Onderstepoort J. vet. Res., 47, 95–100 (1980)

THE HAEMOCYTOLOGY AND HISTOLOGY OF THE HAEMOPOIETIC ORGANS OF SOUTH AFRICAN FRESHWATER FISH. II. ERYTHROCYTES AND THROMBOCYTES OF *CLARIAS GARIEPINUS* AND *SAROTHERODON MOSSAMBICUS**

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ABSTRACT

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 (1980).

This paper describes the light-microscopic appearance of both the erythrocytes and the thrombocytes and their developmental stages of catfish (*Clarias gariepinus*) and Mozambique bream (*Sarotherodon mossambicus*). Apart from some minor differences in the shape and staining affinities of the polychromatophilic erythrocytes, the erythrocytic series is similar in catfish and bream. The thrombocytes and the reactive stages of thrombocytes of both species are similar to those of birds. A possible mechanism of erythro- and thrombopoiesis is postulated.

Résumé

L'HÉMOCYTOLOGIE ET L'HISTOLOGIE DES ORGANES HÉMOPOIÉTIQUES DU POISSONS D'EAU DOUCE SUD-AFRICAINS. II. ERYTHROCYTES ET THROMBOCYTES DU CLARIAS GARIEPINUS *ET DU* SAROTHERODON MOSSAMBICUS

Cet exposé décrit l'apparence microscopique des erythrocytes et des thrombocytes et leurs stades de développement du posson chat (Clarias gariepinus) *et la brème du Mozambique* (Sarotherodon mossambicus).

A part quelques différences mineures dans la forme et les affinités colorantes des erythrocytes polychromatophiliques, la série erythrocytique est similare chez le poisson chat et la brème. Les thrombocytes et les stades réactifs des thrombocytes des deux espèces sont similairies à celles des oiseaux. Un éventuel mécanisme d'erythro- et thrombopoïèse est postulé.

INTRODUCTION

Several authors (Lieb, Slane & Wilber, 1953; Jakowska, 1956; Srivastava, 1968; Ellis, 1976) have referred to the problems encountered with the nomenclature of the blood cells of fish. Ellis (1976) pointed out that the terminology was adopted from mammalian haematology and that it was often based on vague morphological similarities in Romanovsky stained preparations. Jakowska (1956) proposed a system for naming the various blood cells which is based on those cell types that can be recognized with certainty in smears stained with Wright's stain. This system was adopted by Weinreb (1963) and Pitombeira & Martins (1970) and is also used in this study.

The purpose of this paper is to describe and illustrate the erythrocytes and thrombocytes of the catfish (*Clarias gariepinus*) and the Mozambique bream (*Sarotherodon mossambicus*) to augment the present inadequate knowledge of the blood cytology of freshwater fish in the Republic of South Africa.

MATERIALS AND METHODS

Fish were collected and acclimatized as described by Boomker (1979).

Catfish were bled directly from the caudal artery by inserting a sterile hypodermic needle into the mid-ventral line of the caudal peduncle at the level of the posterior margin of the anal fin and directing it dorsally until a drop of blood appeared. Bream were fully anaesthetized before the caudal peduncle was severed.

Blood smears, made according to standard techniques (Wintrobe, 1947), were rapidly air-dried, fixed in absolute acid-free methyl alcohol, and stained with 5% Giemsa's solution, buffered at pH 6,9–7,0, for 45 minutes.

For supravital staining a drop of 0,002% alcoholic methylene blue was allowed to evaporate on a clean glass slide before a small drop of fresh blood was placed on the stain (Ridgeway, 1956). A cover-slip was placed on the droplet and observations were begun immediately.

To study the developing cells, fish were killed and the haemopoietic organs removed. A small piece of each haemopoietic organ was gently macerated in a drop of 0,8% saline (Smith, Potter & Merchant, 1967) and the resulting suspension smeared onto precleaned glass slides, according to standard techniques used for blood. Impression smears were made by bringing a freshly cut part of the organ into contact with a glass slide (Ashley & Smith, 1963). Both organ smears and impression smears were rapidly air-dried, fixed in absolute methyl alcohol and stained with 5% Giemsa's for 45 minutes.

To prevent disruption of the thrombocytes, 5 mℓ of blood was collected in glass tubes containing either 500 units heparin or 0,05 mℓ 15% ethylenediamine-tetra-acetate (EDTA).

Fifty cells of each type from each of 8 catfish and 4 bream were measured with an ocular micrometer in conjunction with an oil immersion lens at 1 000× magnification. The range and mean of the different cells were calculated from these measurements.

RESULTS

The criteria used to differentiate the various cell types were: cell diameter, nucleus diameter, ration of nucleus to cell diameter, shape, structure, colour and staining intensity of the nucleus and cytoplasm. The linear measurements of the various cells and nuclei are listed in Tables 1 and 2.

Haemocytoblasts

These are large cells, with a prominent nucleus and nucleolus. The cytoplasm is contained within an irregular cell membrane. The nucleus, which is vesicular and centrally situated, stains pale blue and contains a blue nucleolus, the position of which varies. The cytoplasm is finely granular and basophilic, and may contain small vacuoles (Fig. 1). The haemocytoblasts are regarded as the stem cells of all the blood cells and probably originate from the RE cells.

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

Received 3 March 1980—Editor

TABLE 1 Linear measurements of the erythrocytic series of cells of *C. gariepinus* and *S. mossambicus*

Cell type	*C. gariepinus*			*S. mossambicus*		
	Cell diameter*	Nucleus diameter*	Ratio of mean cell diameter to mean nucleus diameter	Cell diameter*	Nucleus diameter*	Ratio of mean cell diameter to mean nucleus diameter
Haemocytoblast	13,1 ±1,5	8,75±0,675	1,497	8,1 ±0,9	6,3 ±0,9	1,285
Small lymphoid haemoblast	6,75±0,9	3,6 ±0,9	1,875	5,85±0,45	6,3 ±3,6	—
Erythroblast	10,2 ±1,2	6,75±0,45	1,5	6,2 ±0,45	5,85±0,2	1,05
Polychromatophilic erythrocyte;						
early	9,0 ±0,9	5,4 ±0,9	1,66	6,75±0,55	3,6	1,875
midstage	8,55±1,05	4,75±0,65	1,80	—	—	—
late	8,55±1,35	4,05±0,45	2,11	7,2 ±0,25	2,7	2,666
Immature erythrocyte	8,55±1,35	3,38±0,225	2,52	—	—	—
Mature 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	—

* Mean ±standard deviation, expressed as μm

TABLE 2 Measurements of the thrombocytes of *C. gariepinus* and *S. mossambicus*

	C. gariepinus		*S. mossambicus*	
	Length*	Width*	Length*	Width*
Cell	5,85±0,45	3,6±0,9	5,4±0,45	4,05±0,5
Nucleus	4,95±0,45	2,7±0,9	4,05±0,45	2,7±0,9

* Mean ±standard deviation, expressed as μm

Small lymphoid haemoblasts

These cells are similar to the haemocytoblasts in shape and structure, but are considerably smaller and have a smaller nucleus (Fig. 2 & 3). They may be distinguished from the lymphocytes in that they have a vesicular nucleus with a nucleolus and a cytoplasm which stains blue and does not contain azurophil granules.

Erythroblasts

These cells are not often encountered in blood smears of either species. The erythroblast that is sometimes present is the same size as or slightly larger than the early polychromatophilic erythrocyte. The cytoplasm is finely granular and is intensely basophilic. A distinct perinuclear halo is usually present. The nucleus is round and vesicular and contains a fine chromatin network which stains dark purple. Nucleoli are not visible. The erythroblasts of bream are morphologically identical with those of catfish (Fig. 11).

Polychromatophilic erythrocytes

Three stages of polychromatophilic erythrocytes are recognizable in catfish, namely, the early, middle and late stages. These stages are differentiated by their increasing eosinophilia and progressive pycnosis.

Early stage polychromatophilic erythrocytes have the shape of the mature erythrocytes, but are larger. The cytoplasm stains blue and is very finely granular. The nucleus is large in comparison with that of the middle and late stages, is round and vesicular, with the chromatin radially arranged (Fig. 4).

Middle stage polychromatophilic erythrocytes are slightly smaller than those in the previous stage, and the cytoplasm stains greyish-blue. The nucleus is smaller and the chromatin more compact than that of the previous stage (Fig. 5a).

Late stage polychromatophilic erythrocytes are slightly larger than the immature erythrocytes, though they may be of equal diameter. They are round or slightly oval in shape. The cytoplasm is homogeneous and stains lightly eosinophilic. The nucleus is slightly more vesicular than that of the immature erythrocyte (Fig. 6).

In bream, early and late stage polychromatophilic erythrocytes, with a large range of intermediate forms, can be discerned. The early stage polychromatophilic erythrocytes are morphologically identical with those of catfish, but are slightly smaller. The cytoplasm of late stage polychromatophilic erythrocytes stains slightly more basophilic than that of the immature erythrocytes. The nucleus is larger and more vesicular than that of the immature erythrocytes and already shows a degree of polymorphism.

The intermediate stages show the whole range of sizes and colour changes of cytoplasm and nucleus that can be expected between the early and late stage polychromatophilic erythrocytes. Some of the polychromatophilic series of erythrocytes were occasionally seen in various stages of mitosis.

Immature erythrocytes

These cells are characterized by a nucleus which is slightly larger and more vesicular than that of the mature erythrocytes. In catfish these cells are round, with a round nucleus (Fig. 5b), whereas in bream they are oval with a polymorphic nucleus. The cytoplasm has the same staining intensity as the mature erythrocytes.

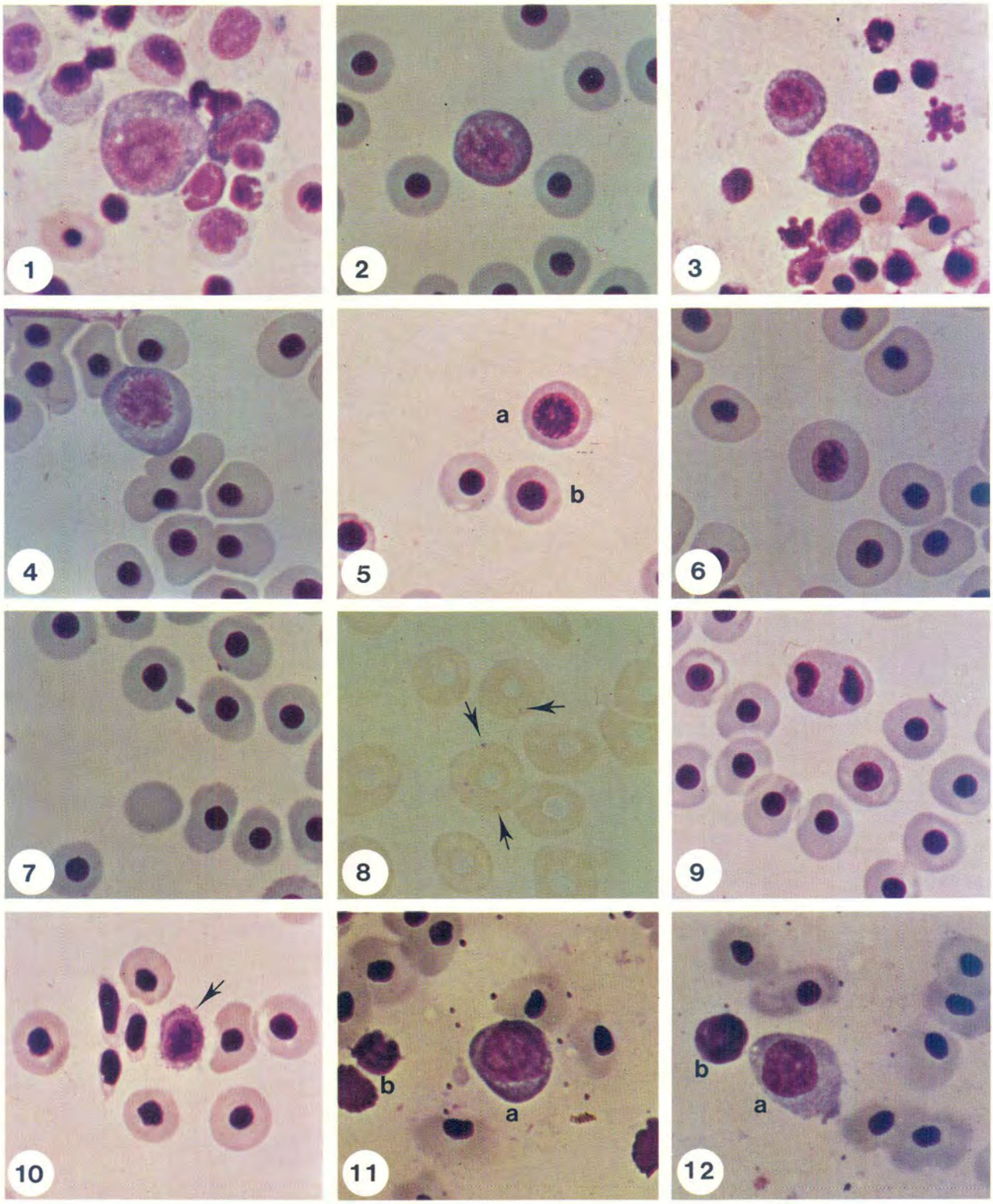


FIG. 1 A haemocytoblast in an impression smear of the mesonephros of catfish; Giemsa, ×1200

FIG. 2 A small lymphoid haemoblast in circulating blood of catfish; Giemsa, ×1 200

FIG. 3 Two small lymphoid haemoblasts in an impression smear of the mesonephros of catfish; Giemsa, ×1 200

FIG. 4 Transitional form between erythroblast and early polychromatophilic erythrocyte; circulating blood of catfish; Giemsa, ×1 200

FIG. 5 (a) Midstage polychromatophilic erythrocyte and (b) immature erythrocyte in circulating blood of catfish. The third cell is a mature erythrocyte; Giemsa, ×1 200

FIG. 6 Late stage polychromatophilic erythrocyte in circulating blood of catfish; Giemsa, ×1 200

FIG. 7 An erythroplastid in blood of catfish; Giemsa, ×1 200

FIG. 8 Erythrocytes of catfish. Arrows indicate small granular remnants of the reticulum; supravital staining, brilliant cresyl blue, ×1 200

FIG. 9 The rare occurrence of mitosis in a mature erythrocyte in blood of catfish; Giemsa, ×1 200

FIG. 10 Thrombocytes in circulating blood of catfish. The elongated cells are in a reactive stage, whereas the cell indicated by the arrow is a pre-reactive stage; Giemsa, ×1 200

FIG. 11 An erythroblast in circulating blood of bream; Giemsa, ×1 200

FIG. 12 Intermediate form of polychromatophilic erythrocyte (a) and small lymphocytes (b) in circulating blood of bream; Giemsa, ×1 200

Mature erythrocytes

The mature erythrocyte of catfish is round to slightly oval and biconvex. The nucleus is smaller than that of immature erythrocytes, round, without a distinct nucleolus, and is situated centrally or slightly eccentrically (Fig. 2–10). With Giemsa's staining, the nucleus is dark purple, and the cytoplasm is pale pink and homogeneous.

The erythrocytes in bream are oval, with a nucleus that may be round, oval or lobed (Fig. 11, 12). The nucleus stains dark purple, while the cytoplasm, which is homogeneous, stains pale pink. No nucleolus is visible.

Even after prolonged supravital staining with both methylene blue and brilliant cresyl blue, no basophilic reticular material could be demonstrated in any of the erythrocytic cells of either species. A number of small blue, round to oval granules were seen, however, in the mature erythrocytes of catfish (Fig. 8).

Abnormal erythrocytes

In both species the erythroplastids are seen as small round to oval eosinophilic masses (Fig. 7). They have the same staining affinities as mature erythrocytes, but lack a nucleus. They are also found in birds (Lucas & Jamroz, 1961) (Fig. 7).

Artefacts, such as distortions, rupture of cells and/or nuclei, were often encountered. Occasionally, chromophobic areas were seen in nuclei or cytoplasm. Haemolyzed cells occur as amorphous purple masses.

Thrombocytes

Normal thrombocytes of catfish vary from round to elongated, and the most commonly encountered shape is oval with an elongated end (Fig. 10). The latter are known as fuse cells (Jordan, 1938) or spindle cells (Lucas & Jamroz, 1961).

The thrombocytes in the blood smears of catfish occur in groups of 2–25. The cytoplasm stains pale pink to pink, is finely granular, and sometimes contains small vacuoles. Most thrombocytes contain a small azurophilic granule, that is, the so-called specific granule (Lucas & Jamroz, 1961; Lewis & Shirakawa, 1962). An eosinophilic spherical structure which stains positive with PAS and is composed of several small granules is sometimes seen. The thrombocyte nucleus is large in comparison with the erythrocyte nucleus. Although mostly oval in shape, bilobed thrombocyte nuclei, in which the 2 halves of the nucleus are connected by a wide bridge, also occur.

Thrombocytes of bream closely resemble those of catfish, except that a more polimorphic nucleus is present in those of bream.

Reactive stages of thrombocytes

Reactive stages occur when clotting of blood takes place.

In the earliest reactive stage, the cell membrane becomes eosinophilic and is clearly visible. The cytoplasm becomes vacuolated and the specific granule becomes distinct. The nucleus rounds off, becomes pycnotic and approximates the size of an erythrocyte nucleus. In the later stages, the cytoplasm becomes progressively smaller, the specific granule gradually disappears and the nucleus becomes very pycnotic (Fig. 10). The final stages are very small; the nuclei stain intensely and are surrounded by fine radiating fibrin threads.

The reactive stages of thrombocytes of catfish and bream are similar to and comparable with those seen in birds (Lucas & Jamroz, 1961).

Discussion

The terminology used by various authors for the stem cell, erythrocytic and thrombocytic series is specified in Table 3. Although not as confusing as that of the leucocytes, the terminology for the erythrocytes and thrombocytes still presents some problems.

Various theories have been formulated regarding the origin of the erythrocytic and thrombocytic series.

Downey (1909), for example, found large mononuclear cells in the intertubular haemopoietic tissue of Polyodon spathula, which he regarded as being the stem cells.

Jordan & Speidel (1924) postulate that the reticuloendothelial (RE) cells give rise to the large lymphocytes, which in turn form the small lymphocytes. The small lymphocytes are the omnipotent cells which give rise to the haemoblasts and thrombocytoblasts.

Yoffey (1929) found both small and large round cells in the spleens of some salt-water fish, and suggested that the small round cells give rise to the erythrocytic series.

Duthie (1939) found both small and large lymphocytes in the haemopoietic organs of several fishes. The small lymphocytes, also known as the small lymphoid haemoblasts, give rise to the blood lymphocytes, thrombocytes and erythrocytes (Duthie, 1939).

Catton (1951) bases his view that the large lymphocytes (large lymphoid haemoblasts) are the precursors of all blood cells on the observations that—

the nucleus of the large lymphoid haemoblast is similar to that of the precursors, the RE cells;

there are intermediate stages between RE cells and large lymphoid haemoblasts;

the large lymphoid haemoblasts have a high mitotic index, and that the large lymphoid haemoblasts are similar to the haemocytoblasts of mammals.

Jakowska (1956) agrees with Catton (1951), but also considers the small lymphoid haemoblast described by Jordan & Speidel (1924) to be a heamocytoblast.

It is evident from Table 3 that the nomenclature adopted by Fijan (1960) is confusing and, as no similarities could be found in this study, his nomenclature is not accepted.

The development of the erythrocytic series of blood cells has been thoroughly studied, and the general opinion is that the large lymphoid haemoblast (haemocytoblast) develops from the RE cells (Jordan & Speidel, 1924; Dawson, 1933; Duthie, 1939; Catton, 1951; Jakowska, 1956). The development into erythroblasts and thrombocytes has been described by the same authors in almost the same detail.

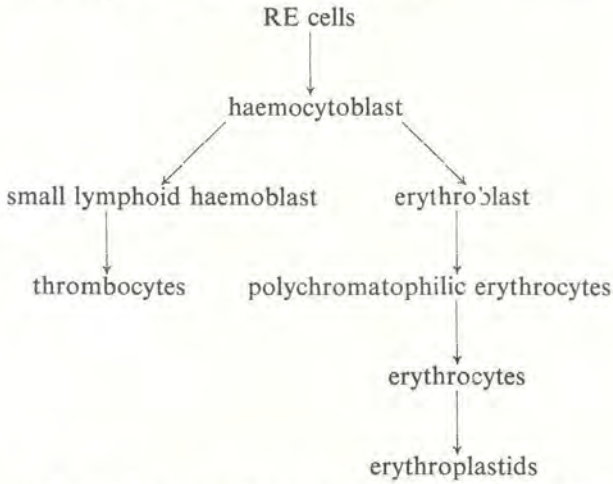
In this study the term "polychromatophilic" erythrocyte is preferred, as it is adopted from avian haematology. The development of the erythrocytic series in birds is similar to that of fish, and the erythrocytes are nucleated in both groups of animals.

TABLE 3 Terminology of the stem cell, erythrocytic and thrombocytic series of blood cells, as used by various authors

Author	Stem cell series	Erythrocytic series	Thrombocytic series
Werzberg, 1911	—	Megalocyte Normoblast Normocyte Erythrocyte	Spindle cell
Jordan & Speidel, 1924	Lymphocyte	Erythrocyte	Spindle cell
Yoffey, 1929	—	Small round cell Immature erythrocyte Erythrocyte	—
Duthie, 1939	Lymphoid haemoblast	Erythroblast Reticulocyte Erythrocyte	Thrombocyte
Catton, 1951	Large lymphoid haemoblast Small lymphoid haemoblast	Pro-erythroblast Erythroblast Reticulocyte Erythrocyte	Thrombocyte
Dombrowski, 1953	—	Erythroblast Erythrocyte	Thrombocytoblast Thrombocyte
Lieb *et al.*, 1953	Large lymphoid haemoblast Small lymphoid haemoblast	Erythroblast Pro-erythrocyte Erythrocyte Degenerated erythrocyte	—
Jakowska, 1956	Large haemoblast Small haemoblast	Erythroblast Erythrocyte Erythroplastid	Thrombocyte
Fijan, 1960	Large lymphoid reticulum cell	Pro-normoblast Basophilic normoblast Polychromatophilic normablasts I and II Erythrocyte Overripe erythrocyte	Thromboblast Thrombocyte
Boyar, 1962	—	Immature erythrocyte Mature erythrocyte Ghost cell	Spindle cell
Weinreb, 1963	—	Erythrocyte	Thrombocyte
Fey, 1966 (a) & (b)	—	Erythrocyte	Thrombocyte
Pitombeira & Martins, 1970	—	Erythrocyte	Thrombocyte
Ezzat *et al.*, 1974	—	Erythrocyte	—
Ellis, 1976	—	Pro-erythroblast Erythroblast Late erythroblast Pro-erythrocyte Young erythrocyte Mature erythrocyte	Thrombocyte
Boomker, 1980	Haemocytoblast Small lymphoid haemoblast	Erythroblast Polychromatophilic erythrocytes Immature erythrocyte Mature erythrocyte Erythroplastid	Thrombocyte

Dombrowski (1953) is of the opinion that thrombocytes develop from thromboblasts. Fey (1963), however, proved, that the thromboblasts described by Dombrowski (1953) were in fact basophils. In this study, no thromboblasts could be identified, and it was found that thrombocytes develop from the small lymphoid haemoblasts. Electron microscopy showed the similarity between thrombocytes and lymphocytes, and it is thought that the thrombocyte is a small lymphocyte that specialized to fulfil a specific function.

The observations reported in this paper suggest the following schematic representation of erythro- and thrombopoiesis in catfish and bream.



From Tables 1 and 2 it can be seen that the sudden decrease in the size of the developing erythrocyte between the haemocytoblast and erythroblast stages is due to the mitotic division of the haemocytoblast into 2 smaller erythroblasts. Mitotic division does not occur again and the formation of the erythrocyte is a maturation process. Only in exceptional cases is mitosis seen in mature erythrocytes (Fig. 9).

The slight increase in the size of the erythrocyte in bream is ascribed to an increase in cytoplasm, a process also described by Catton (1951) in some of the fishes he studied.

In catfish there is a slight decrease in cell size between the immature and mature erythrocytes which probably has to do with the efficiency of gaseous exchange during respiration. A small erythrocyte seems to be more effective in gaseous exchange, as described by Hartman & Lessler (1964). Srivastava & Griffith (1974) found that the erythrocytes of *Fundulus* species, which occurred in salt or brackish water, were smaller than those of fish which occurred in fresh water.

Mature erythrocytes are usually elliptical in shape (Jordan, 1938; Prosser, 1961). Srivastava (1968), however, found that round or slightly oval erythrocytes occurred more frequently in *Clarias batrachus* than the elliptical forms. In this study it was found that round forms are dominant in catfish, whereas elliptical forms were dominant in bream. In addition, the erythrocyte nuclei of catfish are invariably round, whereas those of bream are polymorphic.

According to Wilkins & Clarke (1974), the shape of the erythrocytes of *Clupea harengus* is inversely proportional to the size of the fish, i.e. the larger the fish, the more oval the erythrocytes. Such a relationship has not been found in the 2 species studied in this case.

The colour changes of the erythrocyte cytoplasm during its development are ascribed to the increasing amounts of haemoglobin which replaces the basophilic staining free ribosomes. The more haemoglobin an erythrocyte contains, the more eosinophilic it will stain.

With supravital staining, Ridgeway (1965) found small basophilic granules in the erythrocytes of *Oncorynchus nerka, O. tshawytcha* and *Salmo gairdneri*. Watson, Günther & Royce (1956) found the same granules, and named them mitochondria. In this study the granules were also found in both species examined, and as there is no evidence that mitochandria will absorb a stain, these granules probably represent remnants of the reticulum.

The shape of the thrombocytes in the different species is more or less constant. Lewis & Shirakawa (1962), describing the thrombocytes of some lower vertebrates, found that the cells had a high glycogen content. They also described the clotting of blood and the changes in thrombocytes associated with clotting. The results of this study indicate that the thrombocytes of catfish and bream are morphologically similar and, during clotting, behave in a way similar as that of other fish (Jordan, 1938; Boyar, 1962; Lewis & Shirakawa, 1962; Shepro, Belamarich & Branson, 1966) and birds (Lucas & Jamroz, 1961).

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Printed by and obtainable from the Government Printer, Private Bag X85, Pretoria, 0001