

Cellular Composition of Blood of Marine Teleost, *Gerres filamentosus* (Cuvier), from Visakhapatnam Harbour

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Cytomorphology of immature, mature, and senile erythrocytes and leucocytes of *G. filamentosus* has been studied using Giemsa stained smears. The blood contains nucleated erythrocytes and leucocytes which include non-granulocyte lymphocytes (59.45%), thrombocytes (28.2%), monocytes (4.6%), macrophages (0.3%), and all the 3 types of granulocytes (7.45%), viz. neutrophils, eosinophils, and basophils. Abnormally high percentage of immature erythrocytes and leucocytes, observed in samples collected in March 1977, may be due to environmental stress or pollution. Polymorphy of thrombocytes and phagocytic activity of macrophage cell are noteworthy features. Cytometric measurements of various cell types are made to calculate the nucleocytoplasmic (N/C) ratio.

Haematology of fishes gained recognition in the diagnosis of fish diseases. Composition of blood of fishes is under greater influence of environmental factors compared to terrestrial warm blooded animals and haematological changes due to environmental pollution are of interest. Though information is available on the blood of several fishes¹⁻¹⁴, detailed information on the haematology of fishes from India is very scanty¹⁵.

Differential counts and cytomorphology of blood cells are studied in *Gerres filamentosus* from Visakhapatnam harbour, which is affected by industrial effluents and organic pollution in the form of untreated domestic sewage¹⁶.

Blood from the fish was collected by cardiac puncture with the help of a heparinized syringe in the field. As the fish were of small size (7-10 cm), inadequacy of the blood samples restricted their use to the study of blood smears only. The air dried smears were fixed in methanol for 5 min and then stained by Giemsa's stain using pH 7.2 phosphate buffer. Differential counts and cytometric measurements of cytosome and nucleus of various cell types were made in the laboratory. From the cytometric measurements the nucleocytoplasmic (N/C) ratio of the blood cells which is related to their oxygen carrying capacity, was calculated.

Description of cell types is based on a combination of terms used by earlier workers^{6-11, 14, 15}. The principal cellular components of the blood of *G. filamentosus* are the non-nucleated erythrocytes, thrombocytes, lymphocytes, monocytes, macrophages and the granulocytes like neutrophils, eosinophils and basophils. Also, several developing and degenerating stages, mainly of the erythrocytes, are seen in some smears.

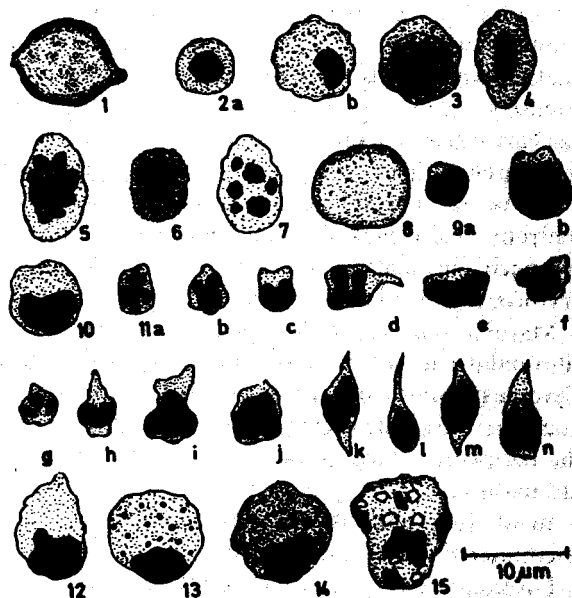
Haemoblasts—It is a large agranular cell containing a large purplish stained nucleus which has uniform reticular pattern. The basophilic cytosome shows pseudopodia (Fig. 1). These cells are absent in the peripheral blood smears except in those of March 1977 samples, and they constitute 0.5% of all the erythrocytes.

Prolymphocytes—These cells (1%) are seen in the smears of March samples only. They are more or less round, having a round or indented nucleus (Fig. 2a). The clear blue staining of the cytosome and the dark violet of the nucleus is similar to that of the mature lymphocytes.

Progranulocytes—They constitute 0.5% of all the erythrocytes and seen only in the smears of March sample. They are more or less spheroid in nature with a kidney shaped, small, eccentric, nucleus. Cytoplasm stains light bluish, and the nucleus stains pinkish violet (Fig. 2b). This is the precursor cell of all the granulocytes.

Erythrocyte series—Cytometric measurements and N/C ratios of various cell types are given in Table 1.

Immature erythrocytes—These are variously known as normoblasts or erythroblasts. As a group these cells constitute normally about 2% of the erythrocytes. Their composition is somewhat abnormal in the March sample.



Figs 1 to 15—Various cell types in the blood of *G. filamentosus* [1 Haemoblast. 2—a, Prolymphocyte; b, progranulocyte. 3 Basophilic Normoblast (BN). 4 Mature erythrocyte. 5 Disintegrated erythrocyte. 6 Disintegrated erythrocyte showing only a 'nuclear shadow'. 7 Senile erythrocyte showing 'exploded nuclei'. 8 Enucleate erythrocyte. 9—a, Small lymphocyte; b, large lymphocyte. 10 Monocyte. 11—a-f, Maturing thrombocytes; g, senile thrombocyte; h-n, mature thrombocytes. 12 Neutrophil. 13 Eosinophil. 14 Basophil. 15 Macrophage.]

Table 1—Cytometric Measurements of Various Cellular Elements of the Peripheral Blood of *G. filamentosus*

Cell type	Cytometric measurements		N/C ratio L × W of nucleus
	Cytosome(C) L × W(μm)	Nucleus(N) L × W(μm)	
			L × W of cytosome
Erythrocyte Series			
Mature erythrocyte	9.51 × 5.51	3.40 × 2.42	0.157
Basophilic normoblast	8.35 × 8	8.00 × 7	0.833
Polychromatocyte	8.35 × 8	8.00 × 7	0.833
Leucocyte Series			
Small lymphocyte	3.65 × 3.65	3.65 × 3.65	1
Large lymphocyte	6.18 × 6.18	6.18 × 5.51	0.892
Small thrombocyte	5.00 × 5	5.00 × 4.34	0.868
Large thrombocyte	8.35 × 5	5.00 × 5	0.598
Monocyte	5.85 × 5.85	5.00 × 4.18	0.614
Macrophage	9.60 × 6.68	—	—
Neutrophil	8.35 × 6.68	5.00 × 4.18	0.376
Eosinophil	8.35 × 8.35	4.18 × 3.34	0.2
Basophil	8.35 × 7.51	5.00 × 4.18	0.335

L = Length; W = Width

The basophilic normoblasts are either roundish or elliptical with a prominent purplish-pink stained nucleus, which is also roundish occupying most of the intensely basophilic cytosome (Fig. 3). The chromatin has cart-wheel shaped arrangement in the nucleus. In the March sample they form 3% of all the erythrocytes.

Cytosome of polychromatocytes is light bluish with centrally placed pinkish stained nucleus with radially arranged chromatin. They also form 3% of all the erythrocytes in March sample.

Mature erythrocytes—These cells are mostly ellipsoidal with a centrally placed elliptical nucleus. Cytoplasm stains orange or reddish yellow and the nucleus is dark purple with dense chromatin (Fig. 4). In the peripheral blood smears 98% of the erythrocytes are mature.

In addition to the above types the following are also encountered rather infrequently.

Disintegrated or senile erythrocytes—A few erythrocytes are seen in various stages of disintegration or senility. The disintegrated erythrocytes (Fig. 5) are also called 'basket' or 'smudge' cells. In the advanced stage of degeneration the nuclear membrane is no longer intact and only the pinkish stained nuclear matter (Fig. 6) is visible in the smears. The senile erythrocytes are also formed by the disintegration of nucleus into small or large roundish pieces in the cytoplasm as if the nucleus has 'exploded' (Fig. 7).

Enucleate erythrocytes—They are roundish with a thin outer membrane enclosing pale red cytoplasm which is slightly granular (Fig. 8). These were described as 'ghost cells' by Boyar¹⁷.

Leucocyte series—Of all the leucocytes nongranular lymphocytes form the major component (59.45%). The other nongranulocytes are (Table 2) thrombocytes, monocytes and macrophages. The granulocytes are represented by the neutrophils and rarely by the eosinophils and basophils (Table 2). N/C ratios for all the leucocytes are given in Table 1. The morphology of different types is given below.

Lymphocytes—Two different types are distinguished according to size—the small (Fig. 9a) and the large (Fig. 9b) forming 51.25 and 8.2% respectively. The shapes and staining characteristics of these cell types are very similar to that of other teleosts⁸.

Monocytes—These are similar in size to the larger lymphocytes but the nucleus, which is small, is either bean shaped or kidney shaped and stains pinkish-violet. It is placed eccentrically in the basophilic cytoplasm (Fig. 10).

Thrombocytes—In accordance with the preference shown by earlier workers^{8,18}, these are included under the leucocytes in the present study. These are functionally equivalent to mammalian blood platelets. Their nuclei may vary greatly in shape. In the same individual and at the same time, they may be round, oval, bilobed or horse shoe shaped. The nucleus stains dark violet or dense purple in mature forms where the clear blue cytosome is either tongue shaped or pointed at one end or both ends (Figs. 11h-n). In the immature or maturing thrombocytes the cytoplasmic content is very little as in small lymphocytes (Figs. 11a-f). Occasionally senile thrombocytes are also seen (Fig. 11g).

Neutrophils—These are the most prominent of all the granulocyte leucocytes constituting about 6.7% of all the leucocytes. They may be called fine

Table 2—Comparison of Differential Count (percentage) of Leucocytes in *G. filamentosus* and *G. cinereus*

Cell type	<i>G. filamentosus</i> (Present study)	<i>G. cinereus</i> (Saunders ⁸)
Large haemoblasts	—	0.1
Small haemoblasts	—	1.1
Small lymphocytes	51.25	35.2
Large lymphocytes	8.2	1.1
Thrombocytes	28.2	54.9
Monocytes	4.6	—
Macrophages	0.3	0.3
Neutrophils	6.7	7
Eosinophils	0.55	0.3
Basophils	0.2	—

granulocytes, but no prominent granules are seen in the present study. The nucleus is usually eccentric, sac like (Fig. 12) and consists of purple staining chromatin, in the delicate grayish blue cytosome.

Eosinophils—These are coarse granulocytes forming about 0.55% of all the leucocytes and are rare in occurrence. The cells are more or less spherical with bluish cytoplasm containing a few purplish orange specific granules. Nucleus is purplish and either bean shaped or spheroid, placed eccentrically in the cytosome (Fig. 13).

Basophils—These are other variety of coarse granulocytes and form only 0.2% of all the leucocytes. Cells are more or less circular in outline with a kidney shaped dark violet nucleus placed eccentrically in the pinkish cytoplasm showing clear blue basophilic granulations all over (Fig. 14).

Macrophages—These are the largest of all the leucocytes, but rare (0.3% only) in their occurrence. Cytosome is mostly basophilic with pseudopodial extensions and contains many delicate blue vacuoles and erythrocyte remains (Fig. 15) suggesting phagocytic activity. No distinct nucleus is present.

Lymphocytes (59.45%) form the major component of all the leucocytes in the blood of *G. filamentosus*. Differential counts of *G. cinereus* (Table 2) show high percentage of thrombocytes. Marked difference between the 2 species of *Gerres* lies in the percentage composition of small lymphocytes and thrombocytes. The former is more in *G. filamentosus* while the latter is more in *G. cinereus*. The difference is perhaps either due to geographical location or environmental conditions. Also the monocytes and basophils are not reported in *G. cinereus*. But the percentage of macrophages, neutrophils and eosinophils are more or less similar in both.

The large (0.1%) and small haemoblasts (1.1%), reported in the blood of *G. cinereus*, are not common to all blood smears of *G. filamentosus*, but are seen in March samples only.

Deviations from the normal composition of the blood cells are not uncommon under conditions of disease or any other kind of physiological stress. Presence of basophilic normoblasts has been reported in diseased fingerlings and yolk sac fry of silver salmon, *Oncorhynchus kisutch*^{2,3} as well as *O. nerka*¹⁹. These basophilic normoblasts (3%) are observed in the present study also in March samples along with other immature cell types such as haemoblasts (0.5%), polychromatocytes (3%), prolymphocytes (1%) and progranulocytes (0.5%). The presence of progranulocytes and prolymphocytes was reported to be of pathological significance¹¹.

In the present study the abnormality observed in the form of high percentage of immature cells in March is

not due to disease as there is no such manifestation on the fishes examined. It is, however, possible that it may be the result of changing conditions in the harbour waters in March like a marked rise (to as high as 36°C) in the surface temperature within the shallow creeks adjoining the north arm of the harbour from which the fish have been collected. This would adversely affect the percentage saturation of dissolved oxygen. Detailed hydrographical and biological observations²⁰ indicate that dissolved oxygen fluctuates diurnally in the harbour waters (0 to 20 mg/litre) and the accumulated organic matter imposes additional stress on fishes particularly during the summer months (March-June) when the rate of decomposition through bacterial action is high.

A definite relationship between changes in environment and variations in the blood counts was already reported²¹.

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