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Parasitic Encapsulation in a Marine Prosobranch: The Role of Agranular Hemolymph Cells

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Cerithidea californica Haldeman, a prosobranch gastropod commonly inhabiting mudflats and estuaries along the southern California coast, serves as the first intermediate host for numerous species of larval Digenea (Maxon and Pequegnat, 1949; Martin, 1955; Yoshino, 1975). As a general rule, natural infections by these parasites do not elicit cellular reactions within their host. However, an exception to this rule has been noted in infections involving sporocysts of *Renicola buchanani*, which appear to elicit a hyalinocyte-mediated encapsulation response in *C. californica*. An ultra-structural account of host capsule formation in response to this parasite is summarized in the following presentation.

HEMOLYMPH CELLS OF *C. CALIFORNICA*

From information provided by recent ultrastructural studies on encapsulation, it appears that hemolymph cells (= leucocytes or amoebocytes) represent the primary constituents involved in capsule formation in mollusks (Rifkin, Cheng, and Hohl, 1969; Cheng and Rifkin, 1970; Sminia, Borghart-Reinders, and van de Linde, 1974; Harris, 1975). Two types of cells, the granular hemolymph cell or granulocyte (Fig. 1, 3) and the agranular hemolymph cell or hyalinocyte (Fig. 2, 4) commonly are found circulating in the hemolymph of *C. californica*. Granulocytes, which measure 9.0 to 15.0 μm in diameter, possess well-defined ectoplasmic and endoplasmic regions of the cytoplasm (Fig. 1, 3), numerous vesicles of smooth endoplasmic reticulum (SER), and pleomorphic electron-dense granules (Fig. 3). Hyalinocytes, besides being smaller in size (4.0 to 8.0 μm in diameter) and in number, are distinguished from granulocytes by a general lack of SER and dense granules (Fig. 2, 4). Little or no cytoplasmic differentiation into endo- and ectoplasmic regions is noted in these cells. Finally, the cytoplasm to nucleus ratio for granulocytes (4.95 ± 1.41, $N=17$) is significantly larger ($P < 0.001$) than that for hyalinocytes (1.11 ± 0.44, $N = 16$).

CAPSULE FORMATION IN *C. CALIFORNICA*

Since experimental infections of *R. buchanani* in *C. californica* were not possible, the progression of capsule development is based on observations of host reactions to sporocysts of varying sizes found in natural infections of this snail. The smallest observed sporocysts of *R. buchanani* (approximately 0.5 mm in length) elicit little host response in the anterior mantle region of *C. californica*, though a small number of granulocytes are present in the immediate vicinity of the parasites (Fig. 5). The presence of karyolytic nuclei and cytoplasmic debris in this area suggests that cells, including granulocytes, which contact the tegument of the parasite may be subject to lytic agents of parasite origin. Granulocytes in the vicinity of young sporocysts frequently are observed phagocytizing debris from lysed host cells.

Numerous hyalinocytes begin to infiltrate the area surrounding sporocysts which measure 1.0 to 1.5 mm in length (Fig. 6). Long, thin pseudopodial processes from encapsulating hyalinocytes extend in a direction perpendicular to the sporocyst's surface, loosely interdigitating with tegumental microvilli. Heterogeneous, electron-dense inclusions commonly develop within hyalinocytes during this stage of capsule formation. Extracellular fibrils also may be present, but are not consistently observed in each capsule.

Hyalinocytes which have aggregated around mature sporocysts (2.5 to 4.0 mm in length) have become horizontally flattened against the parasite's surface forming a compact cellular tunic four to eight cell layers thick (Fig. 7). Pseudopodial processes of hyalinocytes are discernible as they interdigitate in close contact with the sporocyst's tegumental microvilli. The rough endoplasmic reticulum (RER) in these cells is usually well developed and often possesses dilated cisternae. Electron-dense inclusions and cytoplasmic microtubules also appear more numerous in capsular hyalinocytes. Sporocysts of *R. buchanani* are not harmed as a consequence of encapsulation. A normal-appearing tegumental ultrastructure and a vigorous post-encapsulation production of viable cercariae by sporocyst stages attest to this fact.

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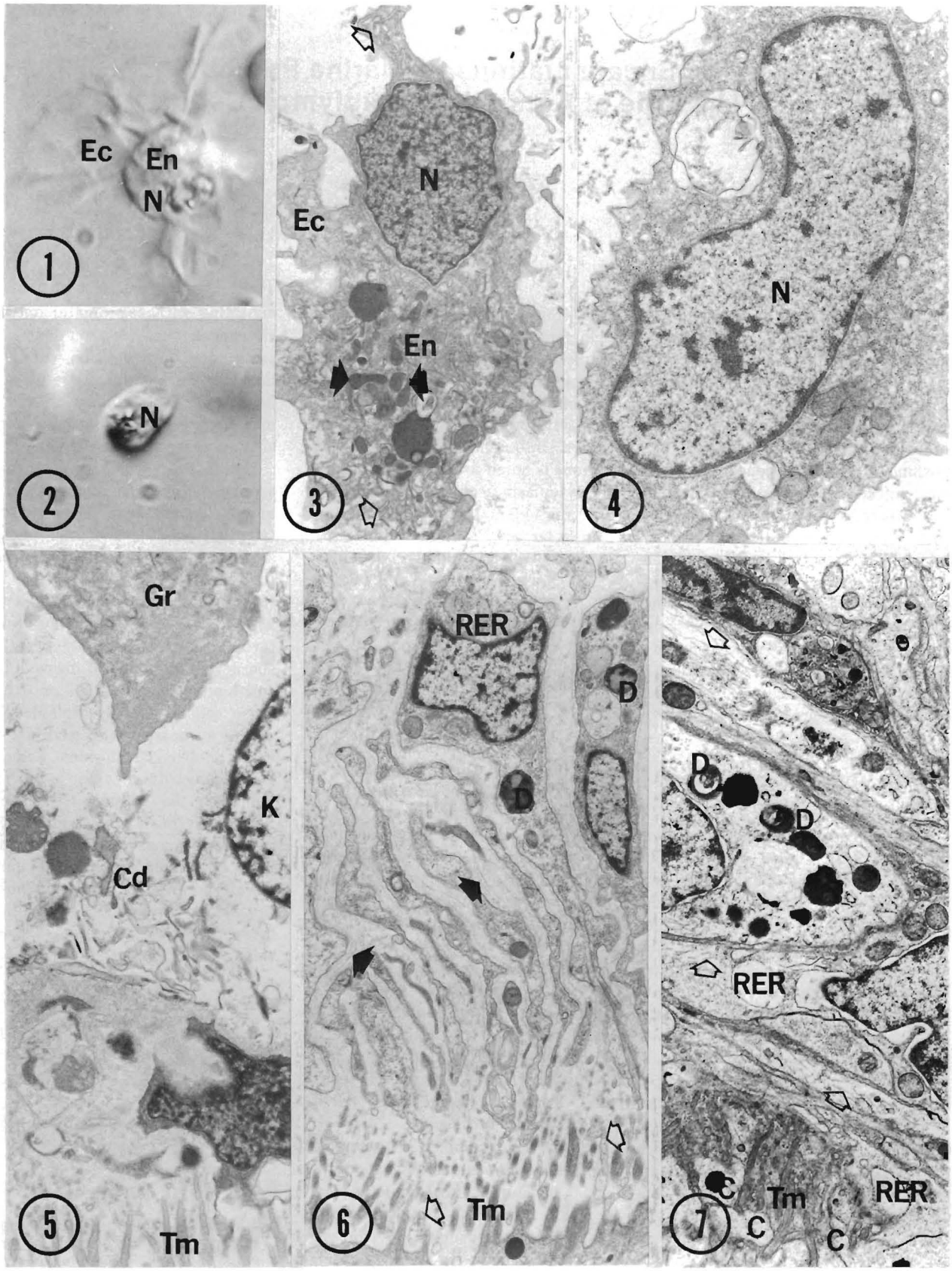
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Figure 1.—Living *C. californica* granulocyte which has been allowed to settle on a glass microscope slide. Nomarski Interference optics. 3,000×. Figure 2.—Living *C. californica* hyalinocyte which has been allowed to settle on a glass slide. Nomarski Interference optics. 3,000×. Figure 3.—Granulocyte in hemolymph circulation of *C. californica.* Note the presence of numerous vesicles of SER (hollow arrows) and electron-dense granules (solid arrows). 8,800×. Figure 4.—Hyalinocyte in hemolymph circulation of *C. californica.* Note the general lack of SER and dense granules. 15,000×. Figure 5.—Pre-encapsulation encounter between host granulocyte (Gr) and 0.5 mm *R. buchanani* sporocyst. Note the karyolytic nucleus (K) and scattered cytoplasmic debris (Cd) from lysed host cell in the immediate vicinity of the sporocyst's tegument (Tm). 10,000×. Figure 6.—Hyalinocytes surrounding *R. buchanani* sporocyst (1.0-1.5 mm in length) at an early stage in capsule formation. Thin cytoplasmic processes from hyalinocytes (hollow arrows) loosely interdigitate with sporocyst microvilli (Tm). Extracellular fibrils are indicated by solid arrows. 8,000×. Figure 7.—Layers of flattened hyalinocytes surrounding a 4.0 mm *R. buchanani* sporocyst. Cytoplasmic processes (C) are intimately bound up with tegumental microvilli (Tm). Cytoplasmic microtubules in hyalinocytes are indicated by hollow arrows. 9,000×.

CONCLUDING REMARKS

Of the two types of hemolymph cells found in *C. californica*, only the hyalinocyte plays an active role in the encapsulation of *R. buchanani* sporocysts. However, whether these cells merely serve as a physical barrier between host and parasite, or whether they actually represent a physiological or biochemical barrier is at present undetermined. The strong development of RER and dense inclusions in capsular hyalinocytes suggests that these cells may be undergoing functional changes, and supports the supposition that hyalinocytes are providing more than a spacial separation between host and parasite tissues. As has been pointed out by Cheng (1976) at this workshop, the ability of cells to react with and eliminate foreign materials may be related to the compatibility of enzymes elaborated by hemolymph cells and the foreign substrates. Certainly the basic biochemical mechanisms which allow for the establishment of a benign relationship between encapsulating hyalinocytes and *R. buchanani* sporocysts are in need of further investigation.

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ABBREVIATIONS USED IN FIGURES

C	cytoplasmic processes
Cd	cytoplasmic debris
D	dense inclusions
Ec	ectoplasmic region
En	endoplasmic region
Gr	granulocyte
K	karyolytic nucleus
N	nucleus
RER	rough endoplasmic reticulum
Tm	tegumental microvilli

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