Braz. J. vet. Res. anim. Sci.,
São Paulo, v. 38, n. 6, p. 273-277, 2001.

Cytochemical aspects of the peripheral blood cells of *Oreochromis (Tilapia) niloticus*. (Linnaeus, 1758) (Cichlidae, Teleostei) - Part II

Aspectos citoquímicos das células do sangue periférico de *Oreochromis (Tilapia) niloticus*. (Linnaeus, 1758) (Cichlidae, Teleostei) - Parte II

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ABSTRACT

Morphologicaly, seven types of cells were identified in the blood of *Oreochromis niloticus*: erythrocytes, thrombocytes, neutrophils, eosinophils, basophils, lymphocytes and monocytes. Glycogen was present not only in the cytoplasm of neutrophils and thrombocytes but also in some lymphocytes and monocytes. The positive reaction for myeloperoxidase and Sudan black was observed in neutrophils and eosinophils. The bromphenol blue method was strongly positive for erythrocytes and eosinophils.

UNITERMS: Blood cells; Cytochemistry; *Oreochromis niloticus*; Fishes.

INTRODUCTION

Interest in fish as laboratory animals has increased considerably in the last few decades. There have been several investigations on their physiology, biochemistry and pathology[17,23,30]. Concerning the haemoichthyology the works of Oria[33] and Phillips[35] and Black et al.[4] acted as pioneers, they established a comparison of the haematological aspect among several species of fish. In the following decades, many studies have been performed to establish the haematology index of teleost fish[6,15,22]. Studies on the erythrocyte lineage cells have been carried out by Mcknight[28]; Ezzat et al.[15]; Imagawa et al.[20] and Ueda et al.[40].

Other authors[6,20,36,40,41,42], have studied fish erythrocyte morphology. In relation to the absolute values of erythrocytes there have been some data that were published by McCarthy et al.[27]; Ribeiro[36]; Oladimeji et al.[32]; Ueda et al.[40] and Veiga[41] wich may be mentioned.

For thrombocyte Ribeiro[36]; Imagawa et al.[20] and Ueda et al.[40] have described differences in their number and morphology. Fish blood thrombocytes have been described as the most abundant blood cells after erythrocytes, very little has been revealed about their morphology features and functional properties, the available data on the percentage of trombocytes in fish peripheral blood being very confused because of identification difficulty between lymphocytes and thrombocytes. Other authors have demonstrated a special interest in the leucocytes of fish with regard to their morphological observations and their absolute values, considering a great diversity of morphological aspects to the some type of leucocyte[5,13,15,16,20,38,40,41]. Therefore the momenclature concerning teleost leucocyte is confusing[14]. Neutrophils have been designated with various synonyms such as heterophils[43], polymorphonuclear leukocyte[2] and type I granulocyte[10]. For the present study, neuthrophil will be the preferred term. A distinction between monocytes and macrophages is also appropriate to clarify the differences between these cells. Monocytes are relatively immature cells present in hemopoietic tissue and circulating blood.

Althrough, it is very important to establish a classification of leucocytes for histopathological examination of disease fish and for experimental study of inflamation on immunopathology, insufficient studies have been mad on several aspects including fine structure research of teleost fish leucocytes.

The cytochemical aspects of blood cells have been described in different species of fishes by Blaxhall and Daisley[5]; Barber amd Westermann[3]; Ribeiro[36]; Caxton-Martins[7]; Doggett et al.[9]; Zinkl et al.[44]; Veiga[41] and Pacheco[34], have been described indifferent species of fishes by have studied this aspect for various species of fish.

However, little is known about the cytochemistry of *Oreochromis niloticus*; so, the present study aimed at investigate the normal morphology of the cellular blood components and the basic data on the cytochemical characteristics for this species.

MATERIALS AND METHODS

Ten *Oreochromis niloticus*, both male and female were kept in aerated water at the environmental temperature. Their weight varied from 135 g to 370 g and their length from 20,0 cm to 27,5 cm. The animals were anesthetized with benzocain 1/10.000

and blood samples (2ml) were taken from caudal vein in the presence of anticoagulant EDTA for the morphological and cytochemical studies. The blood samples were collected from December to March, that is, in summer time, when the temperature of the water was around 20ºC. For the morphological study blood smears were submitted to Wright staining.

For the cytochemical study blood smears were submitted to the following methods: glycogen demonstration by the Periodic Acid-Schiff PAS[29] method and treatment with salivar amylase as a control[26], myeloperoxidase demonstration by the Orto-toluidin method in the presence or absence of hydrogen peroxyde[21], lipidic identification by the Sudan black B[26] method and the Bromphenol blue method[31] for basic protein.

RESULTS AND DISCUSSION

In relation to the morphological elements of peripheral blood of *Oreochromis niloticus*, it was possible to identify the following cells: erythrocytes, thrombocytes, neutrophils, eosinophils, basophils, lymphocytes and monocytes[40]. The criteria used to define the nomenclature of these cells were based on the morphological aspect of the nucleus and the distribution pattern of cytoplasmic granules as well as the staining ability.

The predominant shape of erythrocyte found in *Oreochromis niloticus* was elliptical. The nucleus was also elliptical and centrally located with heterochromatin stained in purple and acidophilic cytoplasm. The immature slapes found by some authors are supposed to be due to the acceleration of erythropoiesis process in different physiological conditions in different species.

The neutrophils were spherical and of several sizes with abundant basophilic cytoplasm showing few azurophilic granules. The spherical nucleus was small, purple and in general eccentrically located. Ribeiro[36] and Doggett and Harris[10] considered the neutrophil as a highly phagocytic cell ingesting both carbon particles and bacteria. The *Oreochromis niloticus* eosinophils were spherical of various sizes, and had abundant cytoplasm filled with large acidophilic granules of different sizes. The purple stained heterochromatin filled all the nucleus which in general was centrally located, but occasionally in an eccentric position. The basophils were spherical, the cytoplasm being rich in strongly basophilic granules of various sizes and metachromatically stained. The nucleus was spherical and purple stained. Sometimes, the nuclear outline could not be distinguished due to the presence of these granules.

There are no conclusive data in the literature about the possible functions of eosinophils and basophils in fish blood. In some fish, eosinophils appear to be plagocytotic[18,39] and accumulate in parasitic infections and in inflammation[24,25].

The spherical lymphocytes were of several sizes, with little basophilic cytoplasm and frequently showed cytoplasmatic blebs, and some azurophilic granules. The nucleus was spherical, purple and filled most of the cell. Ellis[13,14] showed the presence of immunoglobulin molecules attached to the surface of lymphocytes similar to those of the upper vertebrates, suggesting them to be immunocompetent cells. Sigel et al.[37] reported some evidences on the ability of fish lymphocytes to recognize and to respond to human interleukin-1.

The monocytes were predominantly spherical with irregular outline. The cytoplasm was abundant and basophil, with some azurophilic granules. The nucleus with euchromatic feature was large, usually spherical or sometimes reniform and eccentrically. As to the function of monocytes, Imagawa et al.[20] observed their ability to ingest carbon particles thus supporting the results of Ellis[13]; Fergunson[16] and Doggett and Harris[10].

The *Oreochromis niloticus* thrombocytes were elliptical and their nuclei were also elliptical and centrally located. The heterochromatin stained purple. The cytoplasm was slightly acidophilic and showed clear round small areas at the poles. Occasionally some spherical shapes were found.

The cytochemistry study of the blood cells in *Oreochromis niloticus* demonstrated the presence of glycogen not only in the cytoplasm of neutrophils and thrombocytes (Fig. 1) but also in some lymphocytes and monocytes. It is supposed that glycogen mainly in neutrophils and monocytes could be related to the phagocytosis process whose mecanism depends on energy. As to the presence of glycogen in the thrombocytes we believe that this substance represents an energetic source for this cell, since some authors like Ferguson[16] and Imagawa et al.[20], reported the participation of thrombocyte in the phagocytosis mechanism of foreign substances. This process requires the consumption of energy both from endogenous and exogenous source[12].

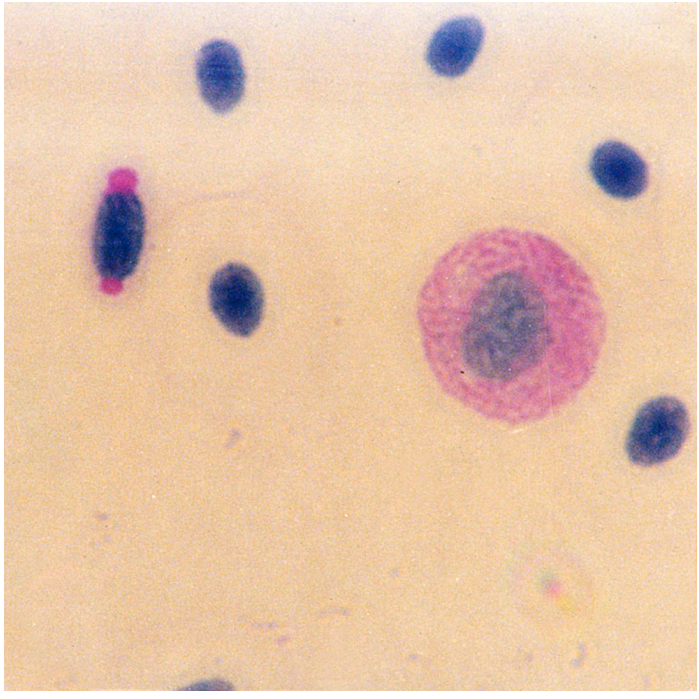

Figure 1
Photomicrography of smears of the peripheral blood of *Oreochromis niloticus*. PAS method + Harris Haematoxylin demonstrating the presence of glycogen in the cytoplasm of thrombocyte and neutrophil 1600X.

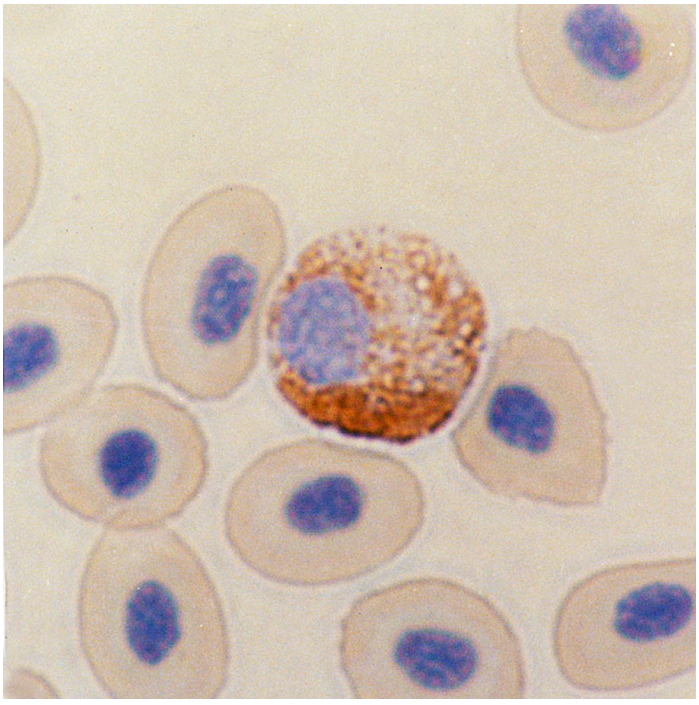

Figure 2
Photomicrography of smears of the peripheral blood of *Oreochromis niloticus*. O-toluidin method – hidrogen peroxyde + Harris Haematoxylin demonstrating the positive reactivity for myeloperoxidase in the cytoplasm of neutrophil. 1600X.

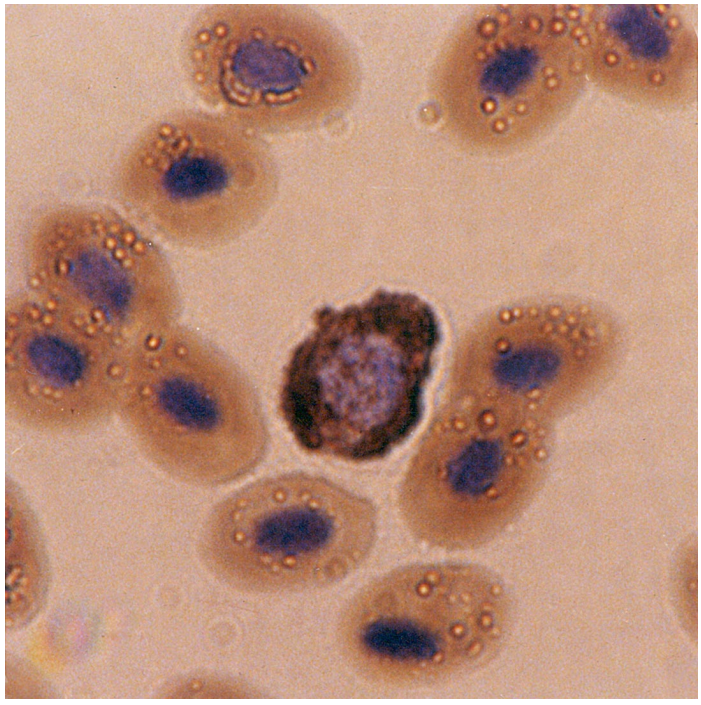

Figure 3
Photomicrography of smears of the peripheral blood of *Oreochromis niloticus*. Sudan black B method + Harris Haematoxylin demonstrating the positive reactivity for the cytoplasmic granules of eosinophil. 1600X.

Imagawa et al.[20] reported in *Cyprinus carpio* the presence of cytoplasmic vacuoles containing superficial microvilli and carbon particles, thus suggesting the participation of thrombocytes in phagocytosis. Catton[6] pointed out the presence of haemoglobin in the thrombocyte cytoplasm, whereas Doggett and Harris[10] reported the participation of thrombocyte in the coagulation process of *Oreochromis mossambicus*.

In relation to the positive reactivity for mieloperoxidase found in neutrophils (Fig. 2) it is certainly related to the known efficient bactericidal system (H2O2-MPO-halide system) used as a defensive mechanism similar to that one observed in mammals[8]. Possibly the eosinophil positive myeloperoxidase activity could be also related to similar oxygen dependent bacteria killing mechanism as suggested by Hoar et al.[19].

The positivity of the Sudan method to the neutrophils and the eosinophils (Fig. 3) to detect phospholipids, is not in agreement with the one found by Doggett et al.[9], who detected sudanophilic granules only in neutrophils, probably due to the differences in membrane constituents among teleost species.

The Bromphenol blue method for proteins was strongly positive for erythrocytes and eosinophils (Fig. 4). In particular, the positivity observed in the eosinophilic granules, may be due to the presence of antilarval substance-like referred classically as major basic protein (MBP)[11] or as eosinophilic cationic protein (ECP)[1] for human.

This research forms the basis of continuing investigations in the functions of these cells in the cellular immune responses.

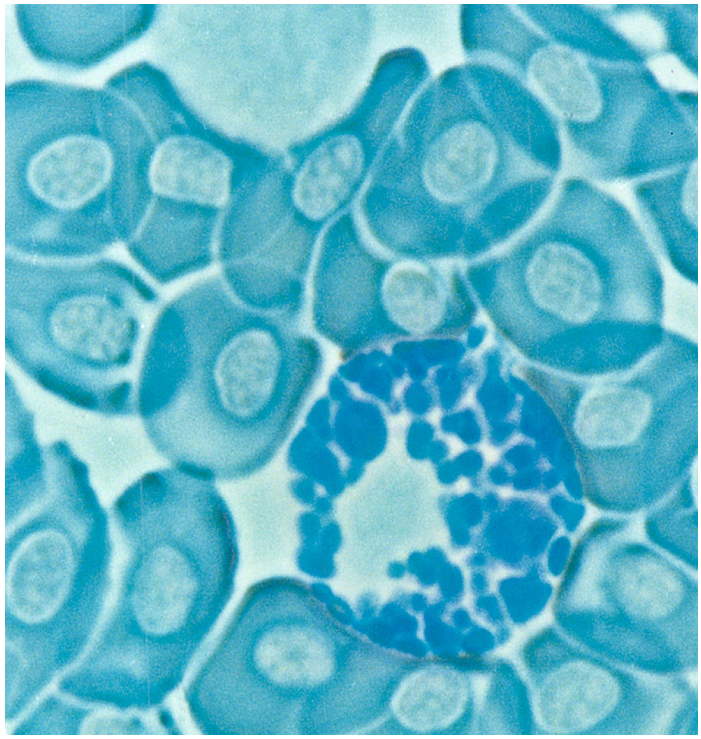

Figure 4
Photomicrography of smears of the peripheral blood of *Oreochromis niloticus*. Bromphenol blue method demonstrating the positivity in the cytoplasm of erythrocytes and eosinophil. 1600X.

RESUMO

Morfologicamente foram identificados no sangue de *Oreochromis niloticus* sete tipos de células: eritrócitos, trombócitos, neutrófilos, eosinófilos, basófilos, linfócitos e monócitos. Em relação aos resultados citoquímicos foi contastada a presença de glicogênio em neutrófilos, trombócitos e em alguns linfócitos e monócitos. Os grânulos citoplasmáticos de neutrófilos e eosinófilos mostraram positividade para mieloperoxidade e Sudan black. O azul de bromofenol foi totalmente positivo em eritrócitos e eosinófilos.

UNITERMOS: Células sangüíneas; Citoquímica; *Oreochromis niloticus*; Peixe.

REFERENCES

1- ABU-GHAZALEH, R. I.; DUNNETTE, S. L.; LOEGERING, D. A.; CHECKEL, J. L.; KITA, H.; THOMAS, L. L.; GLEICH, G. J. Eosinophil granule proteins in peripheral blood granulocytes. **J. Leukoc. Biol.**, v. 52, p. 611-618, 1992.

2- ANDERSON, C. D.; ROBERTS, R. J. A comparison of the affects of temperature on wound healing in a tropical and temperature teleost. **J. Fish Biol.**, v. 7, p. 173-182, 1975.

3- BARBER, D. L.; WESTERMANN, J. E. M. Occurrence of the periodic acid-Schiff positive granular leucocyte (PAS-GL) in some fishes and its significance. **J. Fish Biol.**, v. 12, p. 35-43, 1978.

4- BLACK, E. C.; TUCKER, H. H.; KIRKPATRICK, D. The effect of hemolysis upon the oxigen affinity of haemoglobin in the Atlantic *(Salmo salar)* and landlocked salmon *(Salmo salar sebago).* **J. Fish. Res. Bd. Can.**, v. 23, p. 1575-1780, 1996.

5- BLAXHALL, P. C.; DAISLEY, K. W. Routine haematological methods for use with fish blood. **J. Fish Biol.**, v. 5, p. 771-781, 1973.

6- CATTON, W. T. Blood cell formation in certain Teleost fishes. **Blood**, v. 6, p. 39-60, 1951.

7- CAXTON-MARTINS, A. G. Cytochemical studies of cell population in peripheral blood smears of two west African Teleost. **J. Anat.**, v. 128, p. 269-2676, 1979.

8- COTRAN, R. S.; KUMAR, V.; COLLINS, T. Cellular Pathology I: Cell Injury and Cell Death. In: ———. **Pathologic basic of disease**. London: W. B. Saunders, 1999. p. 1-29.

9- DOGGETT, T. A.; WRATHMELL, A. B.; HARRIS, J. E. A cytochemical and light microscopical study of the peripheral blood leucocytes of *Oreochromis mossambicus Cichlidae.* **J. Fish Biol.**, v. 31, p. 147-153, 1987.

10- DOGGETT, T. A.; HARRIS, J. E. Ultrastructure of the peripheral blood leucocytes of **Oreochromis mossambicus**. **J. Fish Biol.**, v. 33, p. 747-756, 1989.

11- DVORAK, A. M.; FURITSU, T.; ESTRELLA, P.; LETOWRNEAU, L.; ISHIZAKA, T.; ACKERMAN, S. J. Ultrastructural localization of major basic protein in the human eosinophil lineage in vitro. **J. Histochem. Cytochem**, v. 42, n. 11, p. 1443-1451, 1994.

12- EGAMI, M. I. – **Aspectos morfológicos, citoquímicos e haematimétricos do sangue de macaco prego *Cebus apella* (Linne, 1758) submetidos aos raios-X**. 1988. 121 f. Dissertação (Doutorado) – Escola Paulista de Medicina, São Paulo.

13- ELLIS, A. E. Leucocytes and related cells in the plaice **Pleuronectes platessa**. **J. Fish Biol.**, v. 8, p. 143-156, 1976.

14- ELLIS, A. E. The leucocytes of fish: a review. **J. Fish Biol.**, v. 11, p. 453-491, 1977.

15- EZZAT, A. A.; SHABANA, M. B.; FARGHALY, A. M. Studies on the blood characteristics of *Tilapia zilli* (Gervais). I Blood cells. **J. Fish Biol.**, v. 6, p. 1-12, 1974.

16- FERGUSON, H. W. The ultrastructure of plaice *(Pleuronectes platessa)* leucocytes. **J. Fish Biol.**, v. 8, p. 139-412, 1976.

17- FLETCHER, G. L. The effects of capture "stress", and storage of whole blood cells plasma, proteins, glucose and electrolytes of the winter flounder *(Pseudopleuronectes americanus)*. **Can. J. Zool.**, v. 53, p. 197-206, 1975.

18- HINE, P. M.; WAIN, J. M. Observations on eosinophilic granule cells in perotoneal exudates of cells. *Anguilla australis.* **J. Fish Biol.**, v. 34, p. 841-853, 1989.

19- HOAR, W. S.; RANDALL, D. J.; FARRELL, A. P. Fish cells. In: RAGNAR F. **Fish physiology**. London: Academic Press, 1992. p. 15-54.

20- IMAGAWA, T.; HASHIMOTO, Y.; KITAGAWA, H.; KON, Y.; KUDO, N.; SUGIMURA, M. Morphology of blood cells in carp *(Cyprinus carpio L.)*. **Jpn. J. Vet. Sci.**, v. 51, p. 1163-1172, 1989.

21- JACOBS, A. Staining for leucocyte peroxidase. **Lancet**, v. 1, p. 697, 1958.

22- JAKOWSKA, S. Morphologie et nomenclature des cellules du sang des téléostéens. **Rev. D'Hématol.**, v. 11, p. 519-539, 1956.

23- JONASSEN, T. M.; IMSLAND, A. K.; STEFANSON, S. O. The interaction of temperature and fish size on growth of juvenille halibut. **J. Fish Biol.**, v. 54, p. 556-572, 1999.

24- LESTER, R. J. G.; DANIELS, B. A. The eosinophilic cell of the white sucker. *Catostomus commersoni*. **J. Fish Res. Board Can.**, v. 33, p. 139-144, 1976.

25- LESTER, R. J. G.; DESSER, S. S. Ultrastructural observations on the granulocytic leucocytes of the teleoste. *Catostomus commersoni.* **Can. J. Zool.**, v. 53, p. 1648-1657, 1975.

26- LISON, L. Lipides et lipoproteines. In: ———. **Histochimie et cytochimie animales**. Principes et méthodes. Paris: Gauthier-Villars, 1960. v. 2, p. 449-530.

27- McCARTHY, D. H.; STEVENSON, J. P.; ROBERTS, M. S. Some blood parameters of the rainbow trout **(***Salmo gairdneri*, Richardson). I. The Kamloops variety. **J. Fish Biol.**, v. 5, p. 1-8, 1973.

28- McKNIGHT, I. M. A haematological study on the mountain whitefish, *Prosopium williamsoni.* **J. Fish Res. Bd. Canada**, v. 23, p. 45-64, 1966.

29- McMANUS, J. F. A. Histological demonstration of mucin after periodic acid. **Nature**, v. 158, p. 202, 1946.

30- MATUSHIMA, E. R.; MARIANO, M. Kinetics of the inflammatory reaction induced by carrageenin in the swimbladder of *Oreochromis niloticus (Nile tilapia).* **Brazilian Journal of Veterinary Research and Animal Science**, v. 33, n. 1, p. 5-10, 1996.

31- MAZIA, D.; BREWER, P. A.; ALFERT, M. The cytochemical staining and measurement of protein with mercuric bromphenol blue. **Biol. Bull.**, v. 104, p. 57-67, 1953.

32- OLADIMEJI, A. A.; AYANTOYE, A. A.; ESIEVO, K. A. N. Haematological differences between two tropical freshwater fishes, *Oreochromis niloticus* and *Clarias Lazera*. **Rev. Zool Afr. – J. Afr. Zool.**, v. 102, p. 487-492, 1988.

33- ORIA, J. Elementos figurados do sangue de alguns Teleosteos fluviais brasileiros **(**Nematognathas, Characideos, Gymnotideos, Poeciliideos). I. Erythrocytos: formas normaes, formas jovens e formas involuidas. **An. Fac. Med. S. Paulo**, v. 8, p. 43-68, 1932.

34- PACHECO, F. J. **Observações estruturais e ultraestruturais de granulócitos do órgão epigonal e de granulócitos e trombócitos do sangue periférico do tubarão *Rhizoprionodon lalandii* (Valenciennes, 1839) (Elasmobranchii, Carcharhinidae)** 2000. 87 f. Dissertação (Mestrado) - Escola Paulista de Medicina, UNIFESP, São Paulo.

35- PHILLIPS Jr., A. M. The development of anemia in trout fed a synthetic diet and its cure by the feeding of fresh beef liver. **Progve Fish Cult.**, v. 48, p. 11-13, 1940.

36- RIBEIRO, W. R. **Contribuição ao estudo da haematologia de peixes. Morfologia e citoquímica das células do sangue e dos tecidos haematopoéticos do mandi amarelo, *Pimelodus maculatus Lacepede,* 1803**. 1978. 110 f. Dissertação (Doutorado) – Faculdade de Medicina de Ribeirão Preto – Universidade de São Paulo, Ribeirão Preto.

37- SIGEL, M. M.; HAMBY, B. A.; HUGGINS Jr, E. M. Phylogenetic studies on lymphokines. Fish lymphocytes respond to human IL-1 and epithelial cells produce an IL-1 like factor. **Vet. Immunol. Immunopathol.**, v. 12, p. 47-58, 1986.

38- SRIVASTAVA, A. K. Studies on the haematology of certain freshwater teleosts. IV Leucocytes. **Anat. Anz.**, v. 123, S: p. 520-533, 1968.

39- SUZUKI, K. Morphological and phagocytic characteristics of the peritoneal exudate cells in tilapia, *Oreochromis niloticus* (Trewavas), and carp. *Cyprinus carpio.* **J. Fish Biol.**, v. 29, p. 349-364, 1986.

40- UEDA, I. K.; EGAMI, M. I.; SASSO, W. S.; MATUSHIMA, E. R. Estudos hematológicos em *Oreochromis niloticus* (Linnaeus, 1758) (Cichlidae, Teleostei) – Parte I. Braz. **J. Vet. Res. Anim. Sci.**, São Paulo, v. 34, n. 5, p. 270-275, 1997.

41- VEIGA, M. L. **Aspectos morfológicos das células sangüíneas, citoquímicos e ultraestruturais de trombócitos, neutrófilos e eosinófilos de Dourado *Salminus maxillosus*. (Valenciennes, 1840) (Pisces, Characidae)**. 1999. 95 f. Dissertação (Mestrado) – Universidade Federal de São Paulo, Escola Paulista de Medicina, São Paulo.

42- WEINREB, E. L. Studies on the fine structure of teleost blood cells. I. Peripheral blood. **Anat. Rec.**, v. 147, p. 219-225, 1963.

43- YOKOYAMA, H. O. Studies on the origin, development, and seasonal variations in the blood cells of the perch, Perca flavescens. **Wildlife Diseases**, v. 6, p. 1-102, 1960.

44- ZINKL, J. G.; COX, W. T.; KONO, C. S. Morphology and cytochemistry of leucocytes and thrombocytes of six species of fish. **Comparative Hematology International**, v. 1, n. 4, p. 187-195, 1991.

Received: 08/05/2000
Accepted: 31/01/2002