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HEMOCYTES IN SCORPION – *HETEROMETRUS PHIPSONI*

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ABSTRACT
Seven types of hemocytes have been identified in the hemolymph of scorpion *Heterometrus phipsoni* by light and phase contrast microscope: prohemocyte (PR)- 4%, plasmocyte (PL)- 79%, granulocyte (GR)- 11%, sherulocyte (SP)- 2%, adipohemocyte (AD)- 1%,oenocytoid (OE)- 2%, coagulocyte (CO)- 1%. The PR was smallest with large nucleus, PL was polymorphic and abundant. The GR was abundant with cytoplasmic granules, SP was large with spherules. The AD was with variable in size and shape, OE was with less cytoplasmic organelles while CO was very fragile hemocytes. Along with morphology Total and Differential Hemocytes Count (THC and DHC) was also calculated in present investigation. The values of THC were 19500/mm^3 in male, 9600/mm^{3}in non-pregnant female while 11050/mm^3 in pregnant female.

INTRODUCTION

Several important functions have been attributed to hemocytes in arthropoda. They have been reported to be involved in homeostasis (Bang, 1971), storage of glycogen and other nutrients (Johnston *et al.*, 1973). The important role of hemocytes in defense mechanism has been recognized by many authors (Durilant, 1985; Soderhall and Cerenius, 1992). Due to this reasons the study of hemocytes is the most active area of research in invertebrates. Among the arthropods, the insect hemocytes are the centre of attraction. Morphological study of hemocytes of arachnids is very scarce especially in scorpion- living fossil. There are very few published reports regarding the hemocytes of Indian scorpion – Ravindranath (1974), Shah and Patil (2011, 2012), Hence we have undertaken this virgin field for the investigation. In the present investigation we have studied THC and DHC in male, non-pregnant and pregnant female along with types of hemocytes in *H. phipsoni*.

MATERIALS AND METHODS

The scorpions *H. phipsoni* were collected from Vaderu, Taluka - Chiplun and Dist. - Ratnagiri (Maharashtra). These were collected from burrows- their natural habitat, in the morning. These were kept in perforated plastic jars containing hibiscus leaves and fed with cockroaches. The animals were kept in laboratory and maintained for months without any mortality. Hemolymph was collected from the living animal as per the method of Padmanabha (1966). It was collected by aspiration with the help of hypodermic needle through arthroidial membrane of pedipalp. Depending upon the size of animal 1 to 3mL of hemolymph could be easily collected. The technique employed for the study of hemocytes was the modification of those used in the study of vertebrate blood, was developed by Patil and Shah. The qualitative method includes cytological preparation of thin smear of hemolymph and stained by Leishman's stain for light and phase contrast microscopic study. The quantitative method includes THC for which Neubauer's chamber was used. For identification and morphological study different cytological preparations were used. The hemocytes were identified according to key proposed by Gupta (1985).

RESULTS AND DISCUSSION

Light and phase contrast microscopic observations have helped in the identification and quantification of hemocytes population. Seven types of hemocytes were identified in the hemolymph of *H. phipsoni*.

Prohemocyte (PR)

These are slightly larger and round measuring about 5- 13μm in diameter (Fig. 1) . A very small amount of cytoplasm was with granules. The nucleus was large, compact and centrally placed with 3- 10μm in diameter. The nucleus was with compact chromatin and basophilic in nature. The value of DHC was 2- 5 %.

Plasmocyte (PL)

PLs represent unique class of hemocytes in scorpion due to their highly polymorphic nature (Fig. 2) . It is spindle shaped. Generally during cytoplasmic preparation it adheres with slide and formed many cytoplasmic projections. 2% versene acts

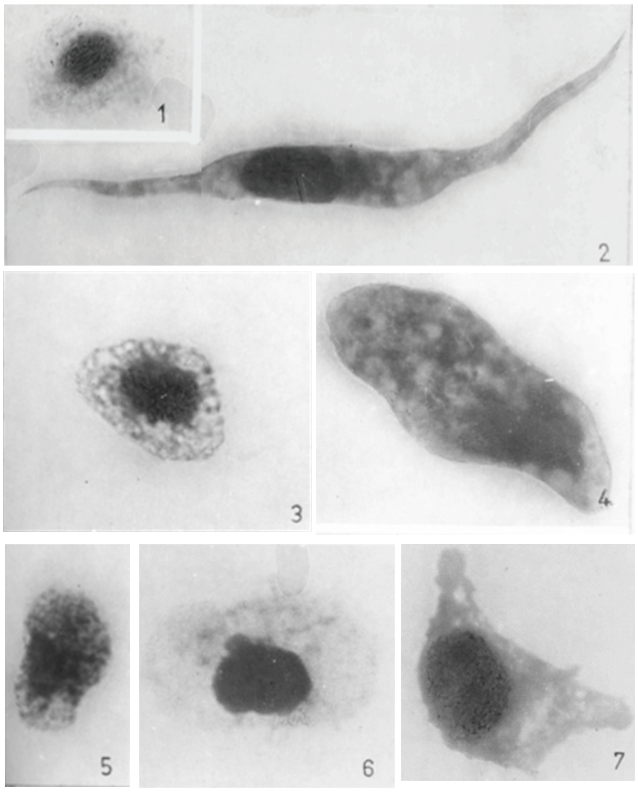

Fig. 1 Hemocyte types of *Mesobathus tamulus phipsoni*
1. prohemocyte(x940) 2. Plasmocyte(x940) 3.Granulocyte (x940) 4.Spherulocyte(x940) 5. Adipohemocyte (x940) 6. Oenocytoid (x940) 7.Coagulocyte (x940)

as a fixative and allow the measurement. The length measures about 10-35 μm and width 6- 12μm. The cytoplasm is abundant and slightly granular. The nucleus was centrally located and elliptical in shape. The size of the nucleus varied between 3-10μm in diameter. PLs constituted a bulk of the hemocytes population- 79 %.

Granulocyte (GR)

These are characterized by the presence of stainable cytoplasmic granules in them (Fig. 3) . These are medium sized cells; the size varies from 6- 15μm. The nucleus is smaller, round, eccentric in position and measuring about 3-8μm. The chromatin was dense and compact. DHC showed that the GRs constitute 11% in total hemocyte population.

Spherulocyte (SP)

Presence of non-refringent spherules in the cytoplasm was the characteristic of spherulocyte (Fig. 4) . These were round or oval in shape and measures about 7- 26μm. The nucleus was centrally placed and completely obscured by the spherules. The nuclear size varied between 3-10μm with dense and compact chromatin. The shrulocytes constituted about 4% in total hemocytes population.

Adipohemocyte (AD)

These were spherical or oval in nature (Fig. 5) . Their size ranged between 8- 40μm. The granules in the ADs were refringent in nature. Sudan Black B staining technique confirmed lipid nature of the granules. Some vacuoles were present in the cytoplasm. Large number of mitochondria was stained by Janus Green B stain. The nucleus was eccentric in position having 3- 11μm in diameter. These hemocytes constitute only 1% in total hemocytes population.

Oenocytoid (OE)

OEs were very fragile and difficult to locate under light microscope, hence studied under phase contrast microscope (Fig. 6) . 2% versene acts as fixative as well as preservative to study the morphological details. These are polymorphic and having 9-40μm diameter. Small granules were present in the cytoplasm. The nucleus was small, eccentric and 3-9μm in diameter. The value of DHC was 2%.

Coagulocyte (CO)

These are also fragile and also studied under phase contrast microscope in fixed and unfixed wet films (Fig. 7). The size and shape was irregular with diameter 13- 35μm. The hemocytes were with granular cytoplasm. The nucleus was centrally placed, having diameter 3- 8μm. The value of DHC was 1%. For THC Neubauer's hemocytometer has been used. The values of THC were, 10500/mm^3 in male, 9600/ mm^3 in non-pregnant female and 10050/ mm^3 in pregnant female scorpion. The female has THC less than male, but interestingly, the pregnant female showed higher THC than male and non-pregnant female scorpion. The PRs have been reported in all arthropoda having larger nucleus and high nucleocytoplasmic ratio indicating its conversion into other types of hemocytes (Arnold, 1952; Srivastava and Richards, 1965). The present investigation does not support this view. In scorpion there is separate hemopoitic organ (Ravindranath, 1974). The PLs were characterized by intense spreading during cytoplasmic preparation. In Diplopoda two types of PLs were observed due to spreading (Xylander and Nevermann, 2006). This difficulty was overtaken by the use of 2% versene in the present investigation. The large number of PLs in the scorpion might be because of their phagocytotic activities. GRs were considered as plesiomorphic hemocytes and have been reported in all major arthropod groups. According to Jalal and Rasoul (2008) GRs play important role during phagocytosis. The granule extrusion was observed in contact with foreign body. It was also reported by Saxena *et al.* (1988) that there was conversion of GRs into SPs and Ads. However this interconversion cannot be ruled out. Hollande (1909) was the first to coin term spherulocyte. These were the transitional hemocytes and converted into ADs and Cos (Gupta and Sutherland, 1966). Jalal and Rasoul (2008) have reported it as a separate functional stage which was supported by the present investigation. Nevermann (1996) have described COs were disintegrating during dropping of hemolymph into fixative and described it as a 'stressed plasmocytes'. In the present investigation it was fond to be separate hemocytes. Xylander and Nevermann also described it as 'valid' hemocytes in Myriopoda. Presence of OEs was also reported in *A.gemmatalis* (Andrade *et al.* 2003). The values of THC indicated, highest in pregnant scorpion than in male and non-pregnant scorpion. The range of hemocytes number was extremely variable, 500/μL^3 in crustaceans to 60000/μL^3 in cockroach (Xylander and Nevermann, 2006). Those species with hard and strongly calcified cuticles or passive protective strategies against predators have fewer hemocytes (Xylander, 2009). In scorpion no such correlation was observed. The large number of

hemocytes may be considered as a primitive characteristic.

REFERENCES

Adrade, F. G., Negreiro, M. C. de. Gregorio, E. A., Mosscardi, F. and Falleros, A. M. F. 2003. Hemocytes of *Anticarsia gemmatalis* (Hubner) (Lepidoptera: Noctudae) larvae: morphological and quantitative studies. *Acta Microscopica*. **12(1):** 59- 63.

Arnold, J. W. 1952. The hemocytes of Mediterran flour moth. *Ephesia kuhhiella* Zella (Lepidoptera: Pyralididae) *Can. J. Zool*. **30:** 352- 364.

Bang, F. B. 1971. A factor in crab amoebocyte, which stimulate in vitro clotting of crab blood. Joun. Invertebr. *Pathol.* **18:** 280-283.

Brehelin, M. and Zachary, D. 1986. Insect hemocytes: a new classification to rule out the controversy. In: Immunity in Invertebrates. (Ed.) Brehelin, M. *Sprnger Verlag , Berlin Heidderg.* 36- 48.

Durliant, M. 1985. Clotting process in Crustacea Decapoda. *Biol. Rev.* **60:** 473- 498.

Gupta, A. P.1985. Cellular elements in hemolymph. In: Comprehensive Insect Physiol. Biochem. and Pharmacology, Gupta, A. P. (Ed.) Pergaman. *Oxford.* **3:** 401- 451.

Gupta, A. P. and Sutherland, D. J. 1966.In vitro transformation of plasmocytes in some insects. *J. Insect Physiol.* **12:** 1369- 1375.

Hollande, A. C. 1909.Contribution a l'etude du sang des Coleopteres, Arch. *Zool. Exp. Gen. (Ser 5)* **21:** 271- 294.

Jalal, J. and Rasoul, S. 2008.The hemocyte types, total and differential count in *Papilio demoleus* L. (Lepidoptera: Papilionidae) during post embryonic development. *Mun. Ent. Zool*. **3:** 199- 215.

Johnston, M. A., Elder, H. Y. and Davies, P. S. 1973. Cytology of *Carcinus* hemocytes and their fuction in carbohy. *Metabolism. Comp. Biochem. Physiol.* **(A) 46**: 569- 581.

Nevermann, L. 1996. Untersuchungen anHemozyten von *Scolopendra cingulata* und *Lithbius forficatus* unter Asepkt zelluarer Abwehrreaktionen Doctoral. *Thesis, University of Giessen. www. nevermanns. de/ hemocytes/*

Padmanabha, N. B. 1967. Perfusion fluid for the scorpion, *Heteromatus fulvipes. Nature.* **213(74)**: 410.

Patil, A. E. and Shah, U. H. 2011. Types of hemocytes in *Mesobuthus tamulus tamulus*. *The Bioscan.* **6(4):** 597-599.

Patil, A. E. and Shah, U. H. 2012. Hemocytes in scorpion *Heterometrus xanthopus*. *The Bioscan.* **7(1):** 139-141.

Ravindranath, M. H.1974. The hemocytes of scorpion *Palamnaeus swammerdami. J. Morphology* **144**:1-9.

Shah, U. H. and Patil, A. E. 2011. The hemocytes types, differential and total counts in *Mesobuthus tamulus concanesis*. *Geobios.* **38(2-3):** 141-144.

Saxena, B. P., Sharma, P. R. and Tikku, K. 1988. Scanning electron microscopical studies of the hemocytes of *Spodoptera litara. Cytologica.* **53:** 385-391.

Srivastava, S. C. and Richards, A. G. 1965. An autobiographic study of the relation between hemocyte and connective tissue in wax moth *Galleric mellonella. Biol. Bull. (Wood hole)* **128:** 337- 345.

Soderhall, K. and Cerenius, l. 1992. Crustacean immunity. *Ann. Rev. Fish. Disease*. **2:** 3- 23.

Xylander, W. E. R. 2009. Hemocytes in Myriopoda (Arthropoda): *A review. ISJ.* **6:** 114- 124.

Xylander, W. E. R. and Nevermann, L. 2006. Hemocytes in Diplopoda and Chilopoda (Arthropoda, Myriopoda). types, structures and numbers. *Scand. J. Entomol.* **53:** 195- 210.