Defense Functions of Granulocytes in the Ridgeback Prawn
*Sicyonia ingentis*

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Classification of crustacean hemocytes has usually been based on subtle morphological features. We use morphological, cytochemical, and functional criteria to classify these cells in the ridgeback prawn, *Sicyonia ingentis*. Two major categories of hemocytes are found, hyaline cells and granulocytes. We previously demonstrated that only hyaline cells initiate coagulation and that two types of granulocytes may be distinguished using morphology and cytochemistry. This study shows that only granulocytes are involved in in vitro phagocytosis and encapsulation of foreign materials. Phagocytosis of the Gram-negative marine bacterium (*Cytodophaga* sp.) was accomplished primarily by small granule hemocytes, rarely by large granule hemocytes, and never by hyaline cells. Phagocytosis was enhanced by prior opsonization of bacteria with cell-free shrimp hemolymph. These results support previous cytochemical observations in which lysosomal enzymes are more abundant in small granule hemocytes than in large granule hemocytes and absent in hyaline cells. In addition, both large and small granule hemocytes, but not hyaline cells, attach to and establish capsules around hyphae of the fungus *Fusarium solani*. This role for granulocytes is supported by our demonstration of prophenoloxidase in these cells. The results of this study allow us to present a classification of shrimp hemocytes combining morphological, cytochemical, and functional criteria.

**KEY WORDS:** *Sicyonia ingentis*; Crustacea; penaeid shrimp; hemocytes; phagocytosis; *Cytodophaga*; encapsulation; *Fusarium solani*.

**INTRODUCTION**

Classification of crustacean hemocytes has been complicated by the abundance of schemes which are based primarily on subtle morphological criteria (see Bauchau, 1981; Martin and Graves, 1985, for review of early classification schemes). In view of the growing interest in hemocyte functions, it is essential to develop a unified classification of crustacean hemocytes which links cell morphology with physiology.

Using a penaeid shrimp (the ridgeback prawn, *Sicyonia ingentis*) as a model for decapod crustaceans, a hemocyte classification scheme has been developed. Based on morphological (Martin and Graves, 1985; Martin et al., 1987) and cytochemical (Hose et al., 1987) criteria, two major categories of hemocytes have been identified. Hyaline cells comprise 50 to 60% of the circulating hemocytes in the shrimp and have from zero to five granules. Shrimp hyaline cells have prominent round, electron-dense deposits filling the cytoplasm. Most hyaline cells contain a small number of distinctive striated granules. The agranular hyaline hemocytes are rare (1 to 10% of the total hemocyte count) and presumably represent immature hyaline cells. Hyaline cells are very fragile and their lysis initiates clotting (Omori et al., 1989). The second major hemocyte category, granulocytes, can also be subdivided into two groups. Granulocytes contain predominantly small (0.4 μm diameter) or large (0.8 μm diameter) granules. These respectively comprise about 30 and 10% of the total number of circulating hemocytes. Neither of the granulocyte types shows significant changes during clotting (Omori et al., 1989).

The purpose of this report is to describe the involvement of hemocytes in phagocytosis and encapsulation of foreign material. This information supports our previous classification of shrimp hemocytes (Hose et al., 1987; Martin et al., 1987) and strength-
ens the functional basis for segregating shrimp hemocytes into two major categories. This research should also provide a foundation for comparative studies on crustacean hemocyte morphology, physiology, and pathology.

MATERIALS AND METHODS

Animals

Shrimp were collected in 80 fathoms of water off the coast of Palos Verdes, California, and maintained in flow-through seawater aquaria at 18°C. Hemolymph was withdrawn from the heart using a 26-G needle attached to a 1 cc syringe.

Bacterial Cultures and Opsonization

The bacterium, *Cytophaga* sp. Isolate Occidental College 1, was selected because of its natural occurrence in seawater, its large size (0.8 μm × 3–4 μm), and its cylindrical shape which allows it to be easily distinguished from the round cytoplasmic granules. The bacterial isolate used in these studies was cultured from the Redondo Marine Laboratory seawater system. Bacteria were grown in marine broth (Difco) for two weeks at room temperature and cell concentrations in stock solutions were determined using a spectrophotometer at 590 nm. Approximately 100,000 bacteria were pelleted by centrifugation (8000g for 1 min) and resuspended in *Sicyonia* culture medium (SCM, see Brody and Chang, 1988) or opsonized in cell-free hemolymph at 6°C for 30 min before addition to the hemocyte cultures. To prepare the opsonization medium, hemolymph (0.4 ml) was rapidly withdrawn, placed into a microfuge tube, and the cells pelleted by centrifugation (8000g for 1 min). The supernatant was stored at 6°C for 5 min and was then inoculated with bacteria.

In Vitro Phagocytosis Experiments

Glass microscope coverslips were placed in the bottom of sterile plastic Petri dishes and covered with 20 ml of SCM. Approximately 1 ml of hemolymph, collected from two to three shrimp, was added to the dishes. The hemocytes were allowed 15 min to settle and attach onto the substrate. Cultures were then inoculated with bacteria (with or without prior opsonization) and incubated at 12°C. After 30 min, 1, 2, or 3 hr, coverslips were removed and examined using phase contrast microscopy. To determine the relative rates of phagocytosis, differential counts of approximately 200 hemocytes were performed and numbers of phagocytic cells (hemocytes containing bacteria within vacuoles) were recorded. Cells were considered dead if nuclear degeneration (pycnosis, karyolysis, or karyorrhexis) was present.

Hemocytes and bacteria were detached from additional slides with a solution containing 0.02% EDTA and 0.05% trypsin in SCM. The detached cells were pelleted (1 min at 8000g) and fixed for examination by transmission electron microscopy (TEM) according to the procedure of Martin et al. (1987). Thick sections (0.5 μm) were stained with methylene blue for LM and thin sections (900 Å) with lead citrate for viewing with a Hitachi HU11A TEM.

Demonstration of Acid Phosphatase Reaction Sites

Monolayers of hemocytes grown on coverslips in SCM were fixed and washed as previously described (Hose et al., 1987). The cells were incubated for 30 min or 2 hr at 37°C in 0.45 μm of filtered Gomori’s solution, pH 5.0 (Hose et al., 1987). The cells were then washed for 10 min in cacodylate buffer and treated for 2 min in a 22.5% solution of ammonium sulfide to visualize the acid phosphatase reaction sites. Hemocytes were examined using LM.

Fungus Encapsulation Experiments

*Fusarium solani* (University of Arizona strain 1623C) was cultured on Sabouraud–dextrose agar. Portions of the culture containing primarily hyphae were cut into 0.5-mm cubes and placed in sterile 15-ml plastic
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centrifuge tubes containing 12 ml of SCM. Approximately 1 ml of hemolymph was added to each tube and cultures were incubated at 12°C. To keep the hemocytes in suspension, the tubes were placed on a rotary mixer (0.5 rpm). To determine the types of hemocytes attaching to the fungus, cell counts were made using phase contrast microscopy following a 1- to 5-min incubation. After this time, hemocyte clumping precluded identification of hemocytes attaching directly to the fungus.

RESULTS

Identification of Hemocytes

Examination of fixed hemolymph reveals two major types of hemocytes, hyaline cells and granulocytes, the latter comprised of small and large granule hemocytes (Figs. 1-3). Hyaline hemocytes are relatively small (4 μm × 6 μm), are agranular or contain few granules with a striated substructure, and have a high nucleocytoplasmic ratio. The cytoplasm is filled with small (50 nm diameter), round, electron-dense deposits as well as some rough endoplasmic reticulum (RER), free ribosomes, and mitochondria. Granulocytes typically contain numerous electron-dense granules with a homogeneous substructure; granule diameters are approximately 0.4 μm in the small granule hemocytes compared to roughly 0.8 μm in the large granule cells. Whereas the granules of small granule hemocytes are dark, those of the large granule cell are refractile and cause the entire cell to appear refractile using phase contrast microscopy. Granulocytes lack the distinctive cytoplasmic deposits of the hyaline cells; instead their cytoplasm contains rough and smooth endoplasmic reticulum, mitochondria, free ribosomes, vesicles, and several Golgi bodies.

In Vitro Phagocytosis Experiments

Phagocytosis of bacteria. Approximately 40% of the hemocytes were dead after a 30-min incubation with bacteria. These were hyaline cells typically enmeshed in strands of clot material. Often they were attached to a single small granule hemocyte or a clump of granulocytes. The granulocytes, in contrast, adhered to and spread on the glass. In the culture series without opsonized bacteria, 31.6% of the small granule hemocytes and 6.2% of the large granule hemocytes had phagocytized at least one bacterium after 30 min of incubation (Table 1). This percentage increased to 77.3% of the small granule hemocytes by 3 hr; obvious large granule hemocytes were rarely observed at this time due to extensive degranulation. The overall percentage of phagocytosis was 63% (No. of phagocytic cells/total No. of viable cells).

Prior opsonization of bacteria increased the percentage of hemocytes ingesting bacteria (Table 1). At 30 min, 28% of viable cells were phagocytic, identical to the percentage observed in the cultures without opsonization. Of the small granule hemocytes, 31.1% were phagocytic; no bacteria were observed in large granule hemocytes. Phagocytosis rates reached a plateau by 2 hr with 89–91% of viable cells ingesting bacteria. As in the nonopsonized treatment, small granule hemocytes were actively phagocytic and large granule hemocytes difficult to identify because of extensive degranulation. Maximal granulocyte death (23–24%) occurred by 2 hr compared to 3 hr for nonopsonized cultures.

Morphology of hemocytes in culture. By 1 hr, both the small and large granule hemocytes formed numerous filopodia and they had undergone noticeable granule exocytosis. Empty vacuoles were present near the cell membrane. By 2 hr, few granules remained in the large granule cells and the cytoplasm was highly vacuolated. Many granulocytes appeared dead. At 3 hr, the percentage of dead granulocytes increased and the clumps of viable granulocytes were linked by clot material. In the control culture, however, good cell viability was maintained in the absence of bacteria. Only 20% of the deposit cells had lysed by 3 hr and all granulocytes appeared viable. Granule loss was accelerated in the opsonized cultures;
TABLE 1
Phagocytosis Rates of Prawn (Sicyonia ingentis) Hemocytes Cultured with Bacteria (Cytophaga sp.)

<table>
<thead>
<tr>
<th>Time</th>
<th>Opsonization</th>
<th>% Phagocytosis</th>
<th>% Dead cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Hyaline cells</td>
<td>Small granule cells</td>
</tr>
<tr>
<td>30 min</td>
<td>-</td>
<td>0.0</td>
<td>31.6</td>
</tr>
<tr>
<td></td>
<td>+ &quot;</td>
<td>0.0</td>
<td>31.1</td>
</tr>
<tr>
<td>1 hr</td>
<td>-</td>
<td>0.0</td>
<td>18.2</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>0.0</td>
<td>60.0</td>
</tr>
<tr>
<td>2 hr</td>
<td>-</td>
<td>0.0</td>
<td>48.1</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>0.0</td>
<td>92.6</td>
</tr>
<tr>
<td>3 hr</td>
<td>-</td>
<td>0.0</td>
<td>77.3</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>0.0</td>
<td>91.4</td>
</tr>
</tbody>
</table>

* Hemocytes were opsonized in cell-free hemolymph at 6°C for 30 min before addition to the cultures.

Free granules were observed at 1 hr. By 2 hr, intact granules were attached to bacteria and clumps of granulocytes were linked by clot material.

Electron microscopy of phagocytosis. Initially, bacteria attached to the surface of the hemocytes (Fig. 4). A clear zone separated the plasma membranes of the bacterium and the granulocyte and probably represents the mucoid capsule of the bacterium. The bacterium was then internalized within a phagocytic vacuole (Fig. 4). The phagosomes may remain separate or fuse with adjacent phagosomes to form larger vacuoles containing several bacteria (Fig. 5). In either case, small vesicles, presumably lysosomes, fused with the phagosome (Fig. 6). LM demonstrated that after 30 min, the phagolysosome stained positive for acid phosphatase. Figure 7 shows a phagosome where vesicles larger than the typical lysosome and similar in size to a small granule appear to have fused with the phagosome and released their contents into the phagosome. Following fusion of vesicles with the phagosome, the bacteria showed signs of degradation. The cytoplasm of the bacteria became electron lucent, their outlines were no longer circular but irregular, and finally all distinctive features of the bacteria were lost. During degradation of the bacteria, the granulocytes also showed signs of degeneration; their cytoplasm became electron lucent, mitochondria and RER dilated, and much of the chromatin condensed (Fig. 8).
Large granule hemocytes rarely contained bacteria. The main changes exhibited by these cells in culture were spreading and granule loss.

**Encapsulation experiments.** When small pieces of fungus were added to hemocytes suspended in culture medium, small and large granulocytes, but rarely hyaline cells, immediately attached to the fungal hyphae at apparently random sites (Fig. 9). Roughly two-thirds of the adherent hemocytes were small granule cells (66.3%), about one-third were large granule cells (31.3%), and deposit cells represented only 2.1% of the total. Compared to their proportions in the circulating hemolymph of this individual (10% large granule hemocytes, 35% small granule hemocytes, and 55% hyaline cells), it appears that large granule hemocytes preferentially attached to the hyphae. Subsequently, granulocytes preferentially bound to attached granulocytes rather than fungal hyphae, producing capsules (Fig. 10) that prevented clear identification of the cell types involved using LM of wet mounts. Sections through these capsules showed that the granulocytes attached to the fungal hyphae and spread around the hyphae (Fig. 11). Since most of the encapsulating granulocytes contained fewer granules than did circulating granulocytes, we speculate that the granules had undergone exocytosis. Although degranulation prevented differentiation between small and large granule hemocytes, the hyaline cells were readily identified by their cytoplasmic deposits and were rarely included in the developing capsules.

**DISCUSSION**

The goal of our research has been to establish a classification scheme for hemocytes of the Crustacea. Using information on shrimp hemocyte morphology, cytochemistry, and physiology, we define a set of criteria which will form the basis of a crustacean hemocyte classification scheme. Previous attempts, based primarily on cell morphology and staining at the light microscopical level, have divided hemocytes into three categories: granulocytes, semigranulocytes, and hyaline cells. Whereas this scheme works well for some species, difficulties arise when comparing cell types in different crustaceans. For example, in some species, the hyaline cells possess distinctive granules (Bodammer, 1978; Martin et al., 1987). In others, however, morphological differences between the categories are not always clear. In addition, there is no way to distinguish maturation stages and the stains often used (such as Giemsa) do not provide information on cell function. As studies on crustacean hemocyte functions become more sophisticated, it is essential to correlate information on physiology with cell morphology.

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**Fig. 4.** Transmission electron micrograph of a small granule hemocyte after 3 hr in culture. Only one granule (G) is visible in the cytoplasm. Two bacteria (B) surrounded by an electron lucent gelatinous capsule are attached to the highly convoluted plasma membrane of the cell. Note the nucleus (N) and the area (ES) which is an extracellular area between two cytoplasmic extensions. ×22,500.

**Fig. 5.** Transmission electron micrograph showing single or multiple bacteria within phagosomes in the cytoplasm of a small granule hemocyte after 3 hr in culture. ×18,500.

**Fig. 6.** Transmission electron micrograph of a single bacterium within a phagosome after 3 hr in culture. Note the nucleus (N), phagosome (P), and small vesicles (arrows) that appear to fuse with the phagosome. ×37,500.

**Fig. 7.** Transmission electron micrograph of a bacterium within a phagosome (P) after 3 hr in culture. Note the two sites (arrows) where something larger than vesicles, possibly granules, have fused with the phagosome and released a flocculent material. ×21,500.

**Fig. 8.** Transmission electron micrograph of a degenerating small granule hemocyte containing a phagosome (P) with a single bacterium (B) after 3 hr in culture. Note the vesiculation and loss of electron density of the cytoplasm, and the condensation of the chromatin (C). ×17,500.
and to incorporate this knowledge into a hemocyte classification scheme.

Using morphological and cytochemical features, we divided shrimp hemocytes into two major categories: deposit cells and granulocytes (Hose et al., 1987; Martin et al., 1987). Current efforts are directed toward defining specific functions for these categories. We recently demonstrated that deposit-containing hyaline cells alone initiate coagulation in this species (Omori et al., 1989). The evidence presented here demonstrates that shrimp granulocytes are responsible for defense against invasive microorganisms by phagocytosis and encapsulation.

Phagocytosis in shrimp is accomplished by the granulocytes, primarily the small granule hemocytes. These cells are distinguished by the presence of numerous small granules, some of which contain lysosomal enzymes. In addition, these cells contain numerous cytoplasmic vesicles that contain acid phosphatase, esterases, and β-glucuronidase (Hose et al., 1987), the hydrolases that apparently degrade the microorganisms within the phagosome. Hemocyte cultures incubated with bacteria and subsequently stained for acid phosphatase show that the vesicles and possibly the granules fuse with the phagosome. Large granule hemocytes, which less frequently phagocytose bacteria, contain less acid phosphatase than the small granule hemocytes but equivalent amounts of β-glucuronidase and esterase. Hyaline cells, which rarely have lysosomes (Hose et al., 1987), never exhibited phagosomes.

Within 1 hr of culture, both large and small granulocytes begin to degranulate and by 3 hr most cells were devoid of granules. Some degranulation was observed in the control culture; however, enhanced granule loss was observed in both small and large granule hemocytes in response to the presence of a Gram-negative bacterium. Söderhäll et al. (1986) also reported bacteria-induced degranulation in separated populations of semigranular and granular hemocytes of the crayfish (Pacifastacus leniusculus) and in granular hemocytes of the crab (Carcinus maenas). Both of these types of hemocyte contain prophenoloxidase (PPO). The loss of granules from shrimp small granule hemocytes apparently results from their fusion with the phagosome and the hydration of their contents. Granules in the PPO-containing large granule hemocytes do not appear to fuse with phagosomes but are readily exocytosed. The release of PPO granules is thought to be the initial event in recognition of the nonself particle and activation of phagocytes (Crossley, 1979; Ratcliffe and Rowley, 1979; Söderhäll et al., 1986).

Maximal rates of phagocytosis were significantly higher in this study (93% after 3 hr) than those previously reported. Published rates typically range from a few percentage to 20% (Paterson and Stewart, 1974; Smith and Ratcliffe, 1978; Goldenberg et al., 1984; Söderhäll et al., 1986). Possible reasons for the observed elevation in the rate of phagocytosis include (1) the use of a different species and a specific shrimp culture medium, (2) incubation at

**Fig. 9.** Light micrograph of small granule (S) and large granule (L) hemocytes attached to fungal hyphae (H) after 2 min of incubation. Even at this magnification, the refractile nature of the large granule hemocyte is obvious. Although the cellular features distinguishing small granule hemocytes from hyaline hemocytes are not apparent at this magnification, the pictured small granule cell is approximately the same size as the large granule hemocyte and thus is significantly larger than a hyaline cell. ×1800.

**Fig. 10.** Light micrograph of a cluster of small and large granule hemocytes, attached to fungal hyphae after 5 min of incubation. ×400.

**Fig. 11.** Transmission electron micrograph showing two fungal hyphae (H) surrounded by extensions of granulocytes after 5 min of incubation. Note the nuclei (N) and granules (G) of the cells. ×19,000.
12°C rather than at 20°C, (3) selective loss of highly labile (but nonphagocytic) hyaline cells in the presence of bacteria, and (4) opsonization in cold cell-free hemolymph rather than room-temperature serum. The only studies achieving higher rates of phagocytosis are those of Vandewalker (1974) using Homarus americanus and Tyson et al. (1974) using the freshwater crayfish, Paracharaeps bicornatus. However, neither study clearly identified the type(s) of hemocytes capable of phagocytosis.

Opsonization of bacteria with cell-free hemolymph significantly increased maximal phagocytosis rates in small granule cells to 93% (compared to 77% without prior opsonization). Oposonic factors in crustaceans are thought to be either products of PPO activation (Smith and Söderhäll, 1983; Söderhäll et al., 1986), or agglutinins, specifically lectins (Paterson and Stewart, 1974; Tyson et al., 1974). Two lectin opsonins have been purified from the lobster (Hall and Rowlands, 1974a, b) and Hartman et al. (1978) have isolated lectins which show different sugar specificities and opsonic properties. Lectin-like agglutinins isolated from a mollusk (Mytilus edulis) demonstrate opsonic properties in vitro (Renwantz and Stahmer, 1983). In contrast, other studies with insects have not shown an enhancement of phagocytosis following incubation with agglutinins (Scott, 1971; Rowley and Ratcliffe, 1980).

Recent studies have focused on the possible opsonic properties of the PPO system. Phenoloxidase itself does not seem to be an opsonin but an intermediate in its formation may be opsonic (Söderhäll et al., 1986). Smith and Söderhäll (1983) observed that phagocytosis of bacteria by hemocyte monolayers was enhanced in the presence of β-1,3-glucan, a PPO activator. However, a similar set of experiments with insect hemocytes showed that while β-1,3-glucan and endotoxin stimulated phagocytosis, only the glucan caused an increase in PPO activity (Ratcliffe et al., 1985; Leonard et al., 1985). An intriguing theory of hemocyte cooperation in phagocytosis has been proposed for insects (Ratcliffe et al., 1984) and more recently for crustaceans (Söderhäll et al., 1986). The latter group suggests that bacterial endotoxin or β-1,3-glucan stimulates labile granulocytes to release PPO granules into the plasma. The intact granules become activated and coat the microorganism, enhancing uptake by phagocytes (hyaline cells in their crayfish and crab systems). We observed attachment of intact granules to bacterial clumps only after a 3-hr incubation when many phagocytes had lysed. Clearly, more information on opsonins and the defensive role of the PPO system is necessary to understand the initiation and modulation of phagocytosis in decapods.

Our phagocytosis experiments also provide further information on the hyaline cells of shrimp which initiate coagulation of the hemolymph. These cells remained viable in culture under bacteria-free conditions, but in the presence of Gram-negative bacteria, all of the hyaline cells lysed within 30 min. This suggests that bacterial products may trigger lysis of these cells. The role of bacterial endotoxins in this response is controversial as it clearly initiates coagulation in some arthropods whereas in others there is no obvious effect (Bang, 1983). In Limulus, bacterial endotoxin has been shown to activate procoagulant enzyme, thereby catalyzing the cleavage of coagulogen to coagulin and the subsequent formation of a clot; 1,3-β-D-glucans can also mediate the coagulation cascade (Durliat, 1985). Previous experiments with shrimp demonstrated that coagulation of shrimp hemolymph may occur under endotoxin-free conditions (Omori et al., 1989), but this does not preclude a role for endotoxin in initiating clotting. In the shrimp, hyaline cells lyse upon contact with seawater, forming circular areas of clotted hemolymph. Bacteria, along with granulocytes, are forced into areas between adjacent expanding clots, thus increasing their chances of being phagocytosed.

The second set of experiments presented
in this paper demonstrates that both small and large granule hemocytes attach to fungal hyphae and initiate encapsulation. Hemocytes attached at apparently random spots along the hyphae and once initial adhesion had been made, other granulocytes quickly attached to form a cluster. Unestam and Nylund (1972) described the same process in greater detail using crayfish hemocytes and the fungal parasite *Aphanomyces astaci* in a simulated "blood stream" under a microscope coverslip. Although granular hemocytes quickly bound to the hyphae, it is not clear if all types of cells were involved. Unestam and Nylund (1972) also discuss the "explosion" of the granular hemocytes and suggest that this process may be involved in a localized coagulation that binds hemocytes to the fungus. We have also observed a rapid degranulation in large granule hemocytes of the shrimp after attaching to fungal hyphae and consider it to be the release of PPO as suggested by Söderhäll et al. (1984) and also of lysosomal enzymes. It is important to distinguish this "explosion" from the explosive lysis of the hyaline cells in the shrimp. Hyaline cells in the shrimp initiate coagulation of the hemolymph; however, they rarely attached to the fungal hyphae nor were they present in the *in vivo* capsules described in another penacid shrimp experimentally infected with *F. solani* (Hose et al., 1984).

Summarizing the data on shrimp hemocytes, three types of circulating cells can be recognized. Hyaline cells are so named because their cytoplasm is filled with dense particles that stain with Sudan Black B. They lyse on exposure to seawater, initiating coagulation of the surrounding hemolymph. Small granule hemocytes are larger than hyaline cells and contain numerous small granules in the cytoplasm. Lysosomal enzymes and PPO are present in the cytoplasm and these cells are the primary phagocytic hemocytes in the shrimp. Small granule hemocytes are also involved in recognition and encapsulation of fungal hyphae. The final category of hemocytes, large granule hemocytes, appears highly refractile using phase contrast microscopy with large granules filling the entire cytoplasm. Large granule hemocytes contain more PPO and less acid phosphatase than do small granule hemocytes. They are less phagocytic than small granule cells but actively involved in fungal encapsulation. This combination of morphology, cytochemistry, and functional studies effectively distinguishes between the main types of hemocytes in the shrimp. It is hoped that this approach will serve as a basis for a unified classification scheme for decapod and possibly all crustacean hemocytes.

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