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Scorpion Prohemocytes: A Comaparative Study

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Abstract: The present study was undertaken to recognize and study the prohemocytes in five species of scorpion- Mesobuthus tamulus tamulus, Mesobuthus tamulus concanensis, Heterometrus xanthopus, Heterometrus phipsoni and Orthochirus bicolor. Light and phase contrast microscopic observations have showed seven distinct types of hemocytes in all five species ; prohemocytes (PRs), plasmocytes (PLs), granulocytes(GRs), spherulocytes (SPs), adipohemocytes (ADs), oenocytoids (ODs) and coagulocytes (COs). Largest PR was observed in Heterometrus xanthopus and smallest in Mesobuthus tamulus concanensis. PR of Heterometrus xanthopus was studied electron microscopically. Along with morphology Differential Hemocytes Count (DHC) was also studied.

Keyword: Scorpion Hemolymph Prohemocytes

INTRODUCTION:

Research on arthropods especially insect hemocytes has received much attention because they are the cells that mediate insect cellular immunity (Huang et al., 2010) and also provide an excellent model system for the study of cell development, differentiation and communication (Ling et al., 2003). During the cellular immune response, hemocytes are able to distinguish self from non-self, reacting pathogens, parasites or eggs by phagocytosis (engulfment of organisms by single hemocytes), multicellular encapsulation and nodule formation (Ling & Yu, 2006; Strand, 2008). A review of literature on arthropod hemolymph and hemocytes reveals that the studies have been restricted to certain selected groups. Among the arachnids, scorpions have remained neglected. Actually, scorpions represent a very primitive surviving arthropod group with a status of "living fossil". These terrestrial animals have survived through millions of years of evolutionary history with little or no change in their organization. There were very few published reports regarding to hemocytes of Indian scorpions- Ravindranath (1974). In the present investigation PRs of five species were compared.

MATERIAL AND METHODS

The different species of scorpions used in the present investigation were collected from different localities of Maharashtra state. Five species were collected from their natural habitat. H. xanthopus and H. phipsoni were collected from their natural burrows while M. tamulus tamulus, M. concanensis and O. bicolor were collected from underneath the stone in areas mentioned in the table No.1.

Table No.1- Scorpion species and locality of collection.

Sr. No.	Name of the scorpion species	Locality
1	*Mesobuthus tamulus tamulus*	Dadaswadi, Tal. Atpadi, Dist. Sangli, Maharashtra, India
2	*Mesobuthus tamulus concanensis*	Vaderu, Tal. Chiplun, Dist: Ratnagiri, Maharashtra, India.
3	*Orthochirus bicolor*	Dadaswadi, Tal. Atpadi, Dist. Sangli, Maharashtra, India.
4	*Heterometrus xanthopus*	Dadaswadi, Tal. Atpadi, Dist. Sangli, Maharashtra, India.
5	*Heterometrus phipsoni*	Vaderu, Tal. Chiplun, Dist. Ratnagiri, Maharashtra, India.

All collections were made in the morning between 7.00a.m. and 10.00a.m.. The males and females of each species were kept separately in perforated jars. A thick layer of soil was placed from the natural habitat. To provide shed, humidity and shelter fresh hibiscus leaves were placed in the jars. The water for drinking and small cockroaches was fed to scorpions. The animals could be maintained for months together without any significant mortality.

Hemolymph was collected from the living animal as per the method of Padmanabha (1967). A hypodermic syringe was inserted through the arthrodial membrane present at the third joint of one of the pedipalps. Depending upon the size of the specimen, the volume of hemolymph collected varied but on an average about 1-3 ml could be easily collected.

The hemocytes were studied in fixed air dried films, stained with Pappenheim's panchrome, Giemsa's and Leishman's stains. Better results were obtained in the preparations stained with Pappenheim's panchrome. Janus Green B was used to observe mitochondria. Sudan Black B and PAS stains were used for observing chemical nature of cytoplasmic inclusions. The ultra structure of hemocyte types was observed with a TEM of H. xanthopus.

RESULTS AND OBSERVATIONS

Prs are small and round. They could be stained in

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varying intensities with the above mentioned stains and could be observed in all preparations, indicating their stable nature. Their average size ranged between 5-13µm. Smallest of them was seen in M. tamulus tamulus and largest in H. xanthopus. Small sized PRs was the characteristic feature of buthid species (M. tamulus tamulus, M. tamulus concanensis and O. bicolor). The PRs of scorpionidae (H. xanthopus and H. phipsoni) were relatively larger.

A very thin layer of cytoplasm was seen in the PRs of all the species. It appeared agranular in M. tamulus tamulus, M. tamulus concanensis and finely granular in O. bicolor, H. xanthopus and H. phipsoni. It gave a weak basophilic reaction in M. tamulus tamulus, M. tamulus concanensis and O. bicolor and was more basophilic in H. xanthopus and H. phipsoni.

TEM study of PRs in H. xanthopus showed that it is usually round or oval and about 6-10 µm in diameter. Plasma membrane is thin and showed vesiculation. Its laminar nature is not visible. A thin homogenous layer of cytoplasm surrounds the nucleus. It contains moderate unbound ribosomes. A large nucleus is characteristic of this hemocyte type. It is concentric in position and about 4-8 µm in diameter. The chromatin appears quite compact. A compact nucleus, large in relation to cell size and mostly located centrally. The nuclei of PRs in M. tamulus tamulus occupy almost 90% space of the cell. The size of the nucleus varied between 3-10µm in diameter (table No. 2). The chromatin was compact. DHC values are as shown in the table No.2. They constituted 5% in M. tamulus tamulus, 3% in M. tamulus concanensis and O. bicolor, 2% in H. xanthopus and 4% in H. phipsoni. Thus the % of PRs ranged 2-5.

Table No. 2- Prohemocyte Characteristics of Different Species of Scorpions

Sr. No.	Species of scorpion	Shape variation		Size variation		Nucleo-cytoplasmic ratio	Position of nucleus	Nature of staining reaction				Differential Hemocyte Count (DHC) (%)
		cell	nucleus	cell	nucleus			Nuclear chromatin	Cytoplasmic inclusions	Cytoplasm	Nucleus	
1	*Mesobuthus tamulus tamulus*	R	R	6-10	4-8	70-80	C	CO	AG	WB	B	05
2	*Mesobuthus tamulus concanensis*	R/O	R	5-9	3-7	80-90	C	CO	AG	WB	B	03
3	*Orthochirus bicolor*	R/E	R	5-10	6-9	80-85	C	CO	FG	WB	B	02
4	*Heterometr-us xanthopus*	R	R	7-13	5-10	80-90	C	CO	FG	B	B	02
5	*Heterometr-us phipsoni*	R	R	8-13	7-10	80-85	C	CO	FG	B	B	04

AG- Agranular, B- Basophilic, C- Central, CO- Compact, E- Elliptical, FG- Fine granules, O-Oval, R- Round, WB- Weakly basophilic

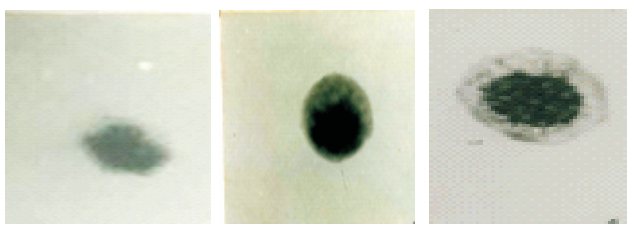
DISCUSSION AND CONCLUSIONS:

In many arthropods, especially the small sized ones, the collection of hemolymph samples presented problems. This is because of the small amount of hemolymph in them. The scorpions investigated presently, however posed no problem in this regard. A sufficient amount of hemolymph could be obtained from each of the scorpion. This was particularly important because a large number of blood films could be prepared from a single specimen and observations could be repeated to add to the accuracy.

The term PRs was first used by Arnold (1952) to describe a group of small, round or oval hemocytes with a thin basophilic layer of cytoplasm containing a exceptionally large nucleus. The PRs have been observed in almost all arthropods with some variations. Most crustaceans, chelicerata, myriopoda, insects and onychophorans have PRs in their hemolymph. But in aquatic chelicerata (Xiphosura) and primitive crustaceans (branchiopoda) possessed no PRs. Among the species of insects, Collembola and Thysanura have also been reported to be lacking the PRs (Gupta, 1979).

Light and electron microscopic observations have showed a very large nucleus and relatively undifferentiated cytoplasm. High nucleo-cytoplasmic ratio reaching to the extent to 90% in M. tamulus tamulus is the indication of the embryonic nature of the cells. An interesting observation have been made in this investigation that buthid species (M.tamulus tamulus, M. tamulus concanensis and O. bicolor) have smaller PRs than those to scorpiondae (H. xanthopus and H. phipsoni).

The controversial questions often raised regarding PRs are: (1) are they are the stem cells that transform into the other hemocyte? And (2) if they are the stem cells are PRs are main post-embryonic source of hemocytes? Although there are substantiating reports that PRs do transform into at least few other hemocyte types. The term “prohemocyte” suggest that these cells give rise to other types, but it has been not yet demonstrated conclusively that all hemocyte types are derived from the PRs. The PRs, in many arthropods were observed to be in different stages of mitosis. This may be the reason that they are regarded as the stem cells responsible for post-embryonic differentiation of other hemocyte types (Gupta, 1985). Such dividing hemocytes were not observed in scorpions in the present investigation. It was the indication of that the differentiation of hemocytes must occur in the hemopoitic tissue. This study confirmed the view of Ravindranath (1974) in the study of Palamnaeus swammerdami . The PRs reside in the hemopoitic organ. According to Arnold (1970) PRs appeared in hemolymph intermittently and often in groups. The differential count of PRs in five species of scorpions ranges between 2-5%. There was possibility that the circulating PRs might have escaped from hemopoitic tissue into the hemolymph. Just why and how is the mystery.



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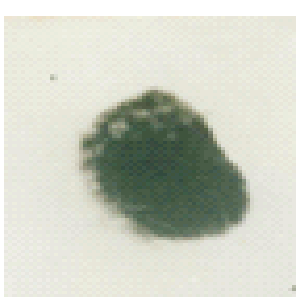

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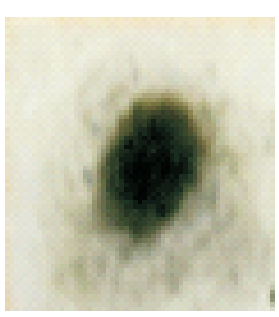

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Fig. 1- Prohemocytes(PRs) (x 940) of 1.M. tamulus tamulus, 2. M. tamulus concanensis, 3. M. tamulus concanensis 4.H. xanthopus 5.Heterometrus phipsoni

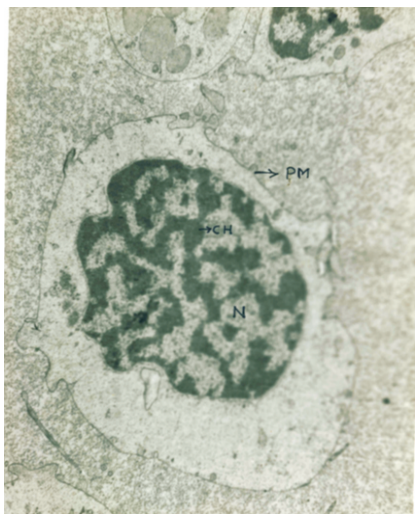

Fig. 2- TEM of PR(x18000) in Heterometrus xanthopus CH- chromatin, N- nucleus, PM-plasma membrane

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