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Cytological Study on the Leukocytes of Selected Fresh Water Fishes of India

Alok Tripathi

Abstract
For the purpose of cytological studies and work out the correlations among the teleost fish species, some 5 fish species are selected *viz Notopterus notopterus, Labeo rohita, Tilapia mossambica, Clarias batrachus,* and *Channa gachua*. The observations regarding hematology are made from peripheral blood by traditional staining method while histological and cytological studies are done from the sections cut of the cephalokidney (head kidney). The cytological observations made through present studies don't show any major difference among the fish species selected, except for the size of cells, which otherwise depend upon the species. The histological observations of the head kidney of fish revealed two clear-cut parts; one that of the ground matrix in which cells are found scattered and the second, which contains encapsulated lymphoid follicles, intermingled with the ground matrix. The lymphoid follicles encapsulated by dense collagenous tissue a tubercular extending from a variable distance into the follicle. Under the present investigation the cytology of all the five cells *viz.* lymphocytes, neutrophils, basophiles, eosinophils and monocytes have been worked out.

Keywords: fish leukocytes, fish lymphocytes, fish neutrophils, fish basophiles, fish eosinophils, ultrastructural studies

1. Introduction
The basal position of fish in vertebrate phylogeny makes them a very attractive option for studies of immune system. Lymphoid organ in fish species of special concern are essential not only for better understanding of their health status and well as to establish a correlation among vertebrates. In spite of systematic diversity [1], all fishes possess two main types of blood cells, erythrocytes (red cells) and leukocytes (white cells), a property shared by the land-living vertebrates, which are derived from early fishlike ancestors. Detailed studies, however, reveal considerable variations in the structure and function of the blood cells between different groups of fishes.

In spite of recent interest in the immune system of fish species regarded as important for aquaculture purposes, very little attention has been paid to the structural features of fish blood cells [2, 3, 4, 5, 6]. The cytological details given by Bodamar [7] for eosinophil, basophils and macrophages studied from peritoneal exudates and some other papers published by Devis and Hynes [8], Ellis [9], Bielek [10] and V´azquez and Guerrero [11]. Rowley el al. [12] has put together a detailed work on fish blood cells illustrated by numerous electron microscopic photos. Comparative hematology is discussed by Andrew [13], Ratcliffe and Millar [14], C and Wales [15], in an atlas of the microscopic anatomy of salmonids, include a chapter on blood cells. Ivanova [16] has illustrated the microscopic morphology of different stages of fish blood cells. Ellis [9] has reviewed works on fish leukocytes dealing with morphology, staining characteristics, physiology, biochemistry, immunology and the relationship of mast cells and eosinophils. Eosinophilic granules without inclusions have also been described in goldfish [8], paddle fish [17] and *Catostomus commersonii* [18]. In *O. niloticus* three granular types were clearly demarcated, granules with homogeneous electron-dense appearance, granules with light electron density dense core and granules with axial light crystalline core El-Saydah *et al* [19]. The intention of present study is to uncover the structural differences and similarities in head kidney among the selected fish species.

2. Materials and Methods
Under present study, some 50 samples of 5 fish species were selected *viz Notopterus notopterus* (Pallas, 1769), *Labeo rohita* (Hamilton, 1822), *Tilapia mossambica* (Peters,

1852), *Clarias batrachus* (Linnaeus, 1758), and *Channa gachua* (Hamilton, 1822). Selected fishes brought to the laboratory and kept in an aquarium for a couple of days. For the morphological observation of blood leukocytes with the help of light microscope, smear of fish blood was prepared and stained as per method given by Anderson, [20]. Size of blood cells has been calculated with the help of micrometer eyepiece in light microscope while simple calculations were used for electron microscopic observations.

Fishes were dissected for the purpose of head kidney and samples were preserved according to need of study. The normal head kidney of healthy *Channa gachua* was taken out and preserved in aqueous Bouin's fluid for 48-72 hours. The tissue was then processed routinely and prepared into paraffin blocks. The blocks of the tissue were cut at 4-6 µm thickness and stained with Delafield's Haematoxylin and Eosin (H.E.) sections were studied under microscopes on different magnification and photographed. Similarly, for cytological observations, the samples for the electron microscopic studies were prepared as per procedure given by Madeley [21]. The tissues of head kidney from the healthy fishes were fixed in C solution and ultra-thin sections were visualized under transmission electron microscope at various magnifications.

3. Results

Observations made under the present study are based on the comparative dimension and morphology of the tissues, cells and cellular organelles. The cells under light microscope appeared as seen in typical blood differential leukocytes count slide except mild variation in their sizes. Under these observations, the leukocytes recognized are neutrophils, basophiles and eosinophils in granulocytes while large and small lymphocytes and monocytes, which were not clearly demarcated from those of monocytes (fig.1). An average variation in size of eosinophils reported from 11.5 to 7.2µm while 12.8-9.6 µm for neutrophils. No size of basophile could be reported. Likewise, an average variation in size for lymphocytes and monocytes was observed from 7-4.8µm and 10.9-8.8 µm respectively (fig.2).

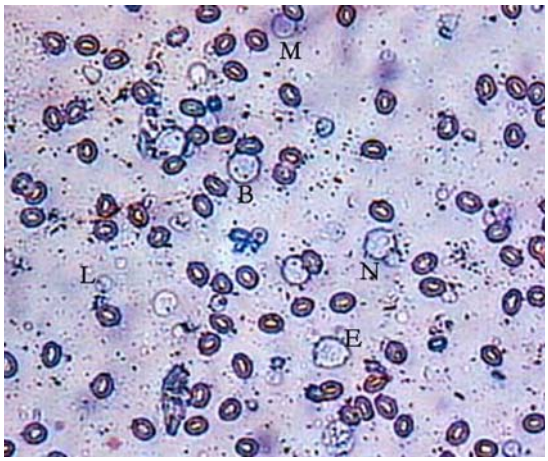

Fig 1: Photograph of light microscope showing the various WBCs after staining (x450).

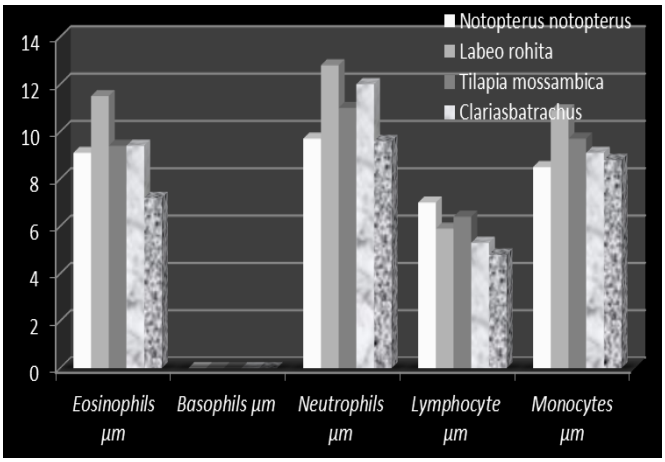

Fig 2: Graph showing the cooperative size of WBCs in the selected fish species.

The histological observations of the head kidney of fish revealed two clear-cut parts; one that of ground matrix in which cells are found scattered and the second, which contains encapsulated lymphoid follicles, intermingled with the ground matrix. The lymphoid follicles encapsulated by dense collagen tissue a tubercular extending from a variable distance into the follicle. The parenchyma of follicles consists of an open mesh-work of reticuline fiber, which provides support for an ever-changing population of lymphocytes. The cortex consists of densely packed lymphocytes. The cells are scattered at their different stage of maturity (Fig.3). The cytological observations made through present studies didn't show any major difference among the fish species selected, except for the mild variations in the size of cells, which otherwise depended upon the species (Figs .4-5).

The cellular structures of some five types of cells are worked out from all the selected fish species, which were recognized as lymphocyte, monocyte, neutrophil, eosinophil and basophile based on their morphological and cytological details. As far as normal cytological organelles are concerned, the cytoplasm of the all five cell types contained typical cell organelles while mitochondria, vacuoles, lysozymes and granules were prominently appeared. Among the ER, rER is rarely encountered. Apart from these cells, some macrophages are also encountered from the lymphoid follicles, during the course of study. The lymphohematopoietic cells are seen scattered randomly throughout stroma of heterogeneous fibroblastic reticular cells among which sinusoidal blood vessels are scattered in the sections. Every hematopoietic cell lineage seems to be differentiated from cell progenitor Antigen Processing Cells (APC) and B-lymphocytes are also seen to be present in the head kidney.

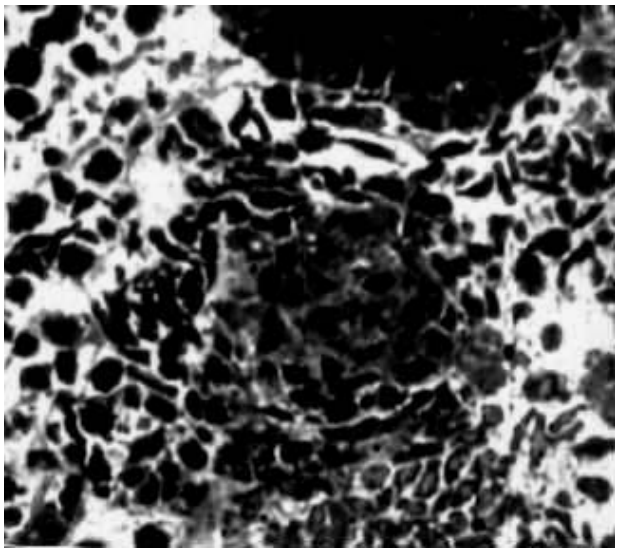

Fig 3: Histological photograph of head kidney (x450)

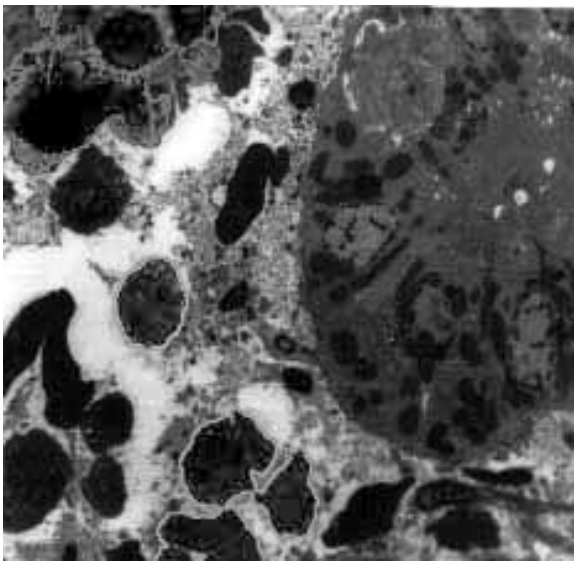

Fig 4: Ultrastructure of *L. rohita* showing the intact and scattered lymphoid cells (x13.5K).

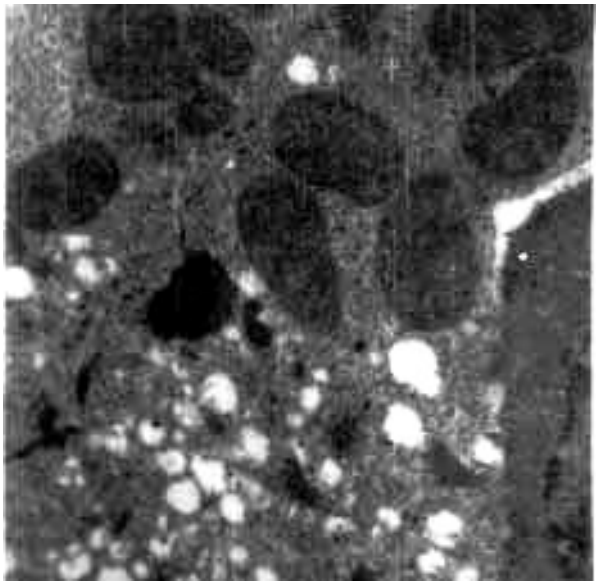

Fig 5: Ultrastructure of head kidney of *N. notopterus* with part of nucleus and other cell organelles (x55.5K)

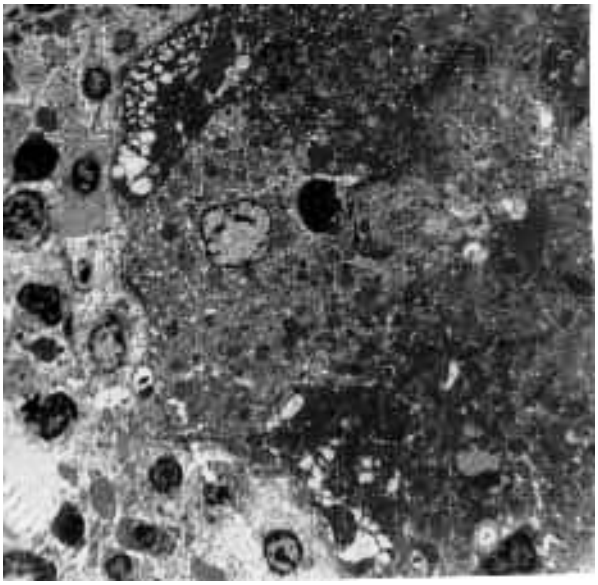

Fig 6: Ultrastructure of lymphocytes cell showing various cell organelles (x54.5K)

3.1 Lymphocytes

Under electron microscope, at low magnification, few lymphocytes like cells with a large nucleus surrounded by large number of mitochondria appeared in the distinct areas of kidney. The lympho-*hematopoietic* cells occur in scattered condition or forming pyroninophilic cell clusters. In some cases, various lymphoid cells are found to be scattered at various stages of their maturity including macrophages, granulocytes and lymphocytes (Fig**.** 6-7).

The size of the lymphocytes studied under present investigations is ranged between the 2.8-3.9 μm. The lymphocytes have round, densely stained nucleus with a pale basophilic non-granular cytoplasm. The nucleus is typically spherical but slightly intended, chromatin moderately condensed, nucleoli is not was not apparent. Cytoplasm contains a few mitochondria, rudimentary Golgi apparatus, a small number of ER and comparatively less number of free ribosome. The cell exhibits small cytoplasmic projection, which with SER appears as microvilli and more in number (Fig. 8)

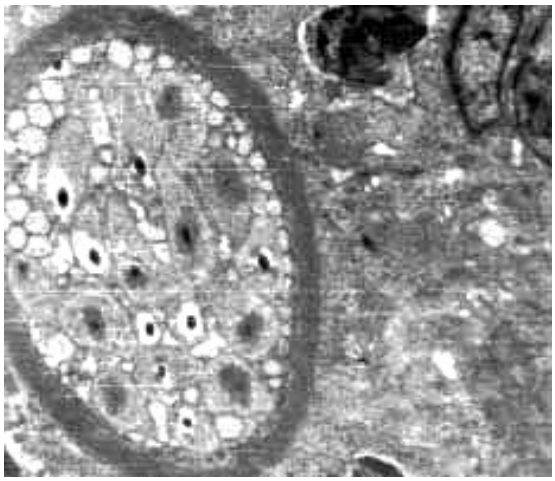

Fig 7: a section of head kidney showing the encapsulated lymphocytes (x11.5K)

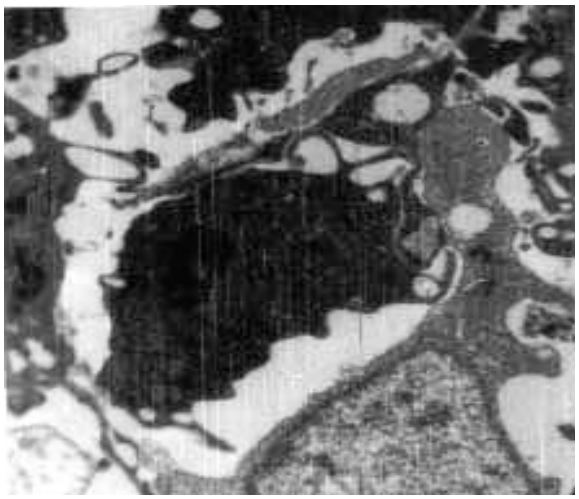

Fig 8: Ultrastructure of a monocytes showing that most of cell covered with a large nucleus (x64.5K).

3.2 Monocytes

Cell observed ultra-structurally in the electron microscope, as they were having a single large eccentrically placed nucleus with comparatively less stained WBC's with shape variable, but there is deep indentation near center, which tends to become more pronounced as cells mature so as to give a pseudo horse shoe shaped or bilobed appearance. Two or more nucleoli were visible. Cytoplasm seems to contain variable number of ribosome and polyribosome and relatively little RER. The Golgi apparatus is well developed and located within centromere in the vicinity of nuclear indentation. Small-elongated mitochondria are prolific. Numerous small pseudopodia extended from the cell reflecting phagocytic ability and amoeboid movement. The cytoplasmic granules of monocyte are electron dense homogenous and membrane bound. The size of the monocytes studied, ranged between the 5.5-8.8μm (Fig.9).

3.3 Neutrophil

Neutrophil had a multilobed nucleus with highly condensed chromatin reflecting low translation. Cytoplasm contains a considerable number of membrane bound granules.

The primary granules are large, spherical and electron dens. The specific granules are more specific, numerous, small, rod shaped with various densities and shape. All other cytoplasmic organelles are scarce, although the cytoplasm is particularly rich in dispensed glycogens. The nuclei did not contain a nucleolus, and the heterochromatin is distributed peripherally or in dispersed patches throughout the nucleoplasm. The endoplasm of these cells contained granules, which varies in size and density. Cytoplasm contains oval mitochondria, a golgi complex and profiles of rough and smooth endoplasmic reticulum membranes, smooth surfaced tubules, vesicles and glycogen. The hyaline cytoplasm observed around the periphery of these cells was most noticeable in surface projections as lamellipodia or ruffles. Mature neutrophils has few appropriate organelle for translation as it has very limited capacity to regenerate along with expanded lysosomal and specific enzymes, which are rapidly depleted by phagocytic activity. The neutrophils are thus incapable of continuous functioning and degenerate after single burst activity (Fig.10).

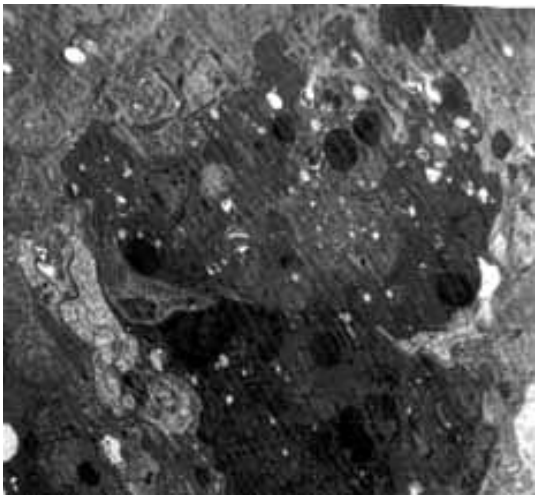

Fig 9: Ultrastructure of monocytes with various cell organelles (x84.5K)

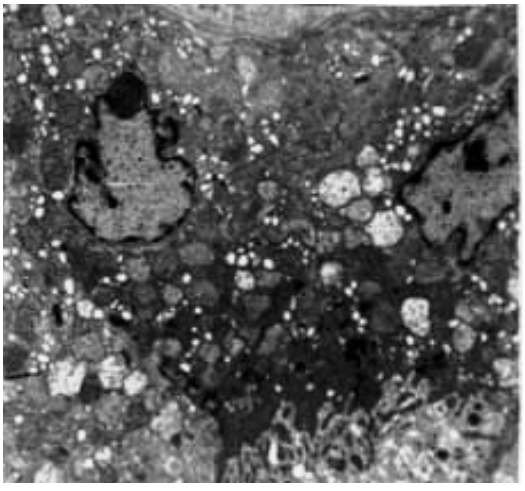

Fig 10: Ultrastructure of neutrophils showing various types and size of granules (x95.5K)

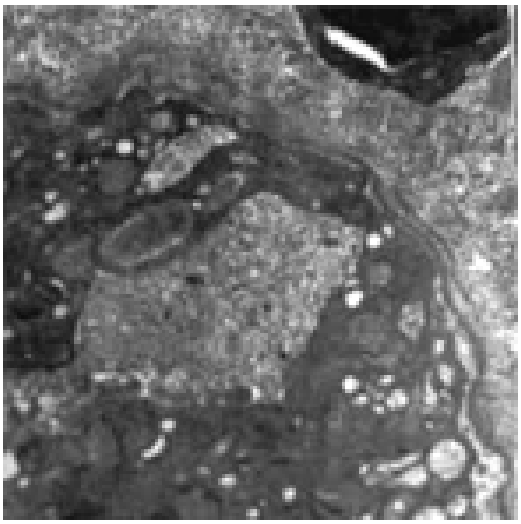

Fig 11: Ultrastructure of eosinophil with various cell organelles (x84.5K)

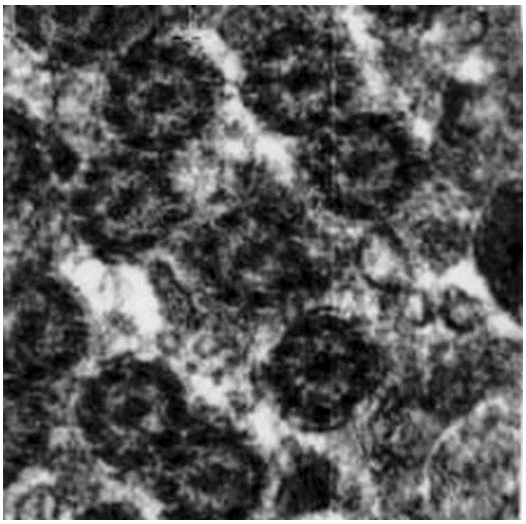

Fig 12: Ultrastructure of granules of eosinophil (x115.5K)

The size of the neutrophils observed, ranged between the 9.6-12.8 µm. Dysfunctional neutrophils are the main cellular constitute of pus and thus referred as pus cells. The paucity of mitochondria and the abundance of glycogen in neutrophils reflect the importance of an aerobic mode of respiration. Neutrophils are highly motile cells moving through the intracellular spaces in a crawling fashion with an undulating pseudopodium typically thrust out in a time of advanced stage.

3.4 Eosinophils

Eosinophils are with large ovoid specific granules each containing elongated crystalloid regular discoidal shape, bilobed nucleus, placed eccentrically in the periphery. The cytoplasm contains characteristic extensive smooth ER. Glycogen is abundant and there is a scattered oval mitochondria dispersed throughout the cell. Their large, densely stained, irregular shaped granules easily recognized them. The granules appeared to be either smooth or lightly stippled (granular). Their nuclei had patches of heterochromatin around their borders and distributed throughout the nucleoplasm. A small nucleolus was infrequently observed. The endoplasm of the cell was composed of granules, small vesicles, many profiles of tubular, SER, a modest amount of RER and a prominent Golgi complex. The observed size of the eosinophils, ranged between the 9.6-12.8 µm (Fig.11-12).

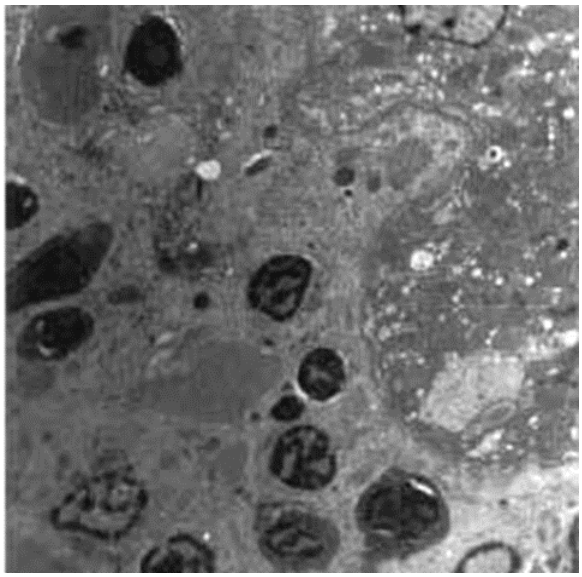

Fig 13: Ultrastructure of basophile showing large portion of nucleus surrounded by the granules (95.5K)

3.5 Basophile

Basophiles have characteristic bilobed nucleus large specific granules are membrane bound round or oval in shape and filled with closely packed electron dense materials. Size of basophiles is larger than eosinophils but less in number. A small population of small granules is also found around the nucleus. Cytoplasm also contains free ribosome, mitochondria and glycogen while plasma membrane exhibit

blunt irregular spaced surface projections. The size of the basophiles observed under present studies ranged between the 9.6-12.8 μm (Fig. 13).

4. Discussion

The cytological observations made through present studies didn't show any major difference among the fish species selected, except for the minor variation in size of cells, which otherwise depend upon the species. The superficial morphological structure of stained cells as appeared under light microscope was as usual as described by various authors. The histological observations of the head kidney of fish revealed two clear-cut parts; one that of ground matrix in which cells are found scattered and the second, which contains encapsulated lymphoid follicles, intermingled with the ground matrix. The lymphoid follicles encapsulated by dense collagen tissue a tubercular extending from a variable distance into the follicle. The parenchyma of follicles consists of an open mesh-work of reticuline fiber, which provides support for an ever-changing population of lymphocytes. The cortex consists of densely packed lymphocytes. The cells are scattered at their different stage of maturity.

Under the present studies, the cytology of blood cells is compared with the observation made by Bodammer [22], Vazquez and Guerrero [11]. Under the present investigation the cellular structure of some five types of cells has worked out from all the fish species, which were lymphocyte, monocyte, neutrophils, eosinophils and basophiles. The cytoplasm of the entire five cells contains typical cell mitochondria, vacuoles, lysozymes and granules but ER especially rER are rarely encountered. The description given here about the occurrence and structure is closely related with Fange [23].

The present cytological structures worked out of fish blood cell are also getting supported by the finding reported by Fange [23]. There are two different type of lymphocytes could be recognized in this work; as a normal cell with the cytoplasm contain mitochondria, ER ribosome and golgi bodies while another one was as antigen presenting cell (APC) which contain dense chromatin fiber in the periphery of the nucleus. The cellular detail of lymphocytes given in this work is supported by Ellis [9], Chihaya *et al* [24].

The structure of monocyte also, described in this work is coincides with the structure given by Bodammer [22]. Cytology of this cell clearly reflecting its phagocytic nature as it contained large eccentrically placed nucleus with comparatively less stained WBC's. Shape was variable, but there is deep indentation near center, which tends to become more pronounced as cells mature and thus give a horseshoe shaped or bilobed appearance. Two or more nucleoli may be visible. Cytoplasm seems to contain variable number of ribosome and polyribosome and relatively little RER. The golgi apparatus is well developed and located within the centromere in vicinity of nuclear indentation. Numerous small pseudopodia extended from the cell reflecting phagocytic ability and amoeboid movement. The cytoplasmic granules of monocyte are electron dense homogenous and membrane bound.

The cells interpreted as eosinophils in this study and their fine structure were similar to that of eosinophils in the blood of *Carassius auratus* [8] *Catostomus commersoni* [18], *https://www.google.co.in/search?q=Scyliorhinus+canicula&oq=Scyliorhinus+canicula&aqs=chrome..69i57j0l5.230j0j9&sourceid=chrome&es_sm=93&ie=UTF-8* [25] and larval *Lampetra* sp. Like the granules found in the eosinophils from the species mentioned above, those from the cells in striped bass lacked the crystalline substructure commonly found in eosinophil granules in mammals. Some workers have suggested the potentially lytic capabilities of these granules in fish eosinophils, and their relationship to the granulocytes of higher vertebrates. The striped bass eosinophils were differentiated from basophils on the basis that the latter are not believed to be phagocytic either in fish [9] or in mammals as reported by various workers.

To mammalian hematologists, the neutrophil is known as an actively phagocytic and mobile cell that responds rapidly to the presence of antigens and/or other inflammatory agents. For students of fish hematology however, the identification and function of these cells have been a difficult and controversial issue. There have been recent and important advances of our understanding in neutrophils in fish. It is also known as polymorphonuclear leukocytes, it is presently accepted that their nuclei show varying degrees of lobulation, depending on the species [9] or on their state of maturation [26]. Their typical oval or elongate granules are normally of one type for a given species and generally present an internal structure that is either striate [27] or crystalline [26]. Like the nucleus, variations in granule structure within neutrophils of a particular species may depend upon the maturity of the cell [10] [26]. The identification parameters of granular leukocytes are specific granules filling the cytoplasm. The main form of specific granules in neutrophils of bony fish of various phylogenetic groups is an elongated granule with different distribution of fibrils or a granule that has crystalloid formed from fibrils. The main form of eosinophil granules was large, electron-dense and homogenous in nature [28].

The appearance of activated cell and/or debris containing macrophages in the healthy fish suggests that the latter were compromised in some way at the time the experiment was conducted. This could have served as a source or stimulus for immature neutrophils that was recovered. The structure of basophiles given in this work is also compared with those of Bodammer [22] and found to be similar.

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