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ULTRASTRUCTURAL STUDIES OF HEMOCYTES IN SCORPION, *HETEROMETRUS XANTHOPUS*

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ABSTRACT
The ultrastructure of hemocytes of scorpion *Heterometrus xanthopus* is studied in this present investigation. Light and phase contrast microscopic observations have shown seven distinct types of hemocytes. However with TEM only six types of hemocytes were observed. The hemocytes observed were – Prohemocytes (PRs), Plasmocytes (PLs), Granulocytes (GRs), Spherulocytes (SPs), Oenocytoids (OEs) and Adipohemocytes (ADs). The PRs were smallest cell type with large nucleus, cytoplasm with few cell organelles and homogenous smooth surface. The PLs were polymorphic, cytoplasm rich with several organelles and cellular surface with several cytoplasmic projections. The GRs were abundant in number and with three types of cytoplasmic granules. The SPs were having cytoplasm rich in spherules. The OEs were with homogenous cytoplasm and with scarce cell organelles. The ADs were with variable size and shape and cytoplasm with fat droplets.

INTRODUCTION

The hemocytes in arthropods, like vertebrate leucocytes, are a mixture of cell types with different morphological and physiological functions (Brookman *et al.,* 1989; Hung and Boucia, 1996). As the hemocytes in scorpion play an important role in physiological functions, the present study has been undertaken. The classification and types of hemocytes in insects were studied by Gupta (1979). He described seven types of hemocytes. The review of literature has shown that the study of hemocytes in scorpion is limited (Ravindranath, 1974; Shah and Patil 2011; Patil and Shah, 2011). These studies were carried out with light and phase contrast microscope. Hence we studied ultrastructure of a Indian scorpion *H. xanthopus*. This paper aims at characterizing morphologically and morphometrically hemocyte types, using electron microscope.

MATERIALS AND METHODS

The scorpions used in the present investigation were collected from Dadaswadi, Taluka- Atpadi and Dist- Sangli and were kept in perforated plastic jars and fed with small cockroaches. The animals were maintained for a month without any significant mortality.

Hemolymph was collected from the living animals as per the method of Padmanabha (1967). Depending upon the size of the specimen, the volume of hemolymph varied, but on an average about 1 to 3 mL could be easily collected. The hemolymph was transferred to a small, clean plastic BEEM capsule served as a container for centrifugation, fixation and dehydration and for block preparation.

After centrifugation hemolymph pellet was formed at 4ºC. After dehydration, the hemolymph pellet was embedded in Araldite. With the help of ultra tome, 600- 900Aº ultra-thin sections were cut with the help of glass knife. The sections were stained by 12.5% alcoholic uranyle acetate, washed with distilled water and observed under JEOL-100S electron microscope at 80 kV.

RESULTS AND DISCUSSION

Seven types of hemocytes were observed with light and phase contrast microscope. With TEM only six type of hemocytes were identified - Prohemocytes (PRs), Plasmocytes (PLs), Granulocytes (GRs), Spherulocytes (SPs), Oenocytoids (OEs) and Adipohemocytes (ADs)

Prohemocytes (PRs) - It was the smallest of all hemocytes in *H. xanthopus* (Fig. 1a). It was usually round or oval and about 6-10 μm in diameter. The plasma membrane was thin and showed vesiculation. The cytoplasm contains a moderate amount of endoplasmic reticulum, few mitochondria and abundant ribosomes. The nucleolus was about 4- 8 μm in diameter with compact nucleus.

Plasmocytes (PLs) – PLs were highly polymorphic and cells present in variety of shapes (Fig. 1b). The size varies from 10-30 μm in length and 3-10 μm breadth. The plasma membrane shows irregular processes and pinocytotic or vesicular invaginations. The cytoplasm was abundant and rich in cell organelles like unbound ribosomes, rod shaped mitochondria and cisternae of endoplasmic reticulum. The nucleus was oval, centrally placed, 4 -10 μm in length and 3-7 μm wide.

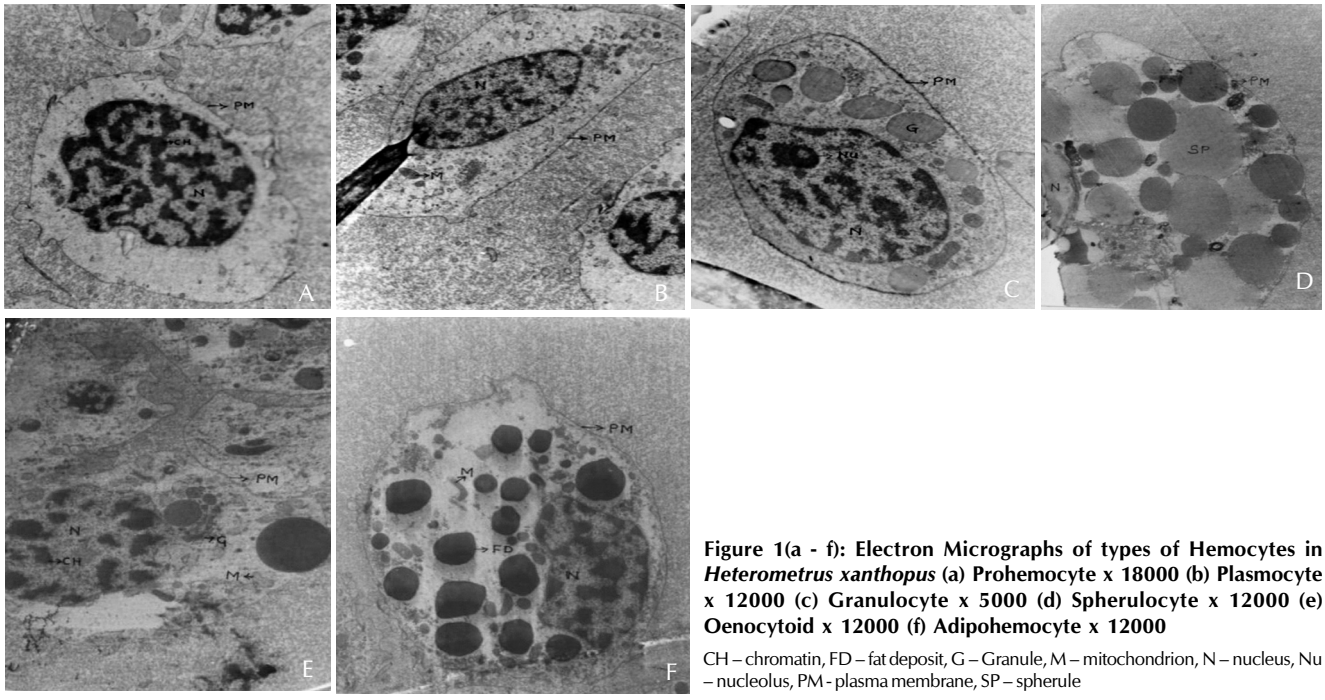

Figure 1(a - f): Electron Micrographs of types of Hemocytes in *Heterometrus xanthopus* (a) Prohemocyte x 18000 (b) Plasmocyte x 12000 (c) Granulocyte x 5000 (d) Spherulocyte x 12000 (e) Oenocytoid x 12000 (f) Adipohemocyte x 12000

CH – chromatin, FD – fat deposit, G – Granule, M – mitochondrion, N – nucleus, Nu – nucleolus, PM - plasma membrane, SP – spherule

The chromatin was scattered.

Granulocytes (GRs) – GRs were easily recognized by presence of stainable granules in the cytoplasm (Fig. 1c). The granules were 1-3 μm in diameter and bounded by thin membrane. However, under the light and phase contrast microscope it was very difficult to discern the structure of cytoplasmic granules. The TEM give a better insight into the nature of granules. These were classified into three types *structureless and electron dense granules; **structureless and thin granular bodies; ***structured granules.

The granules were spherical in shape and are about 10- 15 μm in diameter. The plasma membrane was thin and smooth. Along with granules, free ribosomes were also observed in the cytoplasm. The mitochondria were few in number. The nucleus was irregular in shape and eccentric in position. The chromatin was scattered forming dense clumps near the nuclear membrane.

Spherulocytes (SPs) - These were large in size (10-20 μm long and 5-10 μm in width), ovoid or round in shape and larger than granulocyte (Fig.1d). The plasma membrane was with exocytotic vesicles. The number of intracytoplasmic spherules was quite variable with diameter 1-5μm. The spherules were released into the hemolymph by exocytosis. The cytoplasm contains polyribosome, mitochondria and endoplasmic reticulum. The nucleus was eccentric in position with 5-9μm long and 2-3μm in width.

Oenocytoids (OEs) - OEs were variable in size and shape ranging between 15-45μm in diameter (Fig.1e). The plasma membrane was smooth. The cytoplasm showed irregularly distributed filaments and electron dense granules. Except mitochondria and ribosomes no other cell organelles were present. The nucleus was round measuring about 4- 10 μm in diameter. The chromatin was scattered and generally lies in the periphery.

Adipohemocytes (ADs) – These were also variable in size and shape (Fig.1f). These were spherical or oval and 10- 40 μm in diameter. The plasma membrane was with micro lamellae and exocytotic vesicles. The cytoplasm was with ribosomes, Golgi complex and mitochondria. The cytoplasm contains characteristic fat droplets with diameter 1- 6 μm. The droplets were intensely stained with Sudan Black B indicating lipid nature. The nucleus was comparatively small, eccentric and elongated.

Very little work has been carried out on scorpion hemolymph and hemocytes. Kollamann (1908) has described hemocytes of some non Indian scorpions. For the first time the efforts were made to investigate the ultra structure of scorpion hemocytes.

Based on Jones classification (Jones, 1962) seven types of hemocytes were observed by light and phase contrast microscope. However by TEM preparation only six types of hemocytes were identified.

PRs were having high nucleo-cytoplasmic ratio and considered as stem cells. Jalal and Rasoul (2008) have explained this during development of insect. First instar larva showed highest number of PRs, but as number of GRs and PLs increases, the population of PRs was decreased. Some frequent mitotic figures were encountered in these cells indicating their stem cell nature. But this was not observed in the present investigation. In the scorpion there was separate hemopoitic tissue is present (Ravindranath, 1974).

The plasma membrane of the PLs showed protoplasmic extensions in TEM preparation. The pinocytotic invaginations were also observed indicating phagocytotic nature of these cells. Due to this physiological function large number of PLs was present. Such a high number of PLs in scorpion may also be viewed from the point of their venomous nature and their special dietary requirement.

The GRs are considered as plesiomorphic hemocytes and

were only hemocytes type that has been reported from all arthropod groups. According to electron density of granules are classified into three types. There were conflicting views about the origin of GRs. These are considered as basic unit from which more precisely more structural and functional classes of cells have been developed (Arnold, 1974). The workers in the field do not recognized GRs as separate hemocytes and there was only one report by Devauchelle (1971) indicating GRs as separate hemocytes. The present investigation supports this view.

SPs are observed in all major arthropodan groups except aquatic chelicerata (Gupta, 1979). The spherules were membrane bound, non-refringent. The presence of exocytotic vesicles in the plasma membrane is inductive of their role in histolysis.

OEs were extremely fragile cells. As these were very small in number majority of workers do not consider these as a distinct hemocytes type. The present investigation found these cells with distinct features.

ADs are the most controversial hemocytes. A review of literature shows that ADs occur only in crustacean, myriopoda and insects. These are absent in onychophora (Gupta, 1979). Ravindranath (1974) has also noted the presence of ADs in scorpion *Palamnaeus swammerdami*. These were characterized by cytoplasmic fat droplets.

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