

## THE BLOOD CELLS OF A SEA URCHIN FROM ABOU-KIR COAST ALEXANDRIA, EGYPT.

By

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### خلايا الدم في أحد قنafd البحر من شاطيء أبو قير (الاسكندرية - مصر)

محمد علوي عبد الحميد و محمد حسن منا  
فايز عبدالمقصود شكر و أمال إبراهيم سيف و سامية هانم عيسى

يهدف هذا البحث إلى دراسة الخلايا الدموية في أحد الأنواع الشائعة من قنafd البحر (باراسنتروتوس ليفيدس) على شاطيء أبو قير وذلك باستخدام الميكروسكوب الضوئي والالكتروني .

وقد أوضحت الدراسة تميز الخلايا الدموية إلى ثلاثة أنواع رئيسية هي : الخلايا الليمفية والاميبية والتوتية . وأمكن تقسيم الخلايا الاميبية إلى أميبية هلامية والتهامية وفجوية ومحبيه وتؤكد الدراسة أن الخلايا الأميبية غنية بالمواد السكرية والبروتينية بينما الخلايا الليمفية والتوتية فتوجد بها المواد البروتينية وتتعهد المواد السكرية . كما ناقش البحث الوظائف المحتملة لكل نوع من الخلايا الدموية .

*Key Words: Blood cells, sea urchin, light and electron microscopes*

#### ABSTRACT

The blood cells of a common sea urchin, collected from Abou-Kir region, were investigated using light and electron microscopy. Their distribution, properties, characteristics and histogenesis were determined. On the basis of differences in structure and behaviour towards various dyes the blood cells fall into three basic categories: lymphocytes, amoebocytes and morula-shaped cells. Amoebocytes include the hyaline, phagocytic, vacuolated and granular forms. The probable functions of the blood cell-types, where possible, are suggested.

#### INTRODUCTION

A variety of free cells, known generally as coelomocytes, occur in the coelomic, haemal systems, water-vascular systems and amongst tissues of echinoderm body. These cells have been investigated by numerous workers and the results of these investigations, were well summarized by Hyman (1975). Consequently, Boolootian and Giese (1958 & 1959) listed most of the papers that contributed to the study of echinoderm coelomocytes, that is, all the cell types found in the coelomic fluid, and provided an interpretation of the living cell types as observed with phase-contrast optics. Abraham (1963) and Hetzel (1963 & 1965) have reported some studies on living coelomocytes from echinoids and holothurians, respectively. Also Holland *et al.*, (1965) made an autoradiographic study on the coelomocytes of the sea urchin, *Strongylocentrotus purpuratus* employing tritiated thymidine. He determined the possible sites of coelomocyte (or coelomocyte precursor) proliferation and the transformation of precursor cells into coelomocyte types. Johnson (1969) clarified the presence of

four cell types in *Strongylocentrotus*: phagocytic leukocytes, vibratile cells, red spherule cells and colourless spherule cells.

Several reports of the differentiation of one type of coelomocyte into another type in sea urchins have been given. For instance, Frenzel (1892) believed that coelomocytes differentiated into bladder and filiform amoebocytes. Kindred (1926) reported that bladder and filiform amoebocytes are transformed into colourless spherule amoebocytes, which are subsequently converted to eleocytes. Liebman (1950) held that the vibratile cells, after originating from the peritoneum, transformed into bladder and filiform amoebocytes. Schinke (1950) reported that the colourless spherule amoebocytes, after arising from connective tissue cells of the dermis, possibly differentiated into bladder and filiform amoebocytes.

The disagreement exists among the descriptions of different authors and the diversity of opinions concerning the origin and interrelationships of echinoid coelomocyte types prompted the present investigation using electron microscopy.

## MATERIAL AND METHODS

Specimens of the sea-urchin *Paracentrotus lividus* were collected from Abou-Kir region (Mediterranean Sea) at November, 1988. The specimens were transported to the laboratory in sea water. The axial organ, water vascular system and gut were dissected out of the animals and the prepared for light and electron microscopic investigation.

For light microscopy, pieces of the chosen organs were fixed in 10 formalin, then dehydrated in a series of ethanol and cleared up in terpineol. The material was embedded in paraffin wax, then serially sectioned (5  $\mu$ -thickness) and stained with Ehrlich's haematoxylin and eosin. To reveal the presence of carbohydrates and proteins, periodic acid-Shiff method (Hotchkiss' 1948), alcian blue method (Luna, 1968) and the mercuric bromophenol blue method (Mazia *et al.*, 1953) were used.

For electron microscopy, tissues of the chosen organs were fixed in glutaraldehyde (0.8) for 2 hours at 0°C and postfixed in osmium tetroxide OsO<sub>4</sub> buffered at pH 7.2-7.4 with Na-cacodylate for 2 hours at 0°C. Tissues were then rapidly dehydrated in ethanol and embedded in Epon 812. Ultrathin sections were cut with ultramicrotome using glass knives and stained with lead citrate and uranyl acetate and examined with JEM-100C XII electron microscope.

## RESULTS

A coelomocyte is a generic term for several cell types which circulate in the fluid of the coelomic cavities and also wander freely throughout most tissues and organs of the sea urchin, notably in those of the haemal system. Although several contradictory theories have been given on different organs and tissues as sites of coelomocyte production in sea urchin, it is evident during this work that the axial organ is considered as the site of coelomocyte formation. The previous evidence is primarily supported from the observation that the axial organ, in addition to its cells, has been distended with a huge number of various types of coelomocytes. Thus, the coelomocytes can be easily described from histological sections throughout the axial organ.

On the basis of differences in structure and behaviour towards various days, three distinct cell types were recognized, namely: lymphocytes, amoebocytes and morula-shaped cells. However, this classification is a purely arbitrary one and some of these cells represent stages in the development of others.

### 1. Lymphocytes:

Lymphocytes are the most abundant cell-type in the axial organ. Also, they are found in the water-vascular system and the gut wall. Lymphocytes in their basic form are small, spherical cells (Fig. 1), average approximately 3.2-3.8  $\mu$ m in diameter. Each cell consisted essentially of a single large spherical nucleus, nearly filled the entire cell. Mesh-like aggregations of basophilic material were apparent in the nuclei of these cells. These nuclei are investigated by a thin cytoplasmic envelope. Histochemical reactions indicated that the lymphocytes are essentially composed of proteins (Fig. 2). This was observed by the blue colour produced after treatment with bromophenol blue stain. With PAS and alcian blue stains (Fig. 3 & 4), a weak reaction was obtained indicating that the lymphocytes have a very little quantity of acidic polysaccharide materials.

At the electron microscope level (Fig. 11), the nucleus of lymphocyte has more dense chromatin, heterochromatic mass found along the nuclear envelope and euchromatin associated with the nucleoli. Usually one or two small nucleoli, which are compact electron-dense structure, are occasionally visible in the centre of the nucleus. The chromatin associated with the nucleolus, probably corresponds to the chromosomal region which come in contact with the nucleolus at the beginning of cell division. The cytoplasm of these cells has lacked typical differentiated structures, apart from many free ribosomes, which account for the detection of a large amount of RNA as described by Enden (1960) in these cells. Sometimes, some of the lymphocytes possess small pseudopodia (Fig. 12). Therefore, these cells seem to be the primitive cells and they may give rise to other cell types as have consistently considered by many investigators (George, 1939; Endean, 1960; Hetzel, 1965).

### 2. Amoebocytes:

Series of cells intergrading between lymphocytes and morula-shaped cells were commonly found and collectively termed amoebocytes.

At the light microscope, amoebocytes are large cells usually 9-12  $\mu$ m in diameter and possess irregular outlines. Their shape varies from spherical to oval and sometimes possess polygonal angles. These cells (Figs. 5 & 6) are characterised by their possession of prominent nuclei usually situated in the middle of the cytoplasm or departed to one pole of the cell. The cytoplasm of these cells appears to be clear without inclusions. It was observed that these cells react strongly with PAS while they don't stain with alcian blue (Figs. 7 & 8). Also, they contain a large amount of protein as evidenced by the blue colouration they assumed when the HgCl<sub>2</sub>/bromophenol blue stain was used (Fig. 9). Thus, these cells are formed essentially of conjugated proteins.

Examination at the electron microscope revealed different shapes, size and different constituents in the cytoplasm of these cells. According to these variations, amoebocytes can be divided into four types known as hyaline amoebocytes, phagocytic amoebocytes, vacuolated amoebocytes, granular amoebocytes.

#### 2.1 Hyaline amoebocytes:

This cell (Fig. 13) has an oval shape and contains a relatively large oval nucleus. The nucleus has an envelope consisting of outer and inner membranes. Nuclear pores are seldom seen. The chromatin is dispersed in the nucleoplasm. Also, the heterochromatin fibres are closely pressed in the inner nucleus membranes. The cytoplasm of these cells is finely granular and lack organelles except for few mitochondria which are distributed randomly.

#### 2.2 Phagocytic amoebocytes:

Phagocytic are relatively large cells, having irregular outlines. Long and broad pseudopods radiate from these cells, which contain prominent nuclei (Fig. 14). The nuclear chromatin is densely packed and divided into 3-4 condensed clumps found in the center of the nucleus. Abundant heterochromatin is attached to the inner membrane. Occasionally, large electron-dense inclusions are present in the cytoplasm, which point to a phagocytic activity. This cell-type may be considered as a transition stage results from a gradual increase in the cytoplasm of lymphocytes. At an early stage in this transition, pseudopodia are extended and the cytoplasmic inclusions become apparent. Because of the

presence of pseudopodia, these cells are believed to be phagocytic.

**2.3 Vacuolated amoebocytes:**

Each cell possesses a spherical nucleus and a finely granulated cytoplasm in which a variable number of vacuoles occur. These vacuoles occupy nearly all the cell. Thereby, the nucleus is relatively small and lies between the vacuoles (Fig. 15). The chromatin which is deeply and homogeneously stained and highly condensed, occupies the whole of the much reduced nucleus. The cytoplasm is devoid of organelles, and its most striking feature is the great number of free ribosomes. In some cells (Fig. 16), the smaller vacuoles coalesce to form one large vacuole. In these cells, the cytoplasm contains many small dark granules, a few mitochondria and many ribosomes. This type of cell may be a stage of the developmental stages for the formation of signet ring cell described before by Edean (1960). It is probable that the vacuolated amoebocytes arise directly from lymphocytes as a result of a further increase in cytoplasmic volume and as a result of vacuolization of the cytoplasm.

**2.4 Granular amoebocytes:**

Such type of blood cells has a variable shape and with a round to oval nucleus often showing condensed chromatin. It is characterised by having many granules which fill the cytoplasm (Fig. 17). Granules are homogeneous, moderately electron dense and bordered by a distinct undulating membrane. This cell may be considered as a stage of developmental stages towards the formation of morula-shaped cells.

**3. Morula-shaped cells:**

Morula-shaped cells (Fig. 10) appeared to correspond with the "migratory plasma cells" of Hamann (1883), and the

"colourless amoebocytes with spherules" of Theel (1921) and of Hyman (1955). They also bore a striking resemblance to the "ferrocytes" of the ascidian, *Pyura stolonifera* (Edean, 1955). The morula-shaped cells are more or less spherical to oval cells having a variable number of refractile wedge-shaped globules encased in the cytoplasm. The globules are uniform in size, varying in diameters, and fill the cytoplasm completely, sometimes obscuring the single oval nucleus. The cytoplasm and globules are eosinophilic, while in eccentrically placed nucleus is intensely basophilic. Conventional methods of staining neutral and acidic polysaccharides indicated that the morula-shaped cells devoid of these substances. They usually gave no reaction with PAS and alcian blue stains (Table 1). The morula-shaped cells became dark blue after treatment with mercuric-chloride bromophenol blue indicating the presence of considerable quantities of protein (Fig. 9). It must be noted that, the intense metachromasy with toluidine blue was exhibited by the globules of these cells (Table 1).

At the examination with electron microscope, the cytoplasmic globules (Fig. 18) have showed varying degrees of darkening and varying sizes. Thereby, the globules can be divided to the following types:

- Type I : Few large globules strongly electron-dense. They usually seem to lack any fine structures in the interior.
- Type II : Numerous large globules containing homogeneous material of moderately electron dense.
- Type III : many globules with electron-dense core; and the contour is less dense.

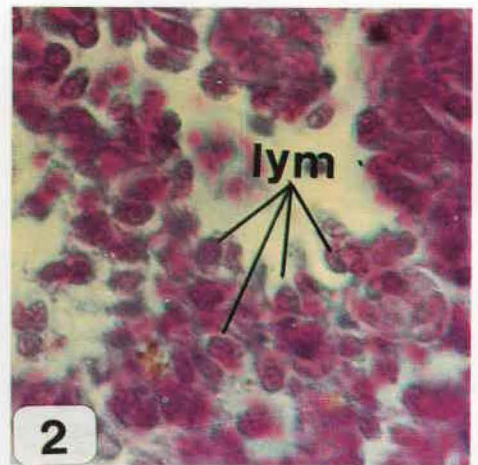
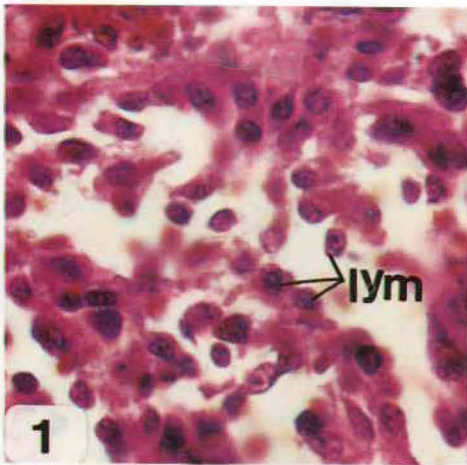
It was observed that morula-shaped cells, identical with those found in the axial organ, occurred also amongst the fibres of the connective tissue in the walls of gut and the walls of tube-feet.

**Table 1**  
Histological and histochemical reactions of the different types of blood cells.

| Dyes used               | Results           |           |                   |           |                     |           |
|-------------------------|-------------------|-----------|-------------------|-----------|---------------------|-----------|
|                         | Lymphocytes       |           | Amoebocytes       |           | Morula-shaped cells |           |
|                         | nucleus           | cytoplasm | nucleus           | cytoplasm | nucleus             | cytoplasm |
| Eosin                   | -                 | +         | -                 | -         | -                   | ++        |
| Ehrlich's haematoxylin  | ++                | -         | ++                | ±         | ++                  | -         |
| PAS                     | ±                 | ±         | ++                | ++        | ±                   | ±         |
| Alcian v. blue PH 2.5   | -                 | -         | -                 | -         | -                   | -         |
| Alcian v. blue PH 1.0   | -                 | -         | -                 | -         | -                   | -         |
| Toluidine blue          | Betametachromasis |           | Betametachromasis |           | Betametachromasis   |           |
| Aldehyde fuchsin        | ++                | ±         | ++                | ±         | -                   | ++        |
| Hgc 12/bromophenol blue | ++                | ++        | ++                | ++        | ++                  | ++        |

The previous convention was utilized to express intensity of staining:

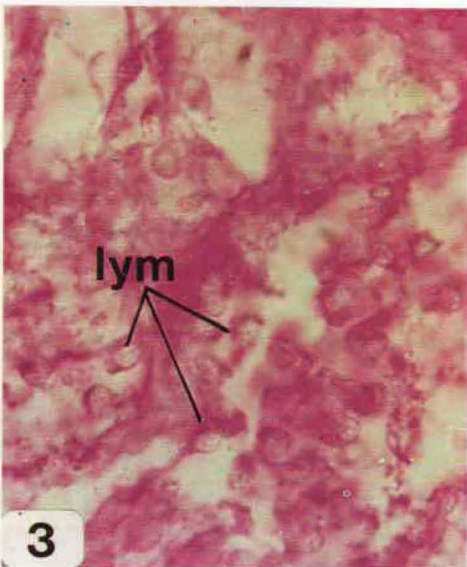
- negative
- ± weak positive
- + definitely positive
- ++ strong positive



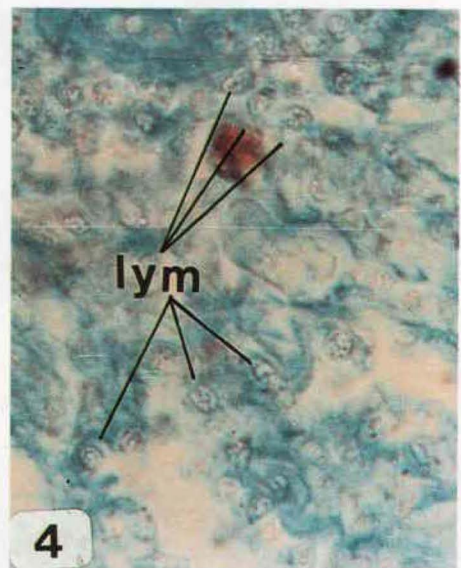
Figs. 1-4: T. S. of the axial organ showing lymphocytes. X1000.

1: Stained with Ehrlich's haematoxylin and eosin.

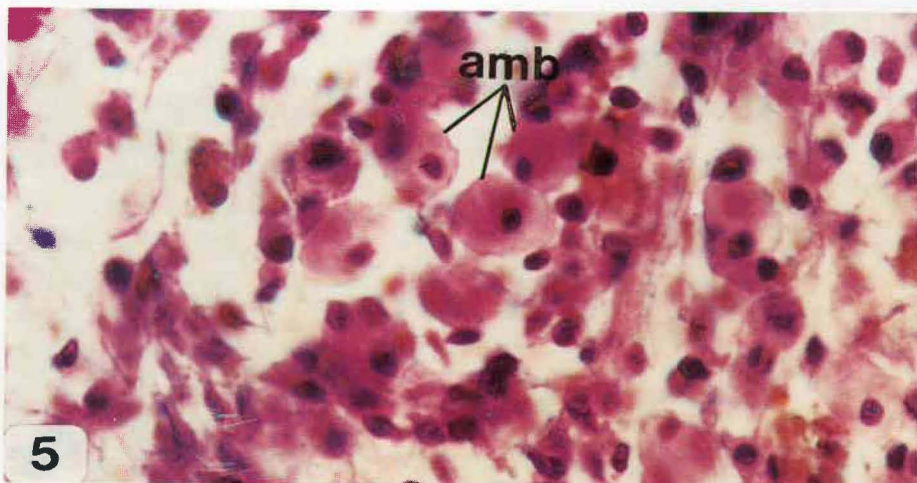
2: Stained with bromophenol blue.



3: Stained with PAS.



4: Stained with alcian blue.



Figs. 5, 7, 8: T. S. of the axial organ showing amoebocytes. X1000.

5: Stained with Ehrlich's haematoxylin and eosin.

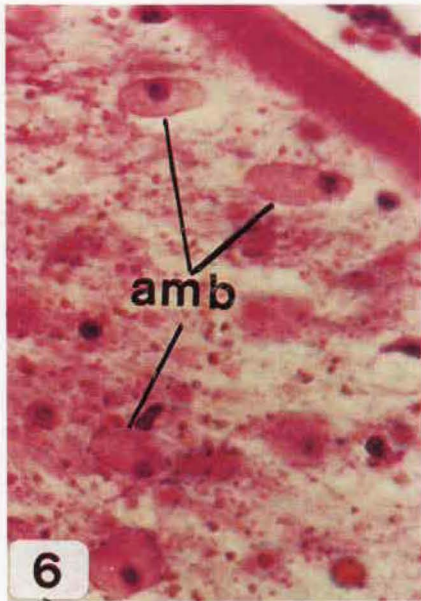
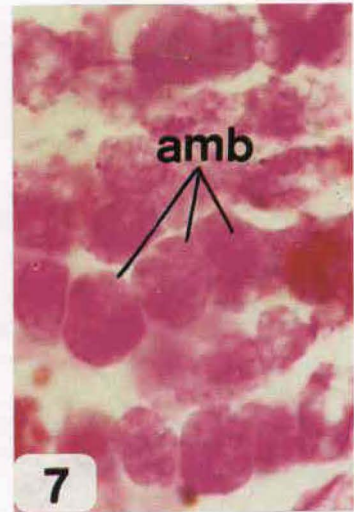
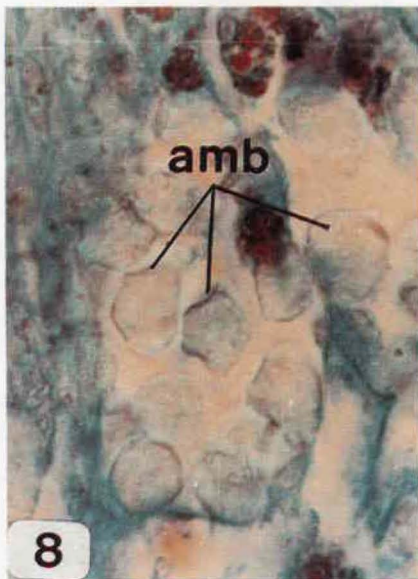


Fig. 6: T. S. of the pharynx showing amoebocytes, stained with Ehrlich's haematoxylin and eosin. X1000.



7: Stained with PAS.

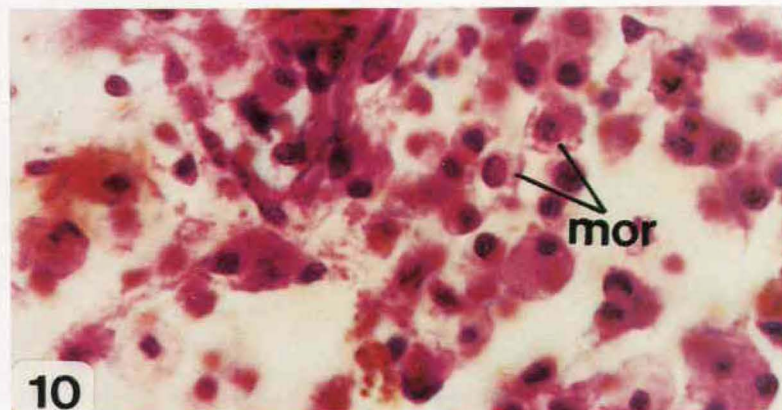


8: Stained with alcian blue.

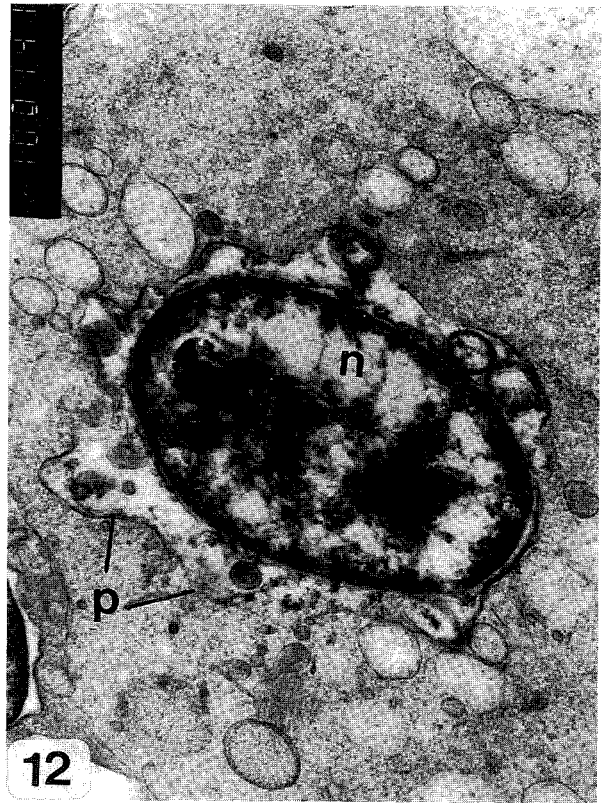


Figs. 9 & 10: T. S. of the axial organ showing morula-shaped cells. X1000.

9: Stained with bromophenol blue.

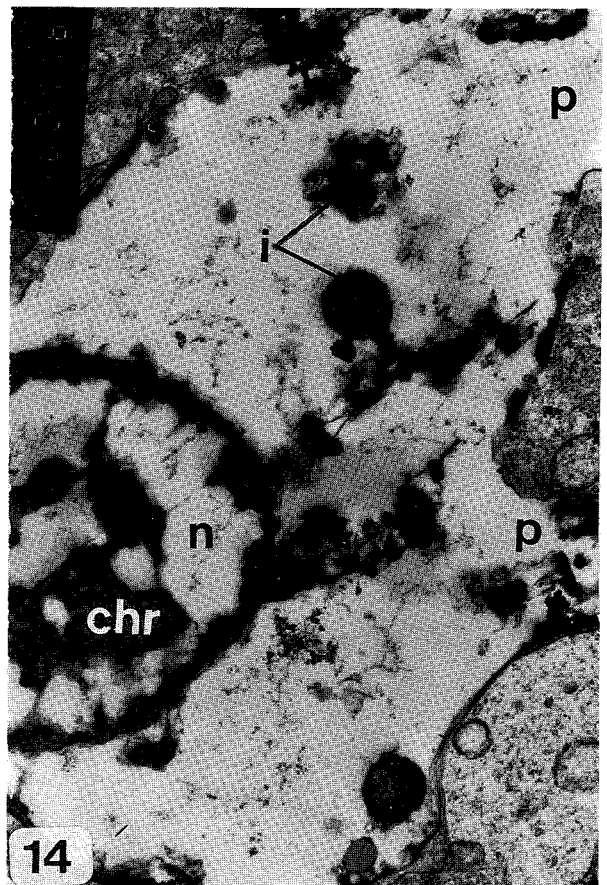
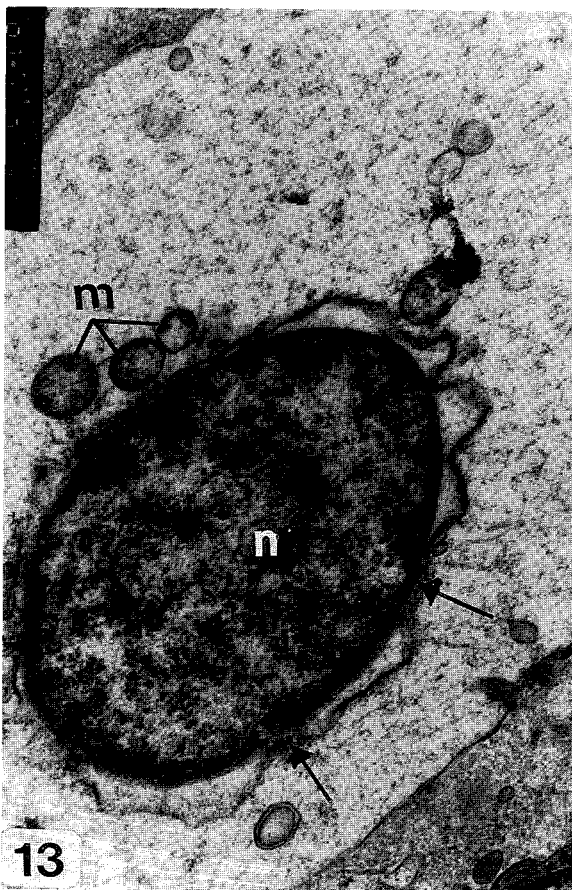


10: Stained with Ehrlich's haematoxylin and eosin.



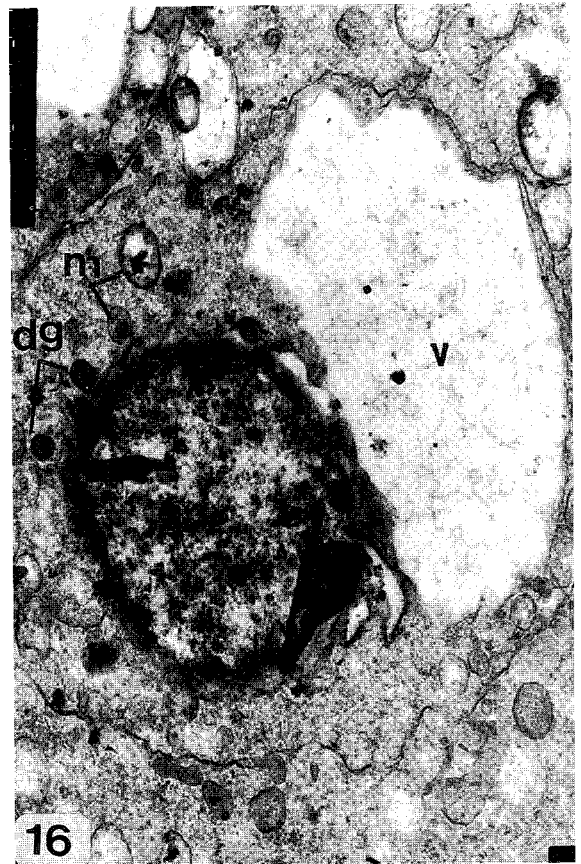
Figs. 11 & 12: Electron micrograph of a lymphocyte cell. 11: Showing nucleolus and ribosomes. X20000.

12: Showing pseudopodia. X10000.



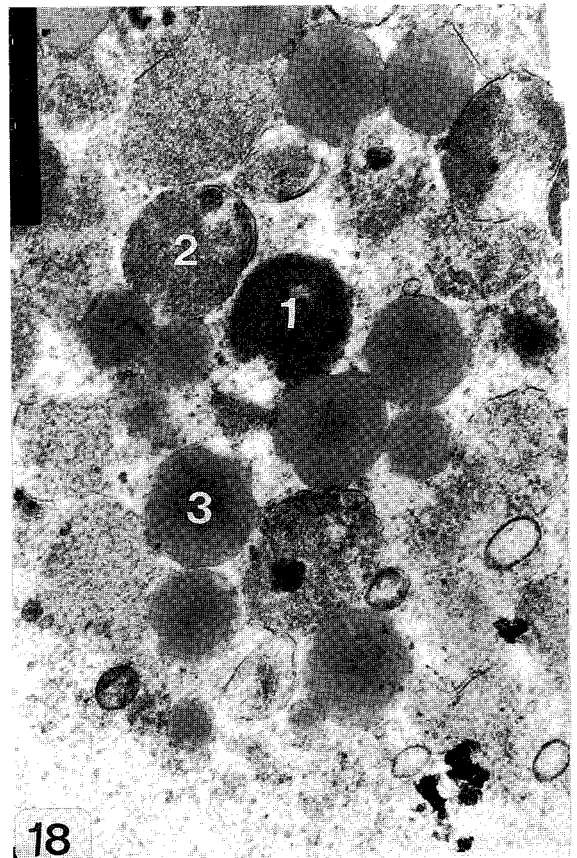
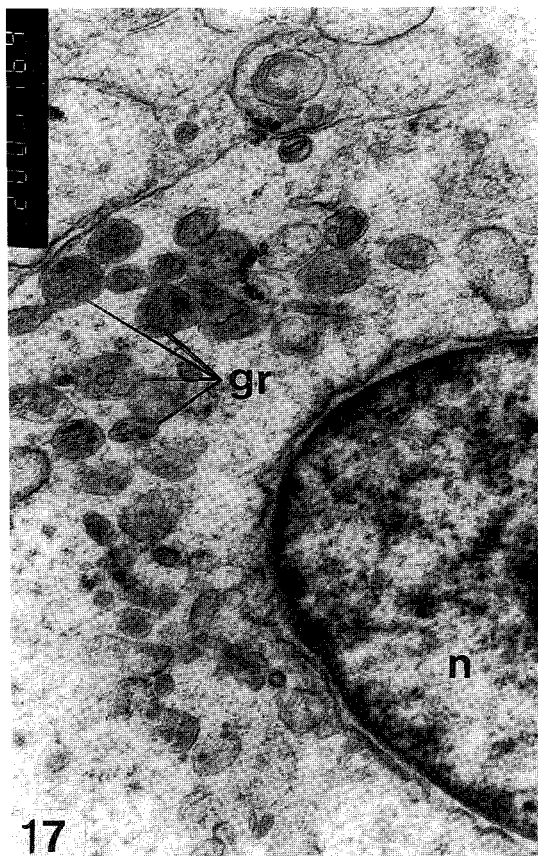
Figs. 13: Electron micrograph of a hyaline amoebocyte showing nuclear pores (arrows). X14000.

Figs. 14: Electron micrograph of a phagocytic amoebocyte showing amoebocyte pseudopodia. X10000.



Figs. 15 & 16: Electron micrograph of a vacuolated amoebocyte showing vacuoles.  
15: X 10 000.

16: X 14 000.



Figs. 17: Electron micrograph of a granular amoebocyte showing granules. X 20 000.

Figs. 18: Electron micrograph of a morula-shaped cell showing the three different types of globules (1, 2, 3). X 14 000.

#### ABBREVIATIONS

amb., amoebocytes; chr., chromatin; dg., dark granules; gl., globules; gr., granules; i, inclusions; lym., lymphocytes, m., mitochondria; mor., morula-shaped cells; n., nucleus; p., pseudopodia; r., ribosomes; v., vacuole.

A comparison between the different types of coelomocytes is summarized in Table (1).

#### DISCUSSION

The coelomocytes of the sea-urchin, *Paracentrotus lividus*, have the same general morphology as those previously reported in echinoids and holothurians (Johnson, 1969; Fontaine and Lambert, 1973, 1977; Mona *et al.*, 1989). The rich variety of cell types found in echinoderm as well as in ascidian blood has led to speculation regarding their developmental interrelationships (Ohuye, 1938; George, 1939; Schinke, 1950; Endean, 1960; Kalk, 1963, 1964; Hetzel, 1965). Based on the available literature and on the findings of the present investigation, one is led to support Ohuye's (1938) conclusion that the various coelomocyte type are possible derived from a common source of stock of stem cells, and that all types of coelomocytes may be produced by direct transformation of the stem cells. Various investigators agree that the lymphocyte is the source of all other blood cell types either directly or indirectly (Ohuye, 1938; Endean, 1960; and Hetzel, 1965; Mona *et al.* 1983).

As proposed by Hetzel (1965), it was noted that lymphocytes may differentiate into amoebocytes and morula-shaped cells. Lymphocytes are presumed to increase in size and produce pseudopodia as they differentiate into phagocytic amoebocytes.

Also, the herein observations indicate that the lymphocytes may form morula cells. The course of this differentiation is believed to be as follows: the hyaline cytoplasm of lymphocytes increases in size and gradually becomes granular forming granular amoebocytes; the granules gradually increase in size and are transformed into the spherules typical of morula cells.

The site of origin of lymphocytes proved to be a problem not easily resolved. Hausman (1931) maintained that they originate from cells of the peritoneal lining of the coelom. This may be so, but would be difficult to prove conclusively. Ohuye (1938) did not rule out the possibility that mesenchymal connective tissue cells may contribute to the formation of lymphocytes in holothurians. Schinke (1950) studied the origin and differentiation of coelomocytes of the echinoid, *Psammechinus miliaris*. This author reported that lymphocytes were formed by direct transformation of cells in the connective tissue matrix of the calcareous tests. In light of all these previous studies, and the observations made in the present investigation, it seems reasonable to assume that lymphocytes to assume that lymphocytes are produced from the peritoneal epithelium which covers the axial organ. The similar appearance, size and the similarity of the nucleus of two cells confirm this assumption.

When considering the functions of the studied coelomocytes, it is well to remember that echinoids, like all invertebrates, often lack organ systems and the cells found in the haemal system may each serve a variety of functions. Hyman (1955) said to the holothurians "it would seem that these animals operate on a very primitive basis, that each organ system covers more than its usual function, and that the amoebocytes play a remarkable role in the economy" and a similar situation appears to be present in the echinoids. Morula cells are referred to as trephocytes by Liebman (1946, 1947) who assigned them the role of food storage. Lymphocytes apparently have no definitely established function, but seem likely to be the stem cell form from which the other coelomocyte type are derived. The amoebocytes are probably involved in phagocytic activity and in nutrition and metabolism as pre-

viously reported by Goodbody (1974). However, the authors feel that these assigned functions of coelomocytes deserve further investigation.

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