Isolation of Human Enterovirus from Mussels

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Mussels maintained in sea water heavily polluted with domestic raw sewage have been found to harbor human enteroviruses.

Several outbreaks of infectious hepatitis have been attributed to consumption of raw shellfish (7), and laboratory studies have shown that various species of mollusks can take up and accumulate viruses (3-9).

In spite of this strong indirect evidence for the role of shellfish as virus vehicles and of the considerable stability of enteroviruses in water (11), there are few reports of human virus isolations from mollusks. Two echovirus 9, one coxsackievirus B4, and three unidentified cytopathic agents were isolated from oysters (Crassostrea virginica) collected in the New Hampshire estuary (8). In Italy, one poliovirus 3 was found in mussels harvested in the harbor of Genoa (10) and three echoviruses (types 3, 9, and 13) were detected in mussels purchased on the city market of Bari (1).

We examined for the presence of viruses samples of mussels (Mytilus galloprovincialis), which are the shellfish most widely consumed in this country.

Mussels, 6- to 7-cm long, obtained from a mussel farm situated in an area of the Leghorn coast removed from gross pollution were examined either directly after harvesting or after a 3-day period in sea water massively polluted by domestic raw sewage (mussels were placed approximately 10 m from a sewage outlet and at a depth of 2 m).

The study was carried out from June to September, the seasonal period during which enterovirus content of sewage reaches maximal levels in this region (2). The mollusks were processed by the procedure described by Metcalf and Stiles (8). After thorough washing of the shells with sterile tap water, the entire contents (mantle cavity fluid and meat) of 10 mussels were aseptically collected, pooled, and homogenized. The homogenate was mixed with an equal volume of ethyl ether and, after 18 hr of incubation at 4 C, the mixture was centrifuged at low speed. The lower aqueous phase was then centrifuged at 104,000 \times g for 2 hr (12); the sediment, suspended in 3 ml of Earle's solution, was seeded into HeLa, HEp-2, and primary human amnion cells (0.25 ml/tube; 4 tubes of each cell type/sample). After 2 hr at 37 C, the culture medium was changed. The tubes were then incubated in roller drums and examined periodically for cytopathic effects until spontaneous degeneration of the cell layers oc-

<table>
<thead>
<tr>
<th>Source</th>
<th>Pools examined</th>
<th>Cytopathic agents isolated on</th>
<th>Virus identified</th>
<th>Bacteria at 37 C (per ml)(^{a})</th>
<th>Coliform (per ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mussel farm</td>
<td>32</td>
<td>HeLa 0</td>
<td>HEP-2 0</td>
<td>Human amnion 0</td>
<td>1 Echovirus 5 2 Echovirus 6 1 Echovirus 8 1 Echovirus 12 1 Coxsackievirus A18</td>
</tr>
<tr>
<td>Heavily polluted sea</td>
<td>36</td>
<td>HeLa 0</td>
<td>HEP-2 0</td>
<td>Human amnion 5</td>
<td>1 Echovirus 5 2 Echovirus 6 1 Echovirus 8 1 Echovirus 12 1 Coxsackievirus A18</td>
</tr>
</tbody>
</table>

\(^{a}\) Bacteriological tests were performed on the initial homogenate.  
\(^{b}\) Mean ± SD.
curred (7 to 8 days for HeLa and HEp-2 and 15 to 18 days for amnion cells). The cell cultures were frozen and thawed and inoculated into fresh tubes (0.25 ml/tube). Two or occasionally three passages were made before the samples were considered free of viruses. Final identification of the isolates was accomplished by neutralization tests.

Sixty-eight pools of mussels were examined. Only the mussels which had been kept for 3 days in an environment subject to gross pollution by domestic sewage led to virus isolation; 5 out of 36 pools of these mussels were found to harbor agents cytopathic for amnion cells (Table 1). The agents isolated were: one echovirus 5, one echovirus 6, one echovirus 8, and one echovirus 12, and one was a mixture of echovirus 6 and coxsackievirus A18.

Our results indicate that mussels grown or temporarily maintained in heavily polluted waters may become contaminated by human enteric viruses and emphasize the health hazards associated with the consumption of raw shellfish.

LITERATURE CITED