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HAEMOCYTES OF COMMON LAND SNAIL FROM LAHORE, PAKISTAN

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Abstract: Two basic types of haemocytes *i.e.*, granulocytes and hyalinocyte were present in the blood of snail, *Bensonies jacquemonti*. Most of the haemocytes observed (97.2%) were granulocytes. Their diameter varies from 18-36 μm. Ectoplasm was not very distinct. The endoplasm have granules of various sizes. The pseudopodia are either filopodia or lobopodia. Each granulocyte forms 2-17 filopodia which are upto 12-62 μm long. Terminal or basal webs showed dichotomous branching or branches on one side. Granulocytes can merge with each other or with hyalinocytes thus loosing their cytoplasmic identity and form large aggregates of cells. The nucleus of granulocytes was rounded to oval, kidney-shaped or lobulated, 9-12 μm in size. Hyalinocytes (2.8%) were rounded cells, without pseudopodia, have diameter 13-42 μm. They differed from granulocytes in the presence of few granules, in having hyaline ectoplasm. The nucleus of hyalinocytes was rounded to oval or somewhat oblong with diameter 9-12 μm. A few spent granulocytes and hyalinocytes were also present. Some haemocytes (2-3%) of *B. jacquemonti* were found binucleated.

Key words: Blood cells, Bensonies jacquemonti, granulocyte, hyalinocyte.

INTRODUCTION

Considering the defensive role of molluscan blood cells against parasites (Cheney, 1971), different parasitologists and malacologists have paid particular attention to them (Cheng, 1975; Sminia, 1981; Bayne, 1983; Ottaviani, 1983). Each cell is delimited by a unit membrane and a compact nucleus developed by a double-membraned nuclear envelop. In large granulocytes nucleolema complex surrounds the nucleolus (Cheng and Rifkin, 1970). The cells have granular or agranular cytoplasm besides a variety of organelles and inclusion, seen under electron microscope (Jeong and Heyneman, 1976; Ruddel, 1971; Feng *et al.*, 1971; Sminia and Barendsen, 1980). These cells cannot be compared with vertebrate erythrocytes functionally or morphologically (Sminia, 1981).

Depending upon the classification system one to four different cell types have been reported in the molluscan blood (Cheng and Guida, 1980a,b; Sminia, 1981; Dikkeboom *et al.*, 1984; Tanveer, 1989, 1990, 1991) and named as leukocytes, lymphocytes, haemocytes, amoebocytes, granulocytes, macrophages and hyalinocytes etc. In Pakistan freshwater snails are of considerable medical and veterinary importance and found throughout the year except a short period in extreme cold and hot (Tanveer and Khan, 1989). Although the work on various aspects of haemocytes of freshwater snails have

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been worked out by Tanveer (1989, 1990, 1991) and Tanveer *et al.* (1995) but terrestrial snails has never been given consideration as far as their blood cell morphology is concerned. It was therefore, considered desirable to undertake such findings in common garden snail *Bensonies jacquemonti* Austin-Goodwin with a view to provide basic information regarding their structure which contribute in the understanding of phagocytosis.

MATERIALS AND METHODS

The snails collected from Jinnah Garden, Lahore, were maintained in the laboratory in large earthen pots half filled with humus soil at temperature 25.0±2.0°C. The snails used in this study measured 1.75±0.33mm - 2.45±0.65mm in shell width. The blood samples were taken out by inserting a micropippette directly into the heart region after removing a part of shell above this region (Guida and Cheng, 1980). The haemolymph was placed on glass slides and left undisturbed for 30 minutes at room temperature. These blood cells were fixed in 1% glutaraldehyde in Sorensen's buffer (pH 7.4) at 5°C and stained with lead haematoxylin and basic fuchsin following Guida and Cheng (1980) and microphotographs were made at an enlargement of X1000.

RESULTS AND DISCUSSION

Two basic types of cells were noted, (1) the granulocytes, (2) the hyalinocytes and several developmental stages or the spent granulocytes and hyalinocytes.

Granulocytes

Granulocytes were 97.2% of the total blood cells examined. These are typical amoeboid cells with highly variable shape depending on the activity, they may appear from rounded to elongated. The ectoplasm is not distinctly separate from the endoplasm. The cytoplasm has some highly pronounced characteristics. Firstly, it contains certain granular structures (Figs.1,2) which provide the basis for the name given to these cells. The cells have a tendency to spread on contact with a solid substratum and the degree of this spreading depends somewhat on the time (for which they are left undisturbed), temperature and the medium in which the cells are kept. It has been observed that in normal saline or in calcium rich media the cells have tendency to become rounded and to withdraw the pseudopodia. However, they spread more quickly in warmer temperatures and in the normal serum. Secondly, they react by throwing out pseudopodia, which may be of various types and shapes. Thirdly, they have remarkable ability to merge with each other (Figs.3,4) so much so that they loose their cytoplasmic identity but the nuclei always remain separate and merging cell can separate subsequently along with their nuclei. This merging may be an extension of their tendency to phagocytose foreign materials. This seems to suggest that in their reaction to solid object, the cells are not able to identify self from nonself and they react in the same manner to another cell of their own type (Fig.4) as they would to a foreign solid object.

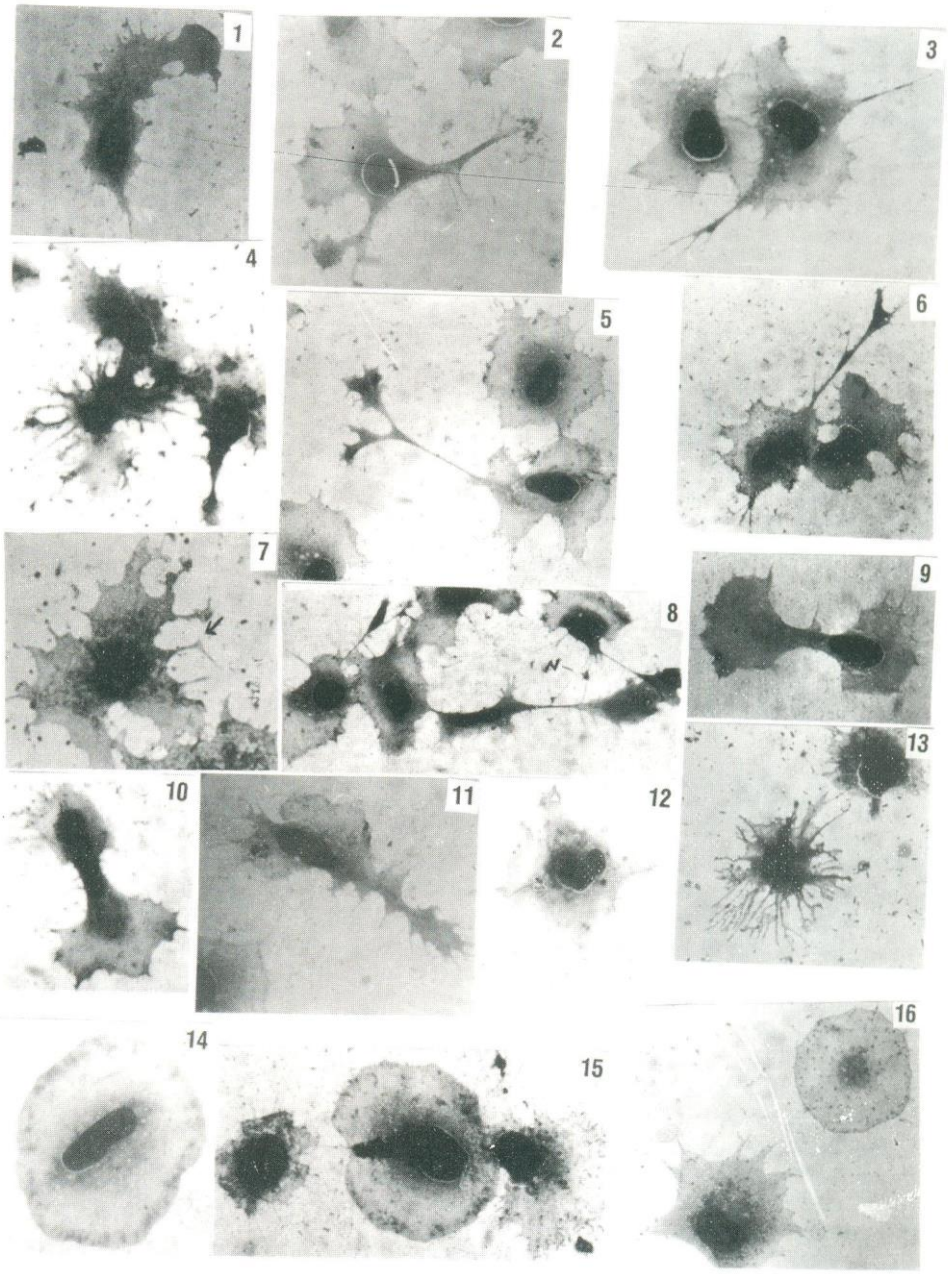
The diameter (μm) of granulocytes and their nucleus measures 26.15 ± 14.28 and 10.45 ± 1.66 respectively. Their respective area $(\mu m)^2$ measures 910.28 ± 325.44 and 101.14 ± 23.21. The cell to nucleus ratio comes to 6:1 (Table 1). It was further observed that 2-3% of the blood cells have double nucleus. The diameter (μm) and area of these cells measured 32.48 ± 19.22 and 9.6 ± 2.03 respectively. Their respective area was measured 1841.34 ± 629.12 and 95.21 ± 28.33, along with 7:1 and 65:1 cell to nucleus ratio.

As can be seen from the measurements given above the size is highly variable and cannot have any taxonomic value, except when the maximum size is considered.

Pseudopodia of granulocytes

All granulocytes of *B. jacquemonti* form pseudopodia which in almost all cases seem to be a reaction to the presence of solid objects (Fig.8) in the vicinity of the granulocytes. This property seems to be lodged in the cytoplasm itself, because a pseudopodium along its course will react exactly in the same manner to the presence of another solid particle with the result that the pseudopodia can behave in various ways. They may branch dichotomously (Fig.5) or form terminal webs (Fig.6) or basal webs (Fig.13). They may bend (Fig.7) as a reaction to strong stimulus from the lateral side. But the pseudopodia wherever and whenever in contact with solid substratum retain property of spreading (Fig.8). The formation of pseudopodium is not restricted to a foreign object only, they will be formed as a reaction to the presence of another homo-haemocyte that ultimately resulted in the formation of large aggregates of cells due to the fusion of cytoplasm as has also been noted by Cheng and Guida (1980), in *Bulinus truncatus rohlfsi* and Tanveer (1989, 1990, 1991), in pulmonate, planorbid and prosobranch snails.

In some cells there was a basal ectoplasmic web (Fig.13). The pseudopodia are generally in the form of filopodia which radiate from the cell and may have a narrow core extending to some distance. The maximum length in pseudopodia observed in this study was 62 μm beyond the margin of the cell body. In several cases the pseudopodia are seen to be dichotomously branched or sometimes the branches may be present on one side only (Fig.3). This obviously depends on the presence of solid particles in that direction as can be seen in Fig.8, showing a solid particle (marked N). Cheng *et al.* (1979) have stated that fine filopodia radiate along the glass substrate and that the cytoplasmic granules may extend along certain pseudopodia. But it is difficult to answer why a pseudopodium will not be formed towards a solid object which is not in the plane of the glass substratum. They are also of the view that the free terminal of all filopodia end in bulbs, this does not seem to be true in all the cases in the granulocytes of *B. jacquemonti*. The maximum size of the pseudopodia in their study is smaller than the pseudopodia of *B. jacquemonti*. In a similar study by Cheng and Guida (1980) the maximum length of pseudopodia is 35 μm and some of the pseudopodia are lobopodia which is also the case in the present study (Figs.9,10). However, the pseudopodia in the present study do not necessarily have a tappered construction as stated by Cheng and Guida (1980a). Ectoplasmic terminal webs and dichotomous branching have also been demonstrated in *B. truncatus rohlfsi*. Sminia (1981) stated that the pseudopodia have a hyaline appearance, this would be dependent on the type of staining, but should also be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expected because the pseudopodia are largely of ectoplasmic structure. Cheng and Guida (1980a) stated that the lobopodia of the granulocytes of *B. truncatus rohlfsi* are of different shapes *i.e.*, rounded bulges, triangular funnels and broad peaks such variations have also been recorded in the present study.

The granulocytes under study occasionally produced 1-40 pseudopodia each. Similar multifilopodial blood cell was also reported by Cheng and Guida (1980) in the stained preparations of *B. truncatus rohlfsi*. They suggested that it was a pathological condition. However, in the present investigation it cannot be said positively particularly in view of the fact that only a few cells were found in the specimens, which otherwise had normal granulocytes and hyalinocytes.

Nucleus of the granulocytes

The nucleus of the granulocytes is variable and it was rounded to oval kidney-shaped or lobulated (Figs.11,12). Sminia (1972) and Tanveer (1989, 1990, 1991) also reported the similar observations. In addition to these many other shapes have also been found in the present study as reported by Jeong and Heyneman (1976). Cheng and Auld (1977) found that nucleus of *B. glabrata* granulocyte are typically subovoid. Whereas Sminia (1977) found in *Lymnaea stagnalis* that the nuclei may be rounded, kidney-shaped or lobulated. The nuclear chromatin according to Cheng and Guida (1980a) was in the form of the interrupted strand or condensed thick strands depending upon the size of the amoebocyte. It appears that when the haemocytes spreads on the substratum the nucleus also spreads with it and it has been proved by Cheng and Guida (1980a) that one or two nucleoli were present in each nucleus.

Cellular inclusions

The vacuolated nature observed in the present finding has been reported by Sminia (1972) along with extensive golgi apparatus as demonstrated by ultrastructure. Carter and Bogitsh (1975) demonstrated lysosome like structure and golgi apparatus. Jeong and Heyneman (1976) showed a few yellow or red coloured inclusions, spherical dark granules, mitochondrial organelles and vacuoles. These vacuoles are not necessarily around the phagocytosed material, although some of the particulate material may be present in the vacuoles as shown by Cheng and Guida (1980a).

Figs.1-16: 1, Granulocytes showing the endoplasmic granules; 2, Granulocyte showing branches of pseudopodium; 3, Merging granulocyte showing the branches of filopodium on one side; 4, Merging granulocyte showing large aggregate of cells; 5, Granulocytes showing dichotomous branching of pseudopodium; 6, Granulocyte showing the terminal ectoplasmic web of pseudopodium; 7, Granulocyte showing the bending of pseudopodium; 8, Granulocytes forming pseudopodia towards a solid particle (marked N); 9, Granulocyte showing lobopodium and oblong nucleus; 10, Granulocyte showing lobopodium and elongated nucleus; 11, Granulocyte showing kidney shaped nucleus and branched pseudopodium; 12, Granulocyte showing lobulated nucleus; 13, Multifilopodial granulocyte showing basal ectoplasmic web; 14, Hyalinocyte showing oblong nucleus and vacuoles around it; 15, Hyalinocyte merging with granulocyte; and 16, Spent granulocyte and hyalinocyte (X1000).

Table 1: **Some morphological characteristics and morphometric data (mean ± S.D.) of stained haemocytes of *Bensonies jacquimonti*.**

Mononucleated granulocytes (n = 55)

Cells		Nucleus		Cells to	Pseudopodia		
Diameter (μm)	Area $(\mu m)^2$	Diameter (μm)	Area $(\mu m)^2$	nucleus ratio	Total Number	Minimum length (μm)	Maximum length (μm)
26.15 ±14.28	910.28 ±325.44	10.45 ±1.66	101.14 ±23.21	6:1	12.12 ±4.22	7.12 ±3.25	48.24 ±12.80

Binucleated granulocytes (n = 55)

Cell		Mother nucleus		Daughter nucleus		Cell to mother nucleus ratio	Cell to daughter nucleus ratio	Pseudopodia		
Diameter (μm)	Area $(\mu m)^2$	Diameter (μm)	Area $(\mu m)^2$	Diameter (μm)	Area $(\mu m)^2$			Total Number	Minimum length (μm)	Maximum length (μm)
32.48 ±19.22	1841.34 ±629.12	9.6 ±2.03	95.21 ±28.33	7.6 ±4.21	86.21 ±22.14	7:1	65:1	10.15 ±4.13	6.24 ±2.48	38.24 ±9.21

Hyalinocytes (n = 22)

Cell		Nucleus		Cell to nucleus ratio
Diameter (μm)	Area $(\mu m)^2$	Diameter (μm)	Area $(\mu m)^2$	
29.35 ±10.21	986.21 ±548.11	10.02 ±1.34	95.24 ±18.21	9:1

Hyalinocyte

2.8% of the blood cells in the present study represented the other distinct cell type which is called the hyalinocyte (Fig.14). The hyalinocyte differ from granulocyte in the presence of a few granules in the cytoplasm, in more or less circular shape in the spread from and in possessing a comparatively hyaline cytoplasm. These also differ from granulocytes in the absence of filopodia and in a slightly high nucleus-cytoplasm ratio. Like granulocytes they can also merge with other hyalinocytes or granulocytes (Fig.15). The nucleus in hyalinocytes has also variable in shape i.e., from rounded to oval to almost oblong. Similar findings have earlier been reported by Cheng and Auld (1977, *Biomphalaria glabrata*), Cheng and Guida (1980a, 1980b, *B. glabrata*), Sminia and Barendsen (1981, *Lymnaea stagnalis*), Schoenberg and Cheng (1981, *B. glabrata*) and Tanveer (1989, 1990, 1991, *Lymnaea acuminata, Indoplanorbis eustus, Physa acuta* and *Bellamaya bengalensis*). For comparison purpose, similar findings for terrestrial snails were not available. The diameter (μm) of hyalinocytes and their nucleus measures 29.35 ± 10.21 and 10.02 ± 1.34 respectively. Their respective area $(\mu m)^2$ measures 986.21 ± 548.11 and 95.24 ± 18.21. Their cell to nucleus ratio was 9:1 (Table 1).

In the present investigation hyalinocytes were less than 3% of the total blood cells while in some earlier reports this ratio was 30% (Renwrantz *et al.*, 1979; Cheng, 1975) and 10% (Schoenberg and Cheng, 1981; Tanveer, 1989, 1990, 1991) in the molluscan blood films. The hyalinocytes in the present study generally conform to above characteristics except that the nucleus-cytoplasm ratio is lower as compared to granulycytes.

Spent granulocytes and hyalinocytes

Occasionally a haemocyte is seen (Fig.16) which is rounded and has few granules and poorly staining cytoplasm and more or less disintegrating nucleus. These probably are spent granulocytes and hyalinocytes. Similar cells have also been seen by Cheng and Guida (1980a) and they suggested them as developmental stages of blood cells.

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