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Echinoderm Coelomocytes

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ABSTRACT

Fu-Shiang Chia and Jun Xing (1996) Echinoderm coelomocytes. *Zoological Studies* **35**(4): 231-254. This paper reviews the progress of studies of echinoderm blood cells (coelomocytes), from publications mostly since 1981; particular attention is paid to recent findings on immunological function, cell culture, and molecular biology of coelomocytes. Some observations made in our own laboratory on coelomocytes of a sea cucumber (*Holothuria leucospilota*) are included.

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Six types of coelomocytes are known in echinoderms. They are the phagocyte, spherule cell, vibratile cell, crystal cell, progenitor cell, and haemocyte. These cells likely originate from mesoderm epithelium of various body parts, and their concentration is (1 to 60) $x10^6$ cells per ml of coelomic fluid.

Echinoderms express both humoral and cellular immunity. Cellular immunity is carried out by phagocytes through phagocytosis, encapsulation, and cytotoxicity. Spherule cells are involved in wound healing and regeneration. Molecular studies indicate that profilin, an actin binding protein in the coelomocytes, can receive and transduce injury signals to other coelomocytes to elicit immune responses.

Evidence supports the idea that the immunological system of echinoderms may be an ancestral form to that of vertebrates. Such evidence includes: the morphological and functional similarities between the echinoderm coelomocytes and vertebrate blood cells; the resemblance of echinoderm brown bodies to the multinucleated giant cells of vertebrates; the inflammation response after challenge by foreign material; and the presence of phagocytosis-regulating agent (interleukin-1-like molecule) in the coelomic fluid.

Some interesting topics in the study of echinoderm coelomocytes are pointed out throughout the text and we hope that they will be useful for researchers who may want to engage in studies of echinoderm coelomocytes.

Key words: Phagocytosis, Immunology, Brown bodies, Coelomic fluid.

INTRODUCTION

Echinoderms are marine invertebrates, including sea lilies (Crinoidea), sea daisies (Concentricycloidea), sea stars (Asteroidea), brittle stars (Ophiuroidea), sea urchins (Echinoidea), and sea cucumbers (Holothuroidea). Some of these can reach 2 m in length (sea cucumbers) or 0.5 m in radius (sea stars) (Chia and Harrison 1994). It is surprising that, for such large animals, the excretory and circulatory systems are poorly developed. Instead, most echinoderms have large and extensive body cavities which are filled with coelomic fluid, and in the fluid there are populations of free cells, collectively called coelomocytes. Coelomocytes are also found among the tissues of various body parts. These cells are believed to carry out various functions including nutrient transport and immune defense that otherwise would be performed by excretory and circulatory systems of animals in other phyla.

Echinoderm coelomocytes have been studied extensively during the past 30 or more years. More than half of the publications are observations of light and electron microscopy (see review by Smith 1981). Among the chief contributors of such studies are Hetzel (1963 1965), Fontaine and his associates (1973 1977 1981), Byrne (1986) and Canicatti et al. (1989). It is now well established that there are 6 types of coelomocytes found in echinoderms: phagocytes; spherule cells; vibratile cells; crystal cells; progenitor cells; and haemocytes. Four of these are thought to have counterparts in vertebrate blood cells: phagocytes to macrophage cells; spherule cells to mast cells; progenitor cells to lymphocytes; and haemocytes to nucleated erythrocytes of lower vertebrates (Fontaine and Lambert 1973, Ratcliffe and Millar 1988).

Intensive research of echinoderm coelomocytes in recent years has been focused on various aspects of immunology. It has been found that echinoderm coelomocytes can secrete antibacterial factors like agglutinins and lysins to immobilize and eventually remove foreign material (Parrinello et al. 1976 1979, Canicatti and Parrinello 1983, Canicatti 1987a, b). At the same time, coelomocytes can also directly phagocytose or encapsulate foreign material (Canicatti 1989a,b 1990a,b, Canicatti and Quaglia 1991).

Much progress has been made since the last comprehensive review of echinoderm coelomocytes by Smith (1981), particularly in the study of immune function. The purpose of this review is to provide an overview of coelomocyte biology, with special emphasis on phagocytosis, cell culture, and the molecular aspects of coelomocytes. We have included some of our own findings in the black sea cucumber *Holothuria leucospilota*. Finally, we have attempted to point out new directions for future research on echinoderm coelomocytes.

ORIGIN OF THE COELOM AND COELOMOCYTES

Although the embryonic development of echinoderms varies among species and among higher taxa, it nevertheless follows the basic patterns of other deuterostomes: indeterminate radial cleavage, coeloblastula, gastrulation by invagination, enterocoelus, and the anus if formed from the blastopore.

The coelomic pouch is derived from the anterior end of the archenteron; it may be in the form of a single anterior pouch, or in the form of a pair of sacs occupying the left and right sides of the

archenteron. Further development of the coelomic pouches differs between the left and right sacs. The sac on the left side elongates and divides into 3 parts: the anterior axocoel, the middle hydrocoel, and the posterior somatocoel. The right coelomic sac divides into 2 parts: the anterior axocoel and the posterior somatocoel. Sometimes there is a right hydrocoel, but it usually degenerates. The left hydrocoel gives rise to the water vascular system which is the key character of all echinoderms. The embryogenesis of the coelomic pouches and their derivatives of coelomic compartments in the adult are summarized in Table 1.

Theories of the origin of coelomocytes remain contradictory. The first population of mesenchyme cells is derived from the tip of the archenteron. The morphology of these free cells resembles that of phagocytes (both petaloid and filopodial forms) (Fig. 1). The function of the mesenchyme cells was thought to be involved with the establishment of larval symmetry, as they are known to aid the formation of stomatodaeum which marks the anterior ventral surface (Crawford and Chia 1978). In sea urchins, the primary mesenchyme cells are involved in the formation of the larval skeleton.

The earlier literature suggested that in adult sea cucumbers, coelomocytes originate from the haemal ring or haemal vessels, the respiratory tree epithelium or the peritoneum (see review by Smith 1981). Hetzel (1965) studied sea cucumber coelomocytes by histological and smear techniques and concluded that lymphocytes (progenitor cells) originate from mesenchyme cells in haemal vessels, and possibly later differentiate directly into haemocytes, amoebocytes (phagocytes) and morula cells (spherule cells). The idea that coelomocytes derive from the connective tissue of the respiratory tree, as suggested by Endean (1958), cannot be generally applied, because many sea cucumbers do not

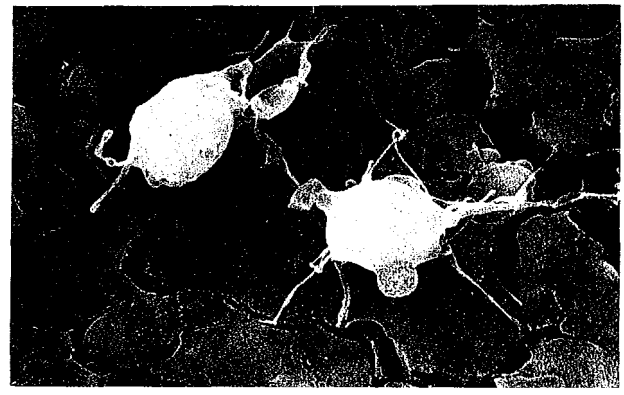

Fig. 1. Scanning electron microscopic photograph of 2 mesenchyme cells attached to the internal surface of the wall of a gastrula of the sea star *Pisaster ochraceous*. Magnification: 1 500X.

Table 1. Derivatives of echinoderm coelomic pouches[a]

	Crinoids	Holothuroids	Echinoids	Asteroids	Ophiuroids
Right					
Axocoel	Not formed	Missing	Lumen of axial gland and aboral coelom into which axial gland extends	Dorsal sac?	Right side of axial sinus
Hydrocoel	Not formed	Missing	Missing	Missing	Missing
Somatocoel	Aboral part of the perivisceral coelom; chambered organ; aboral or genital coelomic canals	Aboral part of the perivisceral coelom; perianal sinus	Aboral part of the perivisceral coelom; periproctal, perianal, and aboral sinuses.	Aboral part of the perivisceral coelom; mesenteries of pyloric caeca; aboral coelom	Aboral part of the perivisceral coelom; aboral sinus
Left					
Axocoel	Axial sinus; hydropore	Stone canal?	Hydroporic canal; ampulla	Axial sinus; inner hyponeural sinus	Left side of axial sinus?; hydroporic canal
Hydrocoel	Water-vascular system; hydroporic canal	Water-vascular system; hydroporic canal	Water-vascular system; hydroporic canal, in part	Water-vascular system; hydroporic canal	Water-vascular system; hydroporic canal
Somatocoel	Oral part of perivisceral coelom; subtentacular coelomic canals	Oral part of perivisceral coelom; peribuccal and peripharyngeal sinuses	Oral part of perivisceral coelom; peripharyngeal cavity and gill cavities	Oral part of perivisceral coelom; outer hyponeural sinus; radial hyponeural sinus	Oral part of perivisceral coelom; periesophageal ring sinus; arm coeloms; peristomial ring sinus

[a]After Meglitsch (1967).

have a respiratory tree. It should be noted also that many sea cucumbers, including *H. leucospilota*, do not have haemocytes. The belief that various coelomocytes are derived from lymphocyte-like progenitor cells is based on the observations of the intermediate forms between progenitor cells and other coelomocytes, but the exact sequence of events leading to the differentiation of various cell types is not known. No mitotic division of any coelomocytes has ever been observed.

Liebman (1950) advanced the idea that coelomocytes originate from the epithelium of the peritoneum in the sea urchin *Arbacia punctulata*. In the sea star *Asterias rubens*, Vanden Bossche and Jangoux (1976) provided evidence that all coelomocytes originate from coelomic epithelium and all organs lined with such epithelium can be considered cytopoietic. This idea has been supported by Maes and Jangoux (1983) who found a pericentriolar complex near the nucleus of the coelomocytes in 2 species of sea stars, *A. rubens* and *Astropecten irregularis*; this complex was somewhat similar to that found in the peritoneal (mesothelial) lining cells. However, a contradiction was given by Panijel et al. (1977) and Brillouet et al. (1981) who reported that in *A. rubens*, the axial organ can produce progenitor cell-like coelomocytes when stimulated by mitogens. Autoradiographic studies in the sea urchin, *Strongylocentrotus purpuratus*, enabled Holland et al. (1965) to discover active cell divisions in the following areas: the visceral peritoneum, peritome peritoneum, hydrocoel peritoneum, Polian vesicle, haemal strands, and dermal connective tissue. He concluded that coelomocytes originate from multiple sites.

The origin of coelomocytes in sea lilies, sea daisies and brittle stars is unknown.

The relationship between various kinds of coelomocytes remains confusing. No convincing evidence is available that the so-called progenitor cell is a stem cell, nor is there evidence that the spherule cell is a product of a phagocyte, although some investigators have made such suggestions (Endean 1958 1966, Hetzel 1965).

It is our opinion that there is no single stem cell type which gives rise to all types of coelomocytes in echinoderms; we support the notion that coelomocytes in echinoderms are derived from the mesoderm epithelium of various parts of the body. Ontogenetic and functional relationships among the coelomocytes are subjects of considerable interest and should be pursued in future research.

MORPHOLOGY OF THE COELOMOCYTES

The morphology of echinoderm coelomocytes has been well documented through studies using light microscopy (especially phase contrast and differentiation interference contrast optics), scanning electron microscopy, and transmission electron microscopy. Six types of coelomocytes in echinoderms have been identified: phagocytes (phagocytic amoebocytes), spherule cells, vibratile cells, crystal cells, progenitor cells, and haemocytes.

Phagocytes

Phagocytes are found in both coelomic fluid and connective tissues (Smiley 1994), and range in size from 10 to 40 μm. They have a round or bean-shaped eccentric nucleus surrounded by cytoplasm which has numerous membrane-bound, electron-dense bodies, vacuoles and vesicles. The Golgi bodies and rough endoplasmic reticulum (RER) of phagocytes are well developed (Fontaine and Lambert 1977, Smith 1981, Canicatti et al. 1989, Smiley 1994), indicating an active secretory activity, probably related to the secretion of lysosomal enzymes. Cytochemical study of phagocytes in *Holothuria polii* has indicated that the cytoplasm reacts to most of the cytoplasmic stains (D'Ancona and Canicatti 1990) (Table 2).

Phagocytes exhibit 2 distinct forms: the petaloid form with bladder-like petals extending 3-dimensionally from the central region (Fig. 2a), and the filopodial form with elongated branched pseudopodia (Fig. 2b). Edds (1980) has suggested that, in the sea urchin *Strongylocentrotus droebachiensis*, the phagocytes can transform from petaloid to filopodial form spontaneously. The transformation begins with the formation of several microspikes at the edge of each petal and is followed by cytoplasmic retraction and active membrane extension. During the extension process, the newly-formed filopodia lengthen actively. Cine film analysis showed that the transformation requires about 15 min (Edds 1980). In addition, the transformation can be induced and enhanced by a transformation-inducing factor, such as 0.1 mg/ml extract of *Strongylocentrotus* egg in a hypotonic solution (50 mM NaCl, 10 mM Tris-HCl, pH 7.8). In this case, all petaloid cells transformed to the filopodial form within 4 to 5 min (Edds 1977c). According to Otto et al. (1979), in *S. purpuratus*, the transformation was reversible although it was rarely completed. However, the reverse transformation from filopodial to petaloid form has never

Table 2. Summary of morphology and reactions to various cytochemical stains of echinoderm coelomocytes

Morphological parameter	Phagocyte		Spherule cell			Vibratile cell	Crystal cell	Progenitor cell	Haemocyte
	Petaloid form	Filopodial form	Type I	Type II	Type III				
Size	10-30 μm diameter	20-40 μm diameter	5-20 μm diameter	5-20 μm diameter	5-10 μm diameter	Cell body: 5-10 μm diameter; Flagellum: 20-50 μm long	2-4 μm long	7-8 μm diameter	10-23 μm diameter
Shape	Petal-like or bladder-like pseudopodia extend from central nuclear region three-dimensionally	Slender and long (up to 50 μm) branched pseudopodia, sometimes stellate	Spherical or ovoid, rough surface, sometimes with microprojections and holes on the cell surface			Spherical with 1 or 2 flagellum	Diamond or rhomboid	Spherical, ovoid or spindle	Biconvex or spherical
Cytoplasmic content	Numerous membrane-bound electron-dense bodies, 6-14 μm diameter; well-developed RER, Golgi bodies and lysosomes		Abundance of tightly-packed membrane-bound electron-dense material	Granules with electron-dense core surrounded by a less dense "shell"	Void of granules, sometimes with residual inclusions	Numerous dark granules of different sizes (0.5-2 μm diameter); well-developed Golgi apparatus	Rhomboidal or star-shaped or spherical inclusions	Lack of elaborated cytoplasmic specialization	Hemoglobin distributed evenly in the cytoplasmic matrix
Nucleus	Spherical or bean-shaped, 4-5 μm diameter with single nucleolus		Regular or irregular-shaped, eccentrically located, 2.5 μm diameter			Large, dominates the cell body	Usually indistinguishable	Large, dominates the cell body	Ovoid, lack of nucleolus in mature haemocyte
Motility in vitro	Actively motile, transforms to filopodial form after phagocytosis or under osmotic shock	Relatively stable, sometimes shows slightly motile	Stable, sometimes amoeboid movement			Active rotation by flagellum lashing	Stable	Stable	Stable
Reactions to various cytochemical stains									
0.1% Toluidine blue ethanol	−	−	+ + +	+	−	n.d.	n.d.	n.d.	n.d.
Alcian blue pH 1	−	−	+ + +	+	−	n.d.	n.d.	n.d.	n.d.
Alcian blue	+ +	+ +	+ +	+	+ + +	n.d.	n.d.	n.d.	n.d.
Stempien's method[a]	−	−	+ + +	−	n.d.	n.d.	n.d.	n.d.	n.d.
PAS	−	−	+	+ + +	+ + +	n.d.	n.d.	n.d.	n.d.
Nimidrin – Schiff	+ +	+ +	−	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Chevremont's method[b]	n.d.	n.d.	+ + +	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Biebrich scarlet	+ +	+ +	+	+	+ + +	n.d.	n.d.	n.d.	n.d.
Sudan black	−	−	+	−	+	n.d.	n.d.	n.d.	n.d.
Lillie's method[c]	−	−	−	−	+ +	n.d.	n.d.	n.d.	n.d.
Schmorl's method[c]	−	−	−	−	+ + +	n.d.	n.d.	n.d.	n.d.
Hueck's method[c]	−	−	−	−	+	n.d.	n.d.	n.d.	n.d.
Acid phosphatase	+	+	+	±	+ + +	n.d.	n.d.	n.d.	n.d.
Alkaline phosphatase	+	+	+	±	+	n.d.	n.d.	n.d.	n.d.
Chloracetate – Esterase	+ + +	+ + +	+ + +	±	+ +	n.d.	n.d.	n.d.	n.d.
Peroxidase	−	−	+ + +	±	+	n.d.	n.d.	n.d.	n.d.
References	See text								

[a]Stempien's method was used to check the existence of acidic groups.
[b]Chevremont's method was used to check the existence of protein – bound SH.
[c]Lillie's method, Schmorl's method, and Hueck's method were used to detect the existence of melanin.
n.d.: not determined.

been documented in any other echinoderm.

Edds (1977a) showed that the petaloid form consists of a loose net of microfilaments while the filopodial form consists of paracrystalline bundles of microfilaments and actin; the formation of the filopodia results from a progressive re-organization of a loose net of actin-containing microfilaments into bundles that are radially oriented (Edds 1977b). Otto et al. (1979) identified a 58 000-dalton actin-organizing protein, fascin, which can organize actin filaments in vitro into linear paracrystalline arrays with a distinct 11-nm banding pattern formed by cross-links between adjacent actin filaments. Therefore, the transformation from the petaloid to filopodial phagocyte is mediated by the actin cross-linking protein, fascin.

Recently, Edds (1993) provided evidence that there are 2 subsets of petaloid phagocytes in the sea urchin *S. droebachieneis* and that they can be separated by a 2-step sucrose gradient centrifugation (the separation medium was 0.75 M and 1.0 M sucrose in buffer). Morphologically, the 2 subsets of cells are quite similar when observed just after separation; they both have 3-dimensionally distributed "petals". However, after they settled and spread, their morphologies were quite distinctive; the subset 1 cells which stayed on the top of the 0.75 M sucrose, were flattened smooth discs with little cytoplasmic elaboration, while the subset 2 cells which banded at the interface of 0.75 M and 1.0 M sucrose, exhibited an irregular polygon outline. In addition, subset 2 cells had numerous small vesicles around the nucleus and some small organelles throughout the central part of the cell. The 2 subsets of cells were also distinguished by biochemical studies of their cytoskeleton-associated proteins.

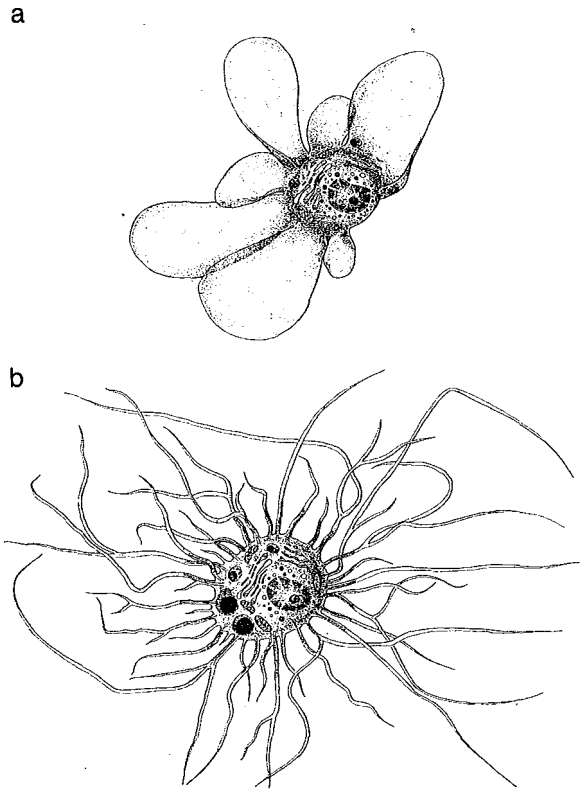

Fig. 2. Diagrammatic presentations of 2 phagocytes in *Holothuria leucospilota*. a: Petaloid form; b: Filopodial form. Note two plastic beads at the lower left side of the cell body in the filopodial cell. Other organelles including nucleus, nucleolus, mitochondria, Golgi bodies, RER, lysosomes, and electron dense bodies are shown.

In our own laboratory, we have observed the morphology of phagocytes of the sea cucumber *H. leucospilota* by using both phase contrast and differentiation interference contrast optics. We separated the petaloid phagocytes from the coelomic fluid by use of a sucrose gradient centrifugation method, and found that, as long as the separated petaloid phagocytes are re-suspended in cell-free coelomic fluid from the same individual, they remain in petaloid form. Our time-lapse Cine analysis showed that the "petals" of the petaloid phagocytes undergo continual collapse and reformation, and that the size and number of "petals" keep on changing. Phagocytosis takes place when a petal encounters a foreign particle. It appeared that the foreign particle was captured by the "petal" and transported slowly toward the nuclear region. The transformation to filopodial form began as soon as the foreign particle was captured. Within 16 min, all petals collapsed and numerous filopodia radiated from the cell. Finally, the cell became a filopodial phagocyte with little motility. However, 70% of the petaloid phagocytes could be induced into filopodial phagocytes within 15 min if they were suspended in an isotonic buffer which contained 0.34 M NaCl, 0.001 M EGTA and 0.01 M Tris-HCl, or in an isotonic anticoagulant solution which contained 0.02 M EGTA, 0.34 M NaCl, 0.019 M KCl and 0.068 M Tris-HCl. It appears that transformation from petaloid to filopodial form is not a spontaneous process in vitro.

Spherule cells

Spherule cells are round or ovoid in shape. Their size range from 8 to 20 μm in diameter. In

H. leucospilota, we found that most of the spherule cells were deformed into a peanut shape in animals which had been kept in an aquarium for 3 months or longer.

The cytoplasm of spherule cells is filled with spherical inclusions (granules) ranging from 2 to 5 μm in diameter, obscuring the irregularly-shaped nucleus (Smith 1981, Smiley 1994). In the sea cucumber *Eupentacta quinquesemita*, the granules of spherule cells are packed tightly against the cell membrane which makes the appearance of the cell resemble a morula (Fontaine and Hall 1981). We have seen the same appearance in the spherule cells of *H. leucospilota*. Histochemical examination showed that the granules contain mucopolysaccharides and protein (Fontaine and Lambert 1977, Canicatti et al. 1989).

Spherule cells have been divided into several types according to different criteria by various authors. Based on color, Endean (1966) recognized red spherule cells in sea urchins due to the presence of echinochromes; colorless, green, or yellow spherule cells were also found in sea lilies and sea cucumbers (see review by Smith 1981). In *H. leucospilota*, the spherule cells have a green color. Based on the ultrastructural characteristics of the granules, Canicatti et al. (1989) recognized 3 types of spherule cells in *H. polii* (Table 2). In Type I cells, granules are homogeneously electron-dense; in Type II cells, granules contain a central electron-dense core surrounded by a less dense shell; and in Type III cells, granules are irregular and seem to be void of content. Cytochemical studies by D'Ancona and Canicatti (1990) indicated that Type I cells are basophilic, with abundant acid and sulfated acid mucopolysaccharides; Type II cells react only slightly with similar stains (Alcian blue and Toluidine blue); and Type III cells are devoid of sulfated mucopolysaccharides although acid mucopolysaccharides are present. In terms of basic protein (using the Biebrich scarlet reaction as a marker), Type III cells show a much higher content than the other two types of spherule cells. In terms of the activities of hydrolytic enzyme (acid phosphatase, alkaline phosphatase, chloracetate-esterase and peroxidase), which are related to lysosomal function, Type I cells show positive peroxidase activity, Type III cells show high acid phosphatase activity, and the activity of Type II cells is uncertain. On the other hand, only Type III cells test positive to Schmorl's reaction, suggesting the presence of melanin, a pigment which might be related to cellular defense (D'Ancona and Canicatti 1990).

In *H. leucospilota*, we have identified all 3 types of spherule cells. Chia and Koss (1994) found Type II cells in a sea star *Henricia leviuscula*. Fontaine and Lambert (1977) reported Type II cells (morula cells) in the sea cucumber *Cucumaria miniata*; they showed that the core is protein and the shell contains mucopolysaccharides.

Spherule cells of *C. miniata* exhibit amoeboid movement (Fontaine and Lambert 1977); movement was initiated by a protrusion of a blunt pseudopodium, followed by flowing of the granules. We observed a similar type of movement in the spherule cells of *H. leucospilota*.

Despherulation (cell membrane breakdown and the escape of granules) of spherule cells often occurs upon fixation (Smith 1981). However, in *H. leucospilota*, we have seen a spontaneous discharge of granules. The function of despherulation has been attributed to wound healing (Smith 1981).

Vibratile cells

Vibratile cells are small (5-10 μm in diameter) spherical cells with a long (20-50 μm) flagellum. Vethamany and Fung (1972) described the ultrastructure of a vibratile cell in the sea urchin *S. drobachiensis*. They reported that the flagellum is 0.3 μm in diameter and extends from the central part of the cell for a length of 20 to 50 μm; the rootlet exhibits a 9 + 2 microtubule pattern.

Vibratile cells move by lashing the flagellum (Johnson 1969a, Bertheussen and Seljelid 1978). It is thought that the vigorous movement may assist the circulation of the coelomic fluid within the body cavity (Smith 1981).

The vibratile cell has been seen to discharge mucoid substances in vitro (Bertheussen and Seljelid 1978) and these substances gelled soon after release. These authors, therefore, suggested that the vibratile cells have the same functional properties as the platelets of vertebrate blood.

Crystal cells

Crystal cells have been found only in sea stars such as *Patiria miniata* and sea cucumbers such as *C. miniata* and *H. leucospilota* (see review by Smith 1981). These cells can be easily recognized by their diamond or rhomboid shape. They are 4 to 5 μm long and 7 to 8 μm wide, and there is a spherical inclusion in the cytoplasm. In *H. leucospilota*, the central part of the cell is green in color. Some authors (Endean 1958 1966, Smiley 1994) thought that the inclusion is crystalline in

nature as it is refractive and disintegrates under slight osmotic stress.

Progenitor cells

Progenitor cells have been found in sea stars and sea cucumbers (Smith 1981). Fontaine and Lambert (1977) believed that this type of cell represents the stem cell of other coelomocytes as they found the transitional forms between this cell type and phagocytes and spherule cells. However, no evidence has been shown to illustrate that the transformation occurs in vitro.

Progenitor cells are small (6-8 μm in diameter), spherical or ovoid in shape, and are characterized by a large nucleus/cytoplasm ratio. Under the light microscope, it can be seen that the large and round nucleus is surrounded by a hyaline cytoplasm. Ultrastructurally, either rough or smooth endoplasmic reticulum are found around the nucleus, dominating the cytoplasm (Fontaine and Lambert 1977, Canicatti et al. 1989).

Some spindle-shaped cells that have been found in sea lilies, brittle stars and sea cucumbers have been thought to be another form of progenitor cells (see review by Smith 1981).

Haemocytes

Haemocytes are mainly found in sea cucumbers although brittle stars appear to have them as well (Smith 1981). In sea cucumbers, haemocytes have been found only in the orders Dendrochirotida and Molpadiida, but not in Aspidochirotida or Apodida. It is not known in the order Dactylochirotida.

Haemocytes are usually 5 to 23 μm in diameter and are biconvex or spherical shaped. The fine structure of haemocytes has been studied by Fontaine and Lambert (1973) and Fontaine and Hall (1981) in the sea cucumbers, *C. miniata* and *E. quinquesemita*, respectively. In both species, haemocytes were numerically dominant. For example, in *E. quinquesemita*, they accounted for 85% to 90% of the total number of coelomocytes. In haemocytes of *C. miniata*, the hemoglobin was found to be evenly distributed in the cytoplasm. Haemocytes were usually stable, but sometimes a cytoplasmic protrusion (bleb) was observed in either freshly collected or fixed cells. The bleb-forming process was microtubule mediated instead of actin microfilament mediated as in phagocytes. It was postulated that bleb formation is related to exocytosis (Fontaine and Lambert 1973).

The major criteria used to distinguish between immature and mature haemocytes in *E. quinquesemita* are cell shape and size and the texture of the cell surface. Mature cells are spheroid shaped while immature ones vary from spheroid to ellipsoid and are smaller; immature cells are usually smooth while mature ones are wrinkled. Ultrastructurally, immature cells have more RER cisternae, mitochondria, lysosomes, and cortical vesiculae than do mature cells (Fontaine and Hall 1981).

Several similarities between sea cucumber haemocytes and the nucleated erythrocytes of lower vertebrates have been listed by Fontaine and Lambert (1973). These include the biconvex shape; the morphology of the nucleus and nucleoli; the distribution of hemoglobin; the presence of a marginal band of microtubules; and the spatial distribution of some main organelles like mitochondria, lysosomes, Golgi bodies, and centrioles. Fontaine and Lambert (1973) provided a diagrammatic illustration of the structures mentioned above.

Among the echinoderms studied, the sea cucumber is the only group to have hemoglobin, and Smiley (1994) has suggested that this might be related to their oxygen-poor benthic environment.

COELOMIC FLUID AND COELOMOCYTE CONCENTRATION

Coelomic fluid

There is no closed or semi-closed circulatory system in echinoderms. The open system includes the perivisceral coelomic system, water vascular system, perihaemal system, and haemal system (Lawrence 1987). All coelomic cavities contain coelomic fluid in which the numerous coelomocytes are suspended.

The coelomic fluid is virtually similar to sea water. However, it is less alkaline and has a slightly higher salinity. For example, in *H. leucospilota* we found that the pH and salinity of coelomic fluid were 7.8 and 34‰, while values for ambient sea water were 8.0 and 30‰, respectively. In addition, compared with sea water, coelomic fluid has a slightly higher content of potassium, protein, reducing sugar and some excretion products like ammonia and urea (Endean 1966, Bertheussen and Seljelid 1978, Smiley 1994). In echinoderms, especially in sea urchins and sea cucumbers, coelomic fluid constitutes a large fraction of the whole body weight (Table 3).

Table 3. Percentage relation of coelomic fluid in the perivisceral coelom to whole body wet weight in some echinoderms

Species	Coelomic fluid percentage of whole body wet weight	Reference
Pisaster ochracens	20.6%	Giese 1966
Patiria miniata	23.0%	Giese 1966
Holothuria leucospilota	30.0%	Xing (unpublished data)
Strongylocentrotus purpuratus	41.5%	Giese 1966
Allocentrotus fragilis	69.5%	Giese 1966

Coelomocyte concentration

The concentration of coelomocytes has been studied mainly in sea urchins and sea cucumbers (Table 4). It is noted that phagocytes are the dominant cell type in species which do not have haemocytes. The coelomocyte concentration and the proportion of each cell type vary greatly, not only from species to species, but also from individual to individual of the same species; perhaps the variation is related to physiological states of the animal. For example, Bertheussen and Seljelid (1978) reported that the coelomocyte concentration in *S. droebachiensis* dropped significantly after the animal was kept in an aquarium for a period of time (exact time not mentioned). We have found that in *H. leucospilota*, coelomocyte concentrations showed no appreciable changes in animals which were kept in the laboratory for 3 months. Vanden Bossche and Jangoux (1976) reported that in the sea star *A. rubens*, a 2 to 9-fold increase of coelomocyte concentration was found 4 d after the injection of foreign particles into the coelom. Canicatti (1989b) found a change of coelomocyte concentration after the injection of formalized sheep erythrocytes in the sea cucumber *H. polii*. The initial control cell concentration was 0.83×10^6 cells per ml of coelomic fluid. The concentration increased 1 d after injection and reached the highest level (2.8×10^6 cells per ml of coelomic fluid) 2 to 4 d after injection. The number of coelomocytes began to decrease on the 5th day and reached the control value on the 10th day, suggesting that there was an immune response similar to that of mammalian inflammation.

It is apparent that studies of coelomocyte concentration are very limited. Based on the limited information, there appear to be great discrepancies on cell density and proportions of different cell types. What is more interesting is that in *Eupentacta quinquesemita*, haemocyte is by far most abundant, yet the same type of cells are not found in many other sea cucumbers (Table 4). Comparative studies of coelomocytes populations in different species of sea cucumbers should yield important information which will allow us to understand further the biology of echinoderm coelomocytes.

We have no knowledge of the fluctuations of coelomocyte concentrations in relationship to reproductive cycles or physiological changes such as autotomy, regeneration, or other environmental stresses.

COELOMOCYTE CULTURE

To date, more than 200 invertebrate cell lines have been established. Only one is from the snail, *Biomphalaria glabrata*; all others are from arthropods. Among marine invertebrates, a number of papers have been published on tissue culture (see review by Machii and Wada 1989), and most of these are on larval mantle tissue of mollusks, especially oysters (Ellis and Bishop 1989). Culturing of echinoderm coelomocytes has been attempted (Kaplan and Bertheussen 1977, Bertheussen and Seljelid 1978, Bertheussen 1979, Harrington and Ozaki 1986, Leclerc et al. 1986), but their efforts could only achieve "keeping coelomocytes in vitro for a period of time in good conditions". One of the major difficulties in culturing echinoderm coelomocytes is in the prevention of cell clotting.

Anticoagulant

In echinoderms, clotting is a quick response to injury, trauma, and contact with non-self materials (Smith 1981). Clotting seems to be the major obstacle in coelomocyte cultures as freshly-drained coelomic fluid can coagulate within a few seconds or minutes. Bertheussen and Seljelid (1978) tried a series of anticoagulants which were known to inhibit clot formation in other animals. These included heparin (50-400 IU/ml); sodium

Table 4. Coelomocyte concentration and percentage of each cell type to the total population of coelomocytes

Species	Coelomocyte concentration (10^6 cells/ml)	Phagocyte (%)	Spherule cell (%)	Vibratile cell (%)	Crystal cell (%)	Progenitor cell (%)	Haemocyte (%)	Reference
Cucumaria plankii	n.d.	n.d.	n.d.	n.d.	n.d.	69	n.d.	Smith (1981)
Cucumaria miniata	60	8.3	0.5	n.d.	n.d.	2.7	90	Fontaine and Lambert (1977)
Eupentacta quinquesemita	60	7-10	<1	n.d.	n.d.	2-3	85-90	Fontaine and Hall (1981)
Holothuria leucospilota	n.d.	10	70	n.d.	5	10	n.a.	Smith (1981)
Holothuria leucospilota	1.1 ± 0.4	46 ± 9	32 ± 8	5 ± 1	2.8 ± 1.1	14 ± 5	n.a.	Xing (unpublished data)
Holothuria polii	0.83	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	Canicatti et al. (1990)
Strongylocentrotus droebachiensis	5-10	64-70	10-19	15-22	n.d.	n.d.	n.a.	Bertheussen and Seljelid (1978)
Strongylocentrotus purpuratus	n.d.	66.3 ± 11.6	19.9 ± 13.1	13.9 ± 9.8	n.d.	n.d.	n.a.	Smith et al. (1992)
Lytechinus pictus	4.25 ± 2.34	14.7	29.8	11.3	n.d.	n.d.	n.a.	Laughlin (1989)
Arbacia puntulata	n.d.	50	50	n.d.	n.d.	n.d.	n.a.	Smith (1981)

Values are shown as mean ± S.D. when applicable.
n.d.: not determined.
n.a.: not applicable.

bisulphite (10 mM); cysteine (15 mM); reduced glutathione (70 mM); 2-mercaptoethanol (50 mM); dithiothreitol (50 mM); n-ethyl maleimide (5 mM); sodium citrate (200 mM); EDTA (30 mM); EGTA (10 mM) and DL-propranolol (0.5 mM). It was concluded that the best one for the sea urchin *S. droebachiensis* is a mixture containing 50 mM mercaptoethanol, 3 mM caffeine, 2 mM TAME (p-tosyl-L-arginine methyl ester) in sea water, at pH 7.4. Two other anticoagulants for sea urchins are also known: 50 mM EGTA, 50 mM Tris-HCl, pH 7.8 (Edds 1977a); and 0.03 M EGTA, 0.5 M NaCl, 0.026 M KCl and 0.1 M Tris-HCl, pH 8 (Otto et al. 1979). Canicatti et al. (1989) described an effective anticoagulant for the sea cucumber *H. polii*: 31 g/l NaCl, 10 mM Hepes and 30 mM EDTA. In our own study, the following solution has worked well for the sea cucumber, *H. leucospilota*: 0.02 M EGTA, 0.34 M NaCl, 0.019 M KCl and 0.068 M Tris-HCl at pH 8.

Separation of different types of coelomocytes

Different types of coelomocytes can be separated by gradient centrifugation based on the differences in their densities. Sucrose gradient, discontinuous sodium metrizoate gradient, and Percoll step gradient are the 3 documented methods of separating coelomocytes. Lindsay et al. (1965) proposed a simple method to separate the major coelomocyte types in the purple sea urchin *S. purpuratus* by using a sucrose-sea water gradient (1.2 M, 1.0 M, 0.85 M and 0.7 M sucrose solutions made in sea water). After centrifugation at 4 100 g for 5 min, 4 coelomocyte types were separated. The 2 upper layers contained petaloid phagocytes; with filopodial phagocytes at the interface of the 2nd and 3rd layers. The 3rd layer was dominated by vibratile cells, and the 4th layer contained spherule cells (Fig. 3a). Edds (1977a) separated the phagocytes from other cell types in the sea urchin *S. droebachiensis* by layering the coelomic fluid on top of a 0.8 M sucrose solution, and centrifuging at 5 000 g for 5 min. He found that the petaloid cells were concentrated at the

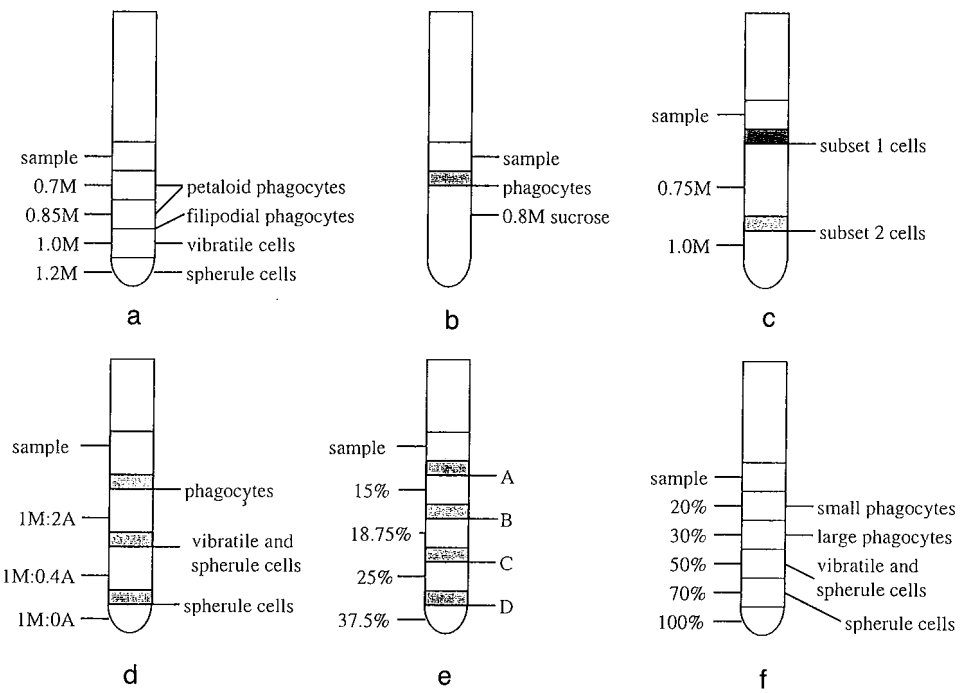

Fig. 3. Diagrammatic presentations of separating different types of coelomocytes by gradient centrifugation. a. Lindsay et al. (1965): Discontinuous sucrose (0.7-1.2 M) gradient; b. Edds (1977a): 0.8 M sucrose solution; c. Edds (1993): Two-step sucrose (0.75 M and 1.0 M) solution. d. Bertheussen and Seljelid (1978): Sodium metrizoate (M) and anticoagulant (A) solution at various ratios; e. Canicatti et al. (1990): Discontinous sodium metrizoate (15%, 18.75%, 25% and 37.5% sodium metrizoate in anticoagulant). A: 84% phagocytes + 13% Type III spherule cells + 3% Type I and II spherule cells; B: 50% phagocytes + 23% Type III spherule cells + 14% Type I spherule cells + 13% Type II spherule cells; C: 58% Type III spherule cells + 18% phagocytes + 12% Type I spherule cells + 12% Type II spherule cells; D: 51% Type II spherule cells + 28% Type I spherule cells + 19% Type III spherule cells + 2% phagocytes; f, Smith et al. (1992): Discontinuous Percoll gradients (20%, 30%, 50%, 70%, and 100% Percoll solutions).

sucrose-coelomic fluid interface (Fig. 3b). We used the same method to separate petaloid phagocytes of *H. leucospilota*. Recently, Edds (1993) discovered that the petaloid phagocytes could be separated into 2 subsets by a 2-step sucrose gradient. The lower layer was 1.0 M sucrose and the upper layer was a 0.75 M sucrose solution. After centrifugation at 5 000 g for 5 min, some cells banded on top of the 0.75 M sucrose layer (subset 1 cells), and another distinct band (subset 2 cells) was found on top of the 1.0 M sucrose layer (Fig. 3c). Subset 1 cells are more condensed than subset 2 cells. The morphological difference between the 2 subsets of cells has already been described in the section "morphology of the coelomocytes".

Three layers of gradient solution, made up by mixing sodium metrizoate and anticoagulant solution in the ratios 1:2, 1:0.4, and 1:0, have been used to separate sea urchin coelomocytes (Bertheussen and Seljelid 1978). After centrifugation at 300 g for 15 min, 3 distinct bands could be collected at the interfaces. The uppermost layer consisted of phagocytes, the middle layer was a mixture of vibratile cells and spherule cells, and the bottom layer contained only spherule cells (Fig. 3d). Canicatti et al. (1990) used sodium metrizoate gradients (15%, 18.75%, 25%, and 37.5%) for separating phagocytes from spherule cells (Fig. 3e).

Smith et al. (1992) recently reported a method of separating different types of sea urchin coelomocytes by using Percoll step gradients. The coelomocyte suspension was loaded on Percoll solutions with concentrations which ranged 20%, 30%, 50%, 70%, and 100%, then samples were centrifuged at 118 g for 15 min. The results indicated that the 20% Percoll solution contained small phagocytes; the 30% layer consisted of large phagocytes; the 50% layer contained vibratile cells with 13.6% contamination by spherule cells; and the 70% Percoll solution contained only spherule cells (Fig. 3f).

In general, phagocytes are the least dense cell type due to their petal-like cytoplasmic extensions, while spherule cells are the most dense coelomocytes as a result of their packed inclusions. Use of a flow cytometer, the instrument used to separate cells of different sizes, is not applicable for echinoderm coelomocytes because of the great overlap in sizes of different coelomocyte types.

Culture medium and culture condition

Among the 6 types of coelomocytes, phagocytes have received the most attention in cell culture because of their immunological properties. A medium used for sea urchin phagocyte culture is the Hepes-sea water medium (Bertheussen and Seljelid 1978). This medium consists of 36 g/l sea water salt; 10 mM Hepes; 10 mg/l phenol red; 10% (vol/vol) minimum essential medium (MEM); 5 mg/l adenosine; 500 mg/l sodium pyruvate; 1 g/l proteose peptone; and antibiotics (50 units/ml penicillin, 50 μg/ml streptomycin and 50 μg/ml tylocine). This medium has similar osmolarity and salinity as those of sea water and sea urchin coelomic fluid. Peptone is proven to be crucial for maintaining the phagocyte culture in good condition for a long period of time. Without peptone, cells can only survive for a couple of days and even the addition of filtered coelomic fluid does not help. Hepes-sea water medium can keep phagocyte cultures in good condition for at least 1 month. Protein content determination and a [^{3}H] thymidine incorporation experiment showed that coelomocytes neither grow nor divide in vitro (Bertheussen and Seljelid 1978).

Leclerc et al. (1986) used a medium consisting of MEM supplied with 36 g/l NaCl and 5×10^{-5} M mercaptoethanol for sea star coelomocyte culture. Harrington and Ozaki (1986) were successful in culturing the phagocytes of the sand dollar *Dendraster excentricus* and the sea urchin *S. purpuratus* by use of the following medium: 1 part distilled water mixed with 9 parts artificial sea water buffered with 10 mM Hepes/sodium carbonate; with a pH of 7.5 and a final concentration of 0.25 mg/ml streptomycin sulfate and 150 units/ml penicillin G. However, for these 2 media, the coelomocytes were kept alive for only 2d and 5d respectively, in their experiments.

Coelomocytes have usually been cultured at 10 °C or 15 °C either in Leighton tubes or culture plates with or without coverslips. Coelomocytes were inoculated on coverslips or wells of the culture plate which were supplied with culture medium. Medium was changed either by transferring the coverslip with the attached cells into fresh medium once a week, or it was replaced within the well once a week (Bertheussen and Seljelid 1978, Bertheussen 1979, Leclerc et al. 1986). There are several advantages in using coverslips, as phagocytes can attach to them, making it more convenient to change the medium. Furthermore, most of the cells attached to coverslips were alive while the dead cells were left suspended in the medium (Bertheussen 1979). This makes it easy to dispose of the dead cells and count the live

ones. In addition, coverslips can easily be handled for observation under a microscope.

Phagocytes of *S. droebachiensis* change shape quickly after the first several hours in culture. They tend to fuse and form large syncytia 4 to 6 h after inoculation. The syncytia are equivalent to giant cells in some vertebrate blood which are also formed by phagocytic cells when challenged by pathogens (Bertheussen and Seljelid 1978).

In our own laboratory, we have had some success in culturing phagocytes of *H. leucospilota*. We separated the phagocytes by following the method of Otto et al. (1979) and by using a medium modified from Bertheussen and Seljelid (1978) in which the concentration of sodium chloride was adjusted to match the osmolarity of the coelomic fluid (842 mOsm). We inoculated 0.4 ml of medium containing phagocytes at 2 concentrations (6×10^5 cells/ml, 4.6×10^7 cells/ml), and placed them into the wells of 24-well culture plates. We then added an equal volume of culture medium to make final cell concentrations of 3×10^5 cells/ml and 2.3×10^7 cells/ml respectively.

We tried 3 types of media: Bertheussen and Seljelid's medium with osmolarity modification, cell-free coelomic fluid and isotonic buffer solution (0.34 M NaCl, 0.001 M EGTA, 0.01 M Tris-HCl, pH 8, as control). The cultures were kept at 10 °C under a normal atmosphere without addition of the medium during the process of culturing. It was found that there was no increase in cell numbers during the 15-d culture period. Furthermore, phagocyte survival is density dependent. In wells with a starting concentration of 3×10^5 cells/ml, the phagocytes in all 3 media died within 10 d. However, in wells with a starting concentration of 2.3×10^7 cells/ml, the phagocytes survived for at least 15 days. Phagocytes in the 3 types of media showed differences in survival time: in the well housing phagocytes with both starting concentrations, the number of cells in the buffer solution decreased much faster than those in the 2 other culture media, indicating the importance of nutrition supplement in culture.

It should be noted that culture of echinoderm coelomocytes is still in the initial stages; we need to further define the culture media and try to culture cell types other than phagocytes, particularly spherule cells, which are also important in immune responses.

IMMUNOLOGICAL FUNCTIONS OF COELOMIC FLUID AND COELOMOCYTES

Invertebrates in general respond to foreign antigens by humoral and cellular immunity. Humoral immunity refers to neutralization or removal of foreign materials by production and secretion of humoral factors. Cellular immunity refers to clearance of foreign material either by phagocytosis, cellular encapsulation, or by specific cell-mediated cytotoxicity (Cooper 1976, Greenberg 1989).

As mentioned in the introduction, 4 types of echinoderm coelomocytes (phagocytes, spherule cells, progenitor cells, and haemocytes) are thought to have counterparts in vertebrate blood cells. For example, the progenitor cells of echinoderms not only closely resemble vertebrate lymphocytes structurally, but also share functional properties: both of them can respond to mitogen and can produce antibody-like molecules (Ratcliffe and Millar 1988). The defense response system of echinoderms is viewed as ancestral to that of vertebrates due to their phylogenetic affinities.

Humoral immunity

Coelomic fluid is believed to fulfill various functions like translocation, excretion, locomotion, as well as protection of the viscera, and, most importantly, humoral immunity (Smith 1981, Lawrence 1987, Smiley 1994). Humoral immunity of coelomate invertebrates has been defined as the process by which foreign microorganisms are neutralized by the synthesis of substances within the coelomic fluid. The "substance" is usually a protein, either a naturally occurring or an induced molecule such as a bacteriocidin, hemolysin or agglutinin, all of which are not unlike vertebrate immunoglobins (Cooper 1976, Parrinello et al. 1976). The immunological function of coelomic fluid has been extensively studied in sea urchins and sea cucumbers (McKay et al. 1969, Parrinello et al. 1976 1979, Bertheussen 1983, Canicatti and Parrinello 1983 1985, Canicatti 1987a, b, Canicatti et al. 1987, Canicatti 1990c, Canicatti 1992). It was found that in *H. polii*, at least 2 specific naturally occurring hemagglutinins exist in the coelomic fluid (Parrinello et al. 1976). Naturally occurring hemolysins were also found in the same animal (Parrinello et al. 1979). Compared with hemagglutinins, hemolysins are smaller in molecular weight, more sensitive to temperature variation and their activities are calcium dependent. Chromatographic studies indicated that the organization of subunits are different between hemagglutinin and hemolysin (Canicatti and Parrinello

1983). After stimuli by foreign materials (injection of formalized sheep erythrocytes), hemagglutinating activity remained constant while the level of hemolysin rose during a 8-d period, indicating that foreign bodies could induce the production of hemolysin but not hemagglutinin (Canicatti and Parrinello 1985). Bertheussen (1983) found that, in *S. droebachiensis*, hemolysin is similar to human complement since those substances that are known to inhibit the function of a human complement also inhibit the function of hemolysin in this animal. Canicatti (1987b), however, provided ultrastructural evidence, indicating that rabbit erythrocyte membranes lysed by *H. polii* coelomic fluid were in the form of irregular holes, and those lysed by human complement were in the form of ring-like holes, suggesting that hemolysin is not the counterpart of a vertebrate complement.

Phagocytosis

All multicellular invertebrates have phagocytes, which are capable of engulfing foreign particles, debris, or microorganisms in a way like an amoeba engulfs food particles; the engulfed material is digested by intracellular enzymes (Davey 1990). Phagocytosis is an important feature of the immune response throughout the animal kingdom; it represents the first line of internal defense (Greenberg 1989). Phagocytosis is usually initiated by the extension and fusion of pseudopodia around the target particle, followed by the formation of a phagosome, an extracellular vesicle. Alternatively, the phagosome can be formed by invagination of the local phagocyte membrane (Bayne 1990). Echinoderms, like other marine benthos, manage to survive in a bacteria-rich marine environment, and this ability may largely depend on their native immune mechanisms, among which phagocytosis is paramount.

Phagocytes are very efficient in recognizing foreign particles (bacteria, cell debris, foreign cells, and inert particles) in the coelomic fluid and clearing them out (see review by Smith 1981). In 2 sea urchins, *S. purpuratus* and *Strongylocentrotus franciscanus*, after exposure to 12 marine bacteria strains, (5 Gram-negative and 7 Gram-positive) as well as a Gram-negative terrestrial insect pathogen in vitro, it was found that phagocytosis occurs only of the Gram-positive bacteria, not of the Gram-negative ones (Johnson 1969b). It was surmised that the Gram-positive bacteria are relatively rare in a marine environment, so they are more foreign to the sea urchin, indicating some response specificity of the phagocytes (see review by Karp and Coffaro 1980). A similar situation was found in the sea cucumber, *Stichopus tremulus*, in which the Gram-positive bacterium, *Gaffkya homari*, was strongly phagocytosed (Johnson and Chapman 1971).

Reinisch and Bang (1971) demonstrated that phagocytes in the sea star *Asterias vulgaris* can remove 2.6×10^7 sea urchin coelomocytes within 1 h when sea urchin cells are injected into the body cavity of the sea star, but it takes 47 h to clear 1.1×10^7 carborundum particles by the same sea star. The difference of the clearance rates indicates that the sea star phagocytes can differentiate among varieties of foreign particles. Brown (1967) investigated the elimination of thorium dioxide particles by coelomocytes of the sea cucumber *Cucumaria stephensoni* by using an autoradiographic technique, and showed that the particles are phagocytosed, and that the phagocytes can be fully laden with particles within 5 h.

Foreign material which has been phagocytosed can be digested and degraded by the phagocyte lysosomes, which contain hydrolytic enzymes, including acid and alkaline phosphatases, β-glucuronidase, acid and alkaline protease, amino peptidase, and lipase. Canicatti and his associates (Canicatti 1988, Canicatti and Miglietta 1989, Canicatti and Roch 1989, Canicatti 1990a, Canicatti and Tschopp 1990, Canicatti 1991, Canicatti and Seymour 1991, Sammarco and Canicatti 1992) studied the lysosomal enzymes in the coelomocytes of the sea cucumber, *H. polii*, and concluded that these enzymes have the capability to degrade various kinds of biological materials. It is thought that β-glucuronidase is possibly related to hydrolysis of acid mucopolysaccharides, a major components of bacterial cell walls and teguments of many parasites.

When indigestible particles are phagocytosed, the phagocytes may undergo a suicidal emigration to the external environment (Bayne 1990). Several discharge pathways have been reported. Reinisch and Bang (1971) found that in the sea star, *A. vulgaris*, the papulae are the main sites of evacuation, and Chia and Koss (1994) provided similar evidence in the sea star *Henricia leviuscula*. Endean (1966) suggested that the madreporite and the alimentary canal are also sites where coelomocytes can pass to the exterior. In the sea cucumber, *C. stephensoni*, autoradiographic studies showed that the phagocytes laden with particles are eliminated from the coelomic fluid by migrating through the walls of the respiratory trees and gut, as well as

via the anterior water vascular system (Brown 1967). In this case, the coelomic fluid was totally free of phagocytes and particles 5 h after injection; the phagocytes did not re-appear until the 4th day after particle injection. In species of sea cucumbers which do not have respiratory trees, ciliary urns are thought to pass the particle-laden phagocytes to the exterior via the body wall (Karp and Coffaro 1980).

Interleukin-1 (IL-1) is an important lymphokine in mammals which is known to regulate certain cellular activities during inflammation. IL-1-like peptide molecules, which have been identified in several invertebrates, including echinoderms, may be involved in the regulation of cellular immunity, such as phagocytosis. Beck et al. (1993) purified the IL-1-like peptide molecules from the coelomic fluid of the sea star, *Asterias forbesi*. It was found that in vitro, if phagocytes are co-incubated with 5 ng/ml of sea star IL-1-like molecules for 30 min prior to mixing with the experimental particles (^{51}Cr-labeled sheep red blood cells), phagocytosis activity will increase by 52%. This effect is inhibited by an antiserum to vertebrate IL-1, indicating that it is the IL-1-like molecules that facilitate phagocytosis; it was also found that 30 min in vitro pre-treatment of phagocytes with 23 μM sodium heparin (a known phagocytosis enhancer in vertebrates) causes a 47% increase of phagocytosis activity in the same species of sea star.

Among vertebrates, opsonins can be used to tag foreign bodies and make them easily recognizable by phagocytes (Davey 1990). In the sea star, *A. forbesi*, IL-1-like molecules were found to behave like opsonin (Beck et al. 1993).

Based on light microscopic observation, Beck et al. (1993) documented the relationship between the incubation time and phagocytosis of latex beads by phagocytes in *A. forbesi*: after 30 min. of incubation, 43% of the phagocytes contained at least two beads; after 60 min, 42% contained at least three beads; by 120 min, 75% of the cells had ingested more than four beads; and the percentage increased to 87% when the incubation time reached 180 min.

Recently, we used a flow cytometric method to quantify the phagocytosis by phagocytes of the sea cucumber *H. leucospilota*. By using fluorescence latex beads (1 μm in diameter), the percentage of phagocytic cells, the number of ingested beads per cell and the total number of beads being ingested were determined simultaneously. We found that 96% of the phagocytes are functionally phagocytic. The phagocytes were tested at bead/cell ratios of 5, 10, 25, 50, 100, and 200 and incubated for two hours. It was found that, at bead/cell ratios of 5, 10, 25, or 50, the percentage of phagocytes ingesting one bead is invariably the highest compared to those ingesting two or more beads. However, at increased bead/cell ratios of 100 and 200, phagocytes ingesting more than three beads become dominant (40% and 62% respectively). About 3.08 million beads were ingested by 0.5 million phagocytes within two hours, indicating that phagocytes are efficient in immobilizing and cleansing foreign particles. Compared with the microscopic examination method, flow cytometry has been proven to be highly efficient in quantifying the work of phagocytosis, and the accuracy of this method has been verified by microscopic examination.

We have also obtained evidence that phagocytosis in *H. leucospilota* is microfilament-mediated since cytochalasin B, a microfilament poison, can effectively inhibit the phagocytosis and the inhibition is dose dependent.

Encapsulation

Encapsulation is another way of clearing unwanted particles such as bacteria, inert materials, foreign tissue implants, and senescent or damaged coelomocytes (Smith 1981, Isaeva and Korenbaum 1990). When the particle is larger than the phagocyte itself, phagocytes can accumulate around the unwanted particles, resulting in encapsulation, which has been considered as "frustrated" phagocytosis (Bayne 1990, Isaeva and Korenbaum 1990). In echinoderms, encapsulation has been documented in Ophiuroidea, Echinoidea, and Holothuroidea, and such encapsulation products are conventionally referred to as brown bodies (Hezel 1965, Canicatti 1989a, Canicatti and Quaglia 1991).

The color of brown bodies is due to the presence of melanin, a pigment associated with the spherical inclusions of spherule cells (Smith 1981). In *H. polii*, both pigmented and unpigmented brown bodies were found in the coelom; and there were comparatively more unpigmented ones. It was postulated that unpigmented brown bodies might represent the earlier unmelanized stage of pigmented ones. Brown bodies were found in both the perivisceral coelom and Polian vesicles (Canicatti 1989a, Smiley 1994). Shinn (1985) reported that brown bodies in the coelom of a sea cucumber were resulted from the encapsulation of intracoelomic parasites (turbellarian flatworms). Brown bodies were roundish, but sometimes appear as a

flattened mass (Canicatti and Quaglia 1991). The size of the brown bodies in *H. polii* varies greatly; the smallest one is 0.1 mm in diameter and the largest one is 4 mm (Canicatti and Quaglia 1991).

Formation of brown bodies can be experimentally induced if carbon particles and formalin-fixed sheep red blood cells are injected into the coelom (Hetzel 1965, Canicatti 1989a). Histologically, these bodies are made of nodules which consist of tight aggregations of phagocytes and spherule cells with foreign materials trapped in the center. In the induced brown bodies, foreign material is surrounded by only one layer of flattened phagocytes (Canicatti 1989a). The aggregation of phagocytes in some brown bodies resembles multinucleated giant cells found in vertebrates.

Encapsulation is probably a more complicated process than simple adhesion of coelomocytes over the foreign particle. In *H. polii*, spherule cells of the brown bodies often despherulate and release acid phosphatase and arysulfatase, suggesting that the digestive capability of the encapsulating structures is similar to that of phagocytosis (Canicatti 1989a).

Cytotoxicity and graft rejection

Cytotoxicity refers to the immunological response in which live infectious organisms are killed by secretion of toxic material without phagocytosis. Many invertebrates such as sponges, corals, earthworms, sea stars, and sea cucumbers are known to use cytotoxicity to reject tissue grafts (Karp and Coffaro 1980, Davey 1990) (Fig. 4). The ability to reject allografts (grafts from different individuals of the same species) has been demonstrated in three classes of echinoderms. Hildemann and Dix (1972) reported that the sea cucumber, *Cucumaria tricolor*, and the sea star, *Protoreaster nodosus*, can reject integumentary allografts; Karp and Hildemann (1976) documented the rejection of allografts in the sea star, *Dermasterias imbricata*; the sea urchin, *Lytechinus pictus* was found to possess a striking ability to reject allografts and the rejection period is shorter than for any other invertebrates or lower vertebrates which have been tested. In addition, the sea urchin is the only known invertebrate to reject 100% of allografts (Coffaro and Hinegardner 1977).

Graft rejection is characterized by loss of pigmentation, contraction of the graft and the ingrowth of host tissue; the histological criteria of rejection is the complete disruption of the graft tissue organization (Karp and Coffaro 1980). These responses have been demonstrated in the sea cucumber *C. tricolor* and the sea star *P. nodosus* (Hildemann and Dix 1972).

Short-term immunological memory has been found in echinoderms. This is demonstrated by making second and third transplants following the first set. The extent and rate of rejection is expressed by the median survival time of the graft (Table 5). For the sea cucumber *C. tricolor* and sea star *D. imbricata*, the first set of transplants was rejected by a chronic response with a longer median survival time (165 days and 213 days, respectively). However, the rejection of the second and third sets of grafts was much more rapid; the median survival time of the grafts decreased dramatically. In vertebrates, the short term immunological memory is attributed to the B-cells (Greenberg 1989). We have no idea what type of coelomocyte functions as the memory cell in echinoderms.

Based on the observation of *C. tricolor*, Hildemann and Dix (1972) proposed that phagocytes, spherule cells, and progenitor cells participate directly in graft rejection, and are responsible for

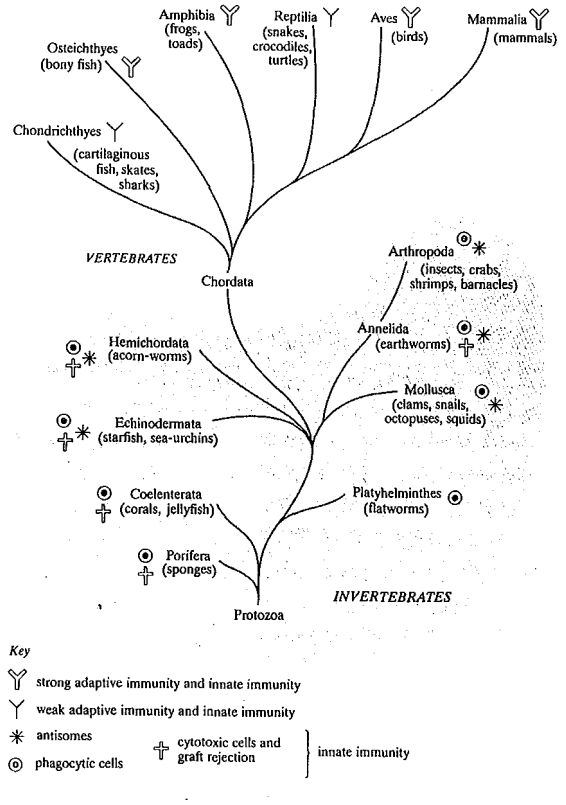

Fig. 4. The immune systems of invertebrate and vertebrate phyla as shown in a phylogenetic tree [After Davey (1990)].

destruction of foreign tissues since these cells infiltrate grafts during the rejection process. A similar phenomenon was documented in *D. imbricata* (Karp and Hildermann 1976). However, Bertheussen (1979) concluded that phagocytes are the only cell type involved in transplant rejection. He separated different coelomocyte types and found that only phagocytes show cytotoxicity to foreign cells. The phagocytes of the sea urchins *S. droebachiensis*, *Strongylocentrotus pallidus*, and *Echinus esculentus*, when cultured on a coverslip, show strong cytotoxicity to allogeneic and xenogeneic coelomocytes.

The disagreement as to the effector cell in transplant rejection is probably due to different experimental conditions. Most of the graft rejection tests in echinoderms were conducted in vivo (Hildemann and Dix 1972, Karp and Hildermann 1976, Coffaro and Hinegardner 1977), while Bertheussen's report (1979) was the only one done in vitro.

IMMUNOLOGICAL RESPONSES TO STRESS

Injury

The soft body surface or protruding organs of some echinoderms can easily be injured. Most of the wound repair studies have been conducted on sea urchins and sea cucumbers. Upon injury, the first defense response is clot formation. Two types of coelomocytes were found to be involved in clot formation. Johnson (1969a) believed that it is vibratile cells which contribute to cell clotting since these cells will discharge their contents in response to injury and this causes the coelomic fluid to gel. Bertheussen and Seljelid (1978) agreed with the above conclusion because after separation of various types of coelomocytes by gradient sodium metrizoate solution, only vibratile cells are observed to discharge some mucoid substance causing coagulation. Bertheussen and Seljelid (1978) proposed that vibratile cells function much as the vertebrate blood platelet. However, Edds (1977a) and Canicatti and Farian-Lipari (1990) believed that phagocytes, especially filopodial phagocytes, play an important role in clot formation. Petaloid phagocytes transform to filopodial phagocytes very quickly upon injury, and the filopodial phagocytes have been observed to connect with other cells by filopodia to form a clot.

Spherule cells were seen to break down rapidly and despherulate at injury sites, possibly producing a collagen material to form the fibrous matrix of the body wall (Coffaro and Hinegardner 1977). Byrne (1986) provided evidence that in the sea cucumber *E. quinquesemita*, spherule cells function as a source of ground substance in the maintenance of the extracellular matrix during regeneration after evisceration. Smith (1983) reported that, when stressed by draining 0.2 to 0.3 ml of coelomic fluid each day on 2 consecutive days, the coelomic fluid of the common Florida sea cucumber *Pentacta pygmea* became a cherry red color while the control samples remained yellow. It was suggested that the red color of the coelomic fluid is the result of a pigment (echinochrome) released from spherule cells under stress (wounding and loss of coelomic fluid).

Spherule cells may play an important role in immobilizing invading microorganisms at wounded sites, with echinochrome acting as a general disinfectant. In the sea urchin, *S. franciscanus*, it was found that many blue-green algae, green algae, diatoms, ciliates, unidentified worm-like organisms, and fungi were attached to the damaged spines. Spherule cells were found in large numbers

Table 5. Allograft rejection in various echinoderms

Species	Allografts	Number of grafts scored	Median survival time (day)	Reference
Cucumaria tricolor (Holothuroidea)	First-set	15	165	Hildemann and Dix (1972)
	Second-set	3	43	
Protoreaster nodosus (Asteroidea)	First-set	3	163	Hildemann and Dix (1972)
Dermasterias imbricata (Asteroidea)	First-set	17	213	Karp and Hildemann (1976)
	Second-set	5	44	
	Third-set	4	8	
Lytechinus pictus (Echinoidea)	First-set	14	35	Coffaro and Hinegardner (1977)
	Second-set	14	12	

[a]After Karp and Coffaro (1980).

at the infected sites, and many of them were seen to release echinochrome (Johnson and Chapman 1970).

Adverse living environment

Mellita quinquiesperforata, an intertidal sand dollar at Cedar Key, Gulf of Mexico (northern Florida USA), was observed to have red coelomic fluid during stormy winter months (Smith 1983), indicating the release of echinochrome from spherule cells. This is probably a protective response to adverse environment or conditions, and it was suggested that the color of the coelomic fluid of this animal can be used as an indicator of environmental stress.

Heavy metals

Some heavy metals in appropriate concentration are essential to the animal in oxygen transport, such as Cu and Fe, and others such as Cu, Co, Mn, Mo, Se, and Zn are parts of enzymatic systems. However, excessive exposure to heavy metals is toxic (George 1990). Koller (1980) have provided a comprehensive review on the effects of heavy metals (Pb, Cd, Hg, Se, Zn, and miscellaneous metals) on mammalian immune functions. It was suggested that lead suppresses most of the immune system while cadmium shows a depressive effect. Mercury suppresses the primary humoral immune response.

Some studies have considered the effects of heavy metals on population dynamics (Sheppard and Bellamy 1974), survival (Sheppard and Bellamy 1974), growth (Dafni 1980), and reproduction and development (Kobayashi 1980, Kristoforova et al. 1984, Lipina et al. 1987) in echinoderms. However, little information is available on the effects of heavy metals on coelomocyte function. Canicatti and Grasso (1988) examined the effect of heavy metals on the immune surveillance system in echinoderms. Of the heavy metals tested, only zinc affected the immune response and the degree of effect varied with concentrations. At 1 mM or higher concentrations, hemolytic activity was depressed but the inhibition was reversible. Upon removal of zinc by dialysis or by addition of a chelating agent (EGTA), the hemolytic activity completely recovered. However, at lower concentrations (0.1 mM, 0.25 mM, and 0.5 mM), zinc did not depress the hemolytic activities, in fact, it slightly enhanced it; it was thought that zinc, at lower concentrations, might function as a stabilizing agent in the hemolytic process. The effect of heavy metals on cellular immunity such as phagocytosis and encapsulation in echinoderms is unknown.

Blood cells of some invertebrates can accumulate heavy metals. For example, in the ascidian, *Ascidia mantula*, large deposits of vanadium in membrane-bound vesicles have been found in vandocytes (a type of blood cell). Similarly, it has been found that in oysters, haemocytes are extremely effective in detoxifying copper and zinc (George 1990). Echinoderm coelomocytes might function much like ascidian vandocytes or oyster haemocytes, since it has been found that some echinoderms can survive in heavy metal-contaminated areas (Papadoula and Kanias 1976, Bohn 1979, Warnau et al. 1995).

Organic pollutants

Massive oil pollution has been known to destroy echinoderm populations. According to Axiak and Saliba (1981), prolonged contact with oil not only decreases the adhesion capacity of sea urchin tube feet to the substrate, but also decreases the response of the spines to mechanical stimuli. Crude oil or petroleum fractions can have some negative effects on the reproduction and development of sea urchins, especially at the late embryo and early larval stages (Crawford and Muto 1977).

Interestingly, populations of littoral echinoderms are favorably affected by moderate organic (domestic) pollution. According to Pearse et al. (1970), a high density sea urchin population was found in an area with a higher organic content from sewage.

However, the effect of organic pollutants on echinoderm immune function is unknown and we believe that this is an area which needs our attention.

MOLECULAR STUDIES OF COELOMOCYTES

Some echinoderms, such as the purple sea urchin *S. purpuratus*, present a model system for a number of molecular studies: oogenesis and development (Raff 1987); regulation of gene expression during development (Guidice 1993); heavy metal response (Harlow et al. 1989, Cserjesi et al. 1992, Bai 1993); and coelomocyte studies (Smith et al. 1992, Smith et al. 1994).

Echinoid molecular biology is far advanced when compared with other extant classes of echinoderms (Smiley 1990), which is likely due to the

fact that the first eukaryotic mRNA was identified from sea urchins (Kedes and Gross 1969). A number of genes have been isolated and characterized from genomic DNA of sea urchins since 1991 (see review by Giudice 1993).

Metallothionein genes which encode the heavy metal-binding protein, metallothionein (MT), have been well characterized in sea urchins. Exposure of animal cells to heavy metals results in an accumulation of MT mRNA. *S. purpuratus* and *L. pictus* have often been used as experimental models in sea urchin MT gene studies. There are approximately 7 MT genes in the genome of *S. purpuratus* and 7 to 10 MT genes in that of *L. pictus* (Durname and Palmiter 1981).

The SpCoel1 gene, a profilin gene, was cloned from the coelomocyte cDNA library in the sea urchin, *S. purpuratus* (Smith et al. 1992). This has been the only gene studied in coelomocytes of echinoderms to date.

The 4-Kb transcript from the SpCoel1 gene has a relatively short (426 nucleotide) open reading frame which encodes a protein showing close similarity in amino acid sequence to profilins in other organisms (from yeasts to mammals). Profilin is a 12 000- to 15 000-dalton cytoplasmic protein that binds to and interacts with actin (Smith and Davidson 1994). Monomer (G-) actin is released from a depolymerizing filament in the form of ADP-G-actin, which is 5 to 10 times less likely to re-incorporate into a polymerizing filament than is ATP-G-actin. Profilin can catalyze the adenosine nucleotide exchange on G-actin, i.e., catalyze the displacement of ADP in ADP-G-actin by ATP to promote filament polymerization (Goldschmidt-Clermont et al. 1992), resulting in cell motility and change of cell shape. Therefore, the elevation of the intracellular profilin level will result in an increase of cell motility and other cellular behaviors related to the cytoskeleton. Profilin actually acts as a regulator of cell shape. It has been shown that when physiologically challenged (by needle puncture, withdrawal and re-injection of self coelomic fluid, or injection of foreign coelomic fluid), profilin transcript shows a significant increase per coelomocyte compared with that of the control (Fig. 5), this suggests that the sea urchin is sensitive to perturbations of the coelom and coelomocytes respond to the challenge by elevation of profilin transcripts. Statistically, however, there was no significant difference of transcript titration among different challenge treatments, indicating that the response of the sea urchin to the disturbance is independent of the degree of injury and challenge. On the other hand, the elevation of the profilin transcripts in the coelomocytes was correlated with the increase of coelomocyte motility (Smith et al. 1992, Smith and Davidson 1994), indicating that profilin is a link between external injury and the cytoskeletal change of coelomocytes.

Smith et al. (1994) found that the transcription of profilin in the egg and cleavage stages is minimal and that it increases substantially at the gastrulation stage in *S. purpuratus*. There were only 5 800 transcripts per embryo at the blastula stage, increased to 11 500 at the midgastrula stage, and to 21 000 by the completion of gastrulation. In situ hybridization showed that the newly synthesized profilin transcripts are localized in mesenchyme cells, which are equivalent to coelomocytes in adults. The increase of profilin gene expression coincides with the initiation of cell migration, filopodial extension, and retraction of the mesenchyme cells during gastrulation, indicating that the embryonically produced profilin plays an important role in regulating the cell shape at early stages of morphogenesis. Moreover, the transcript level of profilin in mesenchyme cells is equivalent to that in activated adult coelomocytes (those being challenged by injury or foreign cells). Thus, it appears that profilin gene expression participates in the mechanism of cytoskeletal activities in both mescenchyme cells of embryos and coelomocytes of adults (Smith et al. 1994).

CONCLUDING REMARKS

There are 6 types of blood cells (coelomocytes) in the body cavity and various tissues of echinoderms: phagocytes, spherule cells, vibratile cells, crystal cells, progenitor cells, and haemocytes; they originate from the mesoderm epithelium of various parts of the body and no stem cell has been found.

Echinoderms deal with pathogenic invasions by means of humoral and cellular immunity responses. Humoral immunity refers to neutralization or removal of foreign materials by secretion of protein substances such as bacteriocidin, hemolysin, and agglutinin. Cellular immunity refers to phagocytosis, encapsulation, and cytotoxicity. Phagocytes function by searching for, capturing and destroying foreign materials. The phagocytosed materials are either digested by the lysosomal enzymes in the phagocytes or discharged to the external environment through the body wall, madreporite, respiratory tree, or water vascular system. Quan-

titative studies show that phagocytosis is positively correlated with the abundance of particles present until a level of saturation is reached. It would be interesting to understand the relationship between saturation level and particle size. We predict that the volume of the particles rather than the number of particles is the limiting factor of the saturation.

There are 2 morphologically distinct phagocytes: motile petaloid phagocytes and non-motile filopodial phagocytes. Hypotonic stimuli as well as phagocytosis can cause transformation from the petaloid to the filopodial form.

Encapsulation is a mechanism for isolating and removing large particles, such as parasites within the body. The encapsulated structure, known as a brown body, consists of phagocytes and spherule cells surrounding the captured object in the center.

Cytotoxicity is involved in the rejection of tissue grafts. A short-term immunological memory has been indicated in echinoderms, but no particular coelomocyte has been identified as the memory cell. There have been no publications on echinoderm cytotoxicity and graft rejection in the past decade, which is unfortunate as we believe this is a rewarding area of research.

In echinoderms, the response to body wall injury includes the formation of a clot by filopodial phagocytes and local production of collagen materials by spherule cells; the fibrous matrix thus formed serves as the ground substance in wound healing and regeneration.

Some echinoderms have been found in areas contaminated by heavy metals. It has been shown

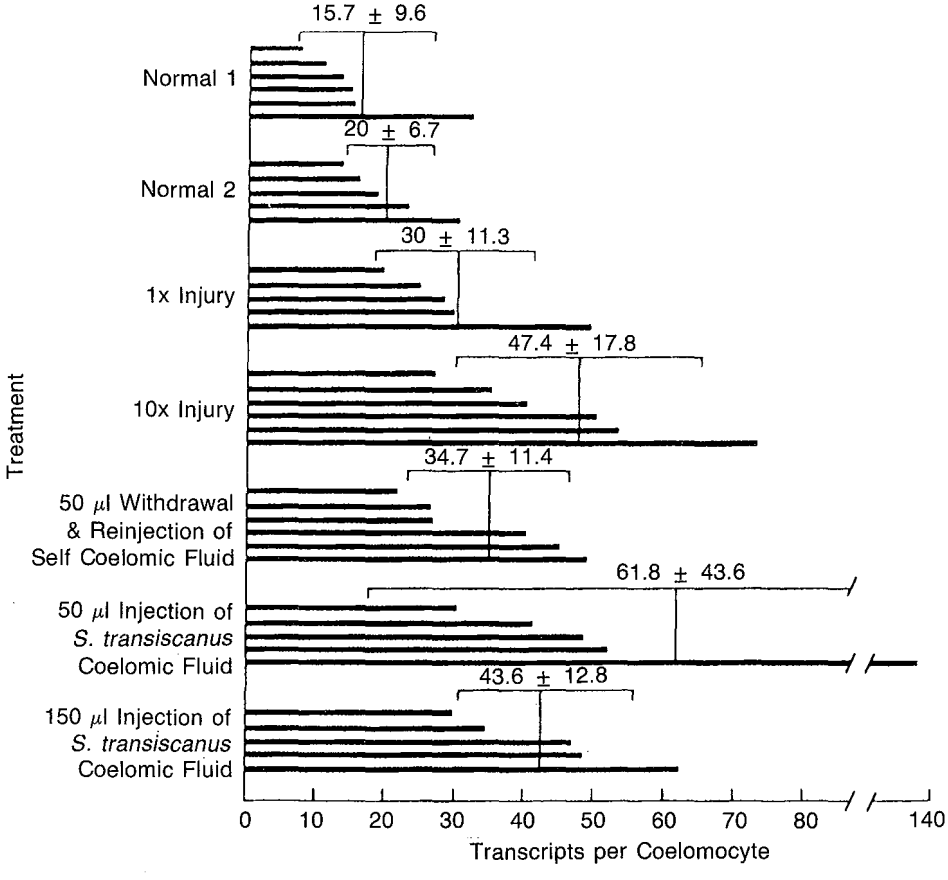

Fig. 5. Profilin transcript prevalences in coelomocytes from individual sea urchins after various treatments. The sea urchins were treated (treatments are listed on the left) on days 1, 2, 4, and 5; the cells were collected 24 h after the last treatment. Probe excess transcript titrations were performed on total RNA isolated from the coelomocytes. The symbols 1X and 10X represent 1 to 10 needle-hole injuries in the peristomial membrane per day on each day of treatment. Means and standard deviations are shown. The p values from Student's t test analysis for each experimental group compared to the combined normal groups are as follows: $p \leq 0.0005$, animals receiving 150 μl of foreign coelomic fluid; $p \leq 0.005$, animals receiving 10 needle-hole injuries per day; $p < 0.025$, animals receiving 1 needle-hole injury per day or those having 50 μl of their own coelomic fluid withdrawn and re-injected of those receiving 50 μl of foreign coelomic fluid (from Smith and Davidson 1994).

that zinc can have a harmful effect on humoral immunity, but the nature of the effect of heavy metals on cellular immunity (phagocytosis and encapsulation) is unknown. We believe that studies on this subject will provide insight into the survival strategy of echinoderms living in polluted areas. The coelomocytes may have the ability to sequester and detoxify heavy metals, much like that of blood cells in ascidians and oyster haemocytes.

Molecular studies of coelomocytes are still in the initial stages and to date only 1 gene (SpCoel1 gene) has been identified from echinoderm coelomocytes. More studies in this area should be profitable as coelomocytes can easily be obtained in large numbers.

To date, most of the publications on echinoderm coelomocyte culture have been on phagocytes. This is because, phagocytes are the most common coelomocytes and they have immunological functions.

Spherule cells are known to be involved in storage, regeneration, and discharge of echinochrome under adverse environmental conditions. However, we have no knowledge about the functional differences among the 3 types of spherule cells. In addition, they also show amoeboid movement which it has been postulated that this maybe related to pinocytosis (cellular drinking). Further studies along this line, especially in vitro examinations are needed.

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Abbreviations used: *A. forbesi*, *Asterias forbesi*; *A. rubens*, *Asterias rubens*; *A. vulgaris*, *Asterias vulgaris*; *C. miniata*, *Cucumaria miniata*; *C. stephensoni*, *Cucumaria stephensoni*; *C. tricolor*, *Cucumaria tricolor*; *D. imbricata*, *Dermasterias imbricata*; *E. quinquesemita*, *Eupentacta quinquesemita*; *H. leucospilota*, *Holothuria leucospilota*; IL-1, interleukin-1; *L. pictus*, *Lytechinus pictus*; MT, metallothionein; MEM, minimum essential medium; *P. nodosus*, *Protoreaster nodosus*; RER, rough endoplasmic reticulum; *S. droebachiensis*, *Strongylocentrotus droebachiensis*; *S. franciscanus*, *Strongylocentrotus franciscanus*; *S. purpuratus*, *Strongylocentrotus purpuratus*.

棘皮動物體腔細胞

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本文綜述了 1981 年以來棘皮動物血液細胞 (體腔細胞) 研究的進展，特別是體腔細胞免疫功能，細胞培養及分子生物學方面的新發現，我們對蕩皮海參 (*Holothuria leucospilota*) 體腔細胞的研究也貫穿文中。

棘皮動物具有六種體腔細胞：吞噬細胞、球狀細胞、顫動細胞、晶體細胞、前體細胞及血細胞。這些細胞源自身體各部分的腔上皮，它們在體液中的濃度爲每毫升一百萬至六千萬細胞。

棘皮動物同時具有體腔免疫及細胞免疫。細胞免疫包括吞噬作用、包囊作用及細胞毒作用，主要由吞噬細胞完成。球狀細胞參與愈傷及再生作用。分子生物學研究表明，體腔細胞內的肌動蛋白結合蛋白質 Profilin 可以接受並傳遞受傷信號，引致其他體腔細胞的免疫反應。

許多證據表明脊椎動物免疫系統可能源自棘皮動物免疫系統。例如：棘皮動物體腔細胞與脊椎動物血細胞具有形態及功能的相似性；棘皮動物的褐體類似於脊椎動物的多核大細胞；棘皮動物遇到異物時會產生類似於脊椎動物的炎症反應；以及棘皮動物體液內存在著類似脊椎動物吞噬作用調節劑 interleukin-1 的物質。

文中還指出了棘皮動物體腔細胞研究中值得深入探討的問題，我們希望這對有意致力於本領域研究的同行有所幫助。

關鍵詞：吞噬作用，免疫學，褐體，體液。

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