Punjab Univ. J. Zool, *Vol.* 1, *pp.* 19—33, 1986.

HAEMOCYTES OF LUCILIA CUPRINA (CALLIPHORIDAE : DIPTERA)

FIRDAUSIA AZAM ALI AND MUHAMMAD ASHRAF NADEEM
Department of Zoology, University of the Punjab, Lahore, Pakistan

ABSTRACT.—Six types of haemocytes were found in the haemolymph of *Lucilia cuprina*. These were prohaemocytes, plasmatocytes, podocytes, vermiform cells, granular cells, and spherule cells. Prohaemocytes were of two distinct sizes and were accordingly divided into two subtypes : microcytes and macrocytes.

INTRODUCTION

Many workers have described the haemocytes of different insects. One of the earliest extensive studies include the work of Yeager (1945) in which he has categorized them into ten main classes in *Prodenia eridania*. Later works include the studies by Rizki (1953); Jones, (1956, 1964, 1965, 1967); Wigglesworth (1959); Hoffman (1970); Lai-Fook (1973) and many others. These authors have divided the haemocytes into different types and there is much confusion regarding the classification. Arnold (1974) and Rowley and Ratcliffe (1981) have given the various synonyms of different types of haemocytes in detail. There can be several reasons for this confusion. For example, the method of fixation can be quite important (Lai-Fook and Neuwrith, 1972; Lai-Fook. 1973). The inherent variability of the haemocytes within the species, the developmental stage in which they are studied, the age of the instar, the difference between the species, their feeding habits and whether they are males or females, could be all important factors. Like many other insect tissues haemocytes can also change their structure and physiology in relation to their moulting cycle as has been emphasized by Locke (1970).

During this work the haemocytes of adult females of different ages were studied and classified using cytological parameters such as appearance and relative size of the nucleus and cytoplasm. The haemocytes of the different developmental stages have been compared and categorized elsewhere.

Among Diptera haemocytes of very few species have been studied and still less is known about cyclorraphan species. Until now haemocytes of only five species belonging to three genera have been described. Rizki (1953, 1957)

described six types for *Drosophila willistoni* and four for *D. melanogaster*. Later on the last species was studied by some other workers, including Whitten (1964) and Nappi and Streams (1969), none of them agreeing completely with Rizki's view. Furthermore, Nappi (1970) studied the haemocytes of *D. eurontus* and described five types.

With respect to *Calliphora erythrocephala* which has been studied by three authors (Rooseboom, 1937; Akesson, 1954; Crossley, 1964) a somewhat similar difference occurs. Similarly in *Sarcophaga bullate*, Jones (1956) and Whitten (1964) found three and five types of categories respectively.

In view of the divergence of views expressed by various authors concerning the haemocytes of this group of Diptera, it was considered that describing the haemocytes of *L. Cuprina* will help in determining their homologies further.

Material ane Methods

Collection and Maintenance of the Flies

The adult female Australian blowflies, *Lucilia cuprina* used in this work were taken from a colony maintained at 27°C to 30°C, 12 hours Photoperiod and relative humidity ranging from 65% to 70%. To avoid any discrepency due to the difference in sex, only females were used. The females at emergence were divided into two groups. The females of group-I were kept throughout on sugar only, while ones of group-II were given *ad libitum* access to protein and sugar source after emergenec.

Examination of Haemocytes

The insects which were selected for haemocyte studies were of the following categories :

1. Newly emerged females, 2. Females belonging to group I : 24-hours, 48-hours, 72-hours old sugar-fed females, 3. Females belonging to group-II : 1-hour, 10-hours, 24-hours, 38-hours, 46-hours, and 72-hours old protein-fed females.

Blood films as well as histological sections of the above developmental stages were prepared. For blood films the whole insects were either exposed for 5-10 minutes to hot glacial acetic acid vapours or submerged in hot water at 60°C for 4-5 minutes. Blood was then forced out though a superficial cut by press-

ing gently the top of the abdomen. A drop of the undiluted haemolymph was thus obtained on a microscope slide, spread by the edge of a coverslip to form a film. These films were air dried and stained with Giemsa's or Wright's stain, cleared in xylene and mounted in Canada Balsam. These two stains gave similar results essentially.

Histological sections to see the distribution and localization of haemocytes were prepared by fixing the insects in alcohlic Bouin's fixative, embedding in Paraffin Wax and cutting at 5 microns. They were stained in Ehrlich's Haematoxylin and counterstained in eosin.

Microscopic studies of the unfixed and unstained haemolymph also proved very helpful when describing the different types of haemocytes. Different types of haemocytes and their nuclei were measured by using an occular micrometer. At least twenty cells of each type were selected at random to give the range of size for each stage.

RESULTS

In the present study the classification of the haemocytes presented by Arnold (1974) and Rowley and Ratchiffe (1981) has predominantly been followed. The account of haemocytes of *Prodena eridania* by Yeager (1945) also proved very useful in determining the various categories of the haemocytes. All the above authors have based their scheme of classification on Jones' (1962) original system of classifying the haemocytes.

In this study the haemocyte classes were first determined for those adult females which had been fed on protein diet. Later on these were compared with larval, pupal and sugar-fed adult females. The larval and pupal haemocyte types have been described elsewhere. The classification was based on staining reaction and cytological parameters such as size, shape and relative size of nucleus and cytoplasm.

Description of the Haemocytes of a Protein-fed Female

The following types of haemocytes were recognised :

(*a*) *Prohaemocytes* (Figs. 1&2) : As described for *Prodenia eridania* (Yeager, 1945) the prohaemocytes of *L. cuprina* are of two distinct size ranges. They are called 'microcytes' and 'macrocytes' for the sake of convenience here. Yeager (1945) has listed them collectively as 'proleucocytoids' but categerized them as

'microcytes' and 'proleucocytes', respectively on the basis of their size. The microcytes in *L. cuprina* are minute cells which are always less than 4 μm, in diameter while the macrocytes vary from 5 to 8.5 μm, when round in shape. Generally they are ellipsoid but sometimes fusiform macrocytes are also seen. They range from 7.5 × 5 μm, to 9.5 × 7 μm. Their nuclei are relatively large and centrally placed occupying most of the cell body varying from 3.5 to 6.5 μm in diameter (Table I). The cytoplasm forms only a thin rim and is usually homogenous. These cells have a compact body, a regular form and a smooth outline. Prohaemocytes are deeply basophilic but macrocytes often have slightly eosinophilic nuclei. The latter are considered prohaemocytes because they are compact, smooth and have large nuclei surrounded by thin bands of cytoplasm. Microcytes and fusiform macrocytes are very often seen in mitotic division while in round macrocytes it is not so commonly seen.

(*b*) *Plasmatocytes* (Fig. 3) : Plasmatocytes are highly polymorphic cells. Although typically they tend to be ovoidal in shape but round, fusiform, spindle-shaped and irregular forms are also common. They are larger than macrocytes and are characterized by having a large round to ovoid, generally centrally placed nucleus surrounded by an equal or larger amount of cytoplasm. The round plasmatocytes vary from 9 to 25 μm, in diameter with nuclei ranging from 4.5 to 15 μm. Ovoidal plasmatocytes range from 10 × 8 μm to 28 × 20 μm. The nuclei of these cells are usually also round but they can be ovoidal too, varying from 4.5 × 5.5 μm to 16 × 12 μm in dimensions and upto 20 μm, in diameter when round (Table 1). Plasmatocytes have a moderately basophilic cytoplasm with eosinophilic nuclei with a distinct nucleolus in some. The cytoplasm is generally very finely granular and stains uniformly but in many cases it contains dark granules of various shapes and sizes. The presence of the granules makes it sometimes difficult to distinguish them from the granular cells. Plasmatocytes have also vacuoles of various sizes in their cytoplasm. The vacuoles are probably the result of release of the granules from the cell body. In stained films scattered granules can be seen in the haemolymph. The vacuoles are larger and more numerous in stained films than in live cells.

The small plasmatocytes are sometimes confused with macrocytes, especially as the only difference that appears between the two is the amount of cytoplasm they have. The former look like those macrocytes that have acquired a bit

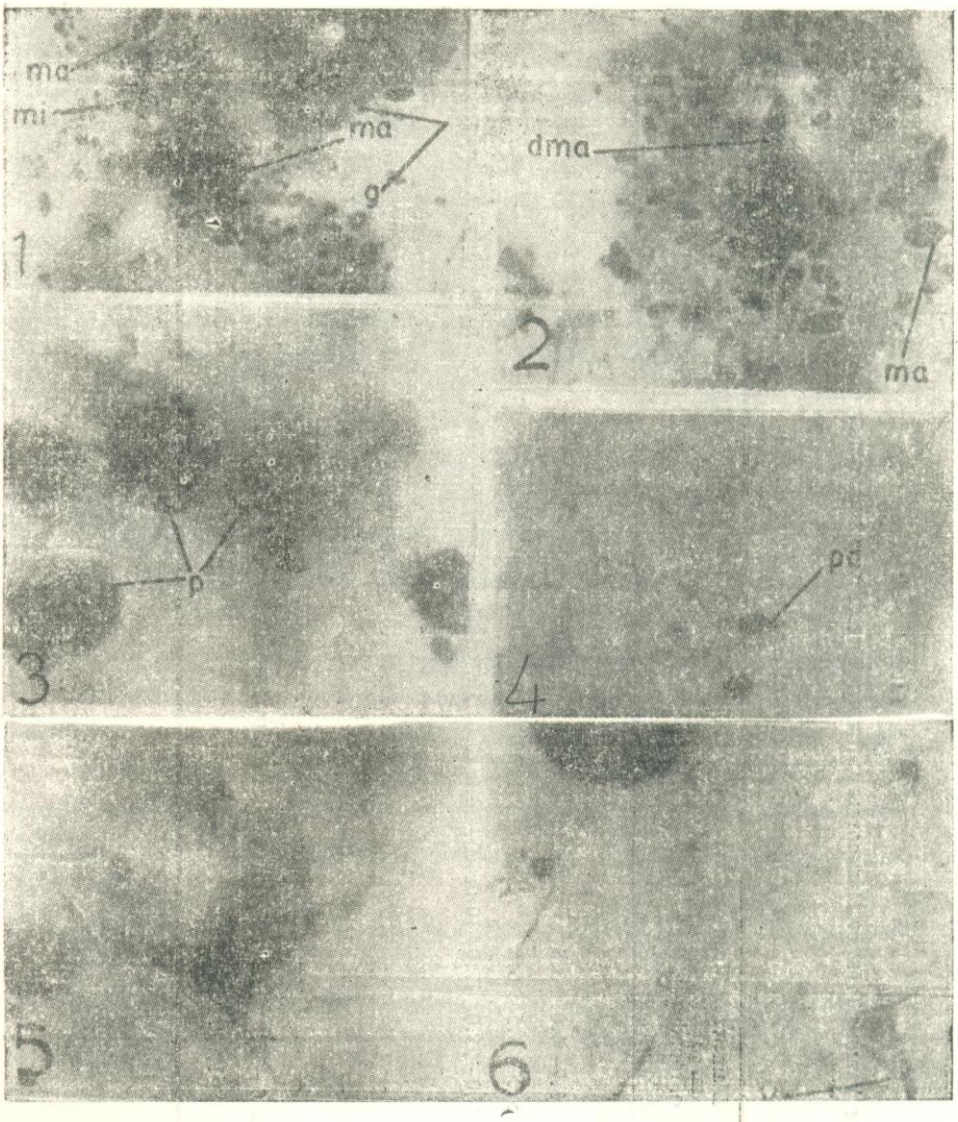

FIG. 1. Microcytes, macrocytes and a granular cell of a protein-fed female. (mi), microcyte; (ma), macrocyte; (g), granular cell. × 1600

FIG. 2. Microcytes and macrocytes of a protein-fed female. (mi), microcytes: (ma) macrocyte; (dma), dividing macrocyte. × 1600

FIG. 3. Plasmatocyte of a protein-fed female. (p), plasmatocyte. × 1600

FIG. 4. Podocyte of a protein-fed female. (pd), podocyte. × 1600

FIG. 5. Podocyte of a protein-fed female. × 1600

FIG. 6. Vermiform cells of a protein-fed female. (v), vermiform cell. × 1600.

TABLE I

DIMENSIONS OF VARIOUS TYPES OF HAEMOCYTES AND THEIR NUCLEI IN THE PROTEIN-FED ADULTS OF *Lucilia cuprina*

(IN μm)

(Mean of 20 cells of each types was taken)

Cell type	Diameter range of round cells	Mean	Cell range of ovoid or any other shape	Mean	Dimension range of round nuclei	Mean	Range of oval nuclei	Mean
Prohaemocytes—								
a-Microytes	3.0—4.0	3.5	3.0x2.0—4.0x3.0	2.5x3.5	2.0—3.0	2.5	—	—
b-Macrocytes	5.0— 8.5	6.5	7.5x5.0—9.5x7.0	8.5x6.0	3.5—6.5	5.0	—	
Plasmatocytes	9.0—25.0	17.0	10.0x8.0—28.0x20.0	19.0x14.0	4.5—15.0	9.8	5.5x4.5—16.0x12.0	10.8x8.3
Podocytes—								
a-Fusiform	—	—	16.0x8.0—25.0x10.0	20.5x9.0	6.0—10.0	8.0	10.0x8.0—12.0x8.0	11.0x8.0
b-Stellate	—	—	16.0x12.0—30.0x20.0	23.0x16.0	6.0—10.0	8.0	10.0x8.0—12.0x8.0	11.0x8.0
Vermiform cells	—	—	16.0x3.0—50.0x6.0	33.0x4.0	—	—	6.0x3.0—9.0x5.0	7.5x4.0
Granular cells	10.0—25.0	17.5	15.0x10.0—35.0x25.0	25.0x17.5	5.0—10.0	7.5	—	—
Spherule cells	20.0—100.0	60.0	25.0x120.0—20.0x80.0	72.5x50.0	10.0—20.0	15.0	—	—

more cytoplasm than is normally the case in prohaemocytes. Binucleate ovoidal plasmatocytes are also seen occasionally. Sometimes these nuclei are equal in size but in other cells they are unequal. Some plasmatocytes with three or four nuclei are also seen. Clusters of variable number of plasmatocytes with intact boundaries are also present. Plasmatocytes with protoplasmic extensions along their periphery are also fairly common.

(*c*) *Podocytes* (Figs. 4 & 5) : These cells have long and tapering cytoplasmic extensions of variable length. The length of these extensions, arms or filipodia varies from 5 to 25 µm beyond the cell membrane. These arms are fixed in position, and are not psudopodial in nature. Some of these cells have fusiform bodies with the two tapering sides extending into long arms. Some of them are stellate with a central mass of cytoplasm with a nucleus, while the arms radiate from this. The cytoplasm of these cells is basophilic and finely granular, while the nucleus is eosinophilic. No vacuoles or darker granules were seen in their cytoplasm, but the nuclei have also round deeply eosinophilic granules in them. Fusiform cells vary from 16 × 8 µm to 25 × 10 µm in dimensions while stellate cells vary from 16 x 12 µm to 30 × 20 µm. Both these types of cells have a round or ovoid nucleus. The round nuclei vary from 6 to 10 µm in diameter while the ovoid nuclei vary from10 × 8 µm to 12 × 8 µm (Table I).

(*d*) *Vermiform Cells* (Fig. 6) : These cells are extemely elongated and thin with finely granular basophilic cytoplasm that extends and tapers into long arms. They vary from 16 to 50 µm in length but their width is very small varying from 3 to 6 µm. The comparatively thick central part sometimes also houses an elongate eosinophilic nucleus ranging from 6 × 3 to 9 × 5 µm in dimensions (Table I).

Some cellls are withoutany apparent nucleus and have only a finely granular cytoplasm in their bodies. This cytoplasm is not so smooth and the granules are not closely packed. These cells seem to be disintegrating because a large amount of granules can also be seen in the haemolymph simultaneously.

(*e*) *Granular Cells* (Figs. 7-10) : Granular cells generally have a smooth and compact body which is usually round or oval with a central more or less spherical nucleus. These cells have a dense basophilic cytoplasmic matrix in which dark granules of different sizes are scattered. The granules are present in all these cells but their number varies. If there are fewer granules

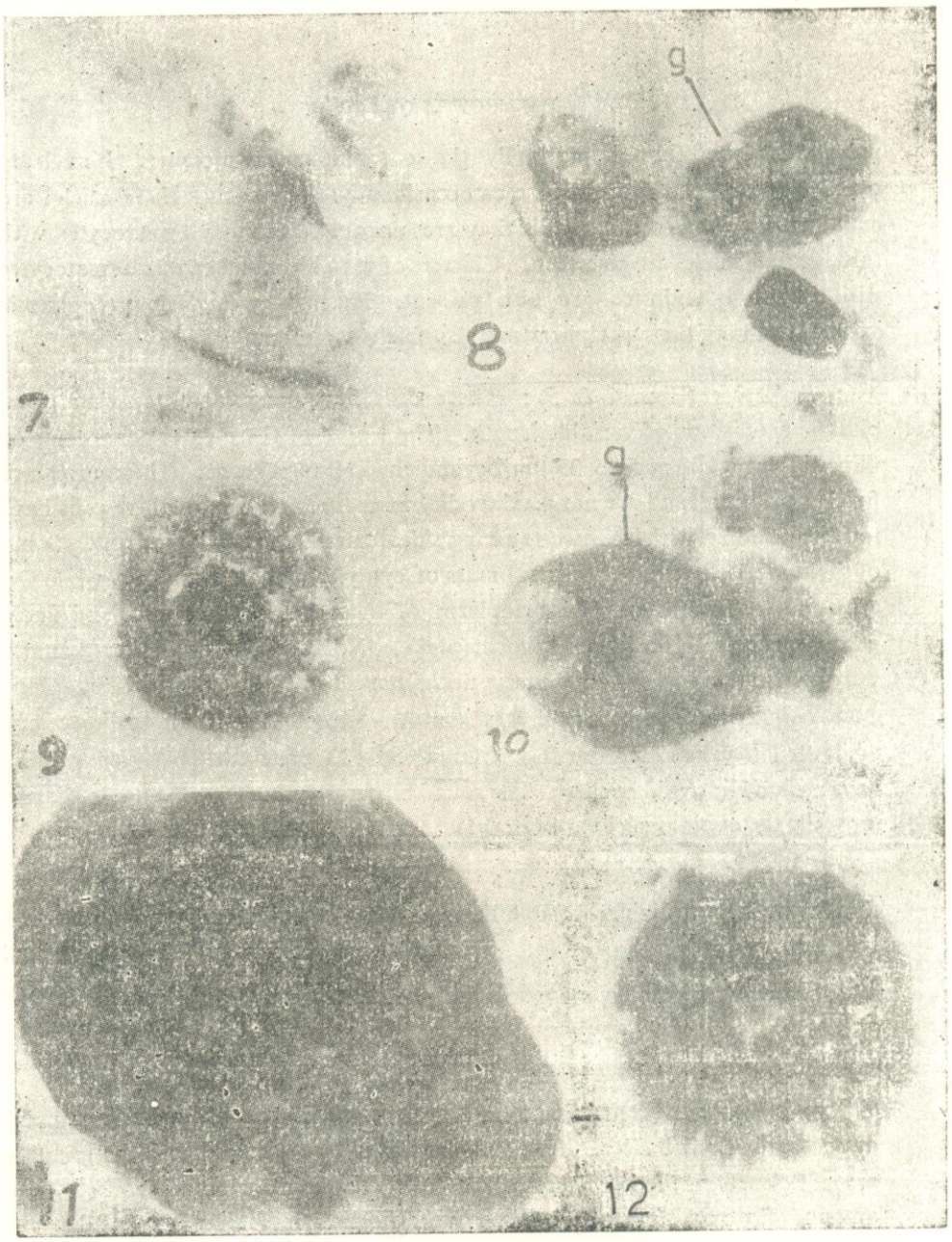

FIG. 7. Vermiform cells of a protein-fed female. (v), vermiform cell ×1600.

FIG. 8. Granular cells of a protein-fed female. (g), granular cell. ×1600

FIG. 9. Granular cells of a protein-fed female. ×1600.

FIG. 10. A binucleate plasmatocyte cell of a protein-fed female. (p), plasmatocyte cell. ×1600

FIG. 11. Immature spherule cell from the haemolymph of a protein-fed female. ×1600

FIG. 12. Mature spherule cell of a protein-fed female. ×1600

they may be scattered randomly in the cytoplasm or located towards the periphery. The size of these granules can vary between 2 and 3 µm in diameter. The cells when round vary from 10 to 25 µm in diameter but when oval they are from 10 to 26 µm wide and 15 to 35 µm long. Their nuclei are eosinophilic and smaller in size as compared to plasmatocyte nuclei and vary from 5 to 10 µm in diameter (Table I). Apart from the granules the cytoplasm also contains vacuoles and vesicles of different sizes. In some stained slides these cells are seen with granules stuck to the outside of the cell membrane and also scattered around the cell body itself as if they have been expelled from it.

(*f*) *Spherule Cells* (Figs. 11 to 14): These cells are very conspicuous because of their large size and spherular inclusions. They are round, ovoidal and sometimes irregular in shape. They vary from 20 to 100 µm in diameter when round and are from 20 to 80 µm wide and from 25 to 120 µm long when ovoidal (Table I). The spherules vary from 2 to 20 µm in diameter. These are membbrane bound and often have themselves 1 to 3 round or rod-like inclusions. Immature spherule cells have fewer spherules which are small in size. Their nuclei are also clear at this stage, but with the increase in their size, the spherule number and size also increase, with the result that the nucleus becomes obscure and the cell boundary becomes distended. The nucleus can also be seen in histological sections where it is quite conspicuous in the centre of the cell body ranging from 10 to 20 µm in diameter. The nucleus is more deeply stained than the cytoplasm. The spherules also have different degrees of basophilia. Some of them stain more deeply than others while some of them do not stain at all. The cell membrane of cells nearing maturation is not always complete but occasionally it is broken and numerous spherules are seen in the vicinity of the cell which have burst out of the membrane. The number of spherules is greatest closer to the cell body but their density decreases away from it. In the fully mature cells the cell boundary gets totally broken and all the spherules become scattered in the haemolymph.

These spherules are round to ovoidal in shape, with clear hyaline bodies having different degrees of basophilia. Their round or rod-like contents stain more deeply blue. These sphrules are very striking feature of the haemolymph and could very well be confused with the crystal cells' or 'Oenocytoids' of many authors (Rizki and Rizki, 1959; Nappi and Streams, 1969; Rowley and Ratcliffe, 1981).

(*g*) *Fixed Haemocytes*: (Fig. 15)

Mostly two types of haemocytes are found attached to various tissues in the histological section of *L. cuprina*. These are plasmatocytes and granular cells. Most of them are found in layers beneath the integument and also between the lobes of the fat body. These plasmatocytes have the same structure and characteristics as described for the free ones. Binucleate cells are more commonly found as fixed cells.

The Haemocytes of a Sugar-Fed Female :

All the types of the haemocytes which have been described above for the protein-fed females were found in the blood of the sugar-fed females also.

Discussion

Although the haemocytes of very few species of Diptera have been described so far, yet there is a lot of controversy regarding their terminology. Even in closely related species different types of haemocytes have been reported. Rizki (1953) described 6 types for *Drosophila willistoni* and later on in 1957 he reported 4 types for *Drosophila malanogaster*, the only two common types between them being plasmatocytes and podocytes. Nappi (1970) described 5 types for *Drosophila euronotus*, some of them he considered the variant forms of plasmatocytes.

In *Lucilia cuprina* the *ad libitum*-fed females were chosen for describing the haemocyte types because in these the hormone supply is not blocked as in the case of sugar-fed females (Applin, 1979 ; Ali and Bokhari, 1982). As described above, 6 morphological types were recognised on the basis of light microscopy. The prohaemocytes were the commonest haemocytes in the newly emerged females and their two subtypes were very distinct. Many workers like Jones (1956, 1965), Nappi (1970), Stang-Voss (1970), Lai-Fook (1973) and Landareau and Grellet (1975) among others have reported the presence of prohaemocytes in insects and they are generally considered the stem cells or germ cells from which other types of haemocytes differentiate (Wiggles worth, 1959; Jones, 1962; Arnold, 1974; Richards and Davies, 1977; Rowley and Ratcliffe, 1981).

In *L. cuprina* they were generally quite distinct from other haemocyte types, particularly the microcytes because of their very small size. Cells comparable

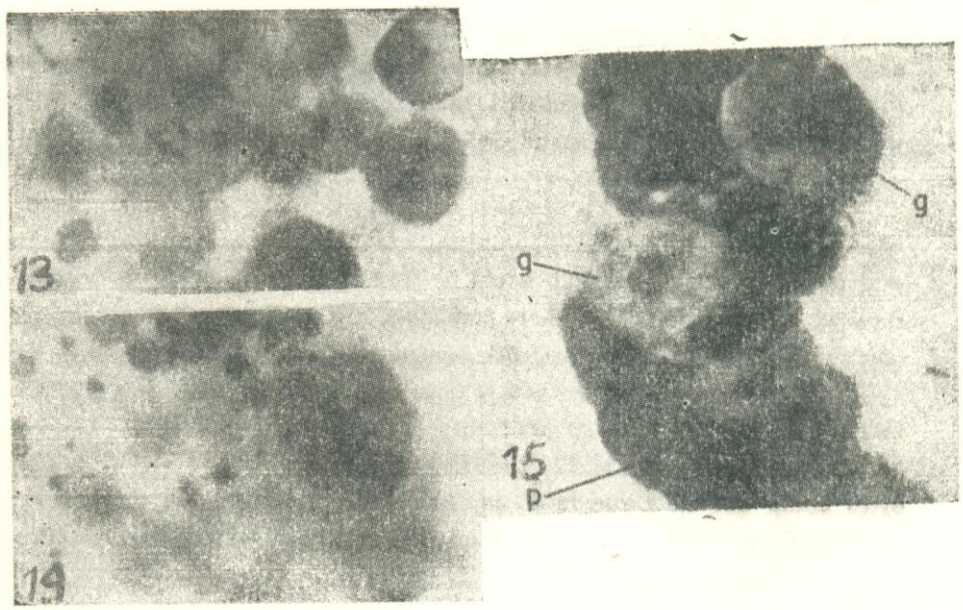

FIG. 13. Spherules from a burst spherule cell from the haemolymph of a protein-fed female. × 1600

FIG. 14. Released spherules of a spherule cell from the blood of a protein-fed female × 1600

FIG. 15. Fixed haemocytes from a histological section of a newly emerged female. (g) granular cell. (p), plasmatocyte. × 1600.

to microcytes of *L. caprina* have also been reported by some other workers like Yeager (1945) and Ashurst and Richards (1964). Most of them were observed undergoing mitosis in the prepared films and were seen continuously moving *in vitro*. Round macrocytes were seldom seen dividing but fusiform macrocytes were sometimes seen undergoing unequal division. In adults they were almost absent.

Plasmatocytes were occasionally seen undergoing division, as has been reported for the larvae of *Drosophila euronotus* (Nappi, 1970). Macrocytes and plasmatocytes were sometimes difficult to distinguish from each other. The only criteria to separate them were the large central nucleus surrounded by a very thin cytoplasmic rim in the former, and sometimes by the presence of vacuoles

and occasional granules in the cytoplasm of the latter. The position of prohaemocytes as stem or germ cells was further established as many intermediate and transitional forms between these two categories could be recognised. Plasmatocytes have been reported almost universally in insects (Wigglesworth, 1959; Jones, 1962; Arnold, 1974; Rowley and Ratcliffe, 1981).

Vermiform cells appear alongwith podocytes in 3-4 day old adults. In these adults many morphologically intermediate forms between plasmatocytes, podocytes and vermiform cells could be seen, thus supporting the view put forward by Nappi (1970) that podocytes are the variant forms of plasmatocytes in the larvae of *Drosophila euronotus*. In *L. cuprina* vermiform cells appeared to be more like the degenerate rather than the variant forms of plasmatocytes. The presence of podocytes has also been reported from other speces of *Drosophila* (Rizki, 1953, 1957), but his and Nappi's (1970) 'lamellocytes' could not be found in *L. cuprina*. Apart from *Diptera* the podocytes and vermiform cells have also been described for some other insects e.g. *Prodenia eridania* (Yeager, 1945).

Granular cells could be a variant form of plasmatocytes, as many intermediate forms between the two could be found in the prepared blood films. For this reason they were sometimes difficult to distinguish from the latter. Their main distinguishing characters were the relatively small nucleus as compared to the size of the cell body and the presence of many dark granules in the cytoplasm. The structure and function of the granules has been discussed by various authors including Neuwirth (1973) and Rowley and Ratcliffe (1981).

Granular cells seemed to undergo degeneration constanly starting first with the disintegration of their cytoplasmic contents. It started with vacuolization of the cytoplasm which was probably the result of the release of granules. In the last stage of degeneration the nuclei became denuded of all cytoplasm and after sometime they themselves broke down and their contents released in the haemolymph.

The spherule cells were quite easily distinguished from granule cells as they had practically no cytoplasm and the cell body was full of basophilic spherules of different sizes. Their spilled out spherules could be seen scattered in the haemolymph They could not be confused with any other cell type in *L. cuprina*. They were the largest cells in the haemolymph, and when immature

their nuclei were quite distinct. Their inclusions had droplets of variable diameters and shapes, which could be seen scattered in the haemolymph when these cells broke down. They looked more like fat cells in *L. curprina*. They could be the extemely mature forms of granular cells in which the granules have greatly increased in size and replaced the cytoplasm. Arnold (1974) also called them a specialized type within the granular haemocyte complex. In *L. cuprina* their released spherules have a hyaline matrix and rod-like or round inclusions, which could be confused with the 'crystal' or 'crystalloid' cells of many authors (Rizki, 1953; Rizki and Rizki, 1959; Nappi and Streams, 1969). In the present study these spherules could not be confused with any other cells as they were seen in their different stages of maturation, release and then degeneration. The considerable controversy regarding their nature has been discussed by several authors like Lai-Fook (1973), Arnold (1974) and Rowley and Ratcliffe (1981). Their burst out contents were scattered about in the haemolymph.

Arnold (1974) and Rowley and Ratcliffe (1981) have considered the crystal cells reported in some other Diptera (Rizki, 1953, 1957; Rizki and Rizki, 1959; Nappi and Streams, 1969) as a special type of oenocytoid. Arnold (1974) has allied oenocytoids with the complex of granular cells. In *L. cuprina* they are distinctly the contents of spherule cells.

In those females where the neurosecretional and hormonal supply from the brain and the corpora allata had been blocked by feeding them on sugar only, (Applin, 1979; Ali and Bokhari, 1982) the haemocyte types were seen as described for *ad libitum*-fed flies.

In *L. cuprina* not all haemocytes were found circulating in the haemocoel. In histological sections many haemocytes were found attached in layers beneath the integument and between the lobes of the fat body, while others were found randomly attached to other tissues of the body. These cells could be a reservoir which can be mobilized during periods of infection or wound healing as suggested by Shapiro (1968). The fixed haemocytes were found to be mostly plasmatocytes or to some extent granular cells.

References

AKESSON, B. 1953. Observations on the haemocytes during the metamorphosis of *Calliphora erythrocephala* (Meig). *Ark. Zool.*, **6** : 203-211.

ALI, F.A. AND BOKHARI, M.A. 1982. Role of medial neurosecretory cells and corpora allata in oogenesis of *Lucilia curprina* (Wied). *Biologia*, **28** : 207-214.

APPLIN, D.G. 1979. Effect of diet on the neuroendocrine system and egg development in the sheep blowfly, *Lucilia sericata*, *Physiol. Ent.*, 6 : 129-134.

ARNOLD, J.W. 1974. The haemocytes of insects. In : *The Physiology of Insecta.* (Ed. by Rockstein) vol. 5, 201-254. Academic Press, New York.

ASHURST, D.E. AND RICHARDS, A.G. 1964. Some histochemical observations on the blood cells of the wax moth *Galleria mellonella* L. *J. Morphol.*, **114** : 247-254.

CROSSLEY, A.C.S. 1964. An experimental analysis of the origins and physiology of haemocytes in the blue blowfly, *Calliphora erythrocephala* (Meig). *J. exp. Zool.*; **.157** : 375-398.

HOFFMAN, J. A. 1970. Orgenes hemotopaietiques du deux insectes orpthopteres, *Locusta migratoria* et *Gryllus bimaculatus* *Z. Zellforsch;* **105** : 451-472.

JONES, J.C. 1956. The haemocytes of *Sarcophaga bullata* (Parker). *J. Morphol.*, **99**: 233-257.

JONES, J.C. 1962. Current concepts concerning insect haemocytes. *Amer. Zool;* **2** : 209-246.

JONES, J. C. 1964. The circulatory system of insects. In : *The Physiology of Insecta.* (Ed. by Rockstein) vol. **3** : 1-107. Academic Press, New York.

JONES, J.C. 1965. The haemocytes of *Rhodnius prolixus* (Stal). *Biol. Bull.*, **.29** : 282-94.

JONES, J.C. 1967. Changes in the haemocyte picture of *Galleria mellonella* (Linnaeus). *Biol. Bull.*, **132** : 211-221.

LAI-FOOK, J. 1973. The structure of haemocytes of *Calpodes ethlius* (Lepidoptera) *J. Morphol.*, **.39** : 79-86.

LAI-FOOK, J. AND NEUWIRTH, M., 1972. The importance of methods of fixation in the study of blood cells. *Can. J. Zool.*, **50:** 1011-1013.

LANDAREAU, J.C. AND GRELLET, P. 1975. Obtention de lignees permanentes d' hemocytes de Blatte. Caracteristiques physiologiques et ultrastructurales. *J. Insect Physiol.*, **2.** : 137-151.

LOCKE, M. 1970. The moult/intermoult cycle in the epidermis and other tissues of an insect *Calpodes ethlius* (Lepidoptera : Hesperiidae). *Tissues and Cells.*, **2** : 197-223.

NAPPI, A.J. 1970. Haemocytes of larvae of *Drosophila euronotus* (Diptera: Drosophilidae). *Ann. Ent. Soc. Am.*, **63** : 1217-1225.

NAPPI, A.J. AND STREAMS, F.A. 1969. Haemocytic reactions of *Drosophila melanogaster* to the parasites, *Pseudocoila mellipes* and *P. bochei. J. Insect Physiol.*, **15** : 1551-1561.

NEUWIRTH, M. 1973. The structure of the haemocytes of *Galleria mellonella* (Lepidoptera). *J. Morphol.*, **39** : 105-124.

RICHARDS, O.W. AND DAVIES, R.G. 1977. *Imm's General Textbook of Entomology*, Vol. 1. 10th ed. Champan and Hall Ltd.,

RIZKI, M.T.M. 1953. The larval blood cells of *Drosophila willistoni. J exp. Zool;* **.23** : 397-411.

RIZKI, M.T.M. 1957. Alterations in the haemocyte population of *Drosophila melanogaster. J. Morphol.*, **.10** : 437-458.

RIZKI, M.T.M. AND RIZKI, R.M. 1959. Functional significance of the crystal cells in the larvae of *Drosophila melanogaster*. *J. Biophys. Biochem. Cytol.*, **5** : 235-240.

ROOSEBOOM, M. 1937. Contribution a l'etude du la cytologie du sang de certains insect ave quelques consideration generales. *Arch. Neer, Zool.*, **2** : 432-559.

ROWLEY, A.F. AND RATCLIFFE, N.A. 1981. In: *Invertebrate Blood cells.* Vol. 2. 421-488 Academic Press, London.

SHAPIRO, M. 1968. Changes in the haemocyte popultion of the wax moth, *Galleria mellonella* during wound healing *J. Insect Physiol.*, **14** : 1725-1733.

STANG-VOSS, C. 1970. Zur ultrastrukture der Blutzellen wiruerbelloser tiere. 1. Uber die Haemacyten der larve de Mehlkafers *Tenebtio moliter L.Z. Zelltorsch.*, **103** : 589-605.

WHITTEN, J.M. 1964. Haemocytes and metamorphosing tissues in *Sarcophaga bullata*, *Drosophila melanogaster* and other cyclorrhaphous Diptera. *J. Insect Physiol*; **10** : 409-428.

WIGGLESWORTH, V.B. 1959. Insect blood cells. *Ann. Rev. Entomol.*, **4** : 1-16.

YEAGER, F.P. 1945. The blood picture of the southern armyworn, (*Prodenia eridania*). *A. Agric. Res.*, 71 : 1-42.