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QUALITATIVE AND QUANTITATIVE STUDIES OF THE HAEMOCYTES OF *LUCILIA CUPRINA* (CALLIPHORIDAE : DIPTERA)

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ABSTRACT.—Early larvae of *Lucilia cuprina* had prohaemocytes, plasmatocytes and granular cells only. Vermiform cells appeared for the first time during the 3rd larval instar and spherule cells during the early pupal life. Sugar-fed females showed a higher percentage of prohaemocytes, plasmatocytes and vermiform cells while granular cells were always less in number as compared to the protein-fed females. During mid-pupal life no proper cells could be identified as the haemolymph was thick with proceeds of histolysis of different body tissues and broken down cells.

INTRODUCTION

Haemocytes of many orders of insects have been classified upto now, but work on dipteran and especially cyclorraphan haemocytes is limited to a few species only. Not only among these few species confusion regarding their terminology occurs, but studies of the haemocytes of the same species by two different workers also reveal it, as is apparent by comparisons made by Nappi (1970). This is partly due to the fact that these species have been studied during different developmental stages without giving due importance to the fact that haemocytes may change or have different functions during different parts of the life cycle of the insect. Lack of quantitative studies during the different developmental stages of an insect also result in this confusion.

The purpose of this work is, firstly to determine the various types of haemocytes that occur in the haemolymph of the different developmental stages of *Lucilia cuprina* and to compare them with those of the adult. Secondly, the order of appearance of different types was observed to study their development and differentiation. Thirdly, differential haemocyte counts were done to observe what kind of haemocytes were predominant at a given stage of life and whether their decrease and increase was correlated with each other.

Material and Methods

The colonies of the Australian blowflies, *Lucilia cuprina* were maintained at 27 °C to 30 °C, 12 hours photoperiod and relative humidity around 70%. The larvae hatched out from the eggs after about 24 hours. Two more moultings occurred at two days interval each. The 3rd instar larvae thus obtained wandered away from the food approximately at the end of the second day but they remained mobile for 4 - 6 hours after which they settled down, and this marked the onset of the pupal life. At this stage of development they were still white, but after about one hour their cuticle started tanning and they assumed the characteristic 'barrel' shape of dipterous puparia. The cuticle darkened and hardened gradually. The adults emerged nearly five days after the start of the pupal life. The larvae were reared on meat throughout their life.

Examination of Haemocytes

The insects which were selected for haemocyte studies were of the following categories : (1) Newly hatched Ist instar larvae, (2) 1-hour, 24-hours and 48-hours old Ist instar larvae, (3) Newly moulted 2nd instar larvae, (4) 1-hour, 24-hours and 48-hours old 2nd instar larvae, (5) Newly moulted 3rd instar larvae. (6) 1-hour, 24-hours and 48-hours old 3rd instar larvae, (7) White pupa, (8) 24-hours, 48-hours, 72-hours and 96-hours old pupae.

Blood films as well as histological sections of the above developmental stages were prepared in the same way as described for the adults of these blowflies (Ali and Nadeem, 1986).

Microscopic studies of the unfixed and unstained haemolymph also proved very helpful when describing the different type 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.

Counting the Haemocytes

Differential haemocyte counts (DHCs) were made in the way usually employed with blood smears. Haemocytes in a smear were found randomly and identified. Approximately 400 cells/developmental stage were classified and a minimum of four insects of a given age were used for this purpose. Those

cells which appeared to be intermediate between the two haemocyte categories were divided equally between the categories following the method employed by Nappi (1970). For DHCs of the sugar-and ad libitum-fed females of different ages the smears already prpeared for describing their haemocytes were used (Ali and Nadeem, 1986).

RESULTS

The classification of larval and pupal haemocytes of *L. cuprina* was based on the same criteria as used in the adults of these blowflies., that is, their staining reaction and cytological parameters such as size, shape and relative size of nucleus and cytoplasm. The terminology is the same as adopted for the adult haemocytes (Ali and Nadeem, 1986).

As the morphological and staining characteristics of the haemocytes of the different developmental stages were found to be the same as those of the adults, so only the haemocyte types and DHCs of these stages are given here. In addition, the DHCs of the haemolymph of the adult female are also given for the sake of comparison and to show the sequence of their appearance qualitatively as well as quantitatively after emergence.

First Larval Instar

In the Ist instar larvae only three types of haemocytes are found, although their percentage varies with age. In the newly hatched larva microcytes make up nearly 97% of the total haemocyte population while macrocytes 2%, plasmatocytes 0.5% and granular cells are also 0.5%. Macrocytes are mostly fusiform and nearly half of them are seen dividing unequally. The diameter of microcytes varies from 2 to 3 μm, while the range of m crocytes is from 4 to 6 μm in length and 2 to 4 μm in width. Plasmatocytes are nearly all round with a diameter range from 6 to 8 μm and nuclei from 4 to 5 μm. Granular cells range from 6 to 12 μm in length and 6 to 8 μm in width with round nuclei ranging from 2 to 4 μm in diameter.

As the larvae grow older the percentage of microcytes decreases gradually with a rise in other haemocyte types accompanied with an increase in the size of the latter types. In 24-hours old larvae the percentage of microcytes has fallen to 80% while macrocytes are 9%, plasmatocytes 10% and granular cells 1%. Towards the end of this larval instar the percentage of microcytes has

fallen slightly and is now about 75%. Macrocytes are 10%, plasmatocytes 12% and granular cells 3%. There is no perceptible increase in their size during this stage. The percentage of different types of haemocytes and their size range is given in Tables I and II respectively.

Second Instar Larva

In the early 2nd instar the percentage of microcytes is 94%, while macrocytes are 3%, plasmatocytes 2% and granular cells 1%. As the larva grows older, microcytes decrease in number accompanied by an increase in other types of haemocytes. In 24-hours old 2nd instar larva, microcytes make up only 40% of the total haemocyte population while macrocytes are 23%, plasmatocytes 35% and granular cells 2%. Towards the end of the 2nd instar, plasmatocytes have increased in number and are upto 55% while microcytes are only 20%. Macrocytes are also 20% and granular cells are now 5% (Table II). The size range of different haemocytes and their nuclei is more or less the same during the whole duration of the 2nd instar larval life and is given in Table III. Many macrocytes are seen undergoing unequal division and some plasmatocytes are also seen in telophase.

TABLE I:—PERCENTAGE OF LARVAL DHC OF *Lucilia cuprina*
(Percentage was determined from a total of 400 haemocytes)

Types of Cells	1st Instar			2nd Instar			3rd Instar		
	Early	Mid	Late	Early	Mid	Late	Early	Mid	Late
Prohaemocytes									
a—Microcytes	97	80	75	94	40	20	50	20	15
b—Macrocytes	2	9	10	3	23	20	5	10	15
Plasmatocytes	0.5	10	12	2	35	55	25	55	25
Vermiform cells	—	—	—	—	—	—	—	—	40
Podocytes	—	—	—	—	—	—	—	—	—
Granular cells	0.5	1	3	1	2	5	20	15	5

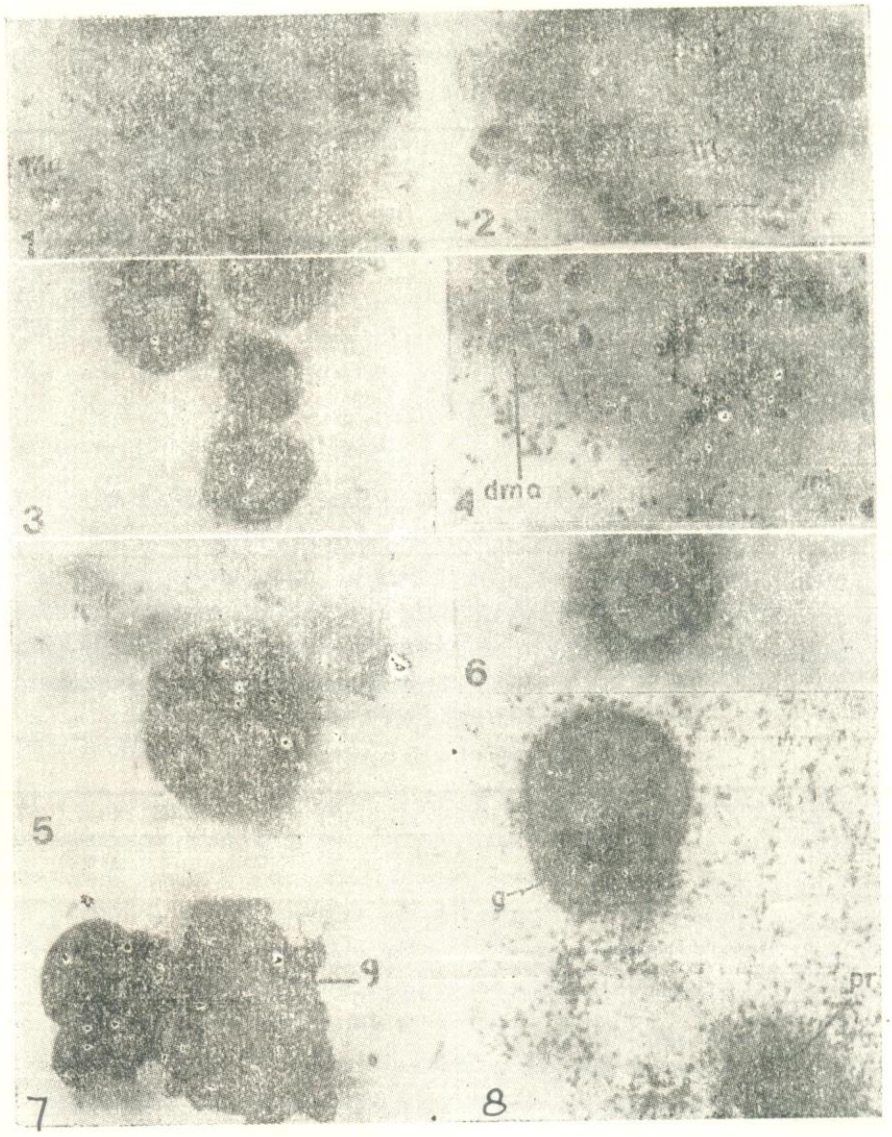

FIG. 1. Prohaemocytes of an early Ist instar larva. (mi), microcyte; (ma), macrocyte, ×1600
FIG. 2. Prohaemocytes of a late Ist instar larva. (mi), microcyte; (ma), macrocyte. ×1600
FIG. 3. Plasmatocytes of Ist instar larva. ×1600
FIG. 4. Prohaemocytes of a 2nd instar larva. (mi), microcyte; (ma), macrocyte. (dma), dividing macrocyte. ×1600
FIG. 5. Plasmatocyte of a 2nd instar larva. ×1600
FIG. 6. Plasmatocyte with vaculated cytoplasm of a 2nd instar larva. ×1600
FIG. 7. Granular cell (g) with vacuolated cytoplasm of a 2nd instar larva. ×1600
FIG. 8. Prohaemocytes and a granular cells of a 3rd instar larva. (pr), prohaemocytes; (g), granular cell. ×1600

TABLE II. DIMENSIONS OF THE VARIOUS TYPES OF HAEMOCYTES AND THEIR NUCLEI IN THE IST INSTAR LARVAE OF *Lucilia cuprina* (in μm)

(Mean of 20 cells of each type was taken)

Cell type	Cell range				Nuclei range			
	Length range	Mean	Width range	Mean	Length range	Mean	Width range	Mean
Prohaemocytes								
a - Microcytes	3—3	3	3—3	3	—	—	—	—
b - Macrocytes	4—6	5	2—6	4	—	—	—	—
Plasmatocytes	6—8	7	6—8	7	4—5	4.5	4—5	4.5
Podocytes	—	—	—	—	—	—	—	—
Vermiform cells	—	—	—	—	—	—	—	—
Granular cells	8—12	10	6—12	9	3—4	3.5	2—4	3

TABLE III. DIMENSIONS OF THE VARIOUS TYPES OF HAEMOCYTES AND THEIR NUCLEI IN THE 2ND INSTAR LARVAE OF *Lucilia cuprina* in (μm)

(Mean of 20 cells of each type was taken)

Type of cells	Cell range				Nuclei range			
	Length range	Mean	Width range	Mean	Length range	Mean	Width range	Mean
Prohaemocytes								
a - Microcytes	3—4	3.5	3—4	3.5	—	—	—	—
b - Macrocytes	4—7	5.5	3—5	4.5	—	—	—	—
Plasmatocytes	6—10	8	6—8	7	4.5—6.5	5.5	4—6	5
Podocytes	—	—	—	—	—	—	—	—
Vermiform cells	—	—	—	—	—	—	—	—
Granular cells	10—16	13	8—16	12	4.5—6.5	5.5	4—6	5

Third Instar Larva (Figs. 8-14)

In the 3rd instar larva also, the percentage of microcytes is highest at the beginning followed by a decline with the accompanied rise in the other haemocyte types. It falls from the initial percentage of 50 to almost 15 towards the end of the instar, while macrocytes rise from the initial 5% to 15% towards the end. Plasmatocytes increase from 25 to 55% in the mid-instar but decrease again towards the end to 25%. Granular cells decrease from the initial 20% to 15% in the mid-instar and to 5% at the end. Vermiform cells form the major component of the haemocyte population towards the end of the 3rd instar and are about 40%. They vary from 15 to 50 μm in length and 4 to 8 μm in width with round or elliptical nuclei. The round nuclei are from 3 to 8 μm in diameter while the elliptical ones vary from 2.5×5 μm to 4×8.5 μm. The dimensions of the various types of haemocytes and their nuclei are given in Table IV.

Haemocytes of the Pupal Haemolymph (Figs. 15-25).

After one hour of the onset of the pupal life the haemolymph is still thin and clear enough so that its haemocytes can be determined quite accurately. At this stage all the haemocyte types present in the adult, with the exception of podocytes, are represented here. During this period the haemolymph has about 7% macrocytes, 2% granular cells, 0.5% spherule and 0.5% vermiform cells while the remainder are all plasmatocytes. Spherule cells appear for the first time during the early pupal life. They are round or ovoid and without any spherules at this stage. They have a clear and slightly basophilic cytoplasm. Their length varies from 35 to 40 μm and width from 25 to 28 μm while their nuclei are from 10 to 12 μm in diameter. Plasmatocytes are the most conspicuous and abundant cells ; they have an eosinophilic nucleus and a basophilic cytoplasm in which small vacuoles are evenly distributed. In almost all of these haemocytes the nucleus is eccentric. Clumping of plasmatocytes and macrocytes has started to take place, but their cell boundaries are clear and no merging of the cytoplasm has yet started. In about 20% plasmatocytes the nuclei have started to degenerate and in some of them vacuoles can be seen. They are polymorphic but mostly round or ovoid. Two or three macrocytes and plasmatocytes appeared to be in mitotic division. Macrocytes are mostly spindle-shaped or round, ranging from 7 to 16 μm in width and 12 to 16 μm in length. Their nuclei are also either round or ovoid, from 4 to 10 μm in

width and 8 to 10 μm in length. Plasmatocytes vary from 8 to 20 μm in width and 12 to 22 μm in length with 6 to 10 μm wide and 8 to 12 μm long nuclei. The round nuclei are from 6 to 12 μm in diameter. Granular cells are slightly bigger in size and range from 14 to 24 μm in width and 20 to 28 μm in length. Their nuclei are also mostly round with a diameter of 10 to 12 μm. Vermiform cells vary from 16 to 40 μm in length and 4 to 6 μm in width. The percentage of occurrence and dimensions of various types of haemocytes for the newly formed pupa are given in Tables V and VI, respectively.

As the pupae grow old there is a perceptable increase in the size of the plasmatocytes as a result of increased vacuolization. Upto 10 hours of the pupal life, the haemolymph is still clear and thin enough so that various haemocyte types can be determined with some accuracy from the prepared blood films. The plasmatocytes make up about 90% of the haemocyte population while macrocyte are 8% and granular cells only 2% (Table V). No other cell types seem to be represented during this stage. The clumping process has somewhat intensified. These clumps now contain only clusters of nuclei with traces of cytoplasm around them. The intact cells sometimes show cytoplasmic processes of different lengths which too have vaculoles. The dimensions of various types of haemocytes for 10-hour pupa are given in Table VII.

After 10 to 15 hours the haemolymph becomes thick and whitish with signs of histolysis. The major components seem to be the spherules from the spherule cells, fat body cells and their released contents, also larval fragments and various other cells which stain too deeply blue to be recognised. Scattered pale pinkish and enlarged nuclei without any cytoplasm can also be seen. These conditions prevail upto about 72 hours of the pupal life. After the start of reorganization of different body tissues haemocytes also reappear. Spherule cells are the first to be recognized; they make their appearance from 50 to 60 hours of the pupal life. By the time the pupa has become 96 hours old, all the cell types including resting and dividing microcytes can be seen. No podocytes or vermiforms cells are found at this stage of development. As the pupa nears emergence and becomes pharate adult the blood picture is more like the newly emerged adult. The percentage of occurrence and dimensions of various types of haemocytes for 96-hour pupa are given in Tables V and VIII respectively. For the period of histolysis they could not he recognised from other contents of the haemolymph, so it was not possible to count them.

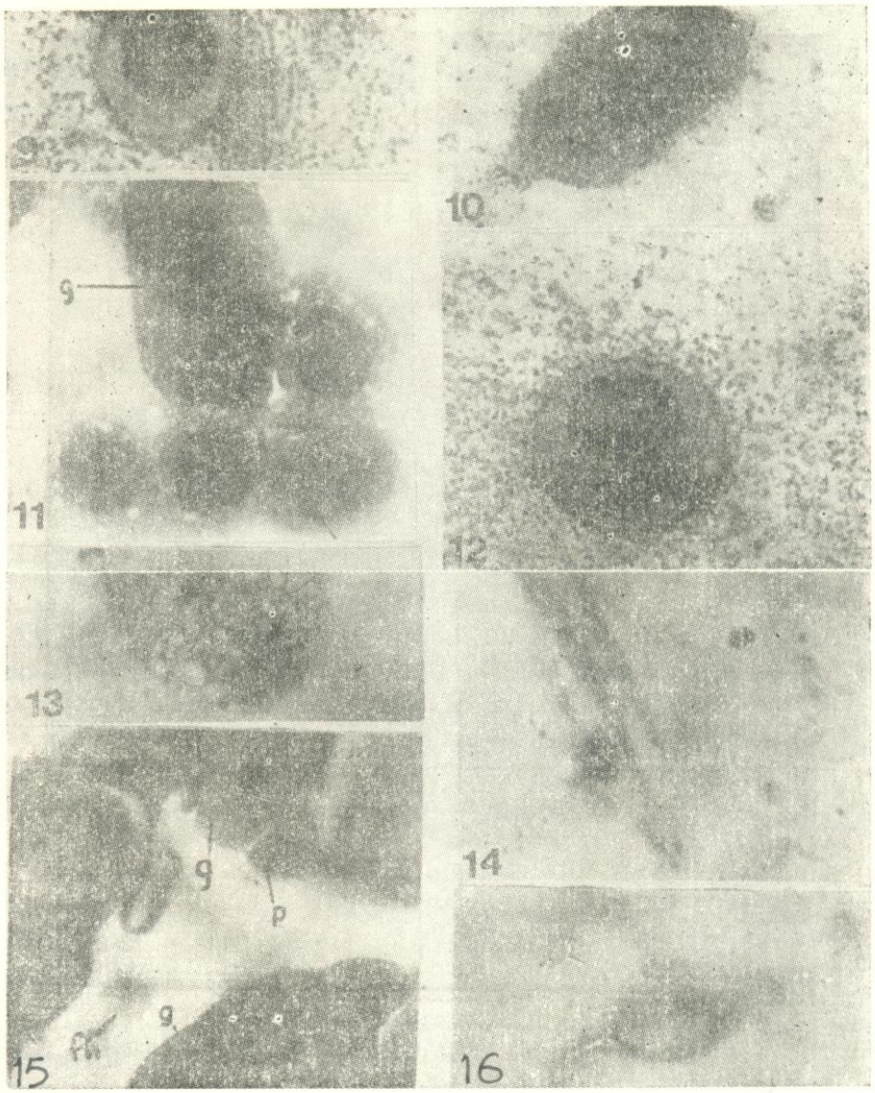

FIG. 9. Plasmatocyte of 3rd instar larva. ×1600.
FIG. 10. Granular cell of a 3rd instar larva. ×1600
FIG. 11. Granular cell and plasmatocytes of 3rd instar larva. (g), granular cell; (p), plasmatocyte. ×1600.
FIG. 12. An intermediate cell between a plasmatocyte and a granular cell from the haemolymph of a 3rd instar larva. ×1600.
FIG. 13. A granular cell with vacuolated cytoplasm in the haemolymph of a 3rd instar larva. ×1600.
FIG. 14. Vermiform cells of a 3rd instar larva. ×1600.
FIG. 15. Fixed haemocytes from a histological section of 3rd instar larva, (g), granular cell; (p), plasmatocyte; (fh), free haemocyte. ×1600.
FIG. 16. Pupal prohaemocyte. ×1600.

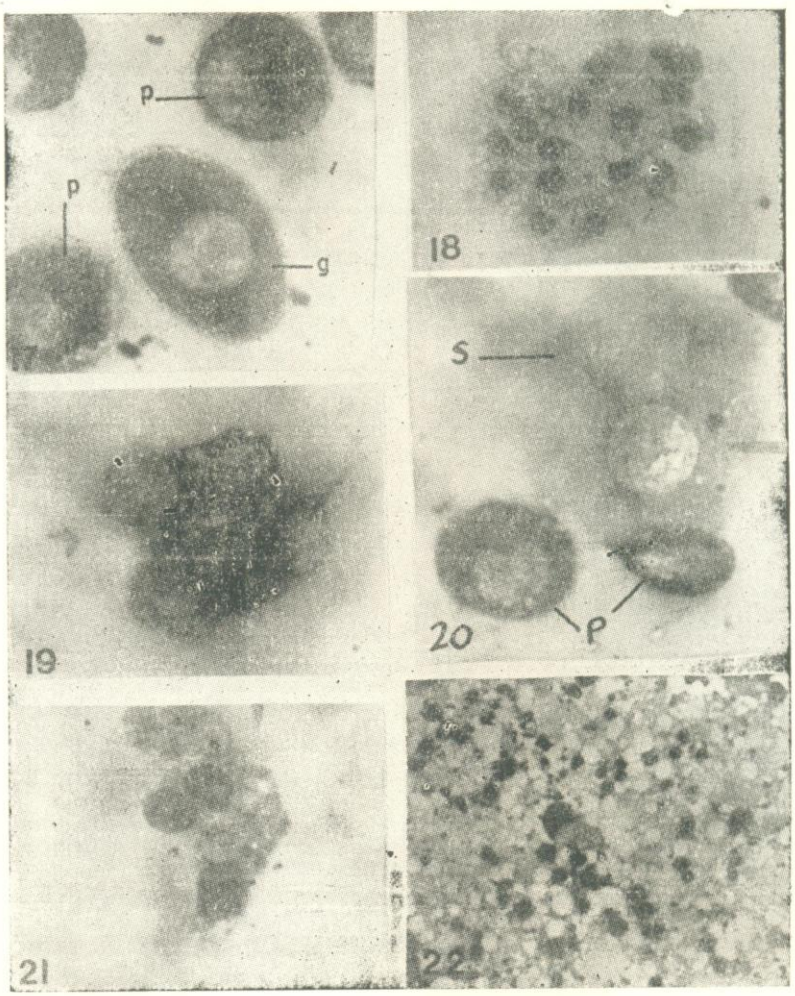

FIG. 17. Plasmatocytes and a granular cell in the haemolymph of 1-hour pupa (p), plasmatocyte ; (g), granular cell. × 1600.

FIG. 18. Aggregating plasmatocytes in the haemolymph of 1-hour pupa. × 800.

FIG. 19. Aggregating plasmatocytes in the haemolymph of 10-hour pupa. × 1600.

FIG. 20. Plasmatocytes (p) and an immature spherule cell (s) from the haemolymph of 1-hour pupa.

FIG. 21. Degenerating granular cell in the haemolymph of 10-hour pupa. × 1600.

FIG. 22. Part of a haemolymph-smear of 24-hour pupa .

TABLE IV : DIMENSION OF THE VARIOUS TYPES OF HAEMOCYTES AND THEIR NUCLEI IN THE 3RD INSTAR LARVAE OF *Lucilia cuprina* (in μm).
(Mean of 20 cells of each type was taken)

Type of cells	Cell range				Nuclei range			
	Length range	Mean	Width range	Mean	Length range	Mean	Width range	Mean
Prohaemocytes								
a - Microcytes	—	—	—	—	—	—	—	—
b - Macrocytes	10—14	12	8—14	11	8—10	9	8—8	8
Plasmatocytes	20—24	22	12—20	16	12—16	14	8—16	
Podocytes	—	—	—	—	—	—	—	—
Vermiform cells	15—50	32.5	4—8	6	5—8.5	6.8	2.5—4	3.3
Granular cells	12—30	21	12—40	18	6—10	8	4—10	7

TABLE V : PERCENTAGE OF THE PUPAE DHC OF *Lucilia cuprina*.
(Percentage was determined from a total of 400 haemocytes)

Type of cells	O-hour pupa	10-hour pupa	96-hour pupa
Prohaemocytes	—	—	
a - Microcytes	—	—	25
b - Macrocytes	7	8	25
Plasmatocytes	90	90	40
Podocytes	—	—	—
Vermiform cells	0.5	—	—
Granular cells	2	2	6
Spherule cells	0.5	—	4

TABLE VI : DIMENSIONS OF THE VARIOUS TYPES OF HAEMOCYTES AND THEIR NUCLEI IN THE NEWLY FORMED PUPAE OF *Lucilia cuprina* (in μm).
(Mean of 20 cells of each type was taken)

Cell types	Cell range				Nuclei range			
	Length range	Mean	Width range	Mean	Length range	Mean	Width range	Mean
Prohaemocytes								
a - Microcytes	—	—	—	—	—	—	—	—
b - Macrocytes	12—16	14.0	7—16	11.5	8—10	9	4—10	7
Plasmatocytes	12—22	17.0	8.20	14	8—12	10	6—10	8
Podocytes	—	—	—	—	—	—	—	—
Vermiform cells	16—40	28.0	4—6	5	—	—	—	—
Granular cells	20—28	24.0	14—24	19	10—12	11	10—12	11
Spherule cells	35—40	37.5	25—28	26.5	10—12	11	10—12	11

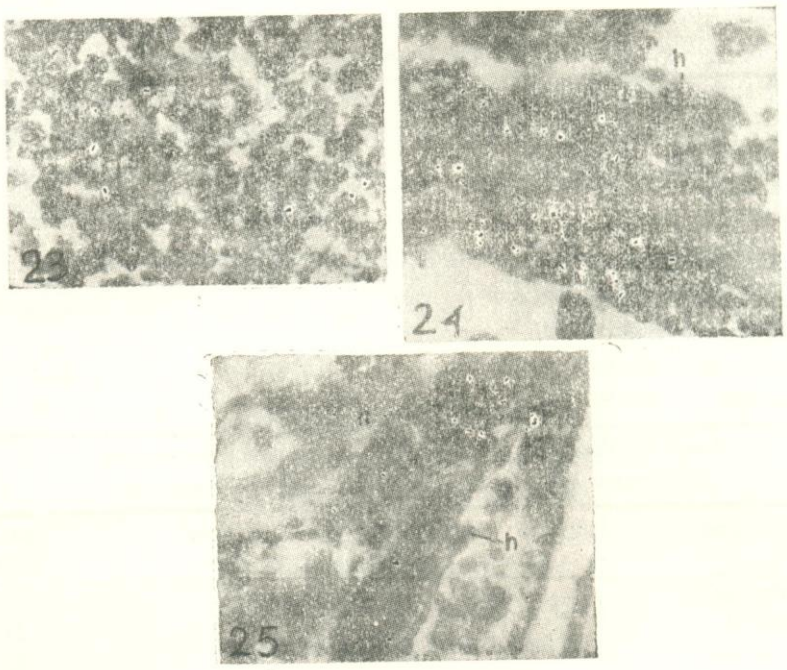

FIG. 23. Part of a haemolymph smear of 48-hour pupa.

FIG. 24. T.S. of a 24-hour old pupa showing body tissues surrounded by haemocytes. (h), × 1600.

FIG. 25. T.S. of 48-hour old pupa showing body tissues surrounded by haemocytes (h). × 1600.

Differential Haemocyte Counts of the Adult Females

The differential haemocyte counts in the adult females also show variation in relation to age. In the newly emerged flies microcytes form almost 90% of the haemocyte population, while macrocytes form nearly 4% of it. At this stage granular cells form about 2% and spherule cells 1% of the haemocyte population. The rest of them are plasmatocytes. Many cells were found to be intermediate between micro-and macrocytes and between macrocytes and plasmatocytes. In the newly emerged flies no other types of haemocytes are seen. The percentage of microcytes decreases with age, *i.e.* in 10-hour old adults microcytes form 60%, macrocytes about 20% and spherule cells about 6% of the total haemocytes. The remainder are all plasmatocytes. Many of them are transitional forms

between the two types of haemocytes. Nearly all the microcytes are seen in mitotic division. In approximately 24-hour old flies the number of plasmatocytes has increased upto 30% and many of them are fusiform and undergoing unequal division. The number of microcytes has decreased to nearly 25% while macrocytes are upto 25% but granular cells have increased in count and are now about 15%. Spherule cells form about 5% of the haemocyte count. As the fly grows old microcytes decrease until in 72-hour old flies they are nearly absent. The main bulk of the haemocyte population is now formed by plasmatocytes vermiform cells, podocytes and granular cells. At this stage plasmatocytes form nearly 50%, granular cells about 25%, vermiform cells about 9%, podocytes 8%, spherules 1% and macrocytes about 2% of the total number of haemocytes counted (Table IX). Apart from these haemolymph also contains spherules from burst spherule cells.

In the sugar-fed females, upto 24 hours of their life, the percentage of different types of haemocytes is more or less the same, but after 24 hours the number of podocytes and vermiform cells is higher than those in the haemolymph of the protein-fed females of comparable age, and microcytes are seen dividing even upto 72 hours of adult emergence. Some haemocyte types are less while others are equal in numbers (Table IX).

Discussion

The haemocytes of *L. cuprina* have been studied quantitatively from the 1st instar larva upto the mature adult to observe the order of appearance of different types. This was also meant to help determining the relationship, if any, among the different types of haemocytes. DHCs under different feeding conditions were done to clarify this relationship further.

Prohaemocytes especially microcytes were found to be the most abundant cells in the early life of the different larval instars, but they decreased in number as an instar became older. This decrease in number was always accompanied by an increase in the percentage of plasmatocytes. This trend continued in the adult females also. In the mature females prohaemocytes made up a very small percetage of the toal haemocyte count. This observation supported the view expressed by many (Wigglesworth, 1959 ; Jones, 1962; Arnold, 1974; Rowley and Ratcliffe, 1981) that they are the germ cells or stem cells from which other types of haemocytes differentiate.

The number of plasmatocytes varies during different periods of life. They form the major component of haemocytes towards the end of the larval instars, but they are maximum in number in the pupa, where they presumably perform the function of phagocytosis, as an extensive amount of histolysis take place during that time. In the histological slides they can be seen adhering to various body tissues thus supporting the view that they are phagocytic in function (Arnold, 1974; Rowley and Ratcliffe, 1981).

Near the end of the pupal life they form almost a layer alongwith granular cells just beneath the new pupal integument. Their position supports the view that they have some function in forming certain parts of the integument (Wigglesworth, 1973).

Unlike prohaemocytes and plasmatocytes, vermiform cells appeared only towards the end of the 3rd instar larva. A low percentage of them was also found in the early pupal life but after that they disappeared and were again seen 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 giving some support to 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 degenerate rather than the variant forms of plasmatocytes. Their contents ultimately scattering in the haemolymph.

TABLE VII : DIMENSIONS OF VARIOUS TYPES OF HAEMOCYTES AND THEIR NUCLEI IN 10-HOUR PUPAE OF *L. cuprina* in (μm)
(Mean of 20 cells of each type was taken)

Cell types	Cell range				Nuclei range			
	Length range	Mean	Width range	Mean	Length range	Mean	Width range	Mean
Prohaemocytes								
a - Microcytes	—	—	—	—	—	—	—	—
b - Macrocytes	12—18	15	8—18	13	10—15	12.5	6—15	12.5
Plasmatocytes	16—30	23	12—30	21	10—19	14	6—18	12
Podocytes	—	—	—	—	—	—	—	—
Vermiform cells	—	—	—	—	—	—	—	—
Granular cells	22—38	30	16—35	30.5	10—15	12.5	8—15	11.5

TABLE VIII : DIMENSIONS OF THE VARIOUS TYPES OF HAEMOCYTES AND THEIR NUCLEI IN 96 - HOUR PUPAE OF *Lucilia cuprina* (in μm)

(Mean of 20 cells of each type was taken)

Cell types	Cell range				Nuclei range			
	Length range	Mean	Width range	Mean	Length range	Mean	Width range	Mean
Prohaemocytes								
a - Microcytes	3—4	3.5	2—4	3.0	2—3	2.5	2—3	2.5
b - Macrocytes	5—8	6.5	5—7	6.0	4—6	5.0	3—4	3.5
Plasmatocytes	10—25	17.5	8—25	16—5	6—18	12.0	5—18	11.5
Podocytes	—	—	—	—	—	—	—	—
Vermiform cells	—	—	—	—	—	—	—	—
Granular cells	18—28	23.0	12—26	—19.0	8—10	9.0	6—10	8.0
Spherule cells	60—128	94.0	50—128	89.0	—	—	—	—

TABLE IX: PERCENTAGE OF THE DHC OF THE FEMALES OF *Lucilia cuprina* IN RELATION TO AGE (PERCENTAGE WAS DETERMINED FROM A TOTAL OF 400 HAEMOCYTES).

Type of cells	0-hour old females		4-hour old females		10-hour old females		24-hour old females		48-hour old females		72-hour old females	
	P-fed	S-fed	P-fed	S-fed	P-fed	S-fed	P-fed	S-fed	P-fed	S-fed	P-fed	S-fed
Prohaemocytes												
a-Microcytes	90	90	70	70	60	60	25	26	—	3	—	3
b-Macrocytes	4	5	15	15	20	23	25	27	8	10	2	3
Plasmatocytes	3	3	8	10	10	10	30	35	40	45	50	60
Podocytes	—	—	—	—	—	—	—	—	10	15	8	11
Vermiform cells	—	—	—	—	—	—	—	—	10	16	9	12
Granular cells	2	1	5	3	8	5	15	10	25	10	25	12
Spherule cells	1	1	1	1	6	6	5	2	1	1	1	1

P—fed = Protein-fed
S—fed = Sugar-fed.

Granular cells could be a variant form of plasmatocytes, as many intermediate forms between the two could be found in the prepared blood films. They were present during the whole life of the fly but their number was very small in the early instars. In the late 3rd instar larva these somewhat increased but they were at their maximum in the adult. In 3-4 day old flies they formed 20% of the total haemocyte population. In the late pupa and adult their released granules could be seen aggregating around them.

Granular cells seemed constantly to undergo degeneration starting first with the disintegration of their cytoplasmic contents. This process apparently started from the late 3rd instar where many degenerating cells could be seen.

Spherule cells were first seen in the early pupae but their number increased in the late pupa and the adult. Their spilled out spherules could be seen scattered in the haemolymph.

Immature spherule cells which were yet without any spheracular inclusions could be very well confused with oenocytoids of other insects like *Calpodes ethlius* (Lai-Fook. 1973), *Galleria mellonella* (Neuwirth, 1973), *Euxoa lutulenta* (Rowley and Ratcliffe, 1981) and others. In the later stages of their maturation they could not be confused with any other type as these cells with their spherules are very distinct with a characteristic appearance. Nappi (1970) has described oenocytoids for the larvae of *Drosophila euronotus*, but in the larvae or pupae of *L. cuprina* no such comparable cells could be detected.

Released spherules could also be confused with the so-called 'crystal cells' or 'crystaloid cells' of some authors like Rizki (1953), Rizki and Rizki (1959), Nappi and Streams (1969). But in the present study such bodies in the haemolymph were distinctly the released products of spherule cells. It seems that the so-called special cells of Diptera which have been termed crystal cells are in reality the released contents of spherule cells. They have been confused with oenocytoids also because of their hyaline cytoplasm as suggested by the classification by Rowley and Ratcliffe (1981).

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) no appreciable difference was found in the haemocyte complex during the first few hours after emergence. But after 24 hours the percentage of plasmatocytes was found to be always greater in

sugar-fed than in protein-fed females, while the granular cells and spherule cells were always less in number in the former as compared to the latter. There was found to be almost no difference in numbers of the other types of haemocytes. Most probably a certain titre of neurosecretions of brain or/and juvenile harmone is needed for the differentiation of granular cells and spherule cells from plasmatocytes. The amount of neurosecretions present in the blood at the time of emergence could be sufficient for the initial differentiation from prohaemocytes. It could also be enough for transformation from plasmatocytes into granular cells or spherule cells during the early adult life. But after that a further supply of secretions is needed for continuation of the transformation process. Lot of further work is needed in fact to establish this hypothesis.

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