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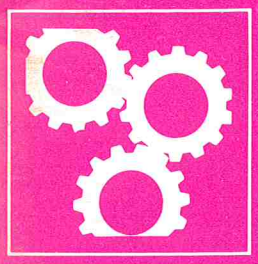

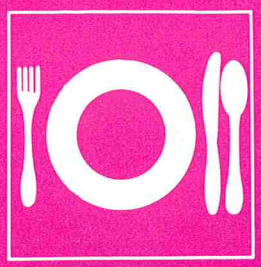

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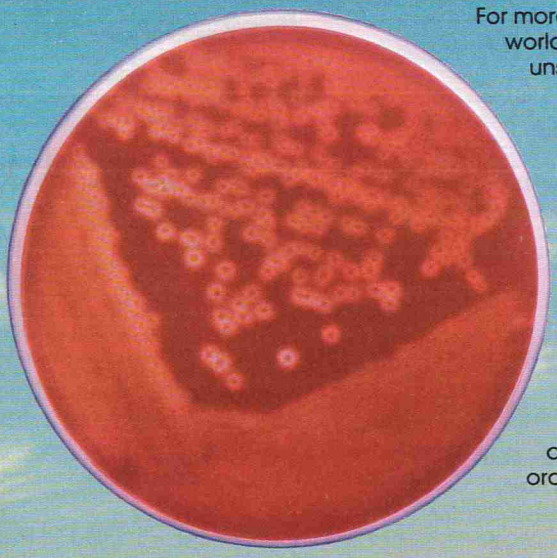

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Cooperative Study of Methods for the Recovery of Enteric Viruses from Shellfish

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(Received for publication April 27, 1979)

ABSTRACT

Ten investigators actively involved in shellfish virology agreed to participate in a methods study. Each participant was given the freedom of choice as to method and cell cultures used. Considerable variation was noted in the number of viruses recovered from six 100-g representative samples. All the methods appeared to detect polioviruses but difficulty was encountered in detecting low levels of coxsackieviruses and echoviruses. The glasswool filtration-hydroextraction method and modifications of the Sobsey method were the most effective techniques for detecting and quantitating virus in this study.

A number of methods have been developed for direct enumeration of viruses in shellfish. These methods basically detect polioviruses and viruses of the coxsackie B group; however, they are less effective for detection of coxsackie A viruses, echoviruses and reoviruses, and do not detect hepatitis or human gastrointestinal viruses. The question was raised as to the limitations of existing virus methods and whether such methods could be used to detect the low levels of enteroviruses that might be encountered in shellfish.

A number of investigators known to be conducting research in shellfish virology were asked to participate in a shellfish methodology study. Each investigator was given complete freedom as to choice of method and types of cell cultures used to detect the viruses. It was anticipated that some procedures might be more effective than others and thus could provide a specific method for a future collaborative study. Eight laboratories participated in the study and one laboratory used three different methods. Therefore, ten methods were studied.

MATERIALS AND METHODS

Crassostrea virginica (oysters), *Mercenaria mercenaria* (hard shell clams), and *Mya arenaria* (soft shell clams) were obtained either from New Hampshire waters or through regular commercial outlets. All shellfish were kept for 17 days in tanks supplied with ultraviolet-sterilized running seawater. At the end of this depuration interval (controlled purification) the shellfish were moved to the Durham campus and placed in seawater holding tanks.

The viruses were either injected into the hepatopancreatic tissue of the shellfish or were added to the overlay water of 4- to 6-liter holding tanks. The pumping process of the shellfish resulted in a natural intake and distribution of the viruses in the animals. Starch granules, which have been shown to enhance virus uptake (Metcalf, et al., in press) were added to the waters along with the stock viruses. In some cases, feces naturally infected with polioviruses 1 and 2 (vaccine strains) were added to the overlay waters. Stock viruses used were polioviruses 1 and 2 (P1, P2), coxsackievirus B3 (CB3), and echovirus 7 (E7). A sewage isolate, echovirus 17 (E17), was passed once in cell culture and used in some experiments.

The shellfish that were added to the holding tanks were allowed to feed for 4 to 6 h. The temperature, salinity and pH of the overlay water were controlled to provide optimum feeding conditions. After the exposure period, the shellfish were removed, washed and dried. Homogenates of the meat and shell liquor were prepared. In addition, lots of shellfish were shucked and injected via the hepatopancreatic tissue and homogenized. Both types of homogenates were portioned into 100-g lots, frozen and forwarded to the seven participating laboratories.

Control homogenates were reserved for virus examination at the control laboratory at the University of New Hampshire. Examinations were made in the control laboratory to confirm the virus types (3) and number(s) present. Isolates recovered from each homogenate by the cooperating laboratory were forwarded to New Hampshire for confirmation. The isolates were passed in cell culture, and serum neutralization procedures were used for identification (2). No attempt was made by the control laboratory to separate virus types when more than one was present in the isolates sent in by the investigators.

RESULTS AND DISCUSSION

The control laboratory boxed two sample sets and held them for a time period equal to the longest shipping time. One set was tested and the second set was frozen until the results were received from all investigators. The second set of samples was then tested for virus content. These data were reported as initial and final results in both Tables 1 and 2. The quantitative recovery data are shown in Table 1. Considerable variation occurred in the number of viruses reported. Part of this difference was due to the examination by some investigators of only 10 to 20 g of the 100-g sample. Uneven distribution of the viruses, with high or low concentrations in the portion examined, could give a false indication of the number in the 100-g sample. Problems with uniform results are frequently encountered when a standard method is not used, and analysis of data produced in this study was affected by a number of variables such as type and passage number of cell cultures used, size of sample examined, volume of inoculum added, type and source of media components, time and temperature of incubation, quantitation methodology, and adaptation of different methods to

[1]*Food and Drug Administration.*
[2]*University of New Hampshire.*

process frozen homogenates of three different types of shellfish.

Examination of data in Table 2 shows variations in recovery effectiveness of 10 to 90% of the seeded virus types. Difficulty was encountered by most investigators in attempting to recover echoviruses. The detection of the polioviruses by most investigators demonstrated that the methods were probably developed in the laboratory using poliovirus as a test organism. The sensitivity of BGM cells to polioviruses was aptly demonstrated.

TABLE 1. *Recovery of viruses from seeded shellfish used in the cooperative study.*

Laboratory code number	Virus totals reported as	Cell cultures used	Samples tested (reported counts/100 g) A Oysters	B Hard shell clams	C Hard shell clams	D Oysters	E Soft shell clams	F Soft shell clams
1	PFU	PMK	0	16,000	14,000	16,000	1,100,000	220,000
2	PFU	BGM-PMK	11	2	0	4	0[a]	0[a]
3	PFU	BGM	113	11	4	17	1	2
	CPE[b]	BGM	18	14	9	NT	8	8
	Total		131	25	13	17	9	10
4	PFU	BGM-PMK	24	0	0	2	1	0
	CPE[b]		45	14	7	4	2	15
	Total		69	14	7	6	3	15
5	CPE	HeLa-BGM						
	Method 1		+BGM[c]	0	0	0	+Hela	+Hela
	Method 2		NT[d]	0	0	+Hela	+Hela + BGM	+BGM
6	PFU	BGM	1040	1700	512	0	0	72
7	PFU	PMK→ BGM and Hep 2	8	9	14	4	3	4
8	MPNCU[e]	BGM	128	79.6	148	14.3	6.42	94
9	PFU	BGM	101	279	102	251	8	52
Control Laboratory Recoveries								
Initial	PFU	BGM	9	6	6	4	6	8
Final	PFU	BGM	22	3	7	17	0[f]	30
	CPE		NT	NT	20	NT	NT	25
	Total		22	3	27	17	0	55

[a]Toxicity.
[b]Monolayers showing CPE expressed as 1 PFU.
[c]CPE, not quantitative.
[d]NT, not tested.
[e]MPNCU-most probable number of cytopathic units =

$$-1\,n\,\frac{\text{Number of negative cultures}}{\text{Number positive cultures}}$$

[f]No isolates recovered by PFU assay in confirmatory test.

TABLE 2. *Identification of isolates recovered from seeded shellfish samples.*

Laboratory code number	Samples tested[a] A	B	C	D	E	F	No. recovered/ No. inoculated
1	NIR[b]	mixture[c]	VINC[d]	P1	VINC	P1	1/8
2	5/9 = P1	1/2 = CB3	NIR	1/2 = P1	NIR	NIR	3/8
	4/9 = VINC	1/2 = VINC		1/2 = VINC			
3	P1	CB3	E7	P1	E17	E7,P2	7/8
4	P1	CB3	E7	P1	P2	P2	5/8
5	P1	NIR	NIR	mixture	VINC	P1	1/8
6	6/6 = P1	4/6 = E7	5/5 = E7	NIR	NIR	2/2 = E7	3/8
		2/6 = P1					
7	13/13 = P1	4/9 = P1	27/27 = P1	1/4 = P1	3/3 = P1	4/4 = P1	2/8
		5/9 = VINC		3/4 = VINC			
8	P1	1/5 = mixture	2/3 = mixture	P1,P2,CB1	VINC	P2	5/8
		4/5 = CB3	1/3 = CB3				
9	P1	P1	mixture	mixture	mixture	P1	2/8
Control Laboratory Recoveries							
Initial	P1	CB3	E7	P1,P2	E17	E7,P2	8/8
Final	P1	CB3	E7	P1	NIR	P2	5/8

[a]Sample A = P1 added to shellfish aquarium seawater - natural uptake.
Sample B = CB3 added to shellfish aquarium seawater-natural uptake.
Sample C = Dilute raw sewage plus E7 inoculated sample. E7 only isolate detected by reference laboratory control test.
Sample D = Raw sewage inoculated sample. Original assay showed P1 and P2 in ratio of 4:1 PFU, respectively.
Sample E = Dilute raw sewage plus E17 inoculated sample. Neither E17 nor any other virus was detected by reference laboratory in control PFU test.
Sample F = P2 added to shellfish aquarium seawater, natural uptake, E7 added to inoculated sample homogenate. E7 was not detected by reference laboratory in control PFU or CPE tests.
[b]NIR = no isolate recovered.
[c]mixture = two or more virus types present.
[d]VINC = virus isolation not confirmed.

The need for some changes in methods is apparent. A sample of at least 100 g should be used as an analytical unit if small numbers of viruses are expected to be present in the shellfish. Most methods were developed to detect viruses in oysters and little cell toxicity was encountered. When soft shell and hard shell clams were examined, toxicity was a serious problem. The use of both cytopathic effect (CPE) and plaque procedures produced on the same sample the most successful results and should be incorporated into future methods development. The type of cell culture system to be used is open to discussion and is limited by cost and virus susceptibility. The BGM line was used successfully in this study, but its susceptibility to natural strains of coxsackieviruses and echoviruses has been questioned (*4*,*5*).

All the methods studied appeared to detect polioviruses. Difficulty was encountered by some investigators in adapting their methodology to analyze 100-g samples. The glasswool filtration-hydroextraction method (unpublished) and modifications of the Sobsey method appeared to detect levels of < 1 PFU/g of shellfish homogenates (*6*). These methods should be examined critically to evaluate their consistency and to determine whether the study conditions favored such methodology.

A limited number of investigators have detected enteroviruses in naturally contaminated shellfish, and the reported levels of contamination were < 10 detectable virus units per shellfish (*1*,*3*). Examination of high quality shellfish resulted in the detection of only an occasional virus unit per shellfish. Because of expected low levels of contamination, the question of the size of the sample to be examined is of importance. The sample size directly influences the effectiveness of virus recovery methodology and the cost of sampling. None of the methods used in this study will detect hepatitis or human gastrointestinal viruses. It is apparent that the methodology studied was limited to detection of only some of the enteroviruses and in some cases to shellfish contamination levels of > 1 PFU/g.

After completion of this study in 1978 a number of the investigators made changes in their procedures which enhanced virus recovery efficiency. Some of these new methods are described in detail elsewhere in this issue of the *Journal*.

ACKNOWLEDGMENTS

We thank all the investigators who participated in this study. We are grateful to Barbara Mullin, Ellen Moulton, and Virgil Jones for their excellent technical assistance. This study was funded in part by Contract No. 223-73-2303 from the U.S. Food and Drug Administration. Feces obtained from children orally immunized with poliovirus vaccine was generously provided by Dr. M. M. Sigel, University of Miami School of Medicine, Miami, Florida.

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possible to contemplate would result from routine depuration of shellfish.

ACKNOWLEDGMENTS

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Uptake and Depletion of Particulate-Associated Polioviruses by the Soft Shell Clam

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ABSTRACT

Human viruses present in shellfish harvesting waters are probably in a particulate or feces-associated state, and are of a low order of magnitude. Under simulated conditions, shellfish were exposed to 5- to 10-fold higher virus concentrations than have ever been detected in New Hampshire estuary waters and examined. Usually less than 10 viruses were bioaccumulated by each soft shell clam, and when the shellfish were allowed to depurate in clean water, viruses were eliminated in 48 to 72 h. A small percentage of the shellfish did not depurate completely, a shellfish characteristic consistently found which is probably related to irregular feeding activity. Depuration or relaying of shellfish should reduce microbial contamination, but there is no guarantee that all shellfish will be virus-free.

All species of the commercially important shellfish have been shown to bioaccumulate enteric viruses from environmental seawater during feeding activities. Elimination of bioaccumulated virus occurs as a natural consequence of entrapment within the fecal mass. Depletion of virus in shellfish via depuration (controlled purification) represents an attempt to encourage or enhance the process of elimination while simultaneously preventing further bioaccumulation.

Studies of depuration effectiveness in oysters and hard shell clams have shown a rapid, initial reduction in numbers, followed by a low level of virus persistence lasting for several days or weeks, depending upon whether animal or bacterial viruses were used (*2,3,4*). With only one known exception which involved a bacterial virus (*1*), all of these studies were carried out with laboratory strains of stock enteroviruses, with numbers varying from a minimum of a few hundred to hundreds of thousands. Little consideration was given to the impact on depuration attributable to use of large numbers of stock viruses. Failure of oysters and hard shell clams to eliminate bioaccumulated virus during depuration was attributed to sequestering of virus in tissue depots, or lack of feeding with its associated movement of virus through the alimentary tract.

New insight into depuration and its potential for depletion of virus was obtained by mimicking as closely as possible the conditions found in estuarine waters polluted by domestic waste discharges. Stools from infants vaccinated with Sabin-type poliovaccine were used as a source of feces-associated natural virus. The stools contained progeny virus adsorbed to fecal particulates. Virus numbers used were low because this was considered representative of the numbers of enteric virus pathogens likely to exist in polluted shellfish growing waters. A maximum of about 100 plaque forming units (PFU) of virus per gallon of shellfish overlay waters is the greatest number of enteroviruses found to date in estuarine waters in New Hampshire, and was found at a point where discharge of raw sewage occurred (unpublished data). The virus concentration was the equivalent of < 0.03 PFU/ml.

Soft shell clams were used because they are extensively consumed in the northeast and no information on virus bioaccumulation and elimination in this species could be found in the literature. A newly developed recovery method capable of detecting the presence of as few as 3 PFU of virus in 100 g of homogenized tissues made it feasible to carry out depuration studies with a degree of accuracy not previously possible. Since depuration effectiveness was considered to be related to virus numbers bioaccumulated, information was needed on how many feces-associated natural viruses would be bioaccumulated by shellfish when contamination levels were about 5 to 10 × greater than the maxima believed to exist in polluted estuaries.

Given the likelihood of the existence of low-level virus carriage states in polluted shellfish, and retention of most of bioaccumulated virus in sites from which removal by depuration seemed feasible, tests of the effectiveness of depuration in a model depuration unit were indicated.

METHODS

A depuration unit was used that was modeled after the Newburyport, MA, plant which has been used for depuration of soft shell clams for many years. Seawater admitted into the unit was irradiated by ultraviolet germicidal lamps, after which it flowed through shellfish holding tanks. The seawater was recycled, passing through a charcoal filter which removed toxic materials. Flow rates equivalent to 1.5 liters per bushel of clams per min were used. Dissolved oxygen values of about 5 mg/liter were estimated for the seawater. A complete description of the methods used has been published (*5*).

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[2]*Food and Drug Administration.*

RESULTS AND DISCUSSION

Enteroviruses bioaccumulation by individual clams is shown in Table 1. The number of PFU bioaccumulated was small regardless of whether virus was presented with cornstarch or feces in numbers varying from 34 to 0.09 PFU/ml of environmental seawater. The efficiency of bioaccumulation of feces-associated natural virus was greater than the cornstarch-associated uptake. The data suggested that while bioaccumulation processes would be efficient in the presence of solids-associated virus, as long as virus numbers were low, numbers bioaccumulated would be low also. Considerable variation in individual clam bioaccumulative capability was shown to occur. This feature raised the possibility that if differences in bioaccumulative capability existed between individual clams, then differences in depurative capability might also occur. The significance of this observation could not be determined, but it seemed reasonable to assume that difference in depurative effectiveness would be of minor importance as long as virus numbers were low and depuration intervals were long enough.

The distribution of feces-associated natural virus in clams was studied to assess the likelihood of virus being sequestered in non-alimentary tract tissue depots. The results of four trials are shown in Table 2. Remaining tissue included mantle, muscle, heart and hemolymph. Almost all of the virus was distributed between siphon and hepatopancreas tissues. Very little was found in remaining tissues. The results indicated that most of the bioaccumulated virus passed into the hepatopancreas via the siphon afferent tubule, and after passage into or through hepatopancreas tissue, exited via the siphon efferent tube. Virus associated with the remaining tissues was minimal in numbers. The data suggested that small numbers of bioaccumulated virus would remain within the alimentary tract or alimentary tract-associated tissues and would not be found in sequestered tissue depot locations. The results also supported the viewpoint that a greater opportunity for effective depuration existed if virus was alimentary tract-associated rather than tissue depot-sequestered.

Shellfish were allowed to bioaccumulate feces-associated virus in seawater tanks over a 20-h period and immediately placed into the depuration unit. The results of three trials are given in Table 3. Bioaccumulation of virus proceeded at different rates and to different levels in the three trials. Depletion of virus also proceeded at different rates. The depuration times required for reduction of virus numbers to non-detectable levels varied from 24 to 48 h, to 48 to 72 h. The vagaries of individual clam depurating efficiency was illustrated in trial 1 where one or more clams in the 72-h test pool failed to function as effectively as clams in other pools tested. This non-pumping activity of a small number of shellfish demonstrates potential problems associated with the possible use of depuration for shellfish harvested from polluted waters.

We believe that depuration as a virus depletion procedure can be used effectively to reduce virus health hazards associated with shellfish. The risk factor represented by depurated shellfish would be significantly less than that associated with non-depurated shellfish. It is not possible to guarantee that depurated shellfish will always be free from virus. However, it does seem that the lowest shellfish-associated virus health hazard risk

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TABLE 2. *Distribution of bioaccumulated feces-associated natural*[1] *virus within* Mya arenaria.

Trial	Tissue distribution of virus (PFU/g)		
	Siphons	Hepatopancreas	Remaining tissue[2]
1	3.8	3.4	1.2
2	4.5	11.7	0
3	6.4	8.1	0.3
4	4.4	3.8	1.7

[1]Feces-associated virus added to seawater to give final concentration of 0.2 PFU/ml. Clams were exposed to virus in overlay water for 6 hours. Ten clams were used in each trial.
[2]Tissues remaining after removal of siphon and hepatopancreas.

TABLE 3. *Depletion of bioaccumulated feces-associated natural*[1] *virus by depuration in* Mya arenaria.

Trial	Bioaccumulation		Elimination	
	Hours	Total PFU	Hours	Total PFU
1	0	—	0	14.2
	4	7.1	24	2.0
	16	4.5	48	0
	20	14.2	72	1.3
2	0	—	0	21.8
	4	2.3	24	5.7
	16	9.7	48	8.2
	20	21.8	72	0
3	0	—	0	33.6
	4	49.1	24	3.8
	16	72.0	48	0
	20	33.6	72	0

[1]Feces-associated virus added to seawater to give a final concentration of 0.2 PFU/ml. Pools of 10 clams were used for each test interval in bioaccumulation and elimination phases of the study.

TABLE 1. *Bioaccumulation of enteroviruses by the soft shell clam* Mya arenaria.[1]

Trial	Solids present[2]	Virus input (PFU/ml)	PFU virus recovered per clam						
			1	2	3	4	5	6	7
1	0	21.0	0	0	0	0	0	—	—
2	Cornstarch	34.0	8	13	0	0	3	—	—
3	Cornstarch	3.0	6	7	5	12	0	—	—
5	Cornstarch	3.0	2	0	0	0	0	—	—
7	Cornstarch	0.2	0	0	0	0	0	—	—
4	Infant feces	0.1	4	9	7	6	1	11	13
6	Infant feces	0.09	3	8	2	4	1	1	0
8	Infant feces	0.17	3	4	3	0	7	5	0

[1]Stock poliovirus 2 used with cornstarch. Natural polioviruses were present in the infant feces.
[2]Cornstarch added in final concentration of 0.01 percent. Feces concentrations varied from 0.3 to 0.4% final concentration.

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A Method for Recovery of Viruses from Oysters and Hard and Soft Shell Clams

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ABSTRACT

A method for recovery of small numbers of enteric viruses from oysters and hard and soft shell clams was developed. As few as 3 plaque forming units (PFU) of virus per 100 g of shellfish homogenate could be detected with an overall accuracy of ca. 60 percent in each of the three species tested.

Studies for detection of enteric viruses in soft and hard shell clams were initiated 3 years ago, anticipating that an oyster-developed method (*1,2*) could be used for effective recovery of small numbers of virus from other types of shellfish. Recovery of small numbers of virus was found to be inconsistent and these data stimulated a search for methods giving more effective virus recoveries from clams. Two methods were developed - one for soft shell clams and one for hard shell clams. The methods were modifications of the oyster-developed method. Using these two methods, it was possible to recover as few as 3 to 5 PFU of virus from the clams in 60% of the trials with both species.

The availability of hand-tailored methods of maximum recovery effectiveness for oysters, hard and soft shell clams is scientifically meritorious and appealing, but it created a number of problems when two or more shellfish species had to be tested for the presence of enteric viruses at the same time. It also was incompatible with the concept of a single standard method which could be used with equal effectiveness for recovery of enteric viruses from all three species.

METHODS

A composite method was developed which could be used effectively for recovery of enteric viruses from each of the three species. More effective recoveries of small numbers of enteric viruses without cell culture cytotoxicity could be obtained than with either the original method of Sobsey et al. (*2*) or an improved version (*3*). The method differed chiefly in (*a*) the mode of separation of virus from shellfish tissues, (*b*) procedures for recovery of separated virus and (*c*) the strategy developed for reconcentration of recovered virus suspension. A complete description of the method is to be published.

Separation of virus was made from the initial supernatant fluid by the combined use of beef extract, alkaline pH, high conductivity and sonication. Recovery of virus was accomplished by adsorption to a beef extract floc formed at acid pH, followed by elution of the precipitate and further clarification of the resulting eluate through treatment with Cat Floc. Reconcentration of recovered virus was achieved by repetition of adsorption of virus to a beef extract floc followed by resuspension of virus in 20 to 30 ml of Na_2HPO_4.

RESULTS AND DISCUSSION

The method represented compromises which permitted reasonably effective recoveries of several enteric viruses from all three shellfish species. Trade-offs were made in which the maximum recovery effectiveness of each of three methods for three species was exchanged in return for a single method of broader applicability for three species. Concessions were made also in which the maximum recovery effectiveness possible to obtain with one virus or group was balanced against the ability to recover a broader spectrum of viruses. For example, large numbers of enteroviruses could be recovered from beef extract precipitates formed at pH 3.5 to 4.0 while reovirus recoveries were best at pH 4.5 to 5.5. Selection of pH 4.0 for precipitation favored enterovirus recovery, although it was not optimal for all members of this group. It did make it possible, however, to recover reasonable numbers of viruses from both groups, even though recovery effectiveness was slightly less for both.

The recoveries made in oysters and hard and soft shell clams are shown in Fig. 1, 2 and 3. In each instance, recovery effectiveness is shown as percent recovery of inputs varying from 3 to 100 PFU per 100 g of shellfish homogenate. Input values represented the actual number of PFU injected into shellfish tissues.

Recovery of better than 50% of 11 to 16 PFU of enteroviruses from oysters shown in Fig. 1 was illustrative of the recovery potential of the method in this species. This sensitivity represented by recovery of such low levels of virus contaminants was considered more important than the percent recovery. The ability to detect and enumerate a significant portion of the virus numbers in oysters will contribute greatly to the value of the method for monitoring this species for potential health hazards. Reovirus recoveries were less satisfactory, but were still effective in detecting about 1 of every 3 viruses present.

The number of viruses recovered from hard shell clams shown in Fig. 2 was about the same as that found with oysters. Percent recoveries for enteroviruses were slightly higher, but input values were also higher than those for

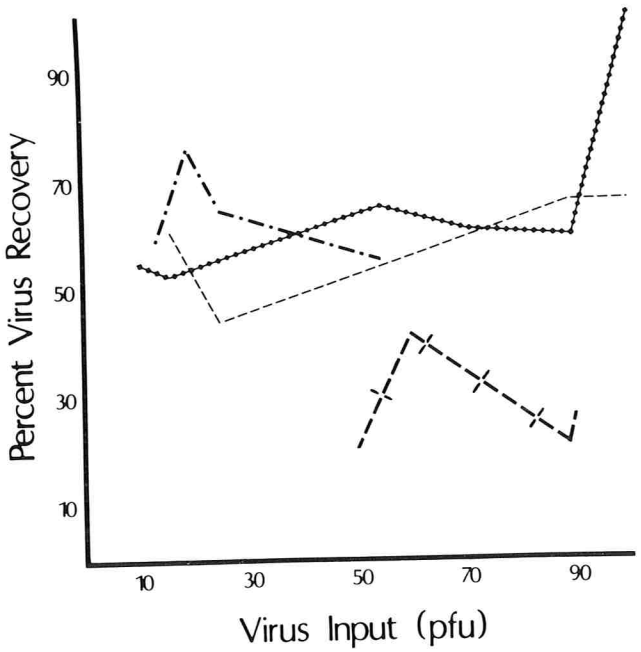

Figure 1. *Enteric virus recovery from oysters. Legend: poliovirus 2* (– – – – –), *echovirus 7* (–•–•–), *coxsackievirus B-1* (– · – · –) *and reovirus 1* (–┼–┼–).

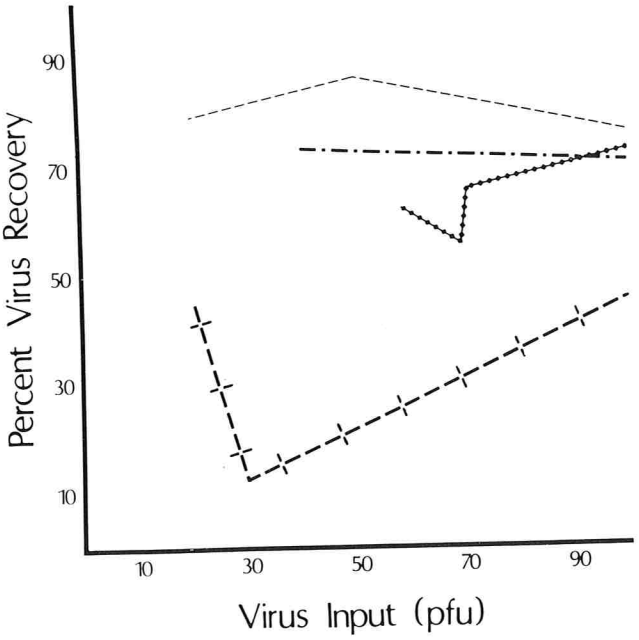

Figure 2. *Enteric virus recovery from the hard shell clam. Legend: poliovirus 2* (– – – – –), *echovirus 7* (–•–•–), *coxsackievirus B-1* (– · – · –) *and reovirus 1* (–┼–┼–).

oysters - hence the challenge to which recovery was put was not as great. Reovirus recoveries were about the same, but the recovery challenge imposed by lower numbers was greater.

Recovery effectiveness with soft shell clams shown in Fig. 3 was greater than that obtained with either of the other two species. Recovery of input PFU's of 8 of 9, 1 of

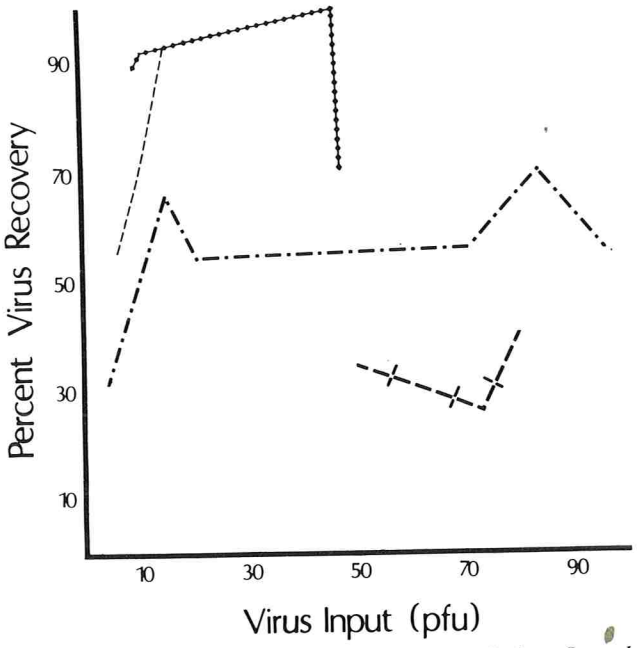

Figure 3. *Enteric virus recovery from the soft shell clam. Legend: poliovirus 2* (– – – – –), *echovirus 7* (–•–•–), *coxsackievirus B-1* (– · – · – · –) *and reovirus 1* (–┼–┼–).

3 and 4 of 7 for test enteroviruses was indicative of satisfactory recovery sensitivity. Reovirus recoveries were less satisfactory, but detection of 1 of every 3 viruses present in moderate numbers was still possible.

It was concluded that the method described was of approximately equal effectiveness in each of the three shellfish species, based upon its sensitivity in recovering significant portions of small numbers of test viruses. Sensitivity was considered more important than the percent recovery. Test samples were virtually free from cytotoxicity for cell cultures. This was especially noteworthy with soft shell clam samples which have been difficult to test because of consistent toxicity problems. The method could be modified to be optimally sensitive for use with oysters or hard or soft shell clams. Modification at one stage would enhance reovirus recovery potential, if this was important. Preliminary studies with adenovirus recoveries to date suggest modifications optimal for reovirus would be optimal for adenovirus.

ACKNOWLEDGMENTS

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Modified Procedure for Extraction of Poliovirus from Naturally-Infected Oysters Using Cat-Floc and Beef Extract

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ABSTRACT

Methods for recovery of poliovirus type 1 (LSc2ab) from naturally-infected oysters (*Crassostrea gigas*) were examined. Extraction procedures analyzed included glycine-saline and polyelectrolyte (Cat-Floc) methods followed by concentration using modifications of an acid precipitation technique. Direct viral assay of shellfish homogenates, when compared to virus recovery following extraction, indicated that substantially fewer viruses were detected in initial homogenates. These data appeared to support the contention that input values based on homogenate assay were inappropriate in determining recovery efficiencies with naturally-infected shellfish. Since absolute efficiencies could not be determined, relative efficiencies using samples from pooled homogenates were used to determine the recovery efficiencies of various extraction procedures. Cat-Floc extraction followed by a beef extract-modified acid precipitation technique resulted in higher virus recoveries than a glycine-saline extraction procedure.

Techniques for extraction and recovery of enteroviruses from shellfish have been greatly improved over the last few years (reviewed by Gerba and Goyal, *3*). As a result there have been more reports of viral isolations from shellfish harvested from both open and closed fishing areas (*2,3,11*). Although extraction procedures appear to be effective, development of a large number of them relied heavily upon use of experimentally infected bivalves for determining recovery efficiencies. Shellfish were infected with viruses by either direct inoculation, or by addition of exogenous viruses to homogenates. Input values were determined by direct assay of the homogenate and used to calculate the efficiency by comparison with the total viruses recovered following extraction. Recovery efficiencies were found to vary greatly depending upon the procedure, shellfish and the type of virus used in the experiment. Sobsey et al. (*10*), employing a glycine-saline extraction procedure followed by ultrafiltration (UF) or acid precipitation (AP), reported an average recovery efficiency of 48% for polio, reo and adenovirus from oyster homogenates. Kostenbader and Cliver (*8*) observed recoveries of 80 to 100% for experimentally-inoculated poliovirus from oysters using a polyelectrolyte-extraction technique. Konowalchuk and Speirs (*7*) reported 50-60% recoveries of Coxsackievirus B5 using an acid precipitation of oyster extracts.

Since enteric viruses are taken up and harbored within the shellfish by mechanisms quite different from artificial infection procedures, questions have arisen as to whether recovery efficiencies observed under experimental conditions truly reflected those which might occur under natural conditions. We therefore initiated a study to determine the recovery efficiencies of enteroviruses from naturally-infected shellfish employing some of the currently used techniques discussed above.

MATERIALS AND METHODS

Virus

Plaque-purified poliovirus type 1 (LSc-2ab) was propagated on low-passage Buffalo Green Monkey kidney cells (BGM) (*1*), and prepared according to the procedure of Jakubowski et al. (*5*). Use of this technique resulted in a monodispersed culture of the virus.

Oysters and oyster infection procedures

Japanese oysters (*Crassostrea gigas*) were provided by Dr. Roger Mann, Environmental Systems Laboratory (ESL), Woods Hole Oceanographic Institution. Lots consisting of from 100-250 animals were exposed to virus-seeded seawater (ca. 10^3 PFU/ml) in the temperature-controlled (18 C) seawater tables of the ESL for 18-24 h. Oysters were then shucked, homogenized in 100-400-g pools, and frozen under dry ice for shipment to Brookhaven National Laboratory where they were stored at -70 C until needed. Uninfected oyster homogenates were also obtained for a seeded virus study.

Virus assay

Shellfish samples were treated with chloroform for 30 min to eliminate contaminating bacteria and fungi, and diluted in appropriate volumes of phosphate-buffered saline solution (pH 7.2). Sample volumes of 0.5 ml were inoculated onto monolayers of BGM cells in 25-cm^2 flasks (4-12 flasks per dilution). Following a 60-min adsorption period with rocking, inocula were decanted and replaced with 4 ml of a neutral red agar overlay (*4*). Flasks were incubated at 36 C and observed for plaque formation for 7 days.

Virus recovery from oysters

Glycine-NaCl (GN). The basic method used was that of Sobsey et al. (*10*). Briefly, shellfish homogenates (usually 100-400 g) were adjusted to a conductivity of $\leq$ 2000 ppm NaCl by addition of cold distilled water, and the pH decreased to 5.0 with 1.2 M HCl, or 0.05 M glycine-HCl (pH 1.5). Following low speed centrifugation, the viruses were eluted from the pellet by resuspension in glycine-NaCl (pH 7.5) at a conductivity of 8,000 ppm. Oysters solids were removed by a second low-speed centrifugation, and the resulting supernatant fluid adjusted to pH 7.5. Neutralized supernatant fluids were then filtered (Millipore, AP25 04700-serum treated), and further concentrated by ultrafiltration, or acid precipitation before assay on cell culture.

Polyelectrolyte flocculation (Cat-Floc, CF). The basic method used was that described by Kostenbader and Cliver (*8*). Oyster homogenates (100 g) were mixed with 500 ml of 0.09 M glycine-NaOH buffer (pH 8.8). Cat-Floc (polydimethyldiallyl ammonium chloride - Calgon Corp.) was then added (10 ml of a 1% Cat-Floc solution/100 g of homogenate), the mixture stirred for 5 min, and allowed to stand for 15 min. Following centrifugation (ca. 9000 × *g*), supernatant fluids were filtered (AP25), and concentrated by ultrafiltration or acid precipitation.

Concentration methods. Ultrafiltrations (UF) were carried out in a 90-mm Hi-Flux cell (Millipore Corp.) equipped with a PTHK ultrafilter with a nominal molecular weight cutoff of 10^5 d. When the virus suspension was reduced to approximately 10 ml, ultrafiltration was halted. The sample was then collected, and the filter rinsed to remove any embedded viruses. Samples were supplemented with 10% fetal bovine serum and frozen at -70 C until assayed. The process was time consuming, requiring 1-2 days for completion.

The initial acid precipitation (AP) method used involved lowering the pH of clarified supernatant fluids to 4.5 with 0.05 M glycine-HCl (pH 1.5). After allowing 15 min for precipitate formation, samples were centrifuged ($9000 \times g$) and the resulting pellets dissolved in 0.1 M Na_2HPO_4 (15-20 ml). All concentrated samples were neutralized to pH 7.2-7.4 and stored at -70 C to await assay.

RESULTS AND DISCUSSION

Unreliability of shellfish homogenates for efficiency measurements.

Initially, we were interested in determining whether the direct assay of exogenously-infected homogenates was a reliable method on which to base recovery efficiency from naturally-infected oysters. Initial homogenate samples were assayed and compared to the total number of viruses recovered following complete processing. The results are presented in Table 1. A comparison of the total PFU recovered from the homogenates with total PFU recovered following processing indicated that, with one exception (exp. #5), a significantly greater number of total viruses were recovered after processing than were observed in direct homogenate assays. Relative recoveries ranged from 0.9 × the homogenate value in exp. #5 to over 4 × in exp. #3. These data indicated that homogenate assays may only represent a small portion of viruses present. If viruses were bound within particles or various organic matrices they might not be detected by simple assay. Extracting or processing the homogenates might then release the trapped viruses. This situation may not apply to artificially-infected shellfish homogenates since artificial infection may produce more surface-adsorbed viruses which could be more easily detected without processing.

Enhancement of virus recovery by beef extract

Since absolute efficiencies based on direct assay of homogenates could not be attained for naturally-infected oysters, we chose to calculate relative efficiencies using large-volume pooled homogenates. Pools of 100 to 200 g of well-mixed homogenates were divided into equal portions and the viruses extracted by various methods. Assuming equal distribution of virus particles within the homogenate, the relative efficiencies of the methods could then be obtained by comparing the total number of viruses recovered by each method.

Before being able to compare relative efficiencies of various extraction methods, recovery techniques had to be modified for efficient virus recovery in naturally-infected oysters. As seen in Table 2, over 90% of the viruses extracted following a glycine-saline procedure were lost to the large volume supernatant fluids during

TABLE 1. *Comparison of the recovery of poliovirus from initial homogenate and final processed oysters.*

Expt. No. (A)	Extraction/clarification method (B)	Concentration method (C)	Total PFU recovered from: Initial homogenate (D)	Total PFU recovered from: Processed[b] oysters (E)	Ratio (E/D)
1	Glycine-NaCl	AP[c]	8.3×10^4	1.2×10^5	1.4
2	Glycine-NaCl	UF[d]	8.3×10^4	1.5×10^5	1.8
3	Cat Floc	AP	4.3×10^4	2.0×10^5	4.6
4	Cat Floc	UF	4.3×10^4	1.7×10^5	3.9
5	Glycine-NaCl	AP	2.4×10^4	2.2×10^4	0.9
6	Cat Floc	AP	2.4×10^4	3.3×10^4	1.3

[a]Recovery based on dilution and direct inoculation of homogenate sample.
[b]Total virus recovered from ultrafilter (UF) or total virus recovered from AP supernatant plus final AP pellet.
[c]Acid precipitation.
[d]Ultrafiltration.

TABLE 2. *Enhancement of virus recovery by beef extract-acid precipitation following glycine-NaCl extraction.*

AP method	Expt. No.	pH	Total virus (PFU) recovered: Supernatant fluid (A)	Total virus (PFU) recovered: Final volume (B)	%[a] Virus lost in supernatant fluid (C)
Without beef extract	1	4.5	1.1×10^5	6.4×10^3	94.6
	2	"	9.2×10^4	7.8×10^3	92.2
	3	"	1.7×10^4	1.9×10^3	96.0
	4	"	2.9×10^4	1.5×10^3	95.1
	5[b]	"	1.9×10^6	2.1×10^5	90.1
3% Beef extract	1	4.5	6.9×10^4	2.9×10^4	70.4
	2	"	4.0×10^4	2.3×10^4	63.5
	3	3.5	2.9×10^4	7.5×10^4	47.2
	4	"	3.0×10^3	3.2×10^4	32.1
6% Beef extract	1	3.5	4.0×10^4	4.0×10^4	50.0
	2	"	2.2×10^4	9.2×10^4	20.4
	3	"	8.3×10^3	1.6×10^4	37.1

[a]% derived by dividing supernatant fluid (A) by total virus (A + B).
[b]Seeded oyster homogenate.

concentration. A single experiment using artificially-infected oysters (exp. #5) also demonstrated the same trend, indicating the problem may not be unique to naturally-infected shellfish.

Since a number of reports have successfully employed beef extract to enhance flocculation of viruses (*6,9*), the acid precipitation technique was modified by employing 3 and 6% beef extract at either pH 3.5 or 4.5. Supplementing the concentration procedure with beef extract somewhat enhanced precipitation of the viruses and decreased the amount lost to the supernatant fluids. Flocculation at pH 3.5 with either 3 or 6% beef extract resulted in better virus flocculation but still left appreciable amounts of viruses remaining in supernatant fluids.

This problem was not unique to the glycine-saline extraction procedure. A similar loss of virus to the supernatant fluids during concentration by acid precipitation was observed following polyelectrolyte extraction with Cat-Floc (Table 3). In the absence of beef extraction, concentration by acid precipitation resulted in 60% of the virus being lost to the supernatant fluids. Supplementing the acid precipitation step with 3% beef extract decreased the loss of virus while the addition of 6% beef extract resulted in only about 5% of the viruses appearing in supernatant fluids.

Comparison of various extraction methods using relative recovery efficiencies

Having modified the extraction-concentration procedures to attain better virus recoveries in the final sample volumes, experiments were initiated to compare the relative efficiencies of the glycine-saline and the Cat-Floc extraction methods. For each experiment, a well-mixed homogenate pool was equally divided and extracted by the appropriate method. Extractions were followed by a concentration step employing the modified acid precipitation technique. The results are shown in Table 4. Comparing the total PFU recovered in each experiment, more viruses were recovered in Cat-Floc-treated samples than in homogenates extracted with the glycine-saline method. In some instances (exp. 1, 4 and 5) the relative efficiency was 5.6, 3.8, and 2.3 × higher, respectively, for Cat-Floc AP than glycine-saline AP. Figure 1 illustrates a flow diagram of the modified Cat-Floc beef extract technique.

Based on the data presented, we have made the following conclusions: (*a*) direct assay of homogenates of naturally-infected oysters do not account for all viruses

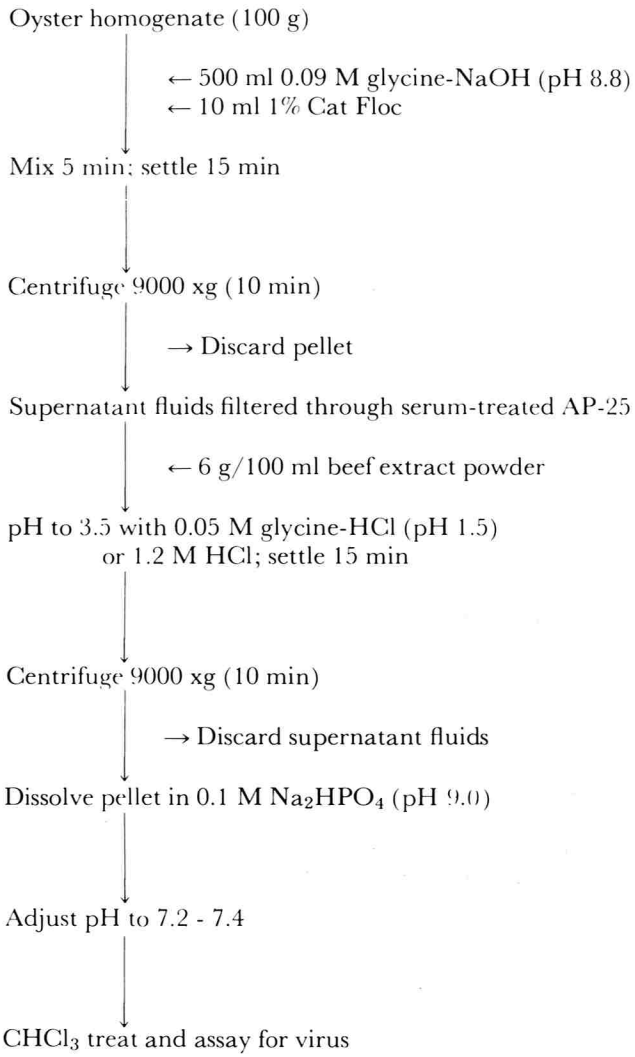

Figure 1. *A schematic diagram of the modified Cat-Floc-beef extract-acid precipitation technique for the recovery of poliovirus from oysters.*

TABLE 4. *Comparison of glycine-NaCl and Cat-Floc methods for virus recovery from common pools of "naturally" infected oysters.*

Pool #	AP method used	Total virus (PFU) recovered by Glycine-NaCl	Cat-Floc
1	No beef extract, pH 4.5	7.8×10^3	4.4×10^4
2	3% Beef extract, pH 3.5	7.5×10^4	8.6×10^4
3	" " "	3.2×10^4	3.6×10^4
4	6% Beef extract, pH 3.5	4.0×10^4	1.5×10^5
5	" " "	9.2×10^4	2.1×10^5
6	" "	1.6×10^4	2.4×10^4

TABLE 3. *Enhancement of virus recovery of beef-extract acid precipitation following Cat-Floc extraction.*

AP method	Expt. No.	pH	Total virus (PFU) recovered: Supernatant fluid (A)	Final volume (B)	%[a] Virus lost in supernatant fluid (C)
Without Beef Extract	1	4.5	6.9×10^4	4.3×10^4	61.2
3% Beef Extract	1	3.5	5.3×10^3	8.6×10^4	5.8
	2	3.5	9.6×10^3	3.5×10^4	21.4
6% Beef Extract	1	3.5	5.8×10^3	1.5×10^5	3.6
	2	3.5	1.3×10^4	2.1×10^5	5.9

[a] % derived by dividing supernatant (A) by total virus (A + B).

present in the shellfish; therefore, it is difficult to determine absolute efficiencies of extraction methods with naturally-infected oysters, (*b*) relative recovery efficiencies can be accurately determined by using well-mixed, large volume, pooled homogenates split into portions and extracted separately, (*c*) in our laboratory, concentration of viruses by unmodified acid precipitation technique was not effective; recovery of virus was increased by supplementing the acid precipitation with 3 or 6% beef extract which increased the amount of floc, (*d*) a greater number of viruses were recovered following use of Cat-Floc than the glycine-saline extraction procedure.

This paper describes the results obtained with only one virus, polio 1 LSc. Preliminary data have indicated the modified Cat-Floc-beef extract technique was superior to the glycine-saline extraction method in the recovery of field strains of polio virus type I, coxsackievirus B3 and echovirus type I from oysters.

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Isolation of Naturally Occurring Enteroviruses from a Variety of Shellfish Species Residing in Long Island and New Jersey Marine Embayments

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ABSTRACT

Shellfish (*Crassostrea virginica* and *Mercenaria mercenaria*) and shellfish-raising waters from a variety of Long Island and New Jersey marine embayments were examined for the presence of human enteroviruses. Little difference in virological quality was noted between areas designated as being open or closed to shellfishing. Viral isolations could not be correlated with coliform counts from identical samples, indicating the need to re-evaluate the use of bacterial standards as indices of the overall sanitary quality of water and shellfish.

Current standards for certification of shellfish and shellfish-harvesting waters are based upon total and fecal coliform counts. While use of these indices has facilitated control of waterborne disease outbreaks of bacterial origin, questions have arisen regarding their use as indicators of overall sanitary quality, with special emphasis on the human viruses. Laboratory studies have indicated significant differences between bacterial and viral survival rates in marine water, and in their uptake and depuration rates in shellfish (*6,10,16,19,26*). Recent field studies have suggested no significant statistical relationship between the occurrences of viruses and coliform bacteria in shellfish and shellfish-raising waters (*8,9,25*).

Documented outbreaks of hepatitis A and non-bacterial gastroenteritis attributed to consumption of raw or partially cooked shellfish taken from sewage-contaminated coastal waters (*5,7,13,14,20,22*) have underscored the need to re-evaluate the limits of a bacterial index for assessment of likely virus hazard. The purpose of the present document is to identify the relationships between coliform bacteria and human enteroviruses occurring in shellfish and shellfish-raising waters located in coastal waters of Long Island and New Jersey. In addition to direct comparisons of coliform and virus concentrations in identical samples, the microbial quality of approved shellfishing areas was compared to that of closed areas.

MATERIALS AND METHODS

Virus concentration

Virus concentrations from water samples were accomplished using a Melnick-Wallis concentrator (*21*). Initial virus extractions from shellfish (1976-1977 study) used the Glycine-NaCl method of Sobsey et al. (*23*). Shellfish extractions in the later (1979) study involved use of a modified method developed in this laboratory. Briefly, shellfish were shucked and homogenized in a blender (60-90 sec). Homogenates were then clarified using the Cat-Floc method of Kostenbader and Cliver (*12*). Virus concentration from oysters was accomplished by a 6% beef extract-supplemented acid precipitation step at pH 3.5 (a modification of the technique of Konowalchuk and Speirs, *11*). Concentrations from clarified clam homogenates involved an unsupplemented acid precipitation at pH 5.0. Resulting pellets from both methods were dissolved in 15 ml of 0.15 M dibasic sodium phosphate (pH 7.2). Final concentrates were frozen at -70 C to await assay.

Enumerations

Viruses from concentrated samples were enumerated by plaque assay on low-passage Buffalo Green Monkey kidney cells (*25*). Isolates were identified using NIAID serum pools (*15*).

Coliform analyses were made by the New York State Department of Environmental Conservation or the New Jersey Department of Environmental Protection using standard 5-tube "most probable number" method (*24*).

RESULTS AND DISCUSSION

The initial studies reported here were undertaken in 1976-1977 as part of a federally-funded 208 water resources study conducted on Long Island. The coastal waters portion of the virus study involved periodic sampling of water and shellfish from beds which had been designated as approved or closed to shellfishing on the basis of coliform analyses. The systems tested included Great South Bay and Oyster Bay.

Great South Bay

Located along Long Island's south shore, Great South Bay is a major source of clams (*Mercenaria mercenaria*) for both New York State and export to other states. The closed area was located several hundred yards from the shoreline (Town of Islip), and the approved area was located approximately 1 mile south of this point.

Viruses were isolated from six of 14 water samples, three each (42.8%) from open and closed areas (Table 1). Isolations from water did not correlate with total or fecal coliform counts, with no viruses being isolated from water samples having the highest coliform counts (2400). Viruses were recovered from both open and closed waters during the month of July. Isolations from clam samples were noted on two occasions from each sampling area (28.5% closed; 40% open). With the exception of the July closed-area sample, little correlation was seen between virus and coliform numbers in clams. On three occasions

(July, April, June) viruses were recovered both from shellfish, and their overlying water during the same month. The major groups identified from these samples (Table 2) included polioviruses (vaccine strain), echovirus and several isolates which could not be identified with the NIAID serum pools. In terms of virus occurrence, there was little difference noted between the approved and closed areas; indeed, the highest virus numbers (800 PFU) were recovered from the waters of the open area.

Oyster Bay

A second shellfishing area studied during the 208 program was Oyster Bay, located on Long Island's north shore. Once again, approved and closed shellfish beds were examined. Viruses were never isolated from the closed waters (Table 3), and on only one occasion (12.5%) from the open waters. The turbidity levels in these waters were extremely high, a factor which may have interfered with the adsorption process, resulting in a reduction of the efficiency of the virus concentrator. Viruses were isolated from two of the approved area oyster samples (*Crassostrea virginica*) (25%), and three of the closed area samples (37.5%). In every instance, viruses were recovered from samples yielding relatively low coliform counts, most of which were below the accepted standard. While the frequency of isolation was slightly greater in the closed area, the highest number of viruses (200) was recovered from open-area samples. Isolates identified from samples included coxsackie and echovirus (Table 4).

Recently (1979), a series of studies were initiated in several established shellfish beds in Long Island and New Jersey waters. Testing included virus assays of shellfish, and coliform analyses of shellfish and shellfish-harvesting waters.

Penataquit Creek

Penataquit Creek (Town of Islip) is a tributary to Great South Bay. The sampling site is within 100 yards of the Great South Bay closed area discussed earlier. At present, the creek is closed to shellfishing. Initial samples were collected during the month of January while the clams (*M. mercenaria*) were still dormant. Viral and coliform recoveries, therefore, likely represented organisms taken up during the previous season. As noted in earlier studies, significant numbers of viruses were recovered in the near absence of coliform organisms (Table 5). In both instances, coliform numbers were well below the recommended standard.

TABLE 2. *Viruses identified from Great South Bay clams and water.*

Month	Sample type	Station	Viruses identified
July	Water	Open	*[a]
"	Water	Closed	*
"	Clam	Closed	Echo type 20
			Echo type 23
August	Water	Open	*
February	Water	Closed	Polio type 2 (vaccine)
April	Water	Open	*
"	Clam	Closed	*
June	Clam	Open	*
"	Water	Closed	Polio type 1 (vaccine)
"	Clam	Closed	Polio type 1 (vaccine)

[a]Isolates from these samples could not be identified using the NIAID serum pools.

Raritan Bay (New Jersey)

A series of samples (*C. virginica* and *M. mercenaria*) was collected from the New Jersey side of Raritan Bay which receives treated and untreated effluents from New York City. Viruses were isolated in significant numbers from all but one sample (Table 5). Once again, coliform counts were inconsistent with virus recoveries.

Raritan Bay (New York)

Samples (*M. mercenaria*) have also been taken from the

TABLE 1. *Coliform and virus isolations from water and clams - Great South Bay.*

Station - Month	MPN of Total coliforms per 100 ml or 100 g		MPN of Fecal coliforms per 100 ml or 100 g		PFU[a] of Virus per 100 gal or 100 g	
	Water	Clam	Water	Clam	Water	Clam
Closed to shellfishing:						
July	430	16,000	75	16,000	400	16
August	110	20	23	< 20	ni[c]	ni
September	93	1,300	4	< 20	ni	ni
February	150	< 20	-[b]	-	440	ni
March	45	50	15	< 20	ni	ni
April	2,400	630	460	20	ni	ni
June	23	220	4	20	110	10
Open to shellfishing:						
July	4	-	4	-	800	-
August	460	-	4	-	120	-
September	93	< 3	< 20	< 20	ni	ni
February	93	20	-	-	ni	ni
March	23	< 20	< 3	< 20	ni	ni
April	150	15	170	130	290	30
June	93	< 3	70	< 20	ni	10

[a]Plaque forming units.
[b]Not done.
[c]None isolated.

TABLE 4. *Viruses identified from Oyster Bay oysters and water.*

Month	Sample type	Station	Viruses identified
July	Water	Open	*[a]
"	Oyster	Closed	Echo type 15
			Echo type 2
November	Oyster	Closed	Coxsackie type B-3
March	Oyster	Open	*
April	Oyster	Closed	*

[a]Isolates from these samples could not be identified using the NIAID serum pools.

New York side of Raritan Bay. Among the samples tested to date is one offering the most dramatic example of the dichotomy existing between coliform counts and virus recoveries. In this sample, five fecal coliforms were recovered per 100 g of shellfish meat, as opposed to 320 total virus PFU (Table 5).

Numerous investigators have reported the isolation of human viruses from shellfish and shellfish harvesting waters (*3,4,6,17,26*). The survey data presented here

TABLE 3. *Coliform and virus isolations from water - oysters - Oyster Bay.*

Station - Month	MPN of Total coliforms per 100 ml or 100 g		MPN of Fecal coliforms per 100 ml or 100 g		PFU[a] of Virus per 100 gal or 100 g	
	Water	Oyster	Water	Oyster	Water	Oyster
Closed to shellfishing:						
July	15	50	15	20	ni[c]	48
August	4	5,400	< 3	270	ni	ni
September	23	1,400	9	90	ni	ni
November	9	< 20	9	< 20	ni	8
February	93	< 20	-[b]	-	ni	ni
March	< 20	70	-	-	ni	ni
April	9	< 20	4	< 20	ni	20
June	2,400	1,300	2,400	220	ni	ni
Open to shellfishing:						
July	1,100	80	9	20	280	ni
August	230	2,400	93	< 20	ni	ni
September	930	1,100	43	60	ni	ni
November	23	< 20	23	< 20	ni	200
February	23	< 20	-	-	ni	ni
March	4	50	-	-	ni	48
April	< 3	70	< 3	< 20	ni	ni
June	15	210	< 3	< 20	ni	ni

[a]Plaque forming unit.
[b]Not done.
[c]None isolated.

TABLE 5. *Virus and bacterial isolations from shellfish - 1979.*

Date	Station	Sample	Median MPN coliforms per 100 g or 100 ml		Virus PFU[a]/100 g
			(Total)	(Fecal)	
January 22	Penataquit Creek	Clam	5	< 2	46
"	"	Water[b]	⩾2400	1600	—[c]
January 31	"	Clam	2	< 2	
"	"	Water[b]	140	70	—
April 11	Raritan Bay (NJ) Area #1	Clam	—	45	32.4
"	"	Water	—	23	—
	Raritan Bay (NJ) Area #2	Clam	—	20	24
"	"	Oyster	—	< 20	7.8
"	Raritan Bay (NJ) Area #3	Clam	—	45	0
"	Raritan Bay (NJ) Area #4	Clam	—	78	108
April 13	Raritan Bay (NY)	Clam	—	5	160
"	"	Water	350	—	—
April 16	Raritan Bay (NY)	Clam	—	7	19.7
" 19	"	Clam	—	< 20	16.8
" 20	"	Clam	—	< 20	62

[a]Plaque forming units.
[b]Samples from which *Salmonella* organisms were isolated (qualitative method).
[c]Not done.

indicated little virological difference between waters and shellfish from areas designated approved or closed on the basis of coliform counts. These findings are in agreement with previous survival studies which demonstrated the extended survival of virus over bacteria in marine and estuarine waters, shellfish and sediments (*1,2,3,18,27*).

Currently-practiced methods for determination of the overall sanitary quality of shellfish have been shown to be inadequate by this and other studies (*6,9,26*). Officials contemplating the need for viral quality assessment in shellfish areas should consider using a human virus index in lieu of bacterial assays.

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Bacterial Indicators and Environmental Factors as Related to Contamination of Oysters by Enteroviruses

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ABSTRACT

Enteric viruses previously have been reported in marine waters and shellfish which met acceptable bacteriological standards for recreational use and shellfish harvesting. Unfortunately, previous data on occurrence of enteric viruses are limited and usually not quantitative. In this report, results of studies conducted along the upper Texas Gulf coast, where a substantial amount of quantitative virological data was collected, are compared to bacteriological indicators and other environmental factors on a statistical basis. A product-moment correlation matrix showed that there was a moderate correlation between viruses in water and total coliforms in water, total coliforms in oysters and fecal coliforms in oysters. However, presence of viruses in water was not found to be correlated with presence of viruses in oysters. The only significant regression coefficient found for the model relating the concentration of viruses in water to bacterial indicators and other environmental variables was concentration of coliforms in oysters. Multiple regression analysis showed that approximately 25% of the variance in the number of viruses detected in water was statistically accounted for by the linear correlation with the total coliforms in oysters. The amount of variation in the number of viruses explained by this indicator, however, was not large enough to make the concentration of coliforms in oysters a good predictor of the concentration of viruses in water. Furthermore, none of the bacterial or other environmental variables was found to be a good predictor of the concentration of viruses detected in oysters. Our failure to find a strong predictive relationship between viruses in marine water and in oysters, and the occurrence of viruses in high frequency in waters which met current bacteriological standards, indicate that these standards do not reflect the occurrence of enteroviruses in marine waters.

Effective control of enteric bacterial disease spread by shellfish has resulted from establishment of bacteriological standards using fecal and total coliform indices as a basis for limiting shellfish harvesting. Much controversy has centered around the adequacy of these standards to reflect a viral disease hazard (*2*). This resulted from the longer survival time and resistance of enteric viruses to disinfectants than indicator bacteria, as well as low numbers necessary to cause infection. A recently reported outbreak in the United States of infectious hepatitis caused by oysters apparently harvested from waters which met national sanitation standards and which were certified for oyster harvesting has caused additional concern (*11*).

At present there is a lack of knowledge as to how widespread is the occurrence of viruses in estuaries and coastal waters. Also, the true incidence of viral diseases transmitted via polluted estuarine and coastal waters is not known. Enteric viruses previously have been reported in marine waters and shellfish which met acceptable bacteriological standards for harvesting (*4*). Unfortunately, data on the occurrence of enteric viruses in marine waters are limited and usually not quantitative. New quantitative methods have become available in recent years for concentration of enteroviruses from large volumes of marine waters (*10*) and shellfish (*13*).

During the last several years the Department of Virology and Epidemiology at Baylor College of Medicine conducted three major field studies on the occurrence of enteroviruses in Galveston Bay, which is located along the upper Texas coast near Houston. We recently reported the results of a study on the presence of enteroviruses in oysters and oyster-harvesting waters of this area (*8*). The current report concerns further statistical analysis of these data and of data collected on marine waters in the same area. It was hoped that such an analysis would provide additional information on the relative importance of environmental factors influencing the presence of enteric viruses in marine waters.

MATERIALS AND METHODS

Oysters and water samples (20-400 liters) were collected from 26 sites in the Galveston Bay area. Samples were collected from shore sites bordering the bay as well as from open bay water several miles from shore and from the nearest source of sewage discharge. A detailed description of the sites can be found in previous studies reported by this laboratory (*7-9*).

The isolation and enumeration of coliforms, fecal coliforms and enteroviruses from water and sediment were conducted by previously described methodology (*7-9*). Salinity, turbidity, pH, temperature and the suspended solids determinations have also been described in detail (*7-9*). Data obtained on the aforementioned parameters and used in the statistical evaluation reported here were summarized previously (*7-9*).

A statistical evaluation of the relationship between the number of viruses and the environmental variables was performed in the following stepwise manner. First, we investigated the relationship of the number of viruses in water with each individual factor representing either bacterial indicators, water quality or characterized harvesting site. These bivariate relationships were approximated by least squares fit of a linear model. Also, the possibility of non-linear relationships was considered and, thus, the least squares fit of an exponential model (logarithmic data transformation) was attempted.

The number of viruses in the water (denoted by y) was further studied as a function of interacting characteristics of water quality and sediment (denoted by x_1, x_2....x_8). Thus, the hypothesized functional dependence

$$y = f(x_1; x_2; x_3; ....x_8) \quad [1]$$

was analyzed by stepwise multiple regression (*12*), using SSPS computer package, version 7.

RESULTS

The only significant regression coefficient found for the concentration of viruses in water to relation to other environmental factors was that with the concentration of coliforms in oysters. The scatterplot of these two variables is presented in Fig. 1. The multiple regression analysis (Table 1) showed that approximately 25% of the variance in the number of viruses detected in water was statistically accounted for by the linear correlation with total coliforms in oysters. However, no variable of 11 factors thought to be potentially controlling the concentration of viruses detected in oysters was found to be statistically significant. The logarithmic transformation of data to take into consideration potential non-linearity of studied relationships did not improve the fit of the models.

The product-moment correlation matrix (see ref. *8*) had shown that there is a moderate correlation between viruses in water and total coliforms in water, total coliforms in oysters and fecal coliforms in oysters. Presence of viruses in water, however, was not found to be correlated with presence of viruses in oysters.

DISCUSSION

Our failure to find a strong predictive relationship between viruses in marine water and oysters, and the occurrence of viruses with high frequency in waters which met current bacteriological standards, indicate that these standards do not reflect the occurrence of enteroviruses in marine waters. Of all the parameters measured in this study, coliform bacteria appear to have the strongest relationship to the presence of enteroviruses. It would appear that further study of this relationship may be warranted. For example, perhaps increasing the volume of seawater analyzed for coliforms or the number of samples analyzed may increase the usefulness of the coliform or even fecal coliform index in predicting the occurrence of enteroviruses in marine waters.

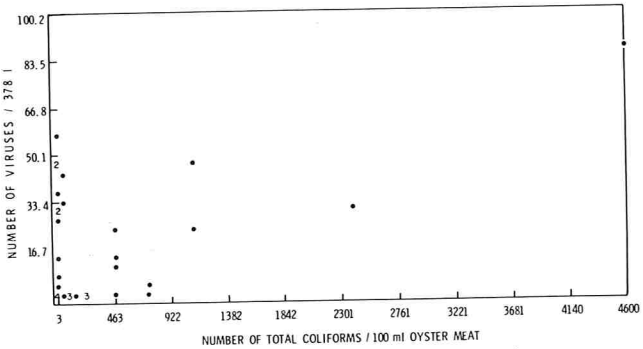

Figure 1. *Scatterplot of the number of total coliforms in oysters per 100 ml as related to the number of viruses found in water per 400 liters ($R = 0.5$; $P < 0.01$).*

Correlations between viruses in water and most probable numbers of total coliforms in water, total coliforms in oysters and fecal coliforms in oysters reported in an earlier study were indicative that some type of relationship may exist between these factors. Integration of the current data with data generated in two previous studies and subsequent statistical analysis in this study failed to strengthen the observed relationship between viruses and bacterial indicators (*5*). The combined data of all studies considered together represented 150 samples of marine water. In the combined studies, viruses were detected 43% of the time in recreational waters considered acceptable as judged by coliform standards (*3*) and 44% of the time when judged by fecal coliform standards. In the study considered here, viruses were detected in waters which met acceptable standards for shellfish harvesting 35% of the time.

Although the multivariate regression analysis showed that the number of viruses detected in water correlated significantly with total coliforms in oysters, the amount of variation in the number of viruses explained by this variable (25%) was not large enough to make one a good predictor of the other. Even if such a relationship was predictive, such an association would be of little value in monitoring efforts, but again indicates some relationship exists between the presence of enteroviruses and coliform bacteria in marine water and oysters.

Without epidemiological data it is difficult to assess

TABLE 1. *Multiple regression of viruses in water and other water quality indicators.*

Variable	Significance of each variable	Multiple R	R square	R square change	Simple R	Overall R	Significance of model
Total coliforms, oysters	0.004	0.499	0.249	0.249	0.499	9.625	0.004
pH	0.111	0.561	0.315	0.066	0.067	6.446	0.005
Salinity	0.212	0.595	0.354	0.039	-0.056	4.939	0.007
Site[a]	0.260	0.621	0.386	0.031	0.067	4.081	0.011
Turbidity	0.248	0.646	0.418	0.033	0.252	3.596	0.014
Total coliforms, sediment	0.480	0.656	0.430	0.012	0.051	3.024	0.024
Fecal coliforms, sediment	0.406	0.669	0.448	0.017	-0.183	2.664	0.036
Fecal coliforms, oysters	0.548	0.676	0.457	0.009	0.484	2.314	0.057
Fecal coliforms, water	0.562	0.682	0.466	0.009	0.477	2.034	0.087
Soluble organics	0.860	0.683	0.467	0.001	0.017	1.749	0.138
Total coliforms, water	0.900	0.683	0.467	0.000	0.495	1.514	0.206

[a]Shore station vs. open bay.

what this discrepancy means in terms of the possible failure of indicator bacteria to represent a viral disease hazard. Epidemiological studies to establish a relationship between viral disease and the presence of viruses in water would be a formidable task, and it is doubtful that such studies would yield meaningful results (*6*). It is felt that current epidemiological methods are not sensitive enough to effectively detect virus disease transmission through water, because clinically observable illness occurs only in a small number of people who become infected and because of the widely varying incubation periods. This fact, and considering the low infective dose of viruses (*12*), has led some to suggest that the presence of enteric viruses in any water is indicative of a potential viral disease hazard (*13*).

Because of their ability to concentrate bacteria and viruses from water during feeding, there is a greater potential risk associated with shellfish consumption than with recreational use of the same water. The suggested microbiological standard in the United States for shellfish-harvesting water requires a median of 70 coliforms per 100 ml, with no more than 10% of the samples exceeding a value of 230 (*11*). Enforcement of this standard has resulted in the apparent absence of shellfish-associated typhoid in this country since 1959 (*3*). However, outbreaks of shellfish-associated infectious hepatitis and nonspecific gastroenteritis continue to occur (*4,11*).

The effect of environmental factors controlling enteric viruses in marine water may be greatly influenced by geophysical parameters (i.e., bottom topography, shoreline contours, water depth, inflow changes, etc.), in that it may be difficult to apply findings of this study to other coastal areas. Clearly, more work is needed on factors controlling the occurrence of viruses in marine waters for the effective management of marine water quality.

ACKNOWLEDGMENTS

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water establishes a potential for shellfish-borne illness. The shellfish control agency must decide how much viable fecal material will be tolerated in waters that produce filter feeding shellfish destined for the raw market, and then it must establish and enforce the appropriate standards. With our present state of knowledge, testing for bacterial and viral pathogens may be an adjunct to, but not a substitute for, the use of indicator organisms for the detection of feces in shellfish waters.

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Glass Wool-Hydroextraction Method for Recovery of Human Enteroviruses from Shellfish

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ABSTRACT

The Glass Wool-Hydroextraction Method was developed to analyze a number of foods for the presence of contaminating human enteroviruses. This method was modified to examine a variety of shellfish, including oysters and hard- and soft-shell clams. The method consistently recovered ca. 50% of viruses inoculated into shellfish at levels of ca. 10 virus units/100 g. In a multilaboratory study, the method successfully detected all but one of the eight test viruses, and the quantitative recoveries compared favorably with the control laboratory data.

For a number of years, we have been developing methods in this laboratory for recovery of viruses from foods (*6,8,9*). The number and complexity of foods in the marketplace presented a formidable challenge to development of a method that could be used to analyze a variety of different foodstuffs. Because of the limited virus susceptibility of cell culture systems, the methods were restricted to detection of human enteroviruses. This decision was made because of the known public health significance of these human viruses and because of past experience with outbreaks shown to be associated with foods contaminated by the food handlers or by human wastes.

In 1975, a collaborative study was made of the glass wool filtration method, and six investigators demonstrated the effectiveness of the method for detection of virus levels of > 10 units/g of ground beef (*6*). However, data from this laboratory and others in the United States and Europe demonstrated that virus levels in contaminated foods were of a low order of magnitude (*2,3,4,7,8*). Therefore the method was modified to detect virus levels of ca. 10 units/100 g of food. This procedure was used to analyze a number of shellfish samples known to be contaminated with human enteroviruses.

METHOD

(*a*) Blend at low speed in a Waring blendor two shellfish for 20 sec at 1:10 dilution of 0.01 M Tricine containing 5 ml of $MgCl_2 \cdot 6H_2O$ (475 g/L) and 5 ml of DEAE-dextran (10 g/L), pH 9.0. Add 1 ml of antifoam emulsion (Dow-Corning, Midland, MI) to each sample before blending. Blend sufficient shellfish to obtain at least 100 g. The pH of the homogenate must be readjusted periodically to ca. 9.0 with 1 N NaOH. A pH test paper (Micro Essential Lab., Brooklyn, N.Y.; VWR Scientific Box 855, Columbus, OH) was used to prevent contamination that might occur with the use of a pH meter.

(*b*) Stir on a magnetic stirrer in an incubator at 37 C for 1 h.

(*c*) Pour the contents into a sterile 150-mm funnel containing 5 g of glass wool that has been pretreated in situ with 100 ml of Tricine, pH 9.0. Attach 8 to 10 ft of sterilized dialysis tubing, 1-1/8 in. in diameter to the base of the funnel.

(*d*) After 0.5 h, or when filtration is complete, rinse with 100 ml of Tricine and depress the glass wool with a tongue depressor to remove excess fluid.

(*e*) Remove the dialysis bag and seal with special closures (Spectrum Medical Industries, Inc.; Cole-Palmer Instrument Co., Chicago, IL). Wet the outside of the dialysis bag with tap water and place it in a large beaker or other container. Add ca. 70 g of polyethylene glycol (20,000 mw) plus 10 ml of water to the container. Place the bag and container in the refrigerator to hydroextract overnight.

(*f*) The following morning, remove the dialysis bag and wash the outside thoroughly with tap water. Add 30 ml of Tricine ($MgCl_2$ and DEAE), pH 9.0. Knead the bag thoroughly by hand; then place it on a flat surface and, using a large (rubber) spatula, squeeze the contents slowly out into a 50-ml (screw capped) centrifuge tube, moving from the lower to the upper portion of the dialysis tubing.

(*g*) Add 1 g of Celite, mix, adjust to ca. pH. 8.5, and centrifuge at 2000 RPM for 0.5 h. (If the sample is expected to be toxic, add 1 part Freon to 5 parts concentrate before centrifugation.)

(*h*) Decant the supernatant fluid into an 8-oz. specimen cup, add antibiotics, and bring the volume to 100 ml with Tricine (*6*). Place it in the refrigerator overnight.

(*i*) The following morning, add 5 ml of inoculum to each of 20 Buffalo green monkey kidney (BGM) cell cultures for the plaque assay; incubate at 37 C. Approximately 4.5 to 5 h later, pour off the inoculum into an additional 20 bottles of BGM cultures (*1*). To the first set of bottles, add 18 ml of the overlay medium, and to the second group, add 15 ml of growth medium (*6*).

(*j*) Incubate, observe cytopathic effect (CPE) and count plaques daily for a period of 14 days.

RESULTS

In early developmental studies, problems were encountered when lipids present in the shellfish solidified at refrigeration temperatures. This problem was especially troublesome during the filtration and hydroextraction procedures. Mixing the homogenate at 37 C for 1 h liquefied the fat and appeared to monodisperse the lipid globules. When the homogenates were cooled, no further globule aggregations were encountered. Occasionally, a fat layer was detected after low-speed centrifugation (step g).

Initially, 30 ml of Tricine (pH 9.0) was used to recover the virus concentrate from the dialysis tubing. This volume was increased to 70 ml to attempt to enhance virus recovery efficiency, but no difference was noted. About 50% of the input viruses were detected with either procedure. Whether the pH is maintained at 8.5 or 9.0 is not critical, since virus recovery was the same when the

pH ranged from 8.0 to 9.0. Therefore pH indicator paper could be used successfully to monitor the sample with a minimum loss of sample volume.

Because of cell culture toxicity associated with some shellfish tissues, the concentrate was diluted to 100 ml, and 5 ml was inoculated onto the cell cultures. It was anticipated that the toxic substance thus diluted would be less likely to produce nonspecific cytopathologic changes on the cell sheet. An additional safeguard was taken by pouring the inoculum into a second culture; we anticipated that any toxicity associated with the inoculum would be removed by the cells in the first bottle. On several occasions the cells in the first bottle were destroyed, but those in the second remained intact during the incubation period.

The addition of 5 ml of concentrate to the cell culture was a change in our normal procedure of adding 1-ml portions to the cell culture. To determine what effect this increase in volume would have on virus adsorption, a study was initiated to monitor the differences in concentrate volume and adsorption times (Table 1). To obtain virus titers comparable to the 1-ml/1-h adsorption procedure, the 5 ml concentrate adsorption time was increased to 5 h. Little if any increase in total virus recovery occurred when the 5-ml concentrate was transferred to a second cell sheet. If cells with a different virus susceptibility were used in the second cultures, different viruses might be detected (*4*).

In sample F, Freon (1 part) was added to the concentrate (5 parts) and removed from the dialysis tubing. This step was taken because two investigators examining the samples in this laboratory had encountered toxicity problems with this sample. Some of the samples were found to be toxic after addition of the concentrates, but sufficient cultures remained unaffected to process the sample.

The effectiveness of the Glass Wool-Hydroextraction Method was demonstrated in a multilaboratory study of methods for recovery of viruses from shellfish meats contaminated with a variety of enteroviruses (*5*). The recovery data obtained using the Glass Wool-Hydroextraction method from this study are shown in Table 2. All but one of the eight test viruses were detected, and the quantitative recoveries compared favorably with the control laboratory data. In all the samples containing Echovirus, the CPE was more sensitive than the plaque technique (PFU) in detecting the presence of virus.

TABLE 1. *Effect of volume of inoculum and adsorption time on virus titer.*

Procedure	No. of tests	Virus titer after 5 h adsorption volume added			Virus titer after 1 h adsorption volume added		
		10 ml	5 ml	1 ml	10 ml	5 ml	1 ml
1. Inoculate-overlay	11[a]	10[b]	10.7	14.2	7.3	10.3	11.3
2. Inoculate, pour off, overlay; 2nd bottles CPE	10	10.5	10.6	11.3	7.5	10.8	10.4
3. Inoculate, pour off, overlay; 2nd bottles CPE, overlay negative CPE	16	11.0	10.8	10.8	5.3	10.3	9.5
4. Inoculate, pour off, overlay; 2nd bottles overlay after 1 h	3	10.3	8.7	15.3	9.0	13.4	13.7

[a]5 bottle cultures used in each test plus an additional set of 5 cultures when inocula were poured off into the 2nd bottle cultures.
[b]Total of plaque numbers plus CPE. Each culture showing CPE was counted as 1 PFU.

TABLE 2. *Recovery of viruses from seeded shellfish.*[a]

Virus numbers reported as	Cell cultures used		A Oysters	B Clams, hardshell	C Clams, hardshell	D Oysters	E Clams, softshell	F Clams, softshell
Glass Wool-Hydroextraction Method	PFU	BGM	113	11	4	17	1	2
	CPE[b]	BGM	18	14	9	ND[c]	8	8
	Total		131	25	13	17	9	10
Types			P1	CB3	E7	P1	E17	E7,P2
Control laboratory recoveries:								
Initial[e] samples	PFU	BGM	9	6	6	4	6	8
Final samples	PFU	BGM	22	3	7	17	0[d]	30
	CPE	BGM	ND	ND	20	ND	ND	25
	Total		22	3	27	17	0	55
Types			P1	CB3	E7	P1,P2	E17	E7,P2

[a]P1, Poliovirus 1; P2, Poliovirus 2; B3, Coxsackievirus B-3; E7, Echovirus, 7; E17 Echovirus 17.
[b]Monolayers showing CPE expressed as 1 PFU.
[c]ND, not done.
[d]No isolates recovered by PFU assay in final test.
[e]Samples were mailed from the control laboratory. After all the investigators had received their samples, the control laboratory analyzed the initial samples; after all investigators had reported their recovery data, the control laboratory analyzed a second set of samples that had been stored at -70 C (final samples).

DISCUSSION

Methods have been developed for recovery of viruses from complex foods with the objectives of quantitative recovery of low levels of virus contaminants and minimal use of costly equipment and apparatus. Another important objective was to develop methods that could be used to analyze a number of food samples during a normal working period. The Glass Wool-Hydroextraction Method utilizes equipment and supplies that would normally be found in a virus laboratory or that could be purchased at a nominal cost. With this method, one investigator can process 10 to 15 shellfish samples a week with a recovery efficiency of ca. 50% when virus levels are ca. 10 units/100 g of shellfish meat.

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to traverse one log cycle; it is the time required for the temperature difference between product and heating or cooling medium to decrease by 90%; f_h identifies the heating parameter.

F, F_0, F_0(BIO), F_0(PHY)

The F-value is the equivalent time at temperature T of a process delivered to a container or unit of product for the purpose of sterilization; it is the common measure of the level of the sterilization process and is calculated using a specific value of z. F_0 indicates that the temperature was 250 F and the z-value was 18 F. F_0(BIO) indicates that the F_0-value was measured biologically; F_0(PHY) that it was determined from data measured physically.

j

Lag factor of the semilogarithmic heating curve for a specific location in a product in a container.

$$j = \frac{\text{(heating medium temperature)} - \text{(Y-intercept temperature)}}{\text{(heating medium temperature)} - \text{(initial product temperature)}}$$

r^2

Statistical correlation coefficient.

z

Measure of the direction of the thermal death time curve, the number of degrees of temperature change necessary to cause the F-value to change by a factor of 10.

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Natural Enterovirus and Fecal Coliform Contamination of Gulf Coast Oysters

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ABSTRACT

The numbers of fecal coliforms and enteroviruses present in oysters and/or their growing waters of two Mississippi reefs were determined over a 12-month period. Bacterial and viral levels reflected the classification of the waters at each location as set by the Mississippi State Board of Health in compliance with the National Shellfish Sanitation Program, but statistically significant correlations between these levels were not observed. Twelve viral isolates were found at an approved oyster harvesting location, eight of which were identified as poliovirus type 1. At the prohibited site, 146 viruses were isolated including poliovirus types 1 and 2, echovirus type 24 and several isolates which remain to be identified. The number of virus isolates from samples from each location represented approximately 35% of the number of plaques observed; however, no consistent ratio of plaque to confirmed virus was demonstrated. The results suggest that the fecal coliform levels in oyster growing waters do not reflect the level of virus contaminaton in either approved or prohibited waters.

The extent to which shellfish growing waters have been polluted by fecal material has been clearly documented in this century by the frequent closure of productive reefs. Since the early 1900's, coliform bacteria have been used to gauge the degree of fecal pollution of water, including marine waters (*25*). Methods for detecting pathogenic bacteria in shellfish or their growing waters are available although the time and expense required may preclude their use in favor of indicator bacteria. In the last 15 years, shellfish viral contaminants have attracted more and more interest as evidenced by the increased number of technical papers dealing with this problem. Compared to bacteria, viruses are not as easily detected nor are the consequences of their presence always understood.

The presence of viruses in shellfish has been documented (*2,8,9,10,17,32,35,36,43*) and usually includes those groups with direct or indirect association with the alimentary tract of man or other homiothermic animals, and whose characteristics permit survival and transmission by feces. The enteroviruses, reoviruses, adenoviruses and hepatitis A virus are considered prime candidates for shellfish contamination. A more complete list and a consideration of the ecological and epidemiological significance of other possible viral contaminants was provided by Carrick and Sobsey (*3*).

Viral epidemics attributed to shellfish ingestion most frequently involve a hepatitis virus (*27,31,33,37,38, 40-42*), usually type A. Some evidence for hepatitis B virus transmission by feces or infected shellfish has been reported (*5,15,32*). At present there is no standard technique for isolating and quantifying hepatitis viruses in feces or shellfish, although several proposed methods of fecal detection are under investigation (*14,16,24,32*). The enteric viruses are more easily isolated by routine virological procedures and could perhaps serve as indicators of viral contamination of shellfish. They are important in that they can produce either acute or chronic disease, but most human infections probably remain subclinical. In certain instances, such syndromes as aseptic meningitis, paralysis, herpangina, pleurodynia, myocarditis, skin rash and coryza may occur. In view of the multitude of problems associated with enteric viral infection, it is surprising that so little information exists which supports or negates the importance of polluted shellfish in the transmission of enteric viral diseases.

Methods for detection of viruses in shellfish usually involve the assay of entire shellfish rather than dissection procedures which are designed to isolate infectious particles associated with the feeding, digestive and excretory systems. Viruses that enter oysters from the surrounding water do not reproduce and are often found in the digestive gland (*4,11*). It is possible for viruses to adhere to shellfish due to the charge differences between virus particles and mucous surfaces (*12*). The effect of bioaccumulation by these mechanisms permits viruses to be concentrated from the growing water at least by a factor of 60 (*36*).

Methods for recovery of viruses from shellfish may or may not employ concentration steps. Procedures that do not involve concentration steps are discussed in references 2, 11, 34 and 35, but are not applicable to the analysis of large quantities of shellfish tissue or are unlikely to demonstrate low level contamination. Recent investigations (*17,22,23,28,-30,45-47*) provided data that may reflect the level of contamination in shellfish tissue. Of the methods reported in those investigations, the Sobsey method (*47*) and subsequent revisions (*45,46*) are most often used and have provided the most consistent results.

In Mississippi, coastal estuaries receive the effluents of sewage disposal facilities. Those effluents have been

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extremely detrimental to the once viable shellfish industry. This report details the results of a study designed to isolate, enumerate and identify viruses from oysters collected from both approved and prohibited shellfish growing areas. Comparative fecal coliform analyses were also performed.

MATERIALS AND METHODS

Sampling procedures

Water and oyster (*Crassostrea virginica*) samples were collected from approved (Pass Christian reef) and prohibited (Graveline Bayou) shellfish growing areas (Fig. 1) from January through December, 1978. Table 1 outlines the types and numbers of samples taken. Samples were collected monthly, with the exception of oysters from Graveline Bayou for virological analysis, which were collected twice monthly. Surface (upper 0.25 m) water samples were collected in sterile wide-mouth jars while bottom samples were collected in a sterile bottle with the aid of a J-Z sampler. Water samples were collected at each of three locations in Graveline Bayou for 3 days before sampling the oysters and on the actual day of oyster sampling. Water samples were taken at three locations on the Pass Christian reef only on the day of oyster sampling. Oysters (3-5 inches long) were harvested with a hand dredge, culled and placed in an insulated box for shipment. All samples were kept at 4 C until processed.

Surface water temperatures were measured in situ with a mercury-in-glass thermometer. Salinity measurements were made on a portion of the water samples collected for bacteriological analysis using an AO Goldberg refractometer (No. 10402). Temperature and salinity data are expressed as averages of three replicate measurements.

Samples analysis

Fecal coliform analyses of water and oyster samples were conducted by methods previously described (*39*). Analyses were normally begun within 3 h after collection.

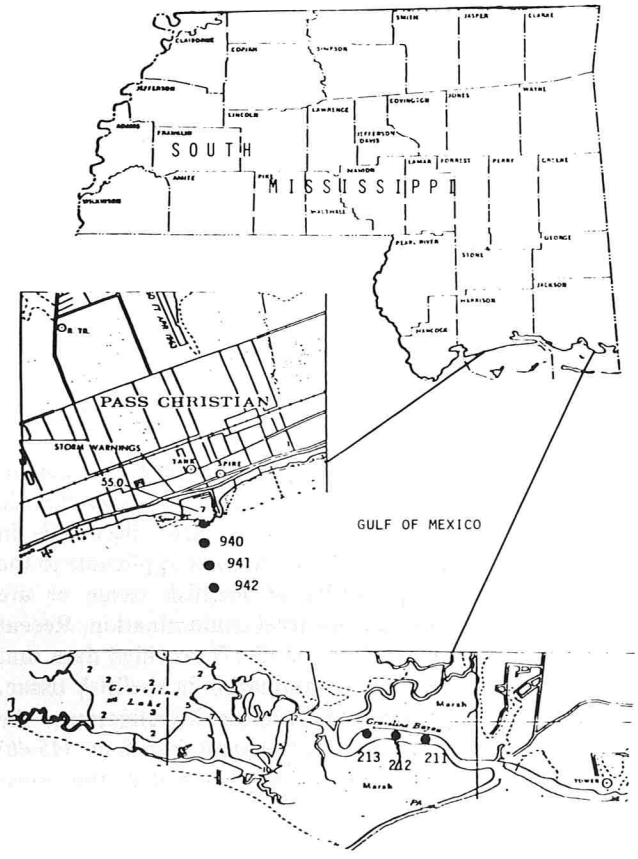

Figure 1. *Sample collection sites.*

TABLE 1. *Nature of samples.*

Location	Type sample	Purpose	Number of samples	Quantity
Pass Christian Reef	Surface water	FC[a]	3	100 ml
	Bottom water	FC	3	100 ml
	Oysters	FC	2	200 ml
	Oysters	V[b]	4	150 ml
Graveline Bayou	Surface water	FC	12	100 ml
	Bottom water	FC	12	100 ml
	Oysters	FC	2	200 g
	Oysters	V	6-7	150 g

[a]Fecal coliform analysis.
[b]Virological analysis.

Oysters (150-g lots) were extracted to determine virus concentration using a modification of the Sobsey procedure (*46*) as shown in Fig. 2.

Tissue culture assay

The Buffalo green monkey kidney cell line (BGM) (*1,6*) was used to analyze all oyster concentrates. Virus samples and/or dilutions (0.2 to 0.5 ml per 25 cm^2 plastic flask) were inoculated onto BGM monolayers (passages 100 to 120) which were incubated for 1 h at 37 C using a rocking apparatus (Bellco) at five rotations per minute. Growth medium for BGM cells consisted of MEM:L15 (1:1), 10% fetal calf serum and 1% L-glutamine (all purchased from Grand Island Biological Company).

Samples were quantitatively assayed by a modification of the plaque method reported by Dahling et al. (*6*). Plaque counts were made on a daily basis for 5 days or until no new plaques appeared for two consecutive days.

Plaque identification

Individual plaques were picked when they were ≥ 1 mm in diameter. A Pasteur pipette, with a bent tip, moistened with 0.05 ml of growth medium was used to transfer an agar plug (area of plaque) to a holding medium (1 ml MEM per tube). Samples were passaged three times in BGM cells with a minimum of one filtration step (0.45 μm). Two blind passages were made of all samples not producing observable cytopathic effect. Plaques identified as viruses were titered and identified serologically (*21*).

Statistics

Bacterial and viral counts in water and oyster samples were subjected to a square root transformation before calculation of linear correlation coefficients (*48*).

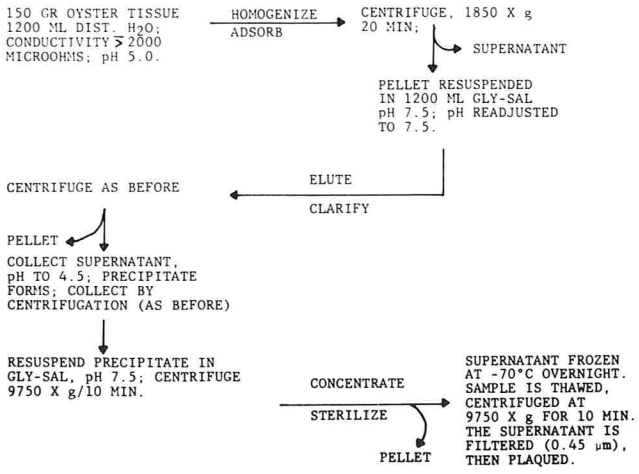

Figure 2. *Oyster extraction procedure for virus isolation.*

RESULTS

This investigation was an extension of a 1976-1977 study designed to compare the viral isolation efficiency of two oyster extraction methods (*13*). The Sobsey

procedure (*47*) was not satisfactory for examination of natural oyster samples and was modified by elimination of the intermediate filtration step and by substitution of the diluent for suspension of the final precipitate. The procedure described in Fig. 2 was tested with seeded oyster samples and yielded recoveries ranging from 61 to 73% (63% average). Oyster concentrates were clear and easy to filter-sterilize before assay. Bacterial and fungal contamination was minimal and individual flasks contained no particulate matter which interfered with visual analysis.

Table 2 presents the results of fecal coliform analyses of water and oyster samples performed during 1978. It is evident that the two areas differ significantly in bacteriological quality. The median fecal coliform MPN for all 71 water samples from the Pass Christian reef was less than 2 per 100 ml with only 8.45% of the samples exceedin an MPN of 43. The Graveline Bayou samples had a median fecal coliform MPN of 23 per 100 ml with 39.9% of the 276 samples exceeding of MPN of 43. These results confirm the approved and prohibited status of Pass Christian reef and Graveline Bayou, respectively.

TABLE 2. *Fecal coliform analysis of water and oyster samples.*

	Pass Christian Reef		Graveline Bayou	
	Water	Oyster	Water	Oyster
Month	Median fecal coliform (MPN/100 ml)	Mean fecal coliform (MPN/100 g)	Median fecal coliform (MPN/100 g)	Mean fecal coliform (MPN/100 g)
January	1400.0	73	1200.0	310.0
February	< 2.0	45	17.0	104.0
March	< 2.0	125	7.8	3,250.0
April	< 2.0	< 20	11.0	765.0
May	< 2.0	< 20	13.0	170.0
June	2.0	61	11.0	44.0
July	3.3	400	130.0	815.0
August	2.0	360	25.0	17,650.0
September	3.3	12,450	28.0	745.0
October	< 2.0	< 20	49.0	230.0
November	17.0	330	170.0	945.0
December	< 2.0	204	22.5	715.0

The fecal coliform counts from oysters (Table 2) also reflected the difference in water quality in those two areas. The median values for all samples taken from Graveline Bayou and Pass Christian were 410 and 78, respectively. There appears to be no apparent relationship between the coliform counts in the water and those in the oysters collected at the same time.

Viral isolates from approved (Table 3) and prohibited (Table 4) oyster samples also reflect the degree of fecal pollution at the two locations examined. Thirty-eight plaque-like isolates from Pass Christian were purified during the 12-month period. Of the 12 plaques confirmed as viruses, eight were identified as poliovirus type 1; four could not be typed. Most of the isolates were picked from April samples but these data could not be correlated with fecal coliform counts of that month.

In comparison, plaque-like isolates from oysters collected from Graveline Bayou totalled 416. Of this number, 146 or 35%, were identified as viruses. Of 55 random isolates identified, 50 were polio type 1, one was polio type 2, one was echovirus type 24 and three were unidentifiable by the procedures employed. Plaque-like isolates were not evenly distributed among the samples for a given month. The percent of positive 150-g samples (Table 4) ranged from 10 to 55%. In July, one of 10 samples contained virus as compared to five of nine samples in October.

TABLE 3. *Viral analysis of approved oysters, Pass Christian Reef.*

Month	# Oysters/ # samples	*	# Plaque-lifke isolates Total/100 g	# Plaques identified as viruses Total/100 g
Jan.	24/2	0	2/0.6	0/0
Feb.	32/2	0	4/1.3	0/0
Mar.	24/2	0	6/2.0	0/0
Apr.	30/2	1	19/6.3	11/3.6
May	21/1	0	0/0	0/0
June	38/2	0	0/0	0/0
July	32/2	0	0/0	0/0
Aug.	36/2	0	2/0.6	0/0
Sept.	33/2	0	0/0	0/0
Oct.	26/2	1	2/0.6	1/0.3
Nov.	21/1	0	0/0	0/0
Dec.	33/2	0	3/1.0	0/0
TOTAL	350/22		38	12

*Number of samples containing confirmed virus isolates.

TABLE 4. *Viral analysis of prohibited oysters, Graveline Bayou.*

Month	# Oysters/ # Samples	*	# Plaque-like isolates Total/100 g	# Plaques identified as viruses Total/100 g
Jan.	139/10	5	88/5.8	31/2.2
Feb.	65/5	1	38/5.0	5/0.7
Mar.	53/3	1	18/3.0	3/0.5
Apr.	56/3	1	5/1.1	3/0.7
May	102/6	3	51/5.6	18/2.0
June	200/13	3	38/1.9	12/0.6
July	180/10	1	44/2.9	23/1.5
Aug.	89/7	3	39/5.0	11/1.4
Sept.	191/10	5	46/2.7	26/1.5
Oct.	129/9	5	15/1.1	10/0.7
Nov.	88/8	1	9/0.8	3/0.3
Dec.	35/3	1	25/5.5	1/0.2
TOTAL	1327/87		416	146

*Number of samples containing confirmed virus isolates.

Generally, in both areas bottom water salinities were higher than surface salinities (Tables 5 and 6). The salinities of the waters over the Pass Christian reef remained fairly constant during the year except for two periods following heavy rainfall when the salinities were significantly reduced. The water salinities in Graveline Bayou ranged from a low of 2.0 to a high of 28 ppt. On one occasion, the salinity fluctuated as much as 15 ppt over the 4-day sampling period.

Temperatures of surface waters at the approved reef ranged from 8 C in January to 31 C in June. The same general trend in temperature fluctuation was observed at Graveline Bayou.

Correlation coefficients which compared fecal coliforms in surface and bottom waters, fecal coliforms in oyster tissue and plaque-like and actual virus isolates from oyster samples are presented in Table 7. A significant ($P \leq 0.1$) positive correlation was found between fecal coliforms in bottom and surface waters but not between those and fecal coliforms in oyster tissue.

TABLE 5. *Physical data on water samples from Pass Christian reef.*

Date	Surface		Bottom
	Temperature[a] (C)	Salinity[a] (ppt)	Salinity[a] (ppt)[b]
1/27/78	8.0	2.0	5.5
2/28/78	14.0	16.0	19.6
3/30/78	19.0	14.6	15.6
4/27/78	21.0	14.6	15.0
5/31/78	30.0	7.0	8.3
6/28/78	31.0	13.8	14.6
7/3/78	28.0	18.0	18.0
8/30/78	29.0	14.6	18.6
9/28/78	27.0	17.3	17.3
11/2/78	23.0	18.0	18.3
11/30/78	17.0	17.6	18.3
12/14/78	11.0	15.3	20.0

[a]An average of three measurements.
[b]Parts per thousand.

Significant correlations between fecal coliforms in waters or oysters and plaque-like or virus isolates in oysters were not observed. Although the number of confirmed viral isolates began as plaque-like isolates, no correlation could be found to indicate a relationship on a month-to-month basis.

When the results of fecal coliform and virus analyses of Graveline Bayou oysters are graphically compared (Fig. 3), they reflect the lack of statistical correlation. The variations observed in three particular months are of interest. In March, the number of fecal coliforms rose while the number of plaque-like and confirmed virus isolates decreased or remained constant. In May, the number of plaque-like and confirmed virus isolates increased, but did not correlate with the decreasing number of fecal coliforms. The numbers of both fecal coliforms and plaque-like isolates increased in August, but the number of confirmed virus isolates remained at approximately the level of the previous month.

DISCUSSION

The two locations chosen for this study were selected because previous observations had shown that each was ecologically, topographically and bacteriologically distinct. The Pass Christian reef lies in open waters of the Mississippi Sound and is not readily influenced by rapid environmental changes. Conversely, Graveline Bayou is greatly influenced by local rainfall and tidal flushing and may change rapidly within the short time period. The maximum sampling effort was expended at this location primarily to increase the probability of virus recoveries. Oyster harvesting has not been permitted in Graveline Bayou since 1975. The bayou begins at the Mississippi Sound and runs 4389 m to Lake Graveline (95 hectares). Bayou depth varies from 0.6-3.7 m. The average sill depth at the bayou's mouth is 15 cm at mean low tide. Several sources of sewage pollution contribute to the closure of Graveline Bayou to shellfishing harvesting: Del Flore treatment plant, 1097 m east of the bayou's mouth and the Gautier Point treatment facility, 3474 m east of Graveline. Septic tanks near the lake and new housing development near the bayou's mouth also

TABLE 7. *Correlation coefficients, Graveline Bayou isolates.*

	FCWS	FCWB	FCO	PLI	VI
FCWS	1.000				
FCWB	0.748**	1.000			
FCO	0.052	0.414	1.000		
PLI	0.079	0.069	0.191	1.000	
VI	0.405	0.446	0.290	0.455	1.000

**P < 0.1 level of significance
FCWS, FCWB, FCO, PLI, VI represent fecal coliform water surface, MPN 100 ml; fecal coliform water bottom, MPN 100 ml; fecal coliform oyster, MPN 100 gr^{-1}; plaque-like isolates, 100 gr^{-1}; viruses identified 100 gr^{-1}.

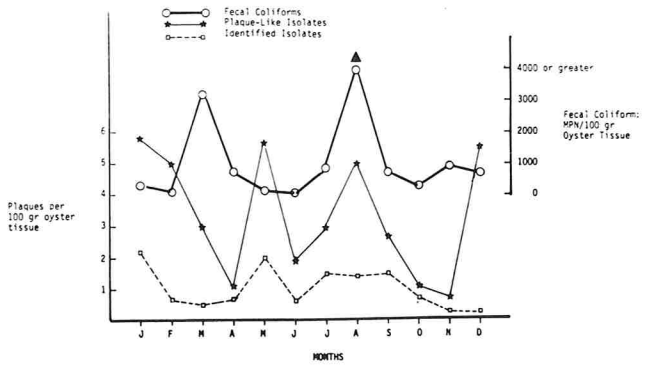

Figure 3. *Fecal coliform, plaque-like isolates and confirmed virus levels per 100 g of oyster tissue. Samples collected in Graveline Bayou, 1978.*

TABLE 6. *Physical data on Graveline Bayou water samples.*

Dates[a]	Surface		Bottom
	Temperature[b] (C)	Salinity[b] (ppt)[c]	Salinity[b] (ppt)[c]
1/24/78 to 1/27/78	9.0 to 12.5	2.0 to 7.0	2.0 to 10.3
2/25/78 to 2/28/78	11.0 to 14.5	6.0 to 12.0	10.3 to 17.6
3/28/78 to 3/31/78	17.5 to 19.5	11.6 to 21.3	17.0 to 22.0
4/25/78 to 4/28/78	19.0 to 23.5	14.6 to 19.6	15.0 to 19.6
5/28/78 to 5/31/78	27.0 to 30.0	4.0 to 5.8	4.8 to 6.0
6/25/78 to 6/28/78	29.0 to 32.0	8.5 to 17.3	10.0 to 17.3
7/31/78 to 8/3/78	28.0 to 32.0	6.2 to 18.6	7.5 to 22.0
8/27/78 to 8/30/78	27.0 to 30.0	14.0 to 16.0	14.0 to 16.0
9/25/78 to 9/28/78	27.0 to 29.0	16.0 to 16.6	16.0 to 16.6
10/30/78 to 11/2/78	21.0 to 23.0	24.0 to 25.6	24.0 to 25.6
11/27/78 to 11/30/78	17.0 to 20.0	21.0 to 22.0	21.0 to 22.0
12/11/78 to 12/14/78	8.0 to 12.0	20.0 to 28.0	22.0 to 28.0

[a]Represents the last 4 days of each month.
[b]An average of three measurements.
[c]Parts per thousand.

contribute sewage. From the sound, sewage enters Graveline by the westerly current drift in conjunction with tidal currents and prevailing southeast winds.

In our literature survey, we could find few previous studies which analyzed the virological content of oysters over an extended period. The lack of such studies is surprising when one considers the inability of the coliform standard to adequately predict increased viral contamination and the possible epidemiological consequences. Undoubtedly hepatitis outbreaks transmitted by shellfish are well documented (*18*), but similar occurrences of enterovirus disease transmission are by their very nature difficult to identify. The ingestion of raw or incompletely cooked oysters presents the potential for enterovirus transmission and the isolation of these viruses from shellfish has been observed on more than one occasion (*17,21,34,35*).

The modifications of the Sobsey extraction procedure, which have been made in our laboratory over the last 3 years, resulted from a desire to process naturally polluted oyster samples. During 1978, a wide seasonal variation of environmental parameters occurred, but no significant changes in the procedure were required. Most problems usually occurred when the final precipitate was suspended. Heavy contamination and/or inability to filter the concentrate before assay demanded the most attention. The more turbid the final concentrate, the more likely that the plaque assay would be adversely affected. When the concentrate was frozen, then thawed, centrifuged and filtered, less than 0.1% of the virus was lost to the precipitate and the filter.

Fecal coliform levels are used to verify the classification of shellfish-growing waters. The present classification system does, in general, protect the public from diseases transmitted by shellfish, but it is by no means considered infallible, especially in regard to the level of viral contamination (*17,20,21*). The problem is compounded by the lack of valid correlations between fecal coliform levels in waters and oyster samples taken simultaneously at the same site (Table 2). One factor which could account for that lack of correlation is salinity which on occasion varied as much as 15 ppt during the 4-day sampling period. Similar fluctuations were noted in the fecal coliform counts in water which in one instance changed by 2 logs in one 3-day period.

The expected rise and fall of surface water temperatures during the year did not correspond to the fluctuation of either fecal coliform or virus levels. At the Pass Christian reef, the highest recorded values for temperature, fecal coliform and virus counts occurred in the months of June, January and April, respectively. The same parameters recorded at Graveline Bayou corresponded to the months of August and January. Although our studies and those of other investigators (*21*) do not indicate correlations between salinity and temperature versus fecal coliforms in water and oysters and virus in oyster, fluctuation of those parameters would affect indicator ratios and could produce significant variation in the data used for sanitary surveys.

The plaque procedure used contributed to the problems of sample assay. For all samples examined, over 60% of all plaques were not of viral origin. This discrepancy could be due to artifacts in the flasks, limited chemical or biological contamination or failure of the isolate to replicate in the BGM cell line. This complication can be avoided using an all-or-none quantal assay in addition to the plaque assay. Studies of minimal viral contamination of oysters that compare all or none versus plaque assay methods would more clearly define the most appropriate method.

Most of the purified viral isolates (85%) was identified as poliovirus type 1. This observation is not unusual (*21*) and probably reflects the wide-spread distribution of oral polio vaccine. What is surprising is the very low numbers of other polio types observed. Perhaps environmental factors or certain aspects of the oyster extraction procedure contributed to the failure to detect viruses that are shed by the fecal route. Although Katzenelson and Kedmi (*26*) did not express this particular concern, they did suggest that additional research be done to develop a cell system with greater potential for multiple-virus assay.

These data again emphasize the need to re-evaluate the use of the coliform standard for verification of shellfish growing waters. As the fecal coliform standard is routinely used, consideration for keeping the total coliform standard should be given since Goyal et al. (*21*) have demonstrated a relationship between viruses in estuarine water and the total coliform counts in water and oysters. The relationships of viruses in shellfish to viruses in estuarine sediments should be defined since recent studies (*7,19,44,49*) confirmed that sediments can contribute large numbers of viruses to the water column and possibly to feeding shellfish.

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Occurrence of Enteric Bacteria and Viruses in Oysters

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ABSTRACT

Enteric bacteria and virus levels were determined in oysters from paired stations that were opened or closed for commercial shellfishing on the basis of total coliform levels in the water. Six pairs of stations were sampled quarterly over a 1-year period. Enteric viruses were found in 3 of 24 50-g oyster samples from closed areas and in none of 23 samples from open areas. *Salmonella* was found in 2 of 47 samples of 40 g each, one from an open and the other from a closed area. Although enteric pathogens of fecal origin were found only in oysters that exceeded the recommended market limit of 230 fecal coliforms per 100 g of meat, the fecal coliform levels in some virus-positive samples were much lower than those in *Salmonella*-positive samples. *Vibrio parahemolyticus* levels were similar in oysters from both open and closed beds, indicating no particular association with fecal pollution. However, there was a marked seasonal variation in *V. parahemolyticus* levels. Total but not fecal coliform levels in oysters from open beds correlated with the occurrence of rainfall 1 or 2 days before sample collection. Neither total nor fecal coliform levels in oysters from closed beds correlated with rainfall. These findings suggest that fecal coliforms levels in oysters are less influenced by rainfall than are total coliforms, and therefore may be a more specific indicator of recent fecal pollution.

The eastern oyster, *Crassostrea virginica,* and other bivalve molluscs are able to concentrate enteric bacteria and viruses from their surrounding water environment during normal filter-feeding activities (*3,7,9*). The ingestion of such contaminated shellfish may lead to illness, and therefore sanitary criteria and standards for fecal contamination of shellfish and their harvesting waters have been established (*4,6,18*).

While the total and fecal coliform standards for shellfish harvesting waters and meats are thought to be adequate for protection from bacterial pathogens of enteric origin, such as *Salmonella,* it has not been established that they adequately reflect the presence in shellfish of either enteric viruses or potentially pathogenic bacteria of natural aquatic origin, such as *Vibrio parahemolyticus* (*2,5,12*). In particular outbreaks of hepatitis A, viral gastroenteritis and *V. parahaemolyticus* food poisoning have occurred due to the ingestion of contaminated shellfish (*1,4,17*). In some recent outbreaks, the incriminated shellfish were apparently harvested from waters meeting present bacterial standards (*10*).

Studies on occurrence of enteric viruses in shellfish and the relationships, if any, between enteric viruses and bacteria in shellfish have been hampered by the previous unavailability of convenient and reliable methods for virus detection in shellfish. However, recent work has led to detection methods for viruses in shellfish that appear to be simple and reliable enough to use in field studies (*14,15*).

The purpose of this present study was to investigate in preliminary fashion the occurrence of indicator and potentially pathogenic bacteria and enteric viruses in oysters obtained from areas that were either approved or not approved for shellfishing on the basis of the total coliform levels in the water.

MATERIALS AND METHODS

Oyster sampling areas

For this study, the North Carolina coast was divided into three main regions, North, Central and South. Oysters were collected from two open and two closed beds from each region, thus representing both a wide geographical distribution and diverse water quality conditions within the state. Open and closed beds were paired stations on each side of a closure line in a single area. The three regions were sampled for oysters on a monthly rotating basis over a 1-year period, so that all four seasons of the year were represented in the sampling for each region.

Oyster collection and processing methods

Oysters were harvested and transported on ice to a field laboratory in Morehead City, N.C. Samples were refrigerated until they were processed, which usually was within 2 days after collection. The samples were scrubbed and shucked aseptically and about 220 g of oyster meat without the liquor were homogenized in a blender. The homogenate was divided into two equal portions for bacterial and viral analysis. The portion for bacterial analysis was further subdivided for the following tests: aerobic plate count (APC), total and fecal coliforms, enterococci, *V. parahemolyticus, Staphylococcus aureus, Salmonella* and *Yersinia.* Bacterial analyses were done by the methods described in the *Compendium of Methods for the Microbiological Examination of Foods* (*16*) except *V. parahemolyticus*, which was enumerated by a recently developed "repair-detection" method (*11*).

The other half of the oyster homogenate was processed for viruses using newly developed methods (*14,15*). The final concentrate was assayed on BGMK cell cultures by a liquid culture, quantal assay technique (*13*).

RESULTS AND DISCUSSION

Microbiological quality of oysters

An overall summary of the microbiological quality of oysters harvested from open and closed waters is shown in Table 1. Oysters from closed beds had higher aerobic plate count, total and fecal coliform, fecal streptococci and *S. aureus* levels than those from open beds. Enteric

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viruses were found in 3 of 24 50-g samples from closed beds but in none of 23 50-g samples from open beds examined so far. Another 50-g portion of each virus concentrate sample is still being assayed. *Salmonella* was found in 2 of 47 40-g samples, one from open and one from closed beds. *Yersinia* was not isolated.

V. parahemolyticus levels were generally similar in oysters from both open and closed beds. This was not unexpected, considering vibrio organisms are indigenous estuarine bacteria and are not specifically associated with fecal pollution (*8,12*). There was a definite seasonal variation in *V. parahemolyticus* levels in oysters, with a strong positive correlation between increasing coastal temperatures and increasing *V. parahemolyticus* levels. Mean *V. parahemolyticus* levels ranged from a low of nearly 0/g during January through March to highs of 1,100/g during June through September. This pattern is consistent with the findings of Kaneko and Colwell (*8*), except maximum *V. parahemolyticus* levels in North Carolina are reached about 1 month earlier than those in the Chesapeake Bay, and probably reflect an earlier warming of the water.

Although aerobic plate counts (APC) were generally higher in oyster samples from closed areas, all of the samples had APC levels well below the market standard of 500,000/g of meat. The two samples with APC levels of 110,000/g were collected 1 day after major rainfall events.

In general, total and fecal coliform levels in oysters were consistent with closure lines based on total coliforms in the water, with a few notable exceptions such as the *Salmonella*-positive sample from an open area. Fecal coliform-to-fecal streptococci (FC:FS) ratios in oysters were intermediate for both areas. In closed areas where there were nearby sources of domestic fecal pollution, the intermediate ratio may reflect the greater survival of fecal streptococci in the marine environment compared to fecal coliforms (*19*). The intermediate FC:FS ratio in open areas may also be explained on this same basis, but it may also be due to fecal contamination from non-human sources.

Coagulase-positive *S. aureus* was isolated with a greater frequency from oysters of closed beds, but there was no correlation between levels of fecal coliforms and *S. aureus* in the oysters ($r = -0.04$ for both open and closed bed samples).

The indicator bacteria levels of oyster samples from closed beds that were either negative or positive for *Salmonella* or enteric viruses are shown in Table 2. No individual samples were positive for both *Salmonella* and enteric viruses. Oyster samples positive for *Salmonella* but not enteric viruses had the highest indicator levels with the fecal coliform recommended market meat limit of 230/100 g being exceeded by factors of 39 and 200. From this, one might conclude that the fecal coliform meat limit provides a substantial safety margin for *Salmonella*. In two of three instances the indicator bacteria levels of virus-positive *Salmonella*-negative samples were not nearly as high as those for *Salmonella*-positive samples and were actually lower than the mean values for samples that were negative for both viruses and *Salmonella*. Fecal coliform levels in two virus-positive samples exceeded the market limit of 230/100 g by factors of only 1.6 and 2, respectively. These limited data suggest that the fecal coliform meat limit provides a much smaller safety margin for enteric viruses in oysters than for *Salmonella*. Furthermore, it should be noted that the extent of virus contamination of the oysters was probably underestimated, because some enteric viruses will not grow in BGMK cells and because the virus concentration method for oysters is only about 50% efficient (*15*).

Influence of rainfall on oyster coliform levels

The relationship between total and fecal coliforms and the occurrence of rainfall 1 or 2 days before oyster collection for both open and closed beds is plotted in Fig. 1. There was a correlation between total and fecal coliforms in shellfish from open beds ($r = 0.74$); however, there was an even stronger correlation between rainfall and oyster total coliforms in open beds ($r = 0.85$). There was no correlation between rainfall and fecal coliforms ($r = -0.14$). These results suggest that rainfall runoff was an important contributor of total but not fecal coliforms in oysters from open beds.

In closed beds there was only a weak correlation between total and fecal coliforms in oysters ($r = 0.56$),

TABLE 1. *Mean microbiological quality of oysters harvested from open and closed beds.*

	OPEN		CLOSED	
Analysis	Log mean	Range	Log mean	Range
	Unit weight		Unit weight	
Aerobic plate count/g	1,500	30 - 52,000	2,400	150 - 130,000
Coliform MPN/100 g	1,200	0 - 110,000	7,000	0 - 110,000
Fecal coliform MPN/100 g	200	0 - 46,000	660	0 - 460,000
Fecal streptococci/100 g	218	0 - 4,000	490	0 - 16,000
V. parahaemolyticus/100 g	3,000	0 - 110,000	3,000	0 - 110,000
S. aureus/100 g	90	0 - 1,000	170	0 - 2,000
Salmonella (+/− 40 g) # Positive / # Tested	1/23		1/24	
Yersinia (+/− 40 g) # Positive / # Tested	0/23		0/24	
Viruses (+/− 50 g) # Positive / # Tested	0/23		3/24	

but neither total nor fecal coliforms correlated with rainfall (r = 0.48 and -0.18, respectively), thus suggesting that the nearby direct wastewater discharges have a more important influence on both types of coliforms in shellfish than rainfall.

These findings support the idea that fecal coliforms are less influenced than total coliforms by non-fecal, environmental factors such as rainfall, and may more accurately reflect levels of recent fecal pollution.

SUMMARY AND CONCLUSIONS

In summary, enteric viruses were found in 3 of 24 50-g oyster samples from non-approved areas and none were found in 23 samples from open areas. *Salmonella* were found in 2 of 47 samples of 40 g each, one from an open and one from a non-approved area. Although enteric pathogens of fecal origin were found only in oysters that exceeded the recommended market limit of 230 fecal coliforms per 100 g of meat, the fecal coliform levels in some virus-positive samples were much lower than those in *Salmonella*-positive samples.

V. parahemolyticus levels were similar in oysters from both open and non-approved beds, indicating no particular association with fecal pollution. However, there was a marked seasonal variation in *V. parahemolyticus* levels.

From the limited data of this study it is not possible to quantitatively establish the relationships between levels of indicator bacteria and pathogens of fecal origin in oysters. In an effort to further define these relationships, we are now studying the occurrence and distribution of enteric bacteria and viruses in shellfish, water and sediment of estuaries receiving a major, point-source discharge of treated sewage effluent.

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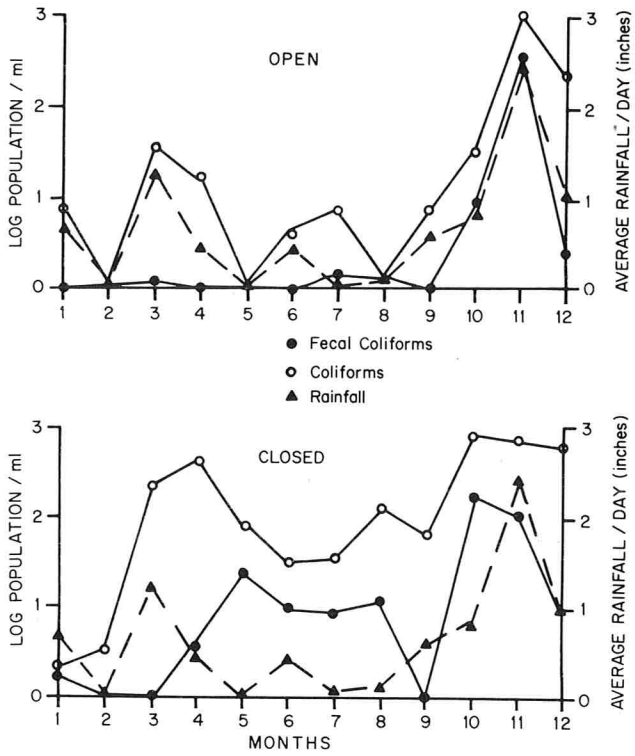

Figure 1. *Relationship of total and fecal coliforms in oysters to rainfall.*

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TABLE 2. *Bacteriological indicator levels relative to enteric pathogen isolations from closed bed oysters.*

Enteric pathogens		APC	Coliforms	Fecal coliforms	Fecal strep.
Virus	*Salmonella*	g	100 g	100 g	100 g
+[a]	+[a]	--	--	--	--
–	+	45,000	110,000	46,000	11,000
–	+	17,000	110,000	9,000	4,000
+	–	600	910	450	1,000
+	–	1,200	15,000	360	100
+	–	130,000	46,000	46,000	16,000
–[b]	–	2,000	6,300	500	340

[a]No individual samples were positive for both *Salmonella* and enteric viruses.
[b]Mean values from all samples negative for both *Salmonella* and viruses.

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Preservation of Cheese and Plain Yogurt by Low-dose Irradiation

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ABSTRACT

Irradiation preservation of Turkish kashar cheese and plain yogurt was studied using very low doses of Co-60 radiation. No side effects were observed below 0.15 Mrad. The number of bacteria killed was directly related to total dose up to 0.02 Mrad, then the effectiveness of the dose decreased. Coating cheese samples with a sorbic acid solution helped in reducing the number of bacteria by about 10-12% in irradiated samples. The shelf-life of irradiated samples stored at refrigerator temperature was almost the same as that of ones coated with sorbic acid but stored at room temperature. Mold formation in irradiated samples took three to four times longer than in nonirradiated ones. With plain yogurt the effect of total dose seemed to be the same as in cheese. Irradiation increased the shelf-life of yogurt three- to four-fold. Preservation by irradiation combined with refrigeration increased the shelf-life about five-fold.

Even though dairy technology plays a leading role in the food industry, only limited investigations have been made to preserve milk products by irradiation. Milk products are quite perishable; it is also difficult to irradiate them without changing their quality. Most work on treating food with ionizing radiation has been carried out on meat products, onions, potatoes, spices, fruits, juices, and related products (*5*). Total doses used in irradiation of these products are usually higher than the permissible dose for milk products (*3*). In milk, the maximum permissible dose giving no side effects was found to be about 0.05 Mrad (*4*). Bongirwar and Kumta (*1*) showed that preservation of cheese was possible at 0.2 Mrad without any off-flavors. Since such a dose is smaller than those used for other foods, milk products can not be preserved for long times by irradiation. However, short term preservation can be successfully accomplished. In addition, one can use irradiation as a supplement to other methods for preservation of milk products.

In this research we aimed to determine if radiation at different doses could be used to extend the shelf-life of cheese and yogurt without changing their quality. Since radiation can be safely used only at very low doses, we compared its effects with other methods like refrigeration and use of a sorbic acid solution (0.2%). Hence preserving cheese and yogurt by refrigeration, sorbic acid and radiation was investigated.

EXPERIMENTAL

In cheese experiments, Cheddar-type aged kashar cheese was employed. It was about 6 months old. Each cheese sample irradiated had dimensions of 5 × 2.5 × 0.5 cm and weighed about 5 g. Each slice was first wrapped with cellophane foil, and then irradiated. The yogurt used in the experiments was made in tubes using fresh milk from a state farm (AOÇ). No additives were used.

The radiation source was Gamma Cell 220 (manufacturer: Atomic Energy of Canada Ltd.). Samples were placed at the center of the cylindrical Co-60 source. The distance of the samples from the source was about 15 cm. The irradiation time was varied from 1 to 20 min. Dose rates used were 0.00026, 0.0021, and 0.00561 Mrad/min, while the maximum total dose used was 0.112 Mrad. The upper limit for the maximum total dose was determined from a preliminary study. It was observed that above 0.15 Mrad both cheese and yogurt showed pronounced fading of color and some off-flavors. So the maximum total dose was kept at about two-thirds of 0.15 Mrad. Surviving bacteria were determined by using the agar plate method (*2*), tryptone glucose yeast agar and incubation at 35 C for 48 h. In each set of experiments, a control sample was employed. Effects of dose rate, total dose, sorbic acid solution, and refrigeration on killing bacteria, and on the shelf-life of cheese and yogurt were investigated.

RESULTS AND DISCUSSION

The effect of dose rate on microbial count of cheese is shown in Fig. 1. The microbial counts were made immediately after irradiation of the cheese. The percent of surviving bacteria decreased exponentially at doses up to 0.001 Mrad/min. Any further increase in the dose was not effective in killing the bacteria. This means that if more than a sufficient number of photons hit the sensitive regions of bacteria, only a certain fraction is necessary to kill them and the rest are not used. Therefore 0.001 Mrad/min is the upper dose rate above which some surplus radiation is not used for killing the bacteria. Moreover, this excess radiation can cause a loss in the nutritional value of cheese. The decrease in number of surviving bacteria after 0.001 Mrad/min (or a total dose of 0.02 Mrad) still shows the dependence of killing rate (i.e. number of bacteria killed per unit time per unit dose) on the probability of collision of photons with bacteria. This dependence is probably due to destruction of less sensitive regions of bacteria at high radiation intensity.

Figure 2 gives the percent increase of bacteria in cheese samples after 1 week of storage at room temperature. The percentages were determined from the number of bacteria at the end of 1 week of storage and the number immediately after irradiation. The percent increase in the number of bacteria was quite small at doses above 0.02 Mrad. The upper curve in Fig. 2 looks like a straight line with a small almost constant slope

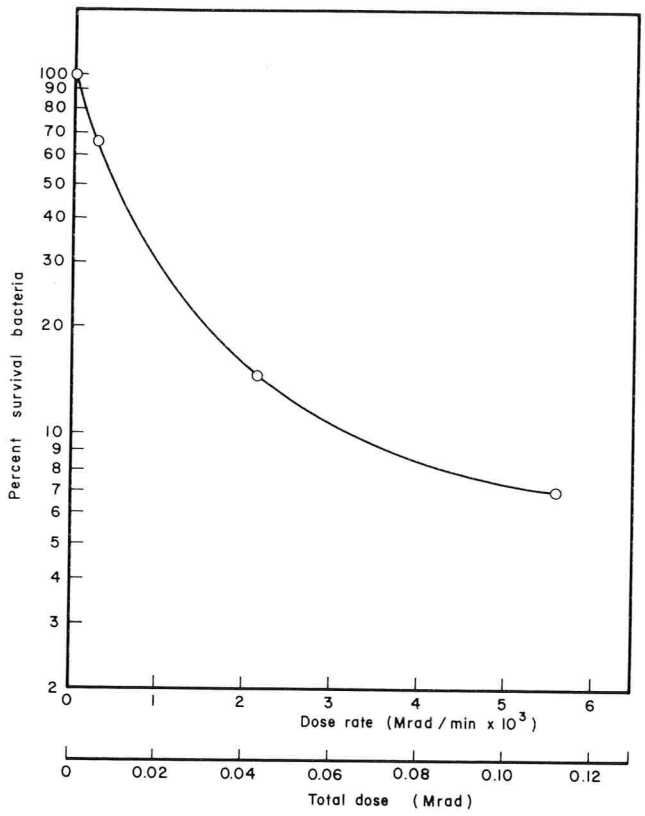

Figure 1. *Radiation effect upon microbial count of cheese at room temperature. (Immediate count after irradiation; irradiation time: 20 min).*

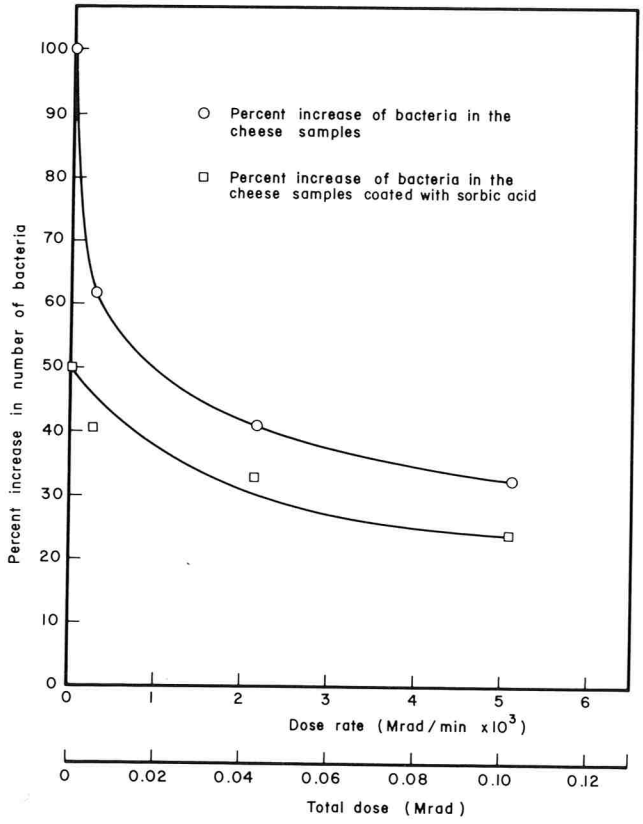

Figure 2. *The percent increase in number of bacteria of cheese in one week of storage (at room temperature) as a function of dose rate. (Irradiation time: 20 min.).*

after 0.02 Mrad. This may have resulted from two causes. One is the possible mutation of bacteria in cheese because of intense radiation, so that the mutated bacteria may not be well adapted to the medium. The second is the decrease in nutrient value of cheese necessary for growth of bacteria. It is again important to note that a dose of 0.02 Mrad was the lower limit for effective decrease in the growth rate of bacteria over an extended time, i.e. 1 week of storage time. The effect of sorbic acid was quite pronounced for nonirradiated samples as is seen from the lower curve in Fig. 2. It reduced the number of bacteria by about 10-12% at the doses used in the experiments.

The effect of storage temperature on number of bacteria in cheese is shown in Fig. 3. The counts were done at the end of 1 week, and the percent survival was based on the nonirradiated samples. Samples stored at refrigerator temperature (6 C) had counts similar to the ones stored at room temperature (22 C) but coated with sorbic acid solution (lower two curves in Fig. 3). As the dose was increased, both sorbic acid and refrigeration became less important. It is also seen from data in Fig. 3 that the change in microbial count was very small after 0.04 Mrad. This was not very clear in Fig. 1 and 2. Even though the effectiveness of dose decreased after 0.02 Mrad, as predicted from Fig. 1 and 2, the bacterial count decreased to a very low value at 0.04 Mrad, beyond which no further reduction occurred. Thus this should be an upper limit for the radiation dose. In practice, one can

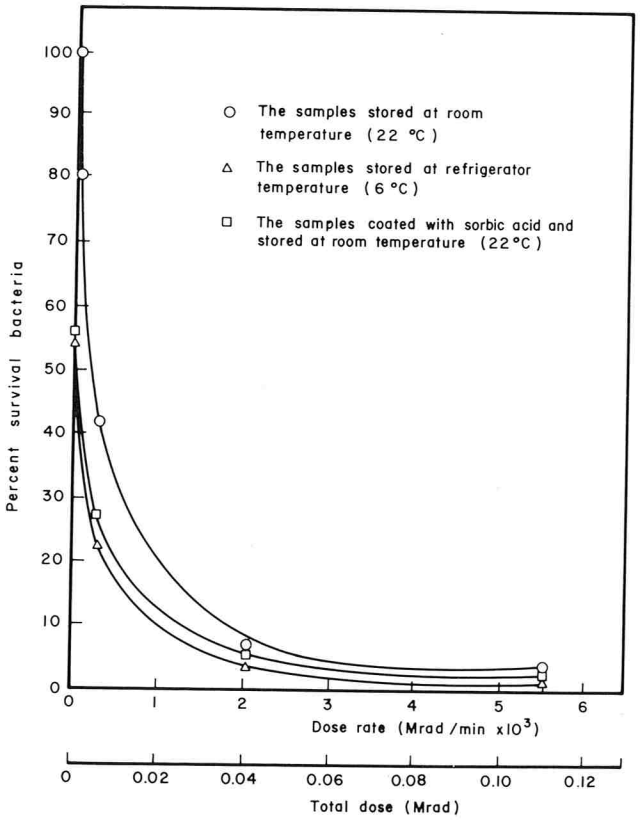

Figure 3. *The effect of radiation and storage temperature upon microbial count of cheese after one week of storage.*

work in the range of 0.02-0.04 Mrad, but lower doses are preferred.

Figures 4, 5 and 6 give the change in the percent survival of bacteria with respect to radiation exposure time. Each figure refers to a different dose rate. In each instance, samples stored at room temperature were compared with the ones stored at refrigerator temperature. As exposure time increased, refrigeration became less important (Fig. 5 and 6). This might be because of destruction of nutrients needed by bacteria. When the dose was reduced to a very low value (Fig. 4), then refrigeration became of real importance. Although the dose rate used in Fig. 6 is more than twice that of Fig. 5, the curves are very similar in each instance. This again implies the need for using a proper dose rate in irradiation; excess radiation can cause a loss in the nutritional value of food. It is important to note that in all instances there was not a substantial decrease in survival of bacteria after about 8 min of irradiation of samples, regardless of the dose rate or the total dose. Thus optimum conditions for irradiation appear to be 0.02-0.04 Mrad total dose with 8 min of irradiation time. The dose rate then comes out to be in the range of 0.0025-0.0050 Mrad/min. To attain highest possible quality of product, one can go down to 0.001 Mrad/min, as discussed earlier, but that will require more irradiation time, and it means more cost in operation.

The change of microbial count of kashar cheese with respect to storage time is shown in Fig. 7. The total dose used was two-thirds of the allowable dose, namely

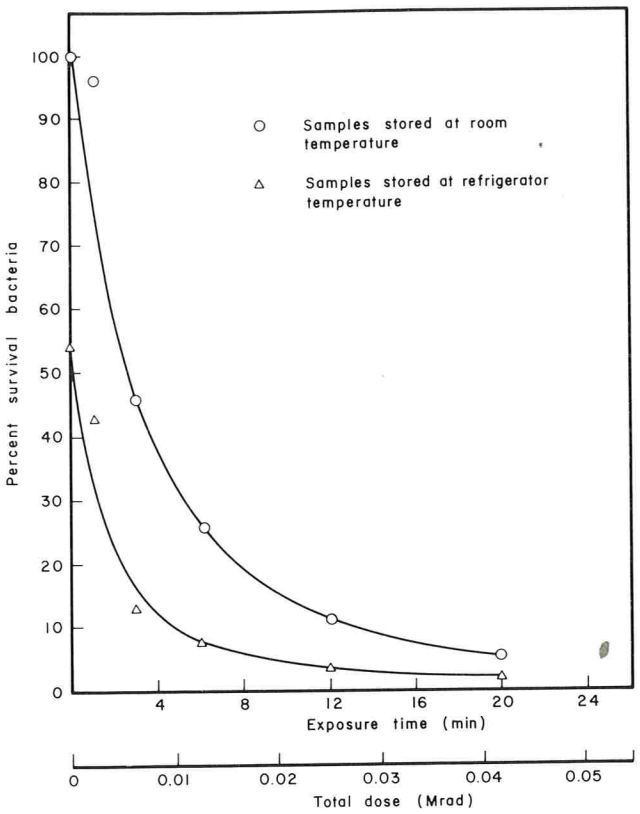

Figure 5. *The effect of total dose at a rate of 0.0021 Mrad/min. dose rate after one week of storage.*

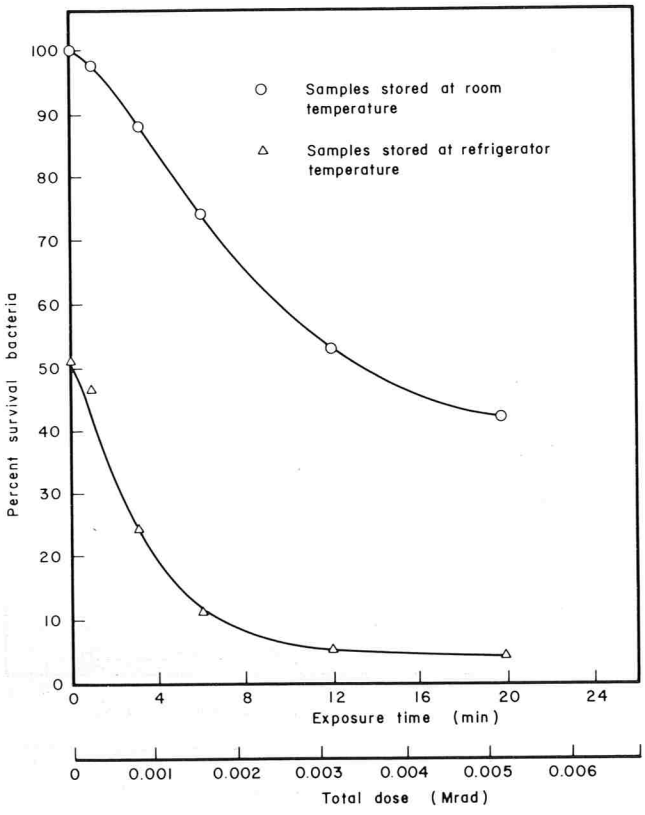

Figure 4. *The effect of total dose at a rate of 0.00026 Mrad/min. dose rate after one week of storage.*

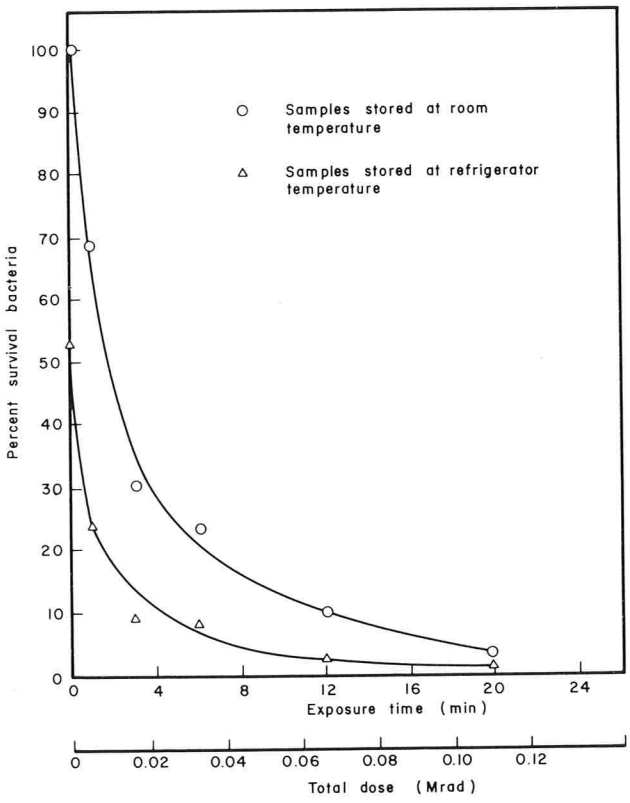

Figure 6. *The effect of total dose at 0.00561 Mrad/min dose rate after one week of storage.*

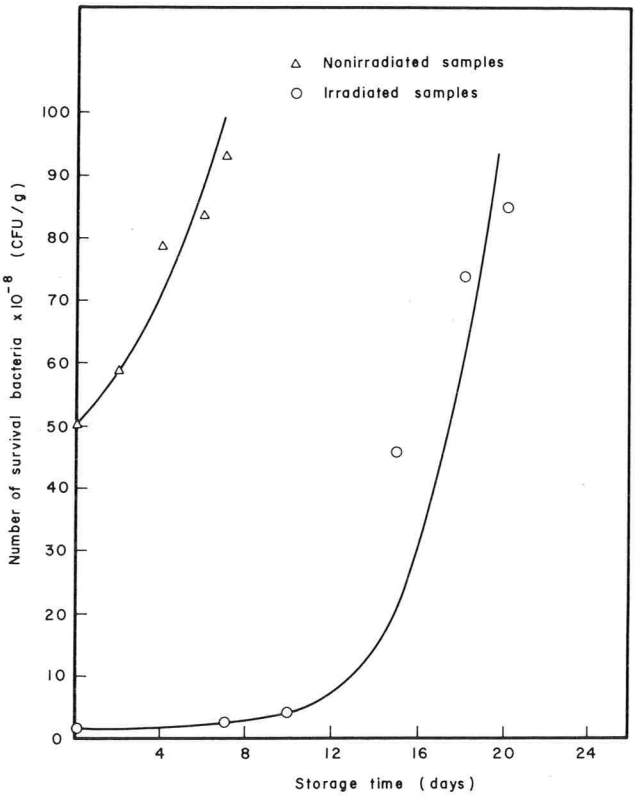

Figure 7. *The change of microbial count of cheese at room temperature with storage time. (Total dose: 0.11 Mrads, dose rate: 0.00561 Mrad/min.).*

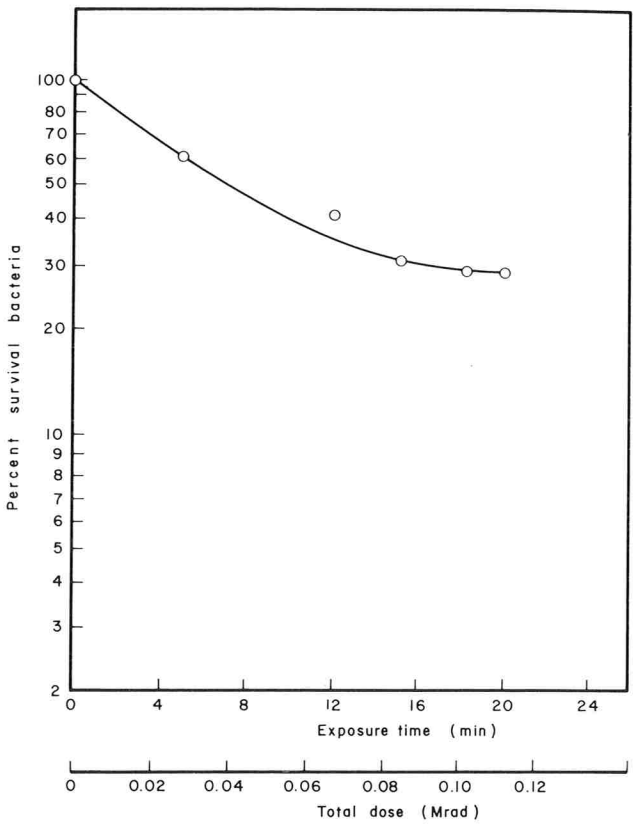

Figure 8. *Effect of irradiation time (i.e. total dose) upon microbial count of yogurt. (Immediate count after irradiation. Dose rate: 0.00561 Mrad/min.).*

0.15 Mrad. The number of bacteria increased very rapidly in nonirradiated samples. In irradiated samples, the rate of increase was very slow until 10 days of storage time. Then a rapid increase started as with nonirradiated samples. It was also observed visually that all nonirradiated samples were moldy after 3-5 days at room temperature, but samples exposed to 0.12 Mrad radiation remained free of mold for 12-15 days.

The effect of radiation on plain yogurt seems to be the same as with cheese. As is seen from Fig. 8, further exposure after 8 min of irradiation became less effective in killing the bacteria. The same result was also observed in cheese. Figure 9 gives the bacterial count with respect to storage time and different preservation conditons. It was observed that nonirradiated plain yogurt stored at room temperature soured in 6 days. The bacterial count by the end of the sixth day was about 5×10^9 CFU/g. The bacterial count of irradiated samples reached this value in 18 days. However, nonirradiated samples stored at refrigerator temperature attained this value in about 15 days. This result is good enough to prefer irradiation over refrigeration. Combined preservation by refrigeration and irradiation extended the shelf-life of yogurt to 29-30 days. It was not possible to show this point in Fig. 9. One can conclude that irradiation extends the shelf-life of plain yogurt 3-fold, whereas a combined process of refrigeration and irradiation extends it 5-fold.

CONCLUSIONS

1. An irradiation dose exceeding 0.15 Mrad results in off flavors and fading of color in kashar cheese and in plain yogurt. Less irradiation caused no difference in taste between irradiated and nonirradiated samples, as determined by a group of people.

2. If the dose rate exceeds 0.001 Mrad/min, there is a surplus fraction of radiation unused in killing the bacteria.

3. A sorbic acid solution reduces the number of bacteria to one-half in nonirradiated cheese samples, but its contribution is about 10-12% in irradiated samples.

4. Refrigeration and sorbic acid have similar effects in preservation of both nonirradiated and irradiated samples.

5. After 0.04 Mrad, neither refrigeration nor sorbic acid made important contributions to preservation by radiation.

6. The optimum conditions of irradiation are as follows: total dose: 0.02-0.04 Mrad, dose rate: 0.0025-0.0050 Mrad/min, irradiation time: 8 min.

7. Irradiation increases the shelf-life of cheese by 4- to 5-fold and of yogurt by 3-fold. Preservation of yogurt by refrigeration and irradiation extends the shelf-life 5-fold.

8. It is better to use radiation as a supplement to other techniques for preservation of milk products since high radiation doses cannot be used.

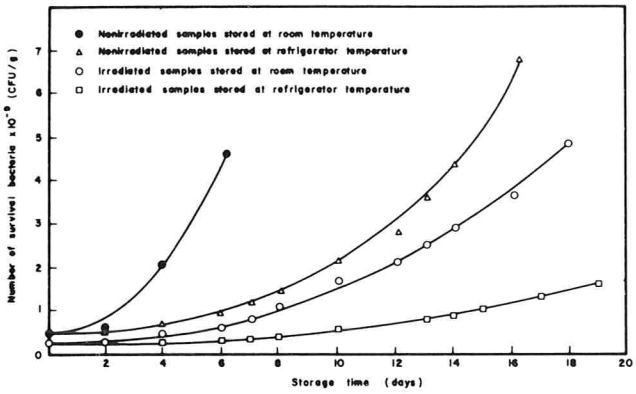

Figure 9. *The change of microbial count of yogurt with storage time. (Dose rate: 0.00561 Mrad/min, total dose: 0.1 Mrad).*

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Upswing Reported In Shigellosis

Several outbreaks of shigellosis have been reported in the Center for Disease Control's *Morbidity and Mortality Weekly Report.*

One outbreak occurred at a children's hospital in Pennsylvania where thirty-two percent of the employees reported being ill. Of 280 employees and visitors complaining of vomiting and/or diarrhea, 51% had positive stool cultures for *Shigella sonnei.* Staffing problems became severe during the outbreak and the hospital was closed to new admissions for a three-day period.

Following analysis of questionnaires distributed to hospital employees to determine symptoms of the disease and places where persons had eaten previous to the outbreak, a strong association was established between the illness and eating in the hospital cafeteria. Based on culture-confirmed cases and controls, significant associations were found between illness and consumption of tuna salad, as well as of foods from the salad bar. A negative association was found between illness and consumption of hot foods at the cafeteria.

One cafeteria employee who worked with all salads and sandwiches in the cafeteria was found to have had diarrhea on the first day of the outbreak. She had been exposed to a child with severe diarrhea the day before onset of her illness. Two peaks in the outbreak were consistent with the one- to two-day incubation period of foodborne shigellosis.

All symptomatic individuals were treated with a five-day course of antimicrobial agents. Cafeteria employees were not allowed to return to work until they had had three negative rectal cultures, taken at one-day intervals at least 48 hours after antibiotic therapy had ended. Other culture-positive employees were allowed to return to work at the hospital 48 hours after completion of therapy.

A second outbreak of shigellosis occurred among persons in the Chinle, Arizona, Service Unit of the Indian Health Service. 158 persons in that unit, which cares for 32,000 inhabitants of a Navaho reservation, were found to have shigellosis. Ages of patients ranged from two months to 102 years. The median age was five years.

Two elderly patients died as a result of dehydration and sepsis. *Shigella flexneri* was responsible for 85% of the cases, with *S. sonnei* accounting for 15%. Shigellosis is normally a self-limiting illness, but antimicrobial agents were used in this case to reduce the severity of symptoms and duration of excretion of the organism.

MMWR editors noted that shigellosis rates on the Navaho reservation are 50-100 times higher than reported in the U.S. population. This is due, generally, to crowded living conditions and a lack of running water (which fewer than 50% of households in the Chinle Unit have). *S. sonnei* is the most frequently indicated organism in shigellosis cases in the total U.S. population, while *S. flexneri* is a more important cause of the illness among the Indians.

The editors also noted that *Shigella* organisms remain a major cause of gastrointestinal illness in the United States. 15,336 cases were reported to CDC in 1978. Transmission is usually from person to person as *Shigella* survive poorly in the environment. In the period from 1961-1978, however, there were 84 reported foodborne outbreaks attributed to *Shigella.* When these occurred, they could almost always be traced to contamination of food by an infected food handler. Food carriers are typically salad or other foods whose preparation requires a great deal of ingredient handling. Foodborne *Shigella* outbreaks tend to be large, with a high attack rate.

Excerpts from "Morbidity and Mortality Weekly Report," Vol. 28, No's. 41, 42, 45, Oct. 19, Oct. 26, and Nov. 16, 1979 issues.

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Measuring Sterilizing Values In Containers of Food Using Thermocouples and Biological Indicator Units [1,2]

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ABSTRACT

Three series of experiments were carried out. Each experiment consisted of six or seven tests where four or five containers were fitted with thermocouples and five were fitted with biological indicator units (BIU). The sterilization value (F_0) delivered to cans of peas in brine was calculated from heat penetration data. The heat penetration data were analyzed for test-to-test reproducibility within each experimental series. Sterilization values for all tests were calculated from the BIU test results. The sterilizing values F_0 (PHY) determined from physical (PHY) heat penetration data were compared with sterilizing values F_0 (BIO) determined using the BIUs, both on the basis of accuracy and variability. The mean F_0 (PHY) - F_0 (BIO) was + 1.2 min. The mean coefficient of variation of the F_0 (PHY) was 0.03 and the F (BIO) was 0.06.

This is the report of a series of experiments carried out to evaluate the performance of thermocouples and plastic rod biological indicator units (BIU) when used to monitor the sterilization process delivered to cans of food heated in a Steritort, both in an agitating and still mode. The Steritort is a process simulator for the FMC Sterilmatic food sterilization machine.

Three series of experiments were carried out at the Green Giant pilot plant facility in Le Sueur, Minnesota. Each experiment consisted of six or seven individual heating tests where four or five containers were fitted with thermocouples and five containers were fitted with biological indicator units.

In addition to the field tests, laboratory tests were carried out to develop a calibration curve for use in the count reduction procedure. These were carried out at the University of Minnesota Environmental Sterilization Laboratory.

The objectives of these studies were to compare the F-value results obtained using thermocouples and biological indicator units when monitoring sterilization processes, and to determine if they are equally effective for agitating and still processes.

MATERIALS AND METHODS

Spores

Bacillus stearothermophilus spores were used. The spores were grown in May, 1975, from American Type Culture Strain 7953, using nutrient agar supplemented with 5 ppm of $MnSO_4$. Incubation was at 55 C for 48 h. The spores were cleaned and suspended in water for injection (USP) and stored at 4 C.

About 2 weeks before filling the rods, the spore suspension was centrifuged and resuspended in 50X standard strength Butterfield's buffer (*1*) in water for injection (USP).

Plastic rod units

The plastic rod biological indicator units (*3*) were prepared in February, 1976. Each rod contained about 0.28 ml of the spore suspension (0.7×10^7 spores). The prepared plastic rod BIUs were stored at 4 C until time of use.

Calibration experiments

The BIUs were calibrated at 121.1 C, using a miniature retort. In each calibration test three randomly-selected rods were heated for 3.8, 5.8, 7.8, 9.8, 11.8 and 13.8 equivalent minutes at 121.1 C. After heating, the rods were cooled in an ice water bath and held in ice water until recovery procedures were started. Three unheated units were analyzed to determine the initial number of spores per unit. The number of surviving spores per BIU was determined using plate count procedures. The recovery medium was soybean casein digest agar with incubation at 55 C for 48 h.

Field test procedures

The BIUs were transported to the plant in ice water in insulated containers. The BIUs were held in the ice water until they were placed in the cans.

To install a BIU or a thermocouple in a can, a hole was punched in the end of the 303 × 406 can. An Ecklund receptacle was then installed in the hole in the end of the container. Needle-type Ecklund thermocouples were installed in the thermocouple cans. Immediately before filling the cans, the plastic rod biological indicator units were screwed into place as shown in Fig. 1.

In experimental series 1 and 2, where cans were agitated during heating, two 15/16-inch-long BIUs were inserted along the center line of the container with the calibrated spores located near the geometric center, the slowest heating zone in the container. In series 3, the cans were not agitated during heating. Therefore, the spores were located near the bottom of the container, the slowest heating zone for a convection heating product. To accomplish this, 4-inch plastic rod units were used and the cans were heated with the receptacle up.

An FMC Steritort was used in all experiments; the heating medium temperature was 254 F. The reel speed was 7.2 rpm in all agitating tests. This reel speed is representative of the reel speed in FMC Sterilmatic processing machines for peas processed in commercial canning plants.

The cans were filled with 11.5 ounces of peas, brine was added until there was a 0.25-inch headspace, and the cans were sealed and then immediately heated.

Series 1. There were six experiments. In A, B, and E, the heating time was 9 min; in C, D and F, the heating time was 11 min (heating time is measured from steam on to steam off).

Series 2. There were seven experiments. In A, E and G, the heating time was 10 min. In C, D and F, the heating time was 12 min, and in B

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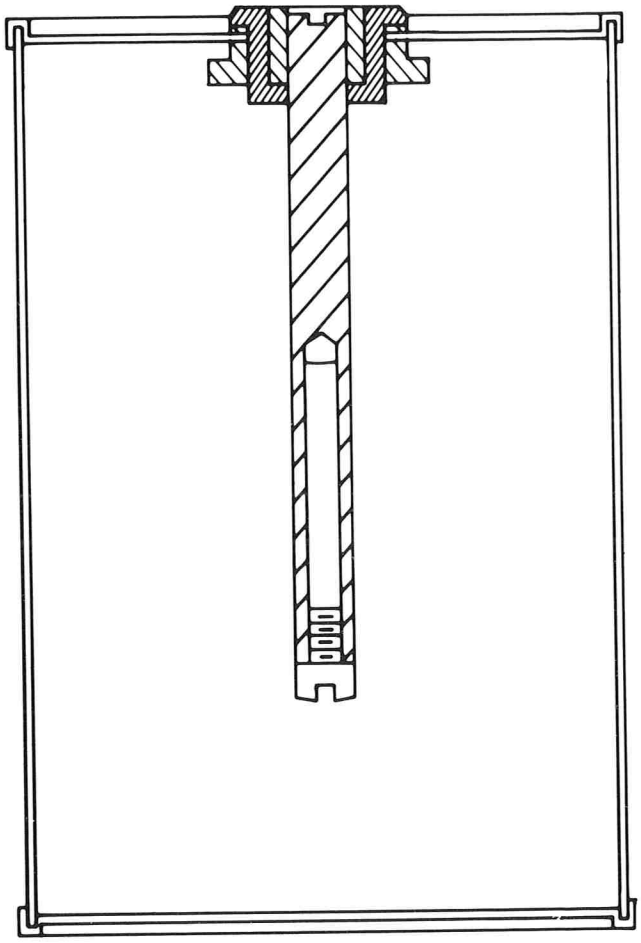

Figure 1. *Cross-section of a can containing a BIU located at the slowest heating zone.*

the heating time was 13.5 min.

Series 3. There were six experiments. The cans of peas were placed on a perforated metal shelf within the Steritort and processed under still conditions. In A, B and E, the heating time was 14 min, and in C, D and F, the heating time was 15 min.

A unique aspect of the experimental program was that within each series the location of can 2 in the Steritort, for example, the thermocouple in can 2 and the connecting harness to can 2, were the same in all six individual experiments. Thermocouple and harness placement were consistent within each series but varied between series.

Since the same thermocouple and measuring system was used, for example, for container 2 in 1A, 1B, 1C, 1D, 1E and 1F, the results can be subjected to an analysis of variance and other statistical tests to determine if the variation among containers, thermocouples and harnesses is random or whether there is bias, suggesting that particular thermocouples yield F_0-values that are greater or less than the average.

Spore recovery procedures

After the heating and cooling process was completed, the cans containing the BIUs were recovered, opened and the BIUs removed. Using a vortex mixer, the BIUs were agitated for 15 sec, opened and the spore suspension removed using a 1.00-ml glass tuberculin syringe. Duplicate 0.1-ml portions of the spore suspension were plated (using the glass syringe) in 100-mm diameter plastic petri plates. The remainder of the spore suspension was deposited as a drop in a sterile empty petri plate. Using an Eppendorf pipettor, duplicate .005-ml portions of the drop were placed in 100-mm diameter plastic petri plates. About 30 ml of soybean casein digest agar were added to each plate. The plates were incubated at 55 C for 48 h and the colonies counted.

Treatment of data

The thermocouple data were recorded on a strip chart by a temperature recording potentiometer. The data were taken off the strip chart, tabulated and then placed on a computer file. The data were analyzed using a computer program that calculated the temperature response parameter f, the lag factor j, and the length of the f-line, determined the correlation coefficient (r^2) of the fit of the f-line to the data and calculated the F_0 (PHY)-value by the General Method. F_0-values were also determined for all container heat penetration tests (CHPT) by two mathematical methods: (*a*) Ball program (5) and (*b*) when sufficient data were available, a program identified as HPSP that was developed in this laboratory. The F_0-values calculated by the Ball Method and by the HPSP program was compared with the F_0-values calculated by the General Method and reported as F_0-value ratios.

To prepare the calibration curve, the mean number of surviving spores per BIU as a function of the equivalent heating time at 121.1 C for each of the two calibration tests was entered into a time share computer program. The best fit second order polynominal was determined and the coefficients used to calculate the number of survivors for the range of sterilizing values over which the BIU was effective. The resulting calibration curve is shown in Figure 2; the survivor data, in the form of the mean and 95% confidence intervals for the two calibration tests, are also shown in Fig. 2.

The F(BIO)-value was calculated from the plate count data. The number of colonies per plate was multiplied by the appropriate dilution factor to obtain the number of surviving spores per BIU. Since duplicate portions were plated for each unit, there were two estimates of the number of surviving spores per BIU. F(BIO)-values were determined on the basis of the count per BIU from the calibration chart shown in Fig. 2. The F-value was obtained by averaging these two values.

The F(BIO)-value was corrected for the difference in the z-value of the spores, approximately 14 F, and the z-value of 18 F to yield an

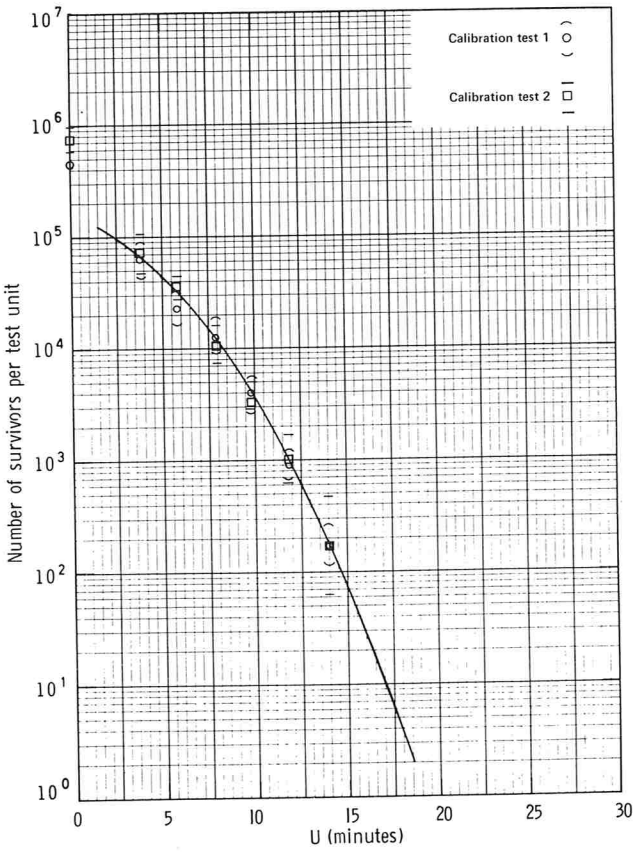

Figure 2. *Calibration curve at 121.1 C for the BIUs used in the three series of experiments; mean data values and their 95% confidence interval are shown.*

F_0(BIO)-value by the method described by Pflug (*4*). The mean F_0(BIO)-value for each experiment was obtained by averaging the F_0(BIO)-value for the five rods in each test.

RESULTS

The heat penetration test results for the three series of experiments are summarized in Table 1. Inspection of the data in Table 1 suggests that the three series of experiments were not identical. In the two series (1 and 2) where the cans were agitated, some environmental factors, probably related to the product, were present since the f_h-value for series 1 was 3.2 min with a coefficient of variation of about 0.03 in contrast to an f_h-value of 3.6 min for series 2 with a coefficient of variation of 0.04. In the non-agitated series 3, the mean f_h-value is 3.7 min and the coefficient of variation of 0.04 is almost identical with the result of the second series where the cans were agitated during heating.

The F_0-value ratios in Table 1 suggest that when the temperature response parameter and lag factor that are determined in the heat penetration data analysis are used to calculate F_0-values, the F_0-values calculated using the Ball program are about 91% of the General Method F_0-values. The HPSP program appears to give F_0-values that compare more closely (98%) to the General Method F_0-values.

In Tables 2, 3 and 4 are shown the General Method F_0-values as a function of container position. An analysis of variance test, Friedman's test (*2*), and an analysis of variance after a log transformation of the data for series 2 and for series 3 all fail to show any significant difference among container positions, thermocouple or thermocouple harness at the 90% level. This is interpreted as meaning that in these experiments there was no bias among position.

TABLE 2. *General method F_0-values calculated for experiment Series 1.*

Thermocouple No., harness and can position	Experiment Number (Heating time) 1A[a] (9 min)	1B (9 min)	1C (11 min)	1D (11 min)	1E (9 min)	1F (11 min)
2	6.2	6.2	9.5	9.8	6.2	10.0
3	6.2	5.8	9.9	9.3	5.9	10.1
4	6.3	6.0	9.2	9.2	6.0	9.8
5	6.1	6.2	9.3	9.5	6.1	10.5
6	6.6	6.2	9.5	9.6	6.9	10.9

[a]Experiment Code and heating time at 255 F.

In series 1, Friedman's test of the data showed differences among positions that are significant at the 90% level but not at the 95% level. The analysis of variance tests of the data (log transformed and not transformed) showed a significant difference at the 95% level but not at the 99% level. Using the Newman-Kuels multiple comparison method, we found that only thermocouples 4 and 6 were significantly different. The results suggest that equipment performance is critical when making heat penetration tests in the Steritort and that a way should be found to validate the temperature measuring systems. In this type of equipment, where units are put on and taken off for each test and because of the agitation conditions, the thermocouple wiring system is subject to continued and deteriorating stress. The researcher must be alert to changes in the condition of either or both the thermocouple harness or the rotating thermocouple connections that can cause a

TABLE 1. *Summary of f, j and F_0-Values for Experiments 1, 2, and 3.*

Experiment number	$\bar{f}_h$ (min)	$\bar{j}_c$	$\bar{f}_c$ (min)	$\bar{j}_c$	F_0-Values (min) Ball	HPSP	GM	F-Value ratios Ball/GM	HPSP/GM
1A	3.1	0.91	5.2	1.13	5.8	6.1	6.3	.92	.97
1B	3.3	0.89	5.4	1.12	5.7	6.2	6.1	.93	1.02
1C	3.3	0.67			8.8		9.5	.94	
1D	3.3	0.84			8.5		9.5	.89	
1E	3.3	0.85	6.3	1.12	5.5	6.1	6.2	.89	.98
1F	3.2	0.90			9.0		10.2	.90	
$\bar{x}$	3.2	0.84						.91	.99
Std. Dev.	0.083	0.089							
Coef. of Var.	0.026	0.106							
2A	3.7	0.91	6.2	1.11	6.3	6.9	6.8	.93	1.01
2B	3.4	0.92			12.9		13.9	.93	
2C	3.6	0.88			10.2		10.8	.95	
2D	3.7	0.88			9.5		10.0	.96	
2E	3.4	0.89	5.6	1.17	6.9	7.4	7.6	.91	.97
2F	3.4	0.87			10.1		10.8	.94	
2G	3.7	1.00	8.1	1.07	5.3	5.9	6.1	.90	1.00
$\bar{x}$	3.6	0.91						.93	.99
Std. Dev.	0.151	0.045							
Coef. of Var.	0.042	0.049							
3A	3.6	0.78			10.2		10.7	.94	
3B	4.0	0.80	3.6	1.36	9.2	9.0	9.5	.97	.95
3C	3.6	0.85			11.5		12.2	.94	
3D	3.7	0.85			11.0		12.1	.93	
3E	3.7	0.76	3.7	1.32	9.9	9.7	10.5	.94	.92
3F	3.8	0.74			11.1		11.9	.94	
$\bar{x}$	3.7	0.80						.95	.94
Std. Dev.	0.151	0.046							
Coef. of Var.	0.040	0.058							

systematic error in the results.

In evaluating the biological indicator results, we will first compare the mean F_0-value results from a group of replicate physical and biological tests, and secondly, we will compare variation within each group of physically and biologically determined results. The biological indicator results for the three series of experiments are summarized in Table 5. The overall performance of the BIUs, as far as measuring sterilizing values, appears to be good as indicated by the difference between the mean F_0 (PHY) and the F_0(BIO)-values of a group of replicate tests. The overall average difference F_0 (PHY) - F_0 (BIO) of the individual tests in series 1 was + 0.9 min, in series 2, + 0.2 min, and in series 3, + 2.4 min. In all three series, the F_0 (PHY)-values are higher than the F_0(PHY)-value more than 10% greater than the F_0(BIO)-values; however, only in series 3 is the mean F_0(BIO)-value. It is possible that some of the differences in the overall performance of the biological measuring system in the three experimental series is due to differences in the recovery media since there was a high degree of consistency within each experimental series. In these experiments, the TSA recovery medium was supplied by the Green Giant Company. It was not from the same lot of medium that was used in tests to develop the calibration curves. Recently, differences have been observed among lots of media. This has led to a program where medium from the same lot is used in both calibration and field tests.

A temperature calibration error of 0.5 C will produce a change in the F_0-value of about 12%. Where we are comparing F_0(PHY)- and F_0(BIO)-values there are two

TABLE 4. *General method F_0-values calculated for experiment Series 3.*

Thermocouple No., harness and can position	Experiment number (Heating time) 3A[a] (14 min)	3B (14 min)	3C (15 min)	3D (15 min)	3E (14 min)	3F (15 min)
1	10.9	9.4	12.5	12.3	9.7	12.1
2	10.6	9.3	11.6	12.0	10.8	11.4
3	11.0	9.7	12.2	12.4	10.9	12.0
4	10.9	9.4	12.4	11.5	10.6	11.6
5	10.4	9.5	12.4	12.2	10.6	12.1

[a]Experiment Code and heating time at 255 F.

TABLE 3. *General method F_0-values calculated for experiment Series 2.*

Thermocouple No., harness and can position	Experiment number (Heating time) 2A (10 min)[a]	2B (13.5 min)	2C (12 min)	2D (12 min)	2E (10 min)	2F (12 min)	2G (10 min)
2	6.3	14.8	11.0	9.7	7.4	10.6	6.0
4	6.7	13.4	11.1	9.8	7.5	10.4	6.1
5	6.7	14.2	10.7	10.3	7.8	11.0	6.1
6	7.4	13.1	10.6	9.9	7.7	11.3	6.1

[a]Experiment Code and heating time at 255 F.

TABLE 5. *Summary of The F_0 (PHY) and F_0 (BIO) results of the three series of experiments.*

Experiment number	Average sterilization value calculated from thermocouple data: F_0 (PHY) General method (min)	Std. dev. (min)	Coef. of var.	Average sterilization value determined by Biological Indicator Units: F_0 (BIO)[a] (min)	Std. dev. (min)	Coef. of var.	F_0 (PHY) - F_0 (BIO) (min)
1A	6.3	.19	.031	5.5	.53	.093	0.8
1B	6.1	.18	.029	4.8	.36	.074	1.3
1C	9.5	.30	.031	8.2	.56	.064	1.3
1D	9.5	.23	.024	8.2	.88	.099	1.3
1E	6.2	.40	.064	6.8	2.43	(.334)[b]	-0.6
1F	10.2	.44	.043	9.1	.48	.048	1.1
			$\bar{x}$ = .0370			$\bar{x}$ = .076	$\bar{x}$ = + 0.9
2A	6.8	.21	.032	6.5	.64	.094	0.3
2B	13.9	.77	.056	13.1	.23	.016	0.8
2C	10.8	.24	.022	10.3	.47	.041	0.5
2D	10.0	.26	.026	9.5	.67	.066	0.5
2E	7.6	.18	.024	6.8	.75	.102	0.8
2F	10.8	.40	.037	11.0	.87	.072	-0.2
2G	6.1	.05	.008	6.8	.59	.080	-0.7
			$\bar{x}$ = .0293			$\bar{x}$ = .067	$\bar{x}$ = + 0.3
3A	10.7	.25	.023	8.9	.50	.052	1.8
3B	9.5	.15	.016	7.4	.36	.045	2.1
3C	12.2	.36	.030	9.5	.26	.026	2.7
3D	12.1	.36	.030	9.9	.38	.034	2.2
3E	10.5	.48	.045	7.6	.45	.055	2.9
3F	11.9	.32	.027	8.9	.53	.055	3.0
			$\bar{x}$ = .0285			$\bar{x}$ = .045	$\bar{x}$ = + 2.4
			$\bar{x}$ (19 tests) = .032			$\bar{x}$ (18 tests) = .062	

[a]Average of five BIU's.
[b]This value was eliminated in the calculation of the mean.

potential sources of error: (*a*) in calibrating the BIUs, and (*b*) in the thermocouple potentiometer system used to gather heat penetration data. Some of the differences among the three series of experiments may have been due to changes in the potentiometer calibration during this approximately 1-month period and changes in the thermocouple harness and thermocouple fittings due to normal heavy usage. It is possible that in the series 3 results, some of the differences were due to errors that might occur in the commutator system that is normally rotating but in this case was not rotating.

The accuracy of the results, as measured by the coefficient of variation, suggests that the F_0-values from time-temperature data vary less than the F_0-values measured by biological indicator units. We are limited in the conclusions we can make because of the complexity of the overall measurement problem. In this measurement situation, we have can-to-can variation that will cause the rate of heating and cooling to vary, and consequently the F_0-value received by the peas in the can will also vary among cans. Also, the thermocouples and BIUs are not in the same cans. The performance of both the thermocouple system and the biological indicator system will vary on a unit-to-unit basis. Any variation in the spore recovery manipulations will be added variation in the BIU system. In considering variation, we are using the thermocouple data as the reference base and are assuming that the difference in variation between the thermocouple-determined data and biologically-determined data are all due to aspects of the biological system. This assumption will produce an inaccuracy since it is almost certain that in both systems there is some error.

The coefficient of variation of the F_0(PHY)-values of the 19 tests ranged from 0.008 to 0.064. The mean coefficient of variation for each series is: 1, 0.037; 2, 0.029; and 3, 0.028. The coefficient of variation is smaller for series 3 (not agitated) than for series 1 or 2 where there was container agitation. The magnitudes of the mean coefficient of variation for both the F_0(PHY) and F_0(BIO) results are interesting in that the coefficients of variation are in consistent order for PHY and BIO measurements in that series 1 had the largest F_0(PHY) and F_0(BIO), and series 3 the smallest coefficient of variation values. Within experiments there does not appear to be any consistency of the coefficient of variation of F_0 (PHY) and F_0(BIO). The coefficients of variation of the F_0(BIO)-values are, in general, larger and vary more widely than for the F_0(PHY) results. The results of test 1E, in terms of its coefficient of variation, appear to be different from all other tests. Inspection of the data sheets suggested that there may have been an error in labeling the petri plates. The data for this experiment are included in Table 5, but they were not included in calculating the average coefficient of variation for the experimental series. The coefficient of variation of the remaining 18 tests ranged from 0.016 to 0.102. The mean overall value, again excluding 1E, was 0.062 min. The results of this study indicate that for peas heated in brine, the mean coefficient of variation of the F_0(PHY) is about 0.03 and for F_0(BIO) is about 0.06. On this basis, if the containers are subjected to identical heat processes and if the average F_0-value is 10 min, 67% of the F_0(PHY) should be between 9.7 and 10.3 min and 67% of the F_0(BIO) should be between 9.4 to 10.6 min.

The results of these experiments indicate that the plastic rod biological indicator units used with the count reduction procedure can be used effectively to determine the sterilizing value delivered to cans of food processed in agitating retorts. Today, we know of no other self-contained monitoring systems that generate data that are as close in agreement with F_0(PHY) as the count reduction biological monitoring system used in the tests described in this report.

In comparing F_0-values calculated from time-temperature data with F_0-values determined by biological indicator units, a greater degree of accuracy is to be expected in the F_0-values calculated from time-temperature data measured by thermocouples than from biological indicator data. The reason for expecting better accuracy from the physical system is that we are measuring temperature and time directly in a laboratory situation, whereas the biological indicator F_0-values are determined in an indirect fashion that includes: (*a*) all of the errors that might be present in the thermocouple temperature-measuring system used to calibrate the biological indicators, and (*b*) all of the additional variation due to the biological measuring system and its sensitivity to a great many uncontrolled environmental factors.

An important attribute of the biological indicator unit system is that it makes possible large numbers of F-value measurements in the same piece of equipment in the same general time period. For example, five, 10 or even 20 containers can be fitted with BIUs and allowed to proceed sequentially through filler, closing machine and retort to monitor the process delivered to a product in an agitating processing machine. The biological indicator units can therefore be used to monitor variations with time in the delivery of the sterilizing value and also may be used to determine systematic variation in the delivery of a sterilizing process as far as location in the processing equipment is concerned. Using replicate BIUs and determining the average F-values from three, five, or more units results in greater accuracy in the estimation of the F-value. The data in Table 5 suggest that if a single, properly-calibrated BIU (that is, without systematic bias) is used, then 95% of the time the calculated F-value will be within 15% of the true, delivered F-value. If three units are used, then 95% of the time the resulting average F-value will be within 9% and if five units are used, 7% of the true value.

SYMBOLS

f, f_h

The temperature response parameter (f) is the time required for the straight line fitted to the log-linear portion of a heating or cooling curve

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Uptake and Elimination of Bacteria in Shellfish*

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ABSTRACT

A general review of knowledge concerning bacterial accumulation and depletion by commercially significant bivalve molluscs is presented. Naturally contaminated shellfish can eliminate fecal coliforms (FC) in 48 h to levels below most market standards over a wide range of environmental conditions when sea water flowing to the molluscs is treated so that fecal coliform levels are indeterminate or marginally determinate as assayed by standard methodology. Most probable number (MPN) enumerations of shellfish depurated for 48 h by the authors yielded a median value of < 18 FC/100 g of oyster (*Crassostrea virginica*) meats with < 10% of the samples exceeding 78 FC/100 g.

The mechanisms of bacterial accumulation and elimination by shellfish are not well-known; however, certain general observations have been made concerning the net effects of such processes at the organismic level. Bacteria in suspension in the ambient water are trapped in mucus on the gill, mantle and labial palp surfaces, then transported by cilia to the labial palps where sorting occurs into rejected components (pseudofeces) and components for ingestion. We have found that naturally contaminated oysters, 4 h after being placed in a depuration plant, show a ratio of 27:1 fecal and 37:1 total coliforms in feces as compared to pseudofeces (*6*). Presumably some of the bacteria are digested or killed while traversing the gut, thus an even higher proportion of coliform bacteria are probably shunted through the gut.

ACCUMULATION

A steady state is known to be rapidly attained for uptake of coliforms, beyond which accumulation in the gut does not occur for a given concentration of the bacteria in ambient water. Maximum levels can be attained in the first 6 h by some individuals, but increased exposure time increases the percent of the population which reaches a steady state. The steady state levels increase as the concentration of bacteria in ambient water is raised, but decrease as the nonbacterial, particulate levels in the ambient water are raised. Presumably the small gut volume can easily be filled with non-cellular particulates such as clay from the water and concentration of the bacteria is prevented (*1*).

Escherichia coli accumulation factors for quahaug clams under optimal conditions were observed by Cabelli and Heffernan (*1*) to be 6.5 to 8.5 while the accumulation factor for soft shell clams was 20 (*2*). Kelley et al. (*12*) found a factor as large as 27.4 for *E. coli* in oysters. Observing oysters under natural estuarine conditions above 15 C, we found values of 3 to 6 for fecal coliforms and 7 to 16 for total coliforms (*6*). However, such data represent only approximations because bacterial levels may fluctuate during the tidal cycle and there is a lag before these variations are reflected in the shellfish.

When temperatures are lowered below the range of optimum physiological activity there is a decline in pumping and filtration activity and thereby an inhibition of accumulation of coliform bacteria but apparently not an inhibition of elimination or inactivation. Thus there is a net loss of coliform bacteria from shellfish (*3,6*). Although adequate data are lacking, temperatures which result in a net loss probably vary depending on species of shellfish and physiological races. Oysters in the southern Chesapeake Bay show a cessation of pumping and biodeposition at about 3 C (*6*); however, below 15 C coliform bacterial accumulation is not pronounced or is absent. We found an accumulation factor of 0.77 for oysters in the range of 1.5 to 15.0 C. Clams in Rhode Island waters accumulate above about 10 C and show a net loss below 10 C, given equal levels of fecal coliforms in the water.

Within shellfish, coliform bacteria are found predominantly in the visceral mass, presumably the lumens of the gut and hepatopancreas. The siphons of clams also show a high level of coliforms (*1*). Other tissues which can be separated and analyzed show levels of coliforms equal to or only slightly higher than those in the ambient water. In preliminary studies, we have found the hemolymph to contain low levels of heterotrophic bacteria (< 100 colony forming units/ml), but it is not known whether any fecal coliforms were present. A few would be expected since phagocytosis by leucocytes is known to occur followed by diapedesis. Transport by the phagocytes may occur into the oyster through the epithelia, thus a few coliforms could be found in transit in the hemolymph.

ELIMINATION [DEPURATION]

As already noted, elimination of coliform bacteria occurs predominantly through the feces rather than the

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pseudofeces, with the role of digestion or deactivation being unknown. As a prerequisite for decline in coliform number we have found that oysters must pump water through the mantle cavity (*6*). If oysters' shells are held shut, with the oyster in or out of the water, fecal coliform levels will decline more slowly in the first 72 h than in oysters which are open and pumping. However, if pumping occurs and the rates are in the range of 1.4 to 10.5 l/h, then there is a fairly uniform and optimum rate of elimination. Elimination below a mean pumping rate of 1.4 l/h was not measured. One might suspect from these observations that deactivation and digestion of coliform bacteria in oysters plays a small role in the first 72 h. In other words, if the oysters pump, they will produce biodeposits and elimination occurs. However, in looking at rates of biodeposition, it was found that biodeposition is not required as a prerequisite for depuration to occur. Oysters showing from zero to the highest levels of biodeposition depurated at essentially the same rates. Therefore, healthy pumping oysters may have the capacity to inactivate or digest significant numbers of coliforms without obvious defecation occurring.

As might be expected, temperature is important in determining elimination rates with responses being a function of species of shellfish as well as physiological race. We have already noted that below 10 C New England hard clams become physiologically inactive with accumulation being more strongly inhibited than elimination, resulting in a steady state level below that of the surrounding water. Between 10 and 20 C the rates of elimination are essentially the same. Soft clams depurate at about the same rates between 8 and 16 C, but at 2 C the rate is markedly less resulting in ca. 1/2 log unit less reduction after 48 h (*2*). We did not determine the lowest temperature at which Chesapeake Bay oysters would depurate but it is known to lie below 11 C (*6*). As observed by Presnell et al. (*15*), Gulf oysters will depurate fecal coliforms to the same levels in 48 h over the range of 16.3 to 28.7 C. They did not test for activity below 16.3 C. We found that Chesapeake Bay oysters will depurate equally as well between about 14 and 29 C.

Effects of the environmental factors of oxygen, salinity, turbidity, flow rates and food have been examined for only a few species of shellfish. Available data are most complete for *Crassostrea virginica* and *Mercenaria mercenaria*. As one might expect, the information indicates that the wide range of conditions which yield growth and survival of a particular race or species of shellfish also result in high depuration rates. Appreciable drops in depuration rates occur below 1.8 mg of dissolved O_2/l in oysters (*6*). Turbidity does not affect depuration rates at turbidity levels as high as 77 mg/l in Chesapeake Bay oysters (*6*), 69.4 mg/l in Gulf of Mexico oysters (*15*) and 25 mg/l in New England clams (*3*). Salinity below 16°/oo slows some Gulf of Mexico oysters and below 7°/oo the rates are highly reduced (*15*). In Chesapeake Bay oysters the rates of depuration were unaffected between 14 and 21.4°/oo (*6*).

Rates of sea water flowing through depuration tanks were found to be unimportant above 1 l/oyster/h (*6*) or 0.5 l/oyster/h (*15*) as long as sediments in the tanks were not stirred into suspension resulting in recontamination of the shellfish. Food concentrations as measured by total chlorophyll levels in the water did not affect Chesapeake Bay oysters over the range of 2.7 to 23.6 mg/l (*6*).

Diseases caused by *Perkinsus marinus* (= *Dermocystidium marinum*) and *Minchinia nelsoni* had no measurable influence on depuration rates at the pathogen levels measured (*6*). In addition, depuration rates did not appear to be influenced by oyster size in the range of 2 to 5 inches.

It has been observed that depuration rates under semi-controlled tank conditions are faster than those observed in the estuaries (*4,13*) even when the estuarine waters contain very low or undetectable levels of coliforms. Reasons for these differences are not known.

Initial levels of fecal coliforms in naturally contaminated oysters can be as high as 39,000 MPN/100 g (mean value for the population being depurated) and still be reduced to values below 50 in 48 h (*6*). The upper limits for reduction to less than 50 FC/100 g in 48 h were not determined by us (Table 1). Higher levels of fecal coliforms represented as *E. coli*, derived from cultures and applied to the oysters under laboratory conditions, are not eliminated below 50 FC/100 g in 48 h (*6,15*); however, such relationships are not likely to be relevant to the naturally occurring situations found in estuaries.

The data concerning rates of depuration of human pathogenic bacteria are conflicting. Metcalf et al. (*13*) found that "bacterial indicators of pollution might be suitable for determining the effectiveness of depuration in removal of pathogens" because they observed that salmonellae were eliminated faster than fecal coliforms when oysters were held in the estuary. Elimination of enteric viruses was less predictable. Janssen (*11*), however, found long residence times in oysters artificially contaminated with high levels of *Shigella flexneri, Francisella tularensis* and *Salmonella typhimurium*. He suggested that fecal coliforms may not be good indicators of depuration of some human pathogens. His studies

TABLE 1. *Summary of fecal coliform levels (MPN/100 g) observed in Chesapeake Bay oysters used in depuration studies.*

Shallow trays (25-36 oysters/tray)		
Elapsed time	0 h	48 h
Median	490	< 18
10% Exceed:	3,300	20
Range	13,300 → 20	1,400 → < 18
Sample size	98	113
Large tanks (ca. 1,400 oysters/tank)		
Elapsed time	0 h	48 h
Median	2,200	< 18
10% Exceed:	13,000	78
Range	130,000 → 78	2,300 → < 18
Sample size	79	117

were conducted using oysters held in closed systems where the water was recirculated past ultraviolet light. Whether the depuration rates were lowered by stressing the oysters in a closed system must be considered. The study is one of basic importance and should be repeated using oysters held in a depuration plant where fecal coliforms have been demonstrated to be eliminated to acceptable levels in acceptable time periods such as < 50 FC/100 g in 48 h.

Chesapeake Bay oysters can be induced to depurate in 48 h to levels which are acceptable in comparison to at least two widely used standards (Table 1). For example, the Microbiological Task Force recommendations at the 7th National Shellfish Sanitation Workshop (1971) were that depurated soft shell clams should not exceed "a fecal coliform MPN of 50/100 g of sample and not more than 10% of the samples should exceed a fecal coliform MPN of 130/100 g of sample." In addition, "fresh or fresh frozen shellfish are generally considered to be satisfactory at the wholesale market if the fecal coliform MPN does not exceed 230/100 g" as a median value (*9*). As can be seen from data in Table 1, both standards are met whether large or small groups of oysters are examined under environmental conditions which are not overly stressful (see above). Shellfish studied by other workers (*2,4,7,8,10,14,15,16*) are found to depurate to levels which are essentially the same although some obtained values, after even 72 h, which were higher than 130/100 g for 10% of the samples (*8,10*).

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Microbiological Standards for Shellfish Growing Areas — What Do They Mean?

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ABSTRACT

Chemical pollutants and shellfish toxins are significant public health concerns in shellfish waters. However, the major concern, in classification of shellfish waters, is the presence of viable fecal material. Indicators of pollution are discussed and the coliform growing area standard and its proper utilization are described.

In reviewing recent literature concerned with isolation and identification of viruses from molluscan shellfish (oysters, clams and mussels), sediments and shellfish growing area waters, the research community in general appears to lack understanding of the shellfish growing area standards and criteria and how they are applied in the classification of shellfish growing area waters.

The purposes of this paper are (*a*) to briefly discuss bacterial indicator groups and the standards which utilize the two most significant members of the indicator groups, and (*b*) to explain the proper use of these standards as they apply to edible species of oysters, clams and mussels.

GROWING AREA CLASSIFICATION

Shellfish growing area classification studies may be divided into two parts, the sanitary survey and the bacteriological survey. The sanitary survey can be compared to the low power objective on a microscope; it provides an overall view of pollution sources, types and volumes of sewage. The bacteriological survey, on the other hand, is analogous to the high power objective. It defines the level of viable sewage organisms in terms of indicator equivalents at any given point in the estuary from the sewage outfall to that point where the indicator can no longer be detected because of dilution, dispersion or the biological and physical dynamics of the estuarine system under study. The indicator groups do not measure total sewage organisms. They measure only those organisms that meet the indicator criteria and have survived wastewater treatment processes and natural die-off. Pathogenic bacteria and viruses, the organisms of greatest public health concern, are among those organisms included in viable sewage that are not measured by the indicator. According to public health tradition, presence of viable sewage as determined by the indicator group is presumptive evidence of the presence of pathogens.

A limited number of shellfish areas in the U.S. have been closed to harvesters because of a variety of chemical contaminants such as mercury, kepone and petroleum products. Although areas in New England, Florida and the Pacific Coast may be intermittently or permanently closed because of shellfish toxins (*1*), the predominant cause of shellfish area closures is the continued use of the estuary as a repository for domestic sewage. Shellfish control agencies do not disregard the possible long-range health effects related to consumption of toxic chemicals in shellfish or the immediate health effects caused by shellfish poisons. However, the major health hazard potential related to shellfish consumption is the continuous or intermittent disposal of domestic wastes into the estuary via the sewer outfalls, the cloaca of our society's villages, towns and cities.

The presence of sewage in the estuary establishes a ground of contention between two opposing forces, the control agency responsible for food protection on one side, and the shellfish industry on the other (*2*). Both of these forces have legitimate positions. The shellfish industry seeks to utilize the maximum amount of the available resource without causing illness to its customers, whereas the control agency must assure that the shellfish beds are not exposed to hazardous or potentially hazardous levels of viable feces and other contaminating materials that could harm consumers.

THE CONTROL AGENCY

To fulfill its responsibilities, the shellfish control agency must have adequate means of detecting and measuring levels of sewage organisms in growing area waters. Although the agency realizes that a zero-tolerance goal is unachievable, it must also determine what level can be tolerated and still maintain both factors of product integrity--safety and quality.

Thus the control agencies conclude that the principal hazard to shellfish consumers is the presence of viable sewage in the growing area and that a pollution-free shellfish environment is inconsistent with the "multiple use" concept of estuaries. The agencies then face the challenge of determining the most effective means of detecting and measuring the hazard, and establishing shellfish growing area standards that will guarantee the public health integrity of shellfish harvested for the

consumer market. Because of the complexities of fresh shellfish marketing and processing practices, and the inability of control agencies to determine the degree of processing the product will receive before it reaches the consumer, the control agency must proceed as though the product will be consumed raw. The growing area standard, or level of sewage permitted in the "approved" growing area, must take this into account. Simply put, how much feces will the control agency and the public tolerate in waters that produce filter-feeding shellfish potentially destined for the raw market?

SANITARY QUALITY OF WATER

A variety of groups of bacteria and viruses have been used or recommended to indicate the sanitary quality of environmental waters. These range from a broad spectrum group such as the aerobic plate count to a narrow spectrum group such as *Escherichia coli* (*2,5*). Of these various groups the most commonly used are the coliform, fecal coliform and fecal streptococcus groups. Historically, the most common bacterial indicator group used by the public health community to determine the presence of sewage has been the coliform group. Improved methodology has resulted in the development of a fecal coliform growing area standard for shellfish waters (*3*). This group is considered to be a more specific indicator for direct fecal contamination and is now being used by some of the state shellfish control agencies.

The coliform standard for "approved" shellfish waters described in Part I of the Manual of Operations of the National Shellfish Sanitation Program (NSSP) (*4*) states that "The coliform median MPN of the water does not exceed 70 per 100 ml and not more than 10% of the samples ordinarily exceed an MPN of 230 per 100 ml for a 5-tube decimal dilution test (or 330 per 100 ml, where the 3-tube decimal dilution test is used) in those portions of the area most probably exposed to fecal contamination during the most unfavorable hydrographic and pollution conditions." The upper 10 percentile limitations were included to account for the variability of the multiple tube fermentation procedure. It was not the intent of the NSSP that shellfish control agencies should permit an area to remain open if the levels of coliforms exceeded the 70-230 standard 10% of the time. Accordingly, "approved" sampling stations should qualify for both sections of the standard, the median MPN of 70, and the 90 percentile MPN of 230.

The question at this point is how the control agency relates the standard to levels of viable sewage. In more precise terms, how much viable fecal material is in water which contains a coliform MPN of 70/100 ml?

It is extremely difficult to relate coliform or fecal coliform values to levels of viable sewage when the elevated levels of indicator organisms are caused by land runoff or other non-point sources of pollution following rainfall. However, when the indicator organisms detected at a specific station can be traced to a point source such as a sewer outfall, the relationship of indicator level to specific volumes of sewage or coliform population equivalent can be determined.

The NSSP uses the value 160×10^9 as the per capita per day contribution of coliforms in municipal sewage. At this level, a single population equivalent of coliforms, or the coliform equivalent to the fecal material produced by one person in one day, would have to be diluted and dispersed in 8 million ft^3 of coliform-free water for that unit volume of water to meet the coliform growing area standard. In more graphic terms, 8 million ft^3 of dilution water would fill a tank with a bottom area the size of a football field to a depth of 177 ft or a cove a quarter of a square mile in an area to a depth of approximately 4 1/2 ft.

At first glance, the standard may appear to be excessively restrictive, but in view of the physical and biological dynamics of estuarine systems, the small size of the sample taken for monitoring purposes, the small number of samples taken per station per year and the wide range of values permitted by the standards, the 70 MPN coliform or 14 MPN fecal coliform standards are believed to represent a reasonable compromise between consumer protection and unreasonable restrictions on resource utilization. It should be emphasized that a 70 MPN median permits approximately 40% of the samples taken from a station to range between 70 and 230, and 10% of the samples to exceed 230. If water quality is monitored on a monthly basis, as is usually true, the level of viable sewge in sampling stations adjacent to the "approved" side of the closure line can exceed the 70 MPN to a considerable degree throughout the year and still meet the standard.

If there were a constant level of pathogens in sewage, a more definitive standard based upon pathogens such as salmonella or enteric viruses might be feasible, but the ratio between the indicator group and pathogens varies with every unit volume of sewage flowing from the outfall. A specific level of feces in wastewater may be relatively free of pathogens at one moment and have a high potential for pathogen transmission through shellfish a moment or so later. However, under optimum pathogen recovery conditions, the high cost of monitoring for pathogens would still have to be considered.

Because of the high costs of monitoring for pathogens, absence of a constant indicator/pathogen relationship, variation in numbers and types of pathogens in sewage, limitations of routine sampling practices and meteorological and hydrographic effects on the physical and biological dynamics of the estuary, the control agency has no reasonable alternative than to test for viable sewage when attempting to establish a shellfish closure line in an estuary.

Both the coliform and fecal coliform indicator groups have been used successfully in the classification of shellfish growing areas. Neither group fulfills all of the desirable characteristics of the ideal indicator. The standards are based upon the public health assumption that the presence of viable fecal material in estuarine

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Poultry-associated Foodborne Disease — Its Occurrence, Cost, Sources and Prevention

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ABSTRACT

Poultry is an important food item in most countries, and production is increasing to satisfy demand. Both chicken and turkey, however, are frequently associated with foodborne disease, with *Salmonella* sp., *Staphylococcus aureus* and *Clostridium perfringens* being the main etiological agents. Inadequate cooking and improper storage of cooked poultry, particularly in foodservice establishments, are major factors in the occurrence of such disease. Since *Salmonella* is recognized as the main pathogen associated with poultry, resulting in high medical care costs and lost productivity of those sick, reduction of this organism in poultry is a major challenge facing the food industry today. Reports from various countries show that high levels of retail carcasses carry *Salmonella*. The significance of this is in the transfer of organisms from the raw birds to cooked poultry and other food products in the kitchen. This is borne out by the fact that the main serotypes involved in human illness are frequently found in poultry and poultry-related material. Processing of the birds may spread *Salmonella* and other pathogens, but clean equipment, healthy workers and efficient rinsing and cooling of carcasses will keep cross-contamination to a minimum. The final products are best preserved by wrapping them in oxygen-impermeable plastic bags which are then refrigerated or frozen. Control programs to reduce salmonellae in poultry comprise rigorous maintenance of *Salmonella*-free primary breeder flocks, elimination of environmental sources of the organism, use of correctly heat-processed feed, establishment of a non-pathogenic gut flora in very young birds and education of the user of the finished products.

POULTRY PRODUCTION

Although chickens and other domesticated and game fowl have been used as food for many centuries, it is only relatively recently that chicken and turkey meat have been available year-round to most people in developed countries. This has been achieved through the broiler industry, begun in North America just before World War II. The aftermath of the same War delayed production in the United Kingdom and Europe until the 1950s and 60s. As a result of the development of the fast-growing broiler bird through selective breeding, use of vaccines and antibiotics to control disease, improved nutrition, automation of the farms and integration of the industry (breeding farms, hatcheries, broiler farms and processors), it is possible to produce meat on a regular basis that is as cheap as, or cheaper than, red meats or fish. Between 1940 and 1961 the production of chickens and turkeys rose by 143% in the United States (*85*). During 1977 in the same country, a total of 3.2 billion broilers were supplied (*117*), and for 1978, it was estimated that 140 million turkeys would be marketed, and that this will increase to 150 million by 1982 (*101*). Poultry production in metric tons in developed and developing countries is shown in Table 1. Dramatic increases between 1970 and 1977 occurred in Oceania (mainly Australia) and East Europe and the U.S.S.R., places with low population increments; and also in the Near East and Latin America, both with greater rises in population during this period. Poultry consumption has increased in most countries where information is available, although not to the same extent as in the United States and Canada; consumption in lb. per capita per year is shown in Table 2.

TABLE 1. *Production of poultry.*[a]

Countries	Production in 1000 metric tons		Percentage increase 1970-1977
	1970	1977	
Developed countries	10870	13939	28.2
North America	6768	7782	15.0
West Europe	3456	4754	37.6
Oceania	130	230	76.9
Other	516	1173	127.3
Developing countries	2124	3820	79.9
Latin America	1003	1947	94.1
Far East	564	763	35.3
Africa	345	512	48.4
Near East	210	594	182.9
Other	2	4	100.0
Centrally planned countries	4679	6635	41.8
Asia (mainly China)	2771	3458	24.8
East Europe and U.S.S.R.	1908	3177	66.5

[a]Based on data from FAO Production Yearbooks 1972 and 1977 (*39,40*).

POULTRY-BORNE DISEASE

Increased consumption of poultry has resulted in an increase of poultry-associated foodborne disease, particularly salmonellosis. Poultry ranks first or second in foods associated with disease in Australia, Canada, England and Wales and fourth in the United States (*128*). Poultry-associated outbreaks compared with total outbreaks reported are shown for four countries in Table 3 and Fig. 1. For England, Wales and the United States, there was an increase in outbreaks in 1968 over those in the previous years, after which figures for England and Wales increased very slightly until 1973-75. From a peak in 1969, outbreaks in the United States dropped to a low in 1973 and rose again in the three succeeding years. The peak in 1975 coincided with one

TABLE 2. *Consumption of poultry.*

Country	Year	Consumption (lb/capita/year)	Reference
CHICKEN[a]			
Canada	1970	35	*103*
Canada	1976	35	*41*
United States	1946	20	*81*
United States	1950	21	*85*
United States	1960	30	*35*
United States	1970	41	*35*
United States	1976	43	*36*
TURKEY			
Canada	1970	10	*103*
Canada	1976	9	*41*
United States	1950	4	*85*
United States	1960	6	*35*
United States	1970	8	*35*
United States	1976	9	*36*
TOTAL POULTRY			
Australia	1966	13	*129*
Australia	1970	20	*129*
Belgium	1966	17	*28*
Belgium	1970	24	*28*
Canada	1970	45	*103*
Canada	1976	44	*41*
Denmark	1976	17	*100*
France	1966	27	*28*
France	1970	32	*28*
Germany (West)	1951	3	*50*
Germany (West)	1971	17	*50*
Italy	1966	16	*28*
Italy	1970	23	*28*
The Netherlands	1966	10	*28*
The Netherlands	1970	13	*28*
Sweden	1970	9	*73*
Sweden	1976	9	*99*
Sweden	1980	14[b]	*73*
United Kingdom	early 1950s	5	*80*
United Kingdom	1968	18	*80*
United Kingdom	1977	26	*81*
United States	1966	43	*35*
United States	1970	49	*35*
United States	1976	53	*36*

[a]Includes broilers and fowl.
[b]Estimated.

occurring in Canada. Although the total number of outbreaks reported varied from year to year, poultry-associated outbreaks and ratios of poultry-associated outbreaks to total outbreaks show similar patterns (Fig. 1).

From 1966 to 1974 five deaths were attributed to contaminated turkey and nine to chicken in the United States (*62*). Only one death, from consumption of chicken sandwiches and egg sandwiches, was reported in Canada between 1973 and 1975 (*57*).

TYPE OF POULTRY ASSOCIATED WITH DISEASE

For England, Wales and Canada, more outbreaks were associated with chickens than with turkey (Table 3). However, the converse was true for the United States (1966-1974), with the average number of cases per outbreak being 100 for turkey and 56 for chicken (*62*). Equivalent figures for Canada (1973-75) were 48 for turkey and four for chicken (*56,57,58*). These figures may mean that only the larger outbreaks in the United States are being reported. For both countries outbreaks associated with turkey involved more persons than those associated with chicken. No case-to-outbreak data are available for England and Wales.

Poultry involved in outbreaks was usually roasted or broiled, and often served with gravy and stuffing, but barbecued and deep-fried chickens were also responsible for illness. Implicated poultry has also been prepared with other foods, such as salads, sandwiches, rolls, casseroles and creamed products.

ETIOLOGICAL AGENTS RESPONSIBLE FOR POULTRY-ASSOCIATED OUTBREAKS

Salmonella caused more poultry-associated outbreaks than any other agent between 1973 and 1975 in three of the four countries compared (Table 4). Outbreaks of salmonellosis were more frequent from turkey (57%) than from chicken (41%). Other major causes of outbreaks were contamination by *Staphylococcus aureus* and *Clostridium perfringens*. *S. aureus* outbreaks involved chicken (66%) much more than turkey (32%), but it was almost the converse for outbreaks of *C. perfringens* (chicken, 43%; turkey, 55%). For the three years compared, *Bacillus* and *Shigella* sp. were each responsible for one outbreak, and single (sporadic) cases were caused by *Clostridium botulinum* and mold (both in chicken pies).

FACTORS THAT CONTRIBUTE TO OUTBREAKS

One question in the investigation of outbreaks that is seldom asked is how contaminating organisms grew to levels sufficient to cause illness. Factors contributing to outbreaks are recorded in Table 5. The most significant one is improper temperature control, during warm or cold storage. Insufficient cooking is also of concern since it may allow survival of pathogens in the finished product. Cooked foods may be contaminated as well through contact with raw poultry or other contaminated foods or materials, such as knives and chopping boards. Pathogens grow better on cooked birds than on raw poultry for two main reasons, lack of microbial competition and thermal breakdown of the tissues.

MISHANDLING OF POULTRY

In Canada and the United States foodservice establishments were mainly responsible for the mishandling of poultry that led to outbreaks (Table 6). In particular, these were institutions (schools, hospitals, prisons, etc.) where turkey was eaten, and restaurants and catering groups where both chicken and turkey were served. Poor hygienic practices in the home, particularly with turkey, also led to several outbreaks; retail food establishments in Canada were also responsible for a few outbreaks. Salmonellosis, *C. perfringens* enteritis and *S. aureus* intoxication resulted from mishandling of both chicken and turkey in most of the main groups of establishments (Table 6).

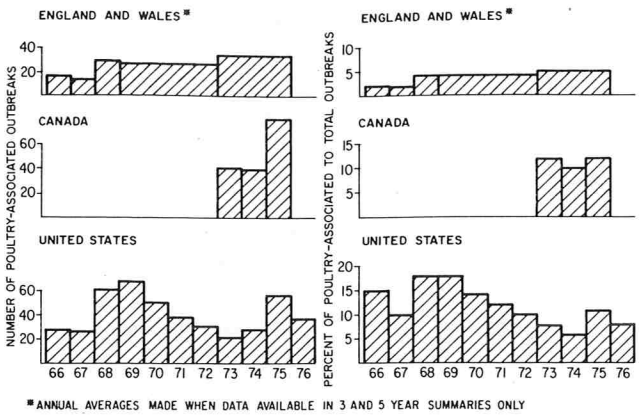

Figure 1. *Poultry-associated outbreaks in four countries.*

In England and Wales, many *Salmonella* and several *C. perfringens* outbreaks occurred in hospitals. Although these data are for all foods, poultry probably played a major role because of its importance as a vehicle of infection in these countries (*135-139*).

No food-processing establishment in any of these countries was involved in outbreaks from 1973 to 1975, but in the United States three outbreaks due to ready-to-eat poultry contaminated with *Salmonella* occurred in 1968 and 1969, and improperly bottled chopped chicken livers caused botulism in 1968 (*62*).

ECONOMICS OF FOODBORNE DISEASE

All cost estimates of foodborne disease to date have been directed towards salmonellosis in North America.

TABLE 3. *Outbreaks in four countries associated with poultry by year of occurrence.*

		Foodborne outbreaks						
Country	Years reported	Total reported	Total with vehicle identified (%)	Total poultry (%)	Chicken (%)	Turkey (%)	Other[a] poultry (%)	Reference
United States	1966-74	2920	2329 (80)	352 (15)	129 (37)	217 (62)	6 (2)	*62*
	1969	371	318 (86)	67 (21)	22 (33)	45 (67)	—	*62*
	1973	307	211 (69)	23 (11)	7 (30)	16 (70)	—	*62*
	1974	456	310 (68)	28 (9)	11 (39)	16 (57)	1 (4)	*62*
	1975	497	378 (76)	56 (15)	35 (62)	20 (36)	1 (2)	*20*
	1976	438	256 (58)	36 (13)	12 (33)	21 (58)	3 (8)	*21*
subtotal	1966-76	3855	2963 (77)	444 (15)	176 (40)	258 (58)	10 (2)	
Canada	1973	343	290 (85)	41 (14)	26 (64)	12 (29)	3 (7)	*56*
	1974	387	324 (84)	40 (12)	25 (63)	14 (35)	1 (2)	*57*
	1975	710	572 (81)	83 (15)	50 (60)	33 (40)	—	*58*
subtotal	1973-75	1440	1186 (82)	164 (14)	101 (62)	59 (36)	4 (2)	
England and	1966	744	98 (13)	17 (17)	NA[b]	NA	NA	*135*
Wales	1967	705	79 (11)	13 (17)	NA	NA	NA	*136*
	1968	792	95 (12)	29 (31)	19 (65)	10 (35)	—	*137*
	1969-72	2833	323 (11)	106 (33)	63 (59)	37 (35)	6 (6)	*139*
	1973-75	1946	334 (17)	103 (31)	57 (55)	46 (45)	—	*138*
subtotal	1966-75	7020	929 (13)	268 (29)	139 (58)[c]	93 (39)[c]	6 (3)[c]	
Total	1966-76	12315	5078 (41)	876 (17)	416 (49)	410 (49)	20 (2)	

[a]Chicken and turkey together, duck, cornish hen and unspecified.
[b]NA = Not available.
[c] = No data for 1966, 1967.

TABLE 4. *Outbreaks associated with poultry by etiological agent, 1973-1975.*

Agent	Poultry	United States[a] outbreaks (%)	Canada[b] outbreaks (%)	England and Wales[c] outbreaks (%)	Total outbreaks (%)
Salmonella sp.	chicken	8 (42)	4 (22)	23 (47)	35 (41)
	turkey	10 (53)	13 (72)	26 (53)	49 (57)
	other	1 (5)	1 (6)	—	2 (2)
	subtotal	19 (100)	18 (100)	49 (100)	86 (100)
Staphylococcus aureus	chicken	9 (60)	13 (52)	15 (94)	37 (66)
	turkey	5 (33)	12 (48)	1 (6)	18 (32)
	other	1 (7)	—	—	1 (2)
	subtotal	15 (100)	25 (100)	16 (100)	56 (100)
Clostridium perfringens	chicken	2 (22)	2 (33)	19 (50)	23 (43)
	turkey	7 (78)	3 (50)	19 (50)	29 (55)
	other	—	1 (17)	—	1 (2)
	subtotal	9 (100)	6 (100)	38 (100)	53 (100)

[a]From Center for Disease Control (*18,19,20*).
[b]From Health Protection Branch (*56,57,58*).
[c]From Vernon (*138*).

TABLE 5. *Factors that contributed to food-borne outbreaks in the United States, England, Wales and Canada.*

Factor	Salmonellosis outbreaks (all foods)[a]		Poultry-borne[b] outbreaks (all agents)
	United States 238 outbreaks 1961-1976	England and Wales 27 outbreaks 1969-1976	Canada 29 outbreaks 1973-1975
Factors affecting growth			
Inadequate cooling	113 (47)[f]	16 (63)	12 (41)
Preparing a day or more before serving[c]	41 (17)	14 (52)	1 (3)
Inadequate hot storage	33 (14)	4 (15)	6 (21)
Using leftovers[c]	9 (4)	0	0
Faulty fermentations	2 (1)	0	0
Process failure			
Inadequate cooking	49 (21)	6 (22)	4 (14)
Inadequate reheating	30 (13)	11 (41)	0
Factors affecting contamination[d]			
Using contaminated raw ingredients	77 (32)	11 (41)	1 (3)
Cross-contamination	50 (21)	7 (26)	1 (3)
Inadequate cleaning of equipment[c]	36 (15)	5 (19)	1 (3)
Infected persons[e]	31 (13)	1 (4)	7 (24)
Unsafe source	2 (1)	8 (30)	0

[a]From Bryan (*14*).
[b]From Health Protection Branch (*56,57,58*).
[c]Indirect factor.
[d]Data not available on contamination from animals to be processed or raw foods of animal origin.
[e]Unknown whether or not infected persons contaminated the food, or were themselves infected because they ate contaminated food.
[f]Percentage.

Based on the costs of three large *Salmonella* outbreaks in the early 1960s, Eickhoff (*37*) postulated that the total economic impact in the United States was $10 to $100 million for one million cases annually. This is substantially in agreement with Bruch (*11*) who reckoned the cost to be between $70 and $140 million for two million cases per year. In 1969, Foster (*42*) estimated that salmonellosis cost American citizens $300 million/year. The estimate of medical costs alone, in 1978, had increased to $1.2 billion/year for 2.5 million cases (*46*). Although these figures are for total salmonellosis, poultry-associated *Salmonella* infections are responsible for a high proportion of these cases (*46,80,100*).

One foodborne outbreak at a restaurant in Minnesota in the early 1970s cost an estimated $28,783, mainly as lost salaries (*72*). Another outbreak in Colorado from cheese (*24*), infected at least 234 persons at a cost of $151,125, most of which was hospitalization costs for 68 persons (58%) and income or productivity losses (26%). Another estimated 32,000 cases associated with this outbreak did not consult a physician; medical and non-medical costs were reckoned at $125 per person for a total of $4,000,000. In these outbreaks, loss in productivity greatly exceeded total medical costs. Extrapolation of these figures would lead to a total salmonellosis cost for the United States considerably in excess of the $1.2 billion indicated by Gangarosa (*46*).

In Canada, salmonellosis has been estimated to cost between $25 and $100 million a year (*100*). Recent analysis of three Canadian outbreaks show that costs per case are high. In Ontario, one outbreak involving 44 cases from *Salmonella*-contaminated eggs in baked goods cost $535 per case with investigational expenses accounting for 50% of this amount (*67*). In Québec, the provisional estimated cost of an outbreak in an hotel was $555 per case, but details are not yet published (*68*): Also in Québec, salmonellosis in a family of five, where fish was the suspected vehicle, cost $806 per case (*68*). About 64% of the costs for this outbreak was for the investigation, including 4 months of follow-up surveillance of fish suppliers.

Although no poultry-borne *Salmonella* illnesses have been economically evaluated, it is assumed that the costs would be the same as for any of the *Salmonella*-contaminated foods so far investigated. Costs of outbreaks are usually considered greater than those of routine inspection, educational programs (*72*) and *Salmonella* surveillance (*24*).

Eradication of *Salmonella* in poultry is one approach to reduce outbreaks, but this could be prohibitively expensive; in Canada the cost has been estimated at $300,018,400 or $1.40 per person per year for 10 years (*38*). For economic and practical reasons, eradication is not feasible nor required, but strict control measures are realistic. The Swedish control program for *Salmonella* in poultry has been in operation for several years, and its costs are no more than $0.5 per person per year (*99*). Another effective control program in Denmark does not prevent two-thirds of the annual broiler production (about 46 million birds) being exported (*98*). These two European programs indicate that stringent control measures are not economically prohibitive.

The economics of foodborne disease caused by other agents has not been studied, but generally it can be assumed that cost per case for infectious agents similar to *Salmonella*, such as *Campylobacter, Shigella, Vibrio* and *Yersinia* spp., will be much the same, whereas the cost per case for toxigenic agents, such as *Bacillus cereus*, *C. perfringens* and *S. aureus* will be less.

SOURCES OF POULTRY CONTAMINATION

Salmonella

Chickens, turkeys and other birds frequently carry *Salmonella*, but few serotypes, e.g., *Salmonella pullorum* and *Salmonella gallinarum*, cause avian clinical illness, except in very young chicks and poults. With the rapid development and expansion of the broiler industry, contamination became much more widespread in the finished raw product. Eggs from the breeder flock may be infected through ovarian transmission or by external contamination of the eggshell (*59*). Also, when chicks and poults ingest contaminated litter or improperly heat-processed feed, infection may occur; *Salmonella* can survive many months in litter, soil, excreta and dust (*12,107*). Unless feed and the environment are strictly controlled, flocks will contain *Salmonella* carriers, although the percentage may vary from flock to flock, e.g., 0-72% of birds in 25 flocks in Canada (*79*). If delays occur between shipping poultry from the farm and slaughtering by the processor, or if adverse weather

conditions occur during transportation, the number of *Salmonella* isolations in carcasses may increase (*110, 141*). The processing of poultry, especially defeathering and evisceration, allows opportunity for *Salmonella* to be spread from bird to bird (*15,25,83,84,87*). Contaminated hands and gloves of processing plant workers also contribute to the dissemination of the organism (*16,120*). The net result is that a high proportion of chickens, turkeys and ducks on the retail market carry *Salmonella* (Tables 7 and 8). The considerable variation in the prevalence of infected carcasses between producing countries is dependent on the sampling method (*9,29,88*), poultry flock chosen and sample size (*79*) and whether fresh, frozen (*5*) or cut-up samples are examined. Many of the countries with a large broiler production appear to have the highest contamination rates; a notable exception is Denmark, which economically produces poultry meat with a very low prevalence of *Salmonella* (*98*).

Freezing of poultry does not kill all *Salmonella* cells (*65*), and, in fact, drip water from thawing is an excellent vehicle for contaminating the environment (*130*). More salmonellae may survive at -20 C than at 1 to -2 C (*43*).

The percentage of poultry-associated salmonellosis has risen in the last decades in relation to salmonellosis from other foods (*23,59,80*), and the number of serotypes accessible to the public has also increased through the expansion of the poultry industry (*53,126*). In 1975 in Canada, Handzel and Laidley (*53*) noted that eight of the top 10 serotypes isolated from poultry sources also occurred in the top 10 serotypes isolated from human specimens, and in England and Wales, according to McCoy (*80*), the number of human incidents due to serotypes other than *S. typhimurium* is coincident with the rise in poultry consumption. In the United Kingdom, *S. bredeney, S. enteritidis, S. heidelberg, S. indiana* and *S. virchow* are established in breeding flocks (*70,80*). Infections from *Salmonella* 4,12:d: –, *S. agona* (*44,109*), *S. cerro, S. eimsbuettel, S. give, S. johannesburg, S. kottbus, S. münchen, S. orion* (*78*) and *S. saint-paul* (*45*) are likely to have risen from contaminated feed. *S. menton, S. thompson* and *S. typhimurium* have often been spread through eggs, but pasteurization of liquid egg has reduced the number of outbreaks from this source (*123*). The significance of contamination in one breeding unit was emphasized by Payne and Scudamore

TABLE 6. *Poultry-associated outbreaks by etiological agent and place of mishandling of poultry, the United States and Canada, 1973-1975.*

Country and product	Agent	Food-service establishments	Home	Retail establishments	Other/unknown	Total
United States[b]						
Chicken	*Salmonella*	4	3	—	1	8 (18)[a]
	S. aureus	5	2	—	2	9 (20)
	C. perfringens	2	—	—	—	2 (5)
	subtotal	11	5	—	3	19 (43)
Turkey	*Salmonella*	5	5	—	—	10 (23)
	S. aureus	3	2	—	—	7 (16)
	C. perfringens	3	3	—	1	5 (11)
	Shigella	1	—	—	—	1 (2)
	subtotal	12	10	—	1	23 (52)
Poultry	*Salmonella*	—	1	—	—	1 (2)
	S. aureus	1	—	—	—	1 (2)
	subtotal	1	1	—	—	2 (5)
Total	*Salmonella*	9	9	—	1	19 (43)
	S. aureus	9	4	—	2	15 (34)
	C. perfringens	5	3	—	1	9 (21)
	Shigella	1	—	—	—	1 (2)
	subtotal	24 (55)[a]	16 (36)	—	4 (9)	44 (100)
Canada[c]						
Chicken	*Salmonella*	3	—	—	1	4 (8)
	S. aureus	5	3	3	2	13 (26)
	C. perfringens	1	—	1	—	2 (4)
	Bacillus	—	1	—	—	1 (2)
	subtotal	9	4	4	3	20 (40)
Turkey	*Salmonella*	7	5	1	—	13 (26)
	S. aureus	5	7	—	—	12 (24)
	C. perfringens	3	—	—	—	3 (6)
	subtotal	15	12	1	—	28 (56)
Poultry	*Salmonella*	1	—	—	—	1 (2)
	C. perfringens	1	—	—	—	1 (2)
	subtotal	2	—	—	—	2 (4)
Total	*Salmonella*	11	5	1	1	18 (36)
	S. aureus	10	10	3	2	25 (50)
	C. perfringens	5	—	1	—	6 (12)
	Bacillus	—	1	—	—	1 (2)
	subtotal	26 (52)	16 (32)	5 (10)	3 (6)	50 (100)

[a]Percentage.
[b]From Center for Disease Control (*18,19,20*).
[c]From Health Protection Branch (*56,57,58*).

(95): *S. enteritidis* and *S. hadar* infection of turkey breeder flocks at one farm resulted in 10 incidents over 8 years involving 185 diagnosed cases and one death. Outbreaks of *S. hadar* associated with poultry continued to occur in England.

Other organisms

S. aureus is present on body surfaces of healthy live chickens to the extent of 10^6/bird; birds suffering from staphylococcal dermatitis are more highly contaminated (10^8 or 10^9/bird) (*31*). If bruising occurs *S. aureus* may

TABLE 7. *Prevalence of* Salmonella *on fresh and frozen chickens.*

Number of samples	Number with *Salmonella*	Percent positive	Country of origin	Date[a]	Reference
26	17	65	The Netherlands[c]	1973	*61*
137	39	29	The Netherlands[d]	1973	*122*
95	61	64	The Netherlands[e]	1975	*112*
298	187	63	West Germany	1975	*112*
146	42	29	West Germany	1975	*91*
88	48	55	Greece	1975	*133*
496	49	9	Greece	1976	*134*
18	8	44	New Zealand[d]	1973	*122*
101	36	36	England	1973	*60*
51	25	50	England	1973	*61*
100[b]	13	13	England	1969-70	*61*
50	24	48	Poland[e]	1975	*112*
2728	293	11	United States[d]	1973	*122*
90	28	31	United States	1974	*26*
240	107	45	United States	1978	*29*
153	27	18	Canada[d]	1973	*122*
108	16	15	Canada	1975	*27*
69[b]	24	35	Canada	1977	*34*
365	143	39	Canada	1976-77	*100*
30	5	17	Belgium[e]	1975	*112*
45	12	27	France[d]	1973	*122*
30	5	17	France[d]	1975	*112*
340	46	14	Bulgaria[d]	1973	*122*
25	2	13	Turkey[e]	1975	*112*
2219	207	9	China[d]	1973	*122*
332	19	6	Hungary[d]	1973	*122*
100[b]	0	0	Denmark[c]	1969-70	*61*
132	12	9	Denmark[c]	1972	*61*
532	20	4	Denmark[d]	1973	*122*
25	0	0	Denmark[e]	1975	*112*
100	9	9	India	1977	*102*
123,355	20	0.016	Sweden	1960-76	*1*
4240	456	11	Five European countries[f]	1975	*25*
Total 136,864	2000	1.5	(median percentage 17)	1960-77	

[a]Date of reference, if actual survey dates are not mentioned.
[b]Cut-up pieces of chicken.
[c]Exported chickens examined in the United Kingdom.
[d]Exported chickens examined in Japan.
[e]Exported chickens examined in West Germany.
[f]Denmark, France, Italy, The Netherlands and the United Kingdom.

TABLE 8. *Prevalence of* Salmonella *in fresh and frozen turkeys, ducks and unspecified poultry.*

Type of poultry	Number of samples	Number with *Salmonella*	Percent positive	Country of origin	Date[a]	Reference
Turkey	58	10	17	United States	1968	*15*
	208	24	12	United States	1968	*16*
	146	18	12	United Kingdom	1962	*32*
	55	4	7	West Germany	1975	*91*
	100	0	0	Northern Ireland	1972	*94*
	119	27	32	Canada	1975	*27*
	1250	145	12	Canada	1978	*79*
Total	1936	228	12 (median percentage 12)			
Duck	53	30	57	West Germany	1976	*104*
	21	18	86	Poland	1973[c]	*61*
	NA[b]	NA	55	United Kingdom	1967-73	*61*
	140	16	11	Northern Ireland	1969	*93*
	597	34	7	Northern Ireland	1972	*94*
Total	911	103	11 (median percentage 55)			
Unspecified	41	3	7	United States	1978	*119*

[a]Date of reference, if actual survey dates not mentioned.
[b]NA = Not available.
[c]Exported duck examined in the United Kingdom.

penetrate inside the damaged tissues, persist for many days, and thus be present during the processing operations (*108*). Although some organisms are washed off during the processing, other strains may contaminate the poultry during defeathering (*48*) or handling (*13*). Genigeorgis and Sadler (*47*) indicated that about 1.6% of poultry livers contained *S. aureus*; 63% of these strains were typable with human type phages. This was confirmed by Kusch and Götze (*66*) who found that 74% of staphylococcal strains isolated from thaw water of frozen chickens, ducks and turkeys were human in origin. Harry (*54*) argues that birds are handled frequently on farms and may become infected with human strains of *S. aureus* in the process. A more recent study, however, has shown that a large proportion of the isolated strains tested for toxins A, B and C produced enterotoxin A, and were non-phage typable by the international set of phages used for testing human strains (*49*). This lack of lysing tends to confirm the conclusion of Bailyozov et al. (*2*) that many of the strains are non-human and probably avian in origin. The conflicting results in the literature on whether the majority of strains on finished poultry carcasses come from human or avian sources may depend on a number of factors: the amount that birds are handled at the farms, the condition of the flocks arriving for processing and hygiene of the workers and equipment at the plant; also the phages used were not identical. Because of growth of competitive psychrotrophic organisms, staphylococci on the surface of raw poultry decrease in numbers upon refrigeration and they are seldom isolated after 12 days of storage (*140*). Therefore, although strains do not grow well on raw poultry, there is opportunity for cross-contamination, and recontamination of cooked products resulting in illness.

C. perfringens spores are present in soil and dust, and have ready access to chicks and poults. In healthy birds, the organism is mostly found in the caeca ($\leqslant 10^5$/g) and colon (*3*), and during processing the organism is frequently present on skin surfaces (*82*). Hall and Angelotti (*52*) found 58% of chicken carcasses at the retail level positive for *C. perfringens*. The cells cannot multiply readily in the presence of oxygen or at cool temperatures. Spores, however, may survive inadequate cooking and grow out during cooling to ambient temperatures. Recontamination of cooked birds from raw poultry, or cross-contamination from hands and equipment can also occur.

Any infectious or toxigenic organism capable of growing in potentially hazardous food can cause illness when conditions are suitable. Thus *C. botulinum, Bacillus* sp. and *Shigella* sp. have been recorded occasionally as poultry-associated food-poisoning agents (see Section on Etiological Agents Responsible for Poultry-associated Outbreaks). *Campylobacter fetus* subspecies *jejuni* and *Yersinia enterocolitica,* recently identified as enteric disease-causing organisms, have been isolated from poultry. The *Campylobacter* species has been found in 3 of 140 (2%) poultry samples in the United States (*116*), in 7 of 50 samples (14%) in England (*113*), in 39 of 63 (62%) samples, also in England (*10*), and in 22 of 110 (20%) samples in Canada (*92*). In Germany, *Yersinia enterocolitica* was isolated from 35 of 121 (29%) samples of chicken (*71*). No illness has yet been linked to consumption of poultry contaminated with these organisms. An outbreak in England, however, indicated that *Campylobacter* caused enteritis typical of the organism in 5 of 29 persons attending a wedding breakfast, consisting of several types of meat, chicken and eggs (*55*). The organism was found in a patient's stool specimen and agglutinins to the same serotype in the sera of all five cases were identified. Although no food was available for examination, subsequent samples of chicken from the same supplier yielded the organism (different serotype), and the authors postulated that contamination of the breakfast food had occurred through cross-contamination by means of a cutting board.

PREVENTION

Salmonella *control in the flocks*

Salmonella is the most significant pathogen transmitted by raw poultry to the kitchen, and practically all research on prevention of poultry-borne human disease is devoted to studies of this organism. The best method to prevent *Salmonella* from contaminating poultry is to maintain *Salmonella*-free breeding flocks (*69,75,106*). In California, a cooperative venture by industry, university and government attempted to produce six successive generations of turkeys that were *Salmonella*-free between 1969 and 1974; most of the few isolations occurring after 3 ½ years of successful operation probably originated from contaminated feed (*143*). Large-scale control programs are operating in the Scandinavian countries. In 1953, a severe outbreak from beef, involving more than 8,000 cases and about 150 deaths.(*1*), generated interest in *Salmonella* control programs in Sweden. The Swedish poultry industry is not large (35 million birds/year) and operates under a state-run, but largely voluntary, veterinary control program. Young birds are screened for infection and flocks with positive birds are destroyed. Strict sanitation on the broiler farm is also maintained (*99*). From 1960 to 1976 the contamination level for one Swedish processing company was 20 of 123,355 poultry samples positive (0.016%) with five isolations only from 79,980 samples (0.006%) from 1965 to 1976. In comparison, from 1961 to 1971, 1.81% of imported chickens (chickens bred by the same company outside Sweden) tested were *Salmonella* positive (*1*). In Denmark, imported breeder flocks are quarantined for 5 months before being declared free of disease, and regular microbiological testing of broiler flocks and feeds is carried out under veterinary supervision (*98*). Outbreaks of salmonellosis in chickens (defined as any *Salmonella* isolation from a flock) decreased from 182 in 1964 to 12 in 1975 (*98*). Table 7 also shows the low contamination

level of Danish chickens. In 1974, relatively few cases of human salmonellosis (368) were reported, of which 258 originated from within, and 110 from outside Denmark with a population of 5,000,000 (*98*). A similar control program in Switzerland has resulted in a 75% reduction of *Salmonella* in poultry since 1971 (*142*).

A different approach to *Salmonella*-free flocks has been developed in Finland. A severe *S. infantis* infection in Finnish broiler flocks was recognized in 1971; this originated from contaminated feed, and at least 277 human cases were identified with the industry suffering severe losses (*89*). As a result of this episode, research was pursued to reduce the *Salmonella* contamination level in poultry. It was found that gut contents from adult roosters given to 1- to 2-day old chicks prevented *S. infantis* infection in 73% of the 26 chicks inoculated orally with the organism at 10^3-10^6 infectious doses (1 cell ≡ 1 dose); all birds in the control group became infected (*89*). The intestinal flora of the chicks becomes established early in life and makes subsequent infection by *S. infantis* and other salmonellae difficult (*142*). Further work led to the use of a mixed culture of unidentified bacteria obtained by enriching digesta from the rooster gut; the mixed culture was added to the chicks' drinking water. These cultures have proven effective in laboratory experiments and show some promise in field trials (*90*). Similar work with *S. typhimurium* and other serotypes has been carried out in Australia (*74*), Canada (*63,105*) and the United States (*118*).

Reduction of *Salmonella* can, therefore, best be attained by the maintenance of *Salmonella*-free breeding flocks, achieved through *Salmonella*-free feeds and rigid control measures. The exclusion of wild birds and rodents from the flocks (*69,101*) also helps reduce exposure of chicks and poults to environmental sources of infection.

Control at the processing plant

At the processing plant, clean equipment and good sanitary techniques are essential (*22,77*). Carcasses should be thoroughly washed (*76,86*) and rapidly cooled by immersion in cold water or slush ice, spraying with cold water or circulation of cold air or other gases (*4*). Some authors claim immersion chilling is unhygienic, allowing organisms to spread from carcass to carcass (*8,50,132*). *Salmonella* contamination is either not reduced (*121*) or actually increases (*83,93*) during the chilling process. *C. perfringens* and *S. aureus* surface contamination of carcasses is also not markedly reduced by chilling (*17*). Work in Denmark with an *E. coli* tracer, however, showed that few cells were transferred from one carcass to another during immersion chilling (*98*). In addition, spray chilling, as recommended by Grossklaus and Lessing (*51*) and Peric et al. (*96*), may not always decrease carcass contamination substantially, as shown with an *E. coli* marker organism (*131*), and with actual *Salmonella* isolates (*97*). To determine which chilling system was the most efficient, the Commission of European Communities (*25*) conducted a survey of processing plants in five countries. The study indicated that properly operated immersion chilling systems do not increase the risk of cross-contamination, and that although slightly fewer *Salmonella*-positive birds were found with air chilling, this method will not reduce the number of carcasses already contaminated. Reduction of contamination before chilling is of more significance than the chilling method used. Economically, water immersion chilling is the most desirable (*115*), and is used by most countries. A code of practice to reduce the amount of cross-contamination during immersion chilling has been agreed to by poultry industries in Denmark, the Netherlands and the United Kingdom (*141*).

Chlorination of chill water can further reduce the microbial count provided the exposure time is sufficiently long (*125,127*). Even levels of up to 114 ppm for many hours, however, may not eliminate all *Salmonella* (*6,33*). In addition, use of chlorine may result in toxic by-products (*30*); consequently, although some countries require chlorination of chill water, others prohibit it (*114*). Oxygen-impermeable plastic films and vacuum packaging usually extend the shelf-life of the product (*7,111*). To reduce the number of pathogens and spoilage organisms, storage of processed raw poultry at 0 C or less is recommended (*5*).

INTERNATIONAL MICROBIOLOGICAL CRITERIA FOR POULTRY

International agreement as to what microbial criteria would be acceptable and how these should be achieved has been under consideration for a few years. Four countries (*124*) and the International Commission on Microbiological Specifications for Foods (*64*) have existing or proposed guidelines for total counts, *Salmonella, S. aureus* and/or indicator bacteria. A FAO/WHO Joint Expert Consultation on Microbiological Specifications for Foods (*124*) has considered the desirability of setting international microbiological criteria, but came to the conclusion that end-product specifications would not in themselves prevent contamination of poultry with *Salmonella*, the main organism of concern, because this usually occurred before the birds entered the processing plants. The Consultation also recognized that foodborne illness from poultry most frequently happened as a result of mishandling after sale (*124*). Therefore, two approaches are advocated: (*a*) use of the Recommended International Code of Hygienic Practice for Poultry Processing (*22*) covering general issues of hygiene on the farm, during transportation and at the processing plant, and (*b*) stress on public educational programs to instruct workers in the home, foodservice and institutional kitchens on safe handling of contaminated raw poultry.

General sanitation guidelines and educational practices are worth recommending, but they are unlikely to deal conclusively with the problem of contamination of

poultry by *Salmonella* and other pathogens. The fact that some countries have very low prevalences for *Salmonella* in poultry indicates that there are ways to deal with the problem. The elimination of infected breeder flocks and the use of uncontaminated feed are measures that can be taken, and governments as well as industries should be active in pursuing these. Unless *Salmonella* contamination of finished birds is reduced the potential for disease remains, and when food handling errors take place infections will continue to occur.

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Foodborne Diseases in the United States Associated with Meat and Poultry

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ABSTRACT

Surveillance data from 1968 to 1977 indicate that meat and poultry and products made from them were vehicles in over 50% of reported outbreaks of foodborne disease. The three most commonly identified vehicles were ham, turkey and roast beef. Ground (cooked) beef, pork, sausage and chicken were also frequently reported as vehicles. These foods were mishandled to the extent that outbreaks resulted in foodservice establishments (65%), in homes (31%) and in processing plants (4%). The most frequently identified factors that contributed to these outbreaks were improper cooling of cooked foods (48%), foods prepared a day or more before serving (34%), inadequate cooking or thermal processing (27%), infected person touching cooked foods (23%), inadequate reheating of cooked and chilled foods (20%), improper hot storage of cooked foods (19%) and cross-contamination of cooked foods from raw foods (15%). Commonly reported foodborne diseases associated with these vehicles were staphylococcal intoxication, salmonellosis, *Clostridium perfringens* gastroenteritis, and trichinosis.

Meat and poultry, and products containing them, were identified as vehicles in more than half of the reported outbreaks in which the vehicle was ascertained from 1968 to 1977, and they may have been ingredients in other foods responsible for additional outbreaks (Table 1). These outbreaks, as exemplified by data from 1973 to 1977, were usually associated with mishandling practices in food service establishments and homes, but occasionally with processed meat and poultry products (Table 2).

FOODBORNE DISEASES

Arizona, Bacillus cereus and its toxin, *Clostridium botulinum* neurotoxins, *Clostridium perfringens, Salmonella, Shigella, Staphylococcus aureus* enterotoxins, group D streptococci, hepatitis A virus, *Toxoplasma gondii, Trichinella spiralis,* monosodium glutamate, niacin, and mercury were reported as etiologic agents of meat- and poultry-borne outbreaks (Table 3). Epidemiology of these diseases, in relation to meat and poultry, is reviewed.

TABLE 2. *Place meat and poultry mishandled to extent that outbreaks resulted in United States, 1973-1977 (37).*

Place	Number	Percent	Percent of known place
Foodservice establishments	322	50	65
Homes	155	24	31
Food processing plants	20	3	4
Unspecified/unknown	150	23	--
TOTAL	647	100	100

Arizonosis

Only a few outbreaks of arizonosis have been reported in the United States (*1*, Table 3). Arizonae are frequently associated with turkeys, and the epidemiology of arizonosis appears to be similar to that of poultry- and egg-associated salmonellosis.

Bacillus cereus gastroenteritis

Few confirmed outbreaks of *B. cereus* gastroenteritis have been reported in the United States. This, no doubt, is influenced by the failure of many investigators and laboratories to seek this agent. Many of the reports of diseases of unknown etiology cited foods served in Chinese-style restaurants as probable vehicles. Many such outbreaks that occurred in England and Wales were caused by *B. cereus* (*105*).

TABLE 1. *Number and percent of foodborne outbreaks reported in the United States, 1968-1977, involving meat and poultry as vehicles (37).*

Year	Number of outbreaks	Number of outbreaks in which vehicle was ascertained	Outbreaks attributed to meat, poultry, or foods containing them		Outbreaks attributed to foods in which meat or poultry may have been an ingredient	
			Number	Percent	Number	Percent
1968	290	226	170	75	11	5
1969	326	275	194	71	15	5
1970	297	206	146	71	14	7
1971	320	249	142	57	11	4
1972	301	247	125	51	18	7
1973	307	237	86	36	21	9
1974	456	312	139	45	13	4
1975	497	379	195	51	33	9
1976	438	226	120	45	33	12
1977	436	269	103	38	56	21
TOTAL	3668	2666	1420	54	225	8

TABLE 3. *Number of meat and poultry products that were reported as foodborne vehicles in outbreaks in the United States, 1968-1977 (37).*

Disease	Beef						Pork				Other meats		Meat, general				Poultry					Totals	
	Beef (specified)	Beef (unspecified, possible roast beef)	Roast beef	Ground beef	Cured beef	Foods containing beef	Pork	Cured pork (ham)	Sausage	Foods containing pork	Lamb	Other meats	Unspecified meats	Luncheon meats	Liver	Gravy	Turkey	Chicken	Duck	Poultry salad	Food containing poultry	Total outbreaks in which meat or poultry were vehicles	Percent of outbreaks in which meat or poultry were proven or suspicious vehicles
Arizonosis																	1					1	25
Bacillus cereus gastroenteritis		1				1				1			1								1	5	39
Botulism				1		5			1			6						2			1	16	14
Clostridium perfringens gastroenteritis	9	11	36	14	1	11	4		1		1		4	1		10 (13)[1]	29	6			1	139	90
Salmonellosis	4	13	22	8	2	3	13	6	4			2	5	3	1	1 (3)	39	15	1	11	4	157	44
Shigellosis															1		1			2		4	8
Staphylococcal intoxication	9	4	7	7	4	4	23	105	7	5	1	2	5	6	2	3	23	16		16	3	252	8
Streptococcal group D gastroenteritis			3	1		1		2	1				1		1		1					11	65
Hepatitis A								1						2								3	9
Toxoplasmosis				1																		1	100
Trichinosis				7			39		42	6		6										100	94
Chemical poisonings	1			3			2			4												10	9
Diseases of unknown etiology	24	44	85	81	11	49	18	77	31	16	5	3	16	22	3	18 (20)	91	52	3	22	22	693	31
Total	47	73	153	123	18	74	99	191	87	32	7	19	32	34	8	32 (36)	185	91	4	51	32	1392	
		226																					
			488					409			26			74					363				
Percent	3.4	5.2	11.0	8.8	1.3	5.3	7.1	13.7	6.3	2.3	0.5	1.4	2.3	2.4	0.6	2.3 (2.6)	13.3	6.5	0.3	3.7	2.3		
		16.2																					
			35.1					29.4			1.9			5.3		4.9			26.1				

[1]Listed in another category, but gravy was served with the food.

Botulism

Outbreaks of botulism usually result because *C. botulinum* is able to survive inadequate time-temperature exposure during thermal processing, and the organisms multiply and produce neurotoxin in the anaerobic environment within cans, jars, plastic bags or in food masses. Commercially prepared meat products which were vehicles during the survey period were spaghetti sauce with meatballs, beef stew, and beef pot pie. Some outbreaks of botulism have been traced to fermented (seal or whale), smoked (fish) or dried (jerky) meat.

Clostridium perfringens gastroenteritis

Animal carcasses and cuts of meat can become contaminated with *C. perfringens* from soil, animal feces or workers' hands during slaughtering and processing. Many organisms that compete with *C. perfringens* are killed when meat and poultry are cooked, but not *C. perfringens* spores. These spores are heat-shocked during cooking, so more of them germinate when temperatures become favorable for growth. Also, E*h* values drop to levels that favor multiplication of *C. perfringens* during cooking. As the temperature of recently cooked meat or gravy drops while holding at room temperature or in warming devices, or during storage in refrigerators, *C. perfringens* is one of the first species to commence multiplication (at about 50 C). Cooked meat can also be contaminated by workers during boning, slicing, grinding, mixing or other handling or when it touches equipment contaminated with *C. perfringens*. Meat, stock and gravy also can become contaminated when they are put into pans. These organisms have a short lag period and can double their number every 8 to 12 min under optimal conditions. If there is time enough during room or warm storage of meat, poultry or gravy, at which the temperature is within a range that *C. perfringens* can multiply, or if these products are stored in large containers in refrigerators (small surface to volume ratio), *C. perfringens* spores may germinate and vegetative cells may multiply to quantities that can cause illness. If such foods are served without being reheated to temperatures sufficient to kill vegetative forms of *C. perfringens*, illness may result. Therefore, when meat-borne outbreaks occur, one or more of the following events usually occurred: (*a*) improper cooling, (*b*) improper hot holding, (*c*) food was prepared a day or more before serving or (*d*) inadequate reheating (*2*).

Salmonellosis

Fowl, cattle, swine, or other food-source animals sometimes become infected or their skin or feet become contaminated while they are on farms. The animals acquire salmonellae from feed and feed ingredients, water and their animate and inanimate environmental contacts. The salmonellae are conveyed to processing plants in or on these animals, usually in fecal material on their feet, skin, feathers, hair or in their intestinal tracts. These organisms can spread to carcasses or cuts of meat during processing. Sometimes these products have been inadequately cooked, so the salmonellae survived. Othertimes, thoroughly cooked products have been processed or prepared in the same room, on the same equipment or by persons who had previously handled these raw products. Occasionally, human carriers of salmonellae are found to have handled the food implicated in a particular outbreak. Such persons often had a history of eating this food; therefore they may have been victims instead of the source of contamination. Also, they often handled the raw meat or poultry which was sometimes contaminated with salmonellae. After cooked foods have become contaminated, there is usually a period of several hours during which they are held either at room temperature or in warmers, or during which they are stored in large pots in refrigerators. Because of these sources of contamination, modes of transfer, and opportunities for survival and multiplication of salmonellae, meat and poultry products are frequently identified as vehicles during foodborne outbreak investigations (*99*).

Factors that have contributed to outbreaks of salmonellosis include improper cooling, contaminated raw ingredients, insufficient temperature or time during cooking, cross-contamination, lapse of a day or more between preparation and serving, inadequately cleaned equipment, improper hot holding and insufficient temperature or time during reheating (*2*).

Shigellosis

Outbreaks of shigellosis occasionally involve meat or poultry which has been sliced, mixed or otherwise handled by persons infected with shigellae who fail to wash their hands after defecating, and soon thereafter handle foods (*1*). Small numbers of shigellae can elicit a diarrheal response (*102,109*). So, infected food workers who have poor personal hygiene can contaminate meat or poultry products with enough of these pathogens (even without subsequent multiplication) to produce illness.

Staphylococcal intoxication

Meat and poultry products become vehicles of staphylococcal enterotoxins when one of the following circumstances occurs. (*a*) Meat or poultry is cooked, which usually kills staphylococci and many competitive organisms. After cooking, meat or poultry is touched during boning, slicing, grinding, mixing or other handling by a person who is harboring staphylococci in his nose, on his skin, or in an infected lesion. The food is then either left unrefrigerated for several hours or put into a large pot or pan which is refrigerated for several hours, during which time *S. aureus* multiplies and elaborates enterotoxin. Epidemiologic data show that improper cooling, preparing foods a day or more before serving and cooked foods being handled by infected persons are the most frequent contributing factors that lead to outbreaks of staphylococcal food poisoning (*2,107*). (*b*) Raw meat or poultry that is contaminated

with staphylococci from lesions or during processing can be the initial source of the organisms whenever there is inadequate thermal processing or cross-contamination. Subsequent temperatures of the contaminated product must be within a range in which staphylococci can multiply for a sufficient period for enterotoxin production. (*c*) Fermented meat can become a vehicle if the raw products are contaminated with enough staphylococci to compete successfully with other organisms. Conditions are such that give enterotoxigenic strains of *S. aureus* an advantage over competitive organisms during the early stages of fermentation. (See section on sausage for a more detailed discussion.)

Streptococcal Group D gastroenteritis

Sporadic outbreaks of enterococcal gastroenteritis have been reported to the Center for Disease Control. Although the epidemiologically implicated foods usually contained large numbers of enterococci, the evidence was insufficient to confirm the causes of the outbreaks. There is conflicting evidence of the pathogenicity of the enterococci (*1,100,101,110,112*).

Hepatitis A

Hepatitis A virus can become meat- or poultry-borne if infected persons (who apparently practice poor personal hygiene) handle products that are not subsequently heat-processed (*98*). Luncheon meat and sliced meat in sandwiches have been epidemiologically identified as vehicles.

Toxoplasmosis

Following a group dinner at a Middle Eastern-style restaurant, four of 15 persons developed toxoplasmosis. Four others had antitoxoplasma antibody titers equal to or exceeding 1:64. All of these persons had eaten a meat dish made from raw beef at the restaurant (*84*). Another outbreak involving five students was attributed to ingestion of rare hamburgers at a dormitory snackbar (*108*).

Trichinosis

The incidence of trichinosis in humans who reside in the United States has decreased during the past three decades. Other indices of this decline appear when comparing studies of historical and current prevalence of trichinellae cysts in humans at autopsy and in swine and pork products. In U.S. citizens, the prevalence of trichinellae cysts at autopsy was 16.1% in the 1940's, but had fallen to 4.2% during the period 1966 to 1970. Prevalence weighted by age (by eliminating those who could have been infected during the previous survey period) was 12.0% in 1940 and 2.2% in 1970 (*117*). In the 1930's, 1% of farm-raised swine were positive for trichinellae, but during the period 1966 to 1970, only 0.13% were positive. During the former period, 11% of garbage-fed swine were positive for trichinellae, and during the later period only 0.5% of garbage-fed swine were positive (*111,115*). The incidence of trichinellae in pork sausage declined from 12.5% in bulk pork sausage and 11.7% in fresh link sausage tested during the period 1944 to 1946 to 0.9% of bulk sausage samples, and 1.5% of fresh link sausage samples during 1953 to 1969 (*114,116*). Only 0.2% of treated (smoked) link sausage samples tested during the later period were positive.

Ingestion of raw and undercooked pork and cross-contamination from raw pork to other meats are the most commonly reported factors that contribute to outbreaks of trichinosis (*2*). During the period 1968 to 1977, for instance, surveillance data show that ingestion of sausage was responsible for 44% and other pork products for 37% of cases of trichinosis. Hamburger and chopped beef were responsible for 8% of the cases, bear meat for 4% and walrus and wild boar for 2% each. The beef products may have become contaminated from pork products through the use of the same meat grinder or by undisclosed mixing of pork into ground beef. During the period 1968 to 1974, cooked meat was reported to be responsible for 23% of cases, undercooked meat for 7%, and uncooked meat for 53%. Those persons who indicated that cooked meat was responsible for their infections could have mistaken rare meat for cooked meat. A temperature of 58 C will kill *T. spiralis* larvae.

Chemicals

The most commonly reported chemical poisoning associated with dishes containing meat in recent years was the so-called Chinese restaurant syndrome, caused by ingestion of excessive amounts of monosodium glutamate. Niacin added to ground meat to mask deterioration caused brief episodes of flushing and itching of the skin, a feeling of warmth, puffing of face and knees and abdominal discomfort to some persons.

Three children of a family developed ataxia, incoordination, loss of vision and depressed consciousness (*47,48*). Three other family members were asymptomatic. The asymptomatic mother gave birth to a blind child whose physical growth was normal, but he was unable to sit up at 1 year of age. Abnormal levels of mercury were detected in serum and hair specimens from the asymptomatic family members as well as from the victims. Epidemiologic investigation disclosed that waste grain had been treated with an organic mercury fungicide and fed to several hogs owned by the affected family. Some of the other hogs had died of an illness characterized by blindness and an unstable gait. One of the hogs was slaughtered and eaten by the family over a period of 3.5 months before the onset of symptoms. A high concentration of mercury was detected in meat from the slaughtered hog, and in samples of grain from which the stricken hogs had been fed.

TYPICAL OUTBREAKS

Meat- and poultry-associated outbreaks that appeared in surveillance reports and professional literature and information about the places of occurrence and factors that contributed to these outbreaks are cited in Table 4. Factors that contributed to these outbreaks are summarized in Table 5.

TABLE 4. *Listing of meat- and poultry-associated outbreaks appearing in surveillance reports and professional literature, 1968-1977.*

Number ill	Vehicle	Place	Contributory factors	Reference
			Botulism	
2	Cooked chicken	Home	Use of leftovers, improper cooling, inadequate reheating	(7)
1	Chopped chicken liver	Processing plant	Inadequate thermal processing	(8)
1	Mikiyak (whale meat)	Home	(Improper fermentation)[1]	(38)
2	Beef stew	Processing plant	Unidentified (inadequate thermal processing)[1]	(71)
3	Beaver tail	Home	Improper fermentation	(77)
1	Beef pot pies	Processing plant/ home	Improper hot holding, inadequate reheating	(90)
			Clostridium perfringens gastroenteritis	
784	Prime rib au jus	Hotel banquet	Holding foods at warm temperatures	(9)
900	Roast beef	Hotel banquet	Contamination by equipment after cooking, holding foods at warm temperatures	(10)
76	Braised beef	School	Prepared one day before serving, improper refrigeration, inadequate reheating	(22)
300	Roast beef	Fast food restaurant	Holding foods at warm temperatures, inadequate reheating	(3)
590	Turkey with gravy	School	Prepared one day before serving, improper refrigeration, inadequate reheating	(6)
25	Turkey with dressing	Airline caterer	Improper thawing of cooked foods	(49)
288	Spaghetti and meat sauce	School	Prepared 2 days before serving, improper refrigeration, inadequate reheating	(59)
146+	Turkey	Buffet meal at factory	Prepared 1 to 3 days before serving, improper cooling, inadequate reheating, improper hot holding	(72)
30	Tenderloin tips	Banquet at restaurant	Prepared 3 days before serving, inadequate reheating	(73)
			Salmonellosis	
300	Roast beef	School	Prepared one day before serving, inadequate cleaning of equipment (slicer), (improper cooling?)	(11)
29	Turkey	Resort restaurant	Prepared one day before serving, (Inadequate cooking, improper cooling), inadequate reheating	(12)
98	Turkey	Church supper	(Inadequate cooking, cross contamination), improper refrigeration	(13)
17	Turkey	Family dinner	Inadequate cooking	(14)
39	Turkey	Restaurant	Inadequate cooking	(15)
37	Smoked ham	Restaurant, (food processor)	(Cross contamination, inadequate cooling)[1]	(16)
73	Flank steak	School	Use of leftovers, inadequate hot-holding, improper construction of cutting boards, inadequate cleaning of equipment	(17)
4	Pork sausage	Home	Prepared several days before serving	(18)
11	Smoked turkey	Processing plant, party	Improper cooling, cross contamination, inadequate reheating	(23)
100	Roast beef	Food processor, church wedding reception	Inadequate cooking, inadequate cooling	(24)
102	Whale meat	Seashore	Ingestion of raw food	(25)
42	Precooked turkey roast	Processing plant	Cross contamination, inadequate reheating, (improper cooling)	(26)
130	Meat balls	Fire company banquet	Prepared one day before serving, inadequate cooking, improper cooling, inadequate reheating	(27)
70	Turkey tetrazzini	Hotel luncheon	Prepared one day before serving, (cross-contamination), inadequate refrigeration, inadequate reheating	(28)
24	Barbecue chicken	Food store	(Inadequate cooking, improper hot-holding)	(29)
526	Turkey salad	Restaurant, national convention	Inadequate cooking, improper cooling	(30)
128+	Turkey	Catered party	Inadequate cooking, improper cooling	(39)
303+	Barbecue pork	Restaurant	Improper storage of raw and cooked pork, inadequate cleaning of equipment	(40)
68+	Cornish hen	Church	(Improper refrigeration)[1]	(41)
130	Cold cuts and other foods	Restaurant	Cross-contamination, inadequate cleaning of equipment (wooden table)	(42)
201	Chickens	Church supper	Inadequate cooking, improper cooling	(43)
35	Turkey	Institutional food service	Prepared one day before serving, inadequate reheating, (inadequate cooling)	(44)
3	Beef jerky	Meat processing plant	Inadequate thermal processing (drying)	(45)
65	Chicken salad	Home birthday party	Prepared one day before serving, cross-contamination, improper refrigeration	(46)
112	Turkey	Summer camp	Inadequate cooking, prepared 2 days before serving, cross-contamination, inadequate cleaning of equipment, (improper cooling)	(50)

165	Turkey	Restaurant catered dinner	Cross-contamination; inadequate cleaning of equipment (cutting board); (improper cooling)	(*51*)
68	Barbecue pork	Restaurant catered dinner (multiple outbreaks)	Cross-contamination; inadequate cleaning of equipment (cutting board); (improper cooling)	(*51*)
54	Escalloped chicken	Nursing home	(Infected worker touching cooked food)	(*52*)
1	Pork/moose-meat salami	Home	Ingesting raw meat	(*53*)
432	Raw ground beef	Homes	Higher attack rates with increased hamburger meals and in those who ate raw beef	(*59*)
17	Chicken salad	Home	Prepared one day before serving	(*61*)
191+	Barbecue brisket	College	Prepared 2 days before serving, inadequate cooking, (improper cooling)	(*66*)
4	Head cheese (pork)	Meat processor	(Inadequacy of cooking?)	(*67*)
57	Roast beef sandwiches	Roadside sandwich bar	Inadequate cleaning of equipment (storage pans)	(*68*)
200+	Precooked roast beef in gravy	Meat processing plant, caterer, parties in homes	Prepared one day or more before serving	(*69*)
468	Turkey salad	School	Prepared one day or more before serving, (inadequate hot-holding)[1], improper cooling	(*74*)
61	Turkey	Church dinner	Prepared one day or more before serving, inadequate cooling, (inadequate cooking)[1]	(*78*)
11	Roast beef sandwiches	Processing plant	Unidentified	(*79*)
43	Raw ground beef	Meat processing plant	Significant association with eating raw meat and purchase from same food chain	(*80,103*)
39	Precooked roast beef	Meat processing plant	Unidentified (inadequate cooking)[1]	(*85*)
7	Precooked roast beef	Meat processing plant	Unidentified (inadequate cooking)[1]	(*85*)
1+	Precooked roast beef	Meat processing plant	Unidentified (inadequate cooking)[1]	(*85*)
20	Precooked roast beef	Meat processing plant	Unidentified (inadequate cooking)[1]	(*85*)
15	Prime rib of beef barbecue ribs	Restaurant	Improper warm storage, improper cooling, (cross-contamination)	(*86*)
43	Precooked roast beef	Meat processing plant	Improper cooking, (additional factors not determined)	(*87*)
181	Precooked roast beef	Meat processing plant	Improper cooking, (additional factors not determined)	(*91,92*)
11	Green spaghetti containing meat	Caterer and vending machine	Inadequate refrigeration, infected worker, prepared one day or more before serving	(*93*)
3	Hot dogs	Fast food establishment	Improper hot-holding, inadequate reheating	(*94*)
41	Precooked roast beef	Meat processing plant	Improper cooking, (additional factors not determined)	(*97*)
			Shigellosis	
440	Turkey salad	School	Unidentified	(*54*)
176	Spaghetti with meat sauce	Military mess	Infected worker, improper holding, inadequate reheating	(*95*)
			Staphylococcal intoxication	
1,364	Chicken salad	Central kitchen for schools	Improper cooling, (infected worker)[1]	(*19*)
143	Ham	Caterer for hospital	Improper cooling, overcrowding, and sanitary deficiencies	(*20*)
28	Pineapple-glazed ham	Food store	Improper hot-holding, infected worker	(*31*)
21	Turkey	Charity bazaar	Prepared one day before serving, (improper cooling)[1], improper hot-holding, handling of cooked food	(*32*)
150	Roast beef	Caterer	Prepared one day before serving, infected worker, (cross-contamination)[1], improper cooling	(*33*)
36	Roast turkey			
800	Barbecue pork	Picnic	Prepared 3 days before serving, improper refrigeration, inadequate reheating, improper hot-holding, infected worker	(*34*)
93	Barbecue pork	Two restaurants of chain	Prepared one day before serving, improper cooling, excessive handling of meat, inadequate hot-holding, infected worker	(*35*)
350	Ham	Community center	Prepared one day before serving, inadequate cooling, improper reheating, inadequate hot-holding, infected worker	(*55*)
7	Genoa salami	Homes	Undetermined	(*56*)
13	Genoa salami	Homes	Undetermined	(*57*)
5	Genoa salami	Homes	Undetermined	(*58*)
1	Uncooked bacon	Meat processing plant	Abscess on bacon	(*62*)

3	Ham	Restaurant	Infected worker	(63)
60	Ham	Restaurant-catered picnic	Prepared one day before serving, inadequate cooling, infected worker	(64)
1	Deviled eggs	Home	Improper cooling, prepared 5 days before serving	(65)
6	Ham	Home	Use of leftovers, improper hot-holding	(70)
196	Ham	Airline caterer caterer	Prepared one day before serving, room temperature storage, improper cooling, infected worker	(81)
81	Chicken salad	Restaurant	Prepared 2 days before serving, improper cooling, infected worker, cross-contamination	(82)
8	Italian dry salami	Meat processing plant	Unidentified	(83)
200	Ham	Restaurant-prepared box lunches on train	Prepared 2 days before serving, (improper cooling, infected worker)[1]	(88)
126	Chicken salad	Church luncheon	Prepared 2 days before serving, improper cooling, infected workers	(89)
			Typhoid fever	
4	Submarine sandwiches (meatball and salami)	Restaurant	Infected workers	(36)
			Taeniasis	
1	Rare and raw beef	Home	Eating rare and raw beef	(21)
			Toxoplasmosis	
2	Raw beef dish	Wedding party	Eating raw meat	(84)
			Chemicals Poisonings	
3	Pork	Home	Feeding animals mercury-contaminated grain, eating sick animals	(48)
			Hepatitis A	
66	Sandwiches	Restaurant	Infected person	(75)
105	Cold meat sandwiches	Department store restaurant	Infected persons touching foods	(76)
18	Luncheon meat submarine sandwiches	Group gathering	Infected person	(96)

[1]Suspected factor but either not proved or not stated in report.

TABLE 5. *Factors that contributed to outbreaks of meat- and poultry-borne diseases (summary of 88 outbreaks, citing such data from Table 4).*

Contributory factor	Confirmed	Suspected	Total	Percent
Improper cooling of cooked foods	30	12	42	48
Prepared a day or more before serving	30	0	30	34
Inadequate cooking or thermal processing	14	10	24	27
Infected person touching cooked food	16	4	20	23
Inadequate reheating of cooked and chilled foods	18	0	18	20
Improper hot storage of cooked foods	16	1	17	19
Cross-contamination of cooked foods from raw foods	9	4	13	15
Inadequate cleaning of equipment	9	1	10	11
Ingesting raw products	4	3	7	8
Use of leftovers	3	0	3	3
Improper fermentation	1	1	2	2
Improper thawing of cooked foods	1	0	1	1
Improper construction of equipment	1	0	1	1
Inadequate processing/preparation space	1	0	1	1
Abscess on meat	1	0	1	1
Feeding animals mercury-treated grain	1	0	1	1
Eating animals that were sick or dying at slaughter	1	0	1	1

IMPORTANT VEHICLES

The three most common vehicles reported during the 10-year period were ham, turkey and roast beef (Table 3). Ground (cooked) beef, pork, sausage, and chicken were also frequently reported as vehicles. Some typical situations that caused these foods to become vehicles are described.

Roast beef

Many of the listings of beef (unspecified) in Table 3 refer to roast beef. Therefore, if these are added to those for roast beef, it becomes the most important vehicle in foodborne disease outbreaks in the U.S. during the last decade. Most of these outbreaks have been traced to roast beef prepared in foodservice establishments. A few

outbreaks of salmonellosis, however, have been traced to commercially prepared, precooked roast beef. (See Table 4).

Precooked roast beef prepared in processing plants. Precooked roasts are often fabricated by putting cuts of beef in plastic casings. They are cooked either on racks in ovens or smoke chambers, in tanks filled with water at 76 C or higher for a few hours, or in smokehouses that are equipped with facilities for injecting steam. Sometimes the roasts are rewrapped after heat processing. In other operations, rounds of beef are roasted in ovens and then wrapped. Afterwards these roasts are frozen and sold to caterers and certain types of foodservice operations, such as delicatessens, where they are thawed and usually sliced for sandwiches.

The roasts that were involved in the outbreaks had not been exposed to time-temperature combinations that would have killed salmonellae. Cross-contamination during packaging or repackaging could have introduced salmonellae after heat processing, however, in some operations. No doubt, although unreported, there were periods during which the roasts were held within a temperature range at which salmonellae could multiply, either in the processing plant before freezing (if frozen) or in delicatessens or other establishments while on slicing machines or during preparation and storage.

As a result of the outbreaks traced to precooked roast beef, the U.S. Department of Agriculture issued an emergency order to heat-process all roasts so that their geometric centers reached 62.8 C. Following results of research, this requirement was modified to include 15 time-temperature combinations for heat treatment (including 53.3 C for 121 min, 57.2 C for 37 min, 60 C for 12 min and 62.2 C for 5 min) which could be used as alternate methods (*106,113*). These time-temperature combinations are based on a 7*D* reduction of six serotypes of *Salmonella* in ground meat implanted in the center of roasts. Cooking to comply with these values, however, will not control post-processing contamination during wrapping or other handling. These time-temperature combinations will probably permit the survival of *S. aureus* if large numbers are present, and spores of *C. perfringens* will definitely survive in and on roast cooked according to these standards.

Roast beef prepared in food service establishments. The above-mentioned time-temperature combinations for cooking beef in processing plants will also kill salmonellae in cuts of beef cooked in foodservice operations, but they will probably do little to prevent foodborne illness associated with roast beef in these establishments. The operations after cooking--hot holding, cooling, reheating--when improperly done, are the most important factors that contribute to outbreaks caused by *Salmonella, C. perfringens*, staphyloenterotoxin, and other agents. During a survey of roast beef preparations in several foodservice establishments, hot-holding procedures would have allowed the survival of vegetative pathogens in the geometric center of 26% and on the surface of 43% of the roasts examined. During cooling, growth of these organisms would have been possible in the geometric center of 83% and on the surface of 79% of the roasts. During reheating, there was a potential for the survival of these organisms in the geometric center of 90% and on the surface of 10% of the roasts (*5*). In these situations, if spores of *C. perfringens* survived cooking, or if they or other pathogens (e.g., staphylococci or salmonellae) were introduced after cooking, outbreaks of foodborne disease could follow. Microbiological surveys in foodservice establishments have shown that *C. perfringens* either survived cooking or reached roast beef after cooking (*3*).

Ground beef

This category includes cooked hamburgers, meatballs, meatloaf, cooked ground beef for Mexican-style foods (e.g., tacos, enchilladas, beef burritos) as well as raw ground meat. Pathogens either can survive cooking or be introduced into the products after they have been cooked. Some of these cooked products are frequently kept in hot-holding devices for several hours. When there are leftovers, they are frequently put into large pots for refrigerated storage. Reheating, if done, is often inadequate to kill the pathogens that multiply during warm or cold storage.

Epidemiologic association has been made between ingestion of raw hamburger and the incidence of salmonellosis caused by tartrate-negative, phagetype 2 strains of *Salmonella typhimurium* (*60*). An association was also made between the frequency of infection by this strain and the frequency of home meals that included hamburger. Groups of persons who had eaten 12 or more hamburger meals per month had statistically significantly higher infection rates than groups that had eaten only four to eight hamburger meals per month. Cooked hamburgers, nevertheless, are rather unlikely vehicles, because they are usually thoroughly cooked and eaten shortly afterwards. Leftovers are not bulky and, if refrigerated, cool rapidly, thereby thwarting the multiplication of any pathogens that survived cooking. The increased rate of illness associated with consumption of hamburgers could have been caused by eating raw or rare hamburgers that happened to contain large numbers of salmonellae. Such gross contamination, however, is uncommon, and, if the meat had been refrigerated, it probably would be accompanied by a large number of competing psychrotrophic bacteria. Inadequate cooking of ground-meat dishes (such as meatloaf, meatballs and casserole dishes) which allowed survival of salmonellae, coupled with inadequate refrigeration of leftovers, could also explain the increase in rates. Cross-contamination from raw hamburger (by equipment or by hands) to other foods which were then improperly stored in home kitchens, may have contributed to the increased rate.

Investigation in two states of an outbreak of salmonellosis caused by an antibiotic-resistant strain of *Salmonella newport* revealed a significant association

between illnesses and the purchase of the meat from stores of a particular grocery chain and the eating of raw hamburger. All the implicated ground meat had been prepared in the same processing plant. Some samples of beef collected from stores within the chain revealed *S. newport* of the same antibiotic-resistant characteristic at a level of 8 to 23 organisms per 100 grams of meat (*80,103*).

Sausage

Pork sausage, eaten raw or inadequately cooked, is the most frequently reported vehicle of trichinosis (Table 3). (The significance of this has been discussed under trichinosis.)

During the survey period, a number of outbreaks of staphylococcal intoxication followed the ingestion of dry, fermented sausage, such as Genoa salami. Some of these products are neither cooked nor smoked, and because they are held at temperatures above 18 C for a few days before being dried at temperatures above 7 C for up to 3 months, they are vulnerable to microbial degradation. A high ratio of staphylococci to competitive organisms in raw products and a favorable temperature for growth during fermentation probably enhanced the growth of staphylococci. Factors that can influence the ratio of staphylococci to these competitors are (*a*) use of meat containing staphylococcal lesions or cheeks contaminated by nasal sinuses, (*b*) freezing which kills fewer staphylococci than their competitors and (*c*) bacteriophage attack of starter cultures of lactic acid bacteria (*104*). Staphylococci are salt-tolerant, grow at low a_w values, grow under either aerobic or anaerobic conditions and produce enterotoxin at temperatures necessary for fermentation. Usual concentrations of sodium chloride, nitrite and nitrate, and initial pH and a_w of the ingredients will not inhibit staphylococci. Smoking causes antioxidant and antimicrobial activities, but these are limited to within a few millimeters from the surface. Staphylococcal enterotoxin is probably formed during the early stages of fermentation, because the combination of low pH, high salt, low a_w and lactic acid bacteria of the final product creates a highly inhibitory environment for growth of pathogens and consequently the production of toxins. These products are quite shelf stable.

Cured pork

Ham was one of the most commonly reported vehicles of foodborne illness during the 10-year period covered in Tables 1 and 3. It is also the most common vehicle responsible for outbreaks of staphylococcal intoxication (Table 3). It is a favorite food for group gatherings, parties and picnics. Ham, because of its salt content (2 to 5%) and consequently relatively low water activity, selectively favors growth of *S. aureus*. It is frequently precooked and therefore contains few competitive organisms. Also, it is often prepared in large quantities, sliced (and possibly contaminated) and held at room temperature or refrigerated in large piles or in large pots. Because ham does not readily spoil, it is often subjected to considerable time-temperature abuse.

Turkey

Turkeys are frequently vehicles in foodborne disease outbreaks (Table 3). They are often contaminated with *C. perfringens, Salmonella,* and *S. aureus* when they enter foodservice establishments and homes. If turkeys are inadequately cooked, these organisms survive; *C. perfringens* spores survive even thorough cooking. Contamination also occurs during boning, slicing, chopping or other handling. Because of their bulk, turkeys and piles of turkey meat cool slowly, allowing pathogenic bacteria an opportunity to multiply (*4*). Turkey leftovers are often inadequately reheated, allowing those organisms that survived cooking, or that were introduced after cooking and subsequently multiplied, to survive.

Chicken

Chicken products -- whole or cut-up, in cold salads, and in hot mixtures -- have been reported as vehicles of foodborne outbreaks. Whole or cut-up chickens are usually cooked adequately, but subsequently become contaminated. The bacterial contaminants multiply during improper cold or hot storage. The cold salads are usually contaminated (frequently with *S. aureus*) during boning, slicing, chopping and mixing. Multiplication (toxin formation in the case of *S. aureus*) occurs during storage at room or warm, outside temperatures or in large pots or bowls during refrigerated storage. Hot mixtures, such as chicken á la king, pot pies or casseroles, are often made of chicken leftover from a previous meal. These leftovers are often stored inadequately which permits bacterial growth, and subsequent inadequate cooking, or reheating allows pathogens to survive.

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20. Bacteriological Evaluation of Retail Ground Beef, Frozen Patties, and Cooked Hamburger, JFP, vol. 40, June, 1977.
21. Bacteriological Quality of Raw and Cooked Hamburger at Retail Level in Tehran, JFP, vol. 40, August, 1977.
22. Satellite Food Service System: Time, Temperature, Microbiological and Sensory Quality of Pre-cooked Frozen Hamburger Patties, JFP, vol. 40, September, 1977.
23. A Bacteriological Survey of Raw Ground Beef, JFP, vol. 40, November, 1977.
24. Incidence of Salmonellae in Raw Meat and Poultry in Retail Stores, JFP, vol. 41, July, 1978.
25. Fate of Salmonella Inoculated into Beef for Cooking, JFP, vol. 41, August, 1978.
26. Bacterial Populations of Ground Beef, Textured Soy Protein and Ground Beef Extended with Soy Protein After 3-10 Days of Refrigerated Storage, JFP, vol. 41, August, 1978.

B. Delicatessen Foods

27. Microbiology of Delicatessen Salads, JMFT, vol. 38, March, 1975.
28. Bacteriological Quality of Deli Foods (Are Standards Needed?), JMFT, vol. 38, June, 1975.
29. Bacteriological Quality of Selected Deli Foods, JMFT, vol. 38, December, 1975.

C. Barbecued Foods

30. Public Health Problem Associated with Barbecued Foods, JMFT, vol. 36, January, 1973.
31. Microbial Quality of Barbecued Chicken from Rotisseries, JMFT, vol. 37, September, 1974.

D. Fresh Salads

32. Microbiological Survey of 3 Fresh Green Salads, JMFT, vol. 39, February, 1976.
33. Lettuce Salad As A Carrier of Microorganisms, JFP, vol. 41, June, 1978.

E. Shell Eggs

34. Effect of Sweating on Chicken Eggs, JMFT, vol. 36, June, 1973.

*Journal of Milk and Food Technology, *published by IAMFES, Inc., Ames, Iowa, until 1977 when it became the* Journal of Food Protection.
**Journal of Food Protection, *published by IAMFES, Inc., Ames, Iowa, beginning with volume 40, 1977.*

35. Microbiology of Eggs, JMFT, vol. 37, May, 1974.

F. Fish and Seafood Products
36. A Microbial Survey of Fresh and Frozen Seafood Products, JFP, vol. 40, May, 1977.
37. Nematodes in Fresh Market Fish of the Washington, D.C. Area, JFP, vol. 41, August, 1978.

G. Sandwiches
38. Incidence of Salmonella in Sandwiches, JMFT, vol. 36, June, 1973.

H. Miscellaneous Foods
39. Aerobic Microbial Flora of Smoked Salmon, JMFT, vol. 36, March, 1973.
40. Staphylococcus Aureus in Commercially Processed Fluid Dairy and Non-Dairy Products, JMFT, vol. 37, June, 1974.
41. Flavor and Shelf Life of Fluid Milk, JMFT, vol. 37, June, 1974.
42. Destruction of *E. coli* and Salmonella in Microwave Cooked Soups, JMFT, vol. 38, January, 1975.
43. Effect of Thawing on Staphylococcus Growth in Frozen Convenience Foods, JMFT, vol. 38, June, 1975.
44. Botulism in Commercially Canned Foods, JMFT, vol. 38, September, 1975.
45. Survival of Enteric Viruses on Fresh Fruit, JMFT, vol. 38, October, 1975.
46. Streptococci in Dried and Frozen Foods, JMFT, vol 39, June, 1976.
47. Bacterial Spoilage of Citrus Products at pH Lower Than 3.5, JMFT, vol. 39, December, 1976.
48. The Microflora of Parsley, JMFT, vol. 39, December, 1976.
49. Bacteriological Evaluation of Luncheon Meats in Canadian Markets, JFP, vol. 40, June, 1977.
50. Microbiology of Mayonnaise and Salad Dressing: A Review, JFP, vol. 40, June, 1977.
51. Failure of *Clostridium botulinum* to Grow in Fresh Mushrooms Packaged in Plastic Film Overwraps with Holes, JFP, vol. 41, May, 1978.
52. Bacteriological Survey of Raw "Soul Foods," JFP, vol. 41, May, 1978.
53. Occurrence of *Bacillus cereus* and Bacteriological Quality of Chinese Take-out Foods, JFP, vol. 41, June 1978.
54. Survey of Bacterial Populations of Bologna Products, JFP, vol. 41, September, 1978.

I. General Information--Food Microbiology/Procedures
55. Vibrio parahaemolyticus Methodology for Isolation from Seafood and Epidemic Specimens, JMFT, vol. 36, February, 1973.
56. Polychlorinated Biphenyls in Man's Food: A Review, JMFT, vol. 36, March, 1973.
57. Effect of Temperature and pH on Growth and Enterotoxin Production by Staphylococcus Aureus, JMFT, vol. 36, May, 1973.
58. Viruses in Foods, JMFT, vol. 36, June, 1973.
59. Influence of Food Environments on Growth of Staphylococcus Aureus and Production of Various Enterotoxins, JMFT, vol. 36, November, 1973.
60. Microbiological Monitoring of the Food Plant - Methods to Assess Surface Contamination, JMFT, vol. 37, July, 1974.
61. Development and Present Status of FDA Salmonella Sampling and Testing Plans, JMFT, vol. 38, June, 1975.
62. Filth in Foods: Implications for Health, JMFT, vol. 38, July, 1975.
63. Microbiological Criteria for Food (Military and Federal Specifications), JMFT, vol. 39, January, 1976.
64. Practical Implications of Injured Microorganisms in Food, JMFT, vol. 39, February, 1976.
65. Salmonellae in Foods, JMFT, vol. 39, May, 1976.
66. Thermal Stability of Enterotoxins in Foods, JMFT, vol. 39, June, 1976.
67. Enrichment and Plating Methodology for Salmonella Detection in Food: A Review, JMFT, vol. 39, September, 1976.
68. Significance of *Clostridium perfringens* in Processed Foods, JFP, vol. 40, May, 1977.
69. Effects of Freezing and Storage on Microorganisms in Frozen Foods, JFP, vol. 40, May, 1977.
70. Temperatures in Home Refrigerators and Mold Growth at Refrigeration Temperatures, JFP, vol. 40, June, 1977.
71. Survey of Microflora on the Top Surface of Vended Canned Drinks, JFP, vol. 40, August, 1977.
72. Evaluation of Quick Bacterial Count Methods for Assessment of Food Plant Sanitation, JFP, vol. 41, April, 1978.
73. Microbiological Criteria for Food, JFP, vol. 41, June, 1978.
74. A Cellulose Sponge Sampling Technique for Surfaces, JMFT, vol. 38, September, 1975.
75. A Procedure for Profiling Temperatures of Dairy Products in Stores, JMFT, vol. 38, December, 1975.

II. Quality Control

1. A Quality Control Program, JMFT, vol. 37, January, 1974.
2. Charting a Safeness Course for the Foodservice Industry, JMFT, vol. 37, April, 1974.
3. Relation of Code Dates to Quality of Milk Sold in Retail Markets, JFP, vol. 40, February, 1977.
4. Guidelines for a Dynamic Quality Control Program in a Changing Market, JFP, vol. 40, June, 1977.
5. HACCP Models for Quality Control of Entree Production, JFP, vol. 40, September, 1977.
6. Concerns, Experiences, Attitudes and Practices of Executives Regarding Quality Control Procedures, JFP, vol. 41, April, 1978.

III. Food Preparation

1. Cooling Rates of Food, JMFT, vol. 36, March, 1973.
2. Procedures for Preparation and Vending of Barbecued Meats Cooked in Rotisseries, JMFT, vol. 37, March, 1974.
3. A Hot Acid Treatment for Eliminating Salmonella from Chicken Meat, JMFT, vol. 37, May, 1974.
4. Hazard Analysis of Clostridium Perfringens in the Skylab Food System, JMFT, vol. 37, December, 1974.
5. Survival of Clostridium Perfringens During Preparation of Pre-cooked Chicken Parts, JMFT, vol. 38, September, 1975.
6. Biblical Food Processing, JMFT, vol. 39, June, 1976.
7. Cooking Inoculated Pork in Microwave and Conventional Ovens, JMFT, vol. 39, November, 1976.
8. Handling Perishable Foods, JFP, vol. 40, May, 1977.
9. Thermal Destruction of Microorganisms in Meat by Microwave and Conventional Cooking, JFP, vol. 40, July, 1977.
10. Effect of Conventional and Microwave Heating on *Pseudomonas putrefaciens, Streptococcus faecalis,* and *Lactobacillus* plantarum in Meat Tissue, JFP, vol. 40, September, 1977.
11. Heating Patterns of Products in Crockery Cookers, JFP, vol. 40, October, 1977.
12. Cook/Chill Foodservice Systems - Temperature Histories During the Chilling Process, JFP, vol. 40, November, 1977.
13. Time-Temperature Observations of Food and Equipment in Airline Catering Operations, JFP, vol. 41, February, 1978.
14. Composition of Selected Commercial Salads from the Retail Market, JFP, vol. 41, July, 1978.

IV. The Retail Food Industry

1. The Food Industry in the 70's, JMFT, vol. 36, February, 1973.
2. Food Distribution in Today's Consumer Climate, JMFT, vol. 36, April, 1973.
3. Recent Trends in Vending, JMFT, vol. 39, January, 1976.
4. The Federal Food Service Program, JMFT, vol. 39, February, 1976.
5. Food Safety in the 70's, JMFT, vol. 39, March, 1976.
6. Know the Score in Your Food Markets, JFP, vol. 40, January, 1977.
7. Evaluation of State Foodservice Programs, JFP, vol. 40, July, 1977.

8. Economics of a Preventive Public Health Program thru Benefit-Cost Analysis, JFP, vol. 40, August, 1977.
9. The Foodservice Industry of the Future, JFP, vol. 41, June, 1978.
10. Environmental Health Surveillance at the 1976 Festival of American Folklife, JFP, vol. 41, August, 1978.

V. Foodservice Manager Training

1. Certification of Food Service Managers, JMFT, vol. 38, March, 1975.
2. The Canadian National Sanitation Training Program, JMFT, vol. 39, May, 1976.
3. Foodservice Manager Certification - The NIFI Program, JFP, vol. 40, March, 1977.
4. Sanitation Training for Food Service Managers, JFP, vol. 40, March, 1977.
5. Evaluation of A Fast Food Management Training Program, JFP, vol. 40, August, 1977.
6. Education and Training to Prevent Problems in Food Protection in the Nation's Capital, JFP, vol. 41, February, 1978.
7. The Need for Standards in Foodservice Sanitation Education, JFP, vol. 41, April, 1978.

VI. Hospitals

1. Food and Other Sources of Pathogenic Microorganisms in Hospitals, JMFT, vol. 37, October, 1974.
2. Microbiology and Hospital Feeding Systems, JMFT, vol. 39, March, 1976.
3. Hospital Food Service - 1978 and Beyond, JFP, vol. 41, June, 1978.

VII. Cleaning and Sanitization

1. Food Soils, Water Hardness and Alkaline Cleaner Formulations, JMFT, vol. 38, March, 1975.
2. Destruction of Food Spoilage, Indicator and Pathogenic Organisms by Various Germicides, JMFT, vol. 39, December, 1976.
3. Potential Health Hazards Associated with Reusable Foodservice Utensils, JFP, vol. 40, February, 1977.
4. Combined and Individual Effects of Washing and Sanitizing on Bacterial Count of Meat, JFP, vol. 40, October, 1977.

VIII. Energy

1. Energy Conservation in the Food Processing Industry, JMFT, vol. 38, November, 1975.

IX. Special Food Topics

1. Health Foods vs Traditional Foods, JMFT, vol. 36, April, 1973.
2. Fish, Shellfish and Human Health, JFP, vol. 40, October, 1977.
3. Food Ingredient Update, JFP, vol. 41, June, 1978.

X. Foodborne Illness

1. Vibrio Parahaemolyticus: An Introductory Statement, JMFT, vol. 36, February, 1973.
2. Vibrio Parahaemolyticus Gastronitus in MD: Clinical and Epidemiologic Aspects, JMFT, vol. 36, February, 1973.
3. Distribution of Vibrio Parahaemolyticus in the Natural Environment, JMFT, vol. 36, Feburary, 1973.
4. The Water Relations of Foodborne Bacterial Pathogens: A Review, JMFT, vol. 36, May, 1973.
5. Prevention of Foodborne Illness by Time-Temperature Control in School Lunch Kitchens, JMFT, vol. 37, August, 1974.
6. The "New Disease" Status of Human Anisakiasis and North American Cases: A Review, JMFT, vol. 38, December, 1975.
7. The Nematodes That Cause Anisakiasis, JMFT, vol. 38, December, 1975.
8. The Natural History of Anisakiasis in Animals, JMFT, vol. 39, January, 1976.
9. The Public Health Implications of Larval Thynnascaris Nematodes from Shellfish, JMFT, vol. 39, January, 1976.
10. Foodborne Disease Outbreaks Traced to Poultry (1966-1974), JMFT, vol. 39, December, 1976.
11. Diseases Transmitted by Foods Contaminated by Wastewater, JFP, vol. 40, January, 1977.
12. Trends in Foodborne Salmonellosis Outbreaks, (1963-1975), JFP, vol. 40, November, 1977.

XI. Special Retail Food Programs

1. Oregon's Experience with Microbiological Standards for Meat, JMFT, vol. 38, August, 1975.
2. A Retailer's Experience with the Oregon Bacterial Standards for Meat, JMFT, vol. 38, August, 1975.
3. Planning Food Protection During the Bicentennial Celebration in the Nation's Capital, JMFT, vol. 39, February, 1976.
4. Microbiology of Mass Feeding Systems, JMFT, vol. 39, March, 1976.
5. Safety in A University Feeding Systems, JMFT, vol. 39, March, 1976.
6. Food Protection in Jails and Prisons, JFP, vol. 40, March, 1977.

XII. Miscellaneous Topics Related to Food Retailing

A. Personnel
   1. Evaluation of a Survey Officer, JMFT, vol. 36, February, 1973.
   2. Comparison of Bacterial Flora on Hands of Personnel Engaged in Food and Non-Food Industries, JMFT, vol. 38, November, 1975.

B. Equipment
   1. Report of the Committee on Food Equipment Sanitary Standards (1973-1974), JMFT, vol. 38, May, 1975.
   2. Evaluation of Household Dishwashing Machines, JMFT, vol. 38, September, 1975.
   3. Health Profession's Attitudes toward Single-Use Food and Beverage Containers, JFP, vol. 40, February, 1977.
   4. Foodservice Equipment - Technological Trends, JFP, vol. 41, June, 1978.

C. Packaging
   1. Effect of Selected Coating Materials on the Bacterial Penetration of the Ovian Egg Shell, JMFT, vol. 36, May, 1973.
   2. A Comparison of Vacuum Packaging Systems and Films on the Physical Characteristics of Beef Cuts, JMFT, vol. 39, November, 1976.
   3. Effect of Various Types of Vacuum Packages and Length of Storage on the Microbial Flora of Wholsale and Retail Cuts of Beef, JMFT, vol. 39, November, 1976.
   4. Trends in Food Packaging for Foodservice, JFP, vol. 41, June, 1978.

D. Transportation
   1. Food Protection During Distribution, JMFT, vol. 36, August, 1973.

Coming Events/News and Events

Feb. 24-29---TENTH ENVIRONMENTAL ENGINEERING IN THE FOOD PROCESSING INDUSTRY CONFERENCE. Asilomar Conference Grounds, Pacific Grove, CA. Sponsored by the Engineering Foundation. Fee: $250, double occupancy, $300 single occupancy. Contact: Engineering Foundation, 345 E. 47th St., New York, NY 10017, 212-644-7835.

Feb. 26-27--KENTUCKY ASSOCIATION OF MILK, FOOD AND ENVIRONMENTAL SANITARIANS, EDUCATIONAL CONFERENCE. Stouffer's Inn, Louisville, KY. Contact: Dale Marcum, 110 Hanley Lane, Frankfort, KY 40601.

March 3-5--GOOD MANUFACTURING PRACTICES FOR THE FOOD INDUSTRY, shortcourse. Sponsored by the Center for Professional Advancement. Contact - Mary Sobin, Dept. NR, Center for Professional Advancement, P.O. Box H, East Brunswick, NJ 08816, 201-249-1400.

March 4-5--VIRGINIA ASSOCIATION OF SANITARIANS AND DAIRY FIELDMEN, DAIRY INDUSTRY WORKSHOP. Donaldson Brown Continuing Education Center, VPI & SU, Blacksburg, VA. Contact: Marshall Cooper, 116 Reservoir St., Harrisonburg, VA 22801.

March 10-12--STATISTICAL QUALITY CONTROL SHORT COURSE FOR THE FOOD PROCESSING INDUSTRY: METHODS AND TECHNIQUES. University of California, Davis. Registration fee: $130. Contact: Robert C. Pearl, Food Science & Technology Dept., Univ. of California, Davis, CA 95616, 916-752-0980.

March 12-14--STATISTICAL QUALITY CONTROL SHORT COURSE FOR THE FOOD PROCESSING INDUSTRY: APPLICATIONS AND DECISION-MAKING. University of California, Davis. Registration fee: $140. Fee for attending this and course listed above is $185. For further information, see entry above.

March 17-19--FOOD MICROBIOLOGY. Three-day course, sponsored by Center for Professional Advancement. Central New Jersey location for course. Registration fee: $490. Contact: Mary Sobin, Information Services, PO Box H, Center for Professional Advancement, PO Box 964, East Brunswick, NJ 08816.

March 17-19--CANADIAN FOOD PLANT SANITATION SEMINAR. Toronto, Ont. Sponsored by American Institute of Baking and Bakery Council of Canada. Contact - Carol Lyon, AIB, 1213 Bakers Way, Manhattan, KS 66502, 913-537-4750.

March 19--INDIANA DAIRY INDUSTRY CONFERENCE. Purdue University. Joint Annual Meeting of the Dairy Technology Societies of Indiana will be at The Trails following Conference. Contact: James V. Chambers, Dairy Technology Extension Specialist, Animal Sciences Dept., Purdue University, West Lafayette, IN 47907.

March 24--IOWA ASSOCIATION OF MILK AND FOOD SANITARIANS, ANNUAL MEETING. Gateway Center Motel, Ames. Contact: Bill LaGrange, Dept. of Food Technology, Iowa State University, Ames, IA 50011.

March 24-26--FOOD MICROBIOLOGY. shortcourse. Sponsored by the Center for Professional Advancement. Contact - Mary Sobin, Dept. NR, Center for Professional Advancement, PO Box H, East Brunswick, NJ 08816, 201-249-1400.

March 24-28--MIDWEST WORKSHOP IN MILK AND FOOD SANITATION. The Ohio State University, Columbus, OH. Contact - J. Lindamood, Dept. of Food Science and Nutrition, 2121 Fyffe Rd., The Ohio State University, Columbus, OH 43210.

March 25--DAIRY INDUSTRY CONFERENCE. Scheman Building, Iowa State University, Ames, IA. Contact - V. H. Nielsen, Dept. of Food Technology, Iowa State University, Ames, IA 50011.

March 25-26--9th ANNUAL WESTERN FOOD INDUSTRY CONFERENCE, Freeborn Hall, Univ. of California, Davis, CA. Contact: John C. Bruhn, Extension Food Technologist, 101 Cruess Hall, Univ. of California, Davis, CA 95616, 916-752-2192.

March 25-27--FLORIDA ASSOCIATION OF MILK, FOOD AND ENVIRONMENTAL SANITARIANS, ANNUAL MEETING, in conjunction with University of Florida. Student Union, Univ. of Florida, Gainesville, FL. Contact: W. A. Brown, 508 Mayo Bldg., Tallahassee, FL 32304, 904-487-1450.

March 26--ONTARIO FOOD PROTECTION ASSOCIATION, ANNUAL MEETING. Holiday Inn, 970 Dixon Road, Toronto, Ont. Contact: Gail Holland, Meat Packers Council of Canada, 5233 Dundas St. W., Islington, Ont. M9B 1A6.

Mar. 26-28---CONFERENCE ON WASTEWATER TREATMENT TECHNOLOGIES FOR THE CONTROL OF TOXIC/HAZARDOUS POLLUTANTS. Stouffer's Cincinnati Towers, Cincinnati, OH. Contact: Kenneth A. Dostal, IERL-Ci, EPA, Cincinnati, OH 45268.

March 26-28--GOOD MANUFACTURING PRACTICES (GMP) FOR THE FOOD INDUSTRY. Three-day course, sponsored by Center for Professional Advancement. Central New Jersey location for course. Registration fee: $490. For further information, see March 17-19 entry.

Mar. 27-28---1980 MEAT INDUSTRY RESEARCH CONFERENCE. Ramada O'Hare Inn, Chicago, IL. Contact: Dr. John Birdsall, Director of Scientific Activities, American Meat Institute, P.O. 3556, Washington, DC 20007.

March 31-April 2--1980 AMERICAN CULTURED DAIRY PRODUCTS INSTITUTE'S "KULTURES AND KURDS KLINIC." Hilton Airport Plaza Inn, Kansas City, MO. National judging contest will be held in conjunction with Klinic. Contact: Dr. C. Bronson Lane, ACDPI, PO Box 7813, Orlando, FL 32854 or Margie Franck, ACDPI, 910 17th St., NW, Washington, DC 20006.

April 7-10--MISSOURI MILK, FOOD AND ENVIRONMENTAL HEALTH ASSOCIATION, ANNUAL MEETING. Ramada Inn, Columbia, MO. Contact: Erwin Gadd, Missouri Division of Health, PO Box 570, Jefferson City, MO 65102, 314-751-2335.

April 13-16--SECOND ANNUAL CONFERENCE ON INDUSTRIAL ENERGY CONSERVATION TECHNOLOGY. Hyatt Regency Hotel, Houston, TX. Co-sponsored by Texas Industrial Commission and the U.S. Dept. of Energy. Contact: M. A. Williams, Technical Program Director, or Gerald Brown, Executive Director, Texas Industrial Commission, Box 12728, Austin, TX 78711, 512-472-5059.

April 14-16---5th ANNUAL FOOD SERVICE SYSTEMS SEMINAR AND EXPOSITION. Sheraton O'Hare Hotel, Chicago, IL. Contact: G. E. Livingston, Food Science Associates, Inc., 595 Fifth Avenue, New York, NY.

April 17-18--ENVIRONMENTAL LAWS & REGULATIONS COURSE. Stouffer's Riverfront Tower Hotel, St. Louis, MO. Two-day course for professionals new to environmental laws field. Contact: Government Institutes, PO Box 5918, Washington, DC 20014, 301-656-1090.

April 21-25---SCANNING ELECTRON MICROSCOPY/1980. McCormick Inn, Chicago, IL. Contact: O. Johari, Scanning Electron Microscopy Inc., P.O. Box 66507, AMF O'Hare, IL 60666.

April 22--UPDATE 80, PROCESS VALIDATION. Toronto, Ont. Sponsored by Toronto Pharmaceuticals Sub-group of the Chemical Institute of Canada. Contact: S. J. Smith, Health Protection Branch, Health and Welfare Canada, 2301 Midland Ave., Scarborough, Ont. M1P 4R7.

April 23-25--55th ANNUAL MEETING, AMERICAN DRY MILK INSTITUTE and 9th ANNUAL MEETING, WHEY PRODUCTS INSTITUTE. Chicago Marriott O'Hare Hotel, 8535 West Higgins Road (at O'Hare Airport), Chicago, IL. Contact: Dr. Warren S. Clark, Jr., Exec. Directo, ADMI and WPI, 130 N. Franklin St., Chicago, IL 60606.

April 27-May 1--ISF/AOCS WORLD CONGRESS. Joint meeting of American Oil Chemists' Society and the International Society for Fat Research. New York Hilton, New York City. Contact - American Oil Chemists' Society, 508 S. Sixth St., Champaign, IL 61820.

April 30---USE AND ABUSE OF FOOD SUBSTANCES---TECHNOLOGICAL AND HEALTH IMPLICATIONS, Pick Congress Hotel, Chicago. Sponsored by Illinois State Medical Society, Chicago Nutrition Association and Chicago Section of Food Technologists. Contact: Therese Mondeika, 312-751-7624.

May 13-15--SOUTH DAKOTA ENVIRONMENTAL HEALTH ASSOCIATION, ANNUAL EDUCATION CONFERENCE. Huron, SD. Contact: Cathy Meyer, Box 903, Mitchell, SD 57301, 605-996-8231 or 605-996-4131.

May 15-16--ENVIRONMENTAL LAWS & REGULATIONS COURSE. Hotel Washington, Washington, D.C. Two-day course on latest regulatory developments. Contact: Government Institutes, PO Box 5918, Washington, DC 20014, 301-656-1090.

June 2-4--USE OF ANIMAL PRODUCTS IN HUMAN NUTRITION. Iowa State University. Three-day symposium sponsored by the Nutrition Foundation, Inc. and Iowa State University Nutrutional Sciences Council. Contact: Dr. Donald C. Beitz, 313 Kildee Hall, Iowa State University, Ames, IA 50011, 515-294-2063.

June 2-5--PENNSYLVANIA DAIRY FIELDMEN'S CONFERENCE. Keller Conference Center, The Pennsylvania State University, University Park, PA 16802. Contact: William Killough, RD 1, Box 393, Conestoga, PA 17516.

June 15-18--75th ANNUAL MEETING, AMERICAN DAIRY SCIENCE ASSOCIATION. Virginia Polytechnic Institute and State University, Blacksburg, VA. Further details will be available once the program is finalized.

June 15-18--AMERICAN SOCIETY FOR AGRICULTURAL ENGINEERS, Summer Meeting. Convention Center, San Antonio, TX. Theme, "Managing Resources in Transition: Agriculture's Challenge for the 80's." Contact: Roger R. Castenson, ASAE, 2950 Niles Road, Box 410, St. Joseph, MI 49085, 616-429-0300.

July 6-11-XI INTERNATIONAL SYMPOSIUM ON PSYCHROTROPHIC MICROORGANISMS IN SPOILAGE AND PATHOGENCITY. Aalborg Universitetscenter, Sohngaardsholmsvej 57, Aalborg, Denmark. Sponsored by International Association of Microbiological Societies, Committee on Food Microbiology and Hygiene. Contact: Secretariat, IAMS, XI International Symposium, Institute of Hygiene and Microbiology, Royal Veterinary and Agricultural University, Bulowsvej 13, DK-1870, Copenhagen V, Denmark.

July 26-31--IAMFES ANNUAL MEETING, Red Carpet Hotel, Milwaukee, WI. Meeting will be held concurrently with Annual Educational Conference of the National Environmental Health Association. Contact: Earl Wright or Jan Richards, IAMFES, PO Box 701, Ames, IA 50010, 515-232-6699.

Aug. 4-8--ADVANCES IN FOOD AND APPLIED MICROBIOLOGY. Summer course, Massachusetts Institute of Technology, Cambridge, MA 02139. Contact: Director of Summer Session, RM E19-356, MIT, Cambridge, MA 02139.

Sept. 29-30--CALIFORNIA ASSOCIATION OF DAIRY, MILK SANITARIANS, ANNUAL MEETING. Sacramento Inn, Sacramento, CA. Contact: John C. Bruhn, Extension Food Technologist, 101 Cruess Hall, Univ. of California, Davis, CA 95616, 916-752-2192.

Oct. 6-8--ENERGY AND THE FOOD INDUSTRY. International Symposium sponsored by Commission Internationale des Industries Agricoles et Alimentaires (CIIA), Madrid, Spain. Contact: CIIA, B.P. 470-08, 75366 Paris Cedex 08.

Oct. 8-9--NEBRASKA DAIRY INDUSTRIES ASSOCIATION, 26th ANNUAL CONVENTION. Regency West, I-680 and Pacific Street, Omaha, NE. Contact: T. A. Evans, Exec. Secretary, 116 Filley Hall, East Campus, University of Nebraska-Lincoln, Lincoln, NE 68583.

Human *Salmonella* Isolates Summarized for 1978

Isolates of *Salmonella* from humans increased about 5% in 1978 over the previous year. This is according to figures reported to the Center for Disease Control in Atlanta, GA.

Massachusetts and Pennsylvania reported increased isolations amounting to almost three-fifths of the overall increase, and increases in *S. enteritidis* and *S. heidelberg* together accounted for almost three-fifths of the total increase. Almost

Age distributions of persons for whom isolates were obtained followed a well-established pattern, the rate highest for infants of about 2 months of age. The rate decreased dramatically through early childhood and held a constant from age 6 half the increased cases attributed to *S. enteritidis* occurred in Massachusetts alone, with the remaining occurring in New York and California. There was no particular state which showed a significantly increased isolation of *S. heidelberg.*

through adulthood.

Ten serotypes which were most frequently reported accounted for three-fourths of the total isolates. These serotypes were *S. typhimurium, S. heidelberg, S. enteritidis, S. newport, S. infantis, S. agona, S. montevideo, S. typhi, S. saint-paul, S. javiana. S. typhimurium* alone accounted for almost 35% of the isolates.

Excerpted from the CDC's *Morbidity and Mortality Weekly Report,* Vol. 28, No. 51, January 4, 1980.

Letters to the Editor

Physical - chemical basis lacks for discussion of results

DEAR SIR:

The article by Clydesdale et al. (J. Food Prot. 42:225-227) is of interest to many food scientists. It lacks however, in terms of critical evaluation of the literature with respect to the physical-chemical basis for the discussion of the results.

One of the first critical evaluations of the loss of natural pigments as a function of water activity (a_w) was by Martinez et al. (J. Food Sci. 33:241, 1968). They showed that oxygen was not a limiting factor but rather diffusion of reacting species was the controlling factor. It was postulated later (Labuza, Crt. Rev. Food Technol. 3:335, 1971) that oxidation reactions should follow a first order plot (i.e. log amount vs. time) and that the rate constant should decrease as a log function with respect to a_w. Based on this, it is not surprising the authors got the results they did. What is unfortunate is that they did not utilize good experimental design in which they could have collected information on the rate constants as a function of both temperature and a_w. Their study is lacking as was the one done by von Elbe on betanine. Lastly, they completely misunderstand basic engineering principles in packaging dynamics as has been extensively published by the Karel group (the classical papers are: Mizrahi et al., J. Food Sci. 35:799, 1970) and Simon et al. (J. Food Sci., 35:749, 1970). What they showed is that dehydrated food systems in packaging materials must be treated as an unsteady state system, in terms of shelf life prediction. The authors of the present paper do not seem to understand this.

Specifically, the following points should have been considered by the authors:

(1) In doing the isotherms, the data should be reported as g H_2O/100 g dry solids, not % moisture which can be confused with wet basis. The number of replicates is not given.

(2) It is imperative that the time to equilibrium be reported, especially for systems that may contain amorphous state carbohydrates, since incongruities may show up as is found for whey powder and milk powder (Berlin - USDA Eastern Regional lab has published over 40 papers on this).

(3) Figure 1 does not show an isotherm, it shows pigment loss.

(4) The authors never reported the WVTR's from Fig. 4 and 5 or stated the published WVTR values or how many replicates were used. The method is simple since weight gain for desiccant in a pouch can be considered to be steady-state for a short time.

$$\frac{\Delta W}{\Delta \Theta} = \text{slope} = \frac{k}{x} A (p_{out} - p_{in})$$

p_{out} = outside vapor pressure at 100 F
= 49.2 mm Hg × 90% RH/100 = 44.2 mm Hg

p_{in} = 0 since desiccant in bag

A = area of pouch - this was never reported. In my calculations below I used a 3 × 5" package, therefore A = 0.0193 m^2.

Therefore, for foil laminate (Fig. 3):

$$\frac{k}{x} = \frac{10}{4.2} \bullet \frac{1}{44.2} \bullet \frac{1}{0.019} = 2.8 \text{ g } H_2O/\text{day m}^2 \text{ mm Hg}$$

WVTR = 125 g H_2O/day m^2

These calculations make the data for Fig. 3 very suspect since foil should have a k/x of less than 0.01. The data for the polycoated paper are more reasonable and are within the published range. The authors basically used the same equation but it is written very confusingly.

$$E = P.A.t.sP$$

The term sP is never defined and I am not sure what it is. Their data also show that after a certain time the line is no longer linear and unsteady state conditions prevail. In actuality there was no need to publish the graphs of weight vs. time since it is just a standard method.

(5) The data from Fig. 1 if plotted as ln (A) vs. time (the figure legends are done in a very unacceptable manner, why is there no regular scale from which others can make some sense) gives a straight line as attached. From this the rate constants can be found: for example, in the strawberry mix the rate constant is 0.216 mg/week which is 0.03 mg/ml/week. I am not sure what per ml means or what ambient on the graph means.

(6) The discussion on page 226 (left bottom to next column) is overdrawing conclusions and shows the authors' lack of understanding of sorption. One cannot compare apples and oranges as they are doing. The sytems do not have the same amount of added coloring mixture per 100 g of cherry beverage mix. There also is no discussion of how much red #2 was added. However, what it shows, as would be expected, the increased gain occurs at 40-50% RH for the added spray dried colors. This is typical of any amorphous form carbohydrate since it has a very open structure, as compared to the solid crystalline sugar that the cherry mix is made of. The latter shows a large gain at about 70% RH, as would be expected. The authors should have consulted and quoted the literature on this. What is obvious from the discussion is that caking occurred, thus there was an amorphous to crystalline change and thus the weight change in doing the isotherms should show a discontinuity when moisture vs. time data are collected.

(7) Taking their data for the isotherm and the permeance from Figure 4, one can calculate the shelf life for a given external condition using the unsteady state equation:

$$\frac{dw}{d\Theta} = \frac{k}{x} A (p_{out} - p_{in})$$

However, since the product gains moisture and thus p_{in} increases with time, dw/dΘ is not constant as the authors mistakenly presume and state (left column, bottom 227). Since they do not report the initial moisture content of the product nor the external conditions, their 200 days is a meaningless value, especially since the foil gains 125 g H_2O/day/m^2. If the cherry pouch contained 12 g and was initially at zero g H_2O/100 g and went to 1.5 g H_2O/100 g to become unacceptable, then using their incorrect method (for poly-paper):

$$\text{shelf life} = \frac{12(0.015)}{10.5 \text{ g} \times 0.019} = \frac{(\text{wt solids})\,(\text{g } H_2O/\text{gained/g solids})}{\text{WVTR} \times \text{area}}$$

$$= 1 \text{ day}$$

I don't know where in the world they got 200 days unless the package was 200 times smaller, i.e., 0.3 in. by 0.25 in., which is impossible. In addition, the pouch with more mix, i.e., strawberry, should have a longer shelf life. Using the unsteady state and integrating, the equation above becomes (if a straight line isotherm is assumed):

$$\ln \frac{m_e - m_i}{m_e - m} = \frac{k}{x} \frac{A}{w_s} \frac{p_o}{b} \Theta$$

m_e = moisture if held with no pouch in external atmosphere (at 70 F 50% RH) = 4 g/100 g

m_i = initial moisture = 0

k/x = as before

w_s = weight dry solids in pouch = 12.0

b = isotherm slope = 0.08 g/100 g

p_o = vp H_2O at 70 F = 18.8 mm Hg

m_c = their cmc = 1.5 g/100 g

$$\ln \frac{4 - 0}{4 - 1.5} = 0.47 = 0.238 \bullet \frac{0.019}{12} \bullet \frac{18}{0.08} \Theta$$

Θ = 5.5 days

It is thus obvious that the reported data for WVTR represent pouches that were leaking since this is too short a shelf life. Cutting the package in half would only increase the shelf life to 10 days.

Overall, I feel that this paper attempted something useful; however, the authors should have consulted with experts in the field with respect to analysis of their data, especially with respect to shelf life predictions.

THEODORE P. LABUZA
Department of Food Science and Nutrition
University of Minnesota
St. Paul, Minnesota 55108

A response from the authors

DEAR SIR:

Thank you for sending us the letter by Dr. T. P. Labuza which contains some interesting yet confusing comments.

For instance, the standard method we used prescribes the use of % moisture rather than g H_2O/100 g dry solids which Dr. Labuza recommends. In fact the points which Dr. Labuza makes are all extremely critical of this standard method, which he initially refuses to accept as standard. Yet, later in his paper under point (5) he states that the method is standard. We were confused by this because if a method is standard then it is our understanding that this implies that it should be followed.

Further, the terms were not explained in detail since the methodology used was referenced. It seemed to us that an interested reader would utilize the references given.

Also Dr. Labuza scorns the use of the term isotherm in Fig. 1. It is our understanding that the root meaning of isotherm is constant temperature, and this is what we used.

We agree with many of the comments made in the 2nd paragraph, but they assume that unlimited amounts of pure pigment are available. This, however, is not the case, and we feel Dr. Von Elbe's excellent work was criticized unfairly since pigment collection is a real problem.

In conclusion, we would like to thank Dr. Labuza for his comments but not for the tone in which they were written.

F. M. CLYDESDALE
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F. J. FRANCIS
Department of Food Science and Nutrition
University of Massachusetts
Amherst, Massachusetts, 01003

Another response

DEAR SIR:

Thank you very much for sharing with me Dr. Labuza's letter regarding an article published by Dr. Clydesdale in the *Journal of Food Protection*. In the letter, Dr. Labuza is critical of the experimental design used in a 2 page article published by J. H. Pasch and myself (J. Food Sci. 40:1145-1146).

As the brevity of the written article might indicate, it was limited in scope, and only dealt with the degradation rate constants of betanine as a function of a_w at 75 C. It would indeed have been advantageous, as Dr. Labuza points out, to obtain rate constants as a function of both temperature and a_w. The limitation imposed in the study certainly was not a lack of awareness of this advantage but was caused by the extreme difficulty in obtaining sufficient amounts of crystalline betanine to conduct additional experiments to vary temperature. In addition, in 1975 we were limited to a rather laborious method of analysis for betanine involving electrophoresis. This analysis required 12 h in contrast to a newer method requiring 9 min and involving HPLC, now routinely used in our laboratory.

The experiments and data reported by us in the mentioned article were done in good experimental design and experimental methodology. I therefore feel that Dr. Labuza's criticism is unjustified.

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Madison, Wisconsin 53706

More information about preliminary incubation

DEAR SIR:

At the annual conference on Interstate Milk Shipments last May, a proposal to substitute a Standard Plate Count (SPC) following Preliminary Incubation (SPC-PI) for two of the four SPCs called for in each 6-month period by the PMO was turned down. Since PI is being used with gratifying success in a number of areas, it would seem that there is a lack of understanding concerning this procedure. This letter attempts to supply useful information concerning it.

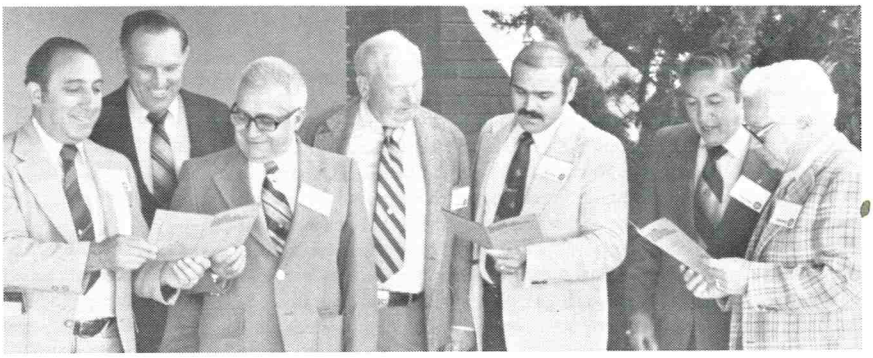
Most persons concerned with quality raw milk production are aware that the SPC fails to indicate where sanitary practices are not being followed. This occurs largely because with bulk milk tanks, little growth of contaminating bacteria takes place. Workers at Iowa State University reported in 1968 that *the Psychrotrophic Bacteria Count (PBC) was the only bacterial test showing a positive correlation with farm practices.* Unfortunately, the PBC calls for incubation for 10 days at 45 F (7 C). To obtain quicker results, Preliminary Incubation (PI) has been suggested, holding samples at 55 F (13 C) for 18 h before plating. At this temperature the udder flora fails to grow, while most contaminants grow well, and the psychrotrophs grow much the fastest! Thus PI helps pinpoint farms in need of improvement, and eventually cuts down the amount of field work, especially if a quality bonus is offered. One California plant, after using PI for a few years, actually found two-thirds of producer samples had counts under 3,000/ml.

In the July issue of the *Journal*, LaGrange showed how SPCs increase during storage and handling in the plant. Only psychrotrophs can grow at recommended storage temperatures; consequently it is vitally important to reduce their number to a minimum. At the IAMFES meeting in Orlando, Barnard reported great improvement in the bacterial quality of raw milk where the SPC-PI tests were performed regularly, so much so that *the manifold increases in SPC when milk is held raw for several days before processing no longer occur!*

It has been objected that PI is unfair to the producer in that his milk is held at a higher temperature than he would hold it. (This has been the practice in Britain since the 1930s.) But if the purpose of doing bacterial tests is to verify production practices, there is no question but that SPC-PI will be much superior for that purpose. It is well to remember that with adoption of farm bulk tanks, deep cooling has often been substituted for efficient cleaning and sanitizing of milk-handling surfaces, the chief source of psychrotrophs. Where equipment is in good sanitary condition, it is rare for a milk sample to show a significant increase in SPC following PI.

C. K. JOHNS
Imperial Harbor, Box 182,
Bonita Springs, Florida 33923

Food Safety, Communication on NY Program



Scenes from the New York and California meetings include, top left: Outgoing New York President William Jordan steps aside as incoming President Charlotte Hinz prepares to address the banquet audience. Top right, John W. Raht, recipient of the Gauhn Memorial Award, thanks the New York Association for his award. Above, California members look over the program prior to their meeting in San Jose. They are, left to right, Pete Benedetti, Morris Holt, Manuel Abeyta, Robert Abel, Joe Miranda, Phil Felix and Pat Dolan.

A need to confront the public's anxieties over the safety of its food was emphasized in one of the two keynote presentations at the Annual Educational Conference of the New York State Association of Milk, and Food Sanitarians (NYSAMFS) in late September.

Dr. Channing Lushbough of Kraft, Inc., in "Openly Confronting Public Anxieties over Food Safety," noted that anxiety, a general discomfort, is compounded by ignorance. "Anxiety tends to dissipate when confronted by facts," he explained. Consumerists, aware of the highly-emotional aspects of food, are often able to play on these aspects, Lushbough noted. But for food manufacturers to do something to their products, as some consumerists claim, which would purposely harm the consumer would be "committing economic suicide," Lushbough said. "Enlighted self-interest leads manufacturers to stimulate repeat purchases in as large a number of consumers for as long as possible," he explained.

An emphasis on the microbiological safety of food produced is important as, "Just a few hours of mishandling in the consumer's home may undo everything that has been done up to that point to protect the safety and quality of the product," Pash noted. "Just doing the job right all along the line isn't enough. We must communicate, inform the consumer.

"The Art of Communicating," by George Pash of New York State Electric and Gas supported Dr. Lushbough's presentation. Pash both educated and entertained the audience as he communicated effectively the need to speak to others in terms they understand. Listening, being specific, and being brief were other key issues Pash highlighted as means to effective communication. Nonverbal communications say a great deal, Pash said. "Sizing people up makes the communication process work better and easier," Pash noted. Part of this "sizing up" process involves interpreting nonverbal cues others are sending, he said.

Listening, also in an important aspect of communications Pash said. "In fact, the art of communications is, essentially, listening," he explained.

The strong program at the conference also included presentations on trends in new product development, trends in food marketing, immersion booster heaters, and detection and enumeration of injured microorganisms. Stress-related illnesses, training laboratory personnel, additives in food products, and use of nitrite in food products also provided the focus for several other sessions. Two presentations of special regional interest, "Kosher Dietary Laws as They Affect the Food Industry," by Dr. Joe Regenstein of the Cornell Food Science Dept.; and "Wine---Its Impact as a Food Commodity," by Dr. Andrew Rice of the Taylor Wine Company, completed the two-day program.

Five NYSAMFS members were honored by the Association at the Awards Banquet.

John W. Raht received the Gauhn Memorial Award for outstanding service and leadership to the New York Association. Dr. Lawrence Conlon was presented the Brooks Memorial Award for contributions to the progress of Association affiliates. The Reich Memorial Award was given to Roger E. Aiken for outstanding service in milk sanitation and quality control. Dr. Robert R. Zall received the Marlatt Award, given in recognition of outstanding service in laboratory technology. The first William V. Hickey Memorial Award was presented to Maurice A. Guerrette for outstanding contributions in food sanitation and Dr. Charles Livak received the Honorary Life Membership.

Californians Discuss Antibiotics

The California Association of Dairy and Milk Sanitarians held its 1979 Annual Conference in conjunction with the California Dairy Industries Association and the Bureau of Milk and Dairy Foods Control. The meeting was held at San Jose, Oct. 22 and 23.

Antibiotics in milk, shelf life of dairy products, and methods for the determination of milk components were subjects discussed in several publications.

"The Relationship Between Iodine in Milk and Use of Teat Dips and 'Backflushing' of Milking Units," was presented by John Bruhn of the University of California in Davis, while several persons addressed "Antibiotics in Milk, How We Keep Them Out." They were Steve Palmer, Dairymen's Cooperative Creamery Association, Doug Melott, Knudsen Corporation, and Gene Senften, California Cooperative Creamery.

Floyd Bodyfelt, Oregon State University, discussed two subjects at the meeting, "Sorbates in Extending Shelf Life of Cheeses, a Fresh Approach," and "A New Scoring System for the Evaluation of Yogurt." Gordan Cheeseman, National Institute for Research in Dairying, England, addressed, "Storage Stability of UHT Products." "Marketing of UHT Products, the Prospects," was Edward Haines' subject. He is with Brik-Pak in Dallas. Clem Pelissier of the University of California, Davis, discussed, "Three Times Milking, It Works and It Doesn't Work, One Person's Perspective."

"The Microwave Oven Procedure for Milk Solids Determination," was seen from two points of view. Bill Green of Chemical Laboratory Services, offered the "state lab perspective," while Steve Palmer of Dairyman's Cooperative Creamery Association, offered the "California industry experience." David McKenna, of Berwind Instruments, Ltd., discussed "The Multispec M, an Infrared Unit for Measuring Milk Components."

The luncheon speaker was Earl Wright, Executive Secretary of IAMFES, who discussed "Professionalism, An Outdated Concept?" He also spoke at a session on "3A Standards, Just What Are They?"

John Cooper, Pacific Gas and Electric, was the banquet speaker. He discussed "Nuclear Energy."

A variety of other topics were presented during the two-day meeting, including on-line standardization for fluid milk, BOD and priorities for milk.

Indiana Sanitarians Meet at Lake Monroe

The resort setting of Lake Monroe, Indiana, provided a beautiful fall backdrop for the 29th Annual Educational Conference of the Indiana Association of Sanitarians (IAS), held in late September.

Subjects of concern and discussion at this year's conference included, "An Industry Concern for Food Safety," by Gale Prince, Director of Quality Control and Sanitation for Eisner Food Stores, Champaign, IL; "Environmental Preservation---Challenge of the 80's" by Herman Koren, Coordinator of Environmental Health Program at Indiana State University; and "Public Legislation and the Legislative Process," by Thomas Dorsey, Director of Grants and Special Projects for the Indiana State Board of Health. Also on the program were "Regulatory Impacts of the New Federal Criteria for Solid Waste Disposal," by Owen Thompson, an Environmental Engineer for the U.S. Environmental Protection Agency; and "Innovative On-Site Waste Disposal Systems in Indiana," by Stephen J. Hudkins, Extension Agronomist at Purdue University.

David Jester, an Indianapolis judge who presides over what is believed to be the only local environmental health court in the country, spoke on "Environmental Health Law." Judge Jester noted that the court hears only cases on environmental health issues. Most cases are heard within thirty days and disposed of within two or three days, Judge Jester noted, which speeds up corrections of environmental violations. Punishment, in itself, has not been so effective in cleaning up environmental problems in Marion County, where Indianapolis and the court are located. "We need to *solve* the problems and have solved some through this court which looked insolvable a year ago," he noted.

Judge Jester also offered counsel on how to approach a court, should that become necessary in following up on some investigation. "You're as good as your attorney, and you can make the job easier for him if you're a professional," he noted. "Know your job. Apply the law uniformly to those you work with.

IAS honored several of its members at its Awards Luncheon and Banquet. Receiving Lifetime Memberships were: Wally Baker, Cliff Bragg, Howard Ellis, Henry Halterman, Gordon McCalment, Bob Nelson, Siegel Osborn, and Archie Rowland. Recipient of the Tim Sullivan Award was Russ Mumma, and Karen Mackowiak was named Indiana's Outstanding Sanitarian. Sanitarian Emeritus Awards were presented to Bob Nelson and, posthumously, to Clifford Beyler. The Southern Chapter of IAS won the Werkowski Award as the top affiliate.

Outgoing President Helene Uhlman gave presidential commendations to IAS members Floyd Bosley, Vernie Seiner, Ed Kiminski and Don Wingstrom.

Officers elected to serve the Association for the coming year included: President, Kevin Burk; President-Elect, Loren Robertson; Vice-President, Steve Creech; Secretary, Mary Peer; Treasurer, Bob Lesley; Auditors, Ted Alexander and Rosie Hansell.

CAST Aflatoxin Report Highlighted

Corn, peanuts, and cottonseed are crops most likely to be contaminated with mycotoxins -- substances produced by molds which are poisonous to humans, livestock, and poultry. Mycotoxins may cause birth defects, spontaneous abortions, tremors, cancer, and other health problems. Mycotoxins cause 18 known diseases of humans and animals and are suspected to cause, or contribute to, 16 others, according to a report released by the Council for Agricultural Science and Technology (CAST), an association of 26 food and agricultural science societies.

"The aflatoxins are the most important and most potent of these natural poisons," says Urban Diener, an Auburn University scientist who chaired the task force of 13 university and government specialists that produced the report at the request of the U.S. Senate Committee on Agriculture, Nutrition, and Forestry. "Aflatoxins occur continuously in small amounts in the U.S. food and feed supply.

"A major concern," says Diener, speaking for the task force, "is the possibility that aflatoxins may contribute to liver cancer in humans." Aflatoxin B_1, the most prevalent of 18 known aflatoxins, is the most potent, naturally occurring, cancer-producing substance known. Presence of this aflatoxin at less than 1 part per billion in the diet of rainbow trout is enough to cause a significant incidence of liver cancer. Humans fortunately are much less sensitive. The average concentration of aflatoxins in the human diet is 0.1 part per billion in the Southeast and 0.011 part per billion in the United States as a whole. "But there is less liver cancer in the Southeast than in the United States as a whole," says Diener, "so we have no evidence to date the aflatoxins are a significant cause of liver cancer in this country." The main evidence for a human cancer hazard comes from certain developing countries in Africa and Southeast Asia, where the aflatoxin content of the diet is as high as 7.4 parts per billion, and the incidence of liver cancer is as high as 13 per 100,000 of population per year.

Both humans and animals may be poisoned if they eat enough grain that is heavily contaminated with aflatoxins. More than 100 human deaths occurred in an area in India in 1974 among residents whose dietary staple was corn contaminated with unusually large amounts of aflatoxins. More than 100,000 turkeys died in England in 1960 from feed contaminated with aflatoxins from peanut meal.

In 1977, 56% of the corn crop in the Southeast was contaminated with aflatoxins at concentrations above the 20 part per billion limit set by the Food and Drug Administration. The concentration in 26% of the corn crop from this area exceeded 100 parts per billion. Feed containing aflatoxins at 100 parts per billion is potentially hazardous to young animals and should not be fed to dairy cows because some of the aflatoxin comes through in the milk as aflatoxin M_1. Many farmers in the Southeast plowed their corn under in 1977 rather than taking a chance on feeding it.

The other main aflatoxin hazard in the Southeast is with peanuts. "The peanut industry watches its products like a hawk," says Diener. "Every year many truckloads cannot be sold for human consumption because they contain more aflatoxin than the 20 parts per billion allowed by the Food and Drug Administration."

In the Southwest, growers had an aflatoxin problem with cottonseed produced in 1977. High temperatures in August and September and relatively high rainfall made conditions in Arizona good for mold development. Some of the milk from dairy cattle fed this cottonseed contained aflatoxins at a concentration exceeding the 0.5 part per billion guideline set by the Food and Drug Administration. "We don't know how much of a human risk this represents," says Diener, "but we need to be cautious. We can't keep aflatoxins totally out of the food supply, but we need to keep contamination down to the lowest feasible level."

The report explains that one of the safety factors that protects the human population from aflatoxins is the variety of foods we eat from different sources. Even if one batch of one food might have more aflatoxin than we could tolerate continuously in our diet, we eat enough other foods with little or no aflatoxin that, overall, we seem to be pretty safe.

Serving with Diener on the task force that prepared the CAST report were John C. Ayres, Department of Food Science, University of Georgia; W. W. Carlton, Department of Veterinary Microbiology, Pathology and Public Health, Purdue University; Richard J. Cole, National Peanut Research Laboratory, U.S. Department of Agriculture, Dawson, Georgia; Leo A. Goldblatt, Southern Regional Research Center, U.S. Department of Agriculture, New Orleans, Louisiana; P. B. Hamilton, Department of Poultry Science, North Carolina State University; Clifford W. Hesseltine, Northern Regional Research Center, U.S. Department of Agriculture, Peoria, Illinois; D. P. H. Hsieh, Department of Environmental Toxicology, University of California at Davis; Elmer H. Marth, Department of Food Science, University of Wisconsin; C. J. Mirocha, Department of Plant Pathology, University of Minnesota; Allan C. Pier, National Animal Disease Center, U.S. Department of Agriculture, Ames, Iowa; R. O. Sinnhuber, Department of Food Science and Technology, Oregon State University; and Jack R. Wallin, U.S. Department of Agriculture, University of Missouri at Columbia.

For a copy of CAST Report 80 entitled "Aflatoxin and Other Mycotoxins: An Agricultural Perspective," write or call CAST, 250 Memorial Union, Ames, Iowa 50011, 515-294-2036 or 2903.

Previous Winners of IAMFES Awards

The following persons have won IAMFES awards since 1970. Please nominate persons for the 1980 awards whom you feel are deserving. Nomination forms will go out soon with 1980 ballots to affiliate and direct members. See the November *Journal* for more information.

Honorary Life Membership

1970---Harold J. Barnum
1971---William V. Hickey
1972---C. W. Dromgold and E. Wallenfeldt
1973---Fred E. Uetz
1974---H. L. Thomasson and K. G. Weckel
1975---A. E. Parker
1976---A. Bender Luce
1977---Harold Y. Heiskell
1978---Karl K. Jones
1979---Dr. Joseph C. Olson, Jr.

Citation Award

1970---Ivan E. Parkin
1971---Dr. L. Wayne Brown
1972---A. Bender Luce
1973---Samuel O. Noles
1974---John C. Schilling
1975---Dr. A. R. Brazis
1976---James A. Meany
1978---Raymond A. Belknap
1979---Harold Thompson

Sanitarian's Award

1971---Shelby Johnson
1972---Ambrose P. Bell
1974---Clarence K. Luchterhand
1975---Samuel C. Rich
1976---Mel Jefferson
1977---Harold Bengsch
1978---Orlowe Osten
1979---Bailus Walker, Jr.

Educator-Industry Award

1973---Walter A. Krienke
1974---Richard P. March (Educator)
1975---Dr. K. G. Weckel (Educator)
1976---Burdet H. Heinemann
1977---Dr. E. H. Marth (Educator)
1978---James B. Smathers (Industry)
1979---Dr. Joseph Edmondson (Educator)

Shogren Award

1972---Iowa Affiliate
1973---Kentucky Affiliate
1974---Washington Affiliate
1975---Illinois Affiliate
1976---Wisconsin Affiliate
1977---Minnesota Affiliate
1978---Florida Affiliate
1979---New York Affiliate

Acknowledgment of Assistance by Reviewers

The Editor thanks members of the Editorial Board for their prompt and conscientious review of the numerous manuscripts that were processed during 1979. Thanks also go to the following persons not on the Editorial Board for their help in reviewing one or more manuscripts during 1979.

R. E. Brackett	R. C. Lindsay
D. O. Cliver	P. A. Lofgren
C. A. Dahl	L. McKay
R. V. Decareau	T. E. Minor
J. F. Frank	V. S. Packard
W. J. Gojmerac	T. Richardson
G. T. Jackson	D. Skogberg
H. W. Jackson	D. A. Stuiber
C. Kloos	S. R. Tatini
W. S. LaGrange	S. Taylor

E. H. MARTH
Editor
Journal of Food Protection

Pieper, Baird and Walter Re-elected BISSC Officers

The 64th meeting of the Baking Industry Sanitation Standards Committee (BISSC) was held in the Crown Center Hotel, Kansas City, MO, in October.

William E. Pieper was unanimously re-elected BISSC Chairman. Pieper has a long history of service to BISSC, having represented the Biscuit and Cracker Manufacturers Association on the BISSC Board of Directors for many years, as well as serving on numerous BISSC Task Committees. He is presently Chairman of the Sanitation Committee of the American Society of Bakery Engineers and represents that organization on the BISSC Board of Directors.

J. Allen Baird was unanimously re-elected BISSC Vice Chairman. Baird, who is Executive Vice President of Mrs. Baird's Bakeries, Inc.,

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Ballots Distributed Soon

Members of IAMFES, both direct and affiliate, will soon receive ballots for the 1980 election. Members will choose a new Secretary-Treasurer of the Association. For details on the two candidates, Archie Holliday and Lee Lockhart, see the December issue of the *Journal of Food Protection*.

Also on the ballot will be proposed amendments to the IAMFES Constitution and By-Laws. They concern adding the Chairman of the Affiliate Council to the Executive Board. Sections affected and the proposed amendments, in italics, are as follows:

Constitution: Article IV, Section 2: The Executive Board shall consist of the President of the Association, the President-Elect, the two Vice-Presidents, the Secretary-Treasurer, the immediate two Past Presidents, and the *Chairman of the Affiliate Council.* The Executive Board shall direct the affairs of the Association. . .

By-Laws: Article II, Section 6, Paragraph F: The Chairman shall preside at all meetings of the Council. He shall appoint all Council committees unless otherwise directed by vote of the Council, and perform such other duties as usually devolve upon the presiding officer or are required of him by the Constitution and By-Laws. *The Chairman shall serve as a member of the Executive Board.*

Please be sure to vote if you are an IAMFES member.

Management Courses Available for Credit

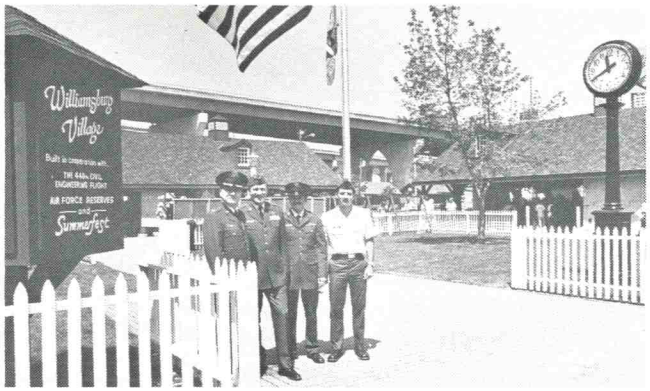
Two independent study courses in management and supervision are available through Indiana State University.

The courses prepare persons who have been newly promoted to management positions for their increased responsibilities. Often persons may be advanced for work ability, knowledge, and personality. What they may lack, due to inexperience, is management skills.

Basic Supervision and Management, Part I, discusses organization and its structure, successful supervision, planning, and selecting employees. Part II works with communication, discipline, performance ratings,

con't p. 164

An Invitation from America's Dairyland:



For professionalism and Gemütlichkeit, plan to attend the 67th Annual Meeting of IAMFES, July 27-31, 1980 at the Red Carpet Hotel, Milwaukee, Wisconsin.

Gemütlichkeit Abendgesellschaft
Monday, July 28, 1980, 6-10 p.m.
Abendgesellschaft = Evening Party
Gemütlichkeit = Well, it's a word not easily defined. Some say it's the feeling Milwaukeeans get when plates are heaped with food, beer is flowing freely, and someone from out of town is there to pick up the check. Come and enjoy, then offer your own definition of Gemütlichkeit!
The IAMFES 1980 meeting will be held in conjunction with NEHA's Annual Educational Conference. Reciprocal admission between IAMFES scientific sessions and NEHA educational sessions has been authorized by the executive boards of both groups.
Don't miss this opportunity to participate in the 67th Annual Meeting of IAMFES!

1980 IAMFES ANNUAL MEETING

Advance Registration Form for the 67th Annual Meeting, July 27-31, 1980, Milwaukee, Wisconsin

MAIL TO: **Richard Rowley, Chairman of Registration**
IAMFES
Bureau of Consumer Protection and
Environmental Health
Milwaukee Health Department
P.O. Box 92156
Milwaukee, Wisconsin 53202

Please check where applicable:

Affiliate Delegate	☐	Speaker	☐
Past President	☐	Host	☐
Executive Board	☐	Non-member	☐

Make checks payable to:
IAMFES 1980 Meeting Fund

Advance register and save - refundable (prior to June 31) if you don't attend

ADVANCE REGISTRATION FEE (prior to July 1)
(All in American currency)

	*Member**	*Spouse of Member*	*Student*
Registration	$20.00	$ 8.00	No chg.
Banquet	15.00	15.00	$15.00
Gemütlichkeit Abendgesellschaft	2.00	2.00	2.00
Total	$37.00	$25.00	$17.00

REGISTRATION FEE AT DOOR
(All in American currency)

	Member	*Spouse of Member*	*Student*	*Nonmember*
Registration	$25.00	$11.00	No chg.	$30.00
Banquet	17.50	17.50	$17.50	22.50
Gemütlichkeit Abendgesellschaft	3.00	3.00	3.00	5.00
Total	$45.50	$31.50	$20.50	$57.00

**Member IAMFES or Wisconsin Dairy Plant Fieldmen's Association*

Name (Member) _____ Spouse _____
Children's First Names and Ages _____
Employer _____
Address _____
City _____ State _____ Zip _____
Means of Transportation _____

RED CARPET HOTEL

5757 So. Howell Avenue
Milwaukee, Wisconsin 53207
Telephone 414-481-8000

Reservations must be received by June 26, 1980
Deposit of 1 night's lodging plus 9% tax or a major credit card number required.
Check in after 1:00 P.M.
Check out time is 12:00 Noon

Arrival Date _____ Departure Date _____
Arrival Time _____ Means of Transportation _____
Name _____ Name _____
Address _____
City _____ State _____ Zip _____

Please check type of accommodation required
_____ Single (one person) $38.00
_____ Double (two persons) $46.00
Family Plan: No charge for children (under 18) when occupying the same room as parents.
Roll-a-way beds available at $4.00 each.

One bedroom and two bedroom suites available.
Courtesy Van operates between Mitchell Field Airport and the Red Carpet Hotel.
Mail directly to: **Red Carpet Hotel,** Reservations
5757 So. Howell Ave.
Milwaukee, WI 53207

One name, two great ideas for better sanitation:

Transflow®

Transflow® tubing

Dairymen across the country rely on Transflow raw milk tubing to protect the wholesomeness and flavor of their product. That's because Transflow tubing is specially designed for the rigid sanitation requirements of the dairy industry.

Transflow tubing cleans quickly and thoroughly. Its inner bore — smoother than rubber tubing and stainless steel pipe — leaves no place for butterfat or milkstone to accumulate. And because Transflow tubing is clear, you can **see** that it's clean and residue-free.

Insist on genuine Transflow M-34R tubing. It complies with FDA and 3-A standards, and is available at local dairy suppliers everywhere.

Transflow® paneling

The Transflow paneling system is fast gaining a reputation as the premier wall and ceiling covering for dairy operations.

The system — panels, moldings and connectors — installs easily with no special tools. Transflow panels are built tough to withstand repeated cleaning, yet stay bright and new-looking for years. They inhibit mold growth, require little maintenance, and never need painting. Like Transflow tubing, Transflow paneling meets FDA and 3-A standards.

Don't accept substitutes — choose Transflow paneling for your operation. When Transflow paneling goes up, maintenance goes down.

33-154

Transflow Hot-Line:
1-800-321-9634

Call toll-free for more information on Transflow paneling and the location of your nearest dealer. Ohio call collect 216-630-9230.

NORTON *PLASTICS AND SYNTHETICS DIVISION*
P.O. BOX 350 AKRON, OHIO 44309 TEL: (216) 630-9230

SOMEONE YOU SHOULD KNOW IN THE DAIRY INDUSTRY

Jim Rahr, Dairy Sanitation Routeman

Jim Rahr works for Botens Dairy Supply, in Cuba, N.Y. and has been a routeman for nine years. Before that he was a dairyman with one of the largest herds in Allegany Co., N.Y. A graduate of New York State Agriculture Technical Institute, and the Surge Training Center in Illinois, Jim offers his views on the value of a dairy route sanitation program.

"For a dairyman trying to make a living from his commitment to the dairy industry, a routeman is his link to the dairy equipment dealership. The routeman is the dealership in the field, sharing the latest information on good milking practices, improved equipment and better sanitation.

Qualities Of a Routeman

"A good routeman needs three basic qualities to help him succeed: Honesty, knowledge of his customer's business, and respect for that customer. A man is only as good as his promise, and if my promise is no good, that's the way I'm perceived. This means when I say I'll deliver a part or merchandise, I make sure I deliver it on time as promised.

"Dairymen expect me to know about their business. Sometimes I feel like a walking encyclopedia, but to help a man do a better job, you have to know the things that can help him. My years as a dairyman helped greatly, and I still keep up on the latest dairy information.

"Respect for the dairyman may be the most important part of a routeman's job. You have to remember the dairyman is a businessman, and his beliefs are part of his livelihood. You might see a way to do something differently to help him out, but you tell him from a position of respect, not superiority.

How We Help

"Since we're bringing the dealership to the dairyman, we can help in a number of ways right there. Our services include:

- Testing the dairyman's water and prescribing the best detergent to meet his needs.
- Making sure service is available to him when his equipment needs attention.
- Leaving enough supplies so the dairyman won't run out and have his operation suffer.
- Delivering supplies in bulk, at the lowest price we can offer.
- Informing him on the latest information which can help improve his operation.
- Checking important details such as vacuum pump oil and vacuum controls to make sure they're working properly.

"In addition, there are some intangible values which only a routeman who's involved with his customer can offer. Like suggesting help from an outside source such as a vet or extension specialist. Understanding the dairyman's thinking helps me serve him better. Once he understands I'm only in business if he is, he knows I want to help him succeed.

"You can't be pushy, but you want the dairyman to understand the importance of things like changing inflations often enough to protect his herd, and using the right products for proper sanitation. I try to think to myself, if I were this dairyman, what would I want to know to help improve my business? This helps me explain things without seeming pushy or like a hardline salesman.

"Another important service which helps both routeman and dairyman is the records kept on supplies used. When I was on the farm, I was a stickler for record keeping and I still believe in it. My records assure the dairyman enough supplies without overstocking. I also know how often inflations need to be replaced.

"We want to help the dairyman help his cows' performance. In my dairy, I had a sign in my parlor which read, 'Every Cow Is A Lady, Treat Her As Such.' I modified that as a motto for our dealership to say, 'We Are The Milking Cow's Friends.' I truly believe a route program is worthwhile for the dairyman and his herd or I wouldn't be in it, and you can take my word on that!"

Babson Bros. Co., 2100 South York Road, Oak Brook, Illinois 60521