Effects of Phenol on Clams

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Adult hard clams (Mercenaria mercenaria) were collected from Rehoboth Bay, Del., in October 1974 and April 1975. Within less than 24 hours they were divided into groups of six; control animals were placed in artificial seawater (25%) while experimental animals were placed in phenol solutions in artificial seawater (25%) with initial concentrations of phenol of 1, 5, 10, 25, or 50 ppm. After 24 hours clams were placed in Bouin's fixative and 4 hours later three transverse sections 0.5-0.7 cm thick were cut from each clam at the level of digestive gland, gonad, and heart. These sections were fixed in Bouin's for an additional 24 hours, then washed, dehydrated in alcohol, cleared in xylol, embedded in Paraplast, sectioned at $10 \,\mu$ m, and stained with Harris' alum, hemotoxylin, and eosin.

Microscopic examination of tissues of control animals revealed that epithelia were intact and free of significant cell damage. Gill epithelium consisted of tall columnar ciliated epithelial cells well-stained and cohesive (Fig. 1). Epithelium of intestine was intact, wellstained and normal in all respects. Digestive diverticular tubules were intact, staining darkly in the basal areas and lightly vacuolated in the distal portions of the epithelial cells. Other tissues (mantle, foot, gonad, heart, kidney) appeared normal and undamaged.

Gills of clams treated with 1 or 5 ppm phenol showed moderate to extensive epithelial necrosis (Fig. 2); hemolymph sinuses were distended and contained an amorphous precipitate (presumably denatured hemolymph protein). Exposure to greater concentrations (10, 25, and 50 ppm) of phenol resulted in greater gill damage. Epithelial necrosis and sloughing was massive, and at 50 ppm the only recognizable gill structure remaining was the chitinous rods (Fig. 3).

Gut epithelia of clams exposed to 1 ppm (or higher) phenol also showed extensive necrosis and sloughing (Fig. 4). The regular palisade structure of normal tissue was disorganized, ciliation was irregular, and patches of epithelium became detached. These effects became more pronounced with increasing concentrations of phenol. Digestive diverticula were affected similarly; extensive sloughing lead to massive disorganization of the digestive tissue (Fig. 5). Initially damage to tubule epithelia might be localized, but in some animals at high concentrations, the damage involved the whole digestive gland. Damage to other tissues was less spectacular and more irregular.

Posterior portions of hind gut epithelium did not seem quite as sensitive as esophagus or stomach, but at 10 or 25 ppm phenol necrosis and sloughing was detected. Mantle connective tissue occasionally was disorganized and distended blood vessels contained precipitated hemolymph, but epithelium seemed little affected. Foot epithelium and muscle seemed unaffected at any phenol concentration tested. Sections through the kidney were not always obtained and it is not clear if that organ was affected. Gonadal tissue was not affected.

It is clear that gill and digestive tract epithelia are damaged by phenol at concentrations of 1 ppm or greater for 24 hours or longer. Experiments are in progress to measure the ability of clams to heal these chemical wounds and to detect subcellular damage at lesser concentrations. The extensive damage to gill and gut tissues thought to be important components of intrinsic defense mechanisms, suggests that such phenol-treated clams may be more susceptible to microbial infection and disease than normal ones. This system may serve as a model for studying host-parasite-environment interactions.



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¹Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.



Figure 1.—Normal gill. Uniform ciliated columnar epithelium and highly vascularized underlying connective tissue. $20 \times$. Figure 2.—Gill of clam treated with 5 ppm phenol for 24 hours. Note lack of epithelial cells and disorganized underlying connective tissue. $20 \times$. Figure 3.—Gill of clam treated with 50 ppm phenol for 24 hours. The filaments have been stripped of their epithelium and only the chitinous supporting rods remain. Underlying connective tissue is necrotic. $20 \times$. Figure 4.—Gut epithelium of clam treated with 1 ppm phenol for 24 hours. The epithelial cells are necrotic and not cohesive; ciliation (c) is reduced. $200 \times$. Figure 5.—Digestive diverticulum of clam treated with 25 ppm phenol for 24 hours. Complete disorganization of absorptive cells is evident (a). Connective tissue is necrotic. $200 \times$.

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