



VITRO STUDY OF THE CIRCULATING HEMOCYTES OF THE BED BUG, CIMEX ROTUNDATUS.

Zoology

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ABSTRACT

A consensus over the hemocyte types, their classification and the terminologies used for their identification has not yet reached to everybody's satisfaction. A diversity in their morphological forms has added further in the confusion. Different workers have identified 6 to 70 hemocyte type. (Arnold, 1979; Gupta, 1979; 1985; Jones, 1962; Price and Ratcliffe, 1974; Sonawane, 1984).

Heteroptera is one of the important insect orders. Only a few reports on their hemocytes are available. Notable amongst them are the contributions by Wigglesworth (1955, 1956), Hamilton (1931), Khanna (1964), Jones (1965), Lai-Fook (1970), Price and Ratcliffe (1974), Zaidi and Khan (1974), Sonawane (1984), More and Sonawane (1989). Most of them have identified five hemocyte types with light microscopy. These are prohemocyte, plasmocyte, granulocyte, spherulocyte, and Oenocytoid. In spite of the great significance of Cimex as an ectoparasite and a vector for some human diseases, it has escaped the attention of the workers in the field. The present communication gives information on the histomorphological characteristics of the circulating hemocytes of Cimex rotundatus. Attempts have also been made to classify them appropriately.

KEYWORDS

MATERIAL AND METHODS

The nymphs and the adults of Cimex rotundatus were collected from the sleeping quarters. The preliminary observations were made on hemocytes by placing a drop of fresh hemolymph on a clean slide which was obtained by amputation of thoracic and/or cephalic appendages. In vitro studies of fixed wet films by phase optics and fixed and stained monolayers with light microscopy were done. The hemocytes of the nymphs and the adults were studied independently. The monolayers were prepared in the following way.

The bugs were immersed in hot water (56°C) for 2 min. The hot water not only served as a partial fixative for the hemocytes but was also useful for cleaning and immobilization of the bug. It also helped to bring more hemocytes in circulation. The bugs were dried up with an absorbent tissue paper. The slides were kept ready with a highly calibrated drop of 2% versene (disodium EDTA) which proved to be the best fixative for the final fixation of the hemocytes. The double fixation, with hot water and 2% versene, resulted in the better preparations of the monolayers. The appendage was amputated under the drop of 2% versene on the slide to avoid an exposure of the hemolymph to the atmosphere. As the hemolymph spreads in the fixative, the hemocytes get adhered to the glass surface, thus forming a monolayer. These slides were air dried and stained separately with Giemsa, Leishman's and Wright's stain for 5 to 7 min. They were then thoroughly washed in tap water (pH 7.8) and rinsed in distilled water. The slides were dehydrated with a brief bath of acetone, cleared in xylene and mounted in DPX. The differential hemocyte counts (DHCs) were determined by counting 200 cells from each preparation. Because of the wide variation among the individual specimens, about 25 bugs (nymphs and the adults separately) were used in the preparation.

OBSERVATION

The hemolymph volume appeared to be more in the nymphs than in the adults and contains more number of the circulating hemocytes. The hemolymph was less granular in the nymphs. In the adults it was more viscous and contained many granules. In both the nymphs and the adults; the fresh unfixed hemolymph expressed on a glass slide coagulates within 2-3 min.

Properly fixed hemolymph observed under phase optics or in light microscope with diaphragm manipulation show vesicular, refractile cells. The differentiation into the cell types however, was difficult, after 5-10 min. the cells attach to the substratum, spread out and reveal intracellular details. Five types of the hemocytes could be identified in the bedbug (Table 1). These were pro hemocytes (PRS), plasmocytes (PLS), granulocytes (GRS), Spherulocytes (SPS), and

Oenocytoids (OES). In addition, thrombocytoids were also observed. Prohemocytes: The PRS were round/oval and their diameter varied between 4-10 μm (figs.3 and 8). The centrally located and intensely stained nucleus occupied most of the cell volume (70 to 90%). Their cytoplasm was dense, homogeneous, agranular and weakly basophilic. It formed a very thin rim at the periphery of the cell around the nucleus. Few of the PRS were found in mitotic division. Transitional forms between PRS and PLS were noticed. They constituted 1-4% of the hemocyte population.

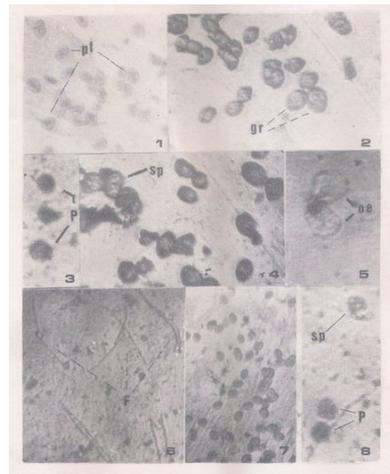


Fig. 1: Plasmocytes (pl) of nymphal Cimex rotundatus in fixed and stained (Wright's) monolayer (X 450)

Fig. 2: Granulocytes (gr) of adult C. rotundatus in fixed and stained (Leishman's) monolayer (X 450)

Fig. 3: Two prohemocytes (p) in the adult haemolymph (X 800)

Fig. 4: Two spherulocytes (sp) in fixed and Giemsa stained film (X 250)

Fig. 5: Oenocytoids (In a group of two) in the adult C. rotundatus. Note eccentric nucleus and thick eosinophilic cytoplasm with eosinophilic inclusions. (X 400)

Fig. 6: Few long cytoplasmic fragments (f) (thrombocytoid fragments). Note the irregular thickness of the fragments (X 450)

Fig. 7: Low power representation of the fixed and stained (Wright's) monolayer, showing plasmocytes as a leading type (X 200)

Fig. 8: One small spherulocyte (Sp) and two prohemocytes (p) of adult bug. Note polychromatic spherules. (X 800)

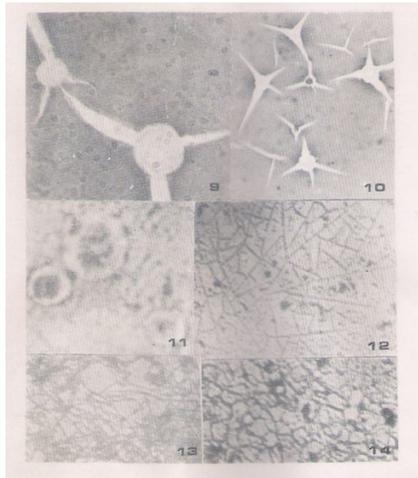


Fig. 9: Two hyaline thrombocytooids in partially fixed haemolymph. Notice the formation of cytoplasmic rays. (X 450)

Fig. 10: Many hyaline thrombocytooids and their fragments. Note the cytoplasmic extensions approaching to neighbouring cells in the process of network formation. (X 250)

Fig. 11: Few thrombocytooids in fixed and stained (Wright's) monolayer showing core of basophilic granule and hyaline periphery. (X 450)

Fig. 12: Pattern of granular coagulation, effect of substances released from thrombocytooids. (X 250)

Fig. 13: Network of coagulation formed by thrombocytooids and their fragments. (X 250)

Fig. 14: Thick coagulum, Note, large number of thrombocytooids, their fragments and few other haemocytes entangled.

Plasmacytes: The PLS were polymorphic in nature. In fresh preparation their amoeboid movements were discernable. In fixed and stained monolayer they appeared round/oval/elliptical in outline (Figs. 1 and 7). Their size varied between 8-14 μm . The nucleocytoplasmic ratio was 30-60%. Their nuclei were round, centrally located and basophilic with evenly distributed chromatin. The cytoplasm was homogeneous and mostly agranular. When granular, they were very fine and were stained eosinophilically. In the wet film preparation PLS were seen attaching to the glass surface and produced pseudopodia like extensions. In the nymphs the PLS constituted 40-60% of the hemocyte population and in the adults they were 20-30%.

Granulocytes: The GRS were oval to round and diameter was 10-10 μm (Fig. 2). The nucleus in them was centrally placed. It was round basophilic and small in size. The nucleocytoplasmic ratio varied between 20-30%. The GRS possessed granules of various sizes. The granules were basophilic in many cells, but in few they were unstained. Their cytoplasm was eosinophilic. The pseudopodia like extensions were not seen. The GRS formed the major class in the adults (45-60%) and in the nymphs they were 20-30%.

Spherulocytes: The SPS were roughly round/oval or spindle shaped (Figs. 4 and 8). Their diameter varied between 10-22 μm . They were usually filled with large granules or spherules of different size. The inclusions were basophilic, acidophilic and the remaining were neutrophilic. The SPS contained central or an eccentric nucleus. It was small and occupied approximately 20-30% space. Often it was obscured by the spherules. The transitional forms between the GRS and SPS were also seen. The SPS constituted about 5-10% cell population. Very often they were seen to release the spherules into the hemolymph by exocytosis.

Oenocytoids : The OES were large oval or pear shaped and their diameter varied between 18-42 μm . Their nuclei were small, eccentrically located and occupied 10-20% of cell column. The cytoplasm was thick in consistency and homogeneous. It contained thick needle like or spherical crystals (Fig. 5) The OES formed 5-8% of cell population and may be dispersed singly or in group of two or three cells. They were the stable cells, not seen lysine even in the unfixed preparation.

Thrombocytooids: Most of them were anucleated. When the nucleus

was present usually it was small and eccentric. The thrombocytooids varied in size, from 4 to 56 μm in diameter. They were spherical. Their cytoplasm contained two types of granules. The core contained intensely basophilic granules and the hyaline granules were seen at the periphery (Fig. 11). In the fresh hemolymph they appeared hyaline, refractile vesicles and showed streaming movements in their cytoplasm. The thrombocytooids were fragile in nature. Immediately after their exposure to the air, cytoplasmic extensions radiated from them. The rays from the adjacent thrombocytes joined to form intricate network of the cytoplasmic threads (fig 10 & 13). The hemolymph in the region became thick and entangled other hemocytes in it. The process was enhanced by the release of their cytoplasmic inclusions into the lymph and it produced a thick coagulum (fig 14). Some thrombocytes got fragmented into small filamentous bodies of different sizes and thickness (fig. 6). The granules from these fragments or may be from the hemolymph precipitated to form the granular coagulum (fig 12). The thrombocytooids in the nymphs were about 10% and in adults their count was 3-5%.

Discussion

The hemocyte morphology appears to be dynamic in nature. This aspect is well discussed by Arnold (1979) and Gupta (1989, 1985). The classification put forward by Price and Ratcliffe (1974) has been adopted presently. In *C. rotundatus*, five morphologically distinct hemocyte types could be identified. They are PRS, PLS, GRS, SPS, and OES. In addition, thrombocytooids could also be identified. Thrombocytooids, however, do not fit in to the classification adopted and hence they are considered separately.

The PLS and / or the GRS constitute the major part of the hemocyte population in a number of insects. In the bedbug, during the nymphal stages, the PLS occur in highest proportion while in the adults their place has been taken over by the GRS. Such change over is probably a functional attribute (Wigglesworth, 1973). During the development of the nymphs the PLS might be involved in some activities suitable for the purpose. The reduction in the PLS number should be also looked from a different angle. In the adults it has been found that the PLS get adhered to the surfaces of the internal organs, i.e. they are taken out of circulation and hence apparently their number goes down. And indirectly this might have led to the increase in the GRS. Possibly of the transformation of the PLS into the GRS can not be overruled (Gupta and Sutherland, 1966; Takada and Kitano, 197; Arnold, 1974). The OES form a distinct hemocyte type. Price and Ratcliffe (1974) are of the opinion that OES are the stable cells. However, Lea and Gilbert (1966) and Nittono (1960) described transformation of the OES into the hyaline cells. Wigglesworth (1973) observed the OES discharging electron dense material into the hemolymph. He was, however, failed to see the ultimate fate of the material. Arnold (1974) claimed that the material ejected from the OES does not result in the gelation of the hemolymph.

Thrombocytooids are yet another category of haemocytes observed in the present study. Such cells have been earlier in *Glossina* (East et al., 1980; Kaaya and Otieno, 1981); in *Calliphora* (Zachary and Hoffmann, 1973); in *Leucilia* are dipterans, crasis is from hymenoptera, whereas *Cimex* is a heteropteran. Although the insects belong to different orders, they all have zoophagous feeding; either haematophagous or microfeeders. The question arises, whether there exists any correlation between food and occurrence of the thrombocytooids. In the red cotton bug, *Dysdercus cingulatus*, occurrence of adiphohemocytes is correlated with the feeding of the bug on cotton seeds (Zaidi and Khan, 1974)

Earlier, the thrombocytooids were reported as hyaline coagulocytes in *L. Sericata* and *C. ignita*; the cell bodies with radiating structures capable of forming coagulation meshwork. The structure of these cells is now evident in *C. Rotundatus*. In nicely fixed and stained monolayer, the cells are spherical, without nucleus or with small eccentric nucleus and with basophilic and hyaline granules. Thrombocytooids in *Glossina* are described as spindle or star shaped, anucleated or with small eccentric nucleus with long cytoplasmic extensions. The thrombocytooids are better identified by their function, rather than by the structure in fresh unfixed hemolymph. The cells are cescicular, hyaline, fragile and immediately get fragmented into small filamentous bodies or produce radiating cytoplasmic extensions to give them star shaped, stellate appearance, further, the rays join each other to form coagulation network.

It is evident that these hemocytes have a role in coagulation. The coagulocytes (=cystocytes) have not been recorded in insects possessing thrombocytoids; then it may be assumed that the thrombocytoids, in the absence of the coagulocytes, take part in the process of coagulation. Although these cells do not have morphological characteristic of ideal coagulocytes, they contain granules of two distinct forms, therefore we presume them as the variant form of the granulocytes. The higher percentage of these cells in the nymphal stages perhaps is the need for sealing the delicate cuticle with higher degree of wear and tear. It is also noteworthy that the number of thrombocytoids increases in repeated hemolymph withdrawal reflecting their role in wound healing. Possibly some of the granulocytes might be transforming in to the thrombocytoids in response to the injury. Thrombocytoids are regarded as the variant form of plasmatocytes (Rowley and Ratcliffe, 1967; Kaaya and Otieno, 1981). On the structural basis the thrombocytoids, although regarded as variant of plasmatocytes or granulocytes, their functional role in haemolymph coagulation is far beyond any doubt.

Studies on the heteropteran hemocytes (Wigglesworth, 1956; Khanna, 1964; Jones, 1965; Lai-fook, 1970; Zaidi and Khan, 1974; Barracco et al., 1987) reveal prohemocytes, plasmatocytes, granulocytes, adipohemocytes and oenocytoids as the distinct types. Present system of classification (Price and Ratcliffe, 1974); however do not consider adipohemocytes as a separate cell type. Unfortunately adipohemocytes are not noticed in the present study.

The occurrence of coagulocytes is noticed in *Nepa cinera* and *Rhodnius prolixus* (Price and Ratcliffe, 1974) but the rate of formation and extent of the "Isles of coagulation" is far less conspicuous. Although coagulocytes are absent in the *Cimex*, the functional analogue, thrombocytoids, variant of granulocytes do occur.

Summary

In vitro studies of the circulating hemocytes of the nymphal and adult bedbug, *Cimex rotundatus* reveal five types of hemocytes; prohemocytes, plasmatocytes, granulocytes, spherulocytes and oenocytoids. In addition, one more category of cells, thrombocytoids were observed in the haemolymph. It is suggested as the variant form of the granulocyte and is functional analogue of the coagulocyte. The occurrence of the thrombocytoids in other insect orders is discussed with the feeding. The characteristics of each type of hemocyte are described on the basis of their histomorphylogy and staining reaction. Differential Haemocyte Count reveals that the plasmatocytes share major bulk of circulating hemocytes in the nymphs, however granulocytes is a leading class in the adult. Occurrence of transitional forms between prohemocytes and plasmatocytes. Granulocytes and spherulocytes is often noticed.

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