Reprinted from the *Indian Journal of Entomology*, Vol. 41, No. 3, 1979, 231-236

Indian J. Ent. **41** (3) : 231-236 (1979)

FREE HAEMOCYTES OF *LIPEURUS LAWRENSIS TROPICALIS* PETERS AN ISCHNOCERAN MALLOPHAGA (SENS. LAT. PHTHIRAPTERA) INFESTING POULTRY BIRDS*

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The freely circulating as well as resting haemocytes have been studied in quite a number of species of insects. The diversity in their morphological forms has invited confusion over their identity and classification The problem of insect haemocytes classification as well as their functional relationship have been reviewed by Wigglesworth (1959) and Jones (1962). Even the haemocytes of a species or those of different species differ in shape, size and in histological details In the absence of one system of classification of blood cells of insects, many systems of insect haemocytes classification and nomenclature have been proposed from time to time by different workers. The information on this aspect is available from the work of Poisson and Pesson (1939), Yeager (1945). Wigglesworth (1956, 1959, 1973), Jones (1962, 1964, 1970), Vostal (1969, 1970) and Arnold (1972). The haemocytes of Mallophaga (Sens. Lat. Phthiraptera) have escaped the attention of workers, and only two types of haemocytes are known in amblyceran species *Laemobothrion percnopteri* (Srivastava, 1974). The present study is an attempt to deal with the histological characteristics and classification of freely circulating haemocytes of an ischnocerous phthirapteran, *Lipeurus lawrensis tropicalis* Peters, an ectoparasite on poultry bird, *Gallus domesticus* Linn.

MATERIALS AND METHODS

The poultry lice, *L. l. tropicalis*, were collected from poultry birds of Banaras Hindu University Agricultural Poultry Farm and a stock of the lice was maintained in incubators at 37±1°C and 90 per cent relative humidity (videmethod given by Agarwal 1967) Blood films were prepared from the haemolymph drops which were obtained by cutting the legs or by making an incision in the terga of the living lice, which were previously washed in insect Ringer's solution and soaked in absorbant tissue. The air dried blood films were either directly stained in Lieshman's stain or first fixed in methanol and then stained in Giemsa or Wright stain. Besides this the heatfixation method of Yeager (1933) was also tried and other fixative like Dobosque Brasil, aquous Bouin's and Carnoys fixative, and other stains like haemotoxyline and eosin were also used. The staining technique of Gray (1954) was adopted for the identification of fat containing cells.

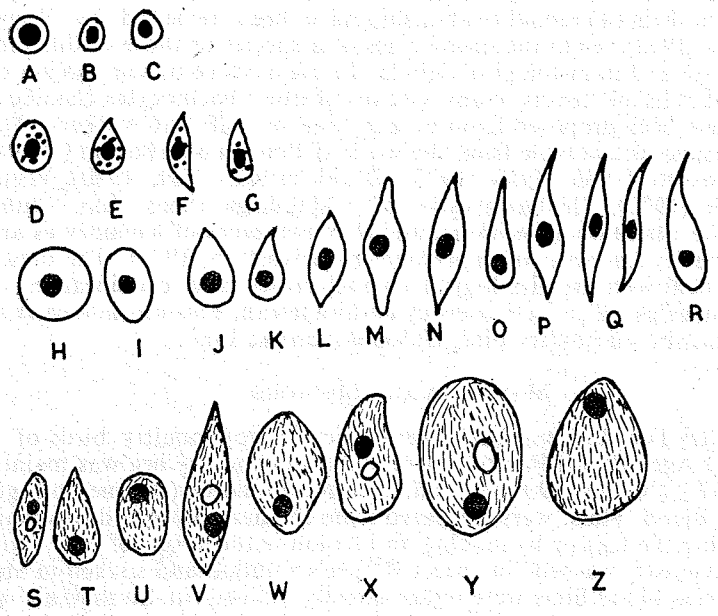
RESULTS AND DISCUSSION

The haemocytes *L. l. tropicalis* have been grouped into four types as : prohaemocytes, plasmatocytes, granular haemocytes and oenocytoids. The preparations fixed in Bouin's fluid and stained in haemotoxyline-eosin gave better histological picture, than those stained in Giemsa's or Lieshman's stains.

Prohaemocytes : These cells are small, rounded, ovoid or pearshaped having centrally located, intensely basophilic, large round to oval nucleus with granular chromatin material around a single excentric nucleolus. The nucleus, in most of these

* Part of the paper was presented at the Asian Congress of Parasitology held at Bombay from 23rd to 26th February, 1978.

cells occupies the entire cell leaving only scanty cytoplasm which is homogeneous and basophilic. There is not much variation in the size of different shaped haemocytes (Table 1). It is also noticed that prohaemocytes are most abundant in the 1st and 2nd instar nymphs, in comparison to their number present in 3rd instar nymph and the adult. (Fig. A—C and Fig. 1).



DIFFERENT TYPES OF HAEMOCYTES OF LIPEURUS LAWRENSIS TROPICALIS PETERS.

A-C PROHAEMOCYTES, D-G GRANULAR HAEMOCYTE, H-R PLASMATOCYTES, S-Z OENOCYTOIDS.

Fig. A to Z

Plasmatocytes : These cells are most abundant type of haemocytes. They are polymorphic and may be round, ovoid, pear or spindle-shaped but the round to ovoid-shaped are most common. Each plasmatocyte has oval centrally located, strongly basophilic, nucleus and abundant lightly eosinophilic cytoplasm. These cells do not tend to send out pny pseudopodium like structure, although sometimes the cytoplasm spread out into one or two spikes. The cytoplasm does not contain any sharply outlined granular inclusion, however 2-5 round to oval nonrefrigerant vacuoles (1.6—4.0 μ in diameter) are seen in some preparations. The size of the plasmatocyte is variable (Table 1). Their number is much more in 3rd instar and adult in comparison to 1st and 2nd nymphal stages (Fig. H-R and Fig. 2).

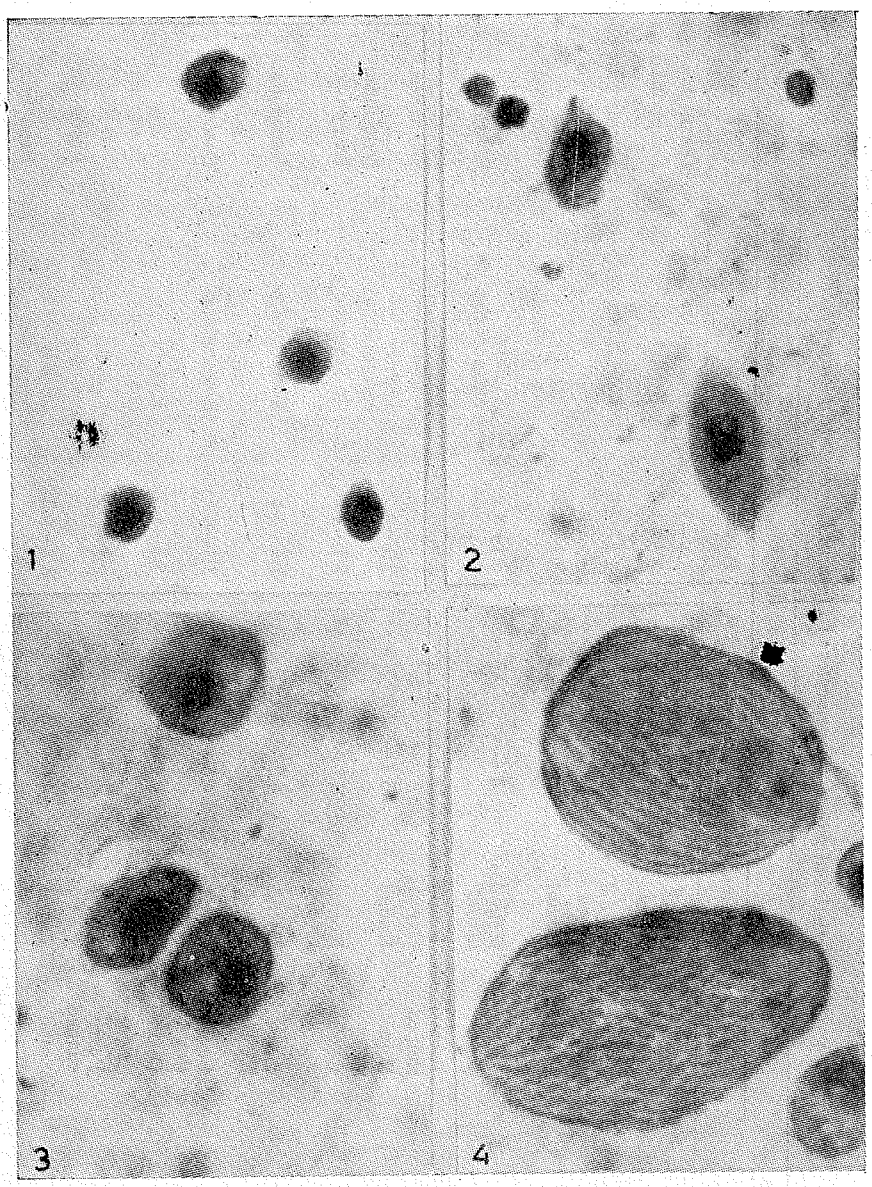


Fig. 1. Photomicrograph of prohaemocytes of *L.l. tropicalis* × 1200
Fig. 2. Photomicrograph of plasmatocyte of *L.l. tropicalis* × 1200
Fig. 3. Photomicrograph of granular hemocytes of *L.l. tropicalis* X 1200
Fig. 4. Photomicrograph of oenocytoids of *L.l. tropicalis* × 1200

Table 1. Characteristics of different haemocytes of *Lipeurus lawrensis tropicalis* Peters

Type of haemocyte	Shape variation		Size variation (μ)		Staining reaction	
	Cell	Nucleus	Cell	Nucleus	Cell	Nucleus
	Round	Round	3.8—4.6	3.1—3.8	Basophilic	Deeply basophilic
	Ovoid	Ovoid	3.1×4.0—4.6	2.5—3.8	Basophilic	Deeply basophilic
Prohaemocytes	Pear shaped	Round or Ovoid	3.1×4.0—4.6	2.5—3.8	Basophilic	Deeply basophilic
	Round	Round	6.2—8.0	3.1—4.6	Lightly eosinophilic	Basophilic
	Ovoid	Round or Ovoid	4.6—6.2×9.3—10.8	3.1—4.6	Lightly eosinophilic	Basophilic
Plasmatocytes	Pear shaped	Round or Ovoid	4.6—5.3×7.8—9.3	3.6—4.9	Lightly eosinophilic	Basophilic
	Spindle shaped	Round	3.1—7.7×12.3—21.7	3.1—4.6	Lightly eosinophilic	Basophilic
	Ovoid	Round or Ovoid	6.2—9.3×9.3—10.8	3.1—4.6	Eosinophilic	Basophilic
Granular	Pear shaped	Ovoid	6.2—9.3×9.3—10.8	3.1—4.6	Eosinophilic	Basophilic
haemocytes	Spindle shaped	Ovoid	4.6—5.3×9.3—12.4	3.1—4.6	Eosinophilic	Basophilic
	Ovoid	Round or Ovoid	6.2—15.5×12.4—31.0	3.8—6.2	Lightly basophilic	Basophilic
Oenocytoids	Pear shaped	Ovoid	6.2—12.5×15.5—15.6	3.8—6.2	Lightly basophilic	Basophilic
	Spindle shaped	Ovoid	4.6—7.7×12.4—31.0	3.8—6.2	Lightly basophilic	Basophilic

Granular haemocytes : These are small, thick, pear or spindle-shaped cells. Their cytoplasm is eosinophilic and contain many sharply outlined granular inclusions (0.2—0 4 μ), which are deeply basophilic and tend to obscure the nucleus. The nucleus is basophilic, spherical or ovoid in shape and usually centrally placed. The cytoplasm in some preparations contain 1—2 vacuoles and is without pseudopodium (Fig. D—G and Fig. 3).

Oenocytoids : The characteristic appearance of oenocytoids is better seen in those preparations which are fixed with acid fixatives and stained in haematoxyline and eosin. These haemocytes are large, thick and conspicuous with excentric nucleus. They may be oval, pear or spindle-shaped. The most common is an ovoid type. Each cell has basophilic cytoplasm filled with characteristic needle shaped crystals, present in most of the cytoplasm. These crystals are packed in such a manner that the small round to oval nucleus is obscured. Furthermore, a differentiation between nucleus and cytoplasm is rather difficult because the nucleus and the cytoplasm are basophilic. In the centre of some of the cells 1-2 vacuoles, measuring 2.75—4.6 μ in diameter are seen. The lysing of oenocytoids has also been noticed. In comparison to other types of haemocytes these haemocytes make up a very small proportion and are better seen in 3rd instars and adult stages. (Fig. S-Z and Fig. 4).

The study of haemocytes of *L.L. tropicalis* is done in the nymphs and adults. The smears made from heat-fixed specimens gave better results than those from unfixed specimens. The identification and classification of different types of haemocytes is done on the basis of staining reaction and the terminology given by Jones (1965) for *Rhodnius prolixes* is adopted. The proleucocyte term is used by Wigglesworth (1933, 1956), while Jones (1965) has preferred the term prohaemocytes for the same type of haemocytes. Zaidi and Khan (1974) have supported Jones terminology on the grounds that haemocytes having small size and scanty cytoplasm in relation to size of nucleus are of transitional nature. Accordingly, these cells can not be considered as leucocytes of the vertebrate blood, and therefore, the term prohaemocyte has been preferred over proleucocytes. The prohaemocytes of *L.l. tropicalis* are similar to leucocytes, proleucocytes or prohaemocytes of other insects and are characteristically the same as in the amblyceran species, *Laemobothrion percnopteri.*

The plasmatocytes of *L. l. tropicalis* are much variable in shape and size, and more or less, of the same nature as in *L. percnopteri* and in other insect species. However, the presence of vacuoles is also noticed in this case. The granular haemocytes are smaller cells, having eosinophilic cytoplasm, which characteristically possess discrete, round to ovoid granular inclusions, which are deeply basophilic. Such cells, though of common occurrence in other insects are not reported to occur in *L. percnopteri.* The oenocytoids, not noticed earlier in Mallophaga, are of common occurrence in *L.L. tropicalis.* These may be scattered singly or seen in clustures and are characterised by the presence of needle-shaped crystals which are packed in entire cytoplasm of the cell. The lysing of oenocytoids has also been noticed. These cells are different in their staining reaction from other types of haemocytes as both the nucleus and cytoplasm are basophilic and the differentiation between the two is difficult.

Summary

The free haemocytes of *Lipeurus lawrensis tropicalis* Peters (Ischnocera : Phthiraptera) are studied. The smears, fixed in Bouins fluid and stained in haemotoxyline-eosin method proved to be better. In all four types of haemocytes *viz.*,

prohaemocytes, plasmatocytes, granular haemocytes and oenocytoids, are reported. Whereas in *Laemobothrion percnopteri* (Amblycera : Phthiraptera) the presence of only two types of haemocytes (proleucocytes, plasmatocytes) was reported by Srivastava (1974).

ACKNOWLEDGEMENTS

The authors are thankful to the Head of the Zoology Department, Banaras Hindu University for laboratory facilities and to State Council of Science and Technology, U.P., Lucknow for financial support under the Scheme No. SCST/4779/BHU (43) 77 sanctioned to Dr. G.P. Agarwal.

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(MS. received : 31-7-1978)