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Experimental Wound Repair in the Freshwater Mussel *Anodonta oregonensis*[1]

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The process of wound repair was studied histologically following surgical incisions in the foot of the freshwater mussel *Anodonta oregonensis*. Wound repair in *A. oregonensis* differed from that in vertebrates and most invertebrates by the lack of a pronounced cellular response. In other respects, however, the general process of healing was similar to that found in most vertebrate and other invertebrate species.

INTRODUCTION

Wound repair is a basic protective defense mechanism in animals that results in the replacement of dead or damaged cells by new healthy cells. This process has been studied in insects (Lazarenko, 1924; Wigglesworth, 1937; Schlumberger, 1952; Day, 1952; Day and Bennetts, 1953; Day and Oster, 1963; Locke, 1966), mollusks (Drew and Morgan, 1910; Kedrovsky, 1925; Zawarzin, 1927; Pauley and Sparks, 1967; Des Voigne and Sparks, 1968), and other invertebrates (Cameron, 1932; Cowden, 1968). Histopathologic observations of the repair process in the freshwater mussel *Anodonta oregonensis* following scalpel incisions in the foot are reported in this paper.

MATERIALS AND METHODS

Freshwater mussels (*Anodonta oregonensis*) were taken from Devil's Lake, Oregon, near Lincoln City, by scuba diving. The mussels were acclimated for several months in the Columbia River and then for 2 weeks in experimental troughs prior to use to ensure that only healthy animals would be used in the experiment. The mollusks were divided into two groups of 80 mussels each. Eighty of the mussels served as untreated controls. The second group of 80 mussels received a surgical incision 1 inch long and approximately ¼ inch deep, which ran laterally along the axis of the foot. The scalpel blade was dipped in isopropyl alcohol and air-dried after each incision to prevent contamination or infection of the subsequent wounds.

Eight mussels were sampled from each of the two groups at the following time intervals post-treatment: 1 d, 2 ds, 4 ds, 8 ds, 16 days, and every 14 days thereafter through 58 days. Cross sections approximately ¼ inch wide and passing through the wound were removed from the anterior visceral mass of the mussels and fixed in methanol. The tissues were dehydrated by standard methods, embedded in Paraplast, and sections were cut at 8 μ. The slides were stained in either hematoxylin and eosin, modified Mallory's trichrome (Pauley, 1967), or modified MacCallum-Goodpasture stain (Pauley and Maulsby, 1967).

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Results

Grossly, no abnormal color was associated with the wounds in *A. oregonensis*. The only change observed was a closed or "healed" appearance of the wounds after 6 weeks. There was, however, a significantly increased mortality in the experimental mussels between the 5th and 23rd day after injury (Fig. 1).

The first observable histological response to the wounds in these mussels was an inward curl (invagination) of one edge of the wound (Fig. 2) in an attempt to close the incision. In many cases, this was the only response observed during the first 8 days. Often complete occlusion of the superficial part of the wound channel was accomplished by this invagination as early as the 1st day after injury. In some mussels an aggregation of hemocytes formed a "clot" in the narrow wound channel. In a few mussels an extremely weak cellular response was observed in the tissues surrounding the wound. However, in most of the mussels, the cellular response observed was an aggregation or layer of hemocytes in the deepest part of the wound. Often the hemocytes were spindle-shaped and had a layered appearance and were accompanied by a few fibroblasts (Fig. 3). As early as 1 day after injury the spindle-shaped hemocytes began to extend up the sides of the wound toward the surface, indicating that wound repair proceeded from the internal or deepest part of the wound toward the surface, as Des Voigne and Sparks (1968) observed in the oyster (*Crassostrea gigas*). A thin layer of hemocytes (two to five cells thick) almost lined the entire wound channel by 4 days, but did not completely line it until the 8th day after injury.

By the 8th day, fibroblasts were prominent among the hemocytes and a few fine, thin strands of collagen had been formed by these cells (Fig. 4). At this time a ciliated epithelium had grown down the sides of the wound and was confluent with the original ciliated columnar epithelium of the foot. New collagen fibers were apparent beneath the incipient epithelium. The new epithelium was cuboidal (Fig. 4) near its confluence with the original foot epithelium, while deeper in the wound it was more flattened and had a squamouslike appearance (Fig. 5).

Collagen formation was slightly more

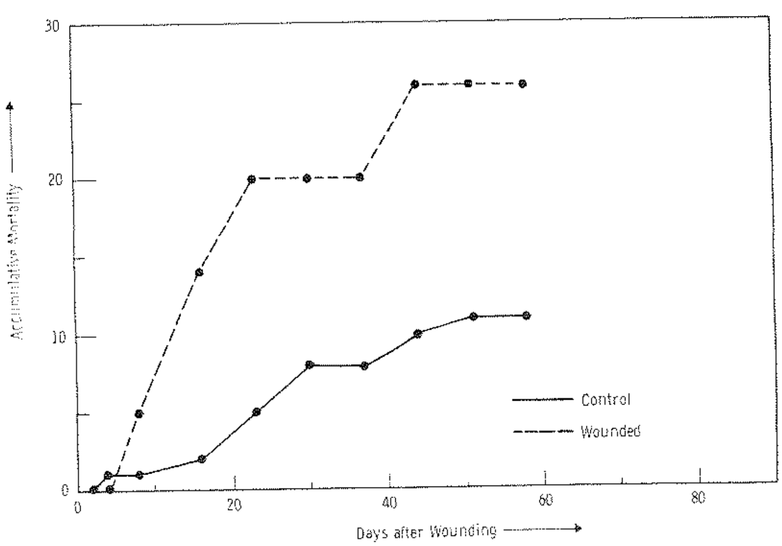

Fig. 1. Accumulative mortality curve for *Anodonta oregonensis*.

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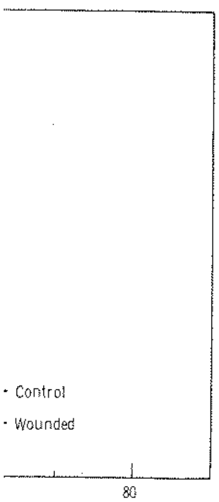

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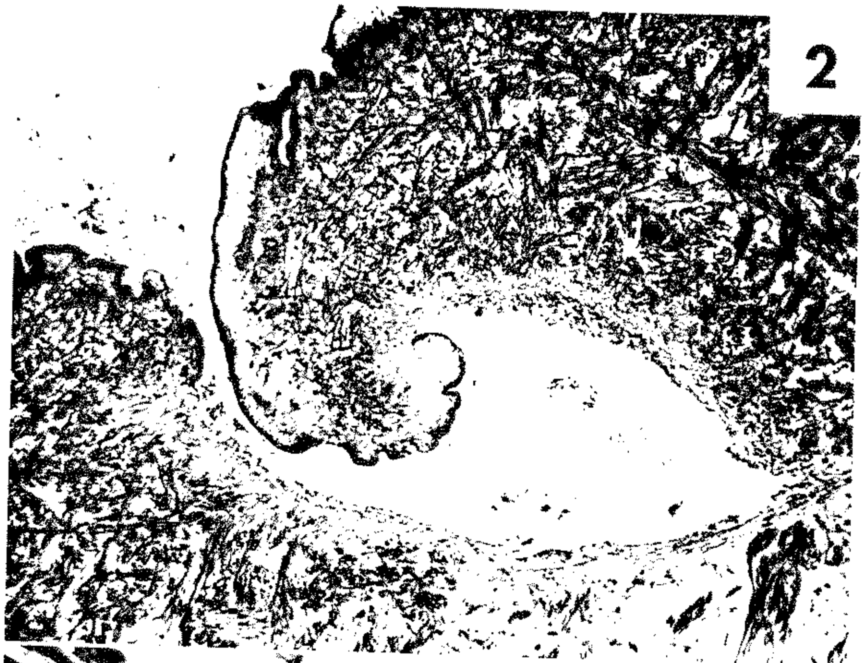

FIG. 2. Invagination of one edge of a wound. Also note hemocytes lining the wound cavity; 8-day wound. Hematoxylin and eosin. 25 ×.

FIG. 3. Layer of spindle-shaped hemocytes (round nuclei) on surface of cut muscles (M). Note a few fibroblasts (elongate nuclei) are present between muscles and hemocytes; 2-day wound. Hematoxylin and eosin. 400 ×.

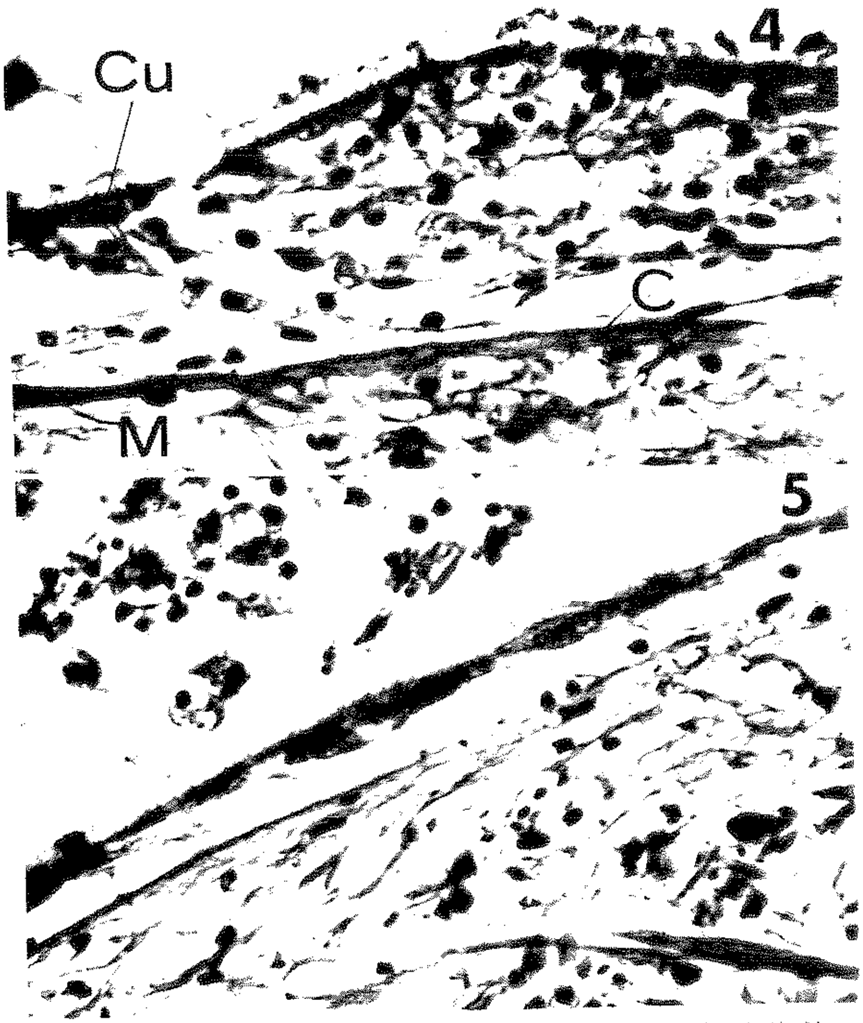

FIG. 4. Fibroblasts are producing a thin layer of collagen (C) over muscles (M). Note ciliated cuboidal epithelium (Cu); 8-day wound. Modified Mallory's trichrome. 400 ×.

FIG. 5. Ciliated epithelium with a squamous-like appearance. Note thin layer of collagen and fibroblasts beneath epithelium; 30-day wound. Hematoxylin and eosin. 400 ×.

prominent by the 16th day beneath areas that possessed a new epithelium. Ciliated cuboidal epithelium continued to grow inward from the original foot epithelium with which it was confluent and nearly lined the entire wound by 30 days (4 weeks). However, as late as 30 days (4 weeks) new epithelium was lacking in some wound channels, in which cases the entire channel was completely lined by spindle-shaped leukocytes 8–10 cells thick.

In most cases after 44 days (6 weeks), the wound channel had a complete ciliated epithelial lining that was sometimes a combination of cuboidal and columnar cells. Beneath the areas of columnar epithelium a network of smooth muscle was formed and the basophilic mucous glands started to regenerate. A complete ciliated columnar epithelium lined the entire wound channel of two of the mussels.

After 58 days (8 weeks), the entire wound channel was lined by a ciliated columnar epithelium (Figs. 6 and 7

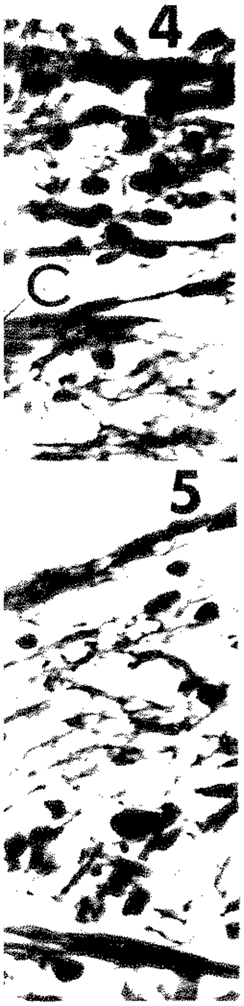

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FIG. 6. Wound completely lined by ciliated columnar epithelium after 58 days. Hematoxylin and eosin. 60 ×.

FIG. 7. Higher magnification of the 58-day wound showing regenerating mucous glands (G.) Hematoxylin and eosin. 160 ×.

FIG. 8. Wound with the epithelium in apposition closing the channel; 44-day wound. Hematoxyl... and eosin. 60 ×.

Smooth muscles and mucous glands were still forming beneath the columnar epithelium. After the wound was entirely lined by columnar epithelium and much of the muscle had been regenerated, the two sides came into apposition, closing the channel (Fig. 8). The juxtaposition of the epithelium apparently caused necrosis of its lower portions, which were removed by phagocytosis (Fig. 9). This subsequently resulted in a shallower and essentially healed wound (Fig. 10).

DISCUSSION

The increased mortality between the 5th and 23d day after injury was probably due to excessive loss of vital fluids from these animals' bodies, as there was no apparent difference between the "gapers" examined and the live animals, except that they had been cut deeper by the scalpel. This disparity in the depth of incisions occurred despite our efforts to treat each individual mollusk in an identical manner. It appears that *A. oregonensis* is capable of successfully repairing surgical incisions if the incisions do not completely penetrate the smooth muscle of the foot into the underlying soft connective tissue. Cowden (1968) also found that the severity of the wound inflicted affected whether or not the sea cucumber *Stichopus badionotus* was able to repair the injury. Wounds passing completely through the body wall caused death in *S. badionotus* while extensive superficial incisions healed without complication.

The immediate response to injury by a mollusk has been termed "inflammation" (Pauley and Sparks, 1965, 1966) with the subsequent reparative process called "wound repair" (Pauley and Sparks, 1967). The early stages of inflammation are usually characterized by a pronounced cellular reaction with a granuloma or fibrous reaction the end result of wound repair. It has been shown that the mollusks (Labbé, 1928, 1930; Stauber, 1950, 1961; Tripp

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FIG. 9. Necrosis of epithelium deep within the wound caused by juxtaposition of the epithelial layers. Also note the phagocytic hemocytes which apparently remove the necrotic debris; 58-day wound. Hematoxylin and eosin. 60 ×.

FIG. 10. Shallow healed wound. Note original line of incision is still visible; 58-day wound Hematoxylin and eosin. 60 ×.

1961, 1963; Cheng, 1967; Feng, 1967; Pauley and Sparks, 1967; Arcadi, 1968; Des Voigne and Sparks, 1968) are capable of efficient wound repair if the injurious agent is nontoxic.

The lack of a pronounced cellular response in the freshwater mussel *Anodonta oregonensis* following injury is unusual, but such response has also been reported absent in an insect (Day and Bennetts, 1953) and in a sea cucumber (Cowden, 1968).

Drew (1910) noted that small incisions made in the foot of the pelecypoda *Cardium norvegicum* healed within 3 weeks by a process similar to that found in mammals. Based on incomplete observations, Pauley and Sparks (1967) estimated that surface wounds in the oyster *Crassostrea gigas* could heal satisfactorily in 2 to 3 weeks. Des Voigne and Sparks (1968) subsequently found that incisions in *C. gigas* heal in about 1 week by a process very similar to that described by Robbins for mammals (1962). Rapid healing was also noted in the sea cucumber by Cowden (1968), where it was essentially complete by the 10th day. The protracted healing period in *A. oregonensis* (6 to 8 weeks) appeared to be due to the severity of the wounds administered to this mollusk.

The epithelization of the wound cavity in *A. oregonensis* before it is filled with connective tissue differs from other mollusks (Drew, 1910; Pauley and Sparks, 1967; Des Voigne and Sparks, 1968). This appears to be related to the lack of early apposition of the edges of the wounds in *A. oregonensis* as was found in *C. gigas* by Des Voigne and Sparks (1968). Kedrowsky (1925) and Zawarzin (1927) found that freshwater mussels (*Anodonta* sp.) combine fibrosis with the formation of an epithelium around foreign bodies. Mackin (1961) observed a similar epithelization in oysters. In *A. oregonensis* it appears as though the epithelium lining the wound cavity proliferated down into the wound channel from the tall columnar epithelium normally covering the foot. Proliferation from preexistent epithelium in the way vertebrate epithelium grows over a wound (Robbins, 1962). In comparing the repair process observed in *A. oregonensis* to that of other invertebrates and mammals we find minor differences. However, there is a similarity in the reparative process in this freshwater mussel and in other animals.

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