Onderstepoort J. vet. Res., 48, 185–193 (1981)

THE HAEMOCYTOLOGY AND HISTOLOGY OF THE HAEMOPOIETIC ORGANS OF SOUTH AFRICAN FRESHWATER FISH. III. THE LEUCOCYTES, PLASMA CELLS AND MACROPHAGES OF *CLARIAS GARIEPINUS* AND *SAROTHERODON MOSSAMBICUS**

J. BOOMKER, Department of Parasitology, Faculty of Veterinary Science, University of Pretoria, P.O. Box 12580, Onderstepoort 0110

ABSTRACT

BOOMKER, J., 1981. The haemocytology and histology of the haemopoietic organs of South African freshwater fish. III. The leucocytes, plasma cells and macrophages of *Clarias gariepinus* and *Sarotherodon mossambicus*. *Onderstepoort Journal of Veterinary Research*, 48, 185–193 (1981).

The various leucocytes, plasma cells and macrophages are described and illustrated. Eosinophils and basophils are lacking in *Clarias gariepinus* but present in *Sarotherodon mossambicus*. The leucocytes of *C. gariepinus* resemble those found in mammals, as do the plasma cells and macrophages of both species. A possible mechanism for their formation is postulated.

Résumé

L'HAEMACYTOLOGIE ET L'HISTOLOGIE DES ORGANES HAEMOPOIETIQUES DU POISSON D'EAU DOUCE D'AFRIQUE DU SUD. III. LES LEUCOCYTES, PLASMOCYTES ET MACROPHAGES DU CLARIAS GARIEPINUS *ET DU* SAROTHERODON MOSSAMBICUS

Les leucocytes différentes, plasmocytes et macrophages sont décrits et illustrés. Les eosinophiles et basophiles sont absents chez le Clarias gariepinus *mais présents chez le* Sarotherodon mossambicus. *Les leucocytes de* C. gariepinus *ressemblent à ceux trouvés chez les mammifères, comme d'ailleurs les plasmocytes et les macrophages des deux espèces. Un mécanisme possible de leur formation est postulé.*

INTRODUCTION

Although the leucocytes of fish show distinct similarities to those of birds and mammals, their nomenclature still presents a problem. Jakowska (1956) suggested that the terminology, particularly that of the leucocytes, be revised. Fish do not have active red bone marrow which is present in Amphibia and higher vertebrates; terms such as "myelocyte", which is a typical cell of the red bone marrow (Dorland, 1968), as well as the terms "myeloblast" and "metamyclocyte" should therefore be avoided (Jakowska, 1956), Jakowska (1956). also suggested that terms such as leucoblast, lymphomyeloblast, lymphoblast, megaloblast and megalocyte should not be used.

Jakowska (1956) proposed a terminology for the leucocytes which is adaptable and causes fewer problems in fish than terms derived from mammalian haematology. It is used by Weinreb (1963) and Pitombeira & Martins (1970) and will also be used as a guide in this study.

The purpose of this paper is to augment the inadequate knowledge on the haemocytology of South African fish by describing the various leucocytes found in the catfish (*Clarias gariepinus*) and the Mozambique bream (*Sarotherodon mossambicus*). In addition, a schematic representation of haemopoiesis is postulated for these fish.

MATERIALS AND METHODS

The acclimatization of the fish, the preparation of blood films and impression smears, and the method of supravital staining of blood cells have been described in previous papers (Boomker, 1979; 1980).

Blood of both fish species was collected in 10 mℓ vacuum tubes containing 500 IU heparin and then centrifuged at 3000 rpm for 10 minutes. The plasma and buffy coat were removed separately and the buffy coat resuspended in a small quantity of 0,85% saline (Smith, Potter & Merchant, 1967). Small drops of this suspension were used to make smears for microscopic examination in the usual manner (Wintrobe, 1947).

Phagocytic leucocytes and macrophages were studied by injecting 2 catfish intraperitoneally with 0,5 mℓ of a dye consisting of equal parts of black india ink and peanut oil. Twenty-four hours later blood smears and impression smears of various organs were prepared and tissue blocks collected for histology. Blood and impression smears were fixed in formalin vapour by inverting them over a Petri dish containing a small quantity of 40% formalin (Ashley & Smith, 1963). The smears were stained with nuclear fast red (Pearse, 1961). Tissue blocks were routinely processed and sectioned, and stained with haematozylin-eosin (HE), Berlin blue (BB) and Schmorl's stain (Boomker, 1979).

Differential counts were made on Giemsa-stained blood smears by identifying the first 200 leucocytes seen (including thrombocytes) in 5 different slides of each of 8 catfish and 5 Mozambique bream. The relative abundance of each cell type was then calculated and expressed as a percentage of the total number of cells counted (Table 1).

Arneth counts, as described by Lucas & Jamroz (1961), were made by counting the nuclear lobes of 100 neutrophils on each of 2 blood smears of 8 catfish and 7 bream (Tables 2 and 3).

Immature leucocytes were studied on Giemsa-stained impression smears of the haemopoietic organs, prepared as described in an earlier paper (Boomker, 1980).

The range and mean of the diameter of 80 cells of each round leucocyte type were calculated on 2 separate blood smears and 3 impression smears of 8 catfish and 7 bream (Table 4).

RESULTS

The criteria used to differentiate the various leucocytes were the conformation, structure and staining affinities of the nucleus and cytoplasm as compared with those of a mature erythrocyte. The size and shape of the cell, as well as the presence, size and staining

* Presented in part as partial fulfilment for the degree M.Sc. in Zoology, at the Rand Afrikaans University, January 1978

Received 14 September 1981

affinities of intracytoplasmic granules were also considered.

The differential leucocyte counts are given in Table 1, and the Arneth counts in Tables 2 and 3. The range and mean values of the dimensions of the round leucocytes as compared with a mature erythrocyte are given in Table 4.

The agranular leucocytes

Small and large lymphocytes occur in both the fish species examined and there is no morphological difference between the lymphocytes found in the haemopoietic organs and those in the circulating blood.

The typical small lymphocyte is smaller than a mature erythrocyte (Table 4). It is usually round and contains a large round nucleus which is surrounded by a thin rim of cytoplasm (Fig. 1). The nucleus stains intensely purplush-blue and the cytoplasm stains pale blue. Sometimes a few, large, purple-staining azurophil granules are present. The cytoplasm is homogeneous, although condensations may impart a slightly granular appearance.

The typical large lymphocyte (Fig. 2) is as big as or slightly bigger than the mature erythrocyte (Table 4). Its nucleus is more vesicular than that of the small lymphocyte, and the chromatin is radially arranged. The cytoplasm stains pale blue and contains a few, large, purple-staining azurophil granules. In some large lymphocytes a clear area is seen adjacent to the nucleus. This area is known as the *Hof* (Lucas & Jamroz, 1961) and represents a large, poorly staining centriole. The *Hof* is seen in about 5% of the larger lymphocytes of both the catfish and the bream.

TABLE 1 Differential counts of the haemocytes of *C. gariepinus* and *Sarotherodon mossambicus*

Cell type	*C. gariepinus*		*S. mossambicus*	
	Range %	Mean %	Range %	Mean %
Thrombocytes	41,5–55,4	48,45	15,4–18,4	16,9
Lymphocytes	12,3–20,3	16,3	26,7–28,6	27,65
Monocytes	1,6–8,9	5,25	4,1–6,5	5,3
Granuloblasts	0–0,1	0,05	0,7–1,4	1,05
Developing cells, neutrophilic series	0,2–20	10,1	1,4–3,1	2,25
Neutrophils	5,0–34,7	19,85	44,3–46,3	45,3
Developing cells, eosinophilic series	—	—	0–0,9	0,45
Eosinophils	—	—	0,8–0,9	0,85
Basophils	—	—	0–0,5	0,25

TABLE 2 Arneth counts of the neutrophils of *C. gariepinus*

Fish No.	Class					Total
	I	II	III	IV	V	
3	41	103	50	6	0	200
4	45	98	45	10	2	200
5	45	105	43	7	0	200
6	40	104	50	6	0	200
7	41	107	45	6	1	200
8	52	100	42	5	1	200
9	43	101	47	9	0	200
10	41	104	49	6	0	200
Mean	21,75%	51,38%	23,18%	3,44%	0,25%	100%

TABLE 3 Arneth counts of the neutrophils of *Sarotherodon mossambicus*

Fish No.	Class						Total
	I	II	III	IV	V	Rings	
1	74	102	22	2	0	0	200
2	74	101	20	2	0	3	200
3	77	101	18	2	0	2	200
4	77	103	20	0	0	0	200
5	74	99	21	2	0	4	200
6	82	96	20	2	0	0	200
7	74	105	19	0	0	2	200
Mean	38%	50,5%	10%	0,7%	0	0,8%	100%

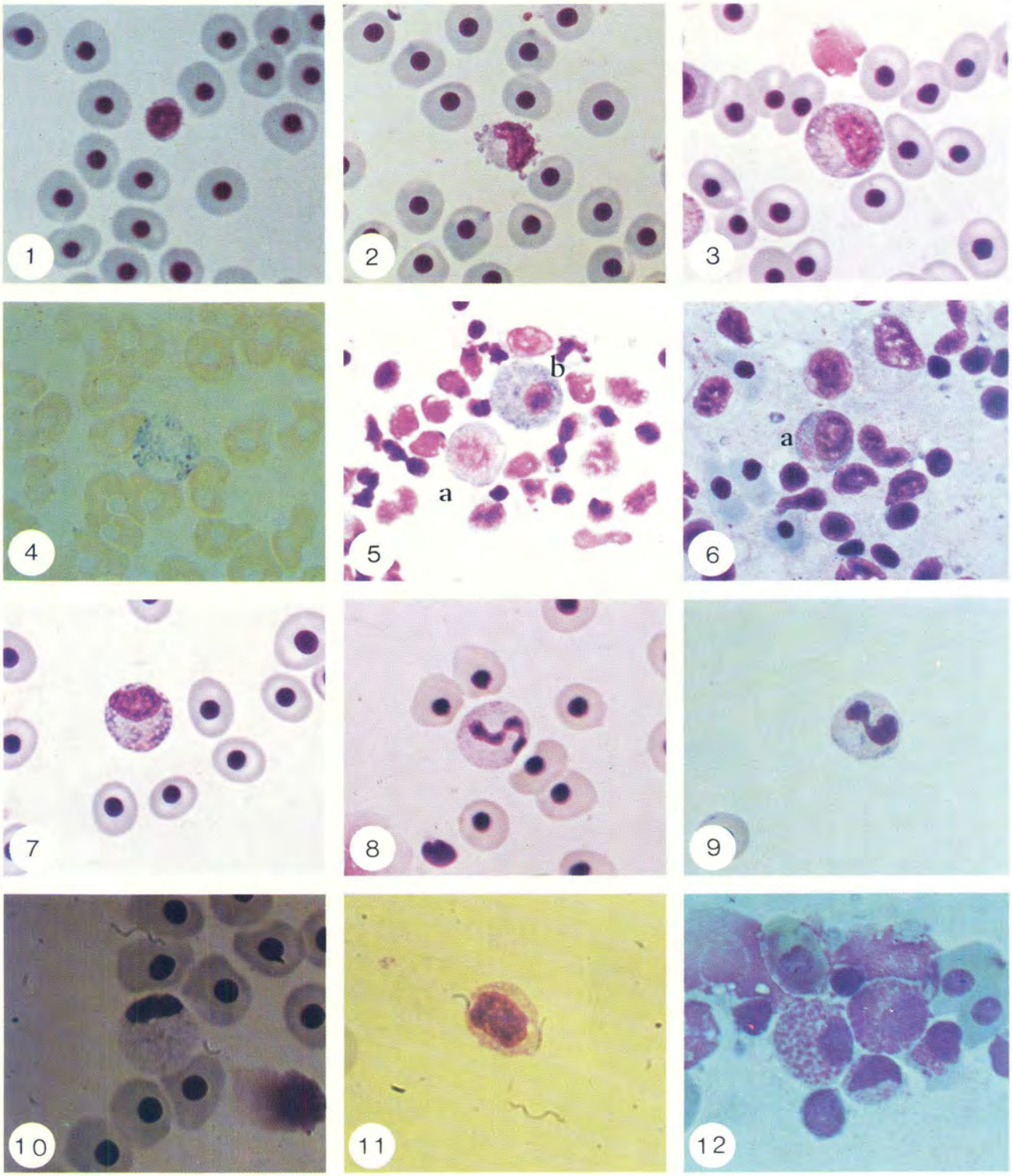


FIG. 1 A small lymphocyte in the circulating blood of the catfish; Giemsa, ×1 000
FIG. 2 A large lymphocyte in the circulating blood of the catfish. Note the pseudopodia and distinct *Hof;* Giemsa, ×1 000.
FIG. 3 A monocyte in the circulating blood of the catfish; Giemsa, ×1 000
FIG. 4 A monocyte in the circulating blood of the catfish. The stain particles do not occur in the area of the *Hof* (centre of cell) or in the nucleus; supravital staining with brilliant cresyl blue, ×1 000
FIG. 5 A granuloblast (a) and a small macrophage (b) in the proncephros of a catfish; Giemsa, ×1 000
FIG. 6 A neutrophilic progranulocyte (a) in the mesonephros of the catfish; Giemsa, ×1 000
FIG. 7 A neutrophilic mesogranulocyte in the circulating blood of the catfish; Giemsa, ×1 000
FIG. 8 A transitional form between the neutrophilic metagranulocyte and the mature neutrophil in the circulating blood of the catfish; Giemsa, ×1 000
FIG. 9 An early mature neutrophil with 2 nuclear lobes in the circulating blood of the catfish; Giemsa, ×1 000
FIG. 10 An aged neutrophil in the circulating blood of the catfish; Giemsa, ×1 000
FIG. 11 A cell believed to be an eosinophilic mesogranulocyte in the circulating blood of the bream; Giemsa, ×1 000
FIG. 12 A group of eosinophils in the mesonephros of the bream; Giemsa, ×1 000

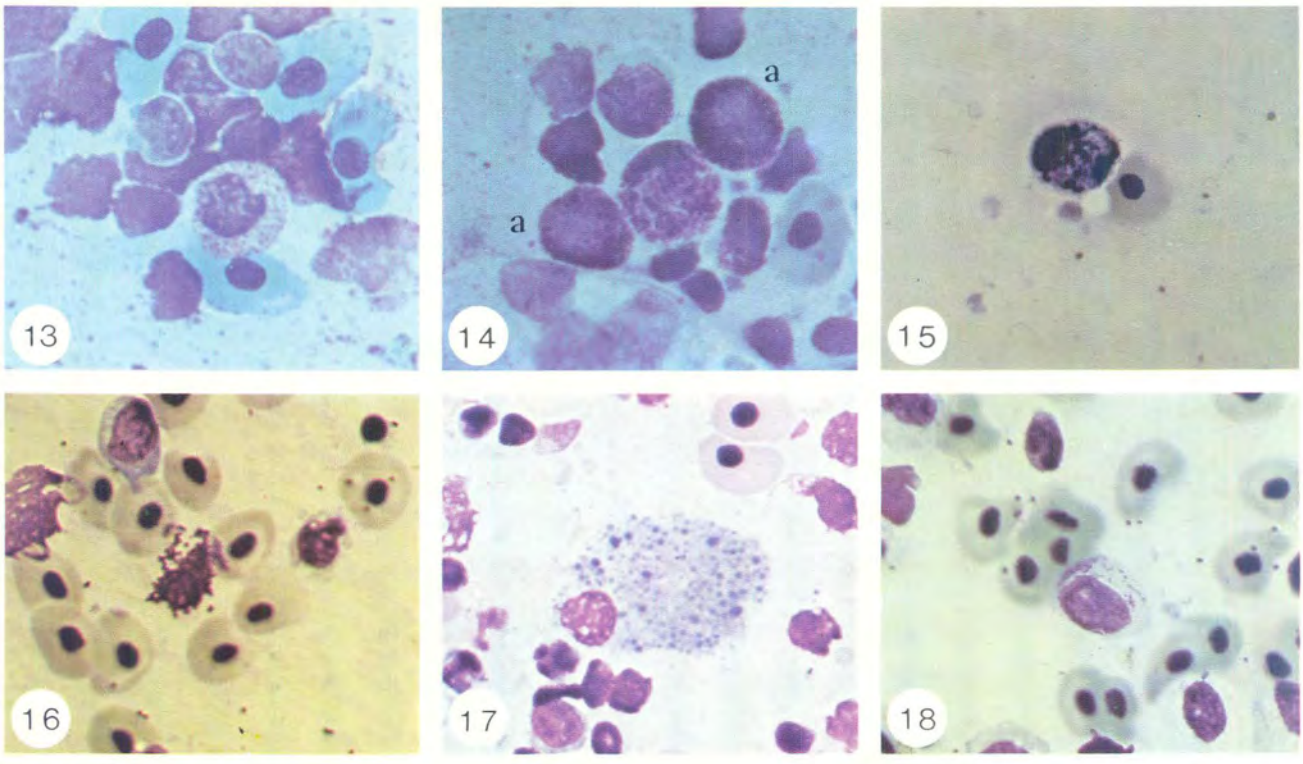


FIG. 13 An eosinophil in the mesonephros of the bream. Compare the size and staining affinities of this cell with those illustrated in Fig. 12; Giemsa, ×1 000

FIG. 14 Two basophils (a) and eosinophils in the mesonephros of the bream; Giemsa, ×1 000

FIG. 15 A basophil in the mesonephros of the bream; Giemsa, ×1 000

FIG. 16 A ruptured basophil in the circulating blood of the bream; Giemsa, ×1 000

FIG. 17 A large macrophage in the spleen of the catfish; Giemsa, ×1 000

FIG. 18 A cell believed to be a monocyte, containing a number of bacteria in the cytoplasm; circulating blood of the bream, Giemsa, ×1 000

TABLE 4 The mean diameter and standard deviation (±) of the leucocytes and immature stages of leucocytes compared with those of the erythrocytes of *C. gariepinus* and *Sarotherodon mossambicus*

Cell type	*C. gariepinus*			*S. mossambicus*		
	Cell diameter μm	Nucleus diameter μm	Ratio of mean cell diameter to mean nuclear diameter μm	Cell diameter μm	Nucleus diameter μm	Ratio of mean cell diameter to mean nuclear diameter μm
Erythrocyte	7,65±1,35	2,93±0,225	2,61	8,55±0,45×5,4±0,9	4,05±1,35×2,25±0,45	
Lymphocyte:						
Small	5,0±0,5	3,6±0,9	1,38	3,6±0,45	2,7	1,333
Large	8,1±1,0	4,6±0,8	1,76	5,4±0,45	4,05±0,3	1,33
Monocyte	11,25±2,25	—	—	9,45±0,45	—	—
Granuloblast	9,9±0,9	4,5±0,9	2,2	7,2±0,45	4,5±0,2×2,7±0,1	—
Neutrophilic series						
Progranulocyte	7,5±0,6	3,15±0,51	2,38	7,2±0,1	4,95±0,45	1,45
Mesogranulocyte	8,5±0,5	—	—	9,0	5,4	1,66
Metagranulocyte	9,0±0,9	—	—	8,1±0,9	—	—
Neutrophil	9,45±1,35	—	—	9,3±0,35	—	—
Eosinophilic series						
Progranulocyte	—	—	—	4,2±0,2	3,4	1,235
Mesogranulocyte	—	—	—	5,4±0,45	—	—
Metagranulocyte	—	—	—	6,75±0,55	—	—
Eosinophil	—	—	—	8,55±1,35	—	—
Basophil	—	—	—	9,0±0,45	—	—

Because of the formation and retraction of pseudopodia, the shapes of both the small and the large lymphocytes may vary considerably (Fig. 2, 19 and 20). The 2 types of lymphocytes, however, may be differentiated on their diameters, the amount of cytoplasm they contain and the morphology of their nuclei.

The developing cells of the lymphocytic series are difficult to subdivide and no attempt has been made to do so.

Monocytes are usually larger than the large lymphocytes, although a certain degree of overlapping occurs (Table 4). A typical monocyte (Fig. 3) is round, although cytoplasmic projections and pseudopodia are often seen (Fig. 21 and 22). The cytoplasm stains pale blue to greyish-blue and may appear finely granular as a result of cytoplasmic condensations. A number of vacuoles occur in the cytoplasm; these may be of equal or unequal sizes. A distinct *Hof*, which is situated in the indentation of the nucleus, is seen in 95% of the monocytes. The *Hof* may have a slightly reticular appearance because of fine cytoplasmic strands which cross it, and it may contain a few granules that stain light red. Some monocytes contain a number of azurophil granules that stain deep purple. The azurophil granules are more numerous, but smaller than those seen in the lymphocytes.

The nucleus of the monocytes is usually situated eccentrically, but may occasionally be central. The central nuclei are round or triangular in shape, whereas those situated eccentrically are bean- or horseshoe-shaped. Nuclei that have 2 distinct lobes connected by a narrow "bridge" are rarely encountered. The chromatin is a delicate network that stains pink or pale purple and contains purple staining condensations.

With supravital staining with brilliant cresyl blue, the monocytes rapidly absorb the stain particles (Fig. 4). The stain particles, however, are not seen in the area of the *Hof*, which confirms the presence of a large centriole.

A cell, believed to be a monocyte that has phagocytosed a number of bacteria, is illustrated in Fig. 18.

The granular leucocytes

Of the 3 types of granular leucocytes seen in mammals, only neutrophils are found in catfish. Bream have neutrophils as well as small numbers of eosinophils and basophils in the haemopoietic organs and circulating blood. The various granular leucocytes are readily identified by the colour of their granules in preparations stained with Giemsa.

The common precursor of the granular leucocytes is the granuloblast (Fig. 5a) which, though resembling an erythroblast, lacks the perinuclear halo. In addition, the cytoplasm is distinctly granular, stains light to dark blue and contains a large number of small, purplish-red granules which are of equal size. The nucleus is round and vesicular, and situated centrally in the catfish; it is elongated to oval in the bream. The nucleolus stains blue and is partially obscured by the chromatin.

The neutrophilic series of granular leucocytes consists of the neutrophilic progranulocytes, the neutrophilic mesogranulocytes, the neutrophilic metagranulocytes, the mature and the aged neutrophils.

The neutrophilic progranulocytes (Fig. 6a) are slightly smaller than mature erythrocytes. They are usually round, but small pseudopodia may give the cells an irregular outline. The cytoplasm stains light blue, is slightly granular, and may contain dark blue cytoplasmic condensations. Neutrophilic granules are scarce, but a number of azurophilic granules may be present. The nucleus is round and usually situated centrally. The chromatin is vesicular, arranged radially, and stains reddish-purple.

Neutrophilic mesogranulocytes (Fig. 7) are slightly larger than neutrophilic progranulocytes. They are round and their cytoplasm stains light blue. Neutrophilic and azurophilic granules occur in more or less equal quantities. The nucleus is fairly large, round,

oval or roughly triangular, and is always eccentrically situated. The chromatin stains purple and is arranged radially.

The neutrophilic metagranulocytes (Fig. 8) are larger than the neutrophilic mesogranulocytes. The cytoplasm is very light blue, and occasionally light pink. It has a granular appearance, owing to the presence of many neutrophilic granules, amongst which a few azurophil granules are scattered. The nucleus is characteristically band-shaped and is situated eccentrically. It is compact and stains purple.

Mature neutrophils of both fish species are larger than mature erythrocytes (Fig. 9). They are usually round, but broad pseudopodia occur in some cells. The cytoplasm contains numerous neutrophilic granules which give the cytoplasm an almost translucent, pale pink appearance. Only a few azurophil granules occur in the cytoplasm, and small vacuoles are occasionally seen. The nucleus is either ring-shaped or polymorphic and contains from 1–5 lobes. The chromatin is dense and stains purple. A nucleolus was not seen.

Variations in the shape of the neutrophils of the catfish and the bream are illustrated in Fig. 23 and 24, and Arneth counts, which indicate the degree of nuclear lobulation, are given in Tables 2 and 3.

Some cells which are present in both catfish and bream (Fig. 10) are believed to be aged neutrophils. They are the same size as the mature neutrophils, but the cytoplasm is very pale blue and difficult to detect. Very few azurophil granules are scattered in the cytoplasm. The nucleus is crescent-shaped (Fig. 10) or may be triangular and situated eccentrically. The chromatin is very dense and stains dark purple.

The eosinophilic series consists of the eosinophilic progranulocytes, eosinophilic mesogranulocytes, eosinophilic metagranulocytes and mature eosinophils. Cells of this series were not found in any of the catfish examined, but were present in small numbers in all of the bream.

The eosinophilic progranulocyte is about half the size of a mature erythrocyte. The cytoplasm is finely granular, stains pink, and contains a few eosinophilic granules. The nucleus is round, vesicular and situated eccentrically. The chromatin is radially arranged and stains reddish-purple.

The eosinophilic mesogranulocyte is slightly larger than the eosinophilic progranulocyte. The cytoplasm stains pale orange-red and contains a number of round, orange-staining granules. The nucleus is round or oval, and is situated eccentrically. The chromatin is radially arranged, more compact than that of the eosinophilic progranulocyte, and stains purple (Fig. 11).

Eosinophilic metagranulocytes are slightly larger than the mesogranulocytes. Their cytoplasm stains very light pink and contains discrete, round, orange-staining granules. The nucleus is oval or elongated, and the chromatin stains purple.

Mature eosinophils are slightly larger than the metagranulocytes (Fig. 12, 13 and 14). The cytoplasm stains very pale pink and the cell is filled with round eosinophilic granules that vary in colour from orange to orange-red. The nucleus is oval or elongated, and sometimes indistinctly lobed. The chromatin is dense and stains purple.

The basophilic series cannot be described in full as too few cells of the immature stages were found. There seems to be distinct polymorphism, and they cannot be assigned to any specific developmental stage.

Mature basophils in circulating blood are slightly larger than the erythrocytes (Fig. 14, 15 and 16). The cytoplasm stains very light blue and contains a large number of dark purple granules. The nucleus is crescent-shaped and is situated eccentrically; the chromatin stains very dark purple.

The plasma cells

The plasma cells represent only a small percentage of cells found in the haemopoietic organs, and they are absent from the circulating blood. They are most abundant in the pronephros and the spleen. They are round cells with a finely granular cytoplasm that stains purple. The nucleus is round or slightly oval and is always situated eccentrically. The chromatin is vesicular and stains purplish-pink.

The macrophages

These cells occur singly or in groups in the haemopoietic organs and are most abundant in the spleen. In HE stained sections they are seen as large, irregularly shaped cells with a pale blue cytoplasm in which a number of golden brown granules occur. In Giemsa-stained smears and sections, the granules vary from pale to dark blue (Fig. 5b and 17). With BB staining the granules stain blue, and brown with Schmorl's, indicating that they consist of iron-containing haemosiderin and iron-free lipofuscin. Macrophages of catfish, injected with india ink, contained the black ink particles in their cytoplasm. The nucleus of the macrophage is small, round and compact, and stains purple.

Discussion

The agranular leucocytes

The lymphocytes of both the catfish and the bream show a distinct similarity to those of other fish species, as well as those of birds and mammals. Fey (1966b) found that there were no histochemical differences between the lymphocytes of fish and those of mammals, and that their post-irradiation reactions were the same. Ellis (1976) and Ferguson (1976) also mention the close similarities between the lymphocytes of fish and those of mammals.

The lymphocytes of the bream are appreciably smaller than those of catfish. This is regarded as interspecific variation, examples of which are also given for other fish species by Catton (1951), Boyar (1962), Weinreb (1963), Pitombeira & Martins (1970) and Ellis (1976).

The variation in the differential counts of the lymphocytes of the fish species examined is also ascribed to interspecific variation. Lieb, Slane & Wilber (1953) found 91% lymphocytes in *Salmo namaycush,* whereas Ezzat, Shabana & Farghaly (1974) found only 61% lymphocytes in *Tilapia zillii.*

In this study it was found that it is sometimes difficult to differentiate between some small lymphocytes and the small lymphoid haemoblasts. The small lymphoid haemoblasts, however, in addition to having a slightly more vesicular nucleus and more cytoplasm, are bigger and have the more primitive appearance of the two.

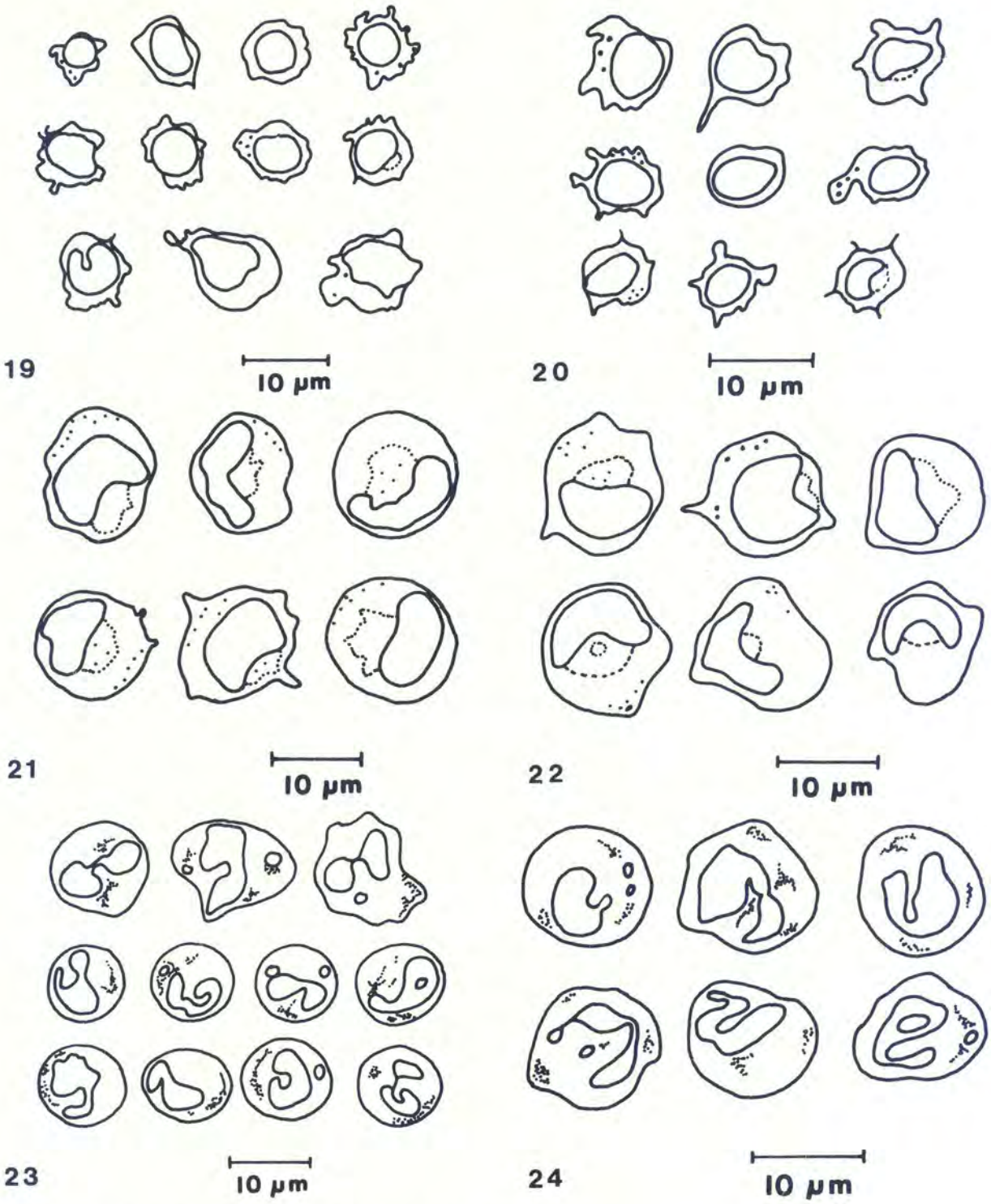


FIG. 19 Variation in the shape and size of the lymphocytes of the catfish
FIG. 20 Variation in the shape and size of the lymphocytes of the bream
FIG. 21 Variation in the shape and size of the monocytes of the catfish
FIG. 22 Variation in the shape and size of the monocytes of the bream
FIG. 23 Variation in the shape and size of the mature neutrophils of the catfish
FIG. 24 Variation in the shape and size of the mature neutrophils of the bream

The development of the lymphocytes has been the subject of controversy amongst various authors. Downey (1909) studied the intertubular renal tissue of *Polyodon spathula* and found small and large lymphocytes, as well as small and large mononuclear cells. He was of the opinion that the lymphocytes develop from the small mononuclear cells.

Jordan & Speidel (1924) regarded the lymphocytes as the omnipotent cells, which function as haemoblasts, and from which the erythrocytes and leucocytes develop. According to Jordan & Speidel (1924), the lymphocytes originate from the reticulum cells and differentiate into small and large lymphocytes.

Jakowska (1956) saw the large lymphoid haemoblast as a haemocytoblast that may occur in any tissue or in the blood. She is of the opinion that the haemocytoblast gives rise to the various white and red cells. In addition, she saw the omnipotent lymphocyte of Jordan & Speidel (1924) as a haemocytoblast, because the lymphocyte is a specialized cell that fulfils a specific function.

The terminology used by Fijan (1960) is confusing (Table 5) and is unacceptable for the purpose of this study.

Downey (1909), Jordan & Speidel (1924), Catton (1951) and Jakowska (1956) are more or less in agreement as to the origin of the lymphocytes. They state that either the small round cell (Downey, 1909) or the small lymphoid haemoblast (Catton, 1951) or the haemocytoblast (Jordan & Speidel, 1924; Jakowska, 1956) develop into the lymphocytes.

The monocytes of the catfish and the bream are also similar to those of birds and mammals. This is apparent from their morphology as well as their reaction to the peroxidase staining method of Graham-Knoll (Fey, 1966b).

The monocytes of the bream are also smaller than those of the catfish, probably because of interspecific variation, as is the case with lymphocytes in mammals.

A number of factors can influence the occurrence and numbers of monocytes. Reznikoff & Reznikoff (1934) found an increase in the numbers of macrophages (monocytes) with intraperitoneal injections of turpentine, and Jakowska (1956) found an increase of monocytes in *Poecilia reticulatus* and *Carassius auratus* suffering from rickettsiosis.

Bloom & Fawcett (1968) are of the opinion that the monocytes develop from lymphocytes, and describe the change from small lymphocytes to monocytes in rabbits infested with *Listeria monocytogenes*. Bloom & Fawcett (1975), however, state that enough proof of a separate line of development of the monocytes in mammals has been found. This is indicated by the work of Virolainen (1968, cited by Bloom & Fawcett, 1975) who found a group of rapidly proliferating cells in the bone marrow of mice. These cells give rise to the monoblasts, which in turn form the monocytes. In both the catfish and the bream no signs of monoblasts could be found in the haemopoietic organs, and, because fish do not have haemopoietically active bone marrow, the older theory seems to be applicable, namely, that monocytes develop from lymphocytes. This view is supported by the findings of Ellis (1976) that a small percentage of monocytes have receptors for antibody-antigen complexes on their surfaces. Lymphocytes have such receptors. If monocytes develop from lymphocytes, it is probable that they have acquired the receptors from the lymphocytes and gradually lose them during their maturation to macrophages. This view is augmented by the work of Chiller, Hodgins, Chambers & Weiser (1969), who found immunocompetent cells in the spleens and pronephroi of trout. They state that lymphocytes, macrophages and plasma cells secrete or produce antibodies to a greater or lesser extent, thereby possibly indicating their common origin.

The granular leucocytes

Except for minor differences, such as size and numbers, the neutrophils of the catfish are similar to those of the Mozambique bream, and the neutrophils of both the fish species are similar to those of mammals.

The differences in the numbers of mature neutrophils between the catfish and the bream are ascribed to interspecific variation, which is also seen in various mammals (Schalm, Jain & Carroll, 1975). Ezzat *et al.* (1974) showed that the numbers of neutrophils of *T. zillii* increase with the age of the fish, and also that females have a higher percentage of neutrophils than males. Because age and sex differences were not taken into account in this study, such a correlation could not be determined. Pitombeira & Martins (1970), however, found a large variation in the numbers of neutrophils of different individuals of *Scomberomorus maculatus,* which they ascribed to intraspecific variation.

TABLE 5 Terminology of the leucocytes, plasma cells and macrophages as used by various authors

Author	Agranular series	Granular series	Other cells
Werzberg, 1911.....	Lymphocyte Small lymphocyte Leucocytoid lymphocyte Lympholeucocyte Amphichromatic lympholeucocyte Monocyte	Oxyphilic granulocyte Neutrophil Eosinophil	Mast cell
Jordan & Speidel, 1924	Small, medium and large lymphocytes	Eosinophil/Pseudo-eosinophil	Macrophage
Yoffey, 1929.....	Small and large round cells	Neutrophil Eosinophil	
Duthie, 1939.....	Lymphocyte	Progranulocyte Granuloblast Neutrophil Progranulocyte Eosinophilic chromophilgranulocyte Progranulocyte Basophilic chromophil granulocyte	
Catton, 1951.....	Lymphocytes of various sizes	Fine granule progranulocyte Fine granule granulocyte Coarse granule progranulocyte Coarse granule granulocyte	

TABLE 5 (*continued*)

Author	Agranular series	Granular series	Other cells
Dombrowski, 1953.....	Lymphocytes of various sizes	Normal and aged leucocytes	
Lieb, Slane & Wilbur, 1953	Small, medium and large lymphocytes Monocyte	Neutrophil	
Jakowska, 1956.....	Small and large lymphocytes Macrophage	Granuloblast Proneutrophil Neutrophilic granulocyte Pro-eosinophil Eosinophilic granulocyte Probasophil Basophilic granulocyte	
Fijan, 1960.....	Lymphoblast Small and large lymphocytes Monocyte	Myeloblast Promyelocyte Heterophilic myelocyte Heterophilic metamyelocyte Heterophilic granulocyte Basophilic myelocytes I, II Basophilic granulocyte	Plasmoblast Plasma cell Macrophage
Boyar, 1962.....	Lymphocyte	Neutrophil Eosinophil Basophil	
Weinreb, 1963.....	Small and large lymphocytes	Neutrophil Eosinophil Basophil	
Fey, 1966a, b.....	Small, medium and large lymphocytes Monocyte	Heterophil Eosinophil Basophil	Plasma cell
Pitombeira & Martins, 1970	Lymphocyte Macrophage	Neutrophilic granulocyte Eosinophilic granulocyte Basophilic granulocyte	
Ezzat *et al.*, 1974.....	Small and large lymphocytes		
Ellis, 1974.....	Lymphoblast Lymphocyte Monocyte	Progranuloblast Granuloblast Neutrophil	Plasma cell Macrophage
Boomker, 1980.....	Small and large lymphocytes Monocyte	Granuloblast Neutrophilic progranulocyte Neutrophilic mesogranulocyte Neutrophilic metagranulocyte Neutrophil Aged neutrophil *Eosinophilic progranulocyte *Eosinophilic mesogranulocyte *Eosinophilic metagranulocyte *Eosinophil *†Basophilic progranulocyte *†Basophilic mesogranulocyte *†Basophilic metagranulocyte *Basophil	Plasma cell Macrophage

* Not found in catfish

† Postulated to occur in bream

There are slight morphological differences between the cells of the neutrophilic series of the catfish and the bream which correspond to those given for other species (Catton, 1951; Boyar, 1962; Weinreb, 1963; Pitombeira & Martins, 1970; Ellis, 1976).

Arneth counts were done by Lucas & Jamroz (1961) for the heterophils of chickens. The Arneth counts for the neutrophils of catfish and bream are given in Tables 2 and 3. For both species the modal is Class II, where 2 nuclear lobes per mature neutrophil are seen. A shift to the left of the modal, that is, to the immature stages, indicates an increased production, i.e. with bacterial infections. A shift to the right, that is to the older neutrophils with more than 2 nuclear lobes, indicates decreased production. The Arneth counts could be of value in determining pathological conditions, especially in those fish species that are farmed commercially.

Authors such as Werzberg (1911), Duthie (1939), Dombrowski (1953) and Topf (1953) described granulocytes as well as cells containing granules that they could not identify as belonging to a specific group of granular leucocytes. Downey (1909) found cells that he names "secretory granulocytes", and Stolk (1957) found oval cells with clavate granules in *Pterophyllum scalare*. According to Duthie (1939), the cells found by Werzberg (1911) did not stain properly, and the granules of those found by Downey

(1909) dissolved during the staining process. Fey (1963; 1966a) found that the "thrombocytoblasts" of Dombrowski (1953) and Topf (1953) were in fact basophils. Pienaar (1962) in his studies on the haemocytology of reptiles regarded mast cells or basophilic mast cells as basophils.

In catfish, no basophils or eosinophils nor their precursors could be found. Some of the catfish used in this study were heavily infested with 3rd stage larvae of *Contracaecum* which were encapsulated in the mesenterium. Although it is generally accepted that most helminth parasites elicit an eosinophilic reaction, no eosinophils could be found either around living or dead nematodes. In bream, small numbers of eosinophils and basophils were encountered, particularly in impression smears of the mesonephros.

Jordan (1938) postulated that basophils were generally lacking in fish, but studies by Jakowska (1956) and also this study indicate that they are present in at least some fish species. Fijan (1960), Boyar (1962), Weinreb (1963), Fey (1963; 1966 a, b), Pitombeira & Martins (1970) and Ezzat *et al.* (1974) all found basophils in the species of fish they studied, but Ellis (1976) and Ferguson (1976) did not find eosinophils or basophils in *Pleuronectes platessa.*

The development of the granular leucocytes has been studied by various authors and, apart from differences in the nomenclature (Table 5), the various cell types have been described in almost the same detail. One must consider, however, that heterophils or neutrophils have been found in all the fish species examined so far by all the authors, whereas oesinophils and basophils are lacking in some. The slight differences in the morphology of the various cells are probably the result of different staining techniques.

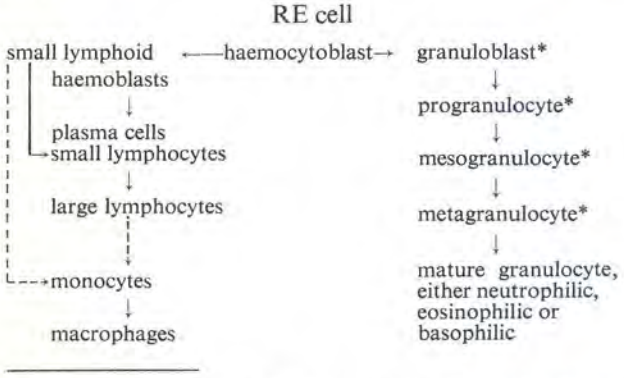
Plasma cells have been found in most of the fish species examined by Chiller *et al.* (1969). Plasma cells were not identified as such by some authors (Jordan, 1938; Duthie, 1939; Catton 1951; Dombrowski 1953). According to Fey (1966b), the methyl green-pyronin reaction is strongly positive for the plasma cells and the presence of γ-globulin is indicative of their presence. Becker, Lovell, Bird, Kelly, Schilling, Solomon & Young (1958), however, were unable to demonstrate the presence of γ-globulin, although plasma cells were present. In addition, it was found in this study that the methyl green-pyronin reaction was unsatisfactory, despite the presence of many plasma cells.

According to Smith, Wivel & Potter (1970), plasma cells seem to develop from the lymphocytes, a phenomenon which was also found in this study.

Macrophages have been described by some authors (Lewis & Lewis, 1926; Jakowska, 1956; Fänge & Gidholm, 1968). These cells occur in both the catfish and the bream and are distinct from all the other cell types in that their granules stain blue-black with Giemsa, brown with HE, and blue with BB and Schmorl's stain. The blue granules seen with the latter 2 staining methods is a characteristic of macrophages.

Three types of macrophages may be distinguished, viz. the blood macrophages or monocytes, the wandering macrophages or histiocytes and the fixed tissue macrophages. The phagocytic properties of each of the 3 types has been illustrated by supravital staining of blood with brilliant cresyl blue and also by the intra peritoneal injection of black india ink. In addition, the nucleus of the monocyte becomes progressively pycnotic as it develops into the final stage, the fixed tissue-macrophage.

A schematic representation of the development of the lymphocytes, monocytes, the various granulocytes, plasma cells and macrophages as believed to occur in the catfish and the Mozambique bream is illustrated below. Solid lines indicate observed processes, while broken lines indicate postulated processes.



* observed for neutrophilic granulocytes, postulated for eosinophilic and basophilic granulocytes

References

ASHLEY, L. M. & SMITH, E. C., 1963. Advantages of tissue imprints over tissue sections in studies of blood cell formation. *The Progressive Fish Culturist*, 25, 93–96.

BECKER, E., LOVELL, R. M., BIRD, J. W., KELLY, J., SCHILLING, J., SOLOMON, S. & YOUNG, N., 1958. Physiology of marine teleosts. II. Haematologic observations. *Physiological Zoology*, 31, 228–231.

BLOOM, W. & FAWCETT, D. W., 1968. A textbook of histology (9th ed.). Philadelphia, London, Toronto: W. B. Saunders Co.

BLOOM, W. & FAWCETT, D. W., 1975. A textbook of histology. Philadelphia, London, Toronto: W. B. Saunders Co.

BOOMKER, J., 1979. The haemocytology and histology of the haemopoietic organs of South African freshwater fish. I. The haemopoietic organs of *Clarias gariepinus* and *Sarotherodon mossambicus*. *Onderstepoort Journal of Veterinary Research*, 46, 217–222.

BOOMKER, J., 1980. The haemocytology and histology of the haemopoietic organs of South African freshwater fish. II. Erythrocytes and thrombocytes of *Clarias gariepinus* and *Sarotherodon mossambicus*. *Onderstepoort Journal of Veterinary Research*, 47, 95–100.

BOYAR, H. C., 1962. Blood cell types and differential counts in Atlantic herring, *Clupea harengus harengus*. *Copeia* 2, 463–465.

CATTON, W. T., 1951. Blood cell formation in certain teleost fishes. *Blood*, 6, 39–60.

CHILLER, J. M., HODGINS, H. O., CHAMBERS, V. C. & WEISER, R. S., 1969. Antibody response in rainbow trout (*Salmo gairdneri*). I. Immunocompetent cells in the spleen and anterior kidney. *Journal of Immunology*, 102, 1193–1201.

DOMBROWSKI, H., 1953. Untersuchungen über das Blut des Karpfens (*Cyrinus carpio* L.) und einiger anderer Süsswasserfischarten. *Biologische Zentralblatt*, 72, 182–195.

DORLAND, 1968. Illustrated medical dictionary (24th ed.). Philadelphia, London: W.B. Saunders Co.

DOWNEY, H., 1909. The lymphatic tissue of the kidney of *Polyodon spathula*. *Folia Haematologia*, 8, 415–495.

DUTHIE, E. S., 1939. The origin, development and function of the blood cells in certain marine teleosts. Part I. Morphology. *Journal of Anatomy*, 73, 396–411.

ELLIS, A. E., 1976. Leucocytes and related cells in the plaice, *Pleuroncetes platessa*. *Journal of Fish Biology*, 8, 143–156.

EZZAT, A. A., SHABANA, M. B. & FARGHALY, A. M., 1974. Studies on the blood characteristics of *Tilapia zillii* (Gervais). I. Blood cells. *Journal of Fish Biology*, 6, 1–12.

FÄNGE, R. & GIDHOLM, L., 1968. A macrophage system in *Myxine glutinosa* L. *Naturwissenschaften*, 55, 44.
FERGUSON, H. W., 1976. The ultrastructure of plaice (*Pleuronectes platessa*) leucocytes. *Journal of Fish Biology*, 8, 139–142.
FEY, F., 1963. Untersuchungen zur vergleichenden Hämatologie niedrer Wirbeltiere. *Folia Haematologia*, 81, 21–29.
FEY, F., 1966 a. Vergleichende Hämozytologie niedrer Vertebraten. III. Granulozyten. *Folia Haematologia*, 86, 1–20.
FEY, F., 1966 b. Vergleichende Hämozytologie niedrer Vertebraten. IV. Monozyten, Plasmozyten, Lymphozyten. *Folia Haematologia*, 86, 133–147.
FIJAN, N., 1960. Die hämopoietische Funktion der Niere bei einigen Süsswasserfischarten. *Acta Anatomica*, 42, 266–267.
JAKOWSKA, SOPHIE, 1956. Morphologie et nomenclature des cellules du sang des teleosteans. *Revue d'Hematologie*, 11, 519–539.
JORDAN, H. E., 1938. Comparative haematology. *In*: Handbook of haematology, ed. H. DOWNEY, London: Hamish Hamilton Medical Books.
JORDAN, H. E. & SPEIDEL, C. C., 1924. Studies on lymphocytes. II. The origin, function and fate of the lymphocytes in fishes. *Journal of Morphology*, 38, 529–549.
LEWIS, M. R. & LEWIS, W. H., 1926. Transformations of mononuclear blood cells into macrophages, epitheloid cells and giant cells in hanging drop cultures from lower vertebrates. *Embryology, Carnegie Institute, Washington*, 18, 95–120.
LIEB, J. R., SLANE, G. M. & WILBER, C. G., 1953. Haematological studies on Alaskan fish. *Transactions of the American Microscopist Society*, 72, 37–47.
LUCAS, A. M. & JAMROZ, C., 1961. Atlas of avian haematology. Agriculture Monograph 25, U.S. Department of Agriculture.
PEARSE, A. G. E., 1961. Histochemistry, theoretical and applied. 2nd ed., London: J & A Churchill.
PIENAAR, U. DE V., 1962. Haematology of some South African reptiles. Johannesburg: Witwatersrand University Press.
PITOMBEIRA, MARIA DA SILVA & MARTINS, J. M., 1970. Haematology of the Spanish mackerel, *Scomberomorus maculatus*. *Copeia*, 1, 182–186.
REZNIKOFF, P. & REZNIKOFF, D. G., 1934. Haematological studies in dogfish (*Mustelus canis*). I. The normal blood picture and the effect of the removal of blood and of turpentine injections. *Biological Bulletin*, 61, 115–123.
SCHALM, O. W., JAIN, N. C. & CARROLL, E. J., 1975. Veterinary haematology, 3rd ed. Philadelphia: Lea & Febiger.
SMITH, A. M., POTTER, M. & MERCHANT, E. B., 1967. Antibody-forming cells in the pronephros of the teleost *Lepomis macrochirus*. *Journal of Immunology*, 99, 876–882.
SMITH, A. M., WIVEL, N. A. & POTTER, M., 1970. Plasmacytopoiesis in the proncephros of the carp (*Cyprinus carpio*). *Anatomical Record*, 167, 351–370.
STOLK, A., 1957. Oval cells with club-shaped inclusions in the connective tissue of the cichlid *Pterophyllum scalare*. *Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen*, 60, 53–60.
TOPF, W., 1953. Über die Blutbildung und die Blutbildungsstätten beim Karpfen (*Cyprinus carpio* L.). *Zoologische Anzeiger*, 150, 91–104.
WEINREB, EVA L., 1963. Studies on the fine structure of teleost blood cells. I. Peripheral blood. *Anatomical Record*, 147, 219–225.
WERZBERG, A., 1911. Studien zur vergleichenden Hämozytologie einiger poikilothermer Vertebraten. *Blood*, 16, 1307–1312.
WINTROBE, M. M., 1947. Clinical haematology. 2nd ed., Philadelphia: Lea & Febiger.
YOFFEY, J. M., 1929. A contribution to the study of the comparative histology and physiology of the spleen with reference chiefly to its cellular constituents. I. In fishes. *Journal of Anatomy*, 63, 314–344.