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Gas-Bubble Disease in the Blue Crab, *Callinectes sapidus*

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Exposure of crabs to water supersaturated with air resulted in formation of gas emboli in the hemal system, which in turn caused localized ischemia. More than one-third of the exposed crabs died during the 2 days following the episode. In surviving crabs, the most severely affected organs and tissues were the gills, heart, and antennal gland. Especially in gills, emboli were still present in apparently healthy crabs 35 days following exposure to water supersaturated with air. Evidence of ischemic injury was focal in character except in the antennal gland, where the epithelium of the labyrinth was sometimes extensively degenerate. Repair processes apparently did not involve hemocytes except for occasional fibroblastic infiltration in damaged gill lamellae.

INTRODUCTION

Gas-bubble disease occurs in aquatic animals that have been exposed to water supersaturated with air or other gases, e.g., O_2. It is sometimes a problem in fish hatcheries (Amlacher, 1961) and also affects invertebrates in captivity. The first published report on gas-bubble disease in fishes also mentioned that various arthropods were affected, including lobsters, king crabs, and pycnogonids (Marsh and Gorham, 1905). These authors remarked on the ability of crustaceans to live for long periods with their blood in a condition resembling foam. Later papers discuss gas-bubble disease in cultured lobsters (Hughes, 1968), in molluscs (Malouf et al., 1972), and in cultured larval and juvenile shrimp (Lightner et al., 1974). The latter paper also reviews the previous occurrence of the disease in invertebrates. Pauley et al. (1967) reported on the histopathology of gas-bubble disease in salmon fingerlings, but to date no publications have described the histopathology of affected invertebrates.

This paper reports on the duration of the disease in the blue crab, recovery of affected animals, and histopathology caused by the formation of gas emboli in the hemal system.

MATERIALS AND METHODS

In late October, 1974, a group of blue crabs, *Callinectes sapidus*, from Chincoteague Bay, Virginia, were placed in the flow-through water system at this laboratory. Late on the second day, water for the flow-through system was switched to an unused line and pressure in the air-filled pipe caused supersaturation of the water with air. A weekend intervened, and on Monday, 25 of 66 crabs were found dead, with most of the remainder sluggish and some moribund. Gills of dead crabs were filled with gas, leading to a presumptive diagnosis of gas-bubble disease. A large male blue crab held in the laboratory for 2 months and several small striped bass, *Morone saxatilis*, in the same flow-through system also died. Hog chokers, *Trinectes maculatus*, were unaffected.

The blue crabs were intermolt animals, 9.5–14.0 cm in width (mean, 12.0 cm). Four of the 36 specimens dissected were juvenile females; the remainder were males. Water temperatures during the first 7 days were 13–15°C and were 16–18°C on days 8–14. There was then a gradual decrease from 13° to 6°C, with slight fluctuations. The temperature was 7°C on the 41st day, when the remaining animals were dissected.

TABLE 1
Gas-Bubble Disease in the Blue Crab

Days in laboratory	Days following gas episode	Animals in good condition	Gas emboli in gills	Gas emboli in general hemal system	Evidence of ischemia	Paramoeba in tissues
0	–	0/3[a]	–	–	–	2/3[b]
1	–	0/2	–	–	–	2/2[c]
5	3	2/6	6/6	0/6	1/6	2/6
6	4	1/3	3/3	2/3	3/3	0/6
7	5	0/6	6/6	6/6	6/6	0/6
13	11	2/4	3/4	4/4	2/4	1/4
22	20	3/3	1/3	2/3	0/3	0/3
29	27	1/1	1/1	0/1	0/1	0/1
37	35	4/4	3/4	1/4	0/4	0/4
41	39	4/4	0/4	0/4	0/4	0/4

[a]Numerator, total number of animals affected; denominator, total number of animals dissected.
[b]All heavy infections.
[c]One heavy infection.

Crabs dissected after exposure to water supersaturated with air were first examined grossly for presence of gas emboli in gills, heart, hemal sinuses, and blood vessels. Three large, easily visible arteries in particular were inspected: the ophthalmic artery, which runs anterodorsally from the heart to supply the eyes, antennules, brain, and anterior epidermis; and the paired antennary arteries, which run anterolaterally to supply the stomach, antennal gland, antennae, and anterior parts of the gonads, hepatopancreas, and epidermis (Pyle and Cronin, 1950). Tissues removed routinely from dissected crabs included heart, brain, thoracic ganglion, gut, hemopoietic tissue, antennal gland, Y organ, gills, hepatopancreas, gonad, epidermis, and the associated connective tissues and muscles. Eyestalks were taken on occasion. Tissues were fixed in Helly's solution and processed routinely for paraffin sectioning. Photographed tissue sections were stained by the Feulgen technique and counterstained with picro-methyl blue (Farley, 1969).

OBSERVATIONS AND RESULTS

On the day of arrival, day 0, one dead and two sluggish crabs were dissected, and on laboratory day 1 two sluggish animals were dissected. *Paramoeba perniciosa* was found in four of the five crabs; three of the infections were heavy (Table 1). Seven other crabs that were not dissected died on days 0–2 (Table 2). Twenty-five crabs died following exposure, late on day 2, to water that had been supersaturated with air. (Henceforth, this event will be termed the "gas episode".) Since the crabs had not been checked during the weekend following the gas episode, the time of death is not known exactly. The differing states of decomposition of the dead crabs suggested that deaths took place over a considerable period of time. Gills of recently dead crabs were inspected, and in all cases the hemal spaces of many of the gill lamellae and stems were filled with gas. Most of the 41 surviving crabs were sluggish on days 3–5 following the episode, and several probably would have died had they not been sacrificed. All crabs dissected on days 20–39 following the episode appeared to be in good condition.

All crabs dissected on days 3–5 and three of four dissected on day 11 following the gas

TABLE 2
Gas-Bubble Disease: Deaths in Nonexamined Crabs

Laboratory day	Number dead
0	2
1	3
2	2
5	25[a]
6	2
7	1
8	1
9	3
12	3[a]
13–41	0

[a]Including those dying over weekend.

episode had gas emboli in the gills, and on days 4–20 the majority also had grossly visible gas emboli in portions of the hemal sinuses, larger arteries, heart, and, occasionally, in the lumen of the midgut. Three animals had light infections of *P. perniciosa* (Table 1).

Some general statements can be made concerning histopathology of gas-bubble disease in the blue crab: There were focal degenerative changes in muscle and nervous tissue caused by ischemia, no fibrocytic response occurred to long-lasting emboli, and no hemocytic response of any variety occurred during the acute phase. Ischemic injury was rare and not severe in epidermis and gut epithelia. Of the organs and tissues studied, the gonads, hepatopancreas, Y organ, and hemopoietic tissue suffered minimal injury, and the gills, heart, and antennal gland were the most severely affected. Connective tissues were mechanically disrupted by gas bubbles but seldom exhibited ischemic injury.

Reference to Table 1 shows, first, with one exception, that cell and tissue injury due to ischemia did not become evident until the fourth day following the gas episode, save in the gills; and, second, that tissue injury and gas emboli in internal tissues occurred concurrently. Later, on days 11–35, emboli were present with minimal evidence of tissue damage. Gas was first visible in the gills and still demonstrable there in most animals after evidence of internal emboli had disappeared, although a bubble was still present in the eye of one crab dissected on day 35 following the episode.

Pathology in the gills apparently was confined to mechanical disruption and displacement of tissues, including the epithelia of the lamellae which, on occasion, were torn loose from the cuticle. During histologic preparation, vacuum infiltration of paraffin caused the gill lamellae to lose the gas and become flattened, or the thin unsupported cuticle tore during sectioning, obscuring the original lesions. Figure 1 shows a gill stem which was only mildly affected. In this case, the central artery was normal, but gas filling the adjoining hemal sinuses caused tissue displacement and disruption. The areas of thickened lamellar epithelia (salt glands) that occur basally on some gills and are active in osmotic regulation were not greatly affected.

Gas bubbles in the heart displaced muscle fibers and caused partial hemal stasis (Fig. 2). Hyaline degeneration of heart muscle occurred focally (Fig. 3), and glial nuclei of the associated nerves, not always present in sections examined, exhibited pycnosis. The nerve fibers themselves were sometimes degenerate (Fig. 4).

Blood vessels were often distended by large gas emboli and accumulated hemolymph (Fig. 5). Hemocytes present in these areas were usually normal, did not appear to be clotting, and had not responded to the presence of gas bubbles. Pycnotic nuclei were occasionally found in large groups of hemocytes.

Hemal spaces and the antennary artery of the antennal gland were often distended with gas. The epithelium of the labyrinth was extensively degenerate, presenting an opaque appearance, and had enlarged, pale nuclei rimmed with chromatin, indicating incipient karyolysis. The lumen of the labyrinth was often obliterated (Fig. 6). Degeneration of the end sac, which interdigitates with the labyrinthal involutions, was less common and was focal. End-sac nuclei were sometimes pycnotic and the internal parts disorganized (Fig. 7), but incipient karyolysis was not common. Normal relationships of the parts of the antennal gland are shown in Figure 8.

Skeletal muscle underwent focal hyaline degeneration (Fig. 9). Hemal spaces distended with gas sometimes occurred in the Y organ, and a few pycnotic nuclei occurred there in occasional specimens. The various parts of the gut were usually not affected, and normal mitotic figures were present in the anterior cecum of the midgut. On one occasion, incipient karyolysis was evident in one small area of the epidermis. Incipient karyolysis was observed in rare nuclei in the acini of the hepatopancreas, but this organ appeared normal except for mild disruption of interstitial tissues due to presence of gas. Pycnosis and incipient karyolysis also occurred in some glial nuclei associated with

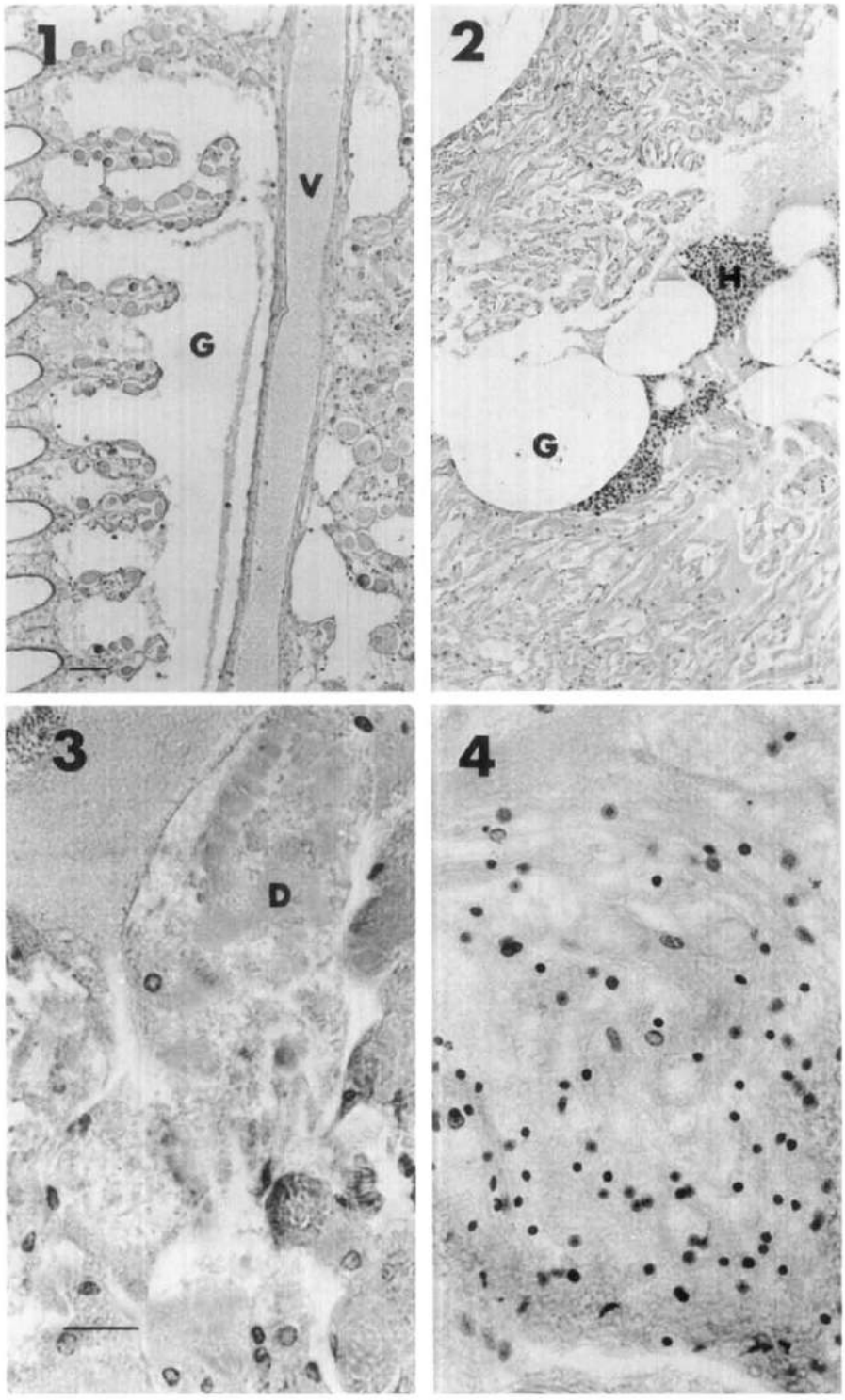

FIG. 1. *Callinectes sapidus:* stem of gill. Gas (G) had disrupted tissues; blood vessel (V) is normal. Line = 100 μm (Figs. 2 and 5 to same scale).
FIG. 2. Heart, showing gas emboli (G) and accumulated hemolymph and hemocytes (H).
FIG. 3. Heart muscle undergoing hyaline degeneration (D). Line = 10 μm (Figs. 4–10 and 12 to same scale).
FIG. 4. Degenerate nerve within the heart. Note the pycnotic nuclei.

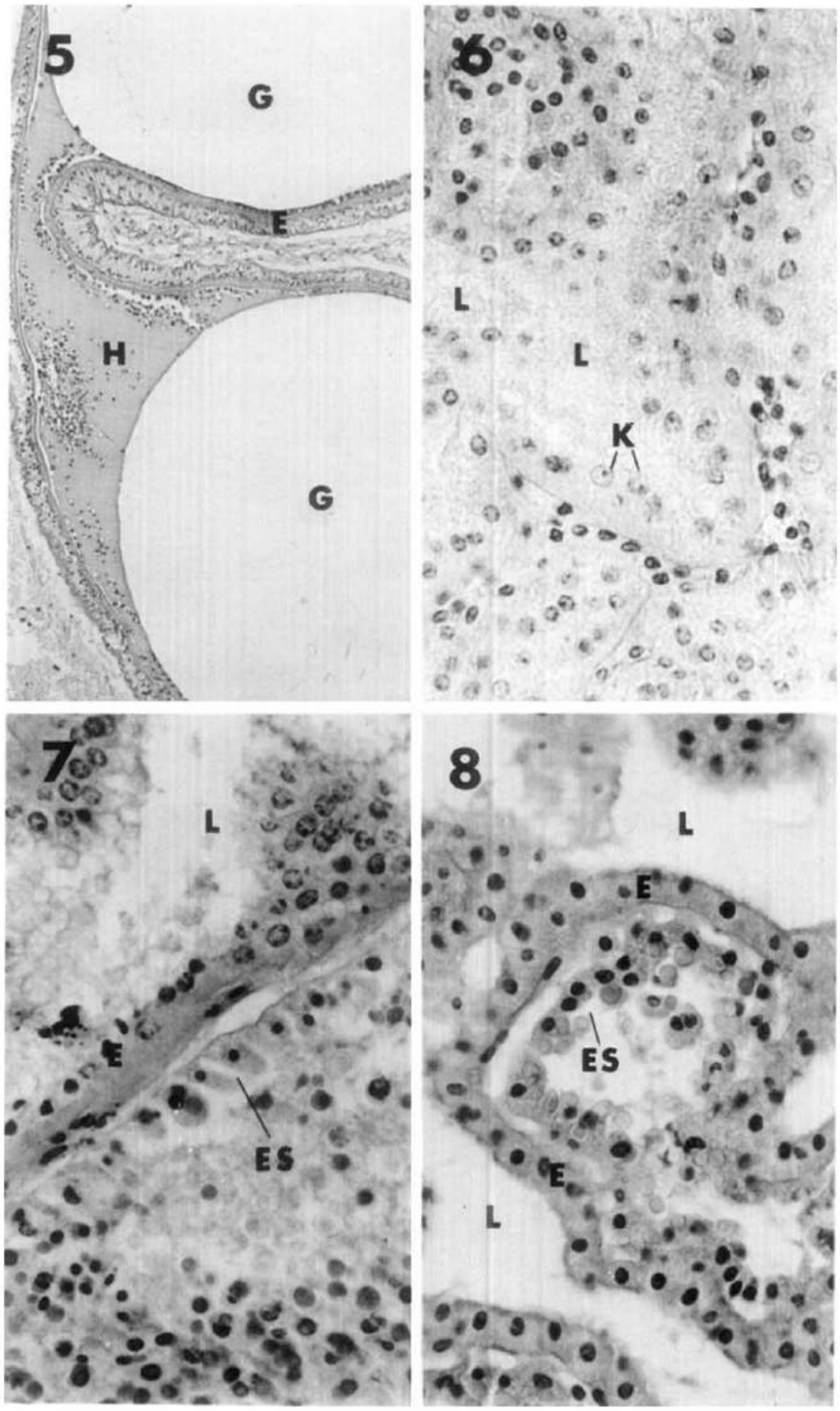

FIG. 5. *Callinectes sapidus:* ophthalmic artery distended with gas emboli (G) and accumulated hemolymph and hemocytes (H). E, endothelium of the vessel.

FIG. 6. Antennal gland showing incipient karyolysis (K) in the labyrinthal epithelium and the obliterated lumen (L).

FIG. 7. Antennal gland showing pycnotic nuclei in the epithelium of the end sac (ES); opaque labyrinthal epithelium (E) and the labyrinthal lumen (L).

FIG. 8. Normal antennal gland. ES, end-sac epithelium; E, labyrinthal epithelium; L, lumen of the labyrinth.

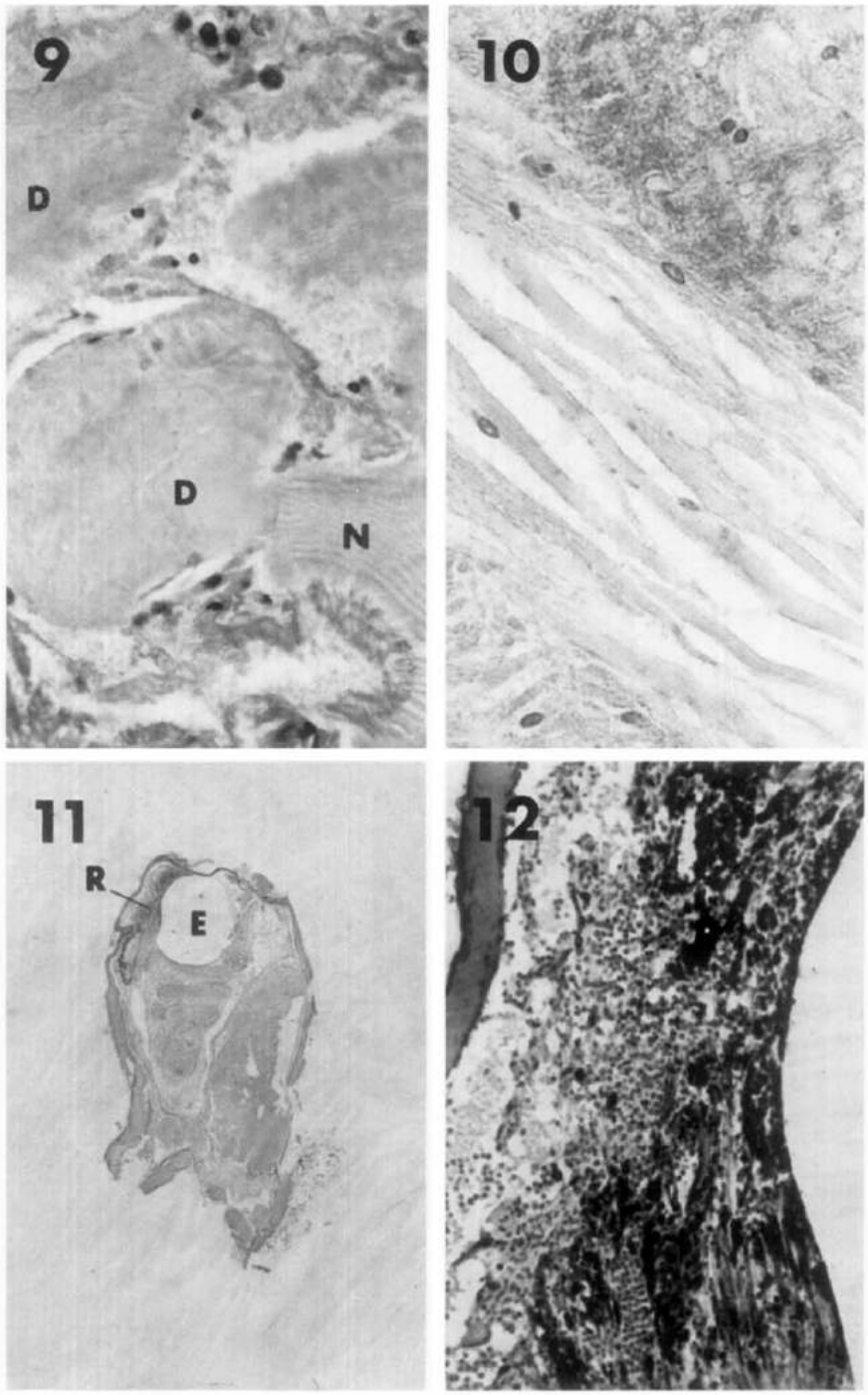

FIG. 9. *Callinectes sapidus*: skeletal muscle undergoing hyaline degeneration. D, degenerate muscle; N, normal muscle.
FIG. 10. Thoracic ganglion showing degenerate hyaline nerve fibers disrupted by gas.
FIG. 11. Eyestalk with a large gas embolus (E) displacing the retina (R).
FIG. 12. An enlargement of Fig. 11, showing the displaced retinal cells.

the brain and thoracic ganglion, and rare nerve tracts disrupted by gas were hyaline in appearance (Fig. 10). A large gas bubble in the eye of a crab dissected on the 35th day had caused compression of the retinal cells, but degeneration and hemocytic response were lacking (Figs. 11 and 12).

Crabs in good condition, dissected on days 11–39 following the gas episode, did not show tissue damage. The only repair process attributable to ischemic or mechanical injury occurred in the gills, where fibroblasts occupied occasional lamellae.

No visible gas emboli or pathology attributable to gas emboli were found in another group of Chincoteague crabs placed in the laboratory water system on the 20th day following the gas episode and dissected on days 20–39 following the episode.

DISCUSSION

Paramebiasis may have contributed to mortalities in the undissected crabs which died the first 2 days following the gas episode. Animals stressed by heavy infections of *P. perniciosa* would be less able to withstand the physiologic insult offered by the gas emboli and resultant ischemia. This suggestion is strengthened by the fact that three of five animals dissected prior to the gas episode had heavy infections of *P. perniciosa*, while the three infected survivors of the gas episode had only very light and presumably nonstressful infections.

The pattern of tissue and organ injury suggests that the major sequelae of gas-bubble disease are: dysfunction of gill epithelia through mechanical disruption and by presence of gas emboli; focal degeneration of the heart muscles and associated pacemaker nerves which, together with gas emboli, would contribute to hemal stasis; and impaired function of the antennal gland.

Rigdon and Baxter (1970) described the development of "spontaneous muscle necroses" in brown shrimp, *Penaeus aztecus*, that were being maintained in poorly aerated water. The muscle lesions were focal and not accompanied by a hemocytic response. If degeneration was not too advanced, recovery and muscle repair took place when the shrimp were placed in well-aerated water. The focal character of muscle degeneration in both hypoxic shrimp and in crabs with gas emboli may demonstrate a basic crustacean response to muscle ischemia. Rigdon and Baxter (1970) found that regeneration of shrimp muscle was preceded by a marked proliferation of sarcolemmal nuclei in the affected muscles. A similar response was not noted in the blue crabs but, since frankly necrotic areas were not seen in the crab muscles, repair may have been a more subtle process there.

All the blue crabs exposed to the water supersaturated with air probably had suffered from gas emboli and tissue injury, including those dissected after the acute phase of the episode. Whatever repair processes occurred in these crabs, overt evidence of the participation of hemocytes was lacking except within the gill lamellae, where a fibroblastic response sometimes occurred.

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