rate constant was 6.413 min^{-1}. Only values from the first segment of each curve are presented because of the small R^2 values calculated for the second segment or tail portion of the curve.

D, z, k (rate constant), and E (activation energy) values were developed as averages from all the constant temperature curves (Table 2) by computer analyses. The z value represents the degrees Celsius required to change the D value tenfold. The first and second segments of the curves were considered separately because of the large difference between the two segments (Table 2). For example, the first segment has a $D_{50\ C}$ value of 30.2 min, and the tail segment has a $D_{50\ C}$ value of 818.6 min.

Table 3 shows D, z, the rate constant (k) and activation energy (E) for each changing temperature trial. The differences between replicate trials did not appear to be significant, especially considering the small number of

TABLE 1. Salmonella typhimurium *Tm-1 inactivation kinetics at constant temperatures.*

Temperature	D[a] (min)	k[b] (min^{-1})	R^2
57 C	2.130	1.081	0.996
	2.667	0.863	0.949
63 C	0.359	6.413	0.967

[a]D = the time of heating at a specific temperature to result in ten percent survival.
[b]k = the rate constant in $min.^{-1}$

TABLE 2. Salmonella typhimurium *Tm-1 inactivation kinetics at constant temperatures from 55 to 63 C.*

Segment	$D^a_{50\ C}$ (min)	z[b] (C)	k[c] (min^{-1})	(J/mol)E[d]
1	30.2	6.5	0.1404	326,671.2
2	818.6	5.4	0.0058	388,914.3

[a]D = the time of heating at a specific temperature to result in ten percent survival.
[b]z = the degrees Celsius required to change the D-value tenfold.
[c]k = the rate constant in $min.^{-1}$
[d]E = activation energy in J/mol.

TABLE 3. Salmonella typhimurium *Tm-1 inactivation kinetics at changing temperatures.*

Temperature rise, Rate (C/h)	$D^a_{50\ C}$ (min)	z[b] (C)	k[c] (min^{-1})	(J/mol)E[d]
6.0	78.6	9.9	0.045	202,395
8.5	82.4	6.7	0.053	301,938
12.5	49.8	9.9	0.071	202,395

[a]D = the time of heating at a specific temperature to result in ten percent survival.
[b]z = the degrees Celcius required to change the D-value tenfold.
[c]k = the rate constant in $min.^{-1}$
[d]E = activation energy in J/mol.

trials. $D_{50\ C}$ values did not vary significantly between the composite trials and *S. typhimurium* Tm-1. Average $D_{50\ C}$ values were 78.6 min for 6.0 C/h, 82.4 min for 8.5 C/h and 49.8 min for 12.5 C/h. The k and E values are also listed. Although mean values of z and E appear to be different among the three heating rates, they could not be statistically differentiated because each value is the average of duplicate determinants.

The constant-temperature data were used to generate or predict changing-temperature curves with computer assistance (Fig. 8). The curve from data on the composite cultures indicates that the rate constants for constant-temperature could not be used to predict the changing-temperature curves. The predicted curve consistently fell short of the actual data. The large difference between the two curves is on the ordinate (N/N_0). The break at the end of the computer-generated curve is due to the effect of the constant-temperature tails.

A comparison of $D_{50\ C}$ values between the changing and constant temperature data shows a considerable difference (Tables 2 and 3). The $D_{50\ C}$ value of 30.2 min at constant temperatures is smaller than any of the $D_{50\ C}$ values for the changing temperature data.

Table 4 shows a comparison of D values observed in four different inactivation media. Evaluation of the results shows that autoclaved ground beef resulted in a higher D value than any of the other three inactivation media. No explanation for this observation was readily evident; however, it did support the earlier statement on

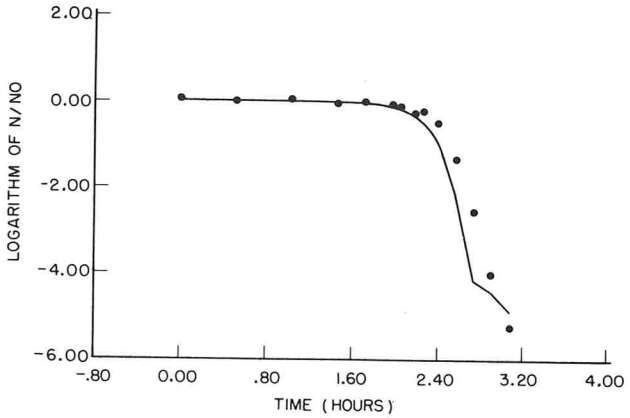

Figure 8. *Theoretical survival curve generated by computer from constant temperature data used to predict survival of* Salmonella typhimurium *Tm-1 exposed to constantly rising temperatures at 8.5 C/h in autoclaved ground beef. Circles are actual data points. N/N_0 determined from CFU/g data.*

TABLE 4. *Comparison of inactivation suspending media on survival of* Salmonella typhimurium *Tm-1*.

Inactivation medium	$D^a_{55\,C}$ (min)	$D^a_{57\,C}$ (min)
Autoclaved ground beef	8.151	—
Typticase Soy Broth with 2% Yeast Extract	8.039	1.432
Brain Heart Infusion	6.618	1.290
Phosphate buffer (pH = 7.0)	—	1.133

[a]D = the time of heating at a specific temperature to result in ten percent survival.

Fig. 6 that data obtained in autoclaved ground beef were useful in evaluating a raw beef system.

A comparison of the heat resistance of cultures incubated for 24, 48 and 72 h with shaking under constant conditions was also made (data not presented). D values showed little difference between the 48-h ($D_{57\,C}$ = 4.095 min) and 72-h ($D_{57\,C}$ = 3.897 min) culture. The $D_{57\,C}$ value for 24 h ($D_{57\,C}$ = 3.149 min) was significantly lower than the other two D values.

A comparison of these *Salmonella* data was made to work done previously with changing temperatures (Fig. 9). The data on growth and survival of *C. perfringens*, an organism also linked with foodborne illness, were reported for changing temperatures by Willardsen et al. (*22*). A comparison of these two curves indicates that if *C. perfringens* is controlled, the *Salmonella* cells are also inactivated. *C. perfringens* growth, which was initiated at about 40 C in this study, ceased at about 55 C, and a large number of *C. perfringens* cells survived above 60 C when exposed to rising temperatures at 8.5 C/h.

Thermal inactivation data from several studies are summarized in Fig. 10. The USDA values are taken from the July 18, 1978, processing ruling (*3*). The USDA values are based on 7D levels of inactivation. The ABC data are D values from the report on fate of salmonella inoculated into beef for cooking cited in the USDA proposal (*7,16*). The changing-temperature curves are based on the D and z values presented above and the changing temperature rate is indicated on each curve. D values

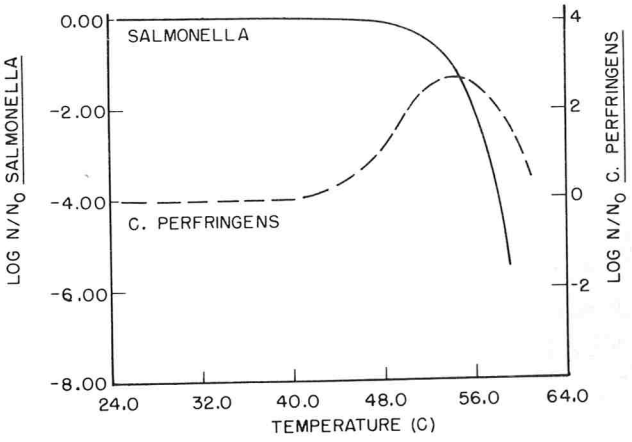

Figure 9. *Comparison of survival of* Salmonella typhimurium *Tm-1 to growth and survival of* Clostridium perfringens *exposed to constantly rising temperatures at 8.5 C/h in autoclaved ground beef. Data on* C. perfringens *from Willardsen et al. (22).*

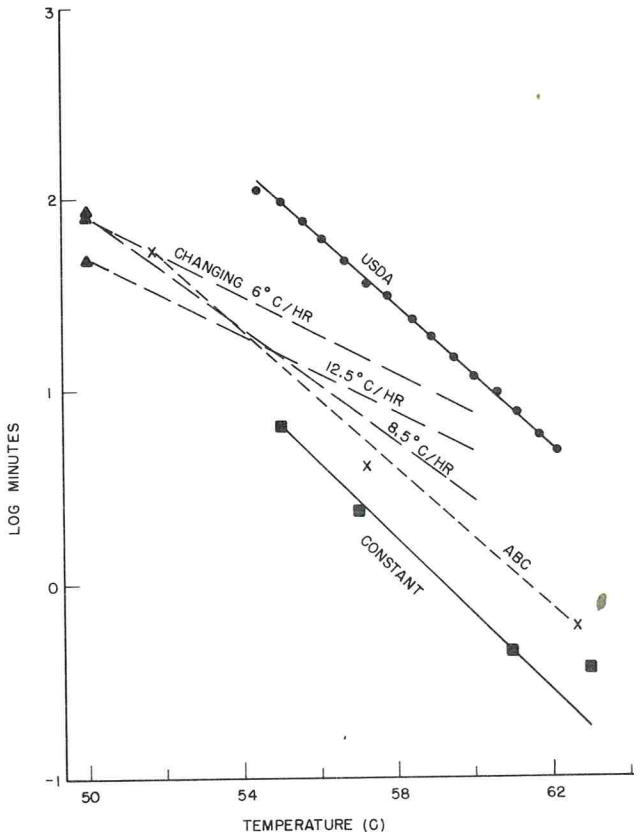

Figure 10. *Comparison of inactivation constants and relationships: (a)* CONSTANT *curve from D values for Salmonella typhimurium Tm-1 exposed to constant temperatures, (b) CHANGING 6 C/h, 8.5 C/h, 12.5 C/h curves from D values for* Salmonella typhimurium *Tm-1 exposed to changing temperatures, (c) ABC curve from D values on salmonellae survival by American Bacteriological and Chemical Research Corporation (7,16), (d) USDA curve from minimum times at indicated temperatures published as cooking requirements by USDA* (3).

from the constant-temperature data are plotted as indicated. Evaluation of these data indicates that the USDA values should be sufficient to inactivate *Salmonella* cells in beef, although our data would suggest that a 7D process might not always be attained with changing temperatures.

On the basis of this work, several observations can be made. By computer simulation, constant-temperature data do not generate curves identical to those observed with changing temperatures. Neither serotype nor heat treatment of ground beef had a major influence on apparent heat resistance of salmonellae. The information used to develop the USDA ruling appears adequate and the prescribed treatments should inactivate *Salmonella* cells in precooked beef roasts. If the growth and survival of *C. perfringens* are controlled, the salmonellae are inactivated as well.

ACKNOWLEDGMENTS

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Extension of Poultry Shelf-Life by Poly (Hexamethylenebiguanide Hydrochloride)

MIR N. ISLAM* and NAFISA B. ISLAM

Department of Food Science and Human Nutrition, University of Delaware, Newark, Delaware 19711

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ABSTRACT

Immersion of freshly processed poultry carcasses in solutions of poly (hexamethylenebiguanide hydrochloride), PHMB, retarded bacterial growth and markedly improved the shelf-life during storage at 2 C. Birds treated with 200, 300, and 400 ppm PHMB had average shelf-lives of 22.9, 25.9, and 26.0 days, respectively, compared to the 10.5 days of shelf-life for water-treated controls. Duncan's multiple range test revealed that the shelf-life differences among PHMB-treated birds were not statistically significant.

The limited shelf-life of fresh poultry has been a serious problem to the poultry industry. Methods for prolonging the freshness of poultry have constantly been sought. These included irradiation, pasteurization, storage in germicidal ice, use of edible coating, and exposure to antibiotics and other chemicals (*7,13*). Mountney and O'Malley (*11*) extended shelf-life of cut-up poultry parts 6 days beyond that of controls by dipping them in solutions of succinic and adipic acids at pH 2.5. However, as the pH was raised the preservative effect diminished and at pH 4.0 no inhibition of spoilage was observed. Heat treatment in combination with succinic acid was shown by Cox et al. (*3*) to retard poultry spoilage, but it resulted in bleached skin and a "cooked" appearance. In-plant chlorination is effective in reducing slime and odor on processing equipment, but it has very little effect on poultry shelf-life when added to the chill-immersion tanks (*10*). The combination of chlorine, succinic acid and heat, extended shelf-life, but caused discoloration of the skin due to partial cooking (*16*). Islam et al. (*7*) screened 53 chemicals and found that chloroacetamide and iodoacetamide extended poultry shelf-life for 3 and 4 days, respectively, at a storage temperature of 5 C. Thomson et al. (*17*) found that 0.5% glutaraldehyde extends poultry shelf-life about 6 days beyond that of controls at 2 C. Arafa and Chen (*1*) obtained similar shelf-life improvement for cut-up broiler parts by dipping in 1% ascorbic acid solution of pH 2.75.

Poly (hexamethylenebiguanide hydrochloride) or PHMB has been used successfully as a biocide for several years, particularly in the European countries. Under the trade name "Vantocil IB" it has been marketed for industrial disinfection (*2*) and for short-term preservation of cattlehides and sheepskins (*4,5*). In addition under the trade name of "Bacquacil SB" it has been marketed for several years as a swimming pool sanitizer (*6*). PHMB alone or in combination with other chemicals also retards development of dental plaque, calculus and caries in animals (*12,14,18*). Strandskov and Bocklemann (*15*) in 1975 obtained a U.S. patent for preservation of beverages such as beer by incorporating PHMB up to a level of 50 ppm.

In view of the above demonstrated applications, PHMB seemed to have a good potential for use as a poultry preservative. Hence, this study was designed to evaluate the effectiveness of poly (hexamethylenebiguanide hydrochloride) in extending the shelf-life of fresh poultry.

MATERIALS AND METHODS

Preparation of PHMB solutions

Distilled water in 14-liter lots was poured into four double-layered 30-gal. size plastic bags. Appropriate amounts of 20% PHMB solution ("Vantocil IB") obtained from the ICI-Americas, Wilmington, Delaware, were added to the water to reach the final concentrations of 0, 200, 300, and 400 ppm, respectively. The plastic bags containing these solutions were placed in rigid containers for easier handling, and then stored at 2 ± 0.5 C. for at least 12 h before use. Portions (about 25 ml) from each of these solutions were withdrawn with sterile pipettes and their pH values determined by a Beckman digital pH meter.

Sample preparation

Twenty-eight fresh broiler carcasses were brought to the laboratory ice-packed in foamed plastic containers from a nearby processing plant. The carcasses were randomly divided into four groups of seven for treatment with the PHMB solutions prepared above. The birds were submerged in respective solutions for 2 h at 2 ± 0.5 C and then drained for 10 min on alcohol-swabbed metal racks. They were then packaged in gallon-size clear polyethylene bags and stored at 2 ± 0.2 C. Three carcasses from each group were designated for sensory evaluation and the remaining four for bacterial count during storage. Bacterial count was limited to the total aerobic psychrotrophs because of their predominance in spoiled chicken (*3,7*).

Sensory evaluation

Since two recent studies (*1,17*) using similar storage conditions have shown that the control chickens remain fresh for at least 10-11 days, the sensory evaluation in this study began after a week of storage at 2 ± 0.2 C. Commencing on the 8th day of storage, and every alternate day thereafter, a five-membered panel consisting of departmental faculty and graduate students evaluated the carcasses for development of off-odor. The following statement appeared on the evaluation forms, "please open each bag, smell the carcass and then rate the off-odor on a 5-point hedonic scale where 1, 2, 3, 4, and 5 represent none, slight, moderate, strong, and very strong, respectively. A score of 3 or less would indicate that the bird is acceptable to you as a potential consumer". This method of evaluation is somewhat similar to that described by Thomson et al. (*17*). When a bird was rated 5 by at least three of the five panels it was removed from the observation.

Shelf-life for each bird was calculated based on the number of days it maintained a sensory score of 3 or less. The shelf-life data were then analyzed as a 4 × 5 factorial in a completely randomized design with the treatments and the panelists as the main effects. This was followed by Duncan's multiple range test to find if there was any statistically significant difference between treatments (*9*). A separate but similar analysis was carried out in a 12 × 5 factorial arrangement with the birds and the panelists as the main effects, to find if there was any difference between the birds within the and between each treatment group.

Total aerobic psychrotroph count

The bacterial densities on the right and left breasts of the broiler carcasses were assumed to be the same based on the earlier studies by Cox et al. (*3*) and Kotula (*8*).

Two of the four carcasses designated for bacterial count were swabbed on the right breast before and after dipping on day 0, and on the left breast on day 5 and 10. The other two carcasses from each group were swabbed subsequently on days 15, 20, 25, and 30. This type of split-sampling within a group was necessary because of the limitation of swabbing space on the same bird. Also, if the same bird was swabbed eight times over a 30-day period, it would have been subjected to a greater degree of contamination. The birds which scored 5 because of their off-odor were swabbed only once beyond their final evaluation day. A 4-cm² area at different locations on the chicken breast was swabbed on each sampling day. Appropriate dilutions were prepared in 0.1% peptone and spread-plated in duplicate on tryptic soy agar (TSA, Difco). The plates were incubated at 7 C for 10 days before enumeration of total aerobic psychrotrophs. Average number of colonies from the four plates in each group were divided by four and reported as log bacterial count per cm².

RESULTS AND DISCUSSION

Total aerobic psychrotroph count

PHMB, at the levels used in this study, had an unusually strong bactericidal effect on the microbial population of the chicken carcasses compared to that of the other potential poultry preservatives reported in the literature (*1,3,7*). This strong bactericidal effect resulted in marked improvement in the shelf-life of PHMB-treated carcasses. The average initial psychrotroph count on the untreated birds was about 10^3 organisms/cm² of skin. These same birds, when swabbed immediately after dipping in PHMB solutions, had so few organisms that no bacterial count could be obtained even at the lowest dilution (10-fold) used. For the control samples there were slight decreases in bacterial count possibly due to the mechanical rinsing effect; however, within the 5th day of storage the log count exceeded its original level (Fig. 1). At the 5th day, the PHMB-treated samples had a very small increase in bacterial growth, but the number of colonies was too few to count except for the 200-ppm group. Hence, to plot growth curves, the unobtainable counts were estimated and linked with dotted lines (Fig. 1).

After the initial reduction in bacterial count, growth curves for all of the groups exhibited a similar lag phase which lasted about 5 days before entering the exponential or the log phase. During this phase the rate of microbial growth for the control group was considerably greater than the growth rates of the flora on PHMB-treated groups. The difference in the rate of growth was partly responsible for the longer shelf-life of PHMB-treated birds. But the main extension of shelf-life

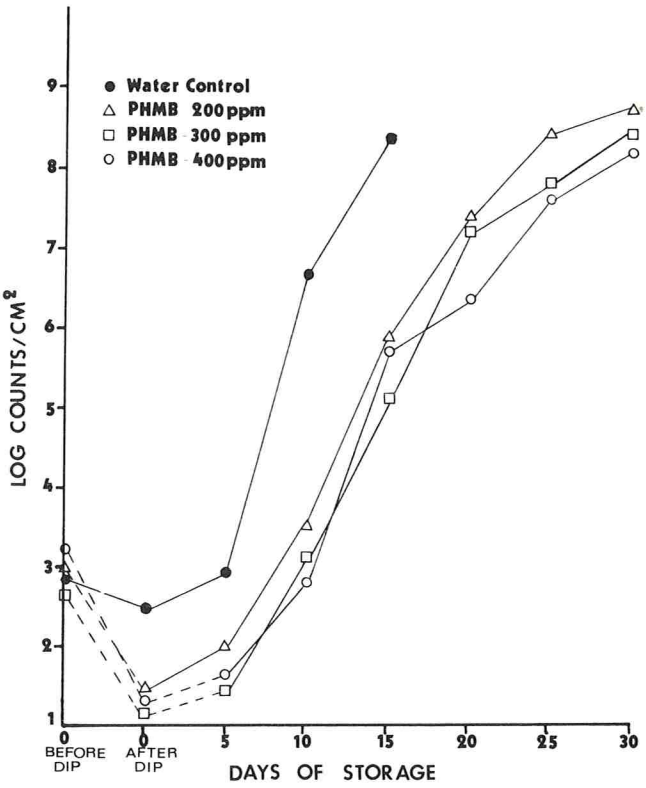

Figure 1. *Total aerobic psychrotrophic counts of poultry carcasses treated with various concentrations of poly (hexamethylenebiguanide hydrochloride) and stored at 2 C. (Points linked with the dotted lines represent estimated counts).*

seems to have originated from the initial bactericidal effect of PHMB.

At each of the sampling intervals, the average bacterial count on the birds dipped in 200 ppm of PHMB was found to be higher than those on the birds dipped in 300 or 400 ppm PHMB. This difference, although very small, was consistent throughout the study. However, there was no such consistent trend in the bacterial counts between the 300- and 400-ppm groups. Overall, the average bacterial counts on all the PHMB-treated birds were very similar despite the large difference in chemical concentration. This suggests that the bactericidal effect of PHMB reached a plateau at about 200 ppm. Perhaps another study with lower concentrations of PHMB would help identify the optimum concentration.

Sensory evaluation

Data on development of off-odor during storage are presented in Fig. 2. The five judgements on each bird were averaged and then the mean score of the three birds per treatment was plotted for each evaluation day. Some of the panel members detected slight off-odor in a few carcasses on the first day of evaluation which indeed was the 8th day of storage at 2 ± 0.2 C. This off-odor, particularly on some PHMB-treated birds, was likened to that of "fresh wheat flour" by two of the panel members. While most of the control samples exhibited strong off-odor on the 10th day of storage, the PHMB-treated samples maintained slight to moderate off-odor until the

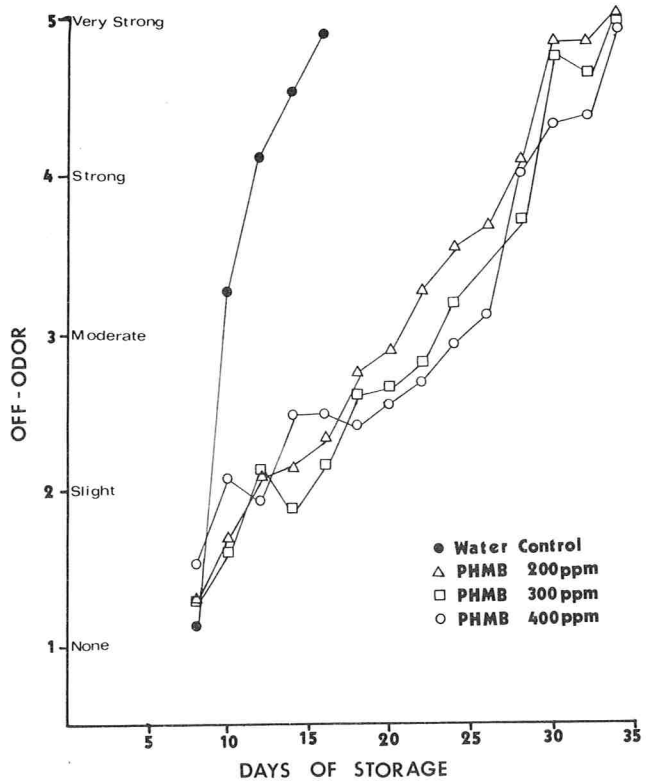

Figure 2. *Off-odor development in poultry carcasses treated with various concentrations of poly (hexamethylenebiguanide hydrochloride) and stored at 2 C.*

20th day. Table 1 lists the shelf-lives of all the 12 birds that were designated for sensory evaluation. These shelf-life data were tabulated based on the number of days the birds maintained a score of 3 or less. Analysis of variance on the 12×5 factorial arrangement showed a highly significant difference ($p < 0.01$) in the shelf-lives of the 12 carcasses. Within the control group, the three birds had shelf-lives of 10.6, 9.6, and 11.4 days, respectively, and these values were considerably lower than the lowest value (19.2 days) with PHMB treatment. Duncan's multiple range test showed that statistically there was no difference ($p > 0.01$) in the shelf-life values of the three control samples. Significant differences ($p < 0.01$) existed among the nine birds within and between the PHMB treatments although there was considerable overlapping (Table 1). Analysis of variance and Duncan's multiple range test on the 4×5 factorial design clearly revealed that there was no significant difference ($p > 0.01$) between the three PHMB treatment in regard to the shelf-life extension. In other words, statistically the shelf-life improvement with 200 ppm was the same as with the 300 or 400 ppm PHMB. This observation on shelf-life seems to be consistent with the effect of PHMB on bacterial count. The effect of 200 ppm PHMB reached a plateau for both bacterial inhibition as well as shelf-life improvement. In view of these findings, another study with lower concentrations of PHMB is being planned.

pH of PHMB solutions

At about 2 C the pH values of the 200, 300, and 400 ppm PHMB solutions were found to be 6.49, 6.39 and 6.34, respectively, compared to the 6.46 value of distilled water used as a control in this study. All of these pH values are well above the minimum required for growth of poultry spoilage bacteria. Hence, the microbial inhibition and the shelf-life extension demonstrated by PHMB was not due to the low pH, as was observed by Mountney and O'Malley (*11*) with succinic and adipic acids.

TABLE 1. *Shelf-life of poly (hexamethylenebiguanide hydrochloride) treated chicken carcasses at storage temperature of 2 C.*

Treatment PHMB (ppm)	Shelf-life (days)[a] Individual carcasses Mean ± S.D.[b]	Overall mean for each treatment
0	10.6 ± .6a 9.6 ± .9a 11.4 ± .9a	10.5x
200	19.2 ± 1.6b 24.8 ± 2.8cd 24.8 ± 5.2cd	22.9y
300	22.4 ± 3.0bc 27.8 ± 3.3de 27.6 ± 2.2de	25.9y
400	29.2 ± 2.5e 27.2 ± 2.5de 21.8 ± 3.5bc	26.0y

[a]Means followed by different letters within each column are significantly different ($p < 0.01$) according to Duncan's multiple range test.
[b]Standard deviation; five judgements on each carcass.

Overall efficacy of PHMB

The mean shelf-lives of poultry using 200, 300 and 400 ppm PHMB were 22.9, 25.9, and 26.0 days, respectively, compared to only 10.5 days shelf-life for the control samples (Table 1). Thus, the PHMB, at levels used in this study, extended poultry shelf-life from 12 to 14 days beyond that of the controls. This degree of shelf-life extension is indeed encouraging since the most recent attempts with other chemicals (*1,7,17*) improved poultry shelf-life a maximum of only 6-7 days. However, one ought to realize that this preservation or the absence of spoilage is based on only the low microbial counts and the lack of off-odor development. No attempt was made in this study to asssess the extent of tissue breakdown due to enzymatic action; also there was no evaluation of the cooking quality of PHMB- preserved chicken. Organoleptic evaluation involving taste, however, cannot be done till there are sufficient toxicological data available on PHMB.

The safety of PHMB is suggested because of its use as a sanitizing agent in swimming pools and for food processing and brewery fermentation equipment. Specific pharmacological and toxicological investigations will be necessary before it can be used as a poultry preservative.

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Colony Count Accuracy Using Selective Media: Analysts Versus Automatic Colony Counters [1,2]

LINDA S. GUTHERTZ* and JOHN T. FRUIN

Letterman Army Institute of Research, Department of Nutrition, Food Hygiene Division, Presidio of San Francisco, California 94129

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ABSTRACT

Using two different methods of counting, a comparison was made of the ability of five analysts to enumerate colonies on selective bacteriologic media. First, analysts manually counted the colonies aided by a Quebec colony counter and a hand tally. This was followed by the counting of colonies on each of three automated colony counters. In this pilot study, the media used included violet red bile agar, KF streptococcal agar, sulfite-polymyxin-sulfadiazine agar, and Standard Methods agar with 2,3,5 triphenyltetrazolium chloride added. The analysts' ability to count colonies on selective media by the manual method was superior to results obtained with the automatic colony counters.

In laboratories doing microbiological analyses of food and dairy products, a significant portion of the workload is represented by the manual counting of colonies on various bacteriologic media. While the *Compendium of Methods for the Microbiological Examination of Foods* (CMMEF) (*4*) makes no suggestion for accuracy standards, *Standard Methods for the Examination of Dairy Products* (SMEDP) (*3*) suggests that laboratory workers should be able to duplicate their own counts on the same plate within 5% and the counts of other analysts within 10%. Provisions are made in the 13th edition of SMEDP for manual counting only. The ability to rapidly and accurately count agar plates with an automatic colony counter (ACC) could represent a considerable labor and cost saving innovation for the laboratory. Fruin and Clark (*1*), in a pilot study, obtained equally accurate counts when analysts and ACCs counted colonies on plates prepared with standard methods agar (SMA).

Since SMA is only one of the media on which colony counts are routinely made, this study was undertaken to compare the plate counting accuracy of analysts and ACCs when other media are used.

MATERIALS AND METHODS

Plates

Throughout the study disposable plastic petri dishes Falcon No. 1029 (Falcon, Oxnard, CA) with a 90-mm inside diameter were used. Using the appropriate dilutions of an overnight broth culture of *Escherichia coli* ATCC 11303, pour plates with violet red bile agar (VRB) were prepared without an agar overlay, and incubated for 24 h at 32 C.

The same dilutions of *E. coli*, prepared above, were plated with Standard Methods agar (SMA/TTC) to which 1 ml of sterile 1% solution of 2,3,5 triphenyltetrazolium chloride per 100 ml of medium had been added before pouring plates.

KF streptococcal agar (KFS) plates were prepared with the appropriate dilution of an overnight broth culture of *Streptococcus faecalis* ATCC 19433 and incubated at 35 C for 48 h.

Sulfite-polymyxin-sulfadiazine agar (SPS) pour plates were prepared from appropriate dilutions of overnight broth cultures of a locally isolated strain of *Clostridium perfringens* type A and of *E. coli* ATCC 11303. Plates were incubated anaerobically for 24 h at 35 C.

Before counting, all plates were screened and given code numbers by the study director.

Analysts

The manual counting of plates, aided by hand tallies and Quebec Colony Counters (American Optical Corp., Buffalo, NY), was accomplished by five analysts having a variety of educational backgrounds and laboratory experience.

Machines

Three machines, generously provided by manufacturers, were used throughout the study. For this study, the machines listed below were randomly designated 1, 2, and 3. (*a*) 3M^{TM} Brand Model 620 Automatic Colony Counter supplied by the 3M Company, St. Paul, Minnesota, (*b*) Model #880 counter equipped with a video monitor supplied by Artek Systems Corporation, Farmingdale, New York, (*c*) Biotran II Automated Colony Counter Model C111 equipped with a video monitor supplied by the New Brunswick Scientific Co., Inc., New Brunswick, New Jersey.

All machines were adjusted so that the colonies counted included those as small as 0.2 mm in diameter for all counts except those of *C. perfringens* on SPS agar. These counts required adjustment of machine sensitivity to 6-mm diameter to provide discrimination between colonies of *C. perfringens* and *E. coli*. Two machines were set to count a 5,000-mm^2 circular portion of each petri plate. The third machine had a fixed counting diameter equal to 5/6 of the surface of the plate.

Treatment of data

The mean of the counts (mean count) for each plate obtained by the five analysts participating in the study was used as the standard to which all counts were compared. Machine counts were multiplied by a compensation factor designed to correct for their tendency to be somewhat lower than manual counts due to masking of colonies around the periphery of the plate. The compensation factor was derived by dividing the sum of the mean counts by the sum of the machine counts.

RESULTS AND DISCUSSION

Laboratories doing routine analyses of food products often count colonies on media other than SMA. Before the use of ACCs becomes a part of the standard analytical method and to justify expenditure for such equipment, the abilities of ACCs to count colonies on other media

[1] *Reprint requests to: Commander, Letterman Army Institute of Research, ATTN: Medical Research Library, Presidio of San Francisco, California 94129.*

[2] *The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.*

should be assessed. This pilot study examined the manual and ACC counts of colonies on VRB, SMA/TTC, KF and SPS agar plates. The distribution of plates of each medium within the various count ranges can be seen in Table 1.

In a study on speed and accuracy in the counting of agar plates, Fruin et al. (*2*) used several statistical procedures to compare the mean of counts by analysts with a true count derived by comparing the agar plate to its photograph. The mean of the analysts' count deviated slightly from the true count; however, it was a consistent, slightly low estimate of the true count and determined to be a practical means of estimating the colony numbers of agar plates. Therefore, the mean of the analysts' counts for each plate counted in this study was used as the standard to which all counts were compared. Table 2 lists the compensation factors used to adjust the machine-produced data.

As stated in SMEDP (*3*) and CMMEF (*4*), a positive presumptive coliform test is indicated when dark red colonies, measuring 0.5 mm in diameter on uncrowded plates having 30 to 150 colonies, appear following 24 ± 2 h of incubation at 32 C on a solid medium such as VRB agar. Presented in Table 3 are the percent of analyst and ACC counts on VRB agar within 5 and 10% of the mean count of plates for each count range. It can be seen that on plates having fewer than 30 colonies, the ACCs were not nearly as accurate as analysts. Within this count range, 41.1% of the counts made by Machine #1 exceeded the mean count by greater than 120%. Of the counts made by Machine #2, 73.3% exceeded the mean by more than 120%, while 45.6% of the counts made by Machine #3 surpassed the mean count greater than 120%. It is noteworthy that if the percent accuracy of counts is considered in 5% increments from 80% through > 120%, that within the count range of 0-29 colonies, the ACCs had significant numbers of counts within all increments. With each machine the greatest percentage of counts exceeded the mean by greater than 120%. When the counts made by analysts are considered in this

TABLE 1. *Number of plates in each count range based on the mean count.*

Count range	Medium			
	VRB	SPS	KF	SMA/TTC
0-29	18	23	32	2
30-99	32	8	4	36
100-199	20	5	0	20
200-299	2	0	0	18
> 300	8	0	0	5
Total plates	80	36	36	81

TABLE 2. *Compensation factors used to adjust machine-produced data.*

Medium	Machine		
	1	2	3
VRB	1.251	1.095	1.304
SPS	1.480	1.653	1.505
KF	1.109	0.842	1.170
SMA/TTC	1.379	1.286	1.248

TABLE 3A. *Percent of colony counts on violet red bile agar within 5% of the mean count.*

Way of counting	Count range				
	0-29	30-99	100-199	200-299	> 300
Analyst					
A	66.7	87.5	90.0	0.0	50.0
B	77.8	56.3	75.0	50.0	25.0
C	55.6	65.6	80.0	50.0	50.0
D	44.4	43.8	65.0	0.0	0.0
E	61.1	50.0	65.0	50.0	25.0
Average	61.1	60.6	75.0	30.0	30.0
Machine					
1	12.2	46.9	48.0	40.0	67.5
2	6.7	32.5	21.0	80.0	37.5
3	7.8	40.6	59.0	10.0	45.0
Average	8.9	40.0	42.3	43.3	50.0

TABLE 3B. *The percent of colony counts on violet red bile agar within 10% of the mean count.*

Way of counting	Count range				
	0-29	30-99	100-199	200-299	> 300
Analyst					
A	83.4	100.0	100.0	100.0	100.0
B	88.9	90.7	100.0	100.0	87.5
C	94.5	96.9	95.0	100.0	50.0
D	72.4	66.3	75.0	0.0	12.5
E	77.8	87.5	90.0	100.0	75.0
Average	83.4	88.3	92.0	80.0	65.0
Machine					
1	23.3	72.5	89.0	60.0	95.0
2	13.3	49.4	58.0	90.0	77.5
3	19.0	68.8	77.0	40.0	75.5
Average	18.5	63.6	74.7	63.3	82.7

respect, most of counts made by Analysts A, B and C ranged from 90 to 110% while counts of Analyst D ranged from 80 to 105% and those of analyst E from 95 to 120%.

It is suggested in SMEDP (*3*) and CMMEF (*4*) that VRB plates not be counted if the colonies exceed 150 in number. In light of this cautionary statement, if the plate count ranges 30-99 and 100-199 are examined, it can again be seen that the analysts outperformed the ACCs with respect to percent of counts within 5 and 10% of the mean count. ACCs performed much more accurately when counts on plates exceeded 300 in number. Growth of too many organisms on a plate prevents formation of typical coliform colonies and results from overloaded plates are often considered unreliable. Therefore, the counting abilities of ACCs within these ranges are not significant when VRB is the plating medium used.

When counting plates, especially those of low dilutions of food products, the decision as to whether or not something is a colony is often difficult to make. Since most bacteria can reduce 2,3,5 tri-phenyltetrazolium chloride, its addition to Standard Plate Count agar has been suggested as a means to alleviate this problem (*4*).Table 4 depicts the percentages of manual and automated colony counts within 5 and 10% of the mean count when SMA/TTC is used as the plating medium. When plates contained colonies numbering between 30 and 299, the machines demonstrated an improvement in the number of counts made which were within 10% of the mean count; however, they still do not appear to equal the accuracy exhibited by the analysts making the manual counts.

KF streptococcal agar appears to present counting problems for both analysts and ACCs. As seen in Table 5, neither analysts nor machines were accurate in counting KF plates with fewer than 30 colonies. When plates contained 30 to 99 colonies, the analysts were able to report counts for all plates which were within 10% of the mean count, while only Machine #1 could come within 10% of the mean count on 75% or more of the counts made on plates within this range.

Table 6 displays the relationship between the mean, manual and machine counts made on SPS agar plates. These plates were prepared with strains of *E. coli* and C. *perfringens* to see if discrimination between the colonies could be made. On this medium, the colonies of the two organisms were of equal size, yet the colonies of *C. perfringens* were surrounded by a zone of sulfide precipitation usually about 6 mm in diameter. To use the ACCs for counts of *C. perfringens* on this medium, the colony size controls on each machine had to be adjusted for the size of the zone surrounding the *C. perfringens* colony. Again, it can be seen that within ranges normally counted on these media, the accuracy of counts made by ACCs could not equal those of the five analysts.

The inaccurate counts made by the machines may be

TABLE 4A. *The percent of colony counts on SMA/TTC within 5% of the mean count.*

Way of counting	Count range 0-29	30-99	100-199	200-299	> 300
Analyst					
A	50.0	88.9	90.0	88.9	100.0
B	100.0	86.1	45.0	55.6	40.0
C	100.0	80.6	80.0	66.7	0.0
D	50.0	66.7	85.0	66.7	60.0
E	50.0	69.4	80.0	83.3	60.0
Average	70.0	78.3	76.0	72.2	52.0
Machine					
1	30.0	30.0	41.0	48.9	68.0
2	10.0	31.1	44.0	71.1	76.0
3	10.0	47.2	52.0	52.2	24.0
Average	16.7	36.1	45.7	57.4	56.0

TABLE 4B. *The percent of colony counts on SMA/TCC within 10% of the mean count.*

Way of counting	Count range 0-29	30-99	100-199	200-299	> 300
Analyst					
A	50.0	97.3	100.0	100.0	100.0
B	100.0	100.0	100.0	94.5	80.0
C	100.0	89.0	85.0	77.8	20.0
D	100.0	98.9	100.0	100.0	100.0
E	100.0	94.4	100.0	100.0	100.0
Average	90.0	95.9	97.0	94.5	80.0
Machine					
1	40.0	75.6	77.0	93.3	98.0
2	40.0	60.0	77.0	95.5	96.0
3	40.0	71.1	73.0	86.6	96.0
Average	40.0	68.9	75.7	91.8	96.7

TABLE 5A. *The percent of colony counts on KF streptococcal agar within 5% of the mean count.*

Way of counting	Count range 0-29	30-99
Analyst		
A	46.9	50.0
B	37.5	100.0
C	46.9	75.0
D	12.5	100.0
E	37.5	75.0
Average	36.3	80.0
Machine		
1	17.5	35.0
2	15.6	15.0
3	21.9	25.0
Average	18.3	25.0

TABLE 5B. *The percent of colony counts on KF streptococcal agar within 10% of the mean count.*

Way of counting	Count range 0-29	30-99
Analyst		
A	75.1	100.0
B	65.7	100.0
C	68.8	100.0
D	28.1	100.0
E	50.0	100.0
Average	57.4	100.0
Machine		
1	40.0	75.0
2	35.6	15.0
3	35.7	45.0
Average	37.1	45.0

in part due to the lack of contrast between colony and growth medium as well as the opacity of the media used. Results of this study seem to indicate that with the selective media routinely used in the food microbiology laboratory, problems are encountered in achieving accurate colony counts when ACCs are used. These results also suggest the need for a more comprehensive study of the use of ACCs in counting colonies on selective media before use of these machines becomes incorporated into laboratory routine.

TABLE 6A. *The percent of colony counts on SPS agar within 5% of the mean count.*

Way of counting	Count range 0-29	30-99	100-199
Analyst			
A	87.0	87.5	60.0
B	73.9	62.5	0.0
C	87.0	87.5	40.0
D	91.3	12.5	0.0
E	87.0	75.0	40.0
Average	85.2	65.0	28.0
Machine			
1	27.0	22.5	12.0
2	5.2	30.0	32.0
3	27.0	35.0	16.0
Average	19.7	29.2	20.0

TABLE 6B. *The percent of colony counts on SPS agar within 10% of the mean count.*

Way of counting	Count range 0-29	30-99	100-199
Analyst			
A	95.7	100.0	100.0
B	95.6	100.0	80.0
C	95.6	100.0	80.0
D	95.6	75.0	60.0
E	95.6	100.0	80.0
Average	95.6	95.0	80.0
Machine			
1	40.0	45.0	32.0
2	22.6	50.0	72.0
3	41.8	60.0	36.0
Average	34.8	51.7	46.7

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Yogurt — A Compositional Survey in the Greater Lansing Area[1]

M. L. RICHMOND, R. C. CHANDAN* and C. M. STINE

Department of Food Science and Human Nutrition, Michigan State University, East Lansing, Michigan 48824

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ABSTRACT

Prompted by numerous consumer inquiries and several reports in the literature, this survey was undertaken to monitor the composition of yogurt in the mid-Michigan market. Forty-seven samples representing six brands were analyzed. Mean values ± standard deviation for the content of protein (Kjeldahl), fat (Mojonnier) and total solids (Mojonnier), pH and net weights were measured. The data are presented by product category, i.e. low-fat flavored, low-fat plain, full-fat flavored, full-fat plain, and cumulated for all samples. Wide variations in chemical composition were observed between and within brands surveyed. Mean values for all flavored samples surveyed (N = 42) were 4.34% protein, 2.34% fat, 25.88% total solids and 4.01 pH. Corresponding values for all plain samples surveyed were 5.68, 2.86, 16.90 and 4.23, respectively. The data show that 25% of all samples analyzed were greater than 6.6% overweight while 10.6% of the yogurts surveyed weighed less than the declared container net weight. Caloric values for flavored yogurts ranged widely. Mean caloric values for flavored, low-fat and full-fat brands were 106 and 121 cal/100 g, respectively. In general, the results indicate that commercial yogurt would benefit from closer composition control.

Yogurt consumption in the United States has increased from 0.11 lb per capita in 1955 to 2.80 lbs per capita by 1977, an increase of nearly 2500%. From 1970-1976 the per capita consumption increased 170% (*4*). In 1976, total sales of yogurt were estimated at 510 million pounds (*9*) and annual sales for 1977 exceeded 600 million pounds (*1*). Initial projections for 1978 indicate that about 0.5% of all fluid milk produced in the U.S. will go into yogurt manufacture (*2*).

Previous yogurt surveys have shown wide variation in the chemical composition of commercial yogurts. Duitschaever et al. (*6*) surveyed 152 yogurts in Ontario, Canada and found that yogurt of uniform composition was generally not available. It was further stated that this was evident both between and within brands surveyed. Net weights ranged widely with a mean overfill of 7.2%. The pH values varied from 3.27-4.53 with a mean value of 3.91. In 1973, Kroger and Weaver (*7*) surveyed commercial yogurts in the Central Pennsylvania area (41 fruit and 3 plain) and found constant overfill to be a major problem in this region also. Samples surveyed were inconsistent in terms of product net weights, with a mean overfill of 6.87%. Also noted in this survey were wide variations in protein, fat, total solids, pH and caloric content. In yet another survey of yogurts in the United Kingdom (*5*), the data revealed appreciable differences in net weight, pH, total solids, fat, protein, ash and sugar content. The survey reported in this paper was made recently in the Greater Lansing Area to assess the chemical composition of various yogurts from commercial markets with the objective of making available meaningful data for use by the industry.

MATERIALS AND METHODS

Samples

Forty-seven samples, encompassing six major brands, were analyzed for total protein, fat, total solids, pH, net weight and caloric content. The samples were purchased in the Greater Lansing area (near MSU) and included both Sundae- and Swiss-style yogurts.

Sample preparation

All containers were weighed to determine their gross weight. The contents were then transferred quantitatively to a Waring blender and mixed at high speed for 3 min to achieve homogeneity (*6,7*) for subsequent analyses.

Product net weight

The gross container weights (yogurt + container) were determined using a Mettler P1000 balance. After transferring and blending as mentioned earlier, the containers, including tops, were rinsed with distilled water and air-dried. The containers were then reweighed and this value was subtracted from the gross weight to obtain the corresponding net weights.

Protein content

Total protein was determined by an official AOAC Kjeldahl method. The factor 6.38 was used in conversion of reduced nitrogen values to protein (*3*). All chemical analyses were done in duplicate.

Fat content

Percentage fat was determined by the Mojonnier modification of the Roese-Gottlieb extraction (*8*).

Total solids

Percentage total solids was determined by the Mojonnier vacuum oven procedure (*8*).

pH

pH of the yogurt samples was measured using a digital pH meter equipped with glass and calomel electrodes (Chemtrix Model 60A). A buffer solution of pH = 4.01 was used for scale calibration.

Caloric content

The caloric value of each sample was determined by calculation as suggested by Kroger and Weaver (*7*), with a slight modification. Calories/100 g = % fat × 8.79 + [% total solids − (% fat + ash)] × 4. Mean ash contents of flavored yogurt and plain yogurt were experimentally determined to be 1.02 and 0.93%, respectively (*10*).

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RESULTS AND DISCUSSION

The results presented herein show mean values plus or minus standard deviation for various parameters. A total of 47 individual samples were analyzed, of which 28 were fruit-flavored. Due to the lower consumption of plain yogurt, only five different commercial brands were available.

Composition of various brands of low-fat flavored yogurt

Examination of the data collected on composition of flavored yogurt reveals considerable variation of a single constitutent within a particular brand. These variations range from wide to minimal and should therefore be of concern from an industrial quality control standpoint. There was also wide variation found in the composition between brands. With low-fat fruit flavored yogurt dominating the market (75-90% of total yogurt sales in the U.S.), it is noteworthy to point out that Brand II (Table 1) ranged widely in terms of its chemical composition; 3.68-4.42% protein, 1.29-1.63% fat, 21.84-27.02% total solids and 3.77-4.14 pH. In terms of the chemical composition, these data are representative of the other brands tested.

TABLE 1. *Chemical composition of various brands of low-fat flavored yogurt*[a].

Product category	Protein (%)	Fat (%)	Total solids (%)	pH
Brand I (N = 5)	4.36 ± 0.22	1.60 ± 0.05	24.80 ± 3.13	4.14 ± 0.11
Brand II (N = 13)	3.97 ± 0.23	1.45 ± 0.10	25.26 ± 1.72	3.98 ± 0.11
Brand IV (N = 6)	4.74 ± 0.08	1.37 ± 0.05	27.25 ± 0.95	4.27 ± 0.11
Brand V (N = 4)	4.33 ± 0.04	2.20 ± 0.13	25.58 ± 1.65	3.99 ± 0.06

[a]Mean ± standard deviation.

The values obtained for full-fat yogurts (not shown) showed wide variation in fat content. The mean values for percent fat of the two brands surveyed were 2.98 and 4.90% with a range of 2.39 to 5.32%. The mean pH of these samples was less than 4.00.

Composition of flavored yogurt

The data in Table 2 compare the mean values of all flavored yogurts, including both low and full-fat brands. These data for low-fat fruit flavored yogurt are in general accord with the published values from Handbook 8-1 (*10*). However, Handbook 8-1 gives values for all types of yogurt except full-fat fruit flavored yogurt. Therefore, no general comparisons can be made for full-fat flavored yogurts. It would seem advisable, with increasing production and consumption of yogurt in the U.S., to make available in the future published data on full-fat flavored yogurt.

TABLE 2. *Chemical composition of various flavored yogurts*[a].

Product category	Protein (%)	Fat (%)	Total solids (%)	pH
Low fat yogurt (N = 28)	4.26 ± 0.35	1.56 ± 0.28	25.83 ± 2.17	4.07 ± 0.16
Full fat yogurt (N = 14)	4.51 ± 0.18	4.01 ± 1.00	26.39 ± 1.58	3.88 ± 0.13
All samples (N = 42)	4.34 ± 0.33	2.34 ± 1.29	25.88 ± 1.99	4.01 ± 0.17

[a]Mean ± standard deviation.

In comparing the data in Table 2, one finds that in addition to the higher fat content, protein and total solids content were also found to be greater in the full-fat brands analyzed. The pH values were comparatively different; the mean pH of full-fat products was 3.88 compared to an average of 4.07 for the low-fat yogurt.

Composition of plain yogurt

Both full- and low-fat plain yogurts surveyed (Table 3) were similar in protein content. Mean values for percent fat, percent total solids and pH were all found to be greater in the full-fat yogurts.

TABLE 3. *Chemical composition of various brands of plain yogurt*[a].

Product category	Protein (%)	Fat (%)	Total solids (%)	pH
Low fat yogurt (N = 3)	5.69 ± 0.73	1.62 ± 0.04	16.25 ± 1.01	4.22 ± 0.17
Full fat yogurt (N = 2)	5.66 ± 0.24	4.71 ± 1.54	17.88 ± 2.61	4.26 ± 0.35
All samples (N = 5)	5.68 ± 0.58	2.86 ± 1.79	16.90 ± 1.99	4.23 ± 0.26

[a]Mean ± standard deviation.

In comparing the data for low-fat flavored and low-fat plain (Table 2 vs. Table 3) the results indicated higher pH, protein and fat content in the low-fat plain yogurt while the flavored low-fat yogurts had larger values for percent total solids. The results were similar for full-fat yogurts (flavored vs. plain) in that pH, protein and fat content were all found to be greater in the plain full-fat yogurt samples analyzed. Mean total solids contents were greater in the full-fat flavored yogurts as would be expected, and mean pH values for full-fat flavored yogurts were notably less than for full-fat plain yogurt.

Net weights of flavored low-fat yogurt

Net weights are shown in Table 4. Overweight seemed to be a common denominator of low-fat flavored yogurt (actually of most yogurt examined) with a mean value for the 28 yogurts surveyed being nearly 5% overweight. Brand I had a mean net product overweight of about 2.2%. Brand II was a striking 7.5% overweight, while Brands IV and V had net product overweights of 2.3 and 1.3, respectively. Considering all low-fat flavored yogurts surveyed, results showed a range from 1.5% under

TABLE 4. *Net weight of various brands of flavored low-fat commercial yogurts.*[a]

Product category	Net weight[b] (g)
Brand I	231.9 ± 3.69
Brand II	244.0 ± 5.15
Brand IV	232.6 ± 2.63
Brand V	229.9 ± 5.83
All samples (N = 28)	237.8 ± 7.78

[a]Mean ± standard deviation.
[b]Declared weight = 227.0 g.

declared container net weight to as high as 12.6% over declared container net weight. In summarizing the data for full-fat yogurts (not shown), Brand IV on the average was 4.7% greater than its declared container net weight, while Brand III averaged only 0.4% overweight. In general, it appears from these data that yogurt consumers are getting more than they are paying for.

Caloric content of yogurt

Table 5 shows estimates of caloric content, cal/100 g, of various commercial yogurts. These values are based on the caloric equation used by Kroger and Weaver (*7*). The mean caloric values for flavored low-fat and full-fat yogurts were 106 and 121 cal/100 g, respectively. Mean values for plain yogurt ranged from 69 cal for low-fat plain to 92 cal/100 g for full-fat plain. From these data it is apparent that low-fat plain yogurt contains 25% less calories than full-fat plain yogurt. Furthermore, it is evident that in relation to low-fat plain yogurt, full-fat and flavored yogurts account for a much increased caloric density on a per container basis. Flavoring addition alone accounted for approximately 30 cal/100 g for both the full and low-fat samples surveyed. Also of interest are the caloric ranges found in flavored yogurts. Low-fat flavored yogurt ranged from 80-120 cal/100 g while full-fat flavored samples ranged from 102-135 cal/100 g. Considering the wide variation in caloric content of market yogurts along with an overfill of almost 5% for all flavored samples surveyed, the caloric content of many yogurts may be substantially greater than the value indicated on the container.

TABLE 5. *Calculated caloric content of various commercial yogurts.*[a]

Product category	Calories/100 g
Flavored:	
Low fat (N = 28)	106 ± 9
Full fat (N = 13)	121 ± 10
Plain:	
Low fat (N = 3)	69 ± 4
Full fat (N = 2)	92 ± 20

[a]Mean ± standard deviation.

The data presented indicate that there is still much variation in yogurt composition not only between brands but within the same brand. Moreover, the results of the analyses obtained in this survey are in general agreement with those reported in the literature (*5,6,7*) in that wide variations were observed in gross composition. Apparently there has been little effort to standardize yogurt during the past 7 years despite the fact that better uniformity in composition and quality would be beneficial to both consumer and producer.

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A Research Note

Comparison of Acidified and Antibiotic-supplemented Potato Dextrose Agar from Three Manufacturers for its Capacity to Recover Fungi from Foods

L. R. BEUCHAT

Department of Food Science, University of Georgia Agricultural Experiment Station, Experiment, Georgia 30212

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ABSTRACT

Six lots of potato dextrose agar (PDA) obtained from three manufacturers were compared for their capacity to recover yeasts and molds from 10 foods. Each lot was tested in two forms, viz., at pH 3.5 and at pH 5.6. The media at pH 5.6 contained 100 ppm each of chloramphenicol and chlortetracycline-HCl. Overall, no single source or lot of PDA was superior to others for enumerating fungi. However, results confirm earlier reports from another laboratory that antibiotic-supplemented PDA performs better than does acidified PDA for determining fungal populations in foodstuffs.

Acidified media traditionally have been used to enumerate yeasts and molds in foods. White and Hood (*12*) were among the first to report that malt agar adjusted to pH 3.4 to 5.8 with lactic acid effectively inhibited growth of bacterial colonies, but did not significantly affect yeast and mold counts of butter. Later studies indicated that at higher pH values, media supplemented with antibiotics were more suitable for recovering fungi from a variety of foods and beverages (*2-7,9,10*). Growth of some yeasts and molds may be retarded at pH below 4, especially if cells have been subjected to environmental stress such as heat (*11*), chilling, or desiccation. Other shortcomings are inherent in acidified media (*8*). Mold colonies have a tendency to spread, there is occasional growth of bacteria, and food particles may precipitate.

In a recent study (*1*), it was observed that potato dextrose agar (PDA) and plate count agar adjusted to pH 3.5 and 5.5 supported colony formation by conidia of *Aspergillus flavus* equally well. Koburger (*4*) attributes the lack of difference in recovery between acidified and unacidified media to the more vigorous condition of laboratory cultures. The possibility also exists that slight differences in formulation of a given recovery medium obtained from various suppliers or produced in different lots by the same supplier may contribute to variations in counts observed at various pH values. To test this hypothesis, a study was conducted in which six lots of PDA manufactured by three companies were compared for their capacity to support colony formation by yeasts and molds.

Ten foods were analyzed using PDA containing no antibiotics at pH 3.5 and containing 100 ppm each of chloramphenicol and chlortetracycline-HCl at pH 5.6 ± 0.2.

MATERIALS AND METHODS

Foods were obtained from local retail stores in the Griffin, Georgia area. Ground beef (fresh), ham hocks, Cheddar cheese, potatoes (hash, frozen), tomatoes (raw), blueberries (frozen), figs (fresh, raw), corn meal, wheat flour, and thyme (dried) were analyzed.

Four 20-g subsamples of each food were each blended (Colworth Stomacher) with 180 ml of sterile deionized water for 2 min. Homogenized foods were further diluted in sterile water and plated in duplicate in six different lots of PDA purchased from three manufacturers: Difco (Detroit, MI), Baltimore Biological Laboratories (Cockeysville, MD), and Oxoid Ltd. (Basingstoke, U.K.). Two lots of PDA had been stored at room temperature for at least 5 yr. Each lot of PDA was acidified to pH 3.5 ± 0.1 with sterile 10% tartaric acid; a second test system consisted of PDA (pH 5.6 ± 0.2) to which 100 ppm each of chloramphenicol and chlortetracycline-HCl were added after sterilization. Plates were incubated at 21 C and colonies were counted after 5 or 6 days.

Means of counts for each food sample were analyzed for significant differences ($P \leq 0.05$) using Duncan's multiple range test.

RESULTS

The foods examined and yeast and mold counts obtained using six lots of modified PDA are listed in Table 1. There is no trend to indicate that a particular source or lot of PDA was superior for supporting colony development. Although significantly higher counts ($P \leq 0.05$) were noted when using a given test lot of PDA for some foods, the same lot was inferior to others for other test foods. Predominant fungal genera varied with the food sample. It is possible that certain types of fungi grew more readily on some lots of PDA than on others. This would have little or no practical significance since it is extremely difficult to predict predominant mycoflora on or in most foods. The choice of manufacturer of PDA, then, is arbitrary at best with regard to superiority for enumerating total yeasts and molds in a variety of foods.

Antibiotic-supplemented PDA was clearly superior to acidified PDA for enumerating yeasts and molds. Considering the six lots of media and 10 food samples,

TABLE 1. *Mean (log_{10}) counts of yeasts and molds per gram of food as determined using six lots of PDA.*

Lot code for PDA	Modification to PDA[a]	Log_{10} viable count per gram[b]									
		Ground beef	Ham hock	Cheese	Potato	Tomato	Blueberries	Fig	Corn meal	Wheat flour	Thyme
A	Acid	3.64b	2.86efg	2.88de	1.20c	1.89bcd	5.95ef	2.52bcd	4.74bc	1.93cde	3.30bc
	Antibiotic	3.81a	3.12cde	3.00abc	1.57a	1.95abcd	6.03bcd	2.61abc	4.85a	2.16abc	3.41ab
B	Acid	3.42cd	2.76g	2.85e	1.36abc	1.93bcd	5.99de	2.32de	4.71c	1.95bcde	3.31abc
	Antibiotic	3.83a	3.40ab	2.91cde	1.36abc	1.84cd	6.03bcd	2.61abc	4.85a	2.07bcd	3.46a
C	Acid	3.50c	2.83fg	2.92bcde	1.28bc	1.95abcd	6.01cde	2.41cde	4.70c	1.87def	3.29bc
	Antibiotic	3.84a	3.36abc	2.96abcd	1.56ab	2.05ab	6.08ab	2,58abc	4.83a	2.36a	3.36ab
D	Acid	3.35d	3.06def	2.97abcd	1.56ab	1.97abcd	6.07abc	2.52bcd	4.75bc	1.66f	3.32ab
	Antibiotic	3.89a	3.42ab	2.99abc	1.30abc	1.93bcd	6.10a	2.70ab	4.80ab	2.18ab	3.34ab
E	Acid	2.83f	2.91defg	2.84e	1.38abc	1.87bcd	5.87gh	2.41cde	4.69c	1.66f	3.17c
	Antibiotic	3.81a	3.17bcd	3.04a	1.32abc	2.03abc	6.00de	2.80a	4.87a	2.13abc	3.36ab
F	Acid	3.18e	2.98defg	2.91cde	1.15c	1.80d	5.89fg	2.17e	3.93e	1.73ef	3.16c
	Antibiotic	3.84a	3.44a	3.01ab	1.51ab	2.12a	5.82h	2.52bcd	4.40d	1.81ef	3.42ab

[a]Potato dextrose agar (PDA) was acidified to pH 3.5 or supplemented with 100 ppm each of chloramphenicol and chlortetracycline-HCl (pH 5.6 ± 0.2).
[b]Means in the same column followed by the same letter are not significantly different ($P \leq 0.05$).

counts obtained on PDA containing 100 ppm each of chloramphenicol and chlortetracycline were significantly higher in 33 of 60 (55%) tests as compared to acidified PDA. In only one test (blueberries, lot F) did acidified PDA support significantly higher numbers of colonies compared to antibiotic-supplemented PDA. No significant differences were noted between acidified and antibiotic media in 26 of 60 (43.3%) tests. Molds present in the foods examined did not spread on various PDA media after 5 days at 21 C to an extent that accurate counting of colonies was difficult.

DISCUSSION

The types of colonies formed on various lots of PDA were similar with regard to genus when considering a specific food; however, colony size was slightly restricted on media from one manufacturer and, for certain foods, pigmentation of colonies differed somewhat on PDA manufactured by a second company. Although specific lots of PDA performed significantly better for enumerating yeasts and molds in certain foods, based on data reported here, one cannot conclude that any one source or lot is superior for all foods. The cost of one of the three manufacturers' products is approximately 30% higher than the other two, a fact that should be considered when purchasing PDA for routine examination of fungal contamination in foodstuffs.

Koburger (*4,5,7*) reported that PDA (pH 7.0) containing 100 ppm of chloramphenicol and chlortetracycline-HCl was superior to acidified (pH 3.5) PDA for enumerating yeasts and molds in a variety of foods. In another study (*6*), he recommended that for routine laboratory analysis pH 5 to 6 is preferred, since the effectiveness of antibiotics used to suppress bacteria is diminished at pH 8 and above. Data reported here confirm the latter report. Upon microscopic examination of selected colonies formed on plates containing antibiotic-supplemented PDA, no bacteria were observed. On the other hand, those fungi unable to initiate growth at pH 3.5 because of impairment induced by previous exposure to adverse environments would presumably be more likely to grow at pH 5.6.

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Conditions for Use of Food Additives Based on a Budget for an Acceptable Daily Intake[1]

SØREN C. HANSEN

The National Food Institute, Mørkhøj Bygade 19, 2860 Soborg, Denmark

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ABSTRACT

Use of food additives is regulated qualitatively in the European Common Market through the EEC directives on food additives, while the concept of Acceptable Daily Intake (ADI) provides a quantitative expression of safe amounts for the guidance of regulatory agencies. It is suggested that a permissible quantity or quantities, the ceiling, should be agreed upon for each permitted additive on the basis of its ADI and in accordance with the procedure described here. The estimation of intake of food and drink starts from the child, who on the basis of body weight has the highest consumption. When dealing with total intake (expressed as energy, weight or volume per kg body weight per day), occupational and climatical variations between adults are largely contained in the difference between child and adult. It is possible to calculate the highest concentration in foodstuffs which is consistent with the ADI, under the assumption that the additive occurs evenly distributed in the whole diet of a child. This concentration is called the primary ceiling. To obtain the technological effect, however, higher concentrations may be needed, and to accomodate this the ceiling may have to be raised. This can be done if the use of the additive can be excluded from or reserved for part of the diet.

The method described here has its origin in talks 20 years ago with people interested in the fluoridation of drinking water. It was in a way fascinating that 1 mg of fluoride per liter of water gave optimal inhibition of dental caries, while 0.5 mg gave very little protection in a population, and 2 mg was causing slight mottled enamel of the teeth in some individuals. How is it possible with the proportions of half-one-two, to move from practically ineffective to optimal and then to "toxic" doses of the chemical fluoride for a population as a whole?

Many other toxic substances occur naturally in foods and beverages, often in amounts such that the margin of safety is rather small. The normal solanine content of potatoes is safe irrespective of eating habits, simply because satiety is reached before a toxic amount is consumed. Potatoes are safe food in Ireland and more so in countries where they are not the staple starchy food. Alcoholic beverages are classed as toxicants, and are taxable if they contain more than 2% ethanol, but levels below that are considered safe. An idea which immediately suggests itself is that thirst is the controlling factor for fluoride and ethanol, the degree of dilution of the toxic substance restricting its intake and being decisive for safety. Similarly the intake of energy-giving foodstuffs and accompanying substances is controlled by appetite.

As is shown below, our knowledge about the physiological quantities, liquid and energy intake, can be used to limit the intake of food additives to amounts considered acceptable by the toxicologists. This procedure may at first sight look unduly restrictive because of the great variety of eating habits and patterns of consumption: An Eskimo selects his diet differently than does a Mediterranean peasant. But nevertheless the nutritionists' message (*8*) about too high a fat, sugar, and alcohol consumption, and their recommendation to increase the cereal consumption has a general address. This leads to the belief that there are many similarities, and these should of course receive primary consideration when conditions of use of food additives are being defined.

Let us take fat consumption and use of antioxidants as examples. A legal provision that relates the permissible level of antioxidants to the fat content of the food commodity in question may aim at toxicological protection of the high-fat consumers with 50% of their energy intake from fat, the average-fat consumers with 40% from fat, or the optimal-fat consumers with only 30% from fat, but such small differences are of minor importance and could be disregarded when dealing with relatively non-toxic substances like food additives. That an antioxidant is at first being proposed for preservation of, e.g., lard only should not lead to legal provisions and limitations of use based on average lard consumption figures because such consumption varies considerably. The lard consumption will probably increase in some part of the population due to the use of the antioxidant, but the percentages for fat energy given above still hold true.

ACCEPTABLE DAILY INTAKE (ADI)

The Joint FAO/WHO Expert Committee on Food Additives (*6,9*) has introduced the concept of zones of acceptable daily intake (ADI) of food additives and applied the concept to compounds under appraisal. The ADI of an additive is established from the results of prolonged feeding experiments with animals. (Some toxicological quarters are now considering the Weekly Intake as more appropriate than the Daily Intake). From

[1]*This paper is a revision of a paper prepared for an EEC-colloquium June 19-21, 1978.*

the level of intake that produces no effect the ADI for man is estimated by application of a safety factor and expressed in miligrams per kilogram of body weight per day:

No effect level in animals	→	*ADI for man*
100 *a* mg/kg b.w./d.		*a* mg/kg b.w./d.

First to be discussed in the following is how knowledge about liquid and food (energy) intake can be used to insure that ADI is not being exceeded.

Intake of beverages

Fig. 1 shows the correlation between the recommended daily intake (*4*) of liquid and age, when the liquid intake is presented on the basis of body weight. It shows a prominent steep decline during the earliest period of life. There is, of course, a certain variation in liquid intake, but for our purpose we need only be concerned with the variation in high intake. If we choose the recommended liquid intake at the age of two, 100 ml per kg body weight per day, as the basis for our calculations of intake we cover the child and we also cover the adult. A 60-kg man is protected even if he drinks up to 6 liters per day which meets his requirement under extreme working conditions (*1*).

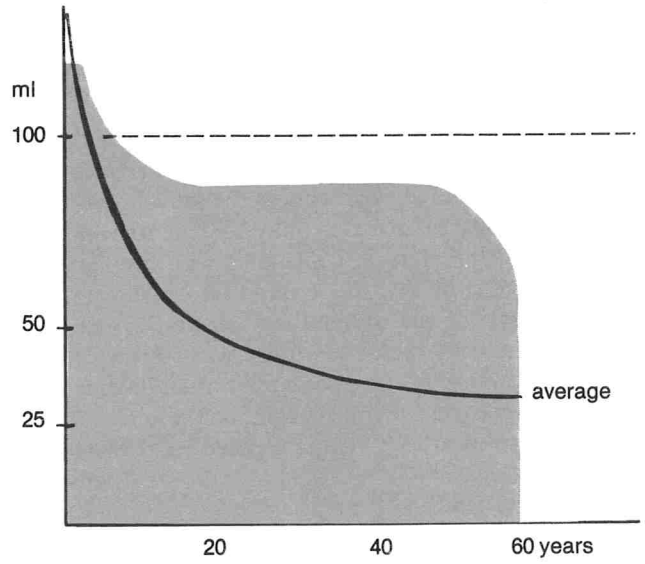

Figure 1. *Variation in liquid intake with age expressed per kg of body weight per day.*

One hundred ml per kg of body weight per day is an important landmark for our further deliberations. This landmark is not likely to emanate from an ordinary consumption survey. The presentation will be just a swarm of randomly scattered points, unless age and body weight of the investigated persons have been recorded.

When ADI is *a* mg/kg b.w./d. this means that any chemical that is added to only beverages is permissible in concentrations of up to *a* mg/100 ml, or 10 *a* mg/liter (= the primary ceiling).

To take an example, the ADI (a) for a food coloring may be 10 mg/kg b.w./d., and it is used only in beverages and in concentrations below 10 *a*, which is 100 ppm (100 mg/liter). The ADI will not be exceeded if a child just quenches its thirst with the beverage and if an adult does not drink more of it than 5-7 liters/day.

Intake of food (energy)

Man needs both drink and food, and food means energy. The next figure (Fig. 2) is drawn up in a similar way as the first one. It shows the correlation between the recommended daily intake (*4*) of energy and age. The energy requirement is presented on the basis of body weight and we note again the remarkable steep decline during the first few years of life which enables us to identify a landmark or starting point for estimation of intake, namely 100 kcal per kg of body weight per day.

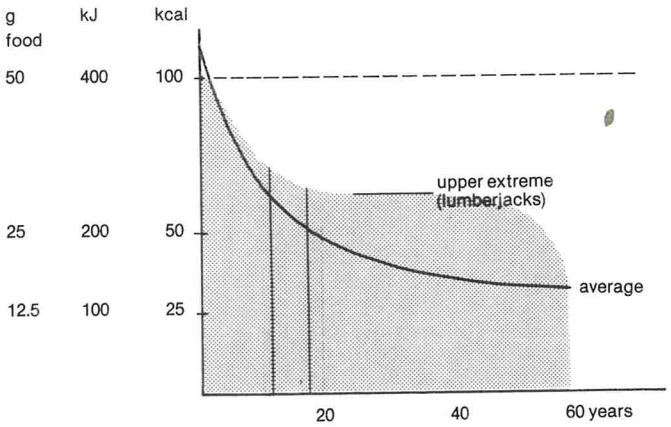

Figure 2. *Variation in energy intake with age expressed per kg of body weight per day.*

The daily energy intake of children per unit of body weight exceeds that of adults. Under very heavy conditions of work, such as those of Canadian lumberjacks, intakes of 60-70 kcal/kg of body weight per day have been recorded (*1*).

Food is eaten largely to satisfy energy requirements. To achieve and maintain proper weight for age, the amount of food consumed over a period must reflect energy needs fairly precisely. For no age group, when energy requirements are met, may the foods contain more of an additive than ADI (*a* mg/kg b.w./d.). For balance, energy intake and the ADI of additives are expressed on the basis of body weight.

How much is 100 kcal (Fig. 3)? It depends on the food we are talking about. For butter and margarine 100 kcal corresponds to 12 g, for sugar it is 25 g and for average food including milk but excluding other beverages, it corresponds to 50 g.

In this context, bulk and the energy density of the food are important. Fruit by itself is not a satisfying food in the sense that it does not readily produce a feeling of satiety, but with sugar and whipped cream it is. New products such as low-fat margarine and mayonnaise, low-sugar marmalade, semi-dried plums, and other semi-moist foods, are readily accepted because they are not too heavy and not too watery. Such foods fit into a dietary

WEIGHT OF 400 KJ (100 KCAL):

50 GRAMS OF AVERAGE FOOD, OR

25 GRAMS OF SUGAR, OR

12 GRAMS OF MARGARINE OR BUTTER

Figure 3. *One hundred kcal are provided by different amounts of food.*

pattern with an energy value of about 400 kJ (kcal) per 50 g.

If a child 1 year of age is used as a reference subject, the ADI is not exceeded if 50 g of food equivalent to 400 kJ does not contain more than the ADI (*a* mg). Except for very low-energy foods, *a* mg of an additive can always be allowed in 50 g of food, i.e., 20 a mg/kg (20 *a* ppm). That is the primary ceiling on the level of use of the additive.

SAFETY MARGIN ALLOWS A FACTOR OF 2 (2 X 20 *a* = 40 *a* ppm)

The ceiling of 20 *a* ppm is based on premises different from those on which the ADI is established (*6*). The 100-fold safety margin is meant to cover, among other things, the difference between children and adults and the variation in food intake among adults. Fig. 2 shows that these variations are largely circumvented in the present approach. It is therefore taken for granted that, except for baby foods, a factor of 2 can be allowed, hence the primary ceiling of 20 *a* can be raised to 40 *a* ppm.

NEW FOOD PRODUCTS AND ADDITIVES

It is generally agreed that an additive should be permitted only when it is needed, and that the amount used should be in accord with good manufacturing practice. Furthermore, if the health authorities would indicate the ceiling to which each additive could be used without endangering public health, the industry would be free to develop new food products within this framework. New products (Fig. 4) might become popular and ingested in substantial amounts, but as the intake of additives would be controlled by appetite and thirst we and our children may eat and drink according to custom, ingesting additives in any amounts up to the ADI. The system is named a budget for ADI, because new products can always be accepted within the ceiling.

The EEC directives on food additives at present contain lists with names of permitted chemicals. Such qualitative rules will need to be worked out with rules for the permitted quantities of each additive. Experience within the EEC framework shows that the horizontal approach (i.e. general rules for all foodstuffs) has advantages over the vertical approach (i.e. specific

Figure 4. *New products containing additives may be developed.*

directives for the various foodstuffs). The horizontal approach can be maintained after setting permitted quantities only if the ADI is budgeted for. The horizontal approach becomes possible if the ceiling is not lower than the technologically effective level. If a full horizon (i.e. that the additive may in principle occur in all foodstuffs) demands too low a ceiling, the additive may be permitted in part of the diet, e.g., in half or a quarter of the horizon. Variations less than 2-fold are not considered.

RESERVED OR EXCLUDED USES OF FOOD ADDITIVES

National food balance sheets prepared by the FAO (*3*) have shown that the diet becomes adjusted to the gross national product by changes in the proportion of energy derived from various sources. Other investigations have shown that in affluent countries the contributions of various food groups to the energy intake of the people are as follows.

Cereals show a downward trend toward 25%
Fat, total, tends to move upward, approaching 50%
Fat, visible, is almost constant at 18%
Meat, fish and *eggs* are approaching 25%
Milk and other dairy products are almost constant at 10-13%
Sugar is moving slowly upward from about 16-18%

If a fully horizontal approach is not possible, the budget should be based on bread groups of foodstuffs akin to the dietary elements (fat, sugar, protein, complex carbohydrate) used in nutritional considerations. On this point, the ADI-budget is at variance with the plan originally suggested from toxicological quarters (*6*) who foresaw detailed food consumption surveys and "book-keeping" on that basis.

ESTIMATED HIGH INTAKE OF CATEGORIES OF BEVERAGES

It is also possible to select categories of beverages which are not likely to be the only beverage in a high consumption situation. Figure 5 presents three alternatives. The first alternative is the fully horizontal

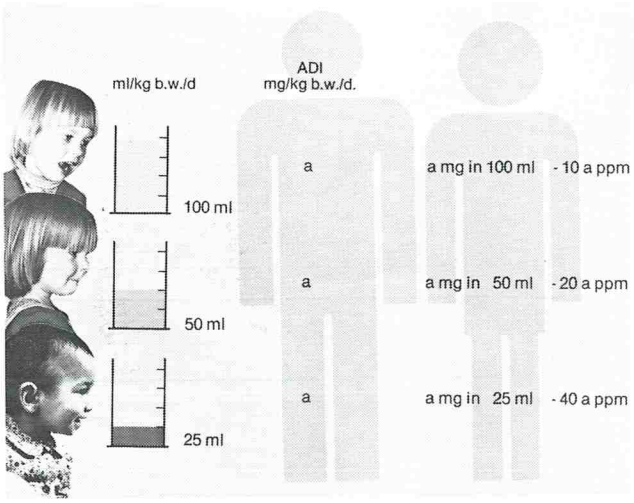

Figure 5. *Three alternatives in consumption of a beverage.*

approach, which assumes an intake of 100 ml/kg b.w./d., and which with an ADI of *a* mg/kg b.w./d. leads to a ceiling of 10 *a* mg per liter, protecting both child and adult. The second alternative foresees a high intake of 50 ml and the third alternative assumes that high intake is only 25 ml/kg b.w./d. If the technological requirement cannot be met under the first alternative, consideration must be given to the next alternative, which allows a doubling of the ceiling, or to the third alternative which allows a redoubling.

Figure 6 contains suggestions as to high intake figures for beer, wine and soft drinks. It should be noted that because of competition in the intake pattern such high intake figures are not additive. If the same substance is of interest in all three categories of beverages 50 ml/kg b.w./d., and not 100, should be used for the calculation. In making the choice between these three alternatives (and no further alternatives seem possible), both judgement and data on beverage consumption should be used. For a man weighing 60 kg, the estimated intake of beer is 3 liters which seems to give reasonable protection.

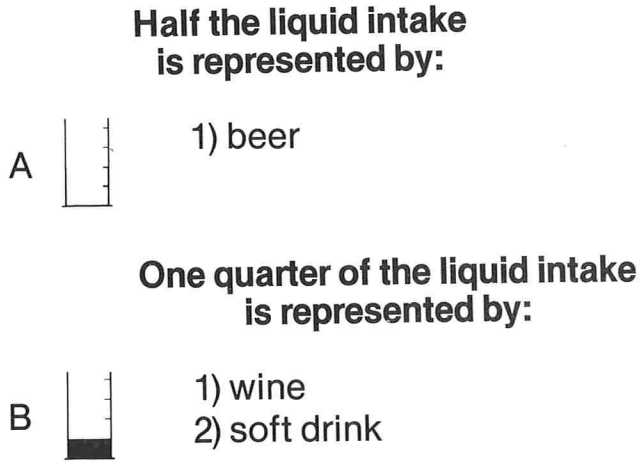

Figure 6. *Values for a high intake of beer, wine, and soft drinks.*

ESTIMATED HIGH INTAKE OF FOOD CATEGORIES

Figure 7 presents three alternatives: 50 g, 25 g and 12 g/kg b.w./d. If the door should be kept open for a technologically promising new additive to be used in a great variety of foodstuffs, it may be wise to pick the first and most cautious alternative. The ceiling is doubled and redoubled in alternatives 2 and 3.

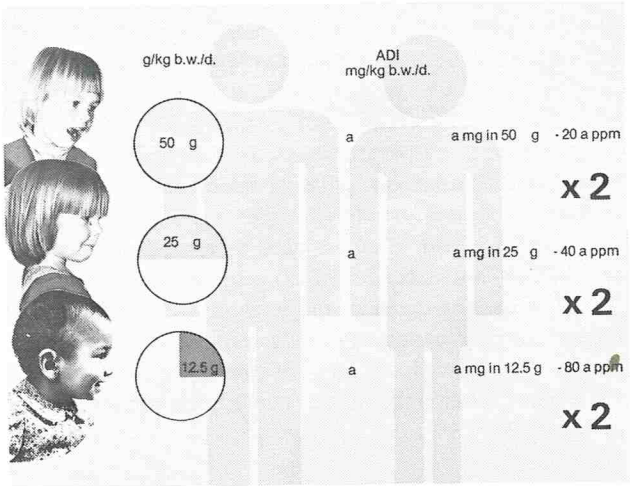

Figure 7. *Three alternatives for intake of food.*

The shade of two adults in Fig. 7 is to remind that there are good reasons to believe that the toxicologists have included the difference between child and adult in the safety margin when they established the ADI. We are therefore unduly cautious, when we base our calculations on the intake of a one-year-old child. A factor of 2 should be permissible.

If 40 *a* mg/kg foodstuff is below the technologically necessary level, an early decision has to made as to what food items should be excluded from the use of the additive, or for which foodstuffs use should be reserved. Figure 8 offers suggestions as to the selection of the most appropriate of the three alternatives for some characteristic components in our diet.

Cereals (or better, starchy foods) make up the main part of the diet in poor countries, but all parts of the world some people eat a substantial amount of bread, potatoes, pizza and pasta. Some years ago a Dutch survey (7) showed that teen-agers going to school by train might get as much as 70% of their energy intake by eating bread. Seventy percent of a teen-ager's intake corresponds to 50 kcal (or 22 g of bread)/kg b.w./d. If, e.g., potato flakes for preparation of mashed potatoes need a certain additive it may be wise to foresee that the same additive will be useful in other kinds of flaked and powdered starchy convenience foods, even in bread-mixes.

The second alternative, 25 g/kg b.w/d., may also be chosen if the food additive in question can be excluded from fresh food, cereal and dairy products.

The third alternative should be considered in the six options listed in Fig. 8. These groups of foodstuffs

Half the energy intake is represented by:

1) CEREALS
2) TOTAL FAT
3) OTHER THAN FRESH FOOD, BREAD AND DAIRY PRODUCTS.

One quarter of the energy intake is represented by:

1) DAIRY PRODUCTS - OTHER THAN MILK
2) VISIBLE FAT
3) SUGAR
4) MEAT, FISH, POULTRY AND EGGS
5) PASTRY AND BREAKFAST CEREALS
6) OTHER THAN FRESH FOODS, BREAD, DAIRY PRODUCTS AND VISIBLE FAT.

Figure 8. *Alternatives that may be chosen from characteristic components of our diet.*

compete for room in the diet as a whole and hence are not additive, but four of the groups together make up a high intake of 25 g/kg b.w./d.

ALLOCATIONS OF ADI TO FOODSTUFFS AND BEVERAGES

If a particular additive is used in both solid foodstuffs and beverages, the ADI must be split into two fractions and the size of each must be decided upon arbitrarily to accommodate the technological requirements as far as possible. (See the example with benzoic acid below). If the ADI is too low to meet both requirements, an administrative decision is necessary so that one additive may be reserved for use in solid foodstuffs and another with the same technological effects for use in beverages.

EXPERIENCE WITH THE BUDGET APPROACH IN DENMARK

In 1973 the Danish administration was instructed to draft a list of permitted food additives (*2*) in accord with the acceptable daily intake values established by WHO. Data on actual intakes were not available and there was no detailed information on the dietary pattern. Information about potential uses and levels of use in different foods was taken from food standards of the *Codex Alimentarius*, industry, the literature and other sources. The findings were presented in data sheets, one for each additive (Fig. 9). It was realized that the information was not complete, that the sheets would require continuous updating and that future developments would necessarily be added. For some additives there were only a few uses, and others were used in a wide variety of foodstuffs.

Benzoic acid (Fig. 9) is a well-known and often used preservative used in foodstuffs. The food industry suggested as permitted levels the figures shown in the first column. Margarine is not on the list, but benzoic acid has in fact been used in margarine in some parts of the world. Most of the figures were quoted from earlier legislation where also the so-called percentage rule applied (see below). The primary ceiling 20 *a* (= 200 ppm) was uninteresting from a technological point of view. To reach technologically effective levels, a factor of 80 or 160 should be used. To remain within the ADI, only one-quarter of the foods and one-quarter of the beverages consumed by a child may contain this additive. It was decided administratively to exclude the use of benzoic acid in milk and meat and their products, bread and visible fat (except mayonnaise). This limited its use so much that the third alternative could apply (the estimated high intake was 12 g of food/kg b.w./d.), and the factor 160 was used. Soft drinks etc. required a special allocation from the ADI and the factor 40 was used.

Data sheet for benzoic acid

	Suggested level ppm	Accepted level ppm
Mayonnaise	1000	1000
Mayonnaise, low-fat	2000	1000
Semi-preserved fish and fish products	2000	1000
Fish and fish products in brine	2000	1000
Tomato purée and ketchup	2000	1000
Pickled fruits and vegetables	2000	1000
Meat products	2000	0
Mustard	2000	1000
Worcestershire sauce	2000	2000
Marmalade, jam	1000	500
Juice, lemonade, etc.	250	200
Confectionary	1000	1000
Cakes and fine baker's ware	1000	1000
Salads and dressings	2000	1000
Non-standardized foodstuffs		500

(a) = 5

CEILING FOR FOODSTUFFS:

160 × 5 = 800 mg benzoic acid/kg food

ADI = 10 mg/kg b.w./d. (conditional ADI).

(a) = 5

CEILING FOR BEVERAGES:

40 × 5 = 200 mg benzoic acid/litre.

Figure 9. *Data sheet for benzoic acid.*

At that time, in 1973, there was a conditional ADI of 10 mg/kg b.w./d. for benzoic acid (*6*); today, however the ADI is only 5 mg/kg b.w./d., as WHO (*9*) has withdrawn all conditional ADI's. It is therefore questionable, whether there will be an allocation at all for beverages (or other foodstuffs).

The levels finally accepted for benzoic acid are a little higher than those calculated (Fig. 9, second column).

PERCENTAGE RULE ABOLISHED

In earlier legislation in Denmark, a rule governing use of mixtures of food additives stated that if the amount of each additive is expressed as a percentage of the maximum amount permitted, the sum should not exceed 100. This rule was abolished except for cases in which the ADI is expressed as a sum of a number of related additives. Abolition of the percentage rule was considered to be essential for estimation of the intake. The rule had forced the authorities to set permissible levels rather high

in older legislation, e.g., 2000 ppm for benzoic acid. Lower figures in new legislation may not necessarily mean stricter rules.

DISCUSSION

National governments and international organizations have already laid down quantitative food additive provisions in food standards. This principle of "first come, first served" that is at present applied in food standardization will eventually conflict with the principle of the ADI because new food products will demand their share of the ADI. New products can probably not be accepted because of a too liberal policy in the past, and because traditional types of foodstuffs have been given ADI-allocations in accordance with eating habits in former times. ADI budgets should therefore be agreed upon and ceilings established for inclusion in horizontal food additives directives.

The main objection to the budget is that the ceilings are unnecessarily low, that they leave too much accommodation for future development and, therefore, are "extra safe." But the alternative, detailed surveys of food consumption, may lead to even lower permissible levels because many high intake figures from such surveys may result in an unrealistically high total. In the philosophy of the budget, however, it is recognized that no one individual can be a high consumer of everything.

The ADI-budget should be considered an adjunct to food consumption surveys in a similar way as budgeting and book-keeping are parallel operations in finance.

The budget is based on broad groups of foodstuffs akin to the dietary elements (fat, sugar, protein, complex carbohydrate) used in nutritional considerations. On this point the ADI-budget is at variance with the plan originally suggested from toxicological quarters who foresaw detailed food consumption surveys and "book-keeping" on that basis.

In the presentation of the budget, it is assumed that certain variations less than 2-fold can be absorbed by the safety margin, but it is debatable whether this is an unduly cautious position. Very few toxicologists (Danish and Swedish) have yet had experience with the budget, and as long as there is no general agreement as to the acceptable variation in food intake it is not meaningful to discuss actual figures. The budget for benzoic acid should therefore be looked at as an illustration. Generally, the budget is not meant to be a dictation, but should be considered for use as a tool in a future dialogue between toxicologists and food technologists (5).

Instead of leaving the initiative with the toxicologists, the food technologists might suggest a ceiling on the level of use and ask for toxicological clearance of the corresponding daily intake. This could facilitate the task of the toxicologists. If then the budget is adminstered in cooperation with toxicologists and food technologists, it might facilitate progress in this important field.

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Milk Intolerance[1]

W. E. SANDINE* and MICHELE DALY[2]

Department of Microbiology, Oregon State University, Corvallis, Oregon 97331

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ABSTRACT

Several causes for milk intolerance are known, of which lactose intolerance is only one. Others include a local (e.g. gastrointestinal) toxic effect caused by one or more milk components and an immunological effect due to hypersensitivity to milk protein(s). Recent publications dealing with these phenomona are cited and the need for physicians, especially pediatricians, to become more knowledgeable in this area is emphasized. Methods to diagnose lactose intolerance are discussed, especially their limitations which likely have resulted in recommendations to too many individuals to restrict milk from their diets.

We hear and read a lot these days about lactose and milk intolerance and so it is fitting that we consider this topic, especially to identify truths, falsehoods and hypotheses. Most of us with a strong allegiance to the dairy industry become defensive when we hear or read that nature's most perfect food may not be quite so perfect after all for some individuals. But we must be honest with ourselves and with others in communicating the many nutritive strong points as well as the milk intolerances that affect some people. We also should be prepared to indicate how this difficulty can be avoided and at the same time allow consumption of many, if not all, dairy products.

A paper published recently (*8*) begins with this statement: "Adverse reactions to cow's milk have been recognized since the time of Hippocrates but with recent immunologic advances and new techniques, a flood of literature has left many pediatricians in a state of confusion, not only in terminology but also in conflicting reports concerning the basic underlying mechanisms of these reactions." Clearly, more research, much of it now in progress, is needed to eliminate this confusion; hopefully, this research will suggest ways in which bovine milk can be modified to eliminate intolerances so that the nutritive properties of milk can be universally available.

MECHANISMS OF INTOLERANCE

At least three mechanisms of gastrointestinal intolerance to cow's milk are known; these are toxic, immunologic and enzymatic (*8*). The latter (i.e. enzymatic) offers the explanation for lactose intolerance which we will consider in some detail. The toxic mechanism suggests that one or more milk components exert a local toxic effect and this phenomenon is the least well understood of the three. It is known, however that a heat-labile protein of cow's milk is involved in about half of all children with severe iron deficiency anemia; a local toxic effect seems likely, leading to gastrointestinal bleeding and protein-losing enteropathy (*27*).

The immune mechanism involves hypersensitivity to bovine milk proteins, and recent studies have shown that there are at least 20 different proteins present in cow's milk. It has been estimated that the incidence of milk hypersensitivity is from 0.3 to 7.0% in children (*6,11*) and symptoms other than gastrointestinal may occur as well (*5,21*).

Deficiencies in the enzyme β-galactosidase or lactase (enzymatic mechanism) in the intestinal lumen of individuals may result in certain symptoms when milk is consumed. In most persons, milk lactose is hydrolyzed in the intestine, releasing glucose and galactose which then enter the blood and are further metabolized. If the lactose is not hydrolyzed, water is drawn into the intestine where bacteria convert the lactose to acids and gas. Diarrhea, flatulence and abdominal cramping result.

INCIDENCE AND DIAGNOSIS

The incidence of lactose intolerance varies with different races and geographical distribution. In the United States it occurs at a rate of 6 to 12% in Caucasians (*15*) but a 60 to 90% rate has been observed among Greeks, Arabs, Jews, black Americans, Japanese, Thai, Formosans and Filipinos (*2*). A recent study (*19*) involving 30 adult Vietnamese entering the U.S. revealed that while all were lactose intolerant, only six had experienced any symptoms when drinking milk. To explain this apparent discrepancy, we need to discuss various methods for diagnosis of lactose intolerance.

There are several different methods being used as diagnostic criteria for lactose intolerance. One method frequently used is to give a fasting individual 50 g of lactose (test dose), measure the blood glucose concentration and wait for symptoms to develop; 25 mg% or more increase in blood glucose is normal (*16*). This method is as an index of absorption and hydrolysis of the lactose. Another method developed (*13*) is to give a fasting individual 300 mg of ethanol per kg of body weight, then 50 g of lactose 15 min later. After 40 min, a

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[2]*Department of Dairy and Food Microbiology, University College, Cork, Ireland.*

capillary blood sample is taken and the galactose level measured. A radioisotope breath test also is in use (*1*) where 1-[^{14}C-]lactose is given orally and $^{14}CO_2$ collected and measured in the breath as a measure of lactase activity; respiratory hydrogen excretion also has been advocated as a test for lactose malabsorption (*9*). Most of the above methods have been criticized for the large amount of lactose given in the tests. Fifty grams of lactose is the amount present in a liter to a liter and a half of milk. It is not the normal dietary pattern to consume so much milk in one sitting, especially on an empty stomach. The lactose administered is in a water solution, whereas milk is a mixture of water, protein, fat, carbohydrate, minerals, and vitamins and often taken with food. Some of these components may have an influence on digestion of lactose. Also the absorption rates of the monosaccharides glucose and galactose should be checked to rule out a monosaccharide malabsorption syndrome. Other problems have arisen with the measurement of the level of glucose in the blood. One study (*16*) indicated that 25% of the normal individuals on a capillary blood test will not show a 20 mg% or more increase in blood glucose. In addition, it has been demonstrated that some normal individuals respond abnormally to the test because they have a slower stomach emptying time (*18*). Thus, the lactose is slower to reach the hydrolytic sites and slower to be absorbed. Another method being used to determine lactose intolerance is the direct determination of the activity of the enzyme lactase using a peroral biopsy technique (*17*). If an individual has less than 2 units of enzyme activity, he is considered lactose intolerant. This method has also become suspect in the light of evidence (*22*) from a survey involving Indian adults and children to determine the incidence of lactose intolerance and the activity of the enzyme lactase. Testing indicated the group had a high incidence both of lactose intolerance and lactase deficiency. The group was then placed on a diet supplemented with skim milk for 4 weeks. At the end of this time, some members of the group showed milder symptoms of lactose intolerance and others had no symptoms at all. The activity of lactase did not increase with the disappearance of symptoms. The mechanism of this adaptation has not been explained. Some members of the group who were diagnosed as lactose-intolerant when given 50 g of lactose could drink a quart of milk a day, if given in 200-ml quantities throughout the day. Based on these data, lactose intolerance is not synonymous with milk intolerance and perhaps unmerited significance has been attached to all of the diagnostic tools mentioned. Diet therapy based on the above diagnostic tests has led to unnecessary restriction of foods from the diets of individuals and Welsh (*26*) has suggested that a more reasonable approach is needed.

Milk intolerances have been mentioned in newspapers recently, especially since publication of Dr. Frank Oski's book *Don't Drink Your Milk* (*20*). His appeal is a solitary one among nutritionists, food and dairy scientistis who are aware that decades of research and human experience have proved that milk and dairy products are relatively inexpensive sources of most of the essential nutrients required each day by man. We retain our daily need for milk and dairy products throughout life, especially to meet the 0.8 to 1.24 g of calcium required by adults each day (*12*). Thus nutritionists still recommend at least three, four, two, three and four glasses of milk per day for children, teen-agers, adults, expectant and nursing mothers, respectively.

There is some evidence that osteoporosis (degeneracy of the bones) may occur as a result of lactose intolerance. A study conducted by Birge et al. (*3*) revealed that nine osteoporotic patients had excluded dairy products from the diet because of gastrointestinal problems when they were consumed. Thus, osteroporosis may have developed because patients had been on a long term calcium-restricted diet due to lactose intolerance. Another study conducted by Condon et al. (*7*) has shown that lactose increases intestinal calcium absorption and decreases urinary calcium and phosphorous excretion.

FERMENTED DAIRY FOODS

A number of reports can be found in the literature which indicate that persons intolerant to lactose, when consumed as milk, may eat fermented dairy products without symptoms (*10,14,23*). It also has been indicated (*4,24*) that sweet or fresh acidophilus milk may be consumed by persons who otherwise cannot drink milk. Thus benefits of this product to some individuals may be more than simply contributing to the desirable bacterial flora of the intestinal tract. Another means of making milk available to lactose intolerant persons is to provide it as a lactose-hydrolyzed product. Lactase is now commercially available and its use for this and other purposes has been described by Vedamuthu (*25*).

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Coming Events

May 15-16--7th ANNUAL ROCKY MOUNTAIN CONFERENCE ON FOOD SAFETY. Ramada Inn, I-70 and Kipling, Denver, CO. Theme: "The Science and Politics of Food Safety." To register in advance, contact: Dick Peterson, U.S. Food and Drug Administration, 500 U.S. Customs House, Denver, CO 80202, 303-837-4918. For additional information, contact: Mary Lou Chapman, Colorado Dept. of Agriculture, 1525 Sherman St., Denver, CO 80203, 303-839-2811.

May 15-17--POWDER AND BULK SOLIDS CONFERENCE/EXHIBITION. Philadelphia Civic Center, Philadelphia, PA. Sponsored by International Powder Institute of London and Chicago. Contact: Industrial & Scientific Conference Management, Inc., 222 West Adams St., Chicago, IL 60606, 312-263-4866.

May 20-22--1979 INTERNATIONAL CHEESE & DELI SEMINAR. Madison, WI. Contact: International Cheese & Deli Seminar, 801 W. Badger Rd., Madison, WI 53713.

May 20-24--CONFERENCE ON INTERSTATE MILK SHIPMENTS, Stouffer Inn, Louisville, KY. Contact: H. H. Vaux, Director, Indiana Food and Drug Division, or John Speer, Milk Industry Foundation, 910 17th St. N.W., Wash., D.C.

May 21-23--WATER SUPPLY ENGINEERING: QUALITY AND TREATMENT. Short course sponsored by Dept. of Environmental Sciences and Engineering and Continuing Education, School of Public Health, University of North Carolina at Chapel Hill. Contact: Dr. Philip C. Singer or Phyllis Carlton, Dept. of Environmental Sciences and Engineering. The University of North Carolina, School of Public Health 201H, Chapel Hill, NC 27514, 919-966-1023.

May 21-24--NSF SEMINARS. Seattle, WA. For more information, contact: Education Service, National Sanitation Foundation, NSF Building, P.O. Box. 1468, Ann Arbor, MI 48106, 313-769-8010.

May 21-24--NALVEX, National Licensed Victuallers and Caterers Exhibition. National Exhibition Centre, Birmingham, England. Contact: British Information Services, 845 Third Avenue, New York, NY 10022, 212-752-8400.

May 22-27--INTERNATIONAL FOOD FAIR. Copenhagen, Denmark. Contact: Bella Center A/S, Center Boulevard, DK-2300 Copenhagen S., Denmark.

May 28-31--NATIONAL CONVENTION OF THE AUSTRALIAN INSTITUTE OF FOOD SCIENCE AND TECHNOLOGY, Theme: "Food, the Consuming Interest." Adelaide, South Australia. Contact: L. Peters, Australian Institute of Food Science and Technology, 13, Bulf Parade, Brighton, South Australia 5048.

June 4-5--NSF SEMINARS, Memphis, TN. For more information, see entry for May 21-24.

June 5--PENNSYLVANIA SANITARIANS ASSOCIATION, Annual Meeting. Keller Conference Center, Pennsylvania State University, University Park Campus, State College, PA 16801. Contact: Sid Barnard, Pennsylvania State University.

June 10-13--INSTITUTE OF FOOD TECHNOLOGISTS 39th ANNUAL MEETING AND FOOD EXPO. Alfonso J. Cervantes Convention and Exhibition Center, St. Louis, MO. Contact: C. L. Willey, Institute of Food Technologists, Suite 2120, 221 N. LaSalle St., Chicago, IL 60601.

June 18-August 23--PRINCIPLES OF TOXICOLOGY, ten-week graduate course, Massachusetts Institute of Technology. Applicants should have background in chemistry, biology, or related science at Master's level or above. EPA Training Grants will provide stipends and tuition for 24 accepted students who must be U.S. citizens. Tuition: $3,000, enrollment restricted to 50 persons. For further details or an application, contact: Linda C. Boyar, Program Administrator, Dept. of Nutrition and Food Science, MIT, Room E18-564, Cambridge, MA 02139, 617-253-7023.

June 23-28 -- NATIONAL ENVIRONMENTAL HEALTH ASSOCIATION ANNUAL EDUCATIONAL CONFERENCE. Charleston, SC. Contact: NEHA, 1200 Lincoln St., Suite 704, Denver, CO 80203, 303-861-9090.

June 24-27--AMERICAN SOCIETY OF AGRICULTURAL ENGINEERS, Summer Meeting. Winnipeg, Manitoba, Canada. Contact: Roger R. Castenson, ASAE, 2950 Niles Road, Box 410, St. Joseph, MI 49085, 616-429-0300

July 30-Aug. 3--ADVANCES IN FOOD AND APPLIED MICROBIOLOGY. Massachusetts Institute of Technology, Cambridge, MA 02139. Program is under the direction of Anthony J. Sinskey, MIT, Professor of Applied Microbiology. Contact: Director of Summer Session, Rm. E 19-356, Massachusetts Institute of Technology, Cambridge, MA 02139.

Aug. 12-16--IAMFES ANNUAL MEETING, Sheraton Twin Towers, Orlando, FL. Contact: E. O. Wright, IAMFES Exec. Sec., P.O. Box 701, Ames, IA. 50010, 515-232-6699, or see registration form in this *Journal.*

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Bacterial Standards for Retail Meats

ROBERT L. WINSLOW

Food Technology Division, Safeway Stores, Inc., Oakland, California 94660

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ABSTRACT

Unsuccessful efforts of the Oregon retail meat industry to comply with the subsequently revoked Oregon bacterial standards for raw meats are reviewed. The satisfactory role of bacterial standards in regulation of pasteurized milk in contrast to their unsatisfactory role in retail raw meats is discussed. It is concluded that bacterial counts on raw meats cannot serve as indicators of: (*a*) health hazards, (*b*) insanitary conditions, (*c*) product spoilage, or (*d*) an aesthetic value of the food. Bacterial standards on raw meats, as applied in Oregon for 4 years, are considered as unjustifiable because they: (*a*) could not accomplish what they purported to do, i.e., reduce public health hazards, (*b*) were not technically feasible, i.e., were unattainable under the conditions of current good manufacturing practice in the industry, and (*c*) were not administratively feasible.

This discussion will deal primarily with bacterial standards on raw ground beef at retail. Lest there be any misunderstanding, I shall use the term "bacterial standards" solely to denote bacterial limits which may serve as the sole basis for legal action against a food product wherein bacterial counts exceed the specified *standard* limits. This differs from "bacterial guidelines" the application of which, may not serve as justification for legal action against the product in which the specified *guideline* limits were exceeded.

From subsequent comments I think you will understand why I do not consider "quality standards" and "bacterial standards" as synonymous terms when applied to raw meats. You will see I am not convinced that bacterial counts necessarily denote quality of raw foodstuffs.

OREGON BACTERIAL REGULATIONS

In May, 1973, the Oregon regulations established as a criminal act the offering for sale of (*a*) raw meats with Aerobic Plate Counts (APC) exceeding 5 million per gram or *Escherichia coli* (MPN) counts exceeding 50 per gram or (*b*) of processed meats with Aerobic Plate Counts exceeding 1,000,000 per gram or *E. coli* (MPN) counts exceeding 10 per gram. Within an 18-month period (September '73 thru February '75), the Oregon compliance program had led to the filing of criminal charges against 24 different Oregon store managers (including four from Safeway) for violation of this regulation.

MEAT DISTRIBUTION SYSTEM

Safeway's meat distribution system in Oregon involved supplying retail stores with bulk ground beef and with primal and sub-primal cuts of beef that had been pre-fabricated at our Clackamas, Oregon USDA-inspected meat breaking plant from USDA-inspected choice beef quarters trucked in primarily from Oregon, Washington and Idaho packers. Some fresh domestic boneless beef was purchased from nearby boners to supplement cow meat that was also boned out in the Safeway breaking plant. The bulk ground beef was prepared at the breaking plant using the USDA Choice trim from the pre-fabrication line together with the boneless cow beef boned out at this plant and/or additional boneless beef purchased from outside sources. The meat was distributed from the breaking-plant to the retail stores in Safeway trucks.

Approximately three-fourths of the ground beef distributed to Safeway's Oregon stores was ground under controlled conditions in the breaking plant in approximately 5,000-lb. batches which were chilled to about 32-34 F with dry ice during blending. It was packaged into 20-lb. "keeper casings" for shipment to the stores. At the stores the product was re-packaged into consumer-sized retail packages. Regular ground beef was re-packaged directly from keeper casings into retail packages, whereas lean ground beef was passed once through the store grinder before final packaging. Trim resulting from breaking of beef primals and sub-primals into retail cuts at the stores, was ground and packaged separately from the warehouse-produced bulk ground beef. From the time the truckloads of beef quarters (or boneless beef) were received at the dock of the breaking plant until finished packages were picked out of the display cases by customers, the meat and its environment were constantly under the control of Safeway personnel. It was felt that better control of temperatures, equipment cleanliness, and sanitation could be maintained in the centralized operation than in approximately 200 individual stores.

SOURCE OF *E. COLI*

By April, 1974 after about a year's experience with the standards, our Portland Retail Division had recognized that consistent compliance with the 50 maximum *E. coli* per gram level in the ground beef was beyond the

capability of our individual store managers (who were being held legally liable for compliance) and that technical assistance was needed. In May, 1974 Safeway Stores, Inc. established a corporate level Quality Assurance Department with the initial assignment of learning how the corporation could meet these and similar bacterial criteria on meats. By September a bacteriological laboratory had been built and equipped in our Portland Division meat breaking plant. This laboratory was staffed by three graduate food technologists, one of whom had earned a Master of Science degree through a study of microbiological aspects of ground beef irradiation before working for about 4 years as Quality Control Supervisor in two of Safeway's milk plants.

Since Safeway had control of the product from time of receipt at the breaking plant, either as quartered beef or as boneless beef, until time of pick-up of the finished package of meat at the store by the customer, it was possible to develop a bacterial monitoring system which pointed up the major source of high *E. coli* counts in the retail packages of ground beef. It was soon found that approximately 30-50% of the lots of bulk ground beef exceeded 50 *E. coli* (MPN) as they were packaged from the breaking plant grinder even before shipping to the stores. Since this incidence was not higher in samples obtained from the retail counters, it was apparent that sanitation and temperature control at the retail stores and during trucking from the breaking plant to the stores were not significant contributors to excessive *E. coli* counts in the retail ground beef.

Our bacteriologists became convinced that the crux of the problem rested with the incidence of *E. coli* on the carcasses as received from the packers. This was indicated by the results of routine equipment sanitation swabbing in the breaking plant, swabbing for *E. coli* on carcasses as unloaded at the receiving dock, and as a result of several special studies.

Based on knowledge gained from the *E. coli* monitoring of carcasses from each supplier, it was found possible to select a very limited number of quarters from select suppliers from which could be prepared ground beef that had about a 95% chance of meeting the Oregon regulations. However, the amount of available beef suitable for this purpose would supply only a very small percentage of our total demand.

BACTERIAL CONTROL MEASURES

Since store managers were being charged with criminal offenses, our bacteriologists recognized that, although the primary source of the problem was beyond our control, all feasible steps must be taken to be certain that our people and our operations were not increasing the problem. At the breaking plant the effectiveness of plant clean-up procedures was regularly checked both visually and bacteriologically (by swabbing of equipment after sanitation) with revisions made in clean-up procedures as indicated by the bacterial checks. A personal hygiene guide developed for guidance of workers was enforced. Every employee of the plant attended a special sanitation seminar designed to give insight into the practical aspects of food bacteriology. Temperature controls were carefully monitored and maintained. To more quickly reduce the heat generated by grinding of the beef, dry-ice was added to the freshly ground product in the blender just before it was packed. Routine bacterial monitoring of the product included swabbing of carcasses and boneless beef on receipt and testing for *E. coli*, APC and *E. coli* counts of freshly packaged bulk packages of ground beef and samples of retail-packaged ground beef from retail meat display cases.

Temperatures of trucks in which meat was delivered to the stores were carefully checked and controlled. Truck scheduling was reviewed and modified as needed to give best possible control during delivery.

At the retail store level a day-long sanitation seminar was held for all Meat Department Managers, Store Managers, District Managers, and Meat Merchandisers. It was presented by the bacteriologists from the laboratory at the breaking plant, the Sanitation Coordinator, an Industrial Engineer, a Meat Merchandiser, and the Retail Operations Manager — all from the Portland Retail Division with the assistance of the Corporate Sanitation Coordinator. Designed as a training course to instruct all operating management personnel in bacterial control of meats at the retail level, the seminar included practical "how to" instructions as well as simplified explanations of food bacteriology and departmental clean-up. Interest at the seminar was very good and management follow-up was excellent with a resultant improvement in bacterial control of the meat at store level.

In an effort to decrease the *E. coli* incidence on incoming carcasses, our bacteriologist made a series of visits to each of our suppliers' packing plants, observing their operations and suggesting ways whereby he felt that each might reduce the *E. coli* on beef quarters being shipped to our breaking plant from their packing plants. This effort met with only very limited success.

RESULTS

In spite of the very considerable efforts of all involved, Safeway was able to routinely accomplish an *E. coli* compliance rate for its bulk packed ground beef of no better than about 60% to 70%. Thus we felt that despite our best efforts, we were unable to attain satisfactory compliance with the Oregon bacterial standards on meats.

In January 1976 a newly appointed State Director of Agriculture in Oregon appointed a blue ribbon meat bacterial standards review committee to review the standards and recommend whether they should be: (*a*) continued as promulgated, (*b*) modified, or (*c*) rescinded. After a year's very thorough study, this committee of consumers, educators, scientists, lawyers, a legislator,

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regulators, and industry representatives concluded the standards should be dropped and the bacterial criteria be applied as guidelines. The committee made eight specific recommendations for use in place of the bacterial standards. In the summer of 1977 the Oregon State Department of Agriculture adopted the recommendations of this committee, including the repeal of their bacterial standards on meat at retail.

PERSPECTIVE

As a food bacteriologist with extensive training and experience in the limited field of dairy bacteriology, I had looked forward to helping develop a program that would bring Safeway's ground beef into compliance with the Oregon standards. I was disappointed that we did not achieve a higher compliance rating.

Our Oregon experience has prompted me to consider why bacterial standards should have proved ineffective on meats in contrast to their extremely important role in the sanitary control of fluid milk products. Suspecting that, like me, many of you have had rather extensive experience with bacterial regulation of milk, I should like to share first my conclusions and then my reasoning with you.

I question the value to the consuming public of such bacterial standards on raw meats as those enforced at the retail store level by the State of Oregon on the grounds that this approach: (*a*) will not increase protection of the consumer against health hazards, (*b*) will not indicate the level of sanitation of the environment to which the meat has been subjected, (*c*) will not indicate either actual or potential spoilage of the meat, and (*d*) can indicate only a falsely-assumed aesthetic property of the meat. Our experience with these Oregon regulations on meats caused us to question: (*a*) the effectiveness of such an approach in accomplishing any of the aforementioned objectives, (*b*) the technical feasibility of the standards in relation to existing practices of the entire meat industry (including feeder, packer, breaker, and distributor), and (*c*) the feasibility of administering such a regulation in an effective compliance program. Apparently, Oregon's "meat bacterial review committee" arrived at similar or related conclusions.

Bacterial counts as health hazard indicators

In considering bacterial counts as health hazard indicators, we are aware that the application of bacterial standards, in combination with certain other techniques, has changed milk from one of our most hazardous agents of foodborne illnesses to one of our safest foods. However, bacteriological regulation of the meat industry cannot be expected to achieve so dramatic a benefit. Thus, diseases originating in the cow as well as those coming from human handlers of milk were often transmitted to the consumer through raw milk. To halt transmission of pathogenic organisms through milk, public health officials required (*a*) sanitary inspection (by regulatory officials) of farms and processing plants, (*b*) pasteurization to destroy any pathogens in the raw milk, (*c*) bacterial monitoring of the pasteurized product to ascertain that it is practically free of coliforms which should be readily destroyed by pasteurization and whose presence indicates probable post-pasteurization contamination of the milk (with the assumption that any post-pasteurization contamination *could* have introduced pathogenic organisms into the pasteurized milk), and (*d*) processing of the product through readily-cleanable, enclosed systems.

In contrast to the former high rate of disease transmission through raw milk, the U.S. Center for Disease Control indicates that few instances of foodborne illness directly attributable to pathogens on raw meats have been reported. Rather, most foodborne illnesses transmitted by meats have been the result of mishandling meats (and/or gravies) that had become contaminated by the pathogen after cooking. This is probably true because most food pathogens are normally unable to grow well in or on raw meats in competition with other bacteria normally present and also, perhaps more important, to the fact that cooking of the meat destroys the pathogenic organisms that may be present. (Few Americans consume raw meat which has not at least been seared on the surfaces - an operation that would destroy the pathogens on most cuts of raw meat.) Furthermore, at present there is no process available to the meat industry (aside from the consumers' cooking of the product immediately before consumption) comparable to pasteurization to guarantee destruction of all pathogens on carcasses, quarters, cuts, or ground meats. Also, it is impossible to cut up meats in an enclosed system and relatively little meat equipment has as yet been designed for easy and automated cleaning.

Thus, whereas the use of bacterial standards has been a very effective aid in the assurance of a safe milk supply for United States consumers, similar standards cannot be expected to guarantee safety of a food such as raw meat which has not been subjected to a bactericidal process that will guarantee destruction of pathogens. Bacterial numbers per se do not — indeed cannot — reflect the degree of hazard involved in consumption of the meat, and therefore their application cannot actually increase the protection of the consumer against a health hazard. Assuming the meat to be palatable, I would have no more concern for consumption of meat which had been properly cooked from product having an Aerobic Plate Count of 100,000,000 per gram with more than 1100 *E. coli* per gram than I would for meat having an Aerobic Plate Count of 10,000 per gram with less than 3 *E. coli* per gram regardless of whether the consumption was to be by myself, by one of my grandsons, or by their great-grandmother!

Bacterial counts as indicators of insanitation

The sanitary conditions of the environment to which a given sample of meat has been subjected cannot be ascertained on the basis of bacterial counts on a specific meat sample obtained from the meat display case of a

retail store. Sanitation standards are probably higher in the dairy industry than in any other segment of the food industry. Yet, even here, neither a dairy bacteriologist nor a regulatory official can ascertain the sanitary conditions under which a given sample of milk was processed solely on the basis of bacterial counts on that specific sample of unknown age and temperature history. To determine sanitary conditions under which milk was produced and processed, it is necessary for the dairy bacteriologist or the regulatory official to conduct a close inspection of production and processing facilities and equipment. As a tool in evaluating sanitation levels in a processing operation, the dairy bacteriologist does not use bacterial counts on individual, isolated samples of unknown history, but rather he studies changes in trends of bacterial counts obtained on samples from various known points throughout the processing system as the trend of the counts varies over days, weeks, and months. The data on any single sample are highly inconclusive. Data on raw milk from the producers must be considered and such milk must be regulated entirely separately from the finished pasteurized product.

Bacterial counts on individual retail packages of raw meat are even less conclusive indicators of the sanitary background of that product. It is impossible to discern, solely from the bacterial count, which of several possibilities may have caused a particularly high bacterial count in a specific package of ground beef. Had the product been ground from particularly low count carcasses, trimmed and boned in the most sanitary manner possible by sanitary employees using cleaned and sanitary equipment with the temperature maintained at 35 F or below for enough days to permit harmless cold-tolerant bacteria to grow to very high numbers? Or had the product perhaps been made from the same low count carcasses by the same well-trained employees in the same manner but for some reason, the product having warmed up to 45-50 F for a while, thus permitting much more rapid growth in a short time of the relatively few organisms originally present? Was the bacterial count high because a very few bacteria had multiplied to a large number either very slowly at low temperatures or more rapidly at higher temperatures? Had the product been contaminated with a particularly large number of organisms so that little growth was necessary to produce the high count? Had the carcass been contaminated at the packer's plant with bacteria from hair, hide, knives, or saws? Was the product contaminated by people or equipment at the meat breaking plant or in the meat department at the store? Were temperatures allowed to get too high at the packer's, the breaking plant, the store, or in transit? Was the product held too long after grinding or prepared from mishandled meat? Was it prepared from intentionally aged beef? A single bacterial count at one particular point in the distribution chain cannot provide the answer to these questions.

Through a study of trends of bacterial numbers in samples taken repeatedly over a prolonged period a meat bacteriologist may be able to develop indications of insanitation which cannot be deduced from isolated sampling at the retail level only. Inspection is then necessary for verification and correction of the inadequacies in the system. This cannot be accomplished solely by establishment of bacterial standards for meats sampled only at retail.

Bacterial counts as spoilage indicators

Neither actual nor incipient spoilage can be deduced from the bacterial count of a specific package of raw meat obtained from the display case of a retail store. Bacteria which grow in foods are of many types. Some, the pathogenic, may cause us to become ill if they are present in or have grown in the food which we eat. Others may cause the food to spoil as they grow in it. In other instances such as cheeses, certain fermented sausages, buttermilk, yogurt, and sour cream the growth of millions to billions of bacterial cells of certain types is essential for production of the desired food. Still other types of bacteria may grow to high numbers in a food without producing any change whatsoever in the palatability or safety of that food. Frequently growth of the desired types of bacteria or of those that cause no apparent change in the food will tend to inhibit growth of the pathogenic and spoilage bacteria.

When bacterial growth in a food is predominantly or virtually exclusively of the spoilage type, the food may become unpalatable by the time the count is hundreds of thousands of bacteria per gram. However, if the growth has been predominantly of desirable or benign types of bacteria, the count may reach hundreds of millions to billions without loss of palatability. In this latter instance, the danger of spoilage or illness may actually be reduced due to inhibition of undesirable bacteria by relatively high numbers of the harmless bacteria!

In dairy bacteriology a relative indication of potential shelf-life of pasteurized milk is obtained by checking bacterial counts on many samples of milk that have been held at 45 F for 5 days to a week before determining the counts. When this is done over a period of weeks and months, establishment of trends of results gives an indication which is valuable to the dairy. However, the approach is not one which has generally proved to be valuable for regulatory purposes and it is unlikely that it would be as valuable for a food such as raw meat which has not been subjected to a bactericidal processing step.

Bacterial counts and aesthetic property of food

In raw meats there is no relationship between bacterial counts and inherent goodness, wholesomeness, or aesthetic value. Publicity in the common news media and from some regulatory agencies would seem to impute some special desirability to low bacterial counts per se — some aesthetic value, if you will. If high bacterial counts actually indicated adulteration of the food with gross amounts of filth there might be some justification for this inference. However, as has already been pointed out, this is not true. It is suggested by many that presence

of *E. coli* in meats denotes fecal contamination, with a frequent inference that it may denote human fecal contamination. There is no arguing the fact that animal feces, both human and non-human, are a very common source of this organism. However, the organism becomes so widespread that its presence in food frequently represents contamination of food with bacteria growing on incompletely cleaned food-contact equipment. Another very plausible source of this organism on meat would be contamination during the skinning operation, wherein knives used for cutting and skinning the hide would carry the organism from the outside of the hide onto the freshly exposed surface meat of the carcass.

Normally the factors that affect the aesthetic properties of food without affecting its safety and nutritive value would include such things as presence of animal hairs, insect fragments, stems, etc. These substances do not increase in numbers after entry into the food, whereas a single *E. coli* organism contaminating a warm carcass could quite conceivably increase to very sizable numbers before the meat was cooled to below 41 F (at which temperature *E. coli* reportedly can multiply in certain foods). Thus neither the presence nor the numbers of these or any other organisms would be indicative of the aesthetic value of the product. No person who savors any fermented food such as cheese, wine, sauerkraut, yogurt, buttermilk, sour cream, or fermented sausages containing billions of microorganisms (bacteria or yeasts) should have any qualms about the consumption of bacterial cells per se.

CONCLUDING COMMENTS

I have shared with you some of our efforts and frustrations in our unsuccessful attempts at satisfactory compliance with Oregon's meat bacterial standards — standards which the state has since seen fit to revoke. Although not reflected in our regulatory compliance rating, we feel that our in-house bacterial monitoring and control programs are of value in improving appearance, shelf life, and loss of raw meats. These experiences have led us to a re-evaluation of the potential role of bacterial standards for raw meats in the regulation of the food industry, leading to our conclusions that bacterial counts on raw meats could not serve as indicators of: (*a*) health hazards, (*b*) insanitary conditions, (*c*) product spoilage, or (*d*) aesthetic value of the food. Based on our Oregon experience we have concluded that bacterial standards on raw meats are not justified because they: (*a*) cannot accomplish what they purport to do, i.e., reduce public health hazards; (*b*) are not technically feasible, i.e., are unattainable under conditions of current good manufacturing practice; and (*c*) are not administratively feasible.

We consider the Oregon State Department of Agriculture's revocation of its bacterial standards for meats, in accordance with the recommendation of its meat bacterial standards review committee, to be a prime example of the consumer benefiting through the cooperative efforts and careful study of a question by affected regulatory agencies, industry, and consumers aided by appropriate legal and scientific guidance.

ACKNOWLEDGMENT

Presented at the 65th Annual Meeting of the International Association of Milk, Food, and Environmental Sanitarians, Kansas City, Missouri, August 13-16, 1978.

Coming Events *con't. from p. 437*

Aug. 13-17--WORKSHOP ON EDUCATIVE PROCESSES IN FOOD MICROBIOLOGY. Sponsored by the Joint American Society for Microbiology/Institute for Food Technologists Committee on Food Microbiology Education. Quadna Resort, Hill City, MN. Contact: E. A. Zottola, Dept. of Food Science and Nutrition, 1334 Eckles Ave., University of Minnesota, St. Paul, MN 55108.

Aug. 29-31--FOURTH INTERNATIONAL IUPAC SYMPOSIUM ON MYCOTOXINS AND PHYCOTOXINS. Co-sponsored by World Health Organization and Swiss Society for Analytical and Applied Chemistry. Lausanne, Switzerland. For participation and poster presentation, contact: Prof. P. Krogh, Dept. of Veterinary Microbiology, School of Veterinary Medicine, Purdue University, West Lafayette, IN 47907 or Prof. D. Reymond, IUPAC, Case postale 88, 1814 La Tour de Peilz, Switzerland.

Sept. 10-13--2nd INTERNATIONAL CONFERENCE ON FOOD SERVICE SYSTEMS DESIGN. Harrogate, England, Contact: G. Glew, Catering Research Unit, Procter Dept. of Food Science, The University of Leeds, LS2 9JT, England.

Sept. 10-14--FOOD PROCESSORS ADVANCED MICROBIOLOGY SHORT COURSE. University of California, Davis. Fee $200. Contact: John C. Bruhn, Dept. of Food Science and Technology, University of California, Davis, CA 95616, 916-752-2192.

Sept. 13-14--INTERNATIONAL SYMPOSIUM ON ANIMAL AND HUMAN INFLUENZA. Ecole Nationale Veterinaire D'Alfort, 7, avenue du General de Gaulle, 97 704 Maisons-Alfort cedex, France. Contact: Ch. Pilet, Secretariat of the Dept. of Microbiology, Ecole Nationale Veterinaire d'Alfort.

Sept. 18-20--WESTPACK '79. Convention Center, Anaheim, CA. Contact: Clapp & Poliak, Inc., 245 Park Ave., New York, NY 10017.

Sept. 19-20--NEW YORK STATE ASSOCIATION OF MILK & FOOD SANITARIANS, Annual Meeting. Holiday Inn Arena, 2-8 Hawley Street, Binghamton, NY 13901. Sponsored by NYSAMFS, Cornell University Food Science Dept., New York State Dept. of Health, New York State Dept. of Agriculture and Markets. Contact: R. P. March, 124 Stocking Hall, Ithaca, NY 14853, 256-4550.

Sept. 19-20--WISCONSIN ASSOCIATION OF MILK AND FOOD SANITARIANS, Annual Meeting. Madison, WI. Sponsored by WAMFS, Wisconsin Dairy Plant Fieldmen's Association, Wisconsin Dairy Tech Society, Wisconsin Environmental Health Association, and Wisconsin Institute of Food Technologists. Contact: Don Raffel, 4702 University

Sept. 26-27--SOUTH DAKOTA STATE DAIRY CONVENTION. Downtown Holiday Inn, Sioux Falls, South Dakota 57100. Contact: Shirley W. Seas, Secretary, Dairy Science Department, South Dakota State University, Brookings, South Dakota 57007, 605-688-5420.

Nov. 3-6--1979 AMERICAN MEAT INSTITUTE CONVENTION. McCormick Place and The Conrad Hilton, Chicago. Contact: Judi Winslow, American Meat Institute, P.O. Box 3556, Washington, D.C. 20007, 703-841-2431.

Now 11-15--FOOD AND DAIRY EXPO '79. McCormick Place, Chicago, IL. Contact: Wes Dibbern, Dairy and Food Industries Supply Assoc., 5530 Wisconsin Ave., Suite 1050, Washington, D.C. 20015, 301-652-4420.

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The International Dairy Federation

KENNETH G. SAVAGE

International Dairy Federation, 6661 Tamany Drive, Victoria, B.C. Canada

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ABSTRACT

The International Dairy Federation (IDF), a non-governmental, non-political international organization was established in 1903. The IDF derives its finances from the annual membership fee (current membership - 31 countries). Membership is accorded to countries through their National Committees representing the country's various dairy interests. These Committees are the link between IDF and the member country. The IDF Executive Committee deals with policy matters delegated by the Supreme body of IDF, the General Assembly, and develops proposals for General Assembly consideration. A permanent secretariat under the direction of Mr. P. Staal, Secretary-General, coordinates IDF activities and carries out the current business at the IDF House, Brussels, Belgium. The technical and scientific work of IDF, under the guidance of the Commission of Studies, is carried out in six special Commissions whose subject matter covers all aspects of interest to the dairy industry. The objective of IDF, which is to promote, through international cooperation, solution of scientific, technical and economic problems in the international dairy field, is achieved through the voluntary work of hundreds of the world's dairy industry leaders. This work is done by Groups of Experts or at Annual Sessions, IDF-sponsored Seminars or Symposia and Congresses.

The International Dairy Federation (IDF), which has its headquarters in Brussels, Belgium is the only international body concerned with the total dairy industry in the world. Thirty-one countries are members and are represented in IDF through their national committees. With the exception of the United States of America, which is not a member, these countries include the top 15 milk-producing countries in the world, thus IDF truly represents the world's major dairy interests.

The IDF is a non-profit, non-governmental organization created in 1903 to promote, through international cooperation, the solution of scientific, technical and economic problems in the international dairy field.

The IDF regularly holds annual meetings at which the work of the various Commissions and Groups of Experts is reviewed and discussed and the next year's work is planned. Every 4 years, in conjunction with the annual IDF meeting, there is held, in the host country, an International Dairy Congress. These Congresses, due to their varied technical and scientific makeup and the all-encompassing nature of their agenda, are extremely well attended by those in the dairy industry throughout the world. The recent (June, 1978) Congress in Paris was attended by delegates from over 50 countries.

Membership in IDF is only accorded to the member country through its National IDF Committee, hence this Committee organization is of vital importance.

COMMISSIONS

The General Assembly is the supreme body of IDF. The main administrative and managerial direction is supplied in IDF by the Executive Committee and the Commission of Studies. The operational administration is carried out at IDF headquarters by the Secretary General and his small support staff. Through the Commission of Studies various specific standing Commissions are appointed to deal with the different subject matter before IDF. At the present time, there are the following semi-permanent Commissions entrusted with certain responsibilities:

A. Production, Hygiene and Quality of Milk
B. Technology and Engineering
C. Economics, Marketing and Management
D. Legislation, Compositional Standards, Classification, Terminology
E. Analytical Standards, Laboratory Techniques
F. Science, Education

These special Commissions name Groups of Experts or working parties to deal with specific dairy problems. These Groups of Experts report back to the Commissions which in turn accept, reject or return the reports for further work. Those accepted by the Commissions are in turn forwarded to the Commission of Studies for final action (this usually results in publication of the report). Additionally, the technique of questionnaires, directed to National IDF Committees, is used to obtain information. There is also one other semi-permanent organization associated with IDF. This is the International Milk Promotion Group, which is concerned with promotion of the consumption of milk and milk products throughout its member countries and the ways in which this consumption may be increased.

GROUPS OF EXPERTS

The work of IDF which goes on all year is carried out by Groups of Experts. Each Group, and there are 85 Groups currently active, is composed of a number of highly competent experts in the subject matter under study. These experts are drawn from the top levels of the dairy industry and are nominated by national committees to work on a specific topic. The numbers of experts per group varies but would average about seven. These experts, all of whom give freely of their time and knowledge, usually meet once a year. They conduct the bulk of their work by correspondence and this work,

when completed, is of high quality and of major significance to the dairy industry.

Each of the six IDF Commissions deals with a specific, related group of subjects and meets at the time of the Annual Sessions. At Paris, 66 reports were presented by their respective Groups of Experts in the appropriate Commissions. These papers represented either mid-project or final reports of the Groups of Experts. A number of these were approved for publication by the Commissions.

A partial listing of the subject matter of these reports would include:

A. Standard method for somatic cell counting in milk.
B. Aseptic collection of milk samples - Isolation and identification of mastitogenic organisms.
C. Chemical residues in milk.
D. Code of practice for design and construction of milk collection tankers.
E. Recombination of milk and milk products - Technology and engineering aspects.
F. Methods for improving the quality of heat-treated milk.
G. General code of hygienic practice for the dairy industry.
H. Control of water and waste water in the dairy industry.
I. Forecasting milk supplies.
J. School milk.
K. Composite and modified dairy products.
L. Labelling of dairy products.
M. Yeasts and molds.
N. Coagulose-positive staphylococci.
O. Freezing point of milk.
P. Behavior of pathogens in cheese.
Q. Heat-resistant proteinases in milk.

In addition, during the individual sessions of the Commissions, special papers are presented by invited speakers on subjects which, in a number of instances, appear in the future program of work of IDF.

The system of utilizing the world's knowledge through formation of working groups of experts is the basis for the success story of IDF and, to a large extent, is unique to this organization.

IDF has as its objective the solution, through international cooperation, of scientific, technical and economic problems in the international dairy field. In the seventy-five years of its existence, its success in meeting that objective makes it unique in terms of international organizations.

SOME OBSERVATIONS

There are several observations which I would like to share with you about IDF. These observations stem from my association with IDF since 1969. During this period I have seen IDF as a delegate from Canada, as a member of the IDF Executive, as President of Commission D and, since the Quebec City Sessions in 1976, as President of IDF.

My views, opinions and consequent observations about IDF have naturally changed and have been modified as I became more familiar with the work of the Federation but can, I believe, be objectively expressed as follows:

The first and most lasting impression is that of the truly international make-up of IDF. What IDF possesses, and what I suspect is lacking in so many international organizations, is a clear-cut basis of common interest which transcends national boundaries and with which all members can readily identify. The interest to which, I refer is, of course, the dairy industry.

There is, I suspect, a rather unique common bond in all of us who have made the dairy industry our career. That this exists on a national level is perhaps not too strange but to see this common interest leaping the barriers of distance and language is a rewarding experience.

The next impression that one gains from association with IDF is the very high quality of delegates who attend annual sessions. I refer here to the high level of expertise and the vast repository of knowledge and experience that is available to IDF in the search for solutions to problems of the dairy industry.

Another observation which one can make about IDF is the diversity of interests represented by delegates to Annual Sessions and by those serving on Groups of Experts.

All facets of the dairy industry are represented: production, processing, government, research, education, supplying and marketing. Interests range, as do topics of study. Included are highly specific, highly technical scientific topics such as "peroxide and T.B.A. values in anhydrous milkfat" and economic analysis as embodied in the work dealing with "methods of market demand analysis for the short, medium and long terms."

IDF has something for everyone in the dairy industry and the flow of information, reports and studies from IDF to National Committees is indicative of the diversity of work and the cooperation that exists between all associated with IDF.

I must mention also the association on a personal basis that flows from the work of IDF. The close cooperation between members of groups of experts and the personal rapport which is thereby established between individuals and thus between countries facilitates an easy exchange of ideas - on a number of topics of concern - between the members of IDF. Thus contacts made within the fraternity of IDF expand into lifelong, valuable links.

A final observation but, I believe, an important one. No country is unique in having to face problems and challenges in its dairy industry. The more one sees of the world's dairy industry the more one realizes that problems know no national boundaries and that solutions must be sought by cooperative effort from all sources.

The experience of other countries can be and is of immeasurable value in the search for solutions to problems. We must avoid narrow, parochial thought and must seek answers wherever they can be found. In so

many instances, we find in so doing that there is absolutely no necessity in trying, as we so often feel we must, to re-invent the wheel. The answer lies, in many instances, just over the horizon in the experience of others. IDF provides the international forum for such cooperative exchange of thoughts, experiences and solutions.

NORTH AMERICAN PARTICIPATION

While Canada has been a member of IDF for over 25 years, we did not play an active role until 1970. As a background to our decision in Canada to proceed with active participation in IDF and to develop the sort of national committee structure which could ensure this close involvement, we made a careful assessment of the advantages to Canada of membership in the IDF. Canada shares many common features with the United States, not the least of these being the ability to objectively analyze the alternatives, benefits and costs of any situation and to make appropriate decisions on the basis of this analysis. This we did and came to the strong conclusion that we should be "in" as a member of IDF and "in" on an active basis.

This revitalization in the 1970's led to a reorganization of the Canadian National IDF Committee. A more representative Committee was developed with a better base of financing.

As a result of this membership base, the Committee is in a position to assist, where necessary, with the travel and living costs of its official delegates to IDF annual meetings, its members on Groups of Experts and Permanent Committees and its nominees to scientific symposia and seminars. Also, and of very real importance, the Committee can now develop, on a constantly expanding basis, the means of providing effective communication and utilization in Canada of the results of the work and activities of the IDF.

Currently Canada has members on 18 Groups of Experts and, in making this positive contribution to the international exchange of ideas, has reaped the benefits of involvement and participation to an extent far greater in value than the cost of our annual membership fee.

The United States of America, while not a member, participates in the work of 25 Groups of Experts in Commission E through the involvement of the AOAC on joint IDF/ISO/AOAC standard groups. Additionally, corresponding membership is held on two Groups by American scientists.

Hence to some Americans, albeit a limited few, IDF is a known commodity. A strong body of interest regarding IDF membership exists in the United States of America. Concerns also exist that the development of a National IDF Committee in the United States of America could diminish the domestic authority and autonomy of some member organizations. This has not proven to be true in other IDF member countries.

ACKNOWLEDGMENTS

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Relative Risk Ratios of Foodborne Illness in Foodservice Establishments: An Aid in Deployment of Environmental Health Manpower

O. BENJAMIN KAPLAN[1] and AMER EL-AHRAF[2]*

Department of Environmental Health Services, 1111 East Mill Street, San Bernardino, California 92415 and Department of Health Science and Human Ecology, California State College - San Bernardino, 5500 State College Parkway, San Bernardino, California 92407

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ABSTRACT

By using "relative risk ratios", various types of foodservice establishments were shown to pose significantly different risks of food poisoning. This implies that there is no solid basis for the traditional belief that all foodservice establishments must be inspected a given number of times.

Among the many facets of environmental health services, foodservice establishment inspection occupies a significant place and consumes a large portion of sanitarian manpower. In 1973 more than 12,500 cases of foodborne illnesses were reported, a figure many believe is a gross underestimation of actual incidence (*2*).

A basic purpose of food inspection, enforcement and educational activities remains the control of foodborne illnesses. An administrator who could predict the risk of foodborne illnesses posed by each type of foodservice establishment would be able to increase the surveillance of high-risk establishments and decrease that of low-risk ones. This would result in a more effective deployment of sanitarian manpower and related resources.

Several environmental health studies identify foods most likely to be implicated in disease outbreak, their microenvironments and methods of protecting the health of the public from risk associated with their consumption (*1,3*). However, from a management point of view, it is important to note that there is no available literature which provides reliable quantitative estimates of risk of foodborne illnesses associated with given practices or characteristics of establishments. Indeed, it would be difficult to conduct a valid multivariate analysis of the extremely complex system schematized in Fig. 1. Each one of this system's steps is subject to innumerable socioeconomic, biologic and psychological variables, such as turnover of restaurant personnel (or management), education of same and of the public, natural population-resistance to microorganisms, motivation and ability to report and record food poisoning cases, etc. In addition, each step in Fig. 1 is in itself a complex subsystem consisting of many substeps, and influenced by many variables.

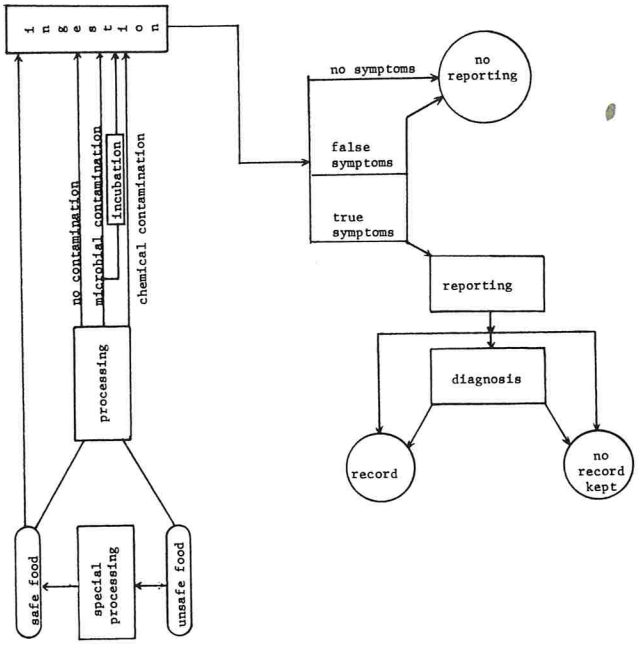

Figure 1. *Schematic of general stepwise processes leading to a recorded case of foodborne illness.*

It may be difficult to estimate the food poisoning risk associated with a given establishment; however, it is possible to estimate the relative risks associated with different *types* of establishments, as this paper will show.

Relative Risk Ratios (RRR) can best be defined in the following way. If establishment A poses a risk of foodborne illness equal to u, and establishments B and C pose risks equal to (respectively) 2u and 5u, then the relative risk ratios for A, B, and C are, respectively, 1, 2 and 5.

Table 1 presents the frequency of foodborne illness outbreaks reported by type of establishment in a major Southern California county. It forms a basis for relative risk ratio calculation. Two things should be noted in this table. One, there was a substantial increase in reported outbreaks after 1974. This might be due to the publicity given to a county-wide foodservice personnel training program introduced by the county environmental health

[1]*Department of Environmental Health Services, San Bernardino.*
[2]*California State College.*

TABLE 1. *Percent of foodborne illness outbreaks reports by establishment.*[a]

Categories	1973		1974		1975		1976	
Fast-Food								
Independent	(7)	15%	(4)	10%	(13)	17%	(16)	17%
Franchise-chain	(10)	21%	(12)	30%	(18)	23%	(20)	22%
Total		36%		40%		40%		39%
Restaurants								
Independent	(16)	35%	(11)	29%	(31)	40%	(28)	30%
Franchise-chain	(9)	19%	(7)	18%	(11)	14%	(14)	15%
Total		54%		47%		54%		45%
Markets								
Independent	(1)	2%	(1)	3%	(2)	3%	(6)	6%
Franchise-chain	(4)	8%	(4)	10%	(3)	4%	(7)	8%
Total		10%		13%		7%		14%
Liquor		0		0		0	(1)	0
Total	(47)		(39)		(78)		(92)	

[a]Numbers in parentheses represent actual numbers of outbreaks.

TABLE 2. *Relative risk ratios of food poisoning outbreak.*[a]

Category of establishment	Approximate number of establishments	y Percent of total establishments	x Percent outbreaks	x/y Relative risk	Relative risk ratio
Fast-food & restaurant	2,500	70	89 ± 4.2	1.27 ± .06	3
Markets	900	25	11 ± 3.6	.44 ± .14	1
Liquor stores	185	5	0.25 ± .86	negligible	negligible
Total	3,600				

[a]Column x represents the calculated mean and standard deviation of percent of outbreaks for years 1973-76, from data in Table 1. The difference between the two given relative risks is highly significant, as the 99% confidence interval for the means in column x/y do not overlap in the least.

unit early in 1975. It is also possible that the increase occurred because of random statistical fluctuations, higher turnover in restaurant personnel, more meals sold, or other reasons. The other thing to be noted is that all *percentages* remain fairly constant from year to year. The percentages are quite insensitive to variation in total number of reported outbreaks or publicity-induced variables, or to other variables which affect all foodservice establishments in the same proportion. The establishments categories and subcategories (independent vs. chain) were not defined operationally, and the data available on the number of establishments were rudimentary. Therefore, the number of establishments in each category is not known accurately. This made it necessary to aggregrate fast-food and restaurant establishments into a single category.

The procedure for calculation of relative risk is shown in Table 2. Since only the approximate total number of fast-food plus restaurant establishments is known, these two categories are merged into one. And since the data refer to reported outbreaks only, relative risk of reported outbreaks is calculated.

The relative risk ratio (last column in Table 2) is obtained by dividing all the relative risks by the smallest relative risk. In this instance, the smallest relative risk corresponds to liquor stores, but it was discarded because it is subject to large inaccuracy (the standard deviation is large compared to the mean) and it is itself negligible.

The term relative risk *ratio* is easier to understand. As seen in Table 2, the average establishment in the category "fast-food and restaurant" is three times more likely to generate a reported outbreak than a food market. This is probably due to the difference in the type and quantity of products sold, and perhaps to management in food sanitation, years in business, and other variables which were not taken into account when Table 1 was prepared.

DISCUSSION AND CONCLUSIONS

Locally, there are highly significant differences in the risk of foodborne illness outbreaks presented by different categories of foodservice establishments. Therefore, there is no logical basis for the traditional rule that all types of establishments must be inspected a given number of times.

ACKNOWLEDGMENT

We are indebted to P. Ryan for allowing us to use his tabulation of outbreak incidence (Table 1).

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A Field Topic

Current Concepts in Brucellosis

FRANCIS J. DRAZEK

Diagnostic Laboratory, New York State College of Veterinary Medicine
Cornell University, Ithaca, New York 14853

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ABSTRACT

Brucellosis continues to be a very devastating disease in the United States and throughout the world. In dairy herds, spread of the disease often results in severe milk losses due to the necessary slaughter of infected animals. Many herds have been depopulated because herd owners have not followed management recommendations to control the spread of infection. Undulant fever is a frequent occurrence in farm families where herds are infected. Increased movement of cattle, including importation of animals, has contributed to its spread. Prevention, control, and eradication have been impeded by present housing and management practices. The general apathy on the part of the owner in not having herd additions blood-tested for brucellosis before entry into the herd is also an important contributing factor.

Brucella abortus, which causes contagious abortion in cattle, was discovered by Bang in Denmark in 1897. The 81 years that have elapsed since his accomplishment have produced much knowledge about the disease, including development of tests for antibodies, bacterial cultures, vaccine, and proper management practices. In spite of this, as much confusion as clarity still exists about control and eradication of the disease in a given herd or geographical area. Allow me to take you on a brucellosis trip, which should provide support for this belief.

Brucellosis is brought into a herd through the purchase of animals of unknown or questionable source. Herd additions should originate from known *Brucella*-clean herds, blood-tested at the time of purchase, then isolated for at least 30 days, retested and found to be negative.

The incubation period of brucellosis may vary from 1 month (or less) to 9 months (or more). A cow may be exposed to *Brucella* following calving and not show any evidence of the disease until she is in the last trimester of the next pregnancy. Another cow may become similarly infected and a blood-reactor in a month. A calf born of an infected cow may not show any evidence of the disease until she aborts at approximately 2 years of age.

Uterine fluid provides an excellent medium for growth of *Brucella*. When an infected cow calves or aborts, this fluid plus the calf or fetus and the afterbirth are saturated with the bacteria. Due to their inquisitive nature, other animals are likely, unless restricted, to come in contact with the tissues or fluid and thus contract the disease. Persons administering to the calvings or abortions can likewise manually carry the disease on their clothing, hands, and footwear. They can infect feed or feed areas or animals they come in contact with.

DIAGNOSIS

Brucellosis is frequently diagnosed in a herd after abortions have occurred. A veterinarian will collect blood samples for serologic (antibody) tests. Standard tube and card tests are generally done on the samples. Abomasal fluid from the aborted fetus provides excellent material for culturing *Brucella*. Individual quarter milk samples also may be collected (aseptically) for culture attempts.

If abortions have not occurred or have been disregarded, brucellosis is often diagnosed by: (*a*) tracéout of the purchase of animals from a source that has revealed brucellosis in other herds: (*b*) a suspicious milkring test in dairy herds, or (*c*) market cattle testing; blood from every adult animal is collected at time of slaughter and tested for brucellosis.

In any of these instances (a, b, or c) the entire herd is subsequently blood-tested. There is no effective cure for brucellosis in cattle. The bacteria live within the body cell. Antibiotics and other treatments are effective where organisms exist between cells. *Brucella* organisms thrive on moisture and contamination. Cold weather (including freezing and thawing) will enable them to survive for weeks or months. The uterus of an infected cow may harbor the organisms for a month or more, discharging some of them almost constantly.

Strong sunlight with a temperature of 80 F or higher and its accompanying drying effect will destroy *Brucella* in a day or less. Cresylic disinfectants are very effective for use on rubber footwear and to disinfect the premises following proper cleaning.

SPREADING BRUCELLOSIS

There is little or no danger of spreading brucellosis from one farm to another by the dairy inspector or inseminator who is cautious and applies good basic judgment. This person should wear rubber footwear and carry a disinfectant, including a pail and scrub brush. Keep away from cows that have calved or aborted or are otherwise discharging uterine fluid which may also be spattered on walls and partitions. A procedure that I have adopted is to disinfect upon arrival at a farm and disinfect before departure. It leaves a good impression with the owner and is an effective disease control measure against the spread of many diseases.

Undulant fever in man is contracted by drinking raw milk or by ministering to calvings or abortions of *Brucella*-infected cows. Slaughter

house workers are a high risk group when they handle *Brucella* reactors. Accidental exposure to *Brucella* vaccines may result in undulant fever.

In cattle, *Brucella* localizes and is generally confined to the uterus, the udder and accompanying lymph nodes. There is no evidence that undulant fever has been contracted by eating meat from infected animals since cooking destroys *Brucella*.

VACCINES

A number of *Brucella* vaccines are used throughout the world. A 45-20 vaccine requires repeated injections. H-38 vaccine is used extensively in some parts of Europe and elsewhere. Research and evaluation of these products in this country have been disappointing. The only vaccine that is available for general use in the United States is Strain 19. It is a live vaccine that is made from *Brucella abortus* Biotype I which is one of the field-strain organisms. It is administered to calves, generally under 6 months of age. Some calfhood-vaccinated animals show a vaccine antibody blood reaction for years or for the life of the animal. It is indistinguishable from blood reactions caused by the field-strain of *Brucella*. This creates a genuine problem in a brucellosis eradication program.

Most people involved in brucellosis programs agree that Strain 19 vaccine protects only two out of three animals that are vaccinated with it. Our experience in New York State has shown that Strain 19-vaccinated animals are just as susceptible as non-vaccinates in a herd outbreak. The following summary illustrates my point.

During a six-month period in 1977, culture attempts were made of tissues from 54 *Brucella* reactors which were found on blood test. All culture attempts were made at the Diagnostic Laboratory of the New York State College of Veterinary Medicine. Of these, 35 (or 65%) yielded *Brucella abortus* Biotype I, 18 (or 51%) had been calfhood-vaccinated with Strain 19, 17 (or 49%) did not show proof of Strain 19 calfhood vaccination, 19 (or 35%) reactors did not yield *Brucella*, 9 (or 47%) of these showed proof of calfhood vaccination with Strain 19, and 10 (or 53%) did not show proof of calfhood vaccination with Strain 19. Three cows aborted in each group of calfhood vaccinates (Strain 19) versus non-vaccinates. Culture attempts were not made on all six of these animals. The owner did not notify us when some of the abortions occurred. All six were *Brucella* blood reactors, however.

Strain 19 adult vaccination of cattle has been used in some of the Southern states, if requested by the owner and approved by State and Federal disease-control officials. Its main benefit seems to be to help cows produce a live calf at or near full term. In my opinion, it contributes little toward the total eradication of brucellosis in the United States, which is the goal of the U.S.D.A. Its use is not permitted in states where the incidence of brucellosis is low. Some of the states that import most of their milk will not accept milk from states that permit use of Strain 19 in adult cattle.

CONTROL

Increased herd size, loose housing and a tremendous increase in the exchange and movement of cattle have all contributed immensely to cattle disease problems. Brucellosis is no exception. Mechanization has enabled farm operations to handle more animals with less help. In a brucellosis outbreak, there can be no short-cuts. The following list of recommendations must be adhered to in the elimination of the disease from infected herds.

I. Pregnant animals should be closely observed for impending abortion or calving morning and night on a daily basis.
   A. Pregnancy exams should be made on every animal of questionable status.
      1. Most *Brucella*-caused abortions occur during the last trimester of pregnancy (6 to 9 months).
      2. *Brucella* abortions may occur as early as 3 to 6 months of pregnancy.
      3. A cow can calve at full term (or beyond), with an apparent normal calf, expel her placenta, milk well, yet be infected with brucellosis.
   B. A cow will generally give a 24-h warning before aborting - sudden bagging and "settling or dropping of cords" followed by discharge and some placenta showing.
   C. All animals that show signs of aborting or calving should be segregated immediately by being tied individually or placed in separate pens.
      1. Areas should be easy to clean and disinfect.
      2. One or two designated (reliable) people should handle all calvings and abortions. They should wear rubber footwear and (if possible) rubber or plastic apron or overalls and disinfect them. Otherwise, they should change their clothes and have same laundered before re-use.
         a. Aborted fetuses should be placed in a plastic bag and buried immediately.
         b. Newborn calves should be removed to an isolated area. They should be carried but not through stable areas. Preferable not to raise any calves until several months of clean tests.
         c. Calving and aborting areas should be thoroughly cleaned and disinfected regularly.
         d. Dogs and cats should be excluded from all barns.
         e. Fresh cows should remain in isolation until they have passed a negative blood test 2 weeks following calving.
         f. Aborted animals that do not show evidence of brucellosis at time of abortion should remain in isolation until after they have been retested at least 2 weeks later, and if still negative,

remain in isolation another 2 weeks and again be tested.

g. Animals that retain their placenta and/or have a discharge following calving should be handled in the same manner as aborted animals (above) until their status is determined.

II. Disinfectants should be placed in areas of entry to mangers and segregation areas. Fresh disinfectant solutions should be made frequently.

Herd owners tell us that these recommendations are too stringent and cannot be implemented. Exposure and spread may continue for months. Eventually they realize that "everything else has failed" and they do enact our recommendations. By then, it is often too late to save much of the herd.

ACKNOWLEDGMENT

Presented at the 65th Annual Meeting of the International Association of Milk, Food and Environmental Sanitarians, Kansas City, Missouri, August 13-16, 1978.

News and Events

Journal of Food Protection now Registered Trademark

IAMFES has received word that the trademark, *Journal of Food Protection*, is registered as of Feb. 6, 1979 with the U.S. Patent and Trademark Office. The mark is registered under the Trademark Act of 1946 and remains in force for 20 years.

Journal of Food Protection was first used March 15, 1976 and it became the name of the official publication of IAMFES with Volume 40, January, 1977. The *Journal* was formerly called the *Journal of Milk and Food Technology*.

HEW Report on State Programs Available

The HEW report, "State Government Activities in Food and Drug Control and Related Programs," is available, free, in single copies to interested persons.

The report is published to provide information on individual states' efforts to develop and maintain comprehensive programs of eliminating unnecessary threats and hazards to consumer health. The report provides a national profile of such aspects of the programs as resources, personnel, legislation, and analytical and inspection services.

To obtain a copy, write:

HEW/PHS/Food and Drug Administration
Division of Federal-State Relations/EDRO
State Services Branch (HFO-310)
Room 15-A-19 Parklawn Building
5600 Fishers Lane
Rockville, MD 20857

New "DRINC® Briefs" Features Quick Test For Penicillin

Dairy Research, Inc. (DRINC) has announced the availability of its latest DRINC® Briefs, Bulletin No. 179. The bulletin describes research and development projects funded by DRINC during the past year.

Articles in the current issue include: The new quick test for penicillin residues in milk which is both accurate and low cost; on-site whey concentrating system for cheese makers producing less than 200,000 lbs. raw whey per day; new milk-based shake dispenser; boosting cheese yield by ultra filtration; the third DRINC technical booklet on properties of dairy ingredients used in confections and other food formulas.

Other current DRINC projects are listed as is information on conditions necessary for project aid.

DRINC is the product and process R&D arm of United Dairy Industry Association, an organization dedicated to increasing the sale of U.S. produced dairy products through its total promotion program. For a copy of Drinc Briefs, No. 179, write: Dairy Research, Inc., 6300 North River Road, Rosemont, IL 60018.

Alberta Association Honors Charles

Walter D. Charles, recently retired as the Supervisor, Edmonton District Office of the Health Protection Branch, was named winner of the 1978 "Sanitarian of the Year" award of the Alberta Association of Milk, Food and Environmental Sanitarians. The award was presented at that group's Annual Meeting.

Charles has served as an active member in numerous committees concerned with use and control of pesticides, dairy and food processing and sanitation, and in committees involved with chemical and microbiological quality of food products.

In addition to his many professional activities, Charles found time to be active in church activities, the Boy Scout organization and committees concerned with combatting drug abuse.

Please Note...

Additional news items appear elsewhere in this issue, at the ends of research articles. Please check the table of contents for the location of the calendar, the new Editorial Board members, and excerpts from recent CDC *Morbidity and Mortality Weekly Reports.*

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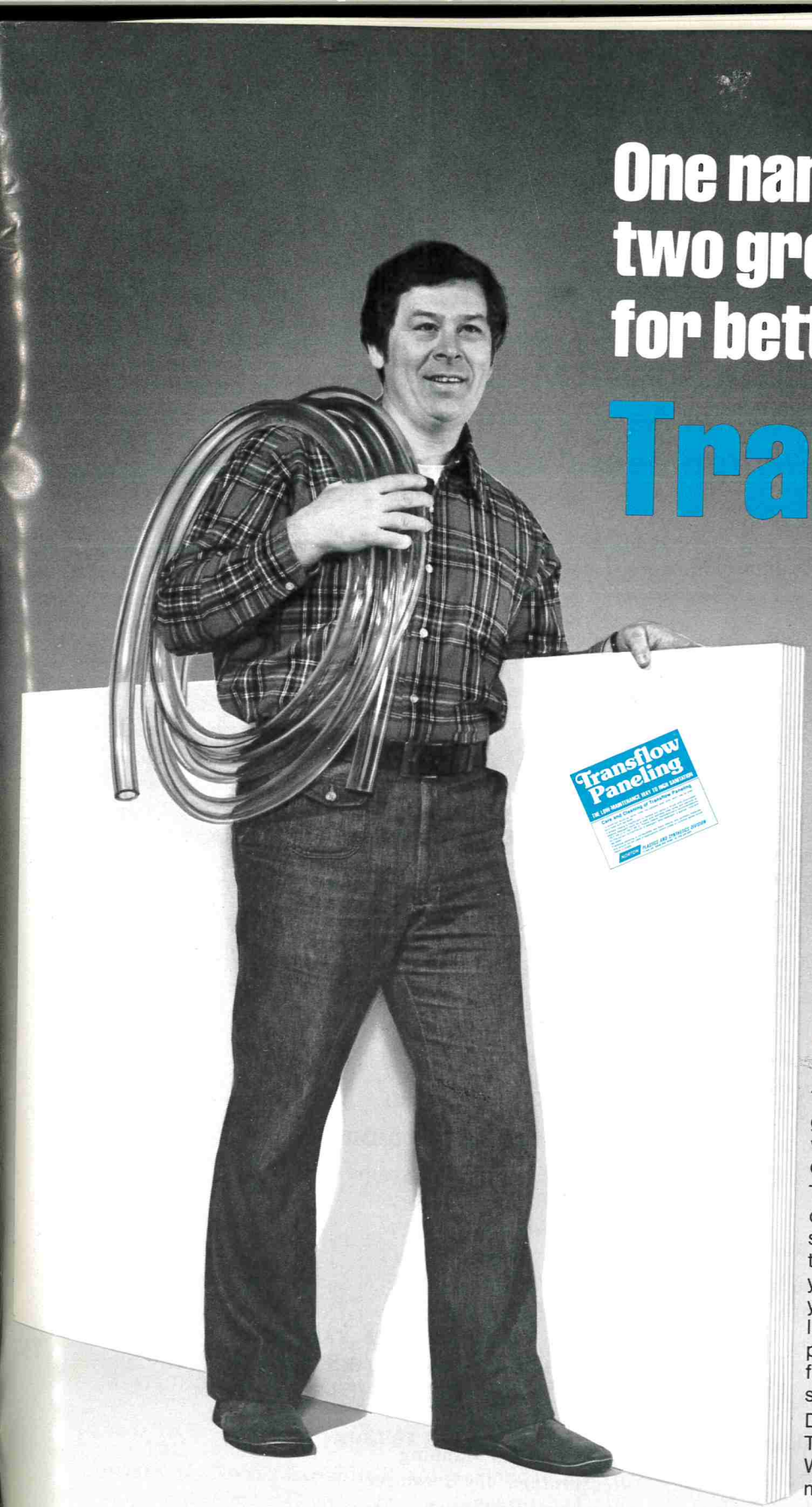

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PROGRAM

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

In cooperation with
Florida Association of Milk, Food and Environmental Sanitarians

Sheraton Twin Towers **Orlando, Florida**

August 12-16, 1979
Howard E. Hutchings
President
I.A.M.F.E.S., INC.

REGISTRATION TIMES

Sunday, August 12 - 1:00 P.M. - 5:00 P.M.
Monday, August 13 - 8:00 A.M. - 5:00 P.M.
Tuesday, August 14 - 8:00 A.M. - 5:00 P.M.
Wednesday, August 15 - 8:00 A.M. - 5:00 P.M.
Thursday, August 16 - 8:00 A.M. - 12:00 Noon

REGISTRATION FEES

	Advanced	*At Door*
Registration Fee	$18.00	$23.00
Student	No Charge	No Charge
Spouse	7.00	10.00
Children's Activities	6.00	10.00
Banquet	15.00	17.00

IAMFES OFFICERS AND EXECUTIVE BOARD

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JOURNAL OF FOOD PROTECTION

Editor: Elmer H. Marth, Madison, Wisconsin
Managing Editor: Earl O. Wright, Ames, Iowa
Associate Editor: Jan Richards, Ames, Iowa

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President ..... Lupe Wiltsey
Past President and Secretary-Treasurer ..... Tom Hart
Vice President ..... William Brown
Director ..... Dick Holtsclaw
Director ..... Luther Lockaby
Director ..... John Koburger
Director ..... William Isbell
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Harry Haverland
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Assistant: Bill Brown
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Transportation, Spouse and Children's Activities: Ray McCown
Assistant: Mary McCown
Milk Breaks: Debbie Miller
Door Prizes: Dave Fry

Meeting Rooms: Tom Hart, Jr.
Photography: Dietrick Wolfframm, George Baumieste
Publicity: C. Bronson Lane
Auditor: Luther Lockaby
Assistant Auditor: John Koburger

AFFILIATE COUNCIL OFFICERS

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Secretary: Archie Holliday

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David D. Fry	Executive Board
Henry Atherton	Executive Board

SATURDAY, AUGUST 11, 1979

4:30 - 7:30 p.m. Executive Board — Florida Keys

SUNDAY, AUGUST 12, 1979

9:00 a.m. - Noon Executive Board — Florida Keys
1:00 - 5:00 p.m. Registration — Convention Center Lobby
1:00 - 4:00 p.m. Journal Management Committee, Pete Read, Chairman — Pinellas
1:00 - 5:00 p.m. Committee on Communicable Diseases Affecting Man, Frank Bryan, Chairman (Sunday through Thursday) — Volusia
1:00 - 4:00 p.m. Council of Affiliates, Clair Gothard, *Chairman,* Archie Holliday, *Secretary* — Hillsborough & Sarasota
3:00 - 5:00 p.m. Executive Board (Executive Board should schedule part of this time to attend part of Council meeting) — Florida Keys

MONDAY, AUGUST 13, 1979

All day, Aug. 13-15 Wisconsin Affiliate - Cape Canaveral
7:00 - 9:00 a.m. Executive Meeting (Breakfast Meeting) — Florida Keys
8:00 a.m. - 5:00 p.m. Registration — Convention Center Lobby
9:00 a.m. Applied Laboratory Methods Committee, A. Richard Brazis, *Chairman* — Dade
9:00 a.m. Milking Machine Manufacturers, Ed Chibica, *Chairman* (Breakfast Meeting) — Pinellas
9:00 a.m. - Noon Farm Methods Committee, Dale Termunde, *Chairman* — Lake Room
9:00 a.m. - 5:00 p.m. Food Equipment/Sanitary Standards Committee, Karl Jones, *Chairman* — Indian River
9:00 a.m. Council of State Sanitarians Registration Agencies — Hillsborough
10:00 a.m. - Noon Foodservice Journal Committee, Dee Clingman, *Chairman* — St. Johns Room
10:00 a.m. - Noon Sanitarians Joint Council, Karl Jones, *Chairman* — Duval
1:00 - 4:00 p.m. National Conference on Interstate Milk Shipments — Sarasota
Food Equipment/Sanitary Standards Committee — Indian River
Ladies' Hospitality — Palm Beach
Speakers' Hospitality — Third Floor Parlor
Local Arrangements #1 — Dade
Local Arrangements #2 — Broward

Afternoon — General Session — Seminole
W. Kempa, Presiding

1:25 p.m. DOOR PRIZE
1:30 p.m. INVOCATION — Sam Noles, Past President, IAMFES
1:35 p.m. ADDRESS OF WELCOME — Bob Graham, Governor of Florida
1:55 p.m. PRESIDENTIAL ADDRESS — Howard Hutchings
2:15 p.m. AGRICULTURE'S CHALLENGE IN FLORIDA — Doyle Connor, Commissioner of Agriculture, State of Florida

3:00 p.m. MILK BREAK

3:15 p.m. CONSUMER IMPACT — R. Alexander Grant, Special Assistant for Consumer Affairs, HF-7, FDA, Parklawn Building, Rockville, Maryland

4:00 p.m. IMPLEMENTATION OF A PLAN TO ACHIEVE RECIPROCITY BETWEEN FOODSERVICE MANAGER CERTIFICATION PROGRAMS — C. Dee Clingman, Director, Food Protection Programs, National Institute for the Foodservice Industry, Chicago, Illinois

4:20 p.m. PROTEOLYTIC AND LIPOLYTIC ACTIVITIES OF SOME TOXIGENIC AND NONTOXIGENIC ASPERGILLI AND PENICILLIA — S. M. El-Gendy and E. H. Marth, Dept. of Food Science, Univ. of Wisconsin, Madison, WI

4:40 p.m. ANNOUNCEMENTS

MONDAY EVENING, AUGUST 13, 1979
W. Kempa, Presiding

7:00 - 9:00 p.m. MILK SANITATION, INTERSTATE MILK SHIPPERS — Pinellas-Hillsborough
H. H. Vaux, Discussion Leader, Chairman, National Conference on Interstate Milk Shipments, Indiana State Board of Health, Indianapolis, Indiana
SURVIVAL AND GROWTH OF *CLOSTRIDIUM* SPECIES IN THE PRESENCE OF HYDROGEN PEROXIDE — S. M. El-Gendy, T. Nassib, H. Abed-El-Gellel and M. El-Hoda, Dairy Dept., Faculty of Agriculture, Assiut University, Assiut, Egypt.

TUESDAY, AUGUST 14, 1979
Morning — General Session — Seminole
Howard E. Hutchings, Presiding

8:10 a.m. DOOR PRIZE

8:15 a.m. DEVELOPMENT OF A PRODUCTION SPRAY MACHINE FOR THE APPLICATION OF POTASSIUM SORBATE ON BAKED GOODS — C. S. Hickey, Monsanto Company, St. Louis, Missouri

8:35 a.m. FOOD SAFETY THROUGH HAZARD CONTROL — Joe Byrnes, Manager of Quality Control, Kraftco., Chicago, Illinois

9:15 a.m. METHODS FOR THE EFFECTIVE RECOVERY OF *SALMONELLA* OF FISH AND SHELLFISH — J. Y. D'Aoust and R. Gélinas, Health Protection Branch, Health and Welfare Canada, Ottawa, Ontario

9:35 a.m. THE EFFECT OF INTERRUPTED INCUBATION ON THE TOTAL PLATE COUNT OF RAW MILK — K. L. Smith and R. L. Richter, University of Florida, Gainesville, Florida, and Cleo Cook and Doris Marchetti, Florida Dept. of Agriculture and Consumer Services, Tallahassee, Florida

9:55 a.m. MILK BREAK

10:05 a.m. DOOR PRIZE

10:10 a.m. ANNUAL BUSINESS MEETING
1. Report of Executive Secretary
2. Report of Secretary-Treasurer
3. Committee Reports
4. 3-A Symbol Council Report
5. Report of Resolutions Committee
6. Report of Affiliate Council
7. Old Business
8. New Business
9. Election of Officers

TUESDAY AFTERNOON AND EVENING, AUGUST 14, 1979

On your own - transportation provided to:
Disney World
Sea World

2:00 - 3:30 p.m. FLA AFFILIATE BUSINESS MEETING AND AWARDS — Duval, St. Johns, Sarasota & Hillsborough

WEDNESDAY, AUGUST 15, 1979
Morning — Milk Sanitation Section — Pinellas, Hillsborough, Sarasota and St. Johns
William Arledge, Presiding

8:10 a.m. DOOR PRIZE

8:20 a.m. GETTING GOOD PRELIMINARY INCUBATION COUNTS — Sidney E. Barnard, Professor of Food Science Extension, Pennsylvania State University, University Park, Pennsylvania

8:40 a.m. SENSORY, SHELF-LIFE, MICROBIAL, AND CHEMICAL EVALUATIONS OF CREAMED COTTAGE CHEESE TREATED WITH SORBATES — F. W. Bodyfelt, Dept. of Food Science and Technology, Oregon State University, Corvallis, Oregon

9:00 a.m. STACK POURING OF PLATES: A POTENTIAL SOURCE OF ERROR — John A. Koburger, Dept. of Food Science and Human Nutrition, University of Florida, Gainesville, Florida

9:20 a.m. A FIELDMAN'S AND SANITARIAN'S CHALLENGES — Myron P. Dean, Professor, Department of Food Science, University of Wisconsin, Madison, Wisconsin

10:00 a.m. RANCID MILK — Dave Bandler, Assistant Professor, Food Science Extension, Cornell University, Ithaca, NY

10:45 a.m. MILK BREAK

11:00 a.m. ANALYSIS OF ANIMAL FEED INGREDIENTS AND SOIL AMENDMENT PRODUCTS PRODUCED FROM CATTLE MANURE FOR SELECTED TRACE METALS USING ATOMIC ABSORPTION SPECTROPHOTOMETRY — W.

V. Willis, California State University, Fullerton, CA; Amer El-Ahraf, Dept. of Health Science and Human Ecology, California State College, San Bernardino, CA; V. V. Dutt, Monsanto Chemical, St. Louis, MO; Khairy Aref, California Regional Water Quality Control Board, Los Angeles, CA

11:20 a.m. COMMERCIALLY STERILIZED BEVERAGE MILK — Ray Mikaby, Vice-President, Drinc, Rosemont, Illinois.

Food Sanitation Section — Seminole
William Kempa, Presiding

8:10 a.m. DOOR PRIZE

8:20 a.m. RELATIONSHIPS OF MICROBIAL QUALITY OF RETAIL MEAT SAMPLES AND SANITARY CONDITIONS — C. Jane Wyatt and V. Guy, Dept. of Food Science and Technology, Oregon State University, Corvallis, Oregon

8:40 a.m. INHIBITION OF *STAPHYLOCOCCUS AUREUS* BY POTASSIUM SORBATE IN COMBINATION WITH SODIUM CHLORIDE, TBHQ, BHA, AND EDTA — M. C. Robach, Monsanto Company, St. Louis, Missouri

9:00 a.m. THE PRESERVATION OF BAKED GOODS BY SORBIC ACID AND/OR POTASSIUM SORBATE — C. S. Hickey, Monsanto Company, St. Louis, Missouri

9:20 a.m. EDUCATION: THE KEY TO SANITATION PROBLEMS — H. J. Vichary, Training Supervisor, Galveston County Health District, La Margue, Texas

10:10 a.m. THE STATUS OF RETAIL FOOD MARKET ORDINANCE — K. J. Baker, Division of Retail Food Protection, Bureau of Foods, F.D.A., Washington, D.C.

10:45 a.m. MILK BREAK

11:00 a.m. NUTRITIONAL AND MICROBIAL CHANGES DURING THE PRODUCTION OF TOSTONES (FRIED PLANTAINS) — R. J. Alvarez, J. A. Koburger, and H. Appledorf, Dept. of Food Science and Human Nutrition, University of Florida, Gainesville, Florida

11:20 a.m. FOOD SANITATION AND REGULATIONS — (1976 Model Food Code) Don Greenway, Visiting Professor, Miami University, Miami, Florida

Afternoon - Milk Sanitation Section - Pinellas
Lupe Wiltsey, Presiding

1:05 p.m. DOOR PRIZE

1:10 p.m. CORRECTING COLIFORM PROBLEMS OF PASTEURIZED MILK — Sidney E. Barnard, Professor of Food Science Extension, Pennsylvania State University, University Park, Pennsylvania

1:30 p.m. VARIATION IN TOXIN LEVELS AND COUNTS IN EMMENTALER CHEESE CONTAMINATED WITH *STAPHYLOCOCCUS AUREUS* — E. Todd, R. Szabo, H. Robern, T. Gleeson, N. Dickie, M. Akhtar, C. Park, and D. Clark, Bureau of Microbial Hazards, Health Protection Branch, Health and Welfare Canada, Ottawa, Ontario

1:50 p.m. AFLATOXIN IN MILK AND MILK PRODUCTS — Elmer Marth, Professor, Department of Food Science, University of Wisconsin, Madison, Wisconsin.

2:30 p.m. MONITORING OF FOOD SUPPLY — Martha Rhodes, Bureau Chief, Food Division, Fla. Dept. of Agriculture and Consumer Services, Tallahassee, Fla.

3:10 p.m. MILK BREAK

3:25 p.m. INDUSTRY COPING WITH THE AFLATOXIN PROBLEM — Vern Bingham, General Manager, Mountain Empire Dairymen's Association, Thornton, Colorado.

4:10 p.m. SIMPLE TECHNIQUE FOR DETERMINATION OF D VALUES FOR *BACILLUS STEAROTHERMOPHILUS* IN LIQUID SYSTEMS — E. M. Mikolajcik and Kathleen T. Rajkowski, Ohio State University, Columbus, Ohio

4:30 p.m. FERMENTATION OF SOY AND WINGED BEAN MILKS BY LACTIC ACID BACTERIA — L. A. Smoot, V. V. Garcia, and M. D. Pierson, Dept. of Food Science & Technology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia

Food Sanitation Section - Seminole
Dick Jolley, Presiding

1:05 p.m. DOOR PRIZE

1:10 p.m. *ESCHERICHIA COLI* ENTEROTOXIN PRODUCTION IN BEEF BROTH AT 15 to 50 C — J. Lovett, J. M. Bisha, and P. L. Spaulding, Food and Drug Administration, Cincinnati, Ohio

1:30 p.m. TOXIGENIC *YERSINIA ENTEROCOLITICA* IN RETAIL PORK PRODUCTS — D. A. Schiemann and M. Latvala, Ontario Ministry of Health, Toronto, Ontario

1:50 p.m. SOME CHARACTERISTICS OF ACID INJURY AND RECOVERY OF *SALMONELLA BARIELLY* IN A MODEL SYSTEM — L. C. Blankenship, U.S.D.A., Athens, Georgia

2:10 p.m. GROWTH OF *STAPHYLOCOCCUS,* ENTEROTOXIN AND THERMOSTABLE NUCLEASE PRODUCTION IN ANAEROBICALLY INOCULATED CANNED SALMON AND SARDINES — A. K. Stersky, H. Robern, T. Gleeson, C. Park, R. Szabo, and C. Thacker, Bureau of Microbial Hazards,

Health and Welfare Canada, Ottawa, Ontario

2:30 p.m. INVESTIGATION OF WATERBORNE ILLNESS — Frank Bryan, Chief, Foodborne Disease Activity, Training Program, Center for Disease Control, Atlanta, Georgia

3:10 p.m. MILK BREAK

3:25 p.m. SHORT-CHAIN FATTY ACIDS AS SANITIZERS FOR BEEF — E. Z. Quartey-Papafio, R. T. Marshall, and M. E. Anderson, Dept. of Food Science and Nutrition, University of Missouri, Columbia, Missouri

3:45 p.m. SANITIZERS IN THE FOOD INDUSTRY — James J. Jezeski, Professor, Extension Dairy Technologist, IFAS, University of Florida, Gainsville, Florida

4:25 p.m. *B. CEREUS*, A FOODBORNE PATHOGEN — O. W. Kaufmann, Supervisory Microbiologist, State Training Branch, DFSR, EDRO, FDA, Cincinnati, Ohio

WEDNESDAY EVENING, AUGUST 15, 1979

6:00 - 7:00 p.m. RECEPTION — Orange

7:00 p.m. ANNUAL AWARDS BANQUET — Orange

9:00 p.m. INSTALLATION OF OFFICERS AND ENTERTAINMENT

THURSDAY, AUGUST 16, 1979

7:30 a.m. Executive Board Breakfast Meeting — Florida Keys

8:30 a.m. - 5:00 p.m. National Mastitis Council 1979 Summer Meeting — Pinellas-Hillsborough and Sarasota

NATIONAL MASTITIS COUNCIL SUMMER MEETING PROGRAM
August 16, 1979
Sheraton Twin Towers
Orlando, Florida
Pinellas-Hillsborough and Sarasota

Presiding for Morning Program — J. J. Jezeski, University of Florida

8:15 REGISTRATION

9:00 GREETINGS AND REPORT FROM PRESIDENT OF NMC — L. H. Schultz, University of Wisconsin

9:10 PHYSIOLOGICAL EFFECTS OF HEAT STRESS ON LACTATING DAIRY COWS — R. J. Collier, University of Florida

9:35 EFFECTS OF HEAT STRESS ON MASTITIS INCIDENCE AND LEUCOCYTE LEVELS — W. D. Schultze, USDA, Beltsville, Maryland

10:00 Break

10:20 STOP TEST FOR ANTIBIOTIC RESIDUALS IN MEAT: A PROGRESS REPORT — USDA Representative

10:50 CURRENT REGULATORY SITUATION WITH TEAT DIPS, — C. R. McDuff, Economics Laboratory, Inc., St. Paul, Minnesota

11:15 UPDATE ON EUROPEAN POSITION ON IODINE RESIDUALS — W. D. Schultze, USDA, Beltsville, Maryland

11:40 Break for Lunch

Presiding for Afternoon Program — H. H. Van Hord, University of Florida

1:15 APPROACHING THE PROBLEM MASTITIS HERD — S. B. Spencer, Pennsylvania State University

1:50 MASTITIS CONTROL — THE FLORIDA APPROACH

As the Animal Health Advisory Committee Sees It — Jim Acree, D. V. M., Committee Chairman and Dairyman

A Producer's Program — Joe Baker, Dairyman, Sanford, Florida

A Practicing Veterinarian's Viewpoint — Jon Seilar, D. V. M., Orlando, Florida

Break

Field Assistance to Producers by the Cooperative — J. M. Rodriguez, D. V. M., Tampa Independent Dairy Farmer's Association

Motivating Milkers — Barney Harris, Jr., University of Florida

Discussion

4:00 Adjourn

ENTERTAINMENT, MEN AND WOMEN

SUNDAY, AUGUST 12, 1979

6:00 - 7:00 p.m. Early Bird Registration — Convention Center Lobby

MONDAY, AUGUST 13, 1979

Sea World or Buena Vista Shopping

TUESDAY, AUGUST 14, 1979

12:00 Disney World or Sea World

WEDNESDAY, AUGUST 15, 1979

6:00 - 7:00 p.m. Reception — Orange

7:00 p.m. Banquet and Entertainment — Orange

SPOUSES' PROGRAM

MONDAY, AUGUST 13, 1979

Ladies' Hospitality Room — Palm Beach

Noon - 11:00 p.m. Visit to Disney World

TUESDAY, AUGUST 14, 1979

Ladies' Hospitality Room — Palm Beach

Noon - 11:00 p.m. Visit to Disney World

WEDNESDAY, AUGUST 15, 1979

Ladies' Hospitality Room — Palm Beach

Tupperware Museum - Lunch

E-3-A Sanitary Standards for Flow Meters for Liquid Egg Products

Number E-2800

Formulated by
International Association of Milk, Food and Environmental Sanitarians
United States Department of Agriculture
Poultry & Egg Institute of America
Dairy and Food Industries Supply Association

It is the purpose of the IAMFES, USDA, PEIA, and DFISA in connection with the development of the E-3-A Sanitary Standards program to allow and encourage full freedom for inventive genius or new developments. Specifications for flow meters which so differ in design, material and construction, or otherwise, as not to conform to the following standards but which, in the fabricator's opinion are equivalent or better, may be submitted for the joint consideration of the IAMFES, USDA, PEIA and DFISA at any time.

A.

SCOPE

A.1

These standards cover the sanitary aspects of flow meters for liquid egg products, and include that portion of any device integral with the meter such as strainers, temperature sensors and density sensors, which is in contact with the flowing product.

A.2

In order to conform with these E-3-A Sanitary Standards, flow meters shall comply with the following design, material and fabrication criteria.

B.

DEFINITIONS

B.1

Flow Meter: A device to measure the flow of liquid egg products.

B.2

SURFACES

B.2.1

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

B.2.2

Non-Product Contact Surfaces: Shall mean all other exposed surfaces.

B.3

Engineering Plating: Shall mean plated to specific dimensions or processed to specified dimensions after plating.[1]

B.4

Mechanical Cleaning or Mechanically Cleaning: Shall denote cleaning, solely by circulation and/or flowing chemical detergent solutions and water rinses onto and over the surfaces to be cleaned, by mechanical means.

C.

MATERIALS

C.1

All product contact surfaces shall be of stainless steel of the AISI 300 series[2] or corresponding ACI[3] types (See Appendix, Section E.), or equally corrosion-resistant metal that is non-toxic and non-absorbent, except that:

C.1.1

Rotors of turbine-type meters may also be made of non-toxic hardenable, corrosion-resistant stainless metal (400 series stainless steel, or equivalent) or these materials covered with an engineering plating of nickel, chromium or an equally corrosion-resistant, non-toxic metal.

C.1.2

Shafts and sleeve bearings may also be made of non-toxic, corrosion-resistant tungsten carbide.

C.1.3

Rubber and rubber-like materials may be used for gaskets, seals, meter bodies, meter body liners, magnet carriers, meter valve members, coating, rotors, pistons, bearings, shafts and parts used in similar applications. These materials shall comply with the applicable provisions of the "E-3-A Sanitary Standards for Multiple-Use Rubber and Rubber-like Materials used as Product Contact Surfaces in Egg Equipment," Number E-1800.

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[1] *QQ-C-320 B - Federal Specification for Chromium Plating (Electrodeposited) June 17, 1974, 40¢.*
QQ-N-290 A - Federal Specification for Nickel Plating (Electrodeposited) November 12, 1971, 20¢. Both documents available from: Business Service Center, General Services Administration, Seventh and D Streets, SW, Washington, DC.

[2] *The data for this series are contained in the following reference: AISI Steel Product Manual, Stainless & Heat Resisting Steels, April, 1963, Table 2-1, pp. 16-17. Availabe from: American Iron & Steel Institute, 1000 16th Street, NW, Washington, DC 20036*

[3] *Alloy Casting Institute Division, Steel Founders' Society of America, 20611 Center Ridge Road, Rocky River, OH 44116*

C.1.4

Plastic materials may be used for gaskets, seals, meter bodies, meter body liners, magnet carriers, meter valve members, coatings, rotors, pistons, bearings, shafts, and parts used in similar applications. These materials shall comply with the applicable provisions of the "3-A Sanitary Standards for Multiple-Use Plastic Materials Used as Product Contact Surfaces for Dairy Equipment," Number 20-08.

C.1.5

Rubber and rubber-like materials and plastic materials having product contact surfaces that are a coating or a covering, shall be of such composition as to retain their surface and conformation characteristics when exposed to the conditions encountered in the environment of intended use and in cleaning and bactericidal treatment.

C.1.6

Pistons and rotors may also be made of hard rubber (a vulcanized rubber having a ratio of combined sulfur to rubber hydrocarbon in excess of 15% and a Shore A Durometer value in excess of 90) that is non-toxic and relatively resistant to abrasion, will maintain its original characteristics such as form, shape and dimensions and will not affect the product and shall when subjected to the test regimen set forth in the "3-A Sanitary Standards for Multiple-Use Plastic Materials Used as Product Contact Surfaces for Dairy Equipment," Number 20-08, (a) comply with the criteria in Section I (1) and Section I (3), (b) have maximum weight gains as set forth in Section I (2) of 0.30 in the Cleanability Response, and 0.30 in Product Treatment with Solution I and 0.30 in Product Treatment with Solution J.

C.1.7

Where materials having certain inherent functional properties are required for specific applications, such as pistons, shafts, bearings, and rotary seals, carbon, and/or ceramic materials may be used. Ceramic materials shall be inert, non-porous, non-toxic, non-absorbent, insoluble, resistant to scratching, scoring, and distortion when exposed to the conditions encountered in the environment of intended use and in cleaning and bactericidal treatment.

C.1.8

Silver soldered or brazed areas and silver solder or braze material shall be non-toxic and corrosion-resistant.

C.2

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

D.

FABRICATION

D.1

All product contact surfaces shall be at least as smooth as a No. 4 ground finish on stainless steel sheets. (See Appendix, Section F.).

D.2

All permanent joints in product contact surfaces shall be welded or may be silver soldered or brazed if welding is not feasible. All welded or silver soldered areas or product contact surfaces shall be at least as smooth as the adjoining surface.

D.3

The minimum thickness of engineering plating shall be 0.0002 inch for all product contact parts except that when the parts that are to be plated are other than stainless steel, the minimum thickness of the engineering plating shall be 0.002 inch.

D.4

Rubber or rubber-like materials and plastic materials having product contact surfaces that are a coating or covering shall be bonded in such a manner that the bond is continuous and mechanically sound, and so that when exposed to the conditions encountered in the environment of intended use and in cleaning and bactericidal treatment, the rubber or rubber-like material or the plastic material does not separate from the base material.

D.5

All product contact surfaces of meters not designed to be mechanically cleaned shall be easily accessible for cleaning and inspection, either when in an assembled position or when removed. Removable parts shall be readily demountable.

D.6

Meters that are to be mechanically cleaned shall be designed so that all product contact surfaces of the meter and all non-removable appurtenances thereto can be mechanically cleaned and are accessible for inspection.

D.7

All product contact surfaces shall be self-draining except for normal clingage.

D.8

Connections having product contact surfaces shall conform to "E-3-A Sanitary Standards for Fittings Used on Egg and Egg Products Equipment and Used on Sanitary Lines Conducting Egg and Egg Products," Number E-0800, and/or to the applicable provisions for welded sanitary product pipelines found in the "E-3-A Accepted Practices for Permanently Installed Sanitary Product-Pipelines and Cleaning Systems," Number E-60500.

D.9

All internal angles of 135° or less on product contact surfaces shall have minimum radii of 1/4 inch, except those in the main case of a meter and those at the base of teeth in gear type meters. When the radius is

less than 1/32 inch the product contact surface of this internal angle must be readily accessible for cleaning and inspection.

D.10

Gaskets shall be removable. Gasket retaining grooves shall be no deeper than their width. The minimum radius of any internal angle in a gasket retaining groove shall be not less than 1/8 inch, except that the radius may be 3/32 inch where a standard 1/4 inch O-Ring is to be used and the radius may be 1/32 inch where a standard 1/8 inch O-Ring is to be used.

D. 11

There shall be no threads on product contact surfaces.

D.12

Any coil spring having product contact surfaces shall have at least 3/32 inch openings between coils, including the ends.

D.13

If legs are used, they shall be smooth with rounded ends and no exposed threads. Legs made of hollow stock shall be sealed.

D.13.1

The minimum clearance between the lowest part of the base and the floor shall be:

D.13.1.1

four inches on meters with legs designed to be fixed to the floor or meters having a horizontal area of more than one square foot.

D.13.1.2

two inches on meters having a horizontal area of not more than one square foot and not designed to be fixed to the floor.

D.13.2

Bases when used shall be constructed without ribs or flanges and shall have a smooth top and bottom surface.

D.14

Non-product contact surfaces shall be readily cleanable and shall be free of pockets and crevices except those created on the face of a register at the window, ticket slots, pick-off coils, auto stop buttons, reset handles, totalizer holes and similar places.

D.15

Non-product contact surfaces to be coated shall be effectively prepared for coating.

APPENDIX

E.

STAINLESS STEEL MATERIALS

Stainless steel conforming to the applicable composition ranges established by AISI for wrought products, or by ACI for cast products, should be considered in compliance with the requirements of Section C.1 herein. Where welding is involved the carbon content of the stainless steel should not exceed 0.08%. The first reference cited in C.1 sets forth the chemical ranges and limits of acceptable stainless steels of the 300 series. Cast grades of stainless steel corresponding to types 303, 304, and 316 are designated CF-16F, CF-8, and CF-8M, respectively. These cast grades are covered by ASTM[4] specifications A296-67 and A351-65.

F.

PRODUCT CONTACT SURFACE FINISH

Surface finish equivalent to 150 grit or better as obtained with silicon carbide, is considered in compliance with the requirements of Section D.1 herein.

These standards shall become effective May 4, 1979.

[4] *Available from American Society for Testing and Materials, 1916 Race Street, Philadelphia, PA 19103.*

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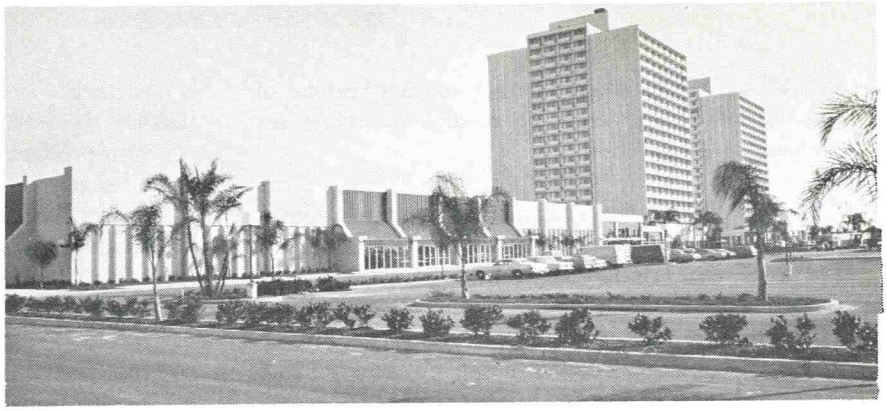

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IAMFES
Florida Dept. of Agriculture & Consumer Services
Division of Dairy Industry
508 Mayo Building
Tallahassee, Florida 32304

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