Reexamination of Hemocytes in Brine Shrimp
(Crustacea, Branchiopoda)

GARY G. MARTIN,* HAN-MING JOSEPH LIN, AND CHAU LUC
Department of Biology, Occidental College, Los Angeles, California

ABSTRACT In 1941, a single type of hemocyte was described in the blood of
the brine shrimp Artemia salina using light microscopy. This condition is unusual
because most crustaceans examined using morphological, cytochemical,
and functional methods have at least two types of hemocytes. Upon
examining A. franciscana, we found a single type of disk-shaped hemocyte,
with a centrally located nucleus and about 15 large (6 μm diameter) granules.
The granules stain for the presence of acid phosphatase and react with
L-DOPA suggesting, respectively, that they are involved in degrading ingested
material and possess the phenoloxidase system. Hemocytes require calcium
for adhesion, bind together to mend small wounds in the body wall, and are
able to phagocytose bacteria. Blood cells of A. franciscana are morphologically
and functionally similar to those of the primitive chelicerate, Limulus poly-
phemus, and both forms have apparently given rise to more advanced taxa
with multiple types of hemocytes. The major difference between the two
species is the presence of the phenoloxidase system in the Crustacea and its
© 1999 Wiley-Liss, Inc.

Key Words: Crustacea; brine shrimp; Artemia; hemocytes

In the early 1940s, Lochhead and Loch-
head (41) described a single type of blood
cell in the brine shrimp Artemia salina using
light microscopy (LM). Other researchers of that time described from one to se-
veral types of hemocytes in various crustaceans (see Martin and Graves, ’85, for
review). Although the classification of hemo-
cytes remains a controversial issue (see
Barnes and Harrison, ’92), recent studies
contributing information on cell function,
cytochemistry, and ultrastructure suggest
that there are two major types of hemocytes,
at least in the decapods (Hose et al., ’90;
Martin and Hose, ’92). Hyaline hemocytes
have a high nucleo-cytoplasmic ratio, lyse
readily on contact with seawater to initiate
hemolymph coagulation, and show the great-
est morphological variation. In some species
granules are absent, whereas in others they
are conspicuous. Granulocytes, on the other
hand, have a low nucleo-cytoplasmic ratio,
do not lyse readily, and phagocytose and
encapsulate foreign material. In most spe-
cies, granulocytes may be subdivided into
small- and large-granule hemocytes based
on the size of the granules. The ratio of these
types of hemocytes varies among species
(Hose et al., ’90), and in some species, such
as Macrobrachium rosenbergii (Vazquez et
al., ’97) and Penaeus japonicus (Tsung et al.,
’89), undifferentiated circulating cells have
also been described.

Most studies on crustacean hemocytes
have been carried out on decapods because of
their relatively large size and importance
in aquaculture. Small- and large-granule he-
omocytes and hyaline cells also appear to be
the common pattern in other groups of malaco-
crancans, such as stomatopods (Ferrero et
al., ’89; Barracco and Amirante, ’92) and
isopods (Alikhian and Naich, ’87; Benjamin
and James, ’87). Variations, however, have
been reported, such as the nine types of
hemocytes in the isopod Ligia exotica (Ravin-
dranath, ’74). In contrast, very little is known
about the hemocytes in amphipods (Steele
and MacPherson, ’81), euphausiids, and many
groups of smaller Crustacea, such as the

*Correspondence to: Dr. Gary Martin, Department of Biology, Occidental College, Los Angeles, CA 90041. E-mail: gmartin@oxy.edu

© 1999 WILEY-LISS, INC.
Remipedia (Felgenhauer et al., '92), Cephalocarida (Hessler and Elofsson, '92), Tantulocarida, and members of the Ostracoda (Maddocks, '92). In the Cirripedia, byline cells and small-granule hemocytes occur in very low numbers (Wade and Walker, '88). Copepods appear to lack hemocytes (Boxshall, '92), and in Branchiura, Overstreet et al. ('92) make a brief reference to "smooth, spindle-shaped, or pear-shaped hemocytes," which contain small granules and polymorphic nuclei. Criel ('91) reviewed the morphology of the branchiopod genus Artemia, but added little new information to the observations of Lochhead and Lochhead ('41). The purpose of our study is to examine the hemocytes in A. franciscana, paying particular attention to properties found useful in understanding the function of hemocytes in decapods and other arthropods.

MATERIALS AND METHODS

Animal maintenance and hemocyte collection

Adult brine shrimp, Artemia franciscana (Class Branchiopoda, Subclass Sarcostraca, Order Anostraca) from San Francisco stocks were purchased at local tropical fish stores and maintained in glass bowls (20 cm diameter × 10 cm deep), containing aerated seawater (38% at 20°C) and small amounts of the green alga Isochrysis galbana as a food source. Although hemocytes are visible through the body wall of living brine shrimp, they are more readily examined when removed from the animal. To obtain hemocytes, 10–20 A. franciscana were rinsed for 10 min in distilled water (dH₂O), blotted dry, and placed on a clean glass slide in a drop (50 μl) of 3% NaCl or fixatives (described below). The animals were sliced in half at the midpoint of the abdomen. After 2 min, the bodies were removed, leaving the hemocytes for examination.

Light microscopical examination of hemocytes

Measurements using phase contrast optics were made of hemocytes in live Artemia franciscana as well as of isolated cells in 3% NaCl or fixatives (described below). The following histological and histochemical stains were performed on hemocyte spreads: hematoxylin and eosin (H&E), periodic acid-Schiff (PAS), and acid phosphatase as described in Hymanson ('67) and Hose et al. ('80). To test for the presence of L-DOPA, hemocytes bound to glass slides were fixed at room temperature in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 15 min and run through three 15-min washes in 0.1 M phosphate buffer. The slides were incubated overnight at room temperature in 0.1% L-DOPA in phosphate buffer and then examined by LM. Because the staining procedure involves transferring the cells through multiple solutions, the glass slides were pretreated with poly-L-lysine (10 mg/ml dH₂O) to decrease hemocyte loss.

Hemocyte spreads were also used for the immunolocalization of α-tubulin according to the procedure of Howard ('91). Hemocytes attached to poly-L-lysine pretreated coverslips were fixed in 4% paraformaldehyde in 0.1 M Amersham (Arlington Hts., IL) phosphat-buffered saline (PBS, pH 7.3) for 10 min. Coverslips were washed six times for 5 min each in PBS containing 0.1% Triton X-100 and 0.5% bovine serum albumin (BSA). Cells were incubated at room temperature for 1 h in 100 μl of monoclonal mouse anti-α-tubulin antibody (T-9026; Sigma, St. Louis, MO) diluted 1:1000 with PBS containing 0.5% BSA. After two 5-min washes with PBS containing 0.5% BSA, hemocytes were incubated overnight at 4°C with FITC-labeled goat antirabbit F antibody (F-9137; Sigma) diluted 1:64 in PBS with 0.5% BSA. Coverslips were finally washed six times for 5 min each in PBS before being mounted on glass slides with a glycerol medium (containing 1 mg/ml p-phenylenediamine to reduce photo-bleaching; Johnson and Aragón, '81) and examined with a Zeiss fluorescence microscope.

Electron microscopy of hemocytes

For electron microscopy, hemocytes were obtained from at least 50 Artemia franciscana as described above in one of the following fixatives: 1) standard fix (2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.8, with 12% glucose); 2) standard fix containing 0.5% tannic acid; or 3) Bell's fix (6% glutaraldehyde in 0.2 M s-collidine, pH 7.4). Hemocytes were pipetted from the glass slides into microfuge tubes where the cells were pelleted by centrifugation at 4000g for 2 min. After a 3-h fixation, hemocytes were washed and postfixed for 1 h in 1% OsO₄ in the same buffer as the fixative and stained
for 1 h in 3% uranyl acetate in 0.1 M sodium acetate buffer. The cell pellets were dehydrated through a series of increasing ethanol concentrations, after which they were infiltrated and embedded in plastic (Spurr, '69). Thick (0.5 μm) and thin (90 nm) sections were stained with methylene blue or lead citrate and examined by light (LM) or transmission electron microscopy (TEM, Zeiss EM 109), respectively.

To examine hemocytes by scanning electron microscopy (SEM), hemocytes were fixed as described above for TEM. Following dehydration, cells were soaked in hexamethyldisilazane (Polysciences, Warrington, PA) for 5 min and air dried. Samples were coated with gold and examined in a Cambridge Stereoscan 360 SEM.

Role of calcium in hemocyte adhesion

Hemocytes were collected as described above on washed glass slides in 50 μl of one of the following solutions: 1) 3% NaCl; 2) 3% NaCl with 10 mM CaCl₂; or 3) 3% NaCl with 0.1 M EDTA. Hemocytes in five randomly selected, 0.16 mm² fields were counted on an Olympus inverted microscope. After allowing the cells to settle for 10 min, the slides were dipped three times in appropriate wash solutions and the number of cells remaining in previously examined fields was counted and expressed as the percent adhesion. Each test was repeated on five slides.

Hemocyte degranulation

Three chemicals were tested for their ability to induce degranulation of Artemia franciscana hemocytes. Hemocyte samples were obtained as described above except the bodies were cut open in a drop (50 μl) of either 3% NaCl containing CaCl₂ (control) or this solution containing 1) 10 μM A23187; 2) 10 ng/ml LPS; or 3) 10 μg/ml laminarin. Hemocytes were examined at 5, 10, and 20 min for morphological changes, in particular, signs of degranulation.

Bacterial phagocytes

Overnight cultures of Bacillus subtilis (in nutrient broth at 37°C) were washed in 3% NaCl and resuspended at a final concentration of 5 × 10⁶ cells/ml. B. subtilis was used because it is relatively large and has been used in previous studies on microbial clearance by crustaceans (Martin et al., '93b, '96). Hemocytes from 20–30 Artemia franciscana were collected on clean glass slides in 3% NaCl and allowed to adhere to the substrate for 10 min. Cultures were then inoculated with 50 μl of bacterial suspension. After incubation at room temperature for 0.5, 1, 2, or 3 h, slides were fixed and examined using phase-contrast microscopy. Replica samples were fixed and examined by SEM.

Assay for phenoloxidase activity

Individual brine shrimp that had been washed in dH₂O and blotted dry were sliced in half along the rim of a microfuge tube containing 1 ml of CAC buffer (0.01 M sodium cacodylate, 5 mM CaCl₂, 50 mM MgCl₂, pH 7.0). After collecting hemolymph from 25, 50, or 100 Artemia franciscana, the hemocytes were pelleted by centrifugation and lysed with a glass rod. Cellular debris was removed by centrifugation (10,000 rpm for 2 min) and the supernatant was used in the following assay modified from Solodovnik and Smith ('83). Supernatant (100 μl), CAC buffer (100 μl), and 0.1% trypsin in CAC buffer (100 μl) were mixed and incubated for 1 h at room temperature. In the negative control, 100 μl of CAC buffer was used in place of the blood sample, while in another test the trypsin solution, which activates the phenoloxidase, was replaced with 100 μl of CAC buffer. After incubation, 100 μl of each sample was transferred to a cuvette containing 500 μl of CAC buffer and 100 μl of 0.4% L-DOPA in CAC buffer. The changes in absorbance due to the activity of phenoloxidase on the L-DOPA substrate were measured using a Beckman DU 640B spectrophotometer at 490 nm. Data were recorded as the change in absorbance per minute, taken 10 min after time zero. Total protein concentration in the samples was obtained using Sigma kit 650-A, which is based on a modification of the Lowry method.

RESULTS

Hemocyte morphology

Although hemocytes can be observed in live Artemia franciscana when lightly compressed between a coverslip and a microscope slide, they are more readily examined when removed from the body and examined by LM (Fig. 1). Hemocytes typically have a disk shape (12–14 μm in diameter), in which the center of the cells is thicker (4 μm) and tapers to a thinner (0.5 μm) circumferential rim. When viewed by SEM, the plasma membrane is normally smooth and shows the contours of underlying cytoplasmic granules.
(Fig. 2). The few short microvilli seen on the cell in Figure 2 probably formed during the short time between severing the body and fixation of the hemocyte as part of the adhesion event described below. The central part of each cell contains the nucleus and granules. The nucleus stains with hematoxylin and with TEM it appears mottled, with large amounts of heterochromatin (Fig. 3). Although the nucleo-cytoplasmic ratio is 24%, the cytoplasm is reduced to the areas between the granules and contains mitochondria, RER, Golgi bodies, and vesicles. Immunofluorescent staining with α-tubulin (Fig. 4) shows a band of microtubules in a circumferential ring around the cell. The microtubules are also seen in TEM, and appear more clearly when the fixative contains tannic acid (Fig. 5).

Hemocytes contain an average of 15 (range 9–27) granules each with an average diameter of 6 ± 1 μm. Granules are colorless with H&E, PAS negative, and show reaction sites for the presence of acid phosphatase. The
granules stain with the Sigma kit for elastin, as does the acellular lining (basal lamina) of the hemocoel. Hemocytes stained with L-DOPA contain a few granules that turn completely dark, while the majority show reactions primarily around the granule rim.

At the TEM level, most granules are homogeneous and electron-dense. In hemocytes prepared with cacodylate-buffered glutaraldehyde, many granules were partially dissolved, which we consider an artifact of inadequate fixation. All fixatives showed the surface of some granules to be smooth, whereas in most the entire membrane or part of the membrane is shown into a series of undulations (Figs. 3, 6). Sections through the periphery of granules show that these undulations are not pits but troughs and ridges over the surface of a granule (Fig. 7). Golgi-derived vesicles bind only to the ridges (Fig. 8). Microfilaments (10 nm diameter) are seen in the troughs of some granules (Fig. 9).

Hemocyte adhesion and degranulation

When Artemia franciscana is placed in a drop of water and its abdomen or thoracic appendages are severed, circulating hemocytes rapidly extend filopodia, binding to one another (Fig. 10) and to the cut surface of the body. Unbound hemocytes are released into the solution, settling and attaching to the slide. When brine shrimp are cut in a drop of 3% NaCl or 3% NaCl with extra calcium (10 mM CaCl₂), 71% and 73% of the cells, respectively, remained attached to the slide after a gentle rinse. These numbers are only slightly reduced from the percentage (81%) of cells released in 3% NaCl and binding to glass slides pretreated with poly-L-lysine to enhance cell adhesion. However, when hemocytes were released into a solution with decreased amounts of calcium (3% NaCl containing 3.7% EDTA), only 34% of the cells remained attached to the slide after rinsing.

When hemocytes circulate within the body or are fixed immediately after removal from the body, they have a smooth plasma membrane. However, hemocytes binding to cut surfaces, each other, or glass slides immediately develop filopodia (Fig. 11). Hemocytes on glass slides gradually flatten and after 20 min approximately 6% show signs of degranulation. Hemocytes on glass slides in 3% NaCl containing the calcium ionophore A23187 show rapid extension of filopodia (Fig. 12) followed immediately by the formation of blebs on the cell surface (Fig. 13) and the release of granules. In many hemocytes, the granules fuse to form a large intracellular compartment (Fig. 14) and a single large bleb protrudes from the cell (Fig. 14). When
Fig. 6. *Artemia franciscana*. TEM of a granule (G) cut through its diameter showing the surface folded into a series of ridges (arrowheads). Scale bar = 0.6 μm.

Fig. 7. Granules (G) in the same cell as in Figure 6 but cut tangential to their surfaces showing that the undulations are actually elongated troughs and ridges (arrowheads). Note Golgi-derived vesicles (V) with electron-dense cores. GB, Golgi body. Scale bar = 0.6 μm.

Fig. 8. TEM showing vesicles (V) with dense cores, presumably from the Golgi body (GB) fusing (arrowhead) with the tips of undulations on the surface of a cytoplasmic granule (G). Scale bar = 0.5 μm.

Fig. 9. TEM showing filaments (arrowheads) in the grooves on the surface of a cytoplasmic granule (G). Scale bar = 0.25 μm.
exocytosis is complete, the cell remnant has an irregular shape and is less than half of its original size. Hemocytes incubated on glass slides with 3% NaCl containing either LPS or laminarin show the same changes as those initiated by A23187, but develop at a slower rate (Fig. 15).

**Bacterial phagocytosis**

In the degranulation experiments, a few bacteria were observed on all slides, presumably released from the severed brine shrimp, despite the 10-min wash in dH2O. To study the ability of hemocytes to phagocytose bacteria, additional bacteria (Bacillus subtilis)
were added to hemocytes released onto glass slides in a drop of 3% NaCl. SEM showed bacteria bound to the tips of filopodia (Fig. 16) and the plasma membrane (Fig. 17). Using LM, phagocytosed bacteria within cytoplasmic vacuoles could be observed after 30 min, but only in less than 5% of the hemocytes examined. After 2 h of incubation, 12% of the hemocytes had phagocytosed bacteria. Fusion of granules within hemocytes and the loss of granules makes the morphology of these cells difficult to interpret, such that the percentage of hemocytes that had phagocytosed bacteria at selected time points was not calculated.

**Phenoxidase activity**

Phenoxidase (PO) activity was determined in samples containing blood from 25, 50, and 100 *Artemia franciscana*. All blood samples showed PO activity, which was responsible for a roughly linear increase in absorbance corresponding to the doubling of brine shrimp contributing hemolymph to the samples (Table 1). Samples with and without trypsin showed little difference in PO activity, and in both cases it was well above control levels.

**DISCUSSION**

Our examination of the hemocytes disclosed a single morphological cell type in *Artemia franciscana*, and suggests that Lockhead and Lockhead ('41) were correct in their original assessment of a single cell type in *A. salina*. This condition is quite unlike that found in the higher crustaceans, where two or more types of hemocytes are present (see Martin and Huse, '92). The single type of hemocyte in *A. franciscana* generally has a disk shape, in which the nucleus and approximately 15 large granules occupy the center of the cell. A thin rim of cytoplasm extends beyond the granules in one plane and, as in other crustacean hemocytes, is supported by a circumferential border of microtubules (Cohen et al., '83). Morphologically, this cell is similar to the granulocytes found in other crustaceans, although the 6-μm diameter granules are larger than those seen in most species (see Martin and Huse, '92). The granules do not stain with hematoxylin, eosin, or PAS, suggesting that they are relatively neutral, with a negligible carbohydrate content. The granules and the acellular lining of the hemocoeol, however, do stain with the "elastin" stains, as shown in other crustaceans (Martin et al., '89). Although this material is not the same as vertebrate elastin, it does have resilient properties that may facilitate blood flow in crustaceans (Shadwick et al., '90). Cells fixed for TEM in glutaraldehyde and cacodylate showed granules missing at least some of their contents; Bell's fixative was the best at preserving granule morphology.

Many granules in *Artemia franciscana* differ from those in other crustaceans by having their surface folded into a series of troughs and ridges. Microfilaments lie in the grooves and may leave the ridges as the only sites where Golgi-derived vesicles may fuse with the forming granules. Since some granules do not show these surface contortions, we suggest that those with smooth surfaces are fully formed and those with the surface folds may be growing. However, we were unable to distinguish growing from mature granules by the nature of their contents. Studies on the interaction of the cytoskeleton and granule maturation deserve future attention.

The granules in *Artemia franciscana* hemocytes are similar to those found in the granulocytes of higher crustaceans (Hose et al., '90; Martin and Huse, '92) in that they contain acid phosphatase. The presence of this enzyme suggests a lysosome-like role in degrading phagocytosed materials. We have
shown that these cells in A. franciscana are capable of binding to and phagocytosing bacteria. Rates of in vitro phagocytosis are quite low, but previous work has shown the importance of serum molecules serving as opsonins to enhance phagocytosis (Paterson and Stewart, 1974; Goldenberg et al., 1984; Hose and Martin, 1989). In this study, the blood of A. franciscana was significantly diluted by the incubation media and no attempts were made to opsonize the bacteria in A. franciscana serum prior to phagocytosis assays. Although Lochhead and Lochhead (1941) did not describe phagocytosis by Artemia hemocytes, they considered it likely because Artemia is closely related to Daphnia, whose hemocytes are capable of phagocytosis (Metschnikoff, 1884). Instead, the Lochheads highlighted phagocytosis by a second cell type, the phagocytic storage cell, which they consider synonymous with fat cells and nephrocytes. These star-shaped cells are con-

### TABLE 1. Phenoloxidase activity in the hemolymph of Artemia franciscana

<table>
<thead>
<tr>
<th>Number of individuals bled</th>
<th>With trypsin activation</th>
<th>Without trypsin</th>
<th>Control (no hemolymph)</th>
<th>Protein concentration (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>0.99 ± 0.01</td>
<td>1.19 ± 0.1</td>
<td>0.05 ± 0.04</td>
<td>0.52</td>
</tr>
<tr>
<td>50</td>
<td>1.97 ± 0.3</td>
<td>2.08 ± 0.6</td>
<td>0.09 ± 0.02</td>
<td>0.96</td>
</tr>
<tr>
<td>100</td>
<td>4.96 ± 0.7</td>
<td>5.45 ± 0.9</td>
<td>0.09 ± 0.05</td>
<td>1.61</td>
</tr>
</tbody>
</table>

*Phenoloxidase activities were determined spectrophotometrically at 490 nm using DOPA as a substrate. Units are defined as the change in absorbance min⁻¹ and are presented as average ± standard deviations from five replicate assays.

*Hemolymph samples were incubated with trypsin to activate prophenoloxidase.

*Hemolymph samples were incubated without trypsin.
siderably larger than hemocytes, are located in the trunk and appendages, take up dyes and India ink, and their large inclusions stain with Sudan black, indicating the presence of fat. The Lochheads observed intact, yet presumably old, hemocytes within these phagocytic storage cells. Although it is unusual for this type of cell to ingest large materials, Tyson ('75) described a spirochete infection in A. salina where the bacteria were found within several cell types, including amoebocytes (i.e., the phagocytic storage cells of the Lochheads). However, because the bacteria were seen in a variety of cell types, their presence may represent a pathological condition rather than effective clearance by specific cells. The Lochheads viewed the hemocytes as being primarily involved with the transport of nutrients to the ovaries, the removal of wastes, and the mechanical sealing of breaks in the body wall. Although the role of the phagocytic storage cells in the phagocytosis of bacteria requires further attention, these cells seem more likely to be involved with ultrafiltration, leaving the hemocytes to ingest the larger particulate materials. Work on the decapods (see Johnson, '87; Factor and Beckman, '90) suggests a separation of functions between cells involved with pinocytosis (branchial podocytes, nephrocytes) and phagocytosis (hemocytes, reserve cells, and fixed phagocytes). Further studies are needed to understand how specialized crustacean cells remove various sized foreign particles from circulation, particularly in the lower crustaceans.

The granules in Artemia franciscana hemocytes also stain with L-DOPA, indicating that they contain prophenoloxidase (PPO). In the decapods, this enzyme is converted into the active phenoloxidase (PO) form by foreign materials, such as β-1,3 glucans and bacterial endotoxins. The activated enzyme sticks to foreign materials, triggering hemocyte attachment, phagocytosis, and encapsulation of foreign particles (Johansson and Söderhäll, '92). Spectrophotometric assays have also demonstrated the presence of PO in A. franciscana blood. Although the small volume of blood we collected precluded direct comparison of PO activity with other crustaceans, PO is present and its activity was roughly proportional to the increase in brine shrimp number used to contribute the hemolymph. Activation of PPO occurred in our assays with and without the presence of trypsin, which is typically used to activate the enzyme in these in vitro assays. Since hemolymph was obtained by slicing the abdomens of A. franciscana and letting it drip into the reaction vials, it is likely that sufficient bacterial products from the gut and/or surface of Artemia also entered our reaction vials to activate the PPO. The activation of PO in turn leads to degranulation of hemocytes, with the subsequent release of more PPO to be activated (Söderhäll and Smith, '86; Liang et al., '92; Söderhäll and Cerenius, '92).

Since the presence of EDTA reduced cell adhesion, hemocytes of Artemia franciscana apparently require calcium in order to bind and spread on glass slides. The release of granules also requires calcium and can be induced by the calcium ionophore A23187, as well as by natural elicitors, such as bacterial endotoxin (LPS) or fungal cell wall β-1,3 glucans (laminarin). These results agree with work on decapods (see Johansson and Söderhäll, '92) and the primitive chelicerate Limulus polyphemus (Armstrong and Rickels, '82; Armstrong, '83; Söderhäll et al., '85). When hemocytes were treated with A23187, many cytoplasmic granules within the cells fused, and as individual granules or the combined mass swelled, they produced blebs on the surface of the cells. These blebs enlarged, and when the contents were released the cell remnants were very small. When hemocytes were treated with LPS or laminarin the same sequence of events occurred, although degranulation took place over a longer period of time.

Although there is some variation in cell size and in the number of granules within each cell, the hemocytes in Artemia franciscana are incapable of division, as is true for decapods. Instead, Lochhead and Lochhead ('41) described clusters of hemocytes at the base of thoracic legs as the sites of hematopoiesis. These areas are occupied by stem and immature cells that are small and lack granules. Agranular cells were not observed in circulation. Although it is not clear if the stem and maturing hemocytes in the hematopoietic tissue are enucleated by collagenous layers, the clusters appear more like the hematopoietic tissue of lobsters (Martin et al., '83a) and crayfish (Chaga et al., '95), than the lamellar tissue of crabs (Johnson, '80) or the tubular system of shrimp (Martin et al., '87).
The single type of blood cell seen in the primitive crustacean *Artemia franciscana* participates in two major internal defense functions: clotting, which mends breaks in the body wall and restricts transport of possible pathogens throughout the body, and the phagocytosis of foreign particles. Although hemocyte classification remains controversial, at least two categories of hemocytes are recognized in more advanced crustaceans, with one specialized for coagulation events and the second for phagocytosis and encapsulation. Tait ('11) described three types of coagulation in the crustaceans. At the extremes were type A, in which the hemocytes aggregate to seal wounds in the body wall, and type C, in which explosive hemocytes release cytoplasmic materials that coagulated the hemolymph to seal wounds. Hemocytes of *A. franciscana* demonstrate type A coagulation, suggesting that this strategy is the primitive condition. Hemocytes may also be involved in a variety of other functions, including hardening of the exoskeleton (Vacca and Fingerman, '83) and transfer of nutrients throughout the body (Johnson et al., '73). The trend of moving from a single type of hemocyte in a primitive crustacean to two or more types in higher crustaceans is repeated in the chelicerates. The primitive representative *Limulus polyphemus*, possesses a single type of circulating hemocyte, whereas more advanced, terrestrial species possess multiple types. Indeed, the morphology of hemocytes in *A. franciscana*, their adhesive properties, their response to bacterial and fungal products, and their ability to phagocytose bacteria are similar to those features for hemocytes of *L. polyphemus*. The major difference between these cells and the evolutionary lines they represent is the presence of the PO system, which is a centerpiece of internal defense in the crustaceans and its apparent absence in the chelicerates.

ACKNOWLEDGMENTS

We thank Katalin Juhasz for help with the histological staining and Alicia Thompson for the use of the SEM facility at the University of Southern California.

LITERATURE CITED


