APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Aug. 1979, p. 275-282
0099-2240/79/08-0275/08$02.00/0

Vol. 38, No. 2

Bioaccumulation and Depuration of Enteroviruses by the Soft-Shelled Clam, *Mya arenaria*

THEODORE G. METCALF,[1]* BARBARA MULLIN,† DANIEL ECKERSON,[1] ELLEN MOULTON,[1] AND EDWARD P. LARKIN[2]

Department of Microbiology, University of New Hampshire, Durham, New Hampshire 03824,[1] and Virology Branch, Division of Microbiology, Bureau of Foods, Food and Drug Administration, Cincinnati, Ohio 45226[2]

Received for publication 4 May 1979

Low levels of feces-associated natural virus, simulating virus numbers estimated to exist in moderately polluted shellfish-growing waters, were used to evaluate the effectiveness of depuration as a virus depletion procedure in soft-shell clams. Depuration effectiveness depended upon the numbers of virus bioaccumulated and whether virus was solids associated. Virus uptake was greatest when viruses were solids associated and pollution levels were equivalent or greater than those likely to be found in grossly polluted growing waters. Virtually all bioaccumulated feces-associated natural virus was deposited within either the hepatopancreas or siphon tissues. Viruses usually were eliminated within a 24- to 48-h depuration period. Dependence upon depuration of clams to eliminate health hazards of virus etiology involved a risk factor not measureable in the study. The greatest reduction of health risks would come from the routine depuration of clams harvested from growing waters of good sanitary quality.

The transfer of shellfish from moderately polluted to clean waters to take advantage of a self-cleansing pumping mechanism of the animal has been practiced before the twentieth century in Europe and, more recently, in North America (8, 9, 25). The self-cleansing mechanism (depuration) has been used to reduce potential public health hazards associated with the consumption of shellfish which might have been exposed to bacterial or viral pathogens. Generally, depuration has been considered to be effective for the reduction or elimination of bacteria, but opinions about its effectiveness for the elimination of viruses are divided (3, 4, 13, 20).

Studies of depuration effectiveness of oysters and hard-shell clams which contained small to large numbers of laboratory strains of enteroviruses usually have shown a rapid initial reduction in numbers, followed by a low-level virus persistence that lasted for several days (14, 17, 19, 23). These findings have raised serious questions about the virus depletion potential of depuration for oysters and hard-shell clams where the usual depuration interval seldom exceeds 48 h.

A review of the literature indicated that no information was available on the bioaccumulation and depletion of naturally occurring human enteric viruses by the soft-shell clam, *Mya arenaria*. The soft-shell clam has been shown to eliminate bacteria, including salmonellae, through depurative action (2). The bivalve mollusk, which is extensively consumed by inhabitants of the northeastern United States, is harvested on a year-round basis and was available for study. A new recovery method developed specifically for soft-shell clam studies made it possible to assess depuration effectiveness through the ability to recover as few as 1 to 5 plaque-forming units (PFU) of enteroviruses from 100 g of shellfish homogenate.

MATERIALS AND METHODS

Viruses and viral assays. Plaque-purified poliovirus, type 2 (strain W-2), and feces-associated natural virus (infant feces collected 5 days post-oral poliomyelitis vaccination) were used in the study. Feces-associated natural virus represented virus progeny from the replication of attenuated, active-vaccine poliovirus in tissues lining the infant gut. The infant feces were kindly supplied by M. Michael Sigel, University of Miami Medical School. The virus content of the feces was titrated at 9.5×10^3 PFU/g. Assays were made by using a specially cloned Buffalo green monkey kidney (BGM) cell line of maximum sensitivity for polioviruses (22). Samples (1 ml of inocula) were added to bottles containing 45-cm^2 cell monolayers. Dilutions were made in isotonic phosphate-buffered saline (PBS), pH 7.2. Inocula were adsorbed for 1 h, and the monolayers were rinsed with PBS and overlaid with

† Present address: Quality Control Laboratory, Corning Glass Co., Oneonta, NY 13820.

20 ml of agar medium (5). PFU were determined from the results of replicate assays 3 to 5 days later.

Preparation of clam samples. Three kinds of samples were prepared: the combined meat and shell liquor of individual clams; separate pools of hepatopancreas, siphon, and remaining tissue from 10 clams; or the pooled meats and shell liquor from 10 clams. Homogenates were prepared by omnimixing the clam pool plus a 6× (pool mass) volume of distilled water for 2 min at high speed. Clam tissues were precipitated at pH 4.5 and a salt concentration of 1,500 μg/ml. Virus was recovered by omnimixing the precipitate at low speed with 300 to 400 ml of 0.1 M glycine (pH 9.5) in the presence of a salt concentration of 10,000 μg/ml. After adjustment of the resulting eluate to pH of 8 and a salt concentration of 8,500 μg/ml, an equal volume of 3% beef extract was added. The pH was adjusted to 3.5, and the virus was precipitated in an organic floc. Virus was recovered by elution with a 5× (pellet mass) volume of 0.15 M Na_2HPO_4, pH 9.5. The eluate was adjusted to pH 7.2 and clarified by addition of a polycationic electrolyte (cat floc), and the volume was reduced to a final 15 to 20 ml by ultrafiltration using an Amicon PM30 membrane filter. The cat floc was kindly supplied by Richard Kenney, Calgon Corp. The final sample (isotonic) was treated with an antibiotic mixture (penicillin, streptomycin, gentamicin, and amphotericin B) or ether if necessary to control microbial contamination.

Bioaccumulation of virus. Clams used throughout the study were purchased from commercial sources and depurated in virus-free seawater for 2 weeks. The shellfish were exposed to test virus in tanks containing 3 to 4 liters of seawater. Virus was added either as stock or natural virus with or without added suspended solids. Cornstarch and the clay kaolinite were used with stock virus. Cornstarch was used to encourage high feeding rates, and kaolinite was used to study the effect of suspended solids-adsorbed virus upon bioaccumulation. Natural virus within infant feces was associated with fecal particulates which were finely suspended when added to seawater. Use of low-level virus inputs was emphasized with both forms of the suspended solid state. Clams usually were exposed to viruses for 2 to 6 h during which time the seawater was agitated and aerated by means of aeration stones. A 20- to 24-h bioaccumulation period was used when depuration immediately followed virus bioaccumulation. Seawater salinity varied between 0.02 to 0.028 g/ml, water temperature varied from 17 to 23°C, and dissolved oxygen values were estimated at 6 mg/liter. Bioaccumulation of virus was reported either as percent accumulation [(total PFU per clam or tissue/total PFU in seawater) × 100] or fold concentration (PFU per g of clam tissue/PFU per ml of seawater).

Depuration of virus. Depuration was carried out in a depuration unit modeled after the soft-shell clam depuration plant located at Newburyport, Mass. The unit consisted of an ultraviolet radiation chamber, a tank within which shellfish were held, and a seawater reservoir. All surfaces in contact with seawater were lined with polyvinylchloride. Seawater was recycled through the unit by means of a pump and connecting pipes. The ultraviolet chamber consisted of two 36-inch (ca. 914 mm) General Electric 30-W germicidal lamps (G30T8), positioned 5 inches (ca. 127 mm) above the seawater flowing through the chamber. Flow rates equivalent to 1.5 liters per bushel of clams per min were used. During operation the unit had a total volume of 60 liters. Fresh seawater was added at 24-h intervals. Toxic materials influencing clam depurative activity were removed by passage of seawater through a charcoal filter.

RESULTS

Bioaccumulation of test virus. The ability of individual clams to bioaccumulate stock and natural enteroviruses was examined in a series of trials in which the numbers of virus in the overlay water varied from 0.09 to 34.0 PFU/ml.

The results in Table 1 showed considerable variability in the bioaccumulative capabilities of individual clams. In general, the greatest degree of bioaccumulation took place in the presence of the greatest numbers of virus when stock virus was used. More virus was bioaccumulated when suspended solids were present. When cornstarch was used, viruses were detected in ca. 38% (5/13) of the clams exposed to 0.2 PFU/ml or less

TABLE 1. *Bioaccumulation of enteroviruses by the soft-shell clam, M. arenaria*[a]

Trial	Solids added[b]	Virus input		PFU of virus recovered per clam						
		(PFU/ml)	(Total PFU)	1	2	3	4	5	6	7
1	None	21.0	63,000	0	0	0	0	0		
2	Cornstarch	34.0	102,000	8	13	0	0	3		
3	Cornstarch	3.0	9,000	6	7	5	12	0		
4	Cornstarch	3.0	9,000	2	0	0	0	0		
5	Cornstarch	0.2	600	0	0	0	0	0		
6	Cornstarch	0.09	270	1	1	1	2			
7	Cornstarch	0.09	270	1	0	0	0			
8	Feces	0.17	510	3	4	3	0	7	5	0
9	Feces	0.17	510	2	1	0	0			

[a] Stock poliovirus 2 used in trials 1 to 7. Feces-associated natural poliovirus used in trials 8 and 9. Clams were exposed for 2 to 4 h.

[b] Cornstarch added in final concentration of 0.01%.

of stock virus. None of these virus-carrying clams had more than 2 PFU/animal. A slightly greater number of clams exposed to natural virus in a feces-associated form was shown to bioaccumulate virus (7/11).

Site of virus deposition. To obtain data on the distribution of bioaccumulated virus within soft-shell clams, a series of trials were made in which siphon, hepatopancreatic, and "remaining" (non-alimentary tract) tissues were tested for their virus content. The results of these tests are given in Table 2.

Viruses were found in all types of tissues examined, but to varying degrees. In the first six trials bioaccumulated virus was found as often in other tissues as in hepatopancreas tissues. In the second study viruses were found in siphons as well as in hepatopancreas tissues. In view of these results much of the virus found in non-hepatopancreas sites in the first trials probably was siphon-associated since "remaining tissues" in these trials included siphons in the test pools.

The numbers of virus bioaccumulated in the first six trials not only were higher than the numbers found in the second six trials, but they also were greater than those reported in Table 1. These differences were the result of two factors: clam size and virus input numbers. When virus numbers per gram of tissue were calculated, there was virtually no difference in bioaccumulative activity. Virus numbers bioaccumulated in the second six trials were very similar to Table 1 data which were obtained with 60 to 120 times less virus than was used in four of the first six trials.

Bioaccumulation of solids-associated stock virus. The ability of clams to bioaccumulate stock virus in the presence of cornstarch and the clay kaolinite was studied in a series of tests in which the clams were exposed to a fresh input of virus at 24-h intervals for 3 days. Fresh seawater and virus was added, after 10 clams had been removed for testing at 24, 48, and 72 h.

No pattern of a constant increase in the numbers of virus recoverable from any of the tissues examined during the entire 96-h test period was detected after examination of the data in Table 3. The PFU of the three tissue pools tested increased slightly at 48 h, but remained constant or declined thereafter. These data indicated that the rate of bioaccumulation exceeded the rate of elimination for about 48 h. After this time the uptake and elimination rates of the two processes were approximately equal in siphon and remaining tissue pools, but not in the hepatopancreatic pool where the rate of elimination exceeded the rate of bioaccumulation. The higher numbers of virus found in hepatopancreatic and siphon tissues emphasized the extent of participation of these tissues in bioaccumulation and elimination activities. In the case of the hepatopancreas, the greater numbers were considered as evidence of an initial virus deposition and concentration at this site. Siphon-associated virus indicated the extent of siphon involvement in the transportation of virus into and out of the clam. Recovery of virus from the remaining (non-alimentary tract) tissues showed that small numbers of virus either escaped from the alimentary tract at a more or less constant rate or were deposited directly in non-alimentary tract tissues. The non-alimentary tract tissues con-

TABLE 2. *Tissue distribution of virus bioaccumulated by M. arenaria*[a]

Trial	Virus input (PFU/ml)	Tissue location of bioaccumulated virus[b]					
		Hepatopancreas		Siphons		Remaining tissues[c]	
		(Total PFU)	(%)	(Total PFU)	(%)	(Total PFU)	(%)
1	12.0	180	0.5			202	0.55
2	14.0	257	0.6			300	0.7
3	5.0	75	0.5			125	0.8
4	2.0	445	7.4			169	2.8
5	2.0	407	6.8			237	4.0
6	1.8	274	5.0			449	8.3
7	0.4	115	9.5	13	1.0		
8	0.5			7	0.46		
9	0.1	8	2.7	5	1.7		
10	0.1	4	1.3	0	0.0		
11	0.2	2	0.3	0	0.0		
12	0.2	0	0.0	5	0.8		

[a] Poliovirus 2 added to seawater containing final concentration of 0.01% cornstarch.
[b] Total PFU represented total virus recovered per pooled tissues from 10 clams.
[c] Remaining tissues for trials 1 to 6 represented all clam tissues minus hepatopancreatic tissues.

TABLE 3. *Bioaccumulation of stock virus by M. arenaria in the presence of cornstarch and kaolinite*[a]

Sample tested	Time of virus bioaccumulation (h)	Trial no.		
		1	2	3
Seawater	0	0.3[b]	0.3	0.4
	24	0.2	0.7	0.4
	48	0.3	0.4	0.5
	72	0.3	0.4	0.4
	96			
Siphons	24	2.3[c]	0.5	7.0
	48	2.5	0.1	15.7
	72	3.7	1.0	10.2
	96	1.3	1.0	14.0
Hepatopancreas	24	0	0	23.2
	48	4.0[c]	1.1	28.7
	72	2.0	1.7	19.0
	96	1.0	2.2	17.7
Remaining tissues	24	0.7[c]	0	3.5
	48	2.0	0	4.2
	72	0.7	0	3.2
	96	0	0.5	4.5

[a] Poliovirus 2 used with cornstarch in trials 1 and 2, and with kaolinite in trial 3. Tissue pools at each test interval were prepared from 10 clams.

[b] Number of viruses in seawater – PFU/ml.

[c] Number of viruses in clam tissue – fold concentration (PFU per gram of clam tissue/PFU per milliliter of seawater).

sisted principally of adductor muscle, mantle lobes, gill structures, and pericardium (heart and vascular sinuses). At the time of these experiments it was felt that the greater numbers of virus found in all tissues in trial 3 were directly attributable to the use of kaolinite even though one could not eliminate the possibility that the greater numbers resulted from an increased clam feeding activity. Data obtained subsequent to these experiments strongly indicated the greater bioaccumulation observed to be the result of a solids-associated virus status. The greater bioaccumulation potential realized with kaolinite indicated that the nature and amount of estuarine-suspended solids could determine the extent of virus bioaccumulation by clams as well as influence the effectiveness of depurative activities.

Bioaccumulation of feces-associated natural virus. Seawater suspension of feces-associated natural viruses adsorbed to fecal particulate matter represented the closest approximation possible to shellfish waters polluted with human natural virus. The extent of virus uptake by the clam after exposure to feces-associated natural virus is shown in Table 4.

Bioaccumulation occurred readily, and concentrations of virus up to 34-fold were measured. The concentrations measured were similar to those found during the continuing 72-h kaolinite-associated virus exposure experiments (Table 3). The time and numbers of test virus to which the clams were exposed in the two sets of experiments suggested feces-associated natural virus was more efficiently bioaccumulated than kaolinite-associated virus. The virus concentrations obtained with feces-associated virus, approximately the same as that with kaolinite-associated virus, resulted with only one-half as much virus applied for one-twelfth as much time.

Data from the two sets of experiments indicated solids-associated virus could be expected to be bioaccumulated more efficiently than nonsolids-associated virus. Some difference in efficiency probably should be anticipated depending upon the identity of the suspended solids involved.

In view of the depurative importance attached to the tissue distribution of bioaccumulated virus, tests using feces-associated virus were carried out in which clams were divided into hepatopancreas, siphon, and remaining tissue pools. Each pool was tested for virus.

The greatest numbers of virus bioaccumulated were found in the siphon and hepatopancreas pools (Table 5). The concentration of virus found in the siphons in the three trials, on the average, was slightly greater than the degree of concentration obtained in the hepatopancreas tissues. However, the siphon mass was about 1.5-fold greater than the hepatopancreas mass, so the total numbers of virus found in the two tissues were about the same. Very low numbers of virus were found outside of the siphon and hepatopancreas tissues. These results indicated that most of the feces-associated natural virus remained within the alimentary tract and presumably would be subject to removal by depuration. The data also showed a degree of efficiency in the bioaccumulation of feces-associated virus which resulted in greater numbers of virus in the clams than had been obtained in previous experiments. This was attributed to a

TABLE 4. *Bioaccumulation of feces-associated natural virus by M. arenaria*[a]

Trial	PFU/g	Virus recoveries bioaccumulation (%)	Concn (fold)
1	3.9	28	19.5
2	6.4	38	32.0
3	6.9	35	34.5

[a] Feces-associated virus added to seawater to give a final concentration of 0.2 PFU/ml. The clams were allowed to feed for 6 h. Ten clams were used in each trial.

greater degree of pumping and feeding activity by the clams used in these trials.

Bioaccumulation and elimination of feces-associated natural virus. Depuration was carried out using clams carrying feces-associated natural virus bioaccumulated during a 24-h exposure period. Pools of 10 clams each were removed at 4, 16, and 24 h, respectively, to determine the numbers of virus bioaccumulated. The remaining virus-carrying clams were depurated for 72 h, with removal of 10 clams after the 24-, 48-, and 72-h periods to determine the rate and extent of virus elimination.

The results of the depuration experiments given in Table 6 indicated that bioaccumulation of virus numbers ranging from 5 to 80 PFU per test pool was shown during the 24-h exposure period. A 4- to 16-h exposure period resulted in maximum bioaccumulation in two out of three trials. In general, the bioaccumulated virus was reduced significantly within the first 24 h of depuration. If failure to detect virus is accepted as evidence of elimination, depuration was complete within 24 h in one trial and 72 h in a second trial. In the third trial the trace of virus found at 72 h followed a negative assay finding at 48 h and may have been caused by a failure of one or two clams in the 72-h pool to depurate.

The potential for elimination of bioaccumulated feces-associated natural virus by depuration was examined in continuing experiments in which the percentage of virus eliminated was calculated. Forty clams were used in each of three trials. Depuration was started immediately after the conclusion of the bioaccumulation phase. Ten clams were removed after a 20-h bioaccumulation interval (0 h depuration period) for determination of the extent of bioaccumulation obtained. Ten clams each were removed at 48, 96, and 144 h for the determination of the extent of depuration achieved. The results of these experiments are given in Table 7.

The maximum rate of depletion of bioaccumulated natural virus took place within the first

TABLE 5. *Tissue distribution of feces-associated natural virus bioaccumulated by M. arenaria*[a]

Trial	Virus in tissues					
	Siphons		Hepato-pancreas		Remaining tissue[b]	
	PFU/g	Concn	PFU/g	Concn	PFU/g	Concn
1	4.4	22.0	3.8	19.0	1.7	8.5
2	4.5	22.5	11.7	58.5	0	0
3	11.2	56.0	2.1	10.5	0.9	4.5

[a] Feces-associated virus was added to seawater to give a final concentration of 0.2 PFU/ml. Clams were exposed to virus in overlay water for 6 h. Ten clams were used in each trial. The amount of virus bioaccumulated is reported as fold concentration.

[b] Clam tissues remaining after removal of siphon and hepatopancreas tissues.

TABLE 6. *Bioaccumulation and elimination of feces-associated natural virus by M. arenaria*[a]

Trial	Bioaccumulation		Elimination	
	h	Total PFU	h	Total PFU
1	0		0	11.1
	4	6.9	24	1.6
	16	5.3	48	0
	24	11.1	72	0
2	0		0	29.5
	4	66.7	24	4.8
	16	80.4	48	0
	24	29.5	72	1.6
3	0		0	19.0
	4	27.0	24	2.1
	16	17.0	48	5.2
	24	19.0	72	0

[a] Depuration of virus-carrying clams carried out in model depuration recycling unit with disinfection of virus by ultraviolet radiation. Feces-associated virus was added to seawater to give a final concentration of 0.2 PFU/ml. Pools of 10 clams were used for each test interval in the bioaccumulation and elimination phases of study.

TABLE 7. *Depletion of bioaccumulated feces-associated natural virus in M. arenaria by depuration*[a]

Depuration (days)	Trial no.					
	1		2		3	
	Virus no. (total PFU)	Virus depletion (%)	Virus no. (total PFU)	Virus depletion (%)	Virus no. (total PFU)	Virus depletion (%)
0	226		303		283	
2	44	80.5	36	88.1	37	87.0
4	12	94.7	16	94.7	23	92.0
6	5	97.8	0	100.0	4	98.6

[a] Forty clams with bioaccumulated feces-associated natural virus were used in each trial. Feces-associated natural virus added to seawater to give a final concentration of 0.2 PFU/ml. Clams were exposed to virus for 20 h. Ten clams were tested for virus at each test interval.

48 h. Eighty to 88% of the virus content of the clam pools was eliminated during this period. The rate of depletion was much slower after 48 h, and elimination of virus within a 144-h (6-day) period was achieved in only one of three trials. Approximately 98 to 99% of the initial virus content was eliminated after 144 h in the other two trials. The data showed a significant reduction of bioaccumulated virus within 48 h in the soft-shell clams even though initial numbers were in excess of those estimated to exist in clams subject to low-level natural virus pollution.

DISCUSSION

The use of feces-associated natural virus in numbers estimated to be equivalent to the greatest numbers likely to be found in moderately polluted shellfish waters in northern New England was instrumental in providing new insights into the effectiveness of depuration as a virus depletion procedure in the soft-shell clam. Feces-associated virus was regarded as an example of a solids-associated virus state which had a decisive influence upon virus bioaccumulation and elimination. The effects observed may be representative of the influence of solids-associated virus in general upon these activities in other shellfish species.

Interpretation of the quantitative aspects of bioaccumulation was made with the knowledge it could be influenced significantly by shellfish exposure conditions. While not all conditions influencing shellfish bioaccumulative processes in a natural estuarine habitat may be known, it seemed highly improbable that clams exposed to 0.2 PFU/ml would bioaccumulate much virus. The low-level virus carriage state found in clams was considered more representative of the virus numbers likely to exist within shellfish subject to occasional virus pollution than numbers reported in previous studies (20). The data from these bioaccumulation experiments using stock virus added to overlay waters were greatly in excess of those found within soft-shell clams. One explanation for the differences was the greater numbers of virus added to overlay waters in these studies. Pollution levels of from 200 to 100,000 PFU per ml were used. Virus concentrations of 10- to 27-fold were obtained in oysters exposed to 300 to 1,000 PFU per ml (20), and 10- to 100-fold in hard-shell clams exposed to 100,000 PFU per ml (23). One study with a reported 1,140-fold concentration of bacterial virus in hard-shell clams used only 0.05 PFU/ml of overlay water (3). Unusually lengthy depuration periods of several days to several weeks were necessary to reduce virus carriage states to low or undetectable levels. The longer reported depuration times may have been caused by the need to eliminate greater numbers of virus or have been the result of using a bacterial virus rather than an animal virus. Differences between these two virus groups and uncertainties about the equivalency of shellfish responses made it difficult to assess how relevant these data were for uptake and elimination of animal viruses by shellfish.

The determination in the present study of the numbers expected for low-level virus carriage provided an idea of how many viruses would have to be removed by depuration. More important than a number per se was the concept advanced that, in order to be effective, virus numbers to be removed through depuration would be low rather than high. Depuration was shown to have the potential to eliminate low or high numbers of virus. Removal of high numbers took longer, which made virus numbers to be removed a critical factor in assessing the effectiveness of depuration. Another critical factor involved the participation or degree of participation of individual clams in feeding activities. Occasionally rates of virus elimination measured during the study were unaccountably slower than expected based upon previous results. These data indicated no reason to expect a significant degree of virus deposition in non-alimentary tract tissues where it might be protected from depuration, or to assume that alimentary tract-located virus would not be eliminated within a 24- to 72-h period. The most plausible explanation for a slow elimination rate was a recognized variability in individual clam depurative effectiveness. One or two non-depurating or ineffectively depurating clams could account for the virus numbers remaining after 48 h. Consideration of virus numbers should include the influence of shellfish size upon bioaccumulation activity. Larger specimens pump more water through gill structures during feeding and have a greater opportunity to bioaccumulate virus. A 50-fold concentration of virus from overlay water with 0.01 PFU/ml would result in 7 PFU within an oyster with a tissue mass of 15 g, whereas bioaccumulation of only 3 PFU would occur under the same conditions in a soft-shell clam with a tissue mass of 6 g.

The decisive nature of the feces-associated virus effect was demonstrated where clams were shown to bioaccumulate virus from overlay waters containing numbers too small to regularly produce virus carriage in the absence of suspended solids. The adsorption of virus to kaolinite which occurred rapidly, and which led to an enhancement of virus bioaccumulation effi-

ciency comparable to that obtained with feces-associated virus, was considered illustrative of the in situ solids-associated status represented by feces-associated virus. Cornstarch essentially was considered an in vitro stimulant of shellfish feeding activity, simulating phytoplankton in situ and helpful therefore in facilitating the bioaccumulation process. An opportunity for adsorption of virus existed, but no evidence of a substantial solids-associated virus effect upon bioaccumulation accompanied the use of cornstarch. Another explanation for an increased bioaccumulation efficiency involves a virus-binding mechanism advanced to explain attachment of virus to shellfish tissues (7). Attachment is considered to occur through an irreversible binding of virus to secreted mucus, followed by ingestion of mucus-bound virus. This concept indicated that a greater likelihood of ingestion of solids-associated virus would exist after release of virus from suspended solids preferentially bound by shellfish mucus. Another explanation is based upon the greater degree of protection against inactivation reported to take place when virus is in a solids-associated form (21, 24). Such virus would be more likely to retain infectivity than freely suspended virus. This difference could result in success or failure in demonstrating that virus bioaccumulation had taken place. The influence of solids-associated virus upon uptake rates in Pacific Coast oysters and the Manila clam was reported by Hoff and Becker (15). The concentration obtained with stock virus in crude and clarified cell culture suspensions was compared. Increases of 10- to 900-fold were obtained with crude suspensions compared to 0.4- to 3.6-fold with clarified preparations. The higher concentrations reported by Hoff and Becker, compared to those obtained in soft-shell clams, were attributed in part to the use of virus pollution levels 1,000- to 3,000-fold greater maintained over a 24-h exposure interval and in part to the different species used. The size of the shellfish may also have been a factor contributing to the greater degree of bioaccumulation reported.

Another aspect of the influence of feces-associated virus upon bioaccumulation and ultimately upon elimination was the tissue distribution of bioaccumulated virus. Earlier studies in which significant numbers of bioaccumulated virus were reported to spread beyond the alimentary tract and into body tissues raised apprehensions that the time required for removal of virus would be increased to a point where depuration would become impractical (6, 17). Bioaccumulation of feces-associated virus by clams resulted in distribution of only a small fraction to non-alimentary tract tissues. There was no indication that this fraction could not be eliminated within the time required for elimination of alimentary tract-associated virus. The location of virus within hepatopancreas tissue was not determined, but judging from the time required for its elimination it was available to depuration activity. If virus was within cellular depots, it was not irreversibly sequestered.

It was concluded that bioaccumulation of small numbers of feces-associated virus represented a different kind of virus carriage situation than that existing when large numbers were involved. The major difference appeared to be the availability of virus to depurative activity. The results of distribution studies of viruses were interpreted as evidence that virus remained chiefly within the alimentary tract.

The extent of the ability of depuration to deplete virus and eliminate virus health hazards in depurated clams involved a risk factor which could not be measured in the study. Shellfish-associated outbreaks involving hepatitis A and gastroenteritis viruses continue to occur, which means that clams, as well as other species located in sewage-polluted waters, continue to bioaccumulate and harbor these viruses. An opinion that the true incidence of shellfish-associated virus diseases has been underestimated infers that an even greater number of virus-carrying shellfish exists than suggested by the number of outbreaks recorded (11). This opinion is based upon the contention that many clinical, and especially silent, cases of these virus diseases escape the attention of public health authorities and go unreported. The opinion also takes into consideration the observation that many clinical cases of infectious hepatitis exist which have not been recognized to be of shellfish-associated virus etiology. Koff et al. (16) reported that epidemic patterns for sporadic cases of infectious hepatitis from unknown sources were found which were linked retrospectively by epidemiological studies after the outbreak to ingestion of steamed clams or raw shellfish.

The failure to recognize the existence of even sizeable outbreaks of shellfish-associated virus disease was well-illustrated by Goldfield (12). An outbreak numbering an apparent 459 cases of clam-associated infectious hepatitis in New Jersey occurred which would have gone entirely unrecognized by classic public health reporting practices. It was only after a postepidemic study of shellfish-associated hepatitis in New Jersey was made that ingestion of raw clams was recognized to be the source of the hepatitis outbreak. The true incidence during the outbreak was estimated at several times the 459 cases

recognized.

An increasing number of cases of nonbacterial gastroenteritis have been attributed to shellfish-associated gastroenteritis viruses such as the rotaviruses, as the result of epidemiological studies and the detection of rotavirus-like particles in fecal samples examined by electron microscopy (1).

It seems plausible to assume that opportunities for carriage of a number of different enteric viruses will continue to exist as long as clams are exposed to fecal pollution. Study data suggested, however, that low-level carriage states may be more common than previously thought. As indicated previously, maximum depuration effectiveness is more likely to be achieved with low-level virus carriage states. An inseparable, as well as imponderable, aspect of depuration lies in the knowledge that not all clams function with equal effectiveness in the elimination of virus. The uncertainty about depuration effectiveness in eliminating the risk of virus health hazards is compounded by the inability to study conveniently and directly "difficult to work with" viruses such as hepatitis and rotavirus which either have been shown as, or implicated as, causal agents in shellfish-vectored virus disease.

While the risk factor from virus health hazards associated with depuration could not be measured in the study, evidence was obtained in support of the contention that the greatest reduction in risk would come from the routine depuration of clams harvested from growing waters of good sanitary quality.

ACKNOWLEDGMENTS

This study was supported by Public Health Service research contract 223-75-2303 from the Food and Drug Administration, Department of Health, Education and Welfare.

LITERATURE CITED

1. **Appleton, H., and M. Pereira.** 1977. A possible virus etiology in outbreaks of food poisoning from cockles. Lancet **i**:780–781.
2. **Arcisz, W., and C. B. Kelly.** 1955. Self-purification of the soft clam, *Mya arenaria*. Public Health Rep. **70**: 605–614.
3. **Canzonier, W. J.** 1971. Accumulation and elimination of coliphage S-13 by the hard clam, *Mercenaria mercenaria*. Appl. Microbiol. **21**:1024–1031.
4. **Crovari, P.** 1958. Some observations on the depuration of mussels infected with poliomyelitis virus. Ig. Med. (Parina) **5**:22–32.
5. **Dahling, D. R., G. Berg, and D. Berman.** 1974. BGM, a continuous cell line more sensitive than primary Rhesus and African Green kidney cells for the recovery of viruses from water. Health Lab. Sci. **11**:275–282.
6. **Di Girolamo, R., J. Liston, and J. Matches.** 1975. Uptake and elimination of poliovirus by West Coast oysters. Appl. Microbiol. **29**:260–264.
7. **Di Girolamo, R., J. Liston, and J. Matches.** 1977. Ionic binding, the mechanism of viral uptake by shellfish mucus. Appl. Environ. Microbiol. **33**:19–25.
8. **Dodgson, R. W.** 1928. Report on mussel purification. Ministry of Agriculture, Fisheries and Food, Fisheries Investigations, ser. II. vol. X, no. 1. London, England.
9. **Fabre-Domerque. M.** 1912. Bacterial purification of oysters standing in filtered artificial seawater. C. R. Acad. Sci. **154**:393–395.
10. **Fabre-Domerque, M.** 1913. Purifying oysters from disease germs. Naturwiss. Umsch. Chem. Ztg. **51**:403–404.
11. **Gerba, C. P., and S. M. Goyal.** 1978. Detection and occurrence of enteric viruses in shellfish: a review. J. Food Protect. **41**:743–754.
12. **Goldfield, M.** 1976. Epidemiological indicators for transmission of viruses by water, p. 70–85. *In* G. Berg, H. L. Bodily, E. H. Lennette, J. L. Melnick, and T. G. Metcalf (ed.), Viruses in water. American Public Health Association, Washington, D.C.
13. **Hamblet, F. E., W. F. Hill, Jr., E. W. Akin, and W. H. Benton.** 1969. Oysters and human viruses: effect of seawater turbidity on poliovirus uptake and elimination. Am. J. Epidemiol. **89**:562–571.
14. **Hedstrom, C., and E. Lycke.** 1963. An experimental study on oysters as virus carriers. Am. J. Hyg. **79**:134–142.
15. **Hoff, J., and R. C. Becker.** 1969. The accumulation and elimination of crude and clarified poliovirus suspensions by shellfish. Am. J. Epidemiol. **90**:53–61.
16. **Koff, R. S., G. F. Grady, T. C. Chalmers, J. W. Mosley, and B. L. Swartz.** 1967. Viral hepatitis in a group of Boston hospitals. III. Importance of exposure to shellfish in a non-epidemic period. N. Engl. J. Med. **276**: 703–710.
17. **Liu, O. C., H. R. Seraichekas, and B. L. Murphy.** 1967. Viral pollution and self cleansing mechanism of hard clams, p. 419–437. *In* G. Berg (ed), Transmission of viruses by the water route. Interscience Publishers, Inc., New York.
18. **Liu, O. C., H. R. Seraichekas, and B. L. Murphy.** 1967. Viral depuration of the Northern quahaug. Appl. Microbiol. **15**:307–315.
19. **Metcalf, T. G. and W. C. Stiles.** 1968. Viral pollution of shellfish in estuary waters. J. Sanit. Eng. Div. Am. Soc. Civ. Eng. **94**:595–608.
20. **Mitchell, J. R., M. W. Presnell, E. W. Akin, J. M. Cummins, and O. C. Liu.** 1966. Accumulation and elimination of poliovirus by the eastern oyster. Am. J. Epidemiol. **84**:40–50.
21. **Moore, B. E., B. P. Sagik, and J. F. Malina, Jr.** 1975. Viral association with suspended solids. Water Res. **9**: 197–203.
22. **Schmidt, N. J., H. H. Ho, J. L. Riggs, and E. H. Lennette.** 1978. Comparative sensitivity of various cell culture systems for isolation of viruses from wastewater and fecal samples. Appl. Environ. Microbiol. **36**:480–486.
23. **Seraichekas, H. R., D. A. Brashear, J. A. Barnick, P. F. Carey, and O. C. Liu.** 1968. Viral depuration by assaying individual shellfish. Appl. Microbiol. **16**:1865–1871.
24. **Stagg, C. H., C. Wallis, and C. H. Ward.** 1977. Inactivation of clay-associated bacteriophage MS2 by chlorine. Appl. Environ. Microbiol. **33**:385–391.
25. **Wells, W. F.** 1916. Artificial purification of oysters. Public Health Rep. **31**:1848–1852.