organs of saline-injected animals, indicating that the injection may not have been sterile.

Using the anti-mouse PCNA, areas of abundant PCNA staining (black-stained nuclei), indicating areas of marked cell proliferation, were identified in the reserve cells of the digestive gland (Fig. 1B), the proliferative epithelial cells of the gill base (2) (Fig. 1C), and in early proliferative phases of reproductive epithelium. As expected, cells of other tissues throughout the body also stained positive for PCNA (*i.e.,* epithelium of the intestine, foot and body wall, and gill plical epithelium), but in much lower numbers. Proliferating hemocytes were identified in the inflammatory cells forming the thick-walled granulomas (Fig. 1D) and rarely in adjacent non-inflammatory cells. In no other areas examined were proliferating hemocytes identified.

These results demonstrate that the epitopes associated with PCNA are conserved between the clam and the mouse, as shown by the positive staining of known proliferative cells in the clam body. Previous studies have shown that hemocytes appear to migrate to areas of infection in bivalves (2, 6). In diseased bivalves, hemocyte numbers appear to increase; the site of origin of these hemocytes has never been determined (2). This study provides evidence that the hemocytes of the hard clam proliferate directly at the inflammatory site, as opposed to a possible bone marrow-like area in the body of the clam, with subsequent migration of hemocytes to sites of infections, as seen in vertebrates.

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Conditions Affecting the Growth and Zoosporulation of the Protistan Parasite QPX in Culture

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Quahog Parasite Unknown (QPX) is a protistan disease of hard clams (*Mercenaria mercenaria*). The QPX organism has been classified in the phylum Labyrinthomorpha (1, 2). Disease resulting from QPX infection has been identified in New Brunswick and Prince Edward Island, Canada; Barnegat Bay, New Jersey; Chatham, Duxbury, and Provincetown, Massachusetts; and three locations in Virginia (1, 2). Mortality from QPX can be severe, with losses especially high in clams just under market size (about 2 years old). An important clinical sign of infection is the occurrence of QPX-infected inflammatory nodules in the mantle.

Whyte *et al.* (3) isolated QPX cells from infected clams; when placed in artificial seawater, these cells produced sporangia and zoospores. Kleinschuster and Smolowitz (2) recently described continuous *in vitro* culture of QPX. QPX was isolated from inflamed mantle nodules and cultured in modified MEM medium at pH 7.2 at 22°C. Mature cultures (after 5 to 10 days) showed thalli, immature sporangia, and mature sporangia containing endospores. Organisms in these stages ranged from 5 to 120 μm in diameter. Endospores released from mature sporangia became the new thalli. Cultured QPX organisms produced, and were embedded in, a thick mucoid material that could be removed intact from the remaining unused culture medium. When placed into sterile seawater, QPX produced motile zoospores within 4 days.

The effects of different environmental conditions on the occurrence of QPX and the resulting disease in the field are unknown. Determination of how environmental parameters affect cultured QPX may help in understanding the pathogenesis of the disease in the field. In this study, the environmental effects of temperature, pH, and salinity were investigated on QPX cells in culture.

Medium (pH 7.2 and salinity 40 ppt) was prepared using the standard methods (2). Modified medium (40 ppt) was prepared at pH 6.0, 7.0, and 8.0 by adjusting pH with 2 *M* HCl and 2 *M* NaOH. Modified medium (pH 7.2) was also prepared at 20, 28, and 34 ppt by proportionally reducing the salt content of the medium and monitoring the resulting solutions with a refractometer. All media were filter sterilized. To test the effects of pH and salinity on the proliferation of QPX in culture, 0.4 ml of two QPX subcultures was placed in a culture flask with 10 ml of each of the three pH variations or four (including standard) salinity variations. Fourteen cultures were created (seven of each subculture). These cultures were incubated for 10 days at 22°C. To test the effects of temperature on QPX growth in culture, the same procedure was followed

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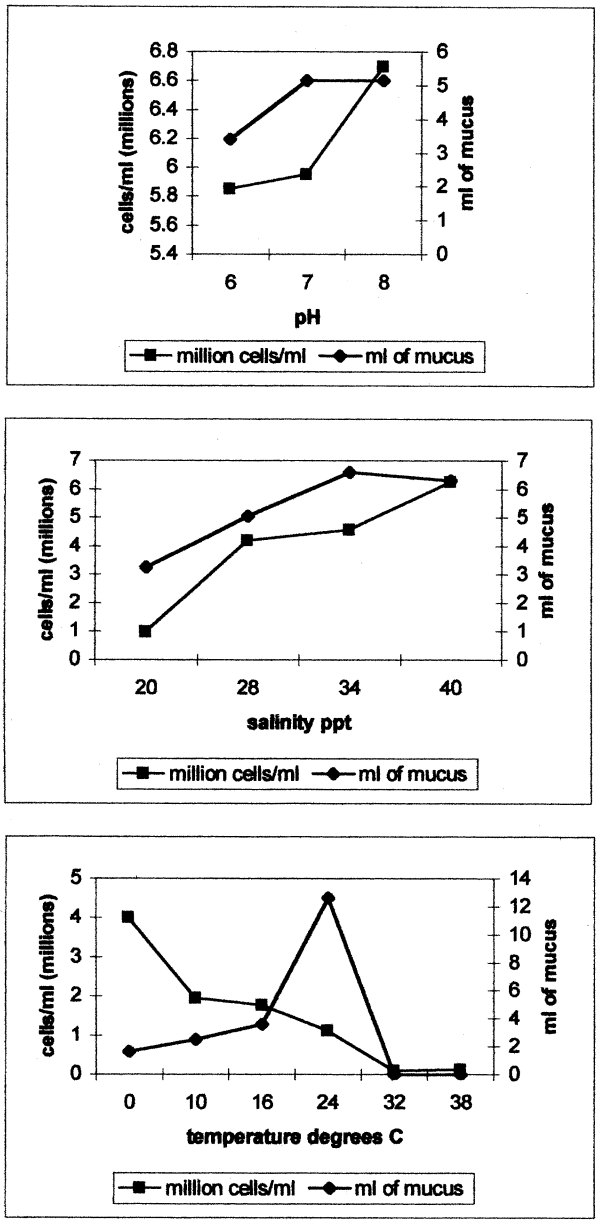

Figure 1. *Growth of QPX as a function of pH, salinity, and temperature as measured by cell count (millions of cells per ml) and volume of mucoid containing QPX material produced (ml of mucus).*

using 10 ml of standard medium to create a total of 12 cultures. One flask of each subculture was incubated at six temperatures (0°, 10°, 16°, 22°, 32°, 38°C) for 10 days.

Concentrations of the mucus containing QPX from each of the four initial subcultures, as well as from the 26 experimental cultures at the end of the 10-day incubation, were determined. The QPX-containing mucus was extracted from the culture medium and measured using a 10-ml pipette. Concentrations of QPX organisms were measured by counting the number of cells per milliliter of a 1:10 saline dilution of the mucus, using a hemocytometer. Initial concentrations of QPX averaged 1.4 million cells per milliliter (range = 1.1 to 1.8 million). Final concentrations and volumes are shown in Figure 1.

QPX concentrations per milliliter and volume of mucus produced both increased with increasing pH and increasing salinity. In culture, the modified MEM medium becomes more acidic as the culture matures. This lower pH may inhibit further growth of the culture. At low salinities, QPX thalli have previously been observed lysing, which may explain the lower cell concentrations at 20 ppt. The cell concentration was highest at 0°C and decreased with increasing temperature. This may represent a thinning out and spreading of the mucus containing the cells with increasing temperature. However, the total number of QPX organisms and total mucus production was greatest at 24°C. The volume of mucus containing QPX produced was low from 0° to 16°C, peaked at 24°C, then declined with increasing temperature. Above 32°C, there was no growth of QPX and no mucus production. Whether QPX will grow above pH 8.0 and above 40 ppt should be investigated.

Proliferation of cultured QPX is best at a temperature of 24°C, pH 7 to 8, and salinities of 28 ppt and above. Such findings are consistent with the field observation of increased infection in the summer and occurrence of QPX disease primarily in high-salinity waters.

Conditions affecting the zoosporulation of QPX were also investigated. Seawater (pH 8.0, salinity 30 ppt) was adjusted to pHs of 6.0, 7.0, and 9.0 (using 2 *M* HCl and 2 *M* NaOH) and to salinities of 20 and 40 ppt (by dilution with distilled water or addition of NaCl), then filter sterilized. Concentrations of 1% and 10% QPX in seawater of each pH and salinity were placed in replicates in a 24-well sterile plate. The plates were incubated at 10°, 16°, 22°, 32°, and 38°C and examined daily for 5 days.

Other researchers (2, 3) have reported zoosporulation in QPX; however, there is reason to believe these may not have been from pure cultures of QPX. This study attempted to replicate those findings; however, no zoospores were observed in repeated trials, even in normal sterile seawater. Whether in fact QPX produces zoospores, as not all members of the phylum do, and under what conditions it does so are important both in the further classification of the organism and in studying transmission of the disease. Therefore, this question deserves further investigation.

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