Preliminary Observations on the Uptake of Poliovirus by West Coast Shore Crabs

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West Coast shore crabs (Pachygrapsus sp. and Hemigrapsus sp.), when in seawater contaminated with poliovirus or allowed to feed on virus-contaminated mussels (Mytilus californianus), were found to accumulate high titers of virus.

It is a proven fact that shellfish (oysters, mussels, and clams) which have been residing and feeding in sewage-polluted waters accumulate pathogenic organisms and can serve as vectors for such virus-induced diseases as infectious hepatitis (5–8). However, there are other invertebrates, such as edible crabs, residing in the same waters which could also become polluted. To date no one has investigated the possibility of these animals accumulating viruses either from seawater or by cross-contamination from feeding on polluted shellfish. Therefore, a preliminary investigation into these possibilities has been initiated in our laboratory.

Two separate series of experiments were conducted. In the first study, Pacific Coast shore crabs, Pachygrapsus marmoratus, with 2.5- to 3-inch (6.3- to 7.6-cm) carapace widths, were placed in 10-gal aquaria containing 3 liters of filtered seawater contaminated with poliovirus Lsc-2ab. They were chosen as test animals because of their ready availability, ease of handling, and similarity of feeding habits to those of edible shore crabs. Virus titer was approximately 1.4 × 10^6 plaque-forming units (PFU)/ml. Samples of seawater and crabs were routinely removed and assayed for virus content at 0, 12-, 24-, and 48-hr intervals. The samples consisted of 5 ml of seawater and eight crabs. Crabs to be assayed were dipped in a 1% hypochlorite solution to inactivate any virus adhering to the carapace surfaces and then were rinsed in sterile distilled water. Samples were readied for assay by preparing 10% (w/v) homogenates of tissue with nutrient broth as a diluent. All homogenates were blended for 1 min at 6,500 rev/min in a Waring Blender. After clarification, serial decimal dilutions were prepared in nutrient broth, and the samples were assayed for virus by plaquing in duplicate monolayer bottles. The assay technique was that of Davis and Dulbecco as modified by Hsuing and Melnick (3). Primary African green monkey kidney tissue was used to prepare monolayers which were grown in 3-oz (ca. 90 ml) prescription bottles.

In the second study, 2-year-old California mussels (Mytilus californianus) were placed in 10-gal aquaria containing 3 liters of filtered seawater contaminated with poliovirus. Virus titer was approximately 3.5 × 10^4 PFU/ml. The shellfish were allowed to become contaminated for 48 hr and then were removed and “sanitized” in 1% hypochlorite. To determine the amount of virus per gram of shellfish tissue, eight mussels were shucked and assayed by the procedure described above. At this time, the mussels contained 2.6 × 10^4 virus PFU/g or, on a per unit weight basis, 75% of the virus present in 1 ml of water. The remaining mussels were opened and placed in aquaria containing 3 liters of filtered seawater and 10 shore crabs. The crabs were allowed to feed on the mussels for a period of 12 hr. At this time, the animals had consumed practically all of the mussel tissue. Samples were removed and assayed at 0 and 12 hr. Routinely, 5 ml of seawater, 10 crabs, and 8 mussels were used as representative samples. The crabs and mussels used in these studies were collected from Pillars Point, Calif., an area free from sewage pollution. Samples were assayed to assure that they were free from virus before being used as test animals.

The results of uptake studies are shown in Fig. 1. There was a continued uptake of virus
from seawater by the crabs during the entire period of the experiment. This uptake varied from $1.8 \times 10^4$ PFU/g at 12 hr to a high of $4.9 \times 10^3$ PFU/g at 48 hr, representing uptakes of 28% and 63%, respectively. These results suggest that the viruses are not merely passed through the crab during respiration but are actively concentrated by these animals.

The results of the feeding experiments are presented in Fig. 2. During the 12 hr they fed upon contaminated mussels, the crabs accumulated 74% to 94% of the virus present in the shellfish. This variation in uptake was probably due to physiological differences and differences in rate of acclimation to the environment (9). These results indicate, however, that crabs can become polluted after feeding on contaminated shellfish.

The results reported are preliminary observations. Additional research is being conducted to determine where the virus is being accumulated and whether virus in crabs can withstand processing (cooking). Johnston et al. (4) suggested that the blood cells of crabs serve a hepatic function. If so, then virus, along with macromolecules such as protein, could be transported to the blood system and hence to edible tissues. DiGirolamo et al. (1) have shown that viruses can survive processing of contaminated oysters. If this is also true of virus in crabs, then contamination of these animals could be a potential public health problem.

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