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Coelomocyte Studies of Three Species of Earthworm *viz Polypheretima elongta, Perionyx sansibaricus and Dichogaster bolaui*

KEYWORDS: Coelomocytes, Polypheretima elongata, Perionyx sansibaricus, Dichogaster bolaui

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ABSTRACT *Morphological features, classification and numbers of coelomocytes were studied in Polypheretima elongata, Perionyx sansibaricus and Dichogaster bolaui. The coelomocytes are divided in to elocytes, granulocytes and amoebocytes. Granulocytes are divided in to two types, granulocytes GI and Granulocyte GII type. And amoebocytes are also divided in to two types, amooebocyte AI type and amoebocyte AII type. Apart from these coelomocytes, coelomic fluid contains numerous transitory cells at different stages of maturation. Coelomcytes present in the coelomic fluid and the blood of worms are same. The total number of coelomocytes per unit volume (ml) of the coelomic fluid was calculated. They differ in different species. Total number of coelomocytes per unit volume of coelomic fluid is 35.22±13.10, 43.73±14.70 and 40.13±03.80 in Polypheretima elongata, Perionyx sansibaricus and Dichogaster bolaui, respectively. Among the three species studied lowest number of coelomocytes are retrieved from largest species i.e., Polypheretima elongata.*

Introduction:
Invertebrates exhibit different immune mechanisms against environmental pathogens. In earthworms, coelomocytes (leucocytes) located in coelomic cavity are responsible for innate cellular immune functions such as phagocytosis and encapsulation against parasites and pathogens. The coelomocyte population has been divided into different subpopulations. The coelomocytes possess immuno-defense related biological functions. The effector cells participate mainly in cellular mechanism but chloragocytes and the granulocytes may produce humoral factors which may mediate the cellular and humoral responses as well. Microbial killing results from the combined action of the phagocytic process with humoral immune factors such as agglutinin(viz., lactin), lysosomal enzymes (viz., acid phosphatases) and various cytotoxic and antimicrobial molecules.

Coelomocytes of Oligochaetes are characterized by a pronounced polymorphism, and their quantitative and qualitative compositions vary depending on environmental factors, individual age and physical condition (Aval, 1959). Earlier attempts at coelomocyte classification based on morphological and functional criteria, have not gained common acceptance. The reason for the lack of a uniform classification system is that the coelomic fluid contains cells at various functional states and various stages of maturation. Cooper and Stein (1981) distinguished three types of coelomocytes in *Lumbricus terrestris* - hyaline amoebocytes, granular amoebocytes and eleocytes. However, Jarosz and Glinski (1997) divided eleocytes, called also chloragogenic cells, into I and II eleocytes.

Eleocytes originate from chloragogenic tissue surrounding the intestine (Jamieson, 1981; Jamieson, 1992; Affar *et al.,* 1998) and are present in all the examined Oligochaete species. Chloragogenic cells are responsible for maintaining a constant *p*H and ionic balance of both coelomic fluid and haemolymph (Prento, 1979). Affar *et al.* (1998) opined that they synthesize extra-cellular respiratory pigments (Needham, 1966, Roots and Johnston, 1966; Fischer, 1977) which are capable of storing endogenous substances, such as glycogen and lipids (Roots, 1957; Roots and Johnston, 1966; Ireland and Richards, 1977) and exogenous substances, such as pigments (Roots and Johnson, 1966; Needham, 1966) or metals (Prento, 1979; Morgan, 1979).

Eleocytes play an important part in immune process in lumbricids, producing bactericidal substances (Valembois *et al.,* 1982; Ville *et al.,* 1995; Milochau *et al.,* 1997), participating in reactions of encapsulation and formation of brown bodies (Cooper and Stein, 1981; Valembois *et al.,* 1992; Valembois *et al.,* 1994). The second type of coelomocytes are amoebocytes among which hyaline and granular cells were distinguished, but till now no unambiguous relation has been found between the hyaline and granular cells, though numerous authors suggest that they may be of common origin (Cooper and Stein, 1981).

Amoebocytes participate in the transport and storage of nutritive substances (Valembois and Cazaux, 1970), coelomic fluid coagulation and wound healing (Byzowa, 1974), immune reactions of humoral system (Cooper and Roach, 1986; Jarosz and Glinski, 1997), cellular defense reactions (Cooper, 1996) phagocytosis (Cossoarizza *et al.,* 1996) as well as encapsulation and nodulation (Valembois *et al.,* 1992, 1994).

Materials and Methods:
Coelomocytes Studies:
Sexually matured earthworms of three species, *Polypheretima elongata, Perionyx sansibaricus* and *Dichogaster bolaui*, were obtained from in and around Gulbarga city; they were used immediately after collection or from culture vessels. Earthworms were not subjected to any controlled condition in the laboratory. Plastic vessels were used for rearing earthworms.

Sieved pasture soil (600 gm) was used as culture medium for the large sized geophagous species *Polypheretima elongata*. Air dried, sieved (1 mm mesh) ground cow dung (200 gm) free from any foreign cocoon was used as culture medium for *Perionyx sansibaricus* which is surface living species inhabiting cow dung heaps. Mixture of sieved cow dung (100 gm) and garden soil (100 gm) was used as culture medium for *Dichogaster bolaui* which is another surface living earthworm.

Extrusion of coelomic fluid for coelomocytes studies:
Coelomic fluid was obtained by means of electric shock (Suzuki and Cooper, 1995), stimulating the earthworms with galvanic current of 6 volts for a duration of 1 minute. The volume of thus obtained coelomic fluid averaged 80µl. The fluid was diluted 1:100 with LBSS (Lumbricus Balanced Salt Solution) (Diogene *et al.,* 1997). Coelomocytes were counted with the chamber method in Neubauer haemocytometer. This gives the coelomocytes density, i.e., cell number per ml. Then this

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was multiplied by factor 3 (volume of extrusion of fluid) giving the cell number per animal.

Total number of coelomocytes = Cell number per ml X 3

A thin smear of coelomic fluid of earthworms was taken on the slides and were dried at room temperature for 24 hours and stained with Leishman's stain. The slides were analyzed in the light microscope and percentage of each type of coelomocytes was calculated on the basis of morphology of coelomocytes.

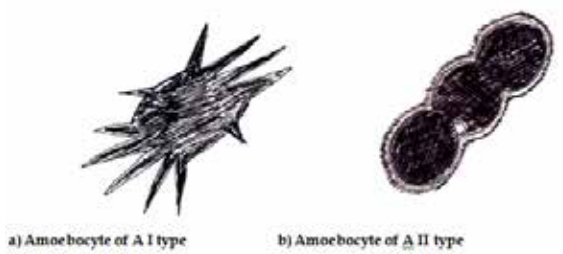
Results:

Ceolomocytes studies: (Fig 1)

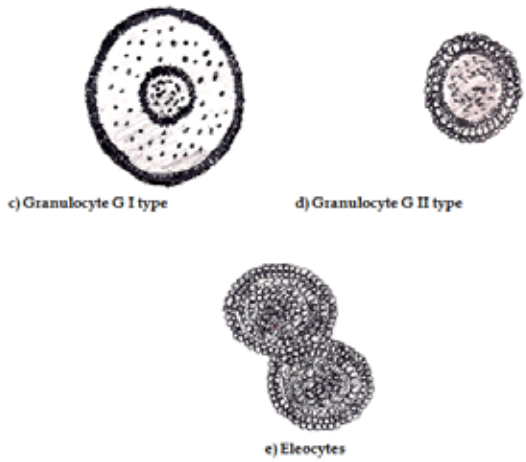
Based on morphological characters, the coelomocytes are classified into three main types: Amoebocytes, Eleocytes and Granulocytes. Approximately amoebocytes constitute 41%, granulocytes constitute 28% and eleocytes constitutes 30% of coelomocytes population in all the three species studied.

The total number of coelomocytes per unit volume (ml) of the coelomic fluid was calculated. They differ in number in different species. The total number of coelomocytes is per unit volume of coelomic fluid 35.22±13.10, 43.73±14.70 and 40.13±03.80 in *Polypheretima elongata, Perionyx sansibaricus* and *Dichogaster bolaui*, respectively.

Amoebocytes constituting the major population of coelomocytes are polymorphic in shape which varies from oval to horse shoe shape. The nuclei are located centrally or peripherally. The cytoplasm contains few granules. The amoebocytes are classified as A I and A II depending on number and shape of cytoplasmic processes.



a) Amoebocyte of A I type b) Amoebocyte of A II type



c) Granulocyte G I type d) Granulocyte G II type

e) Eleocytes

Fig. 1: Coelomocytes of *Polypheretima elongata*

Type A I amoebocytes (Fig. 1a) form number of pseudopodia regularly distributed on the cell periphery and have a form of short lobopodia. Type A II amoebocytes (Fig. 1b) form irregularly distributed pseudopodia, most often concentrated on one pole of the cell and having the shape of long filipodia. Usually type A I amoebocytes are observed dispersed and type A II amoebocytes are observed in groups *in vivo* preparations. The A II amoebocytes are small when compared to A I amoebocytes. The amoebocytes of *Polypheretima elongata* are larger than those of other two species studied.

The number of A I amoebocytes is 20.50±02.30, 19.50±02.30 and 19.90±02.80 respectively in *Polypheretima elongata, Perionyx sansibaricus and Dichogaster bolaui*. A II amoebocytes are 21.20±03.60, 22.20±03.80 and 21.20±03.50 in *Polypheretima elongata, Perionyx sansibaricus* and *Dichogaster bolaui* respectively (Table – 1).

The coelomocytes which contain large number of granules in the cytoplasm are called granulocytes. They constitute 28% of the coelomocytes. They are spherical with centrally located nuclei. Two forms can be distinguished among the granulocytes G I and G II (Fig. 1c & d). G II differ from G I in having characteristic vesicular structure on their surface. These vesicles vary from spherical to club shaped.

Table – 1: Cellular parameters of coelomic fluid of *Polypheretima elongata, Perionyx sansibaricus* and *Dichogaster bolaui*

Name of the species	Number of coelomocytes per/ml	Percent composition of coelomocytes				
			Granulocytes		Amoebocytes	
		Eleocytes	GI	GII	AI	AII
Polypheretima elongata	35.22±13.10	29.80±05.08	13.40±01.90	14.14±02.20	20.50±02.30	21.20±03.60
Perionyx sansibaricus	43.73±14.70	28.80±05.08	14.40±01.80	15.14±02.20	19.50±02.30	22.20±03.80
Dichogaster bolaui	40.13±03.80	29.20±04.08	13.30±01.70	14.15±02.70	19.90±02.80	21.20±03.50

M±SE =Mean ± Standard error

N=Number of samples observed is 20

The number of G I granulocytes is 13.40±01.90, 14.40±01.80 and 13.30±01.70 respectively in *Polypheretima elongata, Perionyx sansibaricus* and *Dichogaster bolaui*. And the number of G II granulocytes is 14.14±02.20, 15.14±02.20 and 14.15±02.70 respectively in the *Polypheretima elongata, Perionyx sansibaricus* and *Dichogaster bolaui* (Table – 1).

Eleocytes constitute about 30% population of coelomocytes in all the three species studied. These cells are round or oval and smaller than amoebocytes and granulocytes. The nuclei are located eccentrically and polymorphic granules are present in the cytoplasm (Fig. 1e). The number of eleocytes is 29.80±5.08, 28.80±5.08 and 29.20±04.08 in *Polypheretima elongata, Perionyx sansibaricus* and *Dichogaster bolaui* respectively (Table – 1) and the number of coelomocytes (per/ml) is 35.22±13.10, 43.73±14.70 and 40.13±03.80 in *Polypheretima elongata, Perionyx sansibaricus* and *Dichogaster bolaui* respectively (Table – 1).

Discussion:

Among the three species studied, (*Polypheretima elongata, Perionyx sansibaricus* and *Dichogaster bolaui*), lowest number of coelomocytes are retrieved from individual largest species *Polypheretima elongata*. In all the species, three types of coelomocytes (eleocytes, amoebocytes and granulocytes) are present. The percentage and number of eleocytes, granulocytes and amoebocytes are species-specific. Our results resemble with that of Agata and Barbara (2003) who stud-

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ied four species of earthworms viz., *Allolobohora chlorotica, Lumbricus terrestris, Dendrobena veneta* and *Eisenia fetida* and concluded that the coelomocytes are specific and their number varies depending upon season.

The clear differentiation in the structure of oligochaeta coelomocytes is associated with their functions, such as phagocytosis (Dales and Kalac, 1992), encapsulation, coagulation of systemic fluid (Valembois *et al.,* 1988), wound healing (Parry, 1975), immune reactions (Dales, 1978; Cooper, 1996; Quaglino, 1996) and transport of nutritive substances (Cooper and Stein, 1981).

In the present study while classifying cells of coelomic fluid of *Polypheretima elongata*, *Perionyx sansibaricus* and *Dichogaster bolaui* morphological character such as shape, size, presence of granules, nuclear location etc were taken into account. The results indicate that the coelomic fluid of the species studied contains three types of cells. They are eleocytes, amoebocytes and granulocytes.

The eleocytes are distinct from the amoebocytes and granulocytes in all the three species.

Occurrence of eleocytes is characteristic of annelids. Some reports indicate that eleocytes are present both in the haemolymph and coelomic fluid (Turska, 1972), while others maintain that they are present only in the coelomic fluid (Andrew, 1965; Dales, 1978).

Eleocytes of all three species of earthworm (*Polypheretima elongata*, *Perionyx sansibaricus* and *Dichogaster bolaui*) like those of other Oligochaete species probably constitute a lineage of coelomocytes which is developmentally and functionally distinct from amoebocytes and granulocytes. Eleocytes of the three species are round or oval cells with small eccentrically located nuclei.

Among the three types of coelomocytes in *Polypheretima elongata*, *Perionyx sansibaricus* and *Dichogaster bolaui,* amoebocytes are the most numerous. Amoebocytes of *Lumbricus terrestris* are also called as leukocytes (Stein and Cooper, 1981), inspite of the lack of any closer morphological functional similarities to vertebrate leukocytes. Likewise, they are often compared to vertebrate macrophages, lymphocytes or lymphoblasts (Stien and Cooper, 1981). In *Lumbricus terrestris*, considering the cellular picture under light microscope, amoebocytes are also termed as basophiles. Other names corresponding to lymphocytes found in the literature are basophilic amoebocytes, young amoebocytes and lymphocytal cell.

In the present study, amoebocytes of all the three species are polymorphic, among which two sub types are distinguished; type A I and type A II depending on differences in distribution and shape of cytoplasmic processes. Amoebocytes of A I type form numerous short lobopodia, regularly distributed on the cell periphery. A II amoebocytes form long filipodia on one pole of the cell. Their centrally or peripherally located nuclei vary in shape, from oval to horse shoe shape.

Based on light microscopic observations two types of granulocytes; G I and G II, are distinguished in all the three species of earthworms viz., *Polypheretima elongata*, *Perionyx sansibaricus* and *Dichogaster bolaui*. The G I granulocytes are smaller in size, have less number of vesicles on their surface. Granulocytes are characterized by vesicles forming on their surface. Large numbers of vesicles are called bleps. Their shape varies from spherical to club shape. Depending upon the degree of maturity of the cell, the vesicles change, increase in volume and finally detach from the cell. They probably have storage function and carry dispensable material. Such bleps are also observed in *Lumbricus terrestris, Eisenia fetida* and *Dendrobena veneta* (Stein *et al.,* 1977; Stein and Cooper, 1981; Anna and Jan, 2001).

SUMMARY AND CONCLUSION:

1) Earthworms are known for their medicinal value since ancient times all over the world.
2) Blood and the coelomic fluid of earthworm contain both haemocytes and coelomocytes.
3) The coelomocytes and haemocytes play an important role in fighting against pathogens. Because of this property the present work is undertaken.
4) The first part of the study is to find out the types of coelomocytes present in the coelomic fluid of the earthworms. The second part of the study comprises the antibacterial activity of the coelomic fluid of the earthworm against pathogenic bacteria.
5) Three types of coelomocytes namely eleocytes, granulocytes and amoebocytes are present in the coelomic fluid of earthworm.Two subtypes of granulocytes are granulocyte I (G I) and granulocyte II (G II). Two types of amoebocytes are amoebocytes I (A I) and amoebocytes II (A II).

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