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Uptake and Elimination of Poliovirus by West Coast Oysters

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Accumulation of poliovirus Lsc-2ab by West Coast oysters was determined by using a stationary seawater system, and depuration was determined by using both stationary and free-flow systems. Results indicate that these shellfish have the same pattern of accumulation and localization of viruses as do East Coast species. However, uptake appeared to occur more rapidly than described for East Coast shellfish. There appeared to be a gradual diffusion of virus from the digestive area into the body. Depuration was found to occur more rapidly and completely under free-flow conditions than in a stationary system.

Outbreaks of infectious hepatitis due to the consumption of shellfish harvested from sewage-polluted waters have been widely reported (5, 8, 12, 15). It has also been shown that shellfish readily accumulate virus from polluted waters (1, 6, 11, 13), and there have been a number of investigations into the mechanisms of virus uptake, retention, and elution by shellfish (1-4, 6, 10, 14). However, most of the reported work has involved shellfish species of the eastern United States or Europe. Little is known of the virus accumulation and depuration capabilities of shellfish species grown on the Pacific Coast of the United States. The shellfish-growing industry of the Pacific Coast states is large, and growing areas are increasingly subject to the hazard of human sewage pollution as the population increases. It seemed important, therefore, that the capacity for uptake and depuration of virus by the two commercially important species of oyster grown on the Pacific Coast be investigated. This paper describes the results of such an investigation.

MATERIALS AND METHODS

Oyster samples. Pacific oysters (*Crassostrea gigas*) and Olympia oysters (*Ostrea lurida*) used in these studies were obtained in lots of 50 to 100 per experiment. After cleaning off shell debris and superficially sanitizing shell surface with 1% hypochlorite, the oysters were placed in 5-gallon (about 19 liters) stainless-steel aquaria containing 3.5 liters of filtered sterile seawater (salinity 28‰). Water temperature was maintained at 13 C by cold-air circulation in a constant-temperature bath. Air hoses were used to provide aeration and circulation of the water.

Viruses. Attenuated poliovirus Lsc-2ab was used as a model system in all experiments. Stock virus was propagated in primary African green monkey kidney cell cultures. The stock virus pool containing approximately 10^8 plaque-forming units (PFU)/ml was kept at -20 C until used.

Tissue culture. Primary African green monkey kidney cell cultures were used throughout the experiments. The cell suspensions were purchased from Baltimore Biological Laboratories. Hanks balanced salt solution containing 0.2% Casamino Acids and 10% calf serum was used for cell growth and Earle medium was used for cell maintenence.

Plaque assays. The assay method used was that of Hsiung and Melnick (7). All samples were run in duplicate, and plaques were counted between the 3rd and 5th days of incubation.

Total uptake studies. To determine virual accumulation by the oysters over a 48-h period, sufficient poliovirus was added to the seawater to yield a total virus count of approximately 1.9×10^4 virus PFU/ml. Samples of seawater, shellfish, and feces were collected at 0-, 12-, 24-, and 48-h intervals. Four to six Pacific oysters (*C. gigas*) or 10 to 12 Olympia oysters (*O. lurida*) were used per sample. Five milliliters of seawater was used as a representative water sample. Oysters were removed from the aquaria, dipped in a 1% hypochlorite solution to sanitize the shell surfaces, and then rinsed in distilled water. Samples not assayed directly were sealed in polymylar pouches and stored at -20 C until used.

Samples were prepared for assay by shucking the oysters as aseptically as possible and preparing 10% (wt/vol) homogenates, using nutrient broth as a diluent. All homogenates were blended for 2 min at 6,500 rpm in a Lourdes homogenizer. Samples were clarified by low-speed centrifugation (1,200 rpm at 10 C) in a Sorvall RC2-B refrigerated centrifuge. Samples were assayed for virus by plaquing in duplicate monkey kidney bottles.

Accumulation and localization studies. To determine the major sites of virus accumulation, the oysters were contaminated in the fashion described above in seawater containing 1.9×10^4 virus PFU/ml. After contamination, the oysters were placed in poly-

mylar bags and frozen for 24 h at -20 C and then allowed to thaw slowly at 22 C. As the shellfish thawed, a fluid sample containing the mantle liquor was collected in a sterile container. The mantle, gills, and palps were dissected out, and then the digestive area and remaining body. After the procedure, the mantle liquor, mantle, gills, and palps were pooled, 10% homgenates in nutrient broth were prepared, and the samples were assayed.

Depuration studies in a stationary system. To determine the ability of the oysters to "self-cleanse" or depurate themselves, they were first exposed to seawater containing approximately 1.5×10^4 virus PFU/ml for 48 h and then placed in aquaria containing filtered, sterile seawater. The filter-sterilized seawater was changed at 12-h intervals. Samples of water, feces, and shellfish were collected at 0, 12, 24, 48, 72, 96, and 120-h intervals. To measure total depuration from the digestive area, the digestive diverticulae were dissected out of the oysters and pooled with the feces. All samples were then prepared and assayed as in previous experiments.

Depuration studies in a free flow system. The stationary seawater system has been reported to be an unsatisfactory means of attaining complete depuration of shellfish (11). Therefore the depuration study was repeated in a free-flow system having a flow rate of 1 liter of filter-sterilized seawater/h. Eight to ten oysters and 5 ml of seawater were routinely drawn from the depuration tank and assayed at 0, 12, 24, 48, and 72 h.

RESULTS

Total uptake studies. The results of total uptake studies using *C. gigas* as a test animal are shown in Fig. 1. Uptake of poliovirus by this oyster was found to reach 46% (4.6×10^3 virus PFU/g) after 12 h of exposure and 88% after 48 h of exposure, at which time the study was terminated.

Contrasted with these results, the initial uptake of poliovirus by *O. lurida* was quite rapid (Fig. 2). Recovery of virus from this oyster was found to be 86% (8.6×10^3 virus PFU/g) at the end of 12 h and 94% after 24 h of exposure.

Accumulation and localization studies. In Table 1 are summarized the results of studies using *C. gigas* as a test animal. The greatest accumulation of poliovirus was found to occur in the digestive area, where 67% of the virus was recovered after 25 h of exposure. A recovery of 1.4% was obtained from the mantle, gills, and palps after the same period of time. Virus titer in the body rose from 0.5% at the end of 24 h to a high of approximately 16% (3.0×10^3) at 48 h. The increase of virus titer in the body followed a partial drop in the number of virus recovered from the digestive area.

In Table 2 are summarized results of studies with *O. lurida*. As in the previous experiment,

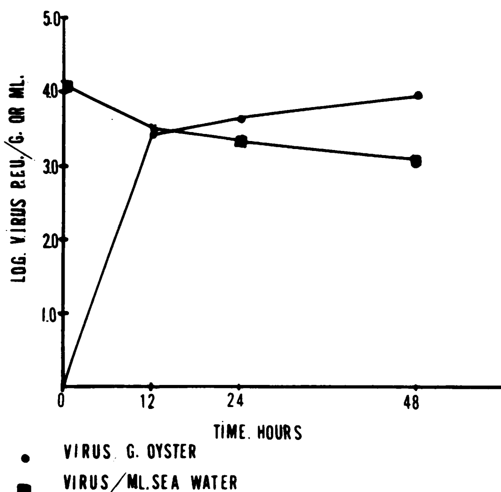

FIG. 1. *Uptake of poliovirus Lsc-2ab C. gigas in contaminated seawater for 48 h. Salinity, 28‰;* temperature, 13 C.

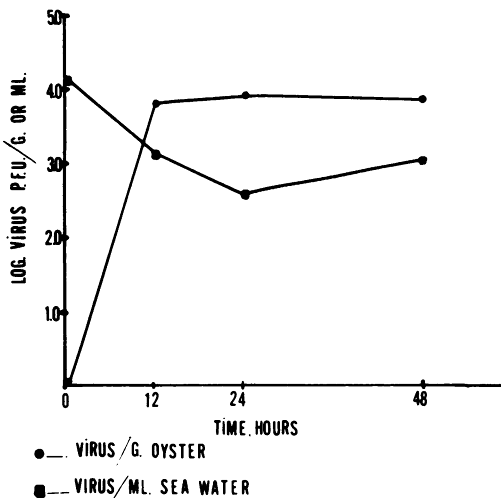

FIG. 2. *Uptake of poliovirus Lsc-2ab by O. lurida in contaminated seawater for 48 h. Salinity, 28‰; temperature, 13 C.*

TABLE 1. *Accumulation of poliovirus by C. gigas under experimental conditions*

Time (h)	Virus (PFU/g or ml of sample)			
	Seawater	Mantle, gills, fluid	Digestive area and feces	Body
0	1.9×10^4	0	0	0
12	1.2×10^4	1.6×10^2	6.8×10^3	6.0×10^1
24	6.0×10^3	3.6×10^2	1.3×10^4	7.4×10^1
48	1.2×10^3	3.0×10^3	1.2×10^4	3.0×10^3

the greatest recovery of poliovirus was made from the digestive area, which had accumulated 71% of the cirus (1.4×10^4) at the end of 24 h of contamination. Concurrently, a 20% recovery of virus was obtained from the mantle, gills, and palps. Virus titer in the body rose from 2% at 12 h to a high of 6.2% (1.3×10^3) at 48 h. This finding indicates a diffusion of virus from the digestive region into the body of the oyster.

Depuration studies in a stationary system. In Fig. 3 are summarized results of depuration experiments with *O. lurida*. Depuration appeared to occur gradually, with 16% (1.6×10^3) of the virus accumulated still remaining in the oyster after 120 h.

In Fig. 4 are summarized results of depuration studies using *C. gigas* as the test animal. As in the preceding study, removal of virus by this oyster appeared to occur gradually for the duration of the study. The experiment was discontinued after 120 h, at which time 3.3×10^3 virus PFU/g, or approximately 21% of the total virus accumulated, was still recovered from the shellfish.

Depuration studies in a free-flow system. In the studies described above, Pacific and Olympia oysters both appeared to depurate poliovirus slowly and incompletely. Therefore, in free-flow experiments only one species *O. lurida* was used as a test animal (Table 3). In all experiments the titer of poliovirus recovered from shellfish before depuration was considered to represent 100% recovery. Contrasted with results under stationary conditions, depuration under free-flow conditions was rapid and efficient. After 24 h of depuration, approximately 24% of the total virus accumulated still remained in the oyster; of this amount 22% was recovered from the body and 2% from the fluid. The study was terminated after 72 h of depura-

TABLE 2. *Accumulation of poliovirus by O. lurida under experimental conditions*

Time (h)	Virus (PFU/g or ml of sample)			
	Seawater	Mantle, gills, fluid	Digestive area	Body
0	2.0×10^4	0	0	0
12	2.8×10^3	3.6×10^3	1.3×10^4	4.0×10^2
24	1.2×10^3	4.0×10^3	1.4×10^4	6.0×10^2
48	1.2×10^3	3.9×10^3	1.3×10^4	1.3×10^3

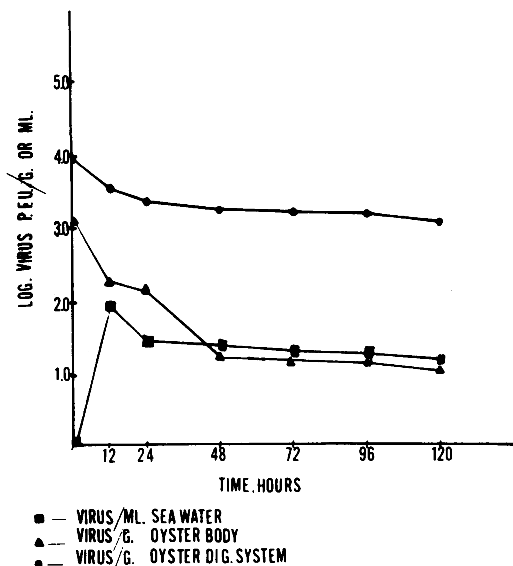

FIG. 3. *Depuration of poliovirus Lsc-2ab by O. lurida in a stationary seawater system.*

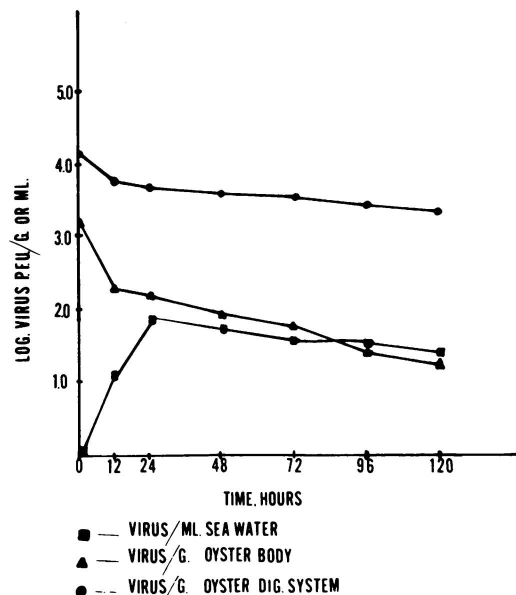

FIG. 4. *Depuration of poliovirus Lsc-2ab by C. gigas in a stationary seawater system.*

TABLE 3. *Depuration of poliovirus by O. lurida in a free-flow seawater system*

Time (h)	Virus (PFU/g or ml of sample)		
	Seawater	Oyster body	Oyster fluid
0	0	3.4×10^3	2.2×10^2
12	2.3×10^1	1.8×10^3	2.0×10^2
24	1.4×10^1	7.8×10^2	6.6×10^1
48	1.0×10^1	1.4×10^2	1.8×10^1
72	9.2×10^1	2.0×10^2	1.0×10^1

tion, at which time less than 1% of the virus accumulated still remained in the oyster.

DISCUSSION

Total uptake studies. Results of these studies indicate that West Coast shellfish accumulate virus in approximately the same quantity as reported for Eastern species. However, there appears to be a difference in rate of uptake between East and West Coast shellfish and between the two species tested. The uptake of poliovirus I by the Eastern oyster *C. virginica* has been reported by Metcalf and Stiles (13) to be 10^5 mean tissue culture infective dose units after 72 h of contamination. Liu et al. (11) have reported a 94% uptake by the Northern Quahaug (*Mercenaria mercenaria*) after the same period of exposure. Although our results show the same percentage of uptake of virus by Western oysters (88 to 94%), this rate of accumulation occurs after 48 rather than 72 h. This finding is important since it suggests that West Coast shellfish can accumulate large quantities of virus in a short time and may have a slightly greater capacity for virus uptake than Eastern species.

Uptake of poliovirus occurs more rapidly in the Olympia oyster (*O. lurida*) than in the Pacific oyster (*C. gigas*), reaching a 94% accumulation at 24 h as opposed to an 88% accumulation by *C. gigas* at 48 h. Therefore, only a short period of exposure would be necessary for *O. lurida* to become highly contaminated.

Accumulation and localization studies. Research by other workers with Eastern shellfish has clearly shown the digestive tract to be the major site of virus accumulation by these species, with the greatest level of contamination occurring a few hours after exposure to polluted seawater (9, 10, 13). Our own results are essentially in agreement with the observations of these workers. The digestive tract of Western oysters, as in Eastern oysters, is the major site of virus accumulation, and maximum uptake of virus occurs within the first 24 h of exposure to contaminated seawater. There also appears to be a gradual diffusion of virus from the digestive tract to the body. Depending upon species, up to 15% of the total virus accumulated may diffuse into the body of the oyster within a 48-h period. This finding is important in considering any commercial depuration process. Depuration times used must be long enough to permit the shellfish to cleanse themselves not only of virus in the digestive area but of residual virus in the body as well.

Depuration studies in a stationary system. Depuration, the process by which shellfish rid themselves of bacteria and viruses, has been proven to occur more rapidly and efficiently in a free-flow rather than in a stationary seawater system (9, 11, 14). Our own results using a stationary system (15% recovery of virus after 120 h of depuration) confirm these findings. Therefore, our results with a stationary system must be considered baseline observations indicating that West Coast shellfish posses the same ability to depurate as do Eastern species.

Depuration studies under free-flow conditions. West Coast oysters depurate viruses rapidly in a free-flow system. Under experimental conditions, the original virus titer in the oysters was reduced by 99% after 72 h of depuration. These observations are in agreement with those of other workers who reported similar results with European mussels, Eastern oysters, and Quahaugs (3, 9, 11, 13). However, to date, all depuration studies with West Coast species have been conducted under rigidly controlled conditions using relatively small sample sizes. How efficient depuration of virus by these shellfish would be under commercial scale operations is yet to be determined. Liu et al. (11) have noted some of the problems likely to be encountered under these conditions.

West Coast shellfish accumulate and depurate viruses in a fashion similar to that of Eastern species. However, the rate of virus uptake appears more rapid in Western oysters. The observed diffusion of viruses into the body of the oysters may prove of significance when considering depuration on a commercial scale. We believe further research is needed in the area of viral depuration by shellfish.

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