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Some Aspects of Haemocytes in *Schizodactylus monstrosus* Drury (Orthoptera: Schizodactylidae)

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Differential haemocyte count in *Schizodactylus monstrosus* (DHC) presents prohaemocytes in nymphs and granular haemocytes in adults as the predominant haemocytes; while total haemocyte count (THC) shows no significant differences in nymphs and adults though females present slightly higher number than males. Thermal stress results in marked increase in THC and relative percentage of almost all categories of haemocytes except granular haemocytes. Haematological stress (haemorrhage) shows increase in THC and relative increase in all cell types except adipohaemocytes and effect of decapitation is shown by an increase in THC and percentage of prohaemocytes and plasmatocytes while decrease in granular haemocytes; number of adipohaemocytes and spherule cells are least affected. The paper deals with the physiological cause of these changes in the haemogram.

Key Words: **Haemocytes, Thermal stress, Haemorrhage, Decapitation,** *Schizodactylus* sp.

Introduction

The freely circulating haemocytes in different insect orders have been reviewed by various workers (Ogel 1955, Choudhuri 1964, Arnold 1972, Wigglesworth 1973, Saxena & Agarwal 1979). The nomenclature, morphological identity, physiological roles and the factors associated with the fluctuations of haemocytes have posed much confusion among the workers (Wcbley 1951, Gupta 1969). Notable fluctuation in the haemogram of an insect serves as an index to various physiological conditions of the individual (Shrivastava & Richards 1965). The study purports to report the haemocyte categories of *S. monstrosus* and the effects of temperature, haematological stress (haemorrhage) and decapitation on the haemogram of this carnivorus, sand-burrowing insect.

Material and Methods

Sexually mature adults and nymphs were collected from the sandy river basin of the Damodar and their sex and moult cycle stages were recorded before collection of haemolymph.

Volume of the haemolymph was measured after Price (1961). Total haemocyte count (THC) was made in phase contrast microscope in a Neubauer haemocytometer without using any anti coagulant. The haemolymph was allowed to fill the chamber directly (in capillary action) about 5 sec after its collection. Morphological observations were made on fresh preparations as well as on fixed stained preparation. Living cells were studied under phase contrast microscope following usual procedure. For other histological studies, air-dried films were either directly stained in Haematoxylene-Eosin or Lieshman's stain or at first fixed in methanol and stained in Giemsa's stain. For the identification of fat-containing cells, staining technique of Gray (1954) was followed. Differential haemocyte count was carried out following usual procedure.

Total and differential haemocyte counts in heat-fixed specimens were carried out after subjecting the insects at 35°C for 10 min. The effect of haemorrhage was determined by drawing out 0.1 ml haemolymph through a micropuncture and making THC and DHC from 4 specimens, after 24 hr Hormonal effects on haemogram were shown by decapitating the adults (male & female) and last instar nymphs (male & female) following the technique of Wigglesworth (1973). In all the experiments haemolymph volume was also measured.

Results

The haemolymph is creamy white; at room temperature (28°C) a drop of haemolymph transforms into a gel in about 200–210 sec and on long exposure, it gets darkened.

Morphology of Haemocytes

According to Jones (1962), the haemocytes can be divided into five types on the basis ot morphological features. The general characteristics of haemocytes are presented in table 1.

Prohaemocytes (figure A): These are small, mostly spherical or fusiform cells, characterised by small amounts of basophilic cytoplasm and the nucleus comprises greater amount of cell volume. Size of the cells varies from 5 to 9μ and that of nucleus 3.8 to 7.9μ; divisional stages are common.

Plasmatocytes (figure B): These are not abundant among circulating haemocytes; usually spherical or ovoid cells, characterised by eccentrically placed nucleus, surrounded by relatively large mass of light basophilic cytoplasm. The cytoplasm contains few non-refractile fine granules. Size of the cells varies from 10 to 13μ and that of nucleus 7.2 to 9.7μ; a few divisional stages are noted.

Granular haemocytes (figure C): These are compact usually spherical, ovoid and spindle shaped cells, with eccentrically placed small nucleus, surrounded by basophilic cytoplasm laden with round, ovoid or rod-shaped granules of variable size. The size of cells varies from 11 to 18μ and that of nucleus 5 to 9μ. Occasionally these cells appear to be binucleate; divisional stages are rare.

Adipohaemocytes (figure D): These are largest haemocytes with spherical or ovoid form, centrally or eccentrically placed nucleus surrounded by basophilic cytoplasm with many refractile droplets or globules of variable sizes. The size of the cells and nuclei are respectively 19 to 22μ and 4 to 8μ; divisional stages are absent.

Spherule cells (figure E): These are round, refractile basophilic haemocytes with many distinct uniformly round acidophilic refractile spherules. They range in size from 5 to 9μ.

Observations made on haemocytes of various age groups reveal that prohaemocytes in nymphs and granular haemocytes in adults are the predominant cell types (table 2). The

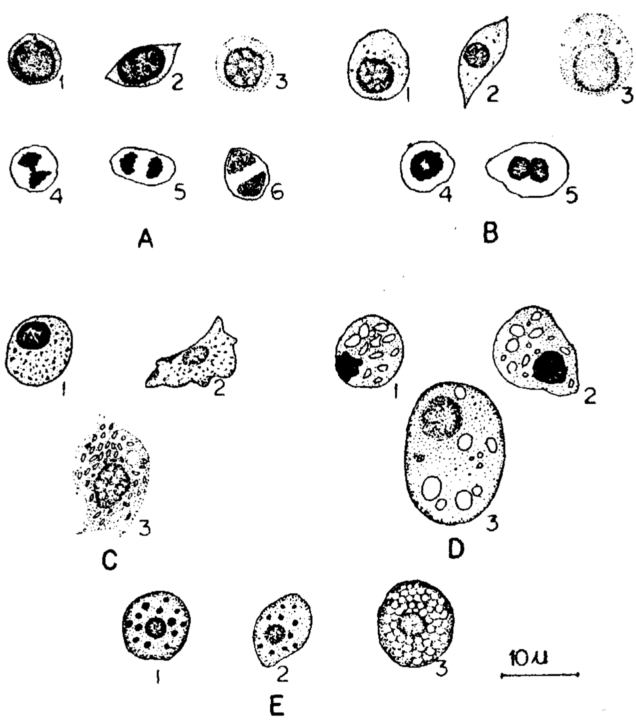

Figure 1 A–E Camera lucida drawings of haemocytes seen under phase contras microscope and stained preparations. *A*, 1–3 Prohaemocytes, 3 (under phase) 4–6 divisional stages; *B*, 1–3 Plasmatocytes, 3 (under phase); 4–5 divisional stages; *C*, 1–3 Granular haemocytes, 3 (under phase); *D*, 1–3 Adipohaemocytes, 3 (under phase); *E*, Spherule cells, 3 (under phase)

magnitude of THC ranges between 16000–18000 cells/mm^3 normally in unfixed specimens but 35000–40000 cells/mm^3 in heat fixed specimens. Marked variation in THC on the basis of age and sex was not noted though females show slight increase in THC (table 2). Thermal stress increased the percentage of all types of haemocytes except granular haemocytes. Effect of haemorrhage was shown to affect both THC and DHC—there was a slight increase in THC and also the percentage of almost all cells except adipohaemocytes (table 2). Decapitation results increase in THC and increase in the percentage of prohaemocytes and plasmatocytes while decrease in granular haemocyte percentage (table 2). Haemolymph volume was shown to be least affected except decrease in heat-fixed and haematological-stressed condition.

Discussion

The observations reported in the present study on the haemocytes of *S. monstrosus* give an idea about the haemogram of a carnivorous orthopteran insect under normal and certain experimental conditions. The normal THC was determined after minimizing the inaccuracies caused due to techniques. Errors in counts caused by diluents or use of glass pipettes were avoided. Significant difference in THC and DHC in normal and heat-fixed specimens is due to the fact that normally a large number of haemocytes remain adhered to tissue surfaces, temperature stress drives them into circulation. Relative increase of all cell types in heat-fixed specimens and abundance of granular haemocytes in fresh specimens explain that this temporary retirement from circulation is also cell specific.

The ambiguities created by previous authors in designating various haemocyte types on the basis of function seem to be more confusing since different functions may be performed by the same cell and apparently different cells may be performing similar functions. The term prohaemocyte applied here was designated as Germinating cells (Cuenot 1896). Though a ready transformation occurs from one cell type to another, occurrence of numerous dividing prohaemocytes consider them as basic cell type which give rise to some other haemocyte categories. Plasmatocytes and granular haemocytes are often considered synonymous (Ravindranath 1978) but their identity in structural and functional aspects are clear. The term adipohaemocytes was misapplied by some authors and considered them to belong to granular

Table 1 *General characters of the haemocytes type of* Schizodactylus monstrosus

Characteristics	Prohaemocytes	Plasmatocytes	Granular-haemocytes	Adipohaemocytes	Spherule cells
Size*	5–9μ	10–13μ	11–18μ	19–22μ	5–9μ
Shape	Spherical or fusiforms, non refractile	Spherical or ovoid	Polymorphic refractile	Spherical or ovoid	Round, refractile
General nature	Intensely basophilic cytoplasm, rarely visible with no cytoplasmic inclusion	Basophilic cytoplasm, with fine granular inclusions	Basophilic cytoplasm with round, ovoid or spindle shaped granules of variable size	Basophilic cytoplasm with refractile droplets or globules of variable size	Basophilic cytoplasm with many distinct uniformly round acidophilic refractile spherules
Nucleus	3.8–7.9μ in size, Central or eccentric in position, occupies greater amount of cytoplasm, round or oval in shape	7.2–9.7μ in size; eccentrically placed, round or oval in shape	5–9μ in size; eccentric in position, round or ovoid in shape, may be binucleate	4–8μ in size; eccentric in position and oval in shape	Ovoid in shape; cytoplasmic granules obscure the position of nucleus
Under phase contrast microscope	blue	blue	purple	Spherules appear colourless, globules are yellowish	purple

*Mean of 25 cells

Table 2 *Haemolymph volume (μl/insect), total haemocyte counts (THC) (cells/mm³; data × 10³) and differential haemocyte counts (DHC) in nymphs (last instar) and adults S. monstrosus in normal (C), heat treated (T), haemorrhage (H) and decapitated (D) conditions.* ±*SE* (*n* = 9)

	NYMPH								ADULT							
	Male				Female				Male				Female			
	C	T	H	D	C	T	H	D	C	T	H	D	C	T	H	D
Haemolymph volume	280	271	193	275	295	289	200	291	320	312	232	316	360	354	275	357
THC	16	35	20	25	17	39	22	26	16.5	41	22	27	18	45	22	29
*DHC**	(±0.5)	(±1.9)	(±0.8)	(±0.7)	(±0.9)	(±1.2)	(±0.7)	(±1.3)	(±0.8)	(±1.9)	(±0.6)	(±0.9)	(±0.8)	(±1.8)	(±1.1)	(±0.7)
Prohaemocytes %	34	40	45	41	35	41	45	39	20	25	29	28	19	21	25	29
Plasmatocytes %	9	10	12	12	10	10	13	12	15	17	17	20	9	10	11	14
Granular haemocytes %	22	12	25	13	25	10	30	14	43	35	46	29	51	44	55	35
Adipohaemocytes %	30	35	13	31	27	35	10	32	18	20	3	20	19	22	5	20
Spherule cells %	2	3	5	3	4	4	2	3	4	3	5	3	2	3	4	2

*Mean of 500 cells

haemocyte complex. The distinct lipoid inclusions of adipohaemocytes proves their morphological and functional identity.

Both the THC and DHC serve as an index to various physiological states and developmental stages of the insect (Hoffmann 1970). Relative abundance of adipohaemocytes in nymphal stages may indicate that in nymphs a major part of haemocyte population is engaged in storage of fat which is less striking in adults. Higher cell counts in females may be due to differences in juvenile hormone (JH) titre between the two sexes, since JH stimulates both production and differentiation of haemocytes (Hoffman 1970). Increase in THC accompanied by the relative increase in plasmatocyte, granular haemocytes, prohaemocytes and spherule cells, and decrease in adipohaemocytes due to haemorrhage is thought to be due to an increase in mitotic activity of the basic cells or the haemopoetic organ to replace the lost cells and also release of haemocytes from tissue surfaces (Jones 1970). This suggests that haemocyte mitotic rate is not coupled with haemolymph volume and total circulating haemocyte number (Shrivastava & Richards 1965). It is also evident from other data on haemolymph volume in relation to THC under different experiments. Harvey and Williams (1961) reported that wound reaction was initiated by a factor generated at the margin of the wound and released into the blood which resulted mitotic activities of the haemopoetic organ.

Changes in THC and DHC in decapitated *Schizodactylus* cogently confirms hormonal roles in maintaining normal haemocytic number. Decapitation which is directly attributed to the removal of neurosecretory cells indirectly affects the titre of ecdysone in the body resulting subsequent increase in THC, prohaemocytes and plasmatocytes and decrease in granular haemocytes number. It may be directly attributed to the low ecdysone titre (Crossley 1964 & Jones 1967).

Thus this investigation, in addition to providing a general account of the haemocytes of *Schizodactylus*, explores the effect of thermal stress, haematological stress and hormones on the haemogram of this insect.

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