Cellular Response to Injury in Penaeid Shrimp

C.T. FONTAINE and D.V. LIGHTNER

ABSTRACT—A review of the cellular response to injury in marine shrimp of the genus Penaeus *is presented. Studies on the inflammatory response of penaeid shrimp have shown six cell forms that respond to injury. These include three forms of hemocytes, fibrocytes, phagocytic cells of the loose connective tissue, and fixed phagocytes lining blood sinuses.*

The cellular defense mechanism is dependent upon the activity of the hemocytes. One type of hemocyte apparently engages in encapsulating foreign material while the other two types phagocytize and eliminate foreign or necrotic material by migrating to the external surface. Later, additional phagocytosis is accomplished by "fixed" phagocytes in the loose connective tissue and by cells that line hemolymph sinuses of the heart, gill, and abdomen.

Collagen-like fibers are typically seen in association with wound healing and in the process of encapsulation in these animals. The dense network of collagen-like fibers and fibrocytes develops in close association with earlier hemocytic encapsulation or deposits. This fibrous tissue is stable, not resorbed, and remains as a permanent "scar."

INTRODUCTION

Within the past 20 years, the possibility of commercial culture of marine invertebrates has gained wide attention and interest, especially with the marine decapod Crustacea belonging to the genus *Penaeus*. On 20 August 1970 there were approximately 75 organizations in the United States involved in shrimp culture including federal, state, institutions, and companies or individuals. In addition, considerable information has been published relating to larval culture, tolerance studies, and pond culture of penaeid shrimp (Johnson and Fielding, 1956; Fujinaga, 1969; Allen, 1963; Zein-Eldin, 1963; Ewald, 1965; Zein-Eldin and Aldrich, 1965; Cook and Murphy, 1966; Zein-Eldin and Griffith, 1966; Cook, 1967; Wheeler, 1967; Zein-Eldin and Griffith, 1967; Aldrich et al., 1968; Cook and Murphy, 1968; Wheeler, 1968; Neal, 1970, 1970b; Mock and Murphy, 1970; and Lindner and Cook, 1971). The primary objective of the Aquaculture Investigation of the National Marine Fisheries Service, Galveston Laboratory, Galveston, Texas, has been to conduct investigations related to the culture of large numbers of penaeid shrimp. As in any intensive culture program, where conditions are also favorable for rapid proliferation of pathogenic organisms, one of the more important areas of consideration is the problem of disease.

The understanding of disease in any organism is dependent upon a basic knowledge of the organism's normal defense mechanisms. Cellular defense reactions have been reviewed in detail for insects by Salt (1970) and for invertebrates other than insects by Bang (1970) and Sparks (1972). A review of the literature, however, reveals very little information on histopathological investigations of marine Crustacea, particularly of the order Decapoda. The lack of recorded histopathological or normal histological data on marine decapod Crustacea is surprising, considering that several genera of this order

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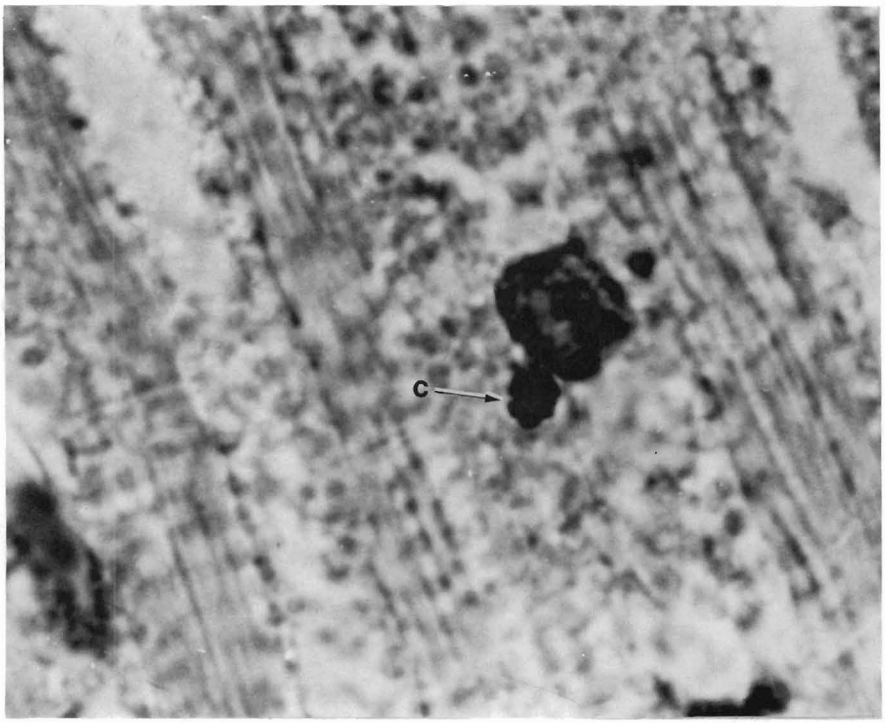

Figure 1.—A phagocytic form of hemocyte that has infiltrated the affected tissue and ingested foreign material (c = carmine). From Fontaine and Lightner, 1974. Hematoxylin, 2500×.

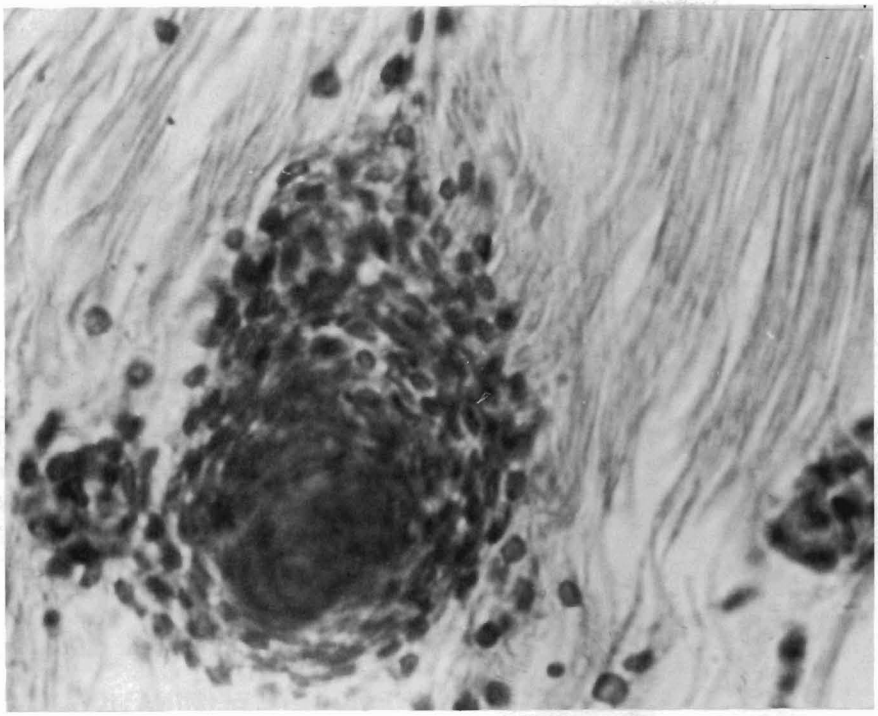

Figure 2.—A large hemocytic encapsulation. The innermost cells have become melanized forming a dark pigmented membrane. From Fontaine and Lightner, 1973. Hematoxylin and eosin, 800×.

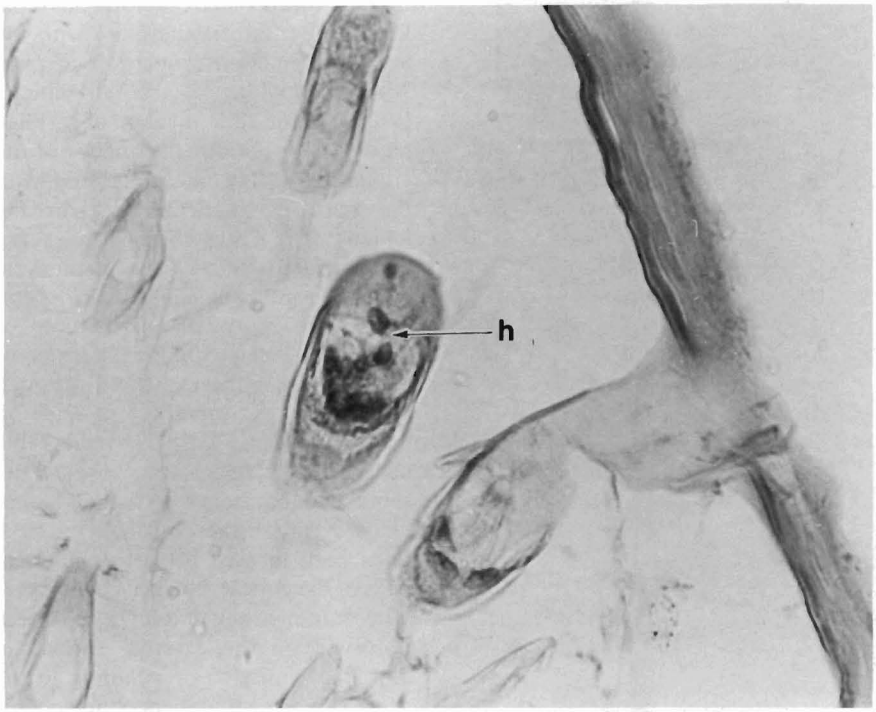

Figure 3.—The bases of setae with hemocytes included through which the phagocytes laden with foreign or necrotic material migrate to the external surface, (h = hemocytes). Hematoxylin and eosin, 650×.

are among the most economically important fishery products in North America. Prior to 1973, documented disease studies of penaeid shrimp were mainly from a parasitological viewpoint and contained little or no histological data (Aldrich, 1964; Iversen and Manning, 1959; Iversen and Van Meter, 1964; Kruse[1], 1959; Baxter, Rigdon, and Hanna, 1970; and Sprague, 1950, 1966). One exception was the histological study on "spontaneous necrosis" (Rigdon and Baxter, 1970).

Little is known about the humoral defenses or responses of penaeid shrimp. Foreign abiotic material injected into the abdominal musculature appears to adhere together to form extracellular clumps (Fontaine and Lightner, 1974). The factor in the hemolymph which causes this agglutination is not known. It is likely that this apparent humoral factor functions to localize the foreign material until cellular responses are initiated.

The subsequent cellular defense mechanisms in penaeid shrimp are dependent upon the activities of at least six cell forms. These include fibrocytes, "fixed" phagocytes in the loose connective tissue, and "fixed" phagocytic cells which line blood sinuses in the gill, heart, and abdominal muscle tissue.

HEMOCYTE FUNCTION IN PHAGOCYTOSIS

The hemocytes of penaeid shrimp typically migrate rapidly to invaded or injured tissue and, depending on particle size, engulf or encapsulate the necrotic or foreign material. Phagocytosis by hemocytes is accomplished by two cell forms that infiltrate the invaded tissue and ingest foreign or necrotic material. One form has eosinophilic cytoplasm and a small basophilic nucleus (Fig. 1). Occasionally, several of these cells that have phagocytized foreign or necrotic material will adhere together forming large multinucleated clumps that closely resemble the large multinucleated foreign body giant cells of other animal groups. A second form of hemocyte is a large macrophage-like cell that also infiltrates invaded tissue and exhibits phagocytosis. These cells possess a large, slightly basophilic nucleus with extensive hypochromatic cytoplasm.

HEMOCYTE FUNCTION IN ENCAPSULATION

Another function of circulating hemocytes is the encapsulation of foreign bodies that are too large for phagocytosis. It is interesting to note (Fontaine and Lightner, 1974) that when carmine particles were injected into the white shrimp, *Penaeus setiferus*, hemocytic encapsulations consisted

[1]Kruse, D. N. A study of the taxonomy, morphology, incidence and biology of the parasites of the commercial shrimp. *Penaeus aztecus* Ives, *P. duorarum* Burkenroad, and *P. setiferus* Linnaeus. M. S. thesis, Florida State University.

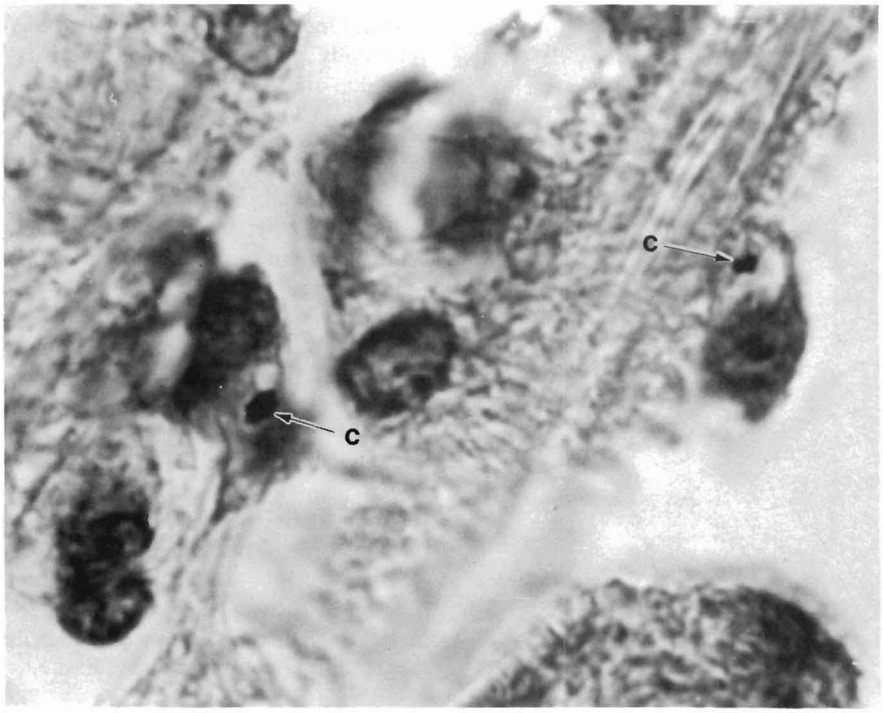

Figure 4.—Fixed phagocytes in blood sinuses of heart that have ingested foreign material (c = carmine). From Fontaine and Lightner, 1974. Hematoxylin, 2500×.

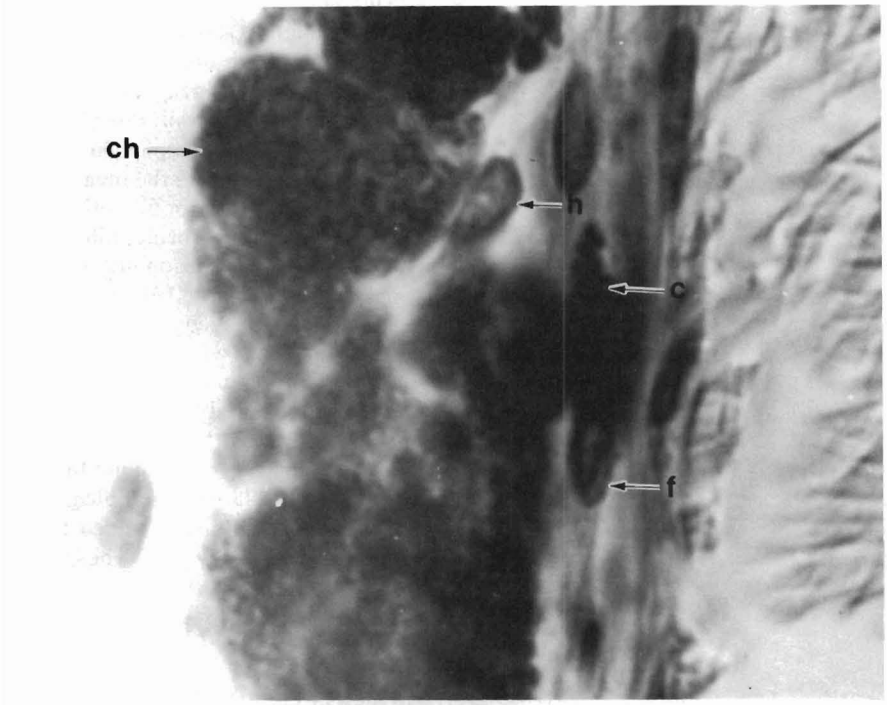

Figure 5.—Free and "fixed" phagocytes in an abdominal blood sinusoid (f = fixed phagocyte, c = carmine, h = free hemocyte, ch = carmine ingested by hemocyte). From Fontaine and Lightner, 1974. Hematoxylin, 2500×.

of necrotic hemocytes with carmine included. None of the cells actively engaged in the process of encapsulating these necrotic phagocytes were observed to contain carmine particles.

The mechanics of the process of encapsulation in penaeid shrimp are similar to that described for insects by Grimstone et al. (1967) and Salt (1970) with hemocytes many cell layers thick forming the encapsulations. The outer cells retain a more normal oval or rounded configuration, while the inner cells become flattened (Fig. 2) and, in later stages are lysed, forming a thick, brown, leathery capsule. These capsules are not resorbed and remain as marks of the foci of encapsulation even though no recognizable hemocytes remain.

The intensity of the cellular response in the process of encapsulation is variable. For example, the response to parasites depends upon the species of parasite and its location within the shrimp. The parasitic nematode, *Contraceacum* sp., elicits little or no cellular response from the shrimp no matter where it is located in the shrimp's body, while the pleuocercoid larvae of the cestode, *Prochristianella penaei*, is destroyed by encapsulating hemocytes if located within the hepatopancreas or muscle. The response to the larval cestode is much less intense if located in the hemocoelic space (Sparks and Fontaine, 1974).

HEMOCYTE ELIMINATION OF FOREIGN MATERIAL

One of the primary sites of elimination of foreign and necrotic material from the shrimp is the gills, with the actual elimination probably occurring with the molting of the gill cuticle. Within 1 hour after injection of carmine particles into shrimp, the lumens of the gill filaments become congested with the particles that are free in the hemolymph. Later, the particles are concentrated into large hemocytic clumps in the gill lamellae.

Another route of elimination of hemocytes laden with foreign or necrotic material appears to be through cuticular pores at the base of setae on the appendages (Fig. 3). The connective tissue and muscle at the base of the setae become congested with hemocytes. Many of these phagocytes have been seen located between epidermal cells of the cuticle. Necrotic hemocytes with carmine included have also been observed on the external surface in close proximity to or adhering to the setae. The great numbers of hemocytes observed in the maxillae, pereiopods, and pleopods where setae are present indicate these areas may be principal sites for eliminating hemocytes laden with necrotic or foreign material.

"FIXED" PHAGOCYTES

The hemolymph chambers or sinuses of the penaeid shrimp are lined with a

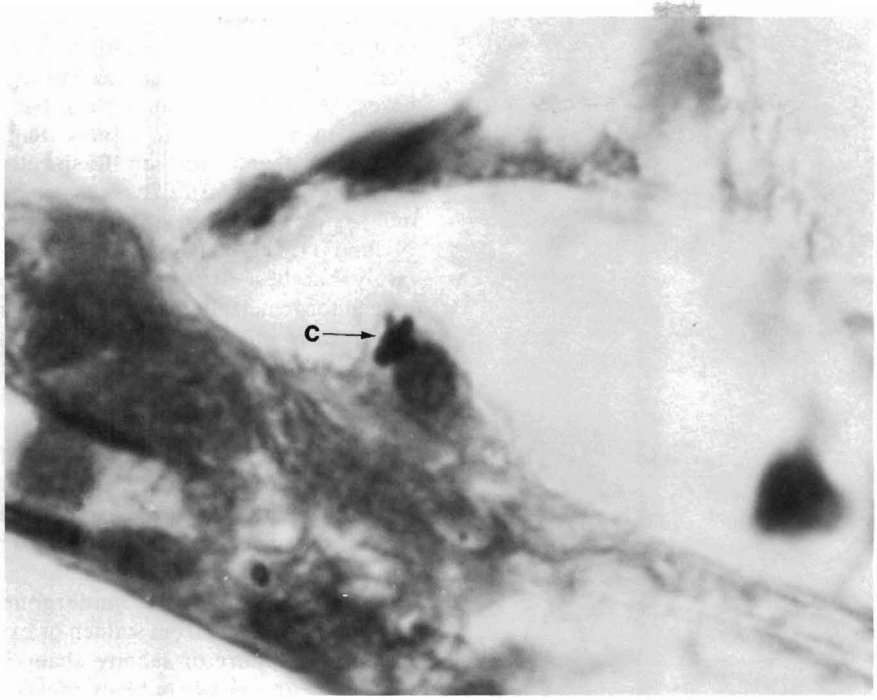

Figure 6.—A "fixed" phagocyte in a gill filament that has ingested foreign material (c = carmine). From Fontaine and Lightner, 1974. Hematoxylin, 2500×.

network of "fixed" phagocytic cells. "Fixed" phagocytes containing ingested foreign material have been observed in the heart (Fig. 4), abdomen (Fig. 5), and the lumen of the gill filaments (Fig. 6). This system of "fixed" phagocytic cells is very similar to that described in insects and may be analogous to the reticuloendothelium of vertebrates (Wigglesworth, 1970). Other fixed phagocytes are the large basophilic cells of the subcuticular loose connective tissue, particularly in the posterio-dorsal portion of the cephalothorax (Fig. 7). These "fixed" phagocytic cells are apparently long lived. Fontaine and Lightner (1974) observed carmine included in phagosomes in these fixed cells 672 hr after injection. This system of fixed phagocytic cells thus accounts for the slow clearance rate of injected stains from penaeid shrimp (Neal, 1969).

FIBROCYTES

In penaeid shrimp wounded with a Petersen disk tag pin (Fontaine and Lightner, 1973), hemocytes infiltrate the area and wall off the pin, forming a dense, brown, leathery membrane. Later, the epidermis involutes into the wound, using the brown pigmented layer as basal support. The involuting epidermis then forms a chitinous layer similar in appearance to the normal external cuticle, thereby effectively relegating the pin to an external rather than an internal position. In association with the hemocytic response, large numbers of fibrocytes infiltrate the area and form a dense network of collagen-like fibers. It was shown in a subsequent study (Fontaine and Dyjack, 1973) that this fibrous tissue is not resorbed, is well organized, stable, and remains as a permanent "scar" (Fig. 8).

Another example of fibrocyte infiltration occurs in the replacement of tissues damaged by the injection of turpentine (Fontaine et al., In press). At 50 days post-injection, large fibrous cysts had been formed at the site of injection by infiltrating hemocytes and fibrocytes (Fig. 9). Heart tissue damaged by the circulating turpentine had been infiltrated by hemocytes and fibrocytes by 240 h post-injection and collagen-like fibers and cellular encapsulations had appeared (Fig. 10). In another study where a strip of polyvinyl chloride was inserted into the abdominal musculature of the white shrimp, *P. setiferus*[2], fibrocytes infiltrated and formed a thick fibrous capsule around the primary hemocytic encapsulation (Fig. 11).

[2]Unpublished study, "An electron microscopic study of capsule formation in penaeid shrimp," on file at the Galveston Laboratory, Gulf Coastal Fisheries Center, NMFS, Galveston, TX 77550.

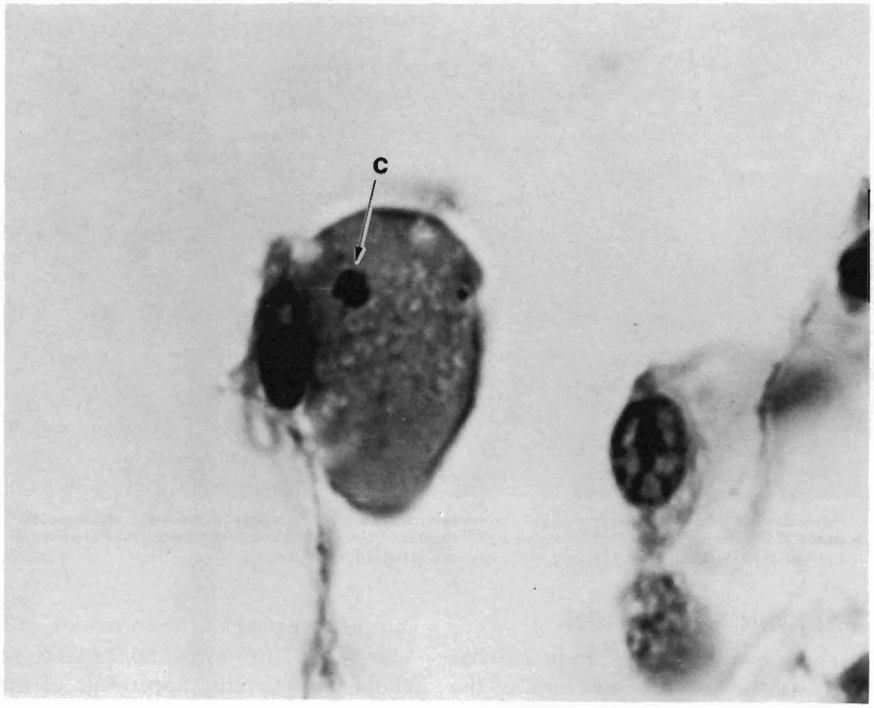

Figure 7.—A large basophilic cell in the subcuticular loose connective tissue of the cephalothorax with ingested foreign material (c = carmine). From Fontaine and Lightner, 1974. Hematoxylin, 2500×.

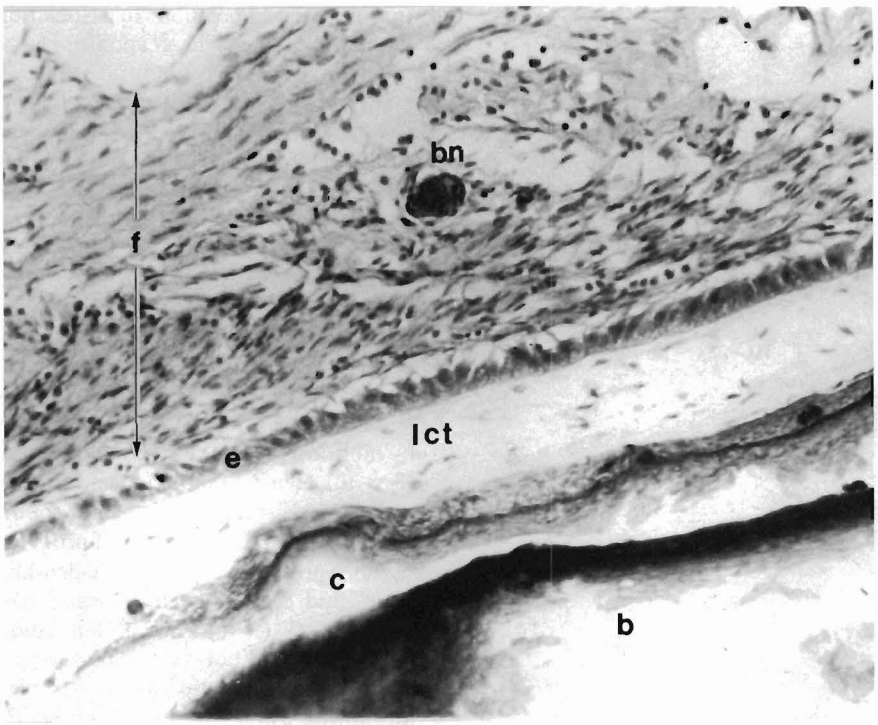

Figure 8.—The wound repair processes 30 days post-tagging with the Petersen disk tag (b = melanized membrane, c = cuticle, lct = loose connective tissue, e = epidermis, bn = brown nodule, and f = fibrous tissue). From Fontaine and Dyjak, 1973. Hematoxylin and eosin, 250×.

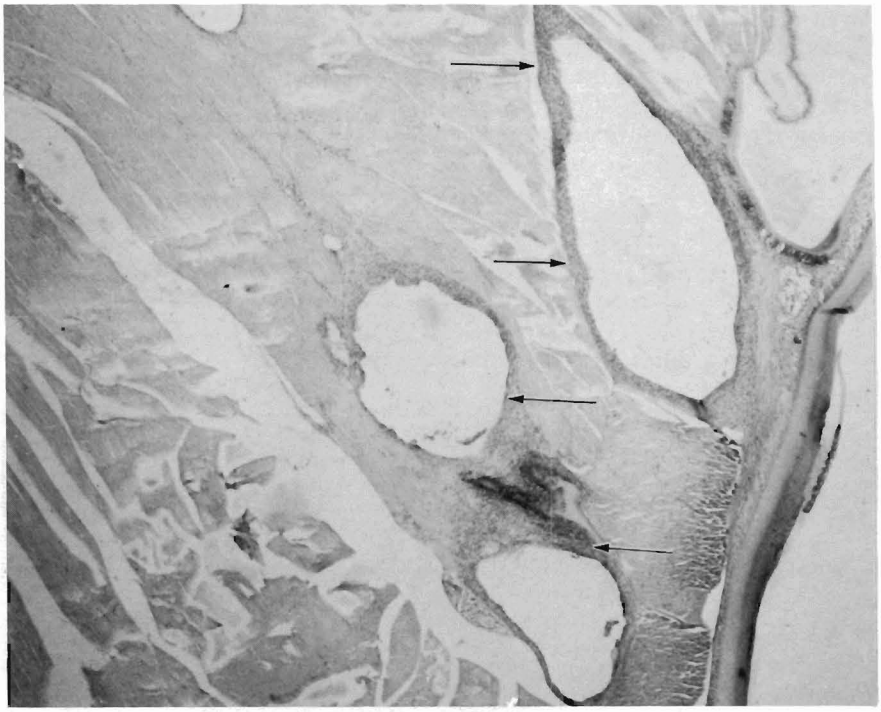

Figure 9.—The inflammatory response in *Penaeus setiferus* to turpentine injected into the abdominal musculature. The response consists of hemocytes and fibrocytes forming a thick matrix of fibrous tissue around the affected area (arrows). From Fontaine et al., In press. Hematoxylin and eosin, 40×.

MELANIN FORMATION

The brown material seen in association with hemocytes in several of the conditions in penaeid shrimp described in this paper has been reported to occur in other crustacea. The formation of a "black cap" from what appeared to be chitin on a severed appendage of the sand flea, *Gammarus* sp., was reviewed by Bang (1970). Sindermann (1971) described brown or "chitinoid" bodies or cysts in the gills as characterizing later stages of a number of crustacean diseases. A similar material has been shown to be melanin in insects (Salt, 1970). In their work on freshwater crayfishes, *Pacifastacus leniusculus* and *Astacus astacus*, Unestam and Nylund (1972) demonstrated conclusively that these decapod Crustacea do indeed form melanin in blood reactions in vitro. They also concluded that both the enzymes and the substrate for the process of melanization originate from the hemocytes. However, the formation of melanin following injury of penaeid shrimp remains to be proven. Dark pigmented material associated with hemocytes reacting to injury in shrimp is presumed to be melanin.

Shrimp which have undergone physiological stress from sudden or extreme temperature or salinity changes form numerous brown or black spots or nodules in the gill filaments. Larval shrimp invaded by the fungus *Lagenidium* sp. (Lightner and Fontaine, 1973) form brown spots in response to fungal hyphae in a manner similar to that described for the crayfish by Unestam and Nylund (1972). The pigmented spots have also been observed and recorded in association with bacterial erosion of the cuticle (Cook and Lofton, 1973; Lightner and Lewis, 1975); in wound repair (Fontaine and Lightner, 1973); after injection of carmine (Fontaine and Lightner, 1974), and turpentine (Fontaine et al., In press); in the capsular formation around an internal PVC tag[3]; and in an inflammatory response within a tumor (Sparks and Lightner, 1973). The black or brown spot syndrome occurs commonly (Fig. 12) and is a clinical sign of disease or injury that has been observed frequently in penaeid shrimp.

LITERATURE CITED

Aldrich, D. V. 1965. Observations on the ecology and life cycle of *Prochristianella penaei* Kruse (Cestoda: Trypanorhyncha). J. Parsitol. 51:370-376.

Aldrich, D. V., C. E. Wood, and K. N. Baxter. 1968. An ecological interpretation of low temperature responses in *Penaeus aztecus* and *P. setiferus* postlarvae. Bull. Mar. Sci. 18:61-71.

Allen, D. M. 1963. Shrimp farming. U.S. Fish Wildl. Serv., Fish. Leafl. 551, 8 p.

Bang, F. B. 1970. Disease mechanisms in crustacean and marine arthropods. Am. Fish. Soc. Spec. Publ. 5:383-404.

Baxter, K. N., R. H. Rigdon, and C. Hanna.

[3]Unpublished data.

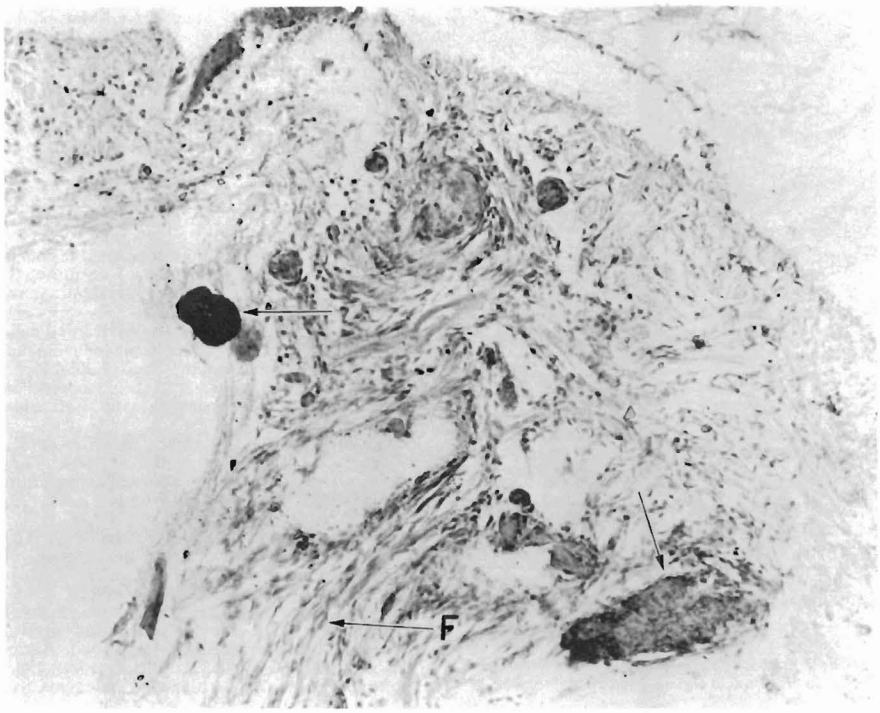

Figure 10.—The cellular response in the heart of *P. setiferus* damaged by turpentine. The response consists of hemocytic encapsulation (arrows) and many collagen-like fibers and fibrocytes (F). From Fontaine et al., In press. Hematoxylin and eosin. 60×.

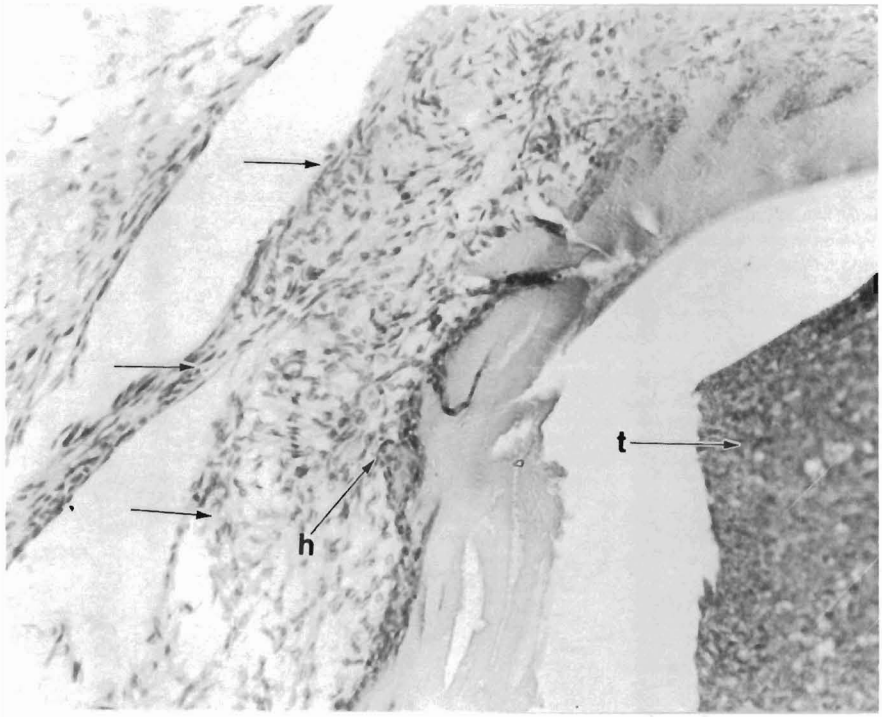

Figure 11.—The cellular response to an internal PVC tag inserted in the abdominal musculature of the white shrimp, *P. setiferus*. The fibrocytes have formed a fibrous capsule around the primary hemocytic encapsulation (arrow). t = plastic insert, h = hemocytes. Hematoxylin and eosin, 200×.

1970. *Pleistophora* sp. (Microsporidia: Nosematidae): A new parasite of shrimp. J. Invertebr. Pathol. 16:289-291.

Cook, D. W., and S. R. Lofton. 1973. Chitinoclastic bacteria associated with shell disease in *Penaeus* shrimp and the blue crab (*Callinectes sapidus*). J. Wildl. Dis. 9:154-159.

Cook, H. L. 1967. A method for rearing penaeid shrimp larvae for experimental studies. FAO (Food Agric. Organ. U. N.) world scientific congress on biology and culture of shrimps and prawns, 12-24 June, 1967, Mexico City, Mexico.

Cook, H. L., and M. A. Murphy. 1966. Rearing penaeid shrimp from eggs to postlarvae. Proc. Southeast Assoc. Game Fish Comm., 19th Annu. Conf., p. 282-288.

_____. 1968. The culture of larval penaeid shrimp. Trans. Am. Fish. Soc. 98:751-754.

Ewald, J. J. 1965. The laboratory rearing of pink shrimp, *Penaeus duorarum* Burkenroad. Bull. Mar. Sci. 15:436-449.

Fontaine, C. T., and R. C. Dyjak. 1973. The development of scar tissue in the brown shrimp, *Penaeus aztecus*, after wounding with the Petersen disk tag. J. Invertebr. Pathol. 22:476-477.

Fontaine, C. T., and D. V. Lightner. 1973. Observations on the process of wound repair in penaeid shrimp. J. Invertebr. Pathol. 22:23-33.

_____. 1974. Observations on the phagocytosis and elimination of carmine particles injected into the abdominal musculature of the white shrimp. *Penaeus setiferus*. J. Invertebr. Pathol. 24:141-148.

Fontaine, C. T., R. G. Bruss, I. A. Sanderson, and D. V. Lightner. In press. The cellular response to turpentine injected into the abdominal musculature of the white shrimp, *Penaeus setiferus*. J. Invertebr. Pathol.

Fujinaga, M. 1969. Kuruma shrimp (*Penaeus japonicus*) cultivation in Japan. FAO (Food Agric. Organ. U.N.), Fish. Rep. 57:811-832.

Grimstone, A. V., S. Rotheram, and G. Salt. 1967. An electron-microscope study of capsule formation by insect blood cells. J. Cell Sci. 2:281-292.

Iversen, E. S., and R. B. Manning. 1959. A new microsporidian parasite from the pink shrimp (Penaeus duorarum). Trans. Am. Fish. Soc. 88:130-132.

Iversen, E. S., and N. H. Van Meter. 1964. A record of the microsporidian, *Thelohania duorara*, parasitizing the shrimp, *Penaeus brasiliensis*. Bull. Mar. Sci. Gulf Caribb. 14:549-553.

Johnson, M.C., and J. R. Fielding. 1956. Propagation of the white shrimp, *Penaeus setiferus* (Linn.), in captivity. Tulane Stud. Zool. 4:175-190.

Kruse, D. N. 1959. A study of the taxonomy, morphology, incidence and biology of the parasites of the commercial shrimp, *Penaeus aztecus* Ives, *P. duorarum* Burkenroad, and *P. setiferus* Linnaeus. M.S. Thesis, Fla. State Univ.

Lightner, D. V., and C. T. Fontaine. 1973. A new fungus disease of the white shrimp *Penaeus setiferus*. J. Invertebr. Pathol. 22:94-99.

Lightner, D. V., and D. H. Lewis. 1975. A septicemic bacterial disease syndrome of penaeid shrimp. Symp.: Dis. Crustacea, Mar. Fish. Rev. 37(5):25-28.

Lindner, M. J., and H. L. Cook. 1971. Progress in shrimp mariculture in the United States. FAO (Food Agric. Organ. U.N.) Fish. Rep. 71:157-162.

Mock, C. R., and M. A. Murphy. 1970. Techniques for raising penaeid shrimp from the egg to post-larvae. Proc. World Mariculture Soc., First Annu. Workshop., p. 143-156.

Neal, R. A. 1969. Methods of marking shrimp. FAO (Food Agric. Organ. U.N.) Fish. Rep. 57:1149-1165.

_____. 1970. Experimentacion en cultivo del camaron. Organizacion de las Naciones Unidas para la Agricultura y la Alimentacion, 14 p.

_____. 1971. Shrimp culture research at the Galveston Biological Lab. Am. Fish. Farmer 2(6):10-11, 18-20.

Rigdon, R. H., and K. N. Baxter. 1970. Spontaneous necroses in muscles of brown shrimp, *Penaeus aztecus* Ives. Trans. Am. Fish. Soc. 99:583-587.

Salt, G. 1970. The cellular defense reactions of insects. Camb. Monogr. Exp. Biol. 16, 118 p.

Sindermann, C. J. 1971. Internal defenses of Crustacea: A review. Fish. Bull., U.S. 69:455-489.

Sparks, A. K. 1972. Reaction to injury and wound repair. *In* Invertebrate pathology: Noncommunicable diseases, p. 20-133. Academic Press, N.Y.

Sparks, A. K., and C. T. Fontaine. 1973. Host response in the white shrimp, *Penaeus setiferus*, to infection by the larval trypanorhynchid cestode, *Prochristianella penaei*. J. Invertebr. Pathol. 22:213-219.

Sparks, A. K., and D. V. Lightner. 1973. A tumorlike papilliform growth in the brown shrimp (*Penaeus aztecus*). J. Invertebr. Pathol. 22:203-212.

Sprague, V. 1950. Notes on three microsporidian parasites of decapod crustacea of Louisiana

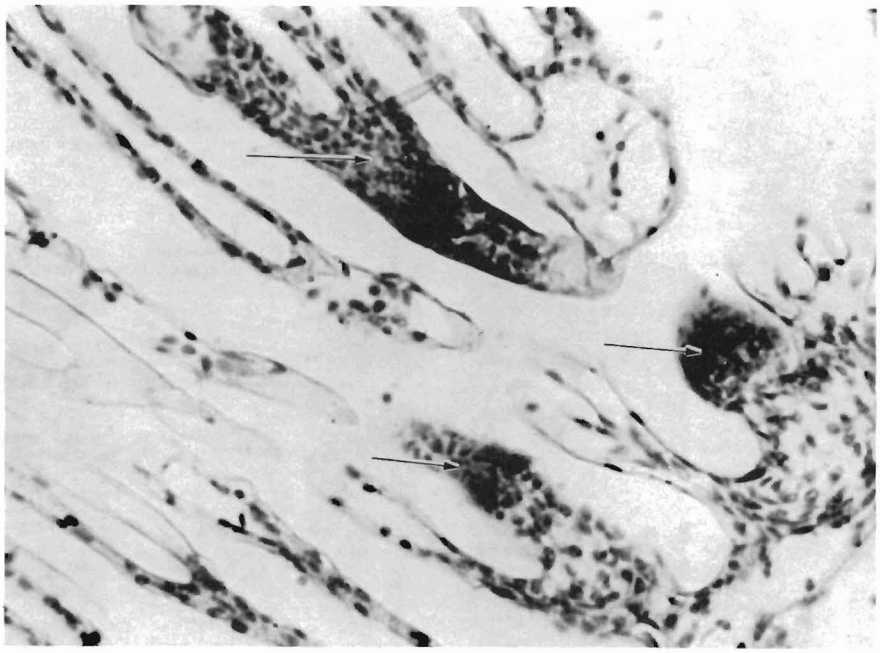

Figure 12.—The cellular response in gills of white shrimp 240 hr after injection of turpentine. These melanized spots or nodules (arrows) are a clinical sign of disease or injury in the penaeid shrimp. From Fontaine et al., in press. Hematoxylin and eosin, 250×.

coastal waters. La. State Univ. Mar. Lab., Occas. Pap. 5:1-8.

———. 1966. Two new species of *Plistophora* (Microsporida, Nosematidae) in decapods, with particular reference to one in the blue crab. J. Protozool. 13:196-199.

Unestam, T., and J.-E. Nylund. 1972. Blood reactions in vitro in crayfish against a fungal parasite, *Aphanomyces astaci*. J. Invertebr. Pathol. 19:94-106.

Wheeler, R. S. 1967. Experimental rearing of postlarval brown shrimp to marketable size in ponds. Commer. Fish. Rev. 29(3):49-52.

———. 1968. Culture of penaeid shrimp in brackish-water ponds. 1966-67. Southeast. Assoc. Game Fish Comm., Proc. 22nd Annu. Conf., p. 387-391.

Wigglesworth, V. P. 1970. The pericardial cells of insects: Analogue of the reticuloendothelial system. J. Reticuloendothel. Soc. 7:208-216.

Zein-Eldin, Z. P. 1963. Effect of salinity on growth of postlarval penaeid shrimp. Biol. Bull. (Woods Hole) 125:188-196.

Zein-Eldin, Z. P., and D. V. Aldrich. 1965. Growth and survival of postlarval Penaeus aztecus under controlled conditions of temperature and salinity. Biol. Bull. (Woods Hole) 129: 199-216.

Zein-Eldin, Z. P., and G. W. Griffith. 1966. The effect of temperature upon the growth of laboratory-held postlarval Penaeus aztecus. Biol. Bull. (Woods Hole) 131:186-196.

———. 1967. An appraisal of the effects of salinity and temperature on growth and survival of postlarval penaeids. FAO (Food Agric. Organ. U.N.) World Sci. Conf. Biol. Cult. Shrimps Prawns, 12-24 June, 1967, Mexico City, Mexico.

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