Detection and Occurrence of Enteric Viruses in Shellfish: A Review

CHARLES P. GERBA* AND SAGAR M. GOYAL

Department of Virology and Epidemiology.
Baylor College of Medicine.
Houston, Texas 77030

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ABSTRACT

During feeding, bivalve mollusks (oysters, mussels and clams) can accumulate pathogenic human enteric viruses when present in sewage-polluted seawater. It has been well established that infectious hepatitis virus is transmitted by consumption of raw or inadequately cooked shellfish. But because of the lack of epidemiologic techniques, transmission of other enteric viruses by shellfish has not been established. Other enteric viruses, such as polio, echo, coxsackie and reo, have been detected in shellfish. Enteroviruses have been detected in shellfish taken from both "open" and "closed" areas, based on bacteriological standards used at present in the United States. Field and laboratory studies have indicated that enteric viruses can survive for long periods in seawater and in shellfish. Recent advances in methodology have led to development of more rapid and less expensive methods for detection of a greater number of enteric viruses in shellfish.

Edible bivalve mollusks of the class Pelecypoda (oysters, mussels, clams) are characterized by two shell valves hinged together at one end and closed by a large adductor muscle to the valves near the other end. These commercially harvested marine organisms are one of the few shellfish which are commonly eaten raw. In view of a great protein shortage in the world, shellfish are assuming a greater nutritive importance. The total world catch of aquatic animals and plants was $656 \times 10^4$ metric tons in 1972 (94), of which 8% were shellfish. The United States presently leads the world in the catch of bivalve mollusks (64).

Bivalve mollusks are filter-feeding organisms, i.e., they sieve out suspended food particles from a current of water passing through the shell cavity. In its quest for food, an oyster may filter as much as 396 gal. (1500 liters) of water per day (2). The amount of water screened by quahogus varies between 0.05 and 0.015 liters per hour per gram of meat (81). The feeding rates, however, depend upon such variable factors as salinity, temperature, particulate matter and availability of suitable food. If the water in which shellfish are feeding contains pathogenic bacteria or viruses, they may become entrapped on the mucous membranes and transferred to the digestive tract. Since the entire shellfish is usually consumed along with the gastrointestinal tract, shellfish may act as passive carriers of human pathogenic microorganisms. The effective control of enteric bacterial disease spread by shellfish has resulted from establishment of bacteriological standards using the coliform and fecal coliform index as the basis for a certification program. Lesser documented and studied are problems associated with transmission of viral disease by shellfish, because of the lack of sensitive techniques for their study. The purpose of this report is to review information on the occurrence of enteric viruses in shellfish and shellfish-growing areas and recent progress in methodology for their detection.

More than 100 types of viruses are present in human feces and may find their way into domestic sewage (32). These include the enteroviruses (polio, coxsackie, echo), reoviruses, adenoviruses, infectious hepatitis, and rotavirus. These viruses cause such illnesses as fever, paralysis, meningitis, respiratory disease, diarrhea, etc. Although in advanced countries water purification processes have reduced the number of waterborne disease outbreaks, viruses are still present even if coliforms are reduced to acceptable levels. Viruses may remain infectious for several weeks or longer after discharge into receiving waters, allowing sufficient time for them to be transported to coastal shellfish-growing areas. Once inside a shellfish, their survival appears to be further prolonged (67).

DISEASE OUTBREAKS ATTRIBUTED TO SHELLFISH

It has been well established that infectious hepatitis virus is transmitted by consumption of raw or inadequately cooked clams and oysters. The first documented outbreak of infectious hepatitis traced to consumption of raw oysters occurred in Sweden in 1955 (54,82). A number of well-documented outbreaks involving over 2,000 cases have been described since then. These outbreaks have been reviewed by Mosley (75) and more recently by Goldfield (33). In Table I are listed outbreaks which have been documented since the latter report.

In nonepidemic times a relationship between
consumption of shellfish and occurrence of hospitalized cases of infectious hepatitis is known to exist (48), indicating that the true incidence of shellfish-associated viral disease is probably greatly underestimated (33). In addition, residents of coastal states rather than inland states seem to have a higher occurrence of infectious hepatitis (13). The predominately occult nature of other waterborne viruses has made it difficult to document their transmission by shellfish, but their occurrence in shellfish is well documented (29, 71, 91) and the potential for disease always exists.

Outbreaks of hepatitis have been attributed to the European oyster (Ostrea edulis) (45, 83), eastern and Gulf coast oysters of North America (Crassostrea virginica) (43), northern quahog clam (Mercenaria mercenaria) (6, 38), the sand clam (Mya arenaria) (36), and mussels (Mytilus edulis) (16). Several recent outbreaks are of particular interest because of new light shed on survival of hepatitis viruses in shellfish.

Recently, a large outbreak of hepatitis A in Houston (270 persons with a history of consuming raw oysters at one of ten Houston restaurants) and a smaller outbreak in Calhoun, Georgia (15 of 225 persons attending one of two seafood dinners sponsored by a Calhoun social club) occurred simultaneously during the fall of 1973. These outbreaks were oyster-associated and oysters incriminated in each outbreak originated from Louisiana. Considered together, these cases represented the second largest recorded outbreak of oyster-associated hepatitis (61).

As a result of intensive investigation, a peak in oyster-associated cases of hepatitis A in Louisiana (37 of 137 reported cases) was shown to coincide with those in Houston and Calhoun. Several theories were put forth to account for the source of contamination in Louisiana oysters and all were discarded. The possibility which gained support from many investigators was that due to flooding in the Mississippi River during April and August 1973 the oyster beds experienced the greatest degree of freshwater intrusion and fecal pollution. Following discovery of a high coliform index, the Louisiana Health Department closed the area for shellfish harvesting in early May; this restriction was lifted on August 20, 1973, following receding of the Mississippi River and falling of coliform counts. The oysters implicated in the outbreaks, however, were not sold until September 16, which meant that at the height of flooding, oysters concentrated virus in their tissues to a level in excess of that existing in the surrounding waters. This indicated that hepatitis virus remained viable for 1-2 months after the receding of the Mississippi River water from the area and at levels sufficient to cause disease.

Dienstag et al. (16) incriminated incompletely cooked mussels (Mytilus edulis) as the vehicle of acute viral hepatitis in seven members of a family of 14. The role of mussels was confirmed epidemiologically as well as serologically by demonstration of rises in titers of serum antibodies to hepatitis A antigen as determined by immune adherence hemagglutination.

Koff and Connelly (46) conducted a prospective, controlled epidemiologic study to determine the modes of transmission of nonepidemic infectious hepatitis cases among patients in 10 Boston Hospitals. Many cases of infectious hepatitis thought to be sporadic fell into epidemic patterns. Ingestion of raw shellfish was significantly more frequent (34/185) in infectious hepatitis patients than in controls (10/185). Only four of the 74 patients who had eaten raw shellfish had a history of contact with jaundiced persons. Also, ingestion of steamed clams (13/104) was more common in patients than in matched controls (2/104); only 3 of the 13 patients had been exposed to jaundiced persons. When considered together, ingestion of steamed clams or raw shellfish was as frequent a potential exposure to hepatitis as was contact with jaundiced persons.

There may also be sporadic cases of infectious hepatitis attributed to ingestion of shellfish. Koff and Connelly (46) reported sporadic cases in New England and Stille et al. (90) in Germany. The study in Germany

<table>
<thead>
<tr>
<th>Year</th>
<th>Source</th>
<th>Place</th>
<th>Number of cases</th>
<th>Remarks</th>
<th>Reference no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1963</td>
<td>shellfish</td>
<td>Boston, Mass.</td>
<td>46</td>
<td>prospective study</td>
<td>48</td>
</tr>
<tr>
<td>1966</td>
<td>shellfish</td>
<td>Frankfurt, Germany</td>
<td>34</td>
<td>—</td>
<td>90</td>
</tr>
<tr>
<td>1968</td>
<td>raw and steamed clams</td>
<td>Rhode Island</td>
<td>3</td>
<td>clams were dug on the Rhode Island shore</td>
<td>28</td>
</tr>
<tr>
<td>1971</td>
<td>oysters from Louisiana</td>
<td>Houston, Tex.</td>
<td>263</td>
<td>—</td>
<td>79</td>
</tr>
<tr>
<td>1973</td>
<td>raw clams</td>
<td>Calhoun, Ga.</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1976</td>
<td>incompletely cooked mussels</td>
<td>Minneapolis, Minn.</td>
<td>1</td>
<td>suspected</td>
<td>37</td>
</tr>
<tr>
<td>1976</td>
<td>cockles</td>
<td>Victoria, Australia</td>
<td>7</td>
<td>confirmed serologically and epidemiologically</td>
<td>16</td>
</tr>
<tr>
<td>1976</td>
<td></td>
<td>Southampton and Chelmsford, U.K.</td>
<td>797</td>
<td>food poisoning due to small, round, virus-like particles</td>
<td></td>
</tr>
</tbody>
</table>
ENTERIC VIRUSES IN SHELLFISH

indicated that consumption of contaminated mollusks accounted for an estimated 19% of hepatitis type A cases in Frankfurt. The German cases were chiefly attributed to eating oysters and mussels from the Mediterranean littoral, especially southern France and Italy. Even so-called sporadic cases may not be truly sporadic, because a common source in two or more cases is often not suspected and goes unnoticed.

In addition to infectious hepatitis, shellfish may also be responsible for outbreaks of nonbacterial gastroenteritis (25,33,63,80). In a series of 33 outbreaks, 797 people suffered from food poisoning during one year in Southampton and Chelmsford, U.K. (7). Epidemiologically, all of the outbreaks were traced to consumption of cockles. Cockles were gathered from waters known to be polluted by sewage and at a time when gastroenteritis was apparently widespread in a nearby area. Small, round, virus-like particles were detected by electron microscopy in a high proportion of fecal specimens from three of the outbreaks. On the basis of morphology, these particles were thought to be the agent of winter vomiting disease but proved serologically distinctive. Again, few investigations have been concerned with outbreaks of nonbacterial gastroenteritis because of the lack of methodology. Recent advances in detection of rotaviruses, a leading cause of diarrhea in infants and apparently adults (27), will undoubtedly make such studies more feasible in the future.

ISOLATION OF VIRUSES FROM SHELLFISH

Several studies on the occurrence of enteric viruses in oysters have been made, and their results are summarized in Table 2. The occurrence of enteric viruses has been reported in M. edulis (24), C. virginica (20,87), and M. mercenaria (57). Viruses have most commonly been isolated from heavily polluted areas closed to shellfish harvesting, but have also been detected in areas approved for shellfish harvesting (29,33). Echinoviruses have been isolated from mussel samples obtained from an Italian market (7). Denis (15) reported recovery of coxsackie A viruses in market samples taken in France, and Fugate et al. (29) reported isolation of poliovirus in oysters imported into the United States from Japan. Thus it appears that enteroviruses may be present in marketed oysters even in developed countries and further investigation appears warranted.

Unfortunately, field studies in the past have been handicapped by the lack of dependable quantitative techniques for detection of enteric viruses and only limited numbers of shellfish could be sampled. Recent developments for detection of enteric viruses in large volumes of water and in shellfish should prove to be an impetus for studies to provide additional insight into occurrence of these pathogens in the marine environment.

Metcalf (66,68a) did the first field studies on occurrence of enteroviruses in shellfish and shellfish-harvesting waters. These studies resulted in the realization that examination of only the water in which the shellfish were growing could not be used as an indication of the presence of viruses in shellfish taken from the same water. This results from the fluctuating nature of water quality within the estuarine environment. The discontinuous patterns of polluted waters are influenced greatly by both tidal and freshwater inflow into the estuary. Thus viruses could be retained by shellfish even after sewage pollution was no longer evident in the area. Human enteric viruses were detected in shellfish beds 7 to 8 miles from the nearest sewage outfall (70a).

In more recent studies, Metcalf et al. (71) carried out parallel examinations of oysters and overlying seawater in Galveston Bay, Texas, for 3 months for the presence of fecal coliforms and enteroviruses. On seven occasions, water samples (25 to 105 gal.) were processed but no virus was isolated. In simultaneously collected oyster samples, however, polioviruses were found on two occasions. Some of the poliovirus type 1 isolates possessed virulent markers, which is significant from a public health viewpoint.

Earlier studies depended on use of "grab" samples or the placement of "gauze pads" in the water being tested for isolation of viruses and the direct assay of crude shellfish homogenates, but more recent developments in methodology allow for quantitative enumeration of enteric viruses in hundreds of gallons of marine water (78) and for the concentration of viruses from large pools of shellfish (89).

Using these new techniques, Goyal et al. (35) conducted a 1-year study on occurrence of enteroviruses in Galveston Bay, along the upper Texas coast. On several occasions, viruses were isolated from 100-gal. (378-liter) water samples and from oysters which were taken from areas open to commercial shellfish harvesting.

In a recent study of shellfish growing waters of Great South Bay, Long Island, New York, enteroviruses were recovered 37.5% of the time in water and oyster samples taken from areas open to shellfish harvesting (90a).

Mahoney et al. (62) found hepatitis B virus, the major cause of serum hepatitis, in hard-shelled clams harvested from a polluted bed on the coast of Maine, where a hospital was discharging sewage nearby. In closed system aquariums, these clams were able to filter and concentrate hepatitis B virus. No replication was demonstrated. Kater et al. (44) detected hepatitis B virus in nine of four soft-shell clams taken from an estuary known to be bacteriologically polluted. Koff and Connelly (46), on the other hand, were unable to detect hepatitis B antigen in approved clam beds (both hard- and soft-shell) in Maine, Massachusetts and Rhode Island. Thus, hepatitis B virus would only appear to be a concern in those areas where untreated or poorly treated hospital wastes are a major source of pollution.

It has been assumed that shellfish act only as passive carriers of virus because no virus multiplication has ever
been demonstrated in them. Convincing proof was provided by Chang et al. (11) who inoculated proflavine-labeled, light-sensitive virus into quahags and examined them subsequently for light-resistant viral progeny. Failure to find such progeny was convincing proof that viruses are only concentrated and do not multiply in shellfish.

**ADEQUACY OF BACTERIOLOGICAL STANDARDS**

It is usually difficult and time-consuming to routinely determine the presence of pathogenic bacteria and viruses in wastewater and shellfish. The fecal coliform index is therefore used as an indicator of sanitary quality because they are normal inhabitants of the gastrointestinal tract of warmblooded animals and are excreted in feces in large numbers. Presence of fecal coliforms in a certain sample is then considered as evidence of recent fecal pollution of that sample. Standards exist in the United States for shellfish and shellfish-growing waters which state that "Most probable numbers of coliforms in water should not exceed 70 per 100 ml and no more than 10% of samples should exceed 230 coliforms per 100 ml" (95). Shellfish meat in itself is required to contain no more than 230 fecal coliforms per 100 g (95). On the basis of these standards, oyster beds have been classified into three separate categories, i.e., closed, approved and conditional.

Recent studies, however, indicate that these standards

<table>
<thead>
<tr>
<th>Reference no.</th>
<th>Species of shellfish</th>
<th>Source</th>
<th>Virus type isolated</th>
<th>% positive</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>66</td>
<td>C. virginica</td>
<td>Little and Great Bay, New Hampshire, known to be polluted with raw sewage</td>
<td>echo 9, coxsackie B4</td>
<td>0 of 10 pools of 10 oysters each</td>
<td>virus was found in 4 miles from the source of pollution</td>
</tr>
<tr>
<td>77</td>
<td>mussels</td>
<td>Genoa Harbor, Italy</td>
<td>polio 3</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>7</td>
<td>mussels</td>
<td>Italian markets of Bari and Parma</td>
<td>echo 3, 9 and 13</td>
<td>30 samples of 3 mussels each</td>
<td>echo 3 isolated in monkey kidney; echo 3 and 9 in human amnion</td>
</tr>
<tr>
<td>68</td>
<td>C. virginica</td>
<td>New Hampshire estuaries (for 4 years)</td>
<td>polio 1, 2, 3; echo 9; reo 1; coxsackie B2, B3, B4</td>
<td>114 of 459 pools</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>Mer. mercenaria</td>
<td>Atlantic coast of U.S.</td>
<td>?</td>
<td>33% of clams, 55% of oysters</td>
<td>area was known to be polluted</td>
</tr>
<tr>
<td>64</td>
<td>C. virginica</td>
<td>area known to be bacteriologically polluted</td>
<td>hepatitis type B antigen</td>
<td>1 of 4</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Mystillus gallo- provincialis</td>
<td>Loughorn coast of Italy</td>
<td>echo 5.6.8.12, coxsackie A18</td>
<td>5 of 68 pools of 10 mussels each</td>
<td>grown or temporarily maintained in polluted water</td>
</tr>
<tr>
<td>70</td>
<td>C. virginica</td>
<td>estuarine inlet of North Atlantic ocean</td>
<td>enterovirus, 30 coliphages</td>
<td>59 of 130 pools positive either for coliphage or enterovirus</td>
<td>treated effluent discharged into the estuary</td>
</tr>
<tr>
<td>15</td>
<td>(a) oysters</td>
<td>Poitiers, France</td>
<td>coxsackie A18 predominant</td>
<td>(a) 7 of 70 pools</td>
<td>12 oysters or mussels in each pool</td>
</tr>
<tr>
<td>71</td>
<td>(b) mussels</td>
<td>Galveston Bay, Texas</td>
<td>polio 1.2</td>
<td>(b) 2 of 10 pools</td>
<td>---</td>
</tr>
<tr>
<td>62</td>
<td>C. virginica</td>
<td>Atlantic coast of Maine</td>
<td>hepatitis type B antigen</td>
<td>100% samples positive from this site but none from other 20 sites</td>
<td>beds were polluted as a result of discharge of untreated sewage from a hospital</td>
</tr>
<tr>
<td>29</td>
<td>C. virginica</td>
<td>(a) Texas Gulf coast</td>
<td>echo 1, polio 1</td>
<td>2 of 17 samples</td>
<td>oysters taken from approved waters were positive on occasion</td>
</tr>
<tr>
<td>74</td>
<td>Mer. californiacus</td>
<td>collected from stations remote from sewage outlets; placed in cages and suspended at various depths below buoys located offshore near outfall diffusers</td>
<td>?</td>
<td>18 of 39 samples</td>
<td>---</td>
</tr>
<tr>
<td>91</td>
<td>C. virginica</td>
<td>Great Bay, New Hampshire</td>
<td>(a) coliphages (b) enterovirus chlamydia, ricketsia, mycoplasma and their phages</td>
<td>80 of 158 pools of 10-12 oysters each</td>
<td></td>
</tr>
<tr>
<td>39</td>
<td>C. virginica,</td>
<td>Chesapeake or Chincoteague Bay, Maryland</td>
<td>polio 2</td>
<td>12 of 158 each</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>Mya arenaria</td>
<td>Galveston Bay, Texas</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>90a</td>
<td>clams and oysters</td>
<td>Great South Bay and Oyster Bay, Long Island, N.Y.</td>
<td>polio 1.2</td>
<td>3 of 22 in open waters; 5 of 24 in closed waters</td>
<td>isolated from beds open for shellfish harvesting</td>
</tr>
</tbody>
</table>

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**TABLE 2. Virus isolation studies on shellfish.**

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cannot be relied upon because coliform bacteria are more sensitive to treatment processes or natural inactivation factors than some of the more resistant enteroviruses (85). Morris et al. (74) calculated that enteric viruses could survive in mussel tissue three to six times longer than coliform bacteria. In several studies, enteroviruses have been isolated from shellfish otherwise having a satisfactory coliform index. Thus, Fugate et al. (29), Vaughn and Landry (90a), and Goyal et al. (35) detected enteroviruses in oysters taken from approved waters. In the second largest outbreak of shellfish-associated hepatitis ever reported, the implicated shellfish were harvested from a bay where the water met national sanitation standards for shellfish growing and was certified for oyster harvesting (61).

Similarly, Metcalf et al. (71) described a study carried out in Galveston Bay in which poliovirus types 1 and 2 were isolated from two of seven pools of oysters tested; the density of fecal coliforms in these two instances was only 7 and 9 per 100 ml of water and 20 and 79 per 100 g of oyster meat, respectively.

In a 2-year study on occurrence of enteric viruses, bacteriological indicators and pathogens along the Texas coast, no significant statistical relationship was demonstrated between virus concentration, bacteriological indicators and pathogens, and a number of various physicochemical characteristics of the water, except pH (34). A strong correlation, however, was found between virus concentration and the presumptive total coliforms in sediments.

Considerable interest has recently been shown toward the possible use of coliphages as an indicator of enteric virus status of shellfish and seawater (52, 53). Indicators are acceptable only if they constantly and accurately reflect the status of the organism concerned. Vaughn and Metcalf (91) described results of parallel examinations of sewage effluent, mud, shellfish and water for coliphages and enteroviruses in a polluted estuarine environment over 3 years. With the exception of mud samples, coliphages occurred more often than enteroviruses in all samples. They doubted the usefulness of the coliphage indicator system for viral presence in estuarine water and shellfish for the following reasons: (a) coliphages were consistently present in raw sewage samples which yielded inconsistent enterovirus isolations; (b) treated effluents were coliphage-positive but enterovirus-negative; (c) many (63%) enterovirus isolations occurred without any phage isolation; (d) replication of phages in estuarine water during the summer months (if proper bacterial hosts are present) makes them unsuitable for use as an indicator system; and (e) in a controlled experiment on comparative uptake of coliphages and enteroviruses by oysters, uptake of coliphage was found to be five- to 30-fold higher than that of enteroviruses (70). They argued that a determination of microbial acceptability of shellfish for human consumption should include assessment of the viral content (70).

Recent developments in virus concentration and rapid techniques for detection of viruses, such as radioimmunoassay, should make monitoring of shellfish for presence of viruses more feasible on a routine basis in the future and could serve as an adjunct to regular bacteriological monitoring programs.

### ACCUMULATION OF VIRUS BY SHELLFISH

Numerous studies (9, 11, 14, 40, 59, 66, 73) have shown that several common species of shellfish rapidly accumulate virus if present in polluted waters. Uptake has been demonstrated in Mediterranean mussels, *Mytilus edulis* (24) and *M. galloprovincialis* (72); European oysters, *O. edulis* (92); eastern and Gulf coast oysters, *C. virginica* (20, 93); hard clams or northern quahog, *Mer. mercenaria* (55); New Zealand marine blue mussel, *M. edulis coteanus* (60); Pacific oysters, *C. gigas* (42); Olympia oysters, *O. lurida* (42); and Manila clams, *Tapes japonica* (42). Maximum accumulation takes place within a few hours, and the level is maintained as long as sufficient virus is present in the surrounding water. It has been demonstrated that shellfish can concentrate virus in their tissues at a level much higher than the surrounding water (35, 57, 73). As the concentration of virus decreases in the water, the viral content in shellfish also starts decreasing. This process is called "depuration" or "purging" (5, 40, 66).

Table 3 shows the results of various experimental studies done on accumulation and subsequent depuration of virus by different species of shellfish. In these experimental systems, shellfish are exposed to known concentrations of virus for a period in aquariums with static or flow-through water supplies. Most workers have demonstrated that maximum concentration of virus occurs in the digestive system of shellfish (20, 54, 56, 57, 63, 66). Transport and uptake rates of virus by shellfish are dependent on a number of factors including turbidity and particulate matter. Hoff and Becker (42) reported that the uptake rate is dependent on the state in which virus exists in natural waters, i.e., whether it is present as free particles or attached to larger particulate matter. They found that the accumulation factor for crude poliovirus was from 10- to 900-fold, whereas for clarified virus preparations, it was only 0.4- to 3.6-fold. Uptake rate is higher in a flow-through than in a static system (59). DiGirolamo et al. (20) demonstrated interspecies differences in the uptake rate when they found that more than 86% of poliovirus present in seawater was concentrated within 12 h by Olympia oysters (*O. lurida*); for the same degree of concentration, Pacific oysters (*C. gigas*) took 48 h. The effect of turbidity on accumulation was demonstrated by Hamblett et al. (39), who showed that the accumulation factor of poliovirus in *C. virginica* was 9.5- to 18.1-fold under conditions of low turbidity (8-24 ppm), whereas it was only 4.2- to 4.6-fold in high turbidity (54-80 ppm). Metcalf and Stiles (68) found that oysters in polluted water at temperatures below 7°C did not accumulate virus. At temperatures above 7°C,
pumping and feeding activity are initiated and viruses are accumulated.

The Florida conch and sea hare (Apelias dactylomela) also accumulate viruses when placed for short periods in water contaminated with poliovirus (86). Conches, a mollusk commercially harvested for food in the Caribbean, accumulate large amounts of virus in their digestive gland and intestine. Viruses could also be detected in the hemolymph. Virus was found to persist at least 8 days after a brief 2-h exposure to contaminated seawater.

Besides mollusks, crustaceans also accumulate virus. DiGirolamo et al. (22) showed that Pacific coast shore crabs (Pachygrapsus sp. and Hemigrapsus sp.) could accumulate high titers of poliovirus when placed in artificially contaminated seawater. When allowed to feed on virus-contaminated mussels (Mytilus californianus), the crabs accumulated 74-94% of the virus present in shellfish.

The same authors (23) demonstrated that the edible west coast crabs — the Dungeness crabs (Cancer magister) and “Red Rock” crabs (C. antennarius) — could accumulate coliphage T4 in high titers if residing in artificially contaminated seawater. They also showed that virus in crabs withstood boiling, which is a normal method of preparing crabs before consumption. The survival rate varied between 2.5 and 20%, depending on

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**TABLE 3. Virus accumulation and depuration studies on shellfish**

<table>
<thead>
<tr>
<th>Reference no.</th>
<th>Virus type</th>
<th>Species of shellfish</th>
<th>Uptake</th>
<th>Virus accumulation factor</th>
<th>Depuration</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>polio 2</td>
<td>mussel (Mer. edulis)</td>
<td></td>
<td></td>
<td>intermittent; 48 h</td>
</tr>
<tr>
<td>40</td>
<td>polio 3 (Saukett)</td>
<td>European oyster (O. edulis)</td>
<td>circulating water for 6 days at 23 C 1-fold</td>
<td>10-fold</td>
<td>1% remaining in 100 h</td>
</tr>
<tr>
<td>66</td>
<td>polio 1 (Chat), coxackie B3 (Nancy)</td>
<td>Eastern oyster (C. virginica)</td>
<td>water</td>
<td>static; 1-fold</td>
<td>95% in 24 h; 100% in 48 h</td>
</tr>
<tr>
<td>56</td>
<td>polio 1 (LSc)</td>
<td>hard clam (Mer. mercenaria)</td>
<td>static; 4-48 h 30-fold in 6 days</td>
<td>static</td>
<td>72-96 h</td>
</tr>
<tr>
<td>43</td>
<td>phage C. gigas</td>
<td>flow-through</td>
<td>3- to 9-fold</td>
<td>10-fold</td>
<td>2-4 days</td>
</tr>
<tr>
<td>73</td>
<td>polio 1 (LSc)</td>
<td>C. virginica</td>
<td>flow-through 1 h</td>
<td>10- to 27-fold</td>
<td>72-96 h</td>
</tr>
<tr>
<td>79</td>
<td>staphylococcus phage C. virginica, Mer. mercenaria</td>
<td>2-3 h</td>
<td>10-fold</td>
<td>static</td>
<td>incomplete in 100 h</td>
</tr>
<tr>
<td>59</td>
<td>polio 1 (LSc-2ab)</td>
<td>hard clam (Mer. edulis ostenul)</td>
<td>4 h; maintained for 3 days</td>
<td>10-fold</td>
<td>72-96 h</td>
</tr>
<tr>
<td>58</td>
<td>polio 1 (LSc-2ab), polio 3 (Leon-12ab), coxackie B4 (POW)</td>
<td>hard clam running water</td>
<td>94% in 73 h</td>
<td>10-fold</td>
<td>72-96 h</td>
</tr>
<tr>
<td>26</td>
<td>polio 3 (Saukett), coxackie A8 (NH; A805)</td>
<td>blue rock mussel (Mytilus californianus)</td>
<td>18-36 h</td>
<td>static</td>
<td>incomplete in 100 h</td>
</tr>
<tr>
<td>38</td>
<td>polio 1 (LSc)</td>
<td>C. virginica</td>
<td>flowing 24 h</td>
<td>9.5- to 18.1-fold at low turbidity; 4.2- to 4.6-fold at high turbidity</td>
<td>48 h</td>
</tr>
<tr>
<td>83</td>
<td>polio 1 (LSc)</td>
<td>C. virginica</td>
<td>flowing 24 h</td>
<td>10- to 100-fold for crude virus; 0.4- to 3.6-fold for clarified virus</td>
<td>48-72 h</td>
</tr>
<tr>
<td>42</td>
<td>polio 1 (LSc)</td>
<td>C. gigas, flowing T. japonica</td>
<td>10- to 900-fold for crude virus; 0.4- to 3.6-fold for clarified virus</td>
<td>2- to 1100-fold</td>
<td>several days to weeks if low levels of virus are accumulated</td>
</tr>
<tr>
<td>9</td>
<td>coliphage 5-13</td>
<td>Mer. mercenaria</td>
<td>flowing</td>
<td></td>
<td>48 h</td>
</tr>
<tr>
<td>22</td>
<td>polio 1 (LSc)</td>
<td>west coast shore crab (Pachygrapsus sp., Hemigrapsus nudus)</td>
<td>25% in 12 h; 63% in 48 h</td>
<td></td>
<td>48 h</td>
</tr>
<tr>
<td>23</td>
<td>coliphage T4</td>
<td>west coast crabs (C. magister, C. antennarius)</td>
<td>58% in 24 h; 73% in 48 h</td>
<td></td>
<td>48 h</td>
</tr>
<tr>
<td>69</td>
<td>coxackie B3 C. virginica</td>
<td>—</td>
<td>90% in 48 h</td>
<td>static</td>
<td>at 9.13 C, 7 days; at 21-22 C, 3 days</td>
</tr>
<tr>
<td>20</td>
<td>polio 1 (LSc)</td>
<td>C. gigas</td>
<td>46% in 12 h; 88% in 48 h</td>
<td>static</td>
<td>21% remained after 120 h</td>
</tr>
<tr>
<td>20</td>
<td>polio 1 (LSc)</td>
<td>Olympia oyster (O. lurida)</td>
<td>86% in 12 h; 94% in 24 h</td>
<td>static</td>
<td>16% remained after 24 h; 24% remained after 24 h; 1% remained after 72 h</td>
</tr>
</tbody>
</table>
the time of boiling. After boiling for 10 and 15 min, the virus survival was 15 and 10%, respectively. It was also postulated that since crabs are not sedentary like bivalve mollusks, they may enter a sewage polluted area either in search of food or with tidal changes and may come back to clean water, where they subsequently act as vectors of viral disease (27).

**MECHANISMS OF ACCUMULATION**

Using aquariums and static seawater, Metcalf and Stiles (67) showed that most viruses were accumulated in the digestive diverticulum of American oysters. Using northern quahogs and poliovirus type 1, Liu et al. (56) confirmed this observation and were also able to detect virus in the stomach, hemolymph and mantle cavity fluids. The digestive tract, though, is the major site of virus accumulation. Up to 15% of the total virus accumulated, depending on the species of shellfish, may diffuse into the body of the oyster within a 48-h period (20). This finding is important in considering depuration processes, since 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.

Results of binding-release studies have shown that viruses become attached to the secreted mucus and then are ingested by shellfish during feeding (21). Attachment of virus to mucus was due to ionic binding and involved the binding of viral particles to sulfate radicals on the mucopolysaccharide moiety of shellfish mucus.

**DEPURATION**

Depuration, or purification, is a mechanical process effected by the physiological functioning of shellfish in clean water (4). Natural purification is also called relaying. When shellfish are relayed on the seabeds in areas where seawater is unpolluted, they unload their fecal contaminants.

Artificial purification or cleansing of shellfish from polluted areas is accomplished by placing them in tanks or basins which are filled with sterilized water. Sterilization of water may be done by filtration, chlorination or ozonation. Shellfish, however, are extremely sensitive to chlorine; even small amounts cause slowing down or complete inhibition of shellfish physiological processes.

The factors affecting the process of purification (depuration) are: (a) Maximum depuration is possible only when environmental conditions are such that the feeding activity of shellfish is optimized. (b) Liu et al. (59) showed that the reduction of salinity to 75% of the original did not have any significant effect on the depuration process, whereas a further reduction in salinity to 50-60% of the original resulted in stoppage of depuration. (c) Efficiency of depuration is a function of temperature (58,59,69). At 5°C, 72-96 h were required for depuration but at 20°C depuration was complete in 24-72 h (58). (d) Depuration occurs more rapidly in a free-flow system than in a static one (20,55,58,73). DiGirolamo et al. (20) observed that after 120 h of depuration in a stationary system, C. gigas still retained 21% of the virus accumulated, while O. lurida retained 16%. Under free-flow conditions, however, depuration was rapid and efficient. Only 1% of the accumulated virus remained in the oysters after 72 h.

The observed unloading of contaminants by shellfish upon being placed in a clean environment has been exploited commercially; oysters harvested from polluted areas are put in fresh, uncontaminated water to allow shellfish to purge themselves of viral contaminants (95). This self-cleaning mechanism appears to be uniformly efficient, but considerable study is still required to define the optimum conditions. The problem encountered in commercial purification is the lack of a source of clean water. For economic reasons, the source of clean water should be close to the polluted oyster-harvesting areas, which is not always possible. Even if a source of clean water is found, it is susceptible to sporadic contamination by overland runoff and by wild and aquatic birds. The remedy may lie in the use of sterilized water. Sterilization by chlorination is feasible but is not optimal because of the reasons mentioned above. Chlorination followed by dechlorination may be a practical means toward this goal.

Use of chlorination has also been advocated to disinfect shellfish. However, it was shown by Galtsoff (30) that the shell movement and the flow of water through the gills was inhibited in the presence of chlorine. Thus, chlorination may cause only external disinfection. Also, chlorine is not a reliable disinfectant for certain viruses (12,84) at concentrations often used. Hedstrom and Lyke (40) observed inactivation of virus in oysters by 0.7-20 ppm of chlorine, indicating that the viruses were retained in the tissues in such a way that no disinfection was possible.

**AQUACULTURE**

Some imaginative approaches have been studied for use of sewage effluents as a nutrient source for production of marine phytoplankton to be used for the aquaculture of marine animals on a commercial scale (65). However, use of sewage effluents in the system indicates the possibility of potential public health risks in the form of human virus transmission. To examine the potential of such a risk, Metcalf (65) did a study of enteroviruses in an aquaculture system developed at the Woods Hole Oceanographic Institute and raised serious questions about its suitability either for tertiary treatment of sewage or culture of shellfish. Despite a 99% reduction in virus titer, the system was judged unacceptable because as many as 1 x 10^4 viruses survived inactivation and appeared daily in discharge waters. Physiologically active juvenile oysters grown in sewage-seawater mixtures were shown to accumulate enteric viruses more or less routinely.

In laboratory experiments it was demonstrated that in the hypothetical aquaculture system under study viruses
could be transmitted through the food chain as follows: shellfish + pseudofeces + sandworms + detrital feeding fin fish. It was also found that lobsters fed virus-contaminated mussels accumulated virus in their alimentary tract and hepatopancreatic tissues. These results indicated that almost complete elimination of virus from sewage effluents would be necessary for their use in the aquaculture of shellfish, if the shellfish are to be marketed for human consumption.

SURVIVAL OF VIRUSES IN MARINE WATER AND SHELLFISH

Field and laboratory studies have indicated that enteric viruses can survive from a few days to over 130 days in marine water. Survival is dependent on a number of factors which include temperature, salinity, type of virus, bacterial antagonism, suspended solids, and pollution. Survival of viruses becomes greatly prolonged once they become associated with sediments (88), and their concentration may be many fold greater in sediments than in the overlying water (31). Generally, viruses survive longer at lower temperature, at low salinity, and in sewage-polluted waters. Several good reviews on virus survival in marine water are available (1).

Metcalf and Stiles (67) have investigated the period of viral retention in the natural environment. The American oyster, poliovirus type 1, a coxsackievirus and an echovirus were used. The experimentally polluted oysters were maintained in a New Hampshire bay for 3 months and samples of shellfish were taken periodically for assay. No reduction of virus titer occurred for 30 days during the winter when the water temperature was about 1 C. As the water temperature rose above 8 C, viruses in the shellfish were rapidly reduced. It was postulated that the shellfish remained dormant until the water temperature reached a critical level. At that point, the oysters began to feed and through deputative activity freed themselves of virus. Temperatures of 4 C or less favored virus retention. Liu et al. (55) suggested that this may serve as an explanation as to why most hepatitis outbreaks occur during the winter and early spring.

Several studies have shown that viruses can survive in shellfish for long periods. Under conditions of dry storage at 5 C, coxsackie and polioviruses were found viable even after 28 days (66). Depending on storage temperature, poliovirus survived in refrigerated oysters for 30 to 90 days (18). In chilled Olympia oysters, poliovirus was reduced by only 10% after 5 days of storage and by 60% after 15 days. Even after 30 days of storage at 5 C, 13% of virus remained viable although the oysters were badly decomposed. In frozen Pacific oysters held at −17.5 C, 91% of added poliovirus was viable after 2 weeks of storage, 40% at 6 weeks, and 10% after 12 weeks. The tendency of the virus to aggregate and its incorporation into shellfish mucus by ionic binding were speculated to be the means by which the virus was able to survive in chilled oysters. It was further shown that not only fresh but also cooked oysters could serve as vectors of viral disease. Between 7 and 10% of poliovirus was still viable after baking, frying, stewing and steaming (18).

DiGirolamo and Daley (17) reported that 29 and 40% of coliphage T4 was recoverable even after 120 days at 8 C from unprocessed and processed (boiled) west coast crabs C. magister and C. antennarius, respectively. When kept at −20 C for 30 days, 35 and 17% of virus survived in unprocessed and processed crabs, respectively. Canzoneri (9) demonstrated that 50-90% of the accumulated coliphage S-13 in oysters was retained during a 2-month period of observation in a free-flowing aquarium, whereas bacteria were eliminated rapidly within 24 h. Metcalf and Stiles (68) stored contaminated oysters at 5 C in estuarine water and found that coxsackievirus B3 and echovirus 5 survived for 4 months under these conditions.

Koff and Sear (47) reported that the internal temperature of soft-shell clams (M. arenaria) did not reach 100 C until 4-6 min of boiling/steaming, whereas their shells were open in only 60 sec. Clams are normally consumed as soon as the shells are opened, not because of hygienic reasons but because of palatability. DiGirolamo et al. (18) showed that 7-13% of added poliovirus survived in oysters even after four commonly used processing methods, i.e., steaming, frying, baking and stewing. It was proved by heat penetration studies that the internal temperature was not sufficient to inactivate all virus present. An outbreak of infectious hepatitis attributed to steamed clams was reported from Massachusetts in 1972 (47). Mosley and Galainbos (76) have advocated the use of boiling temperature for at least 20 min.

Use of ionizing radiation has been proposed recently to eliminate microorganisms from foods. In a preliminary study, DiGirolamo et al. (19) demonstrated that poliovirus 1 in oysters was able to survive relatively high doses of gamma radiation. The rate of survival varied between 7.3 and 87% depending on the dose of radiation and the nature of the sample (whether whole or shucked). To inactivate more than 90% of the virus present, a dose of 400 krad was required, which also resulted in undesirable organoleptic changes.

METHODS FOR VIRUS DETECTION IN SHELLFISH

A number of methods for recovering enteric viruses from natural or laboratory-contaminated oysters, clams and mussels have been described (38,41,66,73). All of these methods begin with homogenization of the whole shucked shellfish or part of it. In earlier methods this homogenate, with or without addition of diluent, was centrifuged to remove solids. The supernatant fluid was then assayed directly or further clarified by treatment with fluorocarbon extraction (73) or ethyl ether extraction (38,66,73). These methods have several shortcomings, which include: (a) lack of significant information on the degree of efficiency of the methods; (b) too large a volume to feasibly assay when attempting
to detect small numbers of viruses; and (c) toxicity to the
animal cells used to assay the virus. Cytotoxic components
present in the homogenate often destroy the cell culture
before viruses can be quantified. This appears to be
especially true with homogenates obtained from clams.

Metcalfe et al. (65) homogenized pools of 10 oysters
followed by low-speed centrifugation. The supernatant
fluid was centrifuged at high speed (160,000 \( \times g \)) for 2 h
and the resulting pellet was resuspended in a small
volume of Hank’s balanced salt solution. Bendinelli and
Ruschi (8) homogenized one part of mussels with an
equal amount of ethyl ether and let it stand at 4 C for
18 h, followed by low-speed centrifugation. The lower
aqueous phase was again centrifuged at 104,000 \( \times g \) for
2 h, and the pellet resuspended in 3 ml of Earle’s
solution.

Metcalfe and Stiles (66) blended pools of 10 oysters for
90 sec; the mixture was centrifuged at low speed, and the
supernatant fluid was mixed with an equal volume of
ethyl ether. This mixture was stored overnight at 5 C and
then centrifuged. The resulting supernatant fluid was
again centrifuged at 125,000 rpm for 2.5 h and the pellet
was resuspended in 4 ml of Hank’s balanced salt
solution.

Morris et al. (74) homogenized the digestive glands
(\( \sim 20 \) g) from 2 kg of mussels. This was followed by
washing with pH 5.5 buffer at low salt concentration to
remove substances toxic to the cells. After low-speed
centrifugation, the sediment was adjusted to pH 3.7 with
0.7% saline buffer to elute virus. The supernatant fluid
recovered after recentrifugation was mixed with fetal calf
serum (FCS) to make a final concentration of 10% FCS.
The efficiency of recovery with poliovirus type 3 was
32-38%.

Konowalchuk and Spiers (49) found that the toxicity
associated with the shellfish extracts became an
important factor in isolation of small numbers of
enteroviruses. They found that even when oyster extract
were diluted to a point where no visible cytotoxicity
occurred, plaque counts were reduced to 50% of the
controls. Much higher toxicities were found for clams
and mussels. Because of the cytotoxicity, the maximum
volume of extract that could be assayed at one time was
1.1 ml for oysters, 0.015 ml for mussels, and 0.006 ml for
clams. Acid precipitation and ether treatment of shellfish
extracts were found to reduce, but not eliminate,
problems with cytotoxicity. In addition, acid
precipitation was found to be effective only with freshly
homogenized shellfish, and at least 50% of the virus
remained in the acid precipitate.

Kostenbader and Cliver (50,51) described a procedure
for detection of enteric viruses from shellfish and other
foods; the procedure involved clarification of the
shellfish homogenate by addition of a polyelectrolyte
(Cat-Floc). This caused flocculation of oyster solids
which were then removed by filtration. The volume of the
filtrate was then reduced by either ultracentrifugation or
ultrafiltration before assay. When \( \sim 70 \) PFU (plaque-
forming units) of poliovirus type 1 were added to oysters,
efficiencies of 86-96% were achieved (50). However,
problems with cytotoxicity still remained. Severe cytotoxi-
city was encountered with extracts of clams (51). These
problems necessitated additional treatments to reduce
these effects and blind passages in the tissue culture
assay system.

Sobsey et al. (69) reported a method which eliminated
the problems of dealing with large volumes of extracts
and of cytotoxicity. They found that by controlling the
pH and salt concentration of the initial oyster
homogenate, viruses could be easily and rapidly
separated from the homogenized oyster tissues by a
procedure involving two basic steps. In the first step
viruses are adsorbed to the oyster solids at pH 5.5 and a
low salt concentration. When this homogenate is
centrifuged at low speed, nearly all of the viruses
sediment with the oyster solids so that the first
supernatant fluid can be discarded. The adsorbed viruses
are then eluted from the sedimented oyster solids by
resuspending the solids in pH 3.5 glycine-buffered saline,
and the virus-free solids are then removed by low-speed
centrifugation. The virus-containing supernatant fluid is
relatively low in turbidity and dissolved and colloidal
organic matter and is easily filtered through a membrane
filter to remove bacteria, molds and other particulate
matter without removing viruses.

The viruses in the resulting filtrate are efficiently
centrifuged to a volume of a few milliliters by
ultrafiltration. Because the final concentrate is of small
volume, the viruses obtained from the entire initial oyster
pool can be inoculated into a small number of cell
cultures.

When this procedure was tested with relatively small
amounts (\( \sim 30 \) PFU) of four different enteroviruses in
experimentally contaminated pools of three oysters
(20-65 g per pool), virus recovery efficiency averaged
63%.

Sobsey et al. (88a) recently reported a modification of
the earlier method (10) in which elution from solids
was accomplished with glycine-saline at pH 7.5 rather than
3.5 and concentration of viruses by acid precipitation.
When this method was tested with oysters and clams
experimentally contaminated with polioviruses, reo-
viruses and adenoviruses, recovery efficiencies averaged
from 25-50% depending on the type of virus.

Acknowledgments

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News and Events

Jan Gauger Richards has accepted the position of Assistant Executive Secretary of the International Association of Milk, Food, and Environmental Sanitarians (IAMFES) and Associate Editor of the Journal of Food Protection, announced Earl O. Wright, Executive Secretary for IAMFES.

Mrs. Richards, a native of Ames, received her B.S. degree from Iowa State University in 1977 with majors in journalism and home economics. She is a member of Phi Upsilon Omicron, professional honorary, and Sigma Delta Chi, professional society. She was communications director for the Dairy Council of Greater Kansas City, Kansas City, Mo., before coming to IAMFES.

Mrs. Richards' responsibilities will include production supervision of the Journal of Food Protection, work with state and national affiliate groups of IAMFES, coordination of workshops and shortcourses sponsored by the Association, and organization of student affiliate groups throughout the United States.

Affiliate Meetings

Sept. 14 and 15. The Minnesota Sanitarians' conference will be held at the Sheraton Inn, Northwest, Brooklyn Park, Minnesota.

Sept. 13 and 14. The Wisconsin Sanitarians will hold their annual conference at the Holiday Inn, Fond Du Loc.

Sept. 20-22. The New York Sanitarians Association will hold their annual conference at Stevensville Country Club, Swan Lake, N.Y.

Sept. 26, 27 and 28. The Indiana Sanitarians' annual conference will be held at Merrillville, Indiana.

Crumbine Award to Arlington Co., Va. Health Bureau

The Environmental Health Bureau of Arlington County, Virginia, which is responsible for ensuring food service sanitation for 170,000 county residents in an area of nearly 30 square miles directly across the Potomac from the nation's capital, has been chosen the winner of the 1978 Samuel J. Crumbine Consumer Protection Award.

This year's award was given for outstanding achievement in a total food and beverage sanitation program embracing such specific elements as effective planning and management, innovative evaluation methods, excellent information and education activities, and sustained program growth and improvement.

The Crumbine Award given to the Arlington County agency consists of a bronze medal and an engraved plate mounted on a walnut plaque. In addition, a bronze medallion is presented to individual agency officials who are directly responsible for the winning program.

Coming Events

University of Maryland dairy products conferences and ice cream short course for 1978-79 are as follows:


March 19-28, 1979. University of Maryland 28th Annual Ice Cream Short Course, Department of Dairy Science, Animal Sciences Center, University of Maryland, College Park, Maryland, 20742.

March 29, 1979. University of Maryland 28th Annual Ice Cream Conference, Center of Adult Education, University of Maryland, College Park, Maryland, 20742.

For information contact Dr. Joseph F. Mattick, Department of Dairy Science, Animal Sciences Center, University of Maryland, College Park, Maryland, 20742. (301) 454-3928.

The National Sanitation Foundation has planned a series of special regional workshops for foodservice equipment designers and manufacturers.

Sept. 12, 1978 Boston, MA
Sept. 14, 1978 Philadelphia, PA
Sept. 25, 1978 Chicago, IL
Oct. 16, 1978 Los Angeles, CA
Oct. 20, 1978 St. Louis, MO
Dec. 5, 1978 Atlanta, GA

The University of Wisconsin-Fau Claire will offer Community Hygiene I and II during the 1978-79 school year. The first semester will be held at Wisconsin Center, 702 Langdon St., Madison, Wis., starting Sept. 1, 1978. The second semester will be held there, also, and will start Jan. 19, 1978. For more information write University of Wisconsin, Eau Claire, Wis.