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STUDIES ON THE ORIGIN, DEVELOPMENT AND FATE OF BLOOD CELLS IN THE TELEOST, CLARIAS LAZERA

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ABSTRACT

Haemopoiesis in Clarias lazera was studied on physiological and cytochemical basis. It was found to be monophyletic since blood cells develop from a common stem cell; the haemocytoblast in the lymphomyeloid tissue of haemopoietic organs. Senile blood cells were also noticed to disintigrate in the circulation and haemopoietic organs.

INTRODUCTION

Many investigators postulated different schemes for the origin and site of stem cells responsible for the formation of blood cells in poikilothermic animals (Jordan and Speidel, 1924; Duthie, 1939; Catton, 1951; Yokoyama, 1960; Watson et al., 1963; Weinreb and Weinreb, 1969; Gardner and Yevich, 1969; Ellis, 1976 & 1977; Mahajan and Dheer, 1979; Cannon et al., 1980; Barber et al., 1981; Hoole and Arme, 1982; El-Feky, 1982; Bergeron and Woodward, 1983; Hightower et al., 1984; Scott et al., 1985; Miller et al., 1986 and Roubal, 1986). However, studies on the haemopoiesis of subtropical fish are very rare and in particular on Egyptian fish species. The aim of this work is to carry out a study on the origin, development and fate of blood cells in the Egyptian catfish, Clarias lazera.

MATERIAL AND METHODS

Fish were collected alive from the unpolluted area of Bab-El-Abid in Lake Mariut, near Alexandria. They were kept for 48 hrs in suitable continuously aerated tanks before examination.

Twelve healthy fishes were examined monthly. Prior to investigation, each fish was measured and weighed. Their lengths ranged between 15-40 cm and weighed between 30-250 gm. Blood smears were made, air dried and fixed in methyl alcohol for 5 minutes and stained by Giemsa, Wright's or panoptic methods. Thereafter, fish were dissected and haemopoietic organs (head kidney, liver and spleen) were removed, cut and applied to clean slides to make tissue imprints. They were fixed in methyl alcohol for 5 minutes, formalin vapour or in a solution of 10 ml formalin and 90 ml methanol, to study periodic Acid Schiff (PAS), Sudan Black B and peroxides reactions respectively (MayHoe et al., 1960 and Pearse, 1972). Sections of the head kidney were made after fixation in 10% neutral formalin and stained using eosin-haematoxylin.

RESULTS

Sections and imprints of the head kidney (Figs. 1,2,5,6,12,13 & 14) showed the presence of stem cells and several developmental stages. The blood smears also showed the presence of some developmental stages in addition to the mature blood cells (Figs. 3,4,7,8,9,10,11,15,16,17,18,19 & 20).

From the study of sections, imprints and smears, it appears that the haemocytoblast in Clarias lazera is the stem cell that arises from a primitive reticular cell which hypertrophies and later separates from the reticular syncytium. The reticular cell (RC) can be seen in areas of blood forming tissue between the uriniferous tubules (Ut, Fig. 1). The haemocytoblasts are formed extravasculary in the stromal areas not within the venous sinusoids. In kidney imrpint preparations (Fig. 2), the outline of the haemocytoblast appears either oval, spherical or irregular. Each cell contains a moderate amount of cytoplasm and a large, centrally located nucleus with one or two nucleoli. The size of the haemocytoblasts varies. It appears that some of the large haemocytoblasts (LHcb) proceed in development towards the granulocytes series, while others form the precursors of erythrocytes series. The medium-sized haemocytoblasts (MHcb) divide to form the small cells (SHcb) which develop into lymphocytes and thrombocytes.

Erythrocytes

The developmental stages of the erythrocyte series are as follows:

a) Pronormoblast

This stage can be recognized in blood smears (Fig. 3, PrN) and kidney imprints (Fig. 5). The large nucleus is still present with thickening of some of the chromatin threads, but no haemoglobin is yet evident.

b) Basophilic Normblast

This stage is often seen in kidney imprints (Fig. 6) and rarely in blood smears (Figs. 3 & 4, BN). It is characterized by homogenous basophilic cytoplasm and a concentric nucleus in which the chromatin forms large clumps. It is smaller in size than that of the pronormoblast, and the nucleus is still large in proportion to the cytostome.

c) Polychromatophil Normblast

As development proceeds haemoglobin appears in the cytoplasm of the erythroblast cell which looses its basophilia. In blood smears and imprints of haemopoietic organs, the cytoplasm may have lighter areas and opaque ones (Fig. 4 PN), and hence the name polychromatophil normoblast. The cell is often rounded and the nucleus is spherical and concentric.


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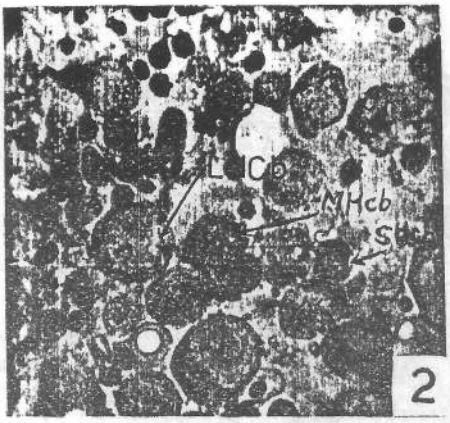
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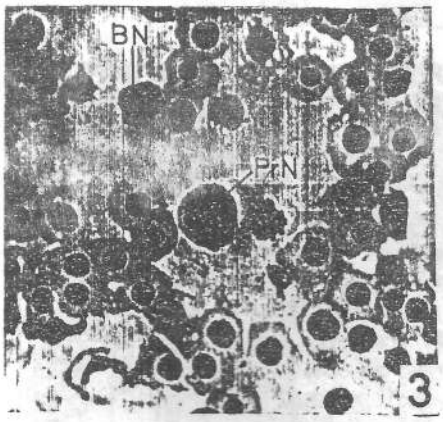
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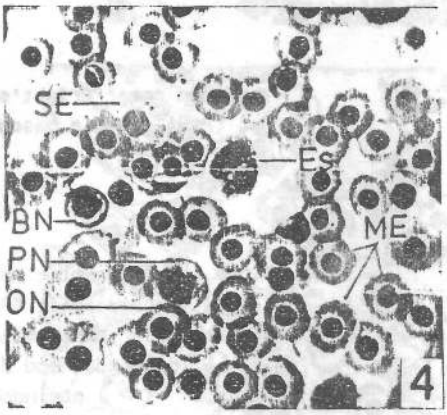
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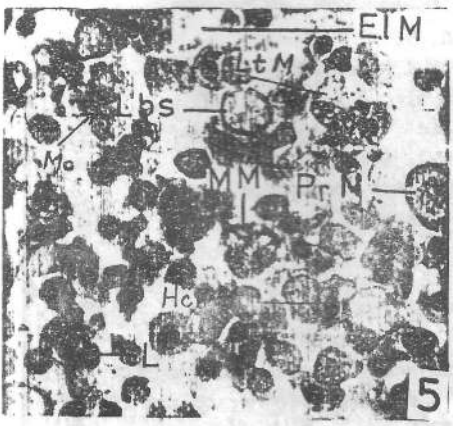
EIM
LtM
Lbs
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MM
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Hc
L
5

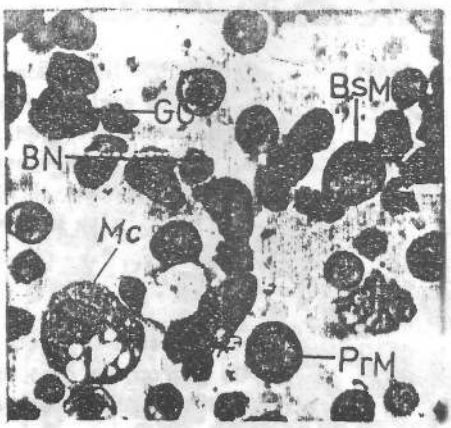

BsM
G
BN
Mc
PrM

FIG. 1.
Formalin -eosin haematoxylin. L.S. in the head kidney showing reticular cells (RC) between renal tubules (UT) and a group of developing cells (DC). X 1250.

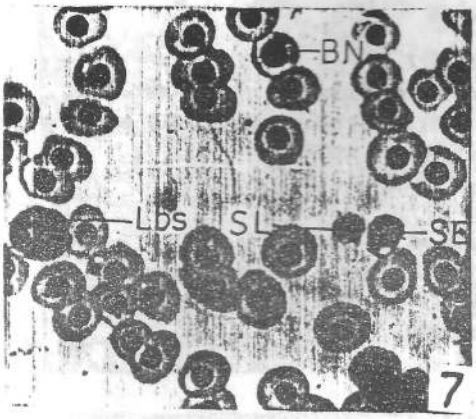
FIG. 2.
Kidney imprints showing large haemocytoblast (LHcb) medium haemocytoblast (MHeb and small hawmocytoblast (SHcb). X 1250.

FIG. 3.
Blood smear-Wright's stain showing a pronormoblast (PrN) and a basophilic normoblast (BN), X 1250

FIG. 4.
Blood smear-Panoptic method showing basophilic normoblast (BN), Polychromatophil (PN), orthrochromatophil normoblast (ON), mature erythrocytes (ME) and large senile erythrocyte distended and deformed cytoblasm (SE), eosinophil (Es). X 1250.

FIG. 5.
Kidney imprints-Sudan Black B, showing + ve granules in both early and late myelocytes (ElM) (LtM) and metamylocytes (MM), while -ve in haemocytoblast (Hcb), lymphoblast (Lbs), lymphocyte (L) and pronormoblast (PrN). Notice a macrophage (Mc). with weak + ve reaction. X 1250.

FIG. 6.
Kidney imprints-Wright's stain showing basophilic myelocyte (BsM), macrophage (Mc) with several vacuoles and promylocyte (PrM). Notice Ghost cell (GC) and basophilic normoblast (BN). X 1250.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SE
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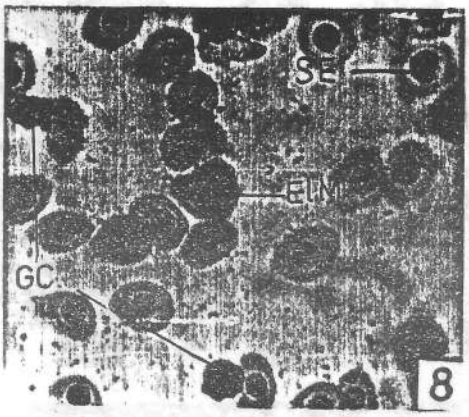
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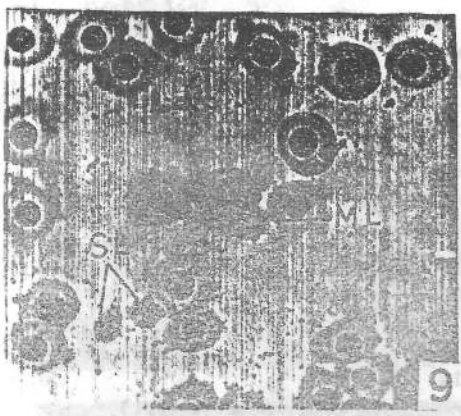
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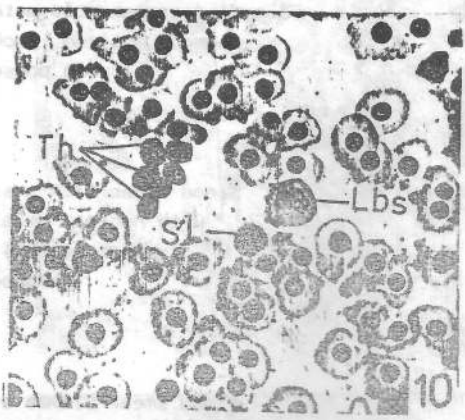
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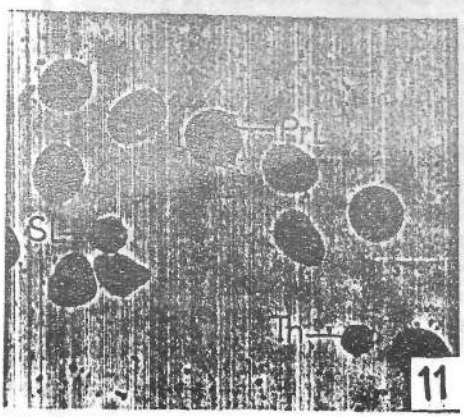
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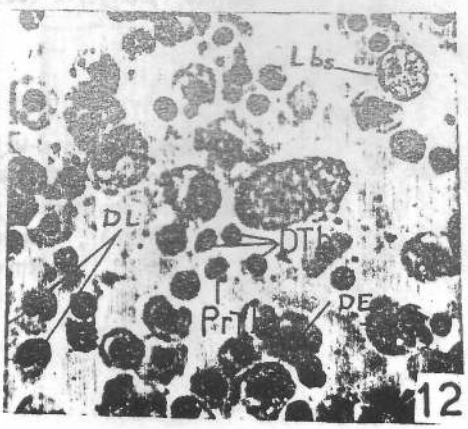

Lbs
DL
DTh
PrL
PE
12

FIG. 7.
Blood smear-Giemsa stain showing a basophilic normoblast (BN), small senile erythrocyte (SE), small lymphocyte (SL) and lymphoblast (Lbs). X1250.

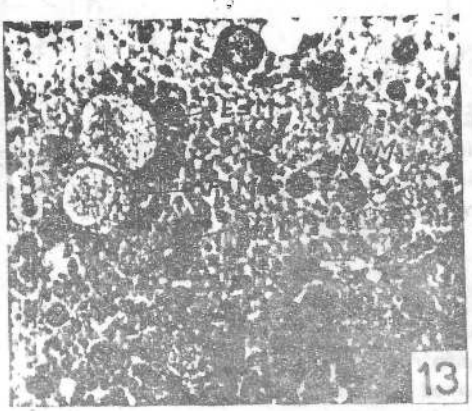
FIG. 8.
Blood smear-Giemsa stain showing poikilocytosis, senile rythrocyte (SE), ghost cells (GC) and early myelocyte (ElM). X 1250.

FIG. 9.
Blood smear-panoptic method showing 2 small lymphocytes (SL), a medium sized lymphocyte (ML). Notice that all the cells posses pseudopodia. X 1250.

FIG. 10.
Blood smear-Giemsa stain showing a lymphoblast (Lbs) with nuclear details and pseudopodia, small old lymphocyte (SL) and a cluster of spherical thromboctye (Th). X 1250.

FIG. 11.
Blood smear-Giemsa stain showing prolymphoctye (PrL), senile lymphocyte (SL) and spherical thrombocyte (Th). X 1250.

FIG. 12.
Kidney imprints-Wright's stain showing developing lymphocytes (DL), developing thrombocytes (DTh). Notice a prothrombocyte (with kidney- shaped nucleus) (Prth) , lymphoblast (lbs) and developing erythrocytes (DE). X 1250.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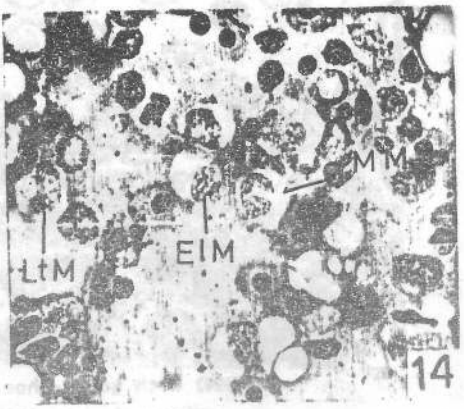
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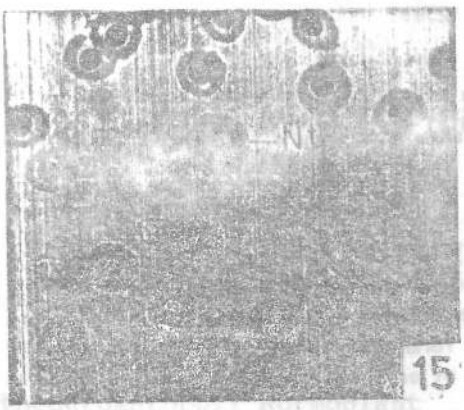
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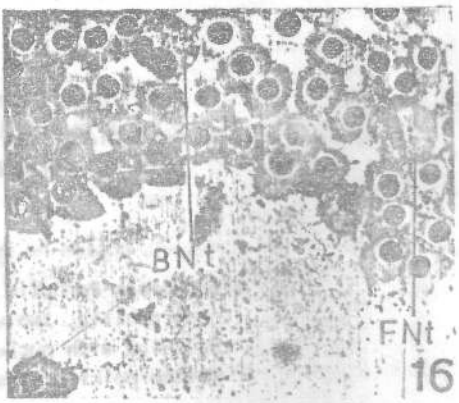
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FNt
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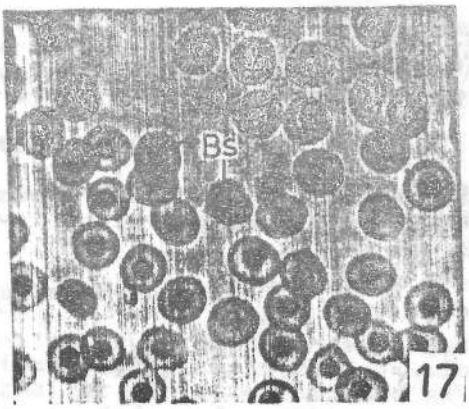
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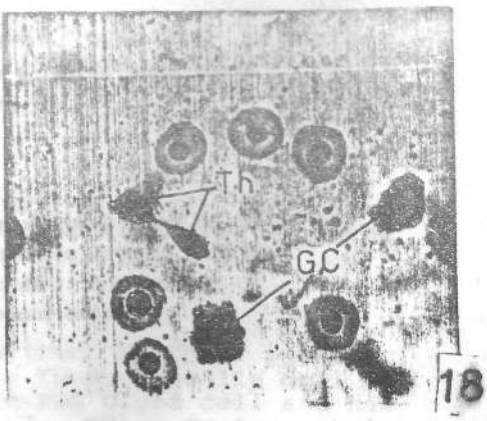

Th
GC
18

FIG. 13.
Kidney imprints-Panoptic method, a neutrophilic myelocyte (NtM), an eosinophilic myelocyte (EsM), promyelocyte (PrM) and haemocytoblast (Hcd). X 1250.

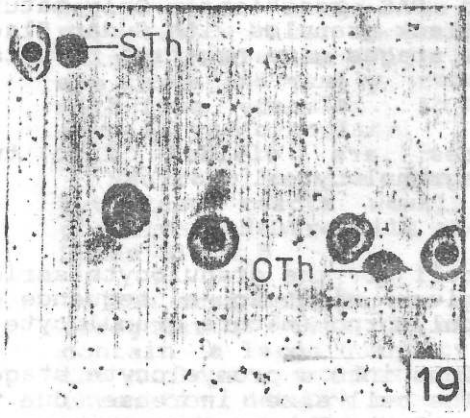
FIG. 14.
Kidney imprints-Wright's stain showing metamyelocytes (MM) with horse-shoe shaped nucleus (stab cell), early myelocyte (ElM) and late myelocyte (LtM). X 1250.

FIG. 15.
Blood smear-Giemsa stain showing a mature filamented neutrophil (Nt). X 1250.

FIG. 16.
Blood smear-Panoptic method showing three mature neutrophilis, two with bilobed nuclei (BNt), the third with nucleus formed of 4 lobes (FNt). Notice poikilocytosis of erythrocyte. X 1250.

FIG. 17.
Blood smear-Wright's stain showing a basophil (Bs). X 1250.

FIG.18.
Blood smear-Panoptic method showing 2 thrombocytes sending several pseudopodia, one of these pseudopodia is long and links the two thrombocytes (Th). Notice ghost cells (GC). X 1250.



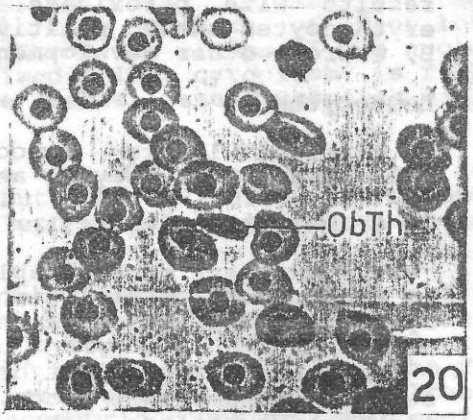


FIG.19.
Blood smear-Panoptic method showing a spherical and an oval thrombocytes (STh) & (OTh). X 1250.

FIG.20.
Blood smear-Panoptic method showing an oblong-shaped thrpmbocyte. (ObTh). X 1250.

d) Orthochromatophil Normoblast

In this stage, the cytoplasm has acquired a further amount of haemoglopin which accounts for its acidophilic or orthochromatic reaction (Fig. 4, ON) but the nuleus is still round with further condensation of chromatin.

e) Mature Erythrocytes

Normal mature erythrocytes contain a centrally located biconvex nucleus and are often round, rarely oval in shape (Fig. 4, ME).

F) Senile Erythrocyte

Senile erythrocytes are characterized by their pycnotic nuclei and cytoplasmic condensation. Thus, a senile cell is much smaller in size than the mature red blood cell and has always a deformed outline (Fig. 7, SE). During their formation, erythrocytes increase in size, stain lightly, loose their normal appearance and show patchy areas which are torn away (Figs. 4 & 8, SE)

It was noticed that all above stages gave negative results with peroxidase and PAS reactions. Only mature erythrocytes showed positive black granules with Sudan Black B, whereas other developmental stages gave negative results.

Leucocytes

Clarias lazera leucocytes are divided into two categories; granulocytes and agranulocytes.

I. Granulocytic leucocytes

The earliest recognizable cell of the granulocyte series is the promyelocyte which gives rise to a sequence of myelocyte, metamyelocyte and polymorph (mature granulocyte).

When a haemocytoblast develops into a promyelocyte stage, several changes take place. The cell size increases due to increased cytoplasm (Fig. 13, PrM). The nucleus assumes an eccentric position, and nucleoli disappear.

In kidney imprints (Fig. 13), the cytoplasm of promyelocyte is pierced in some regions by acidophilic areas which first become evident close to the nucleus and gradually spread irregularly to the periphery of the cell. Thereafter, acidophilic areas enlarge and finally affect the basophilic cytoplasm. Cytochemically the promyelocyte is strongly positive to PAS, Peroxidase and Sudan Black B.

Myelocyte is most abundantly found in the circulating blood. The cytoplasm has lost most of its basophilic nature and possesses a fine spongy appearance. The nucleus contains a coarse network of chromatin, sometimes with heavier clumps. In early myelocyte, the nucleus is oval in shape (Figs. 5 & 14, ElM), while in the late myelocyte, it becomes indented (Figs. 5 & 14, ElM). According to the affinity of the cytoplasmic granules of myelocytes to various days, there are neutrophilic, eosinophilic and basophilic myelocytes. The neutrophilic and eosinophilic myelocytes appear to have similar sizes (Fig.13), where as the basophilic myelocyte is always of a much smaller size (Fig. 6). Myelocytes give positive reactions with PAS and strongly positive reactions with Sudan Black B. With peroxidase reaction, both neutrophilic and eosinophilic myelocytes show positive results, while basophilic myelocyte give a negative one.

In metamyelocyte the nucleus becomes indented and finally attains a horse-shoe shaped (Figs. 5 and 14). The metamyelocytes are of similar size to the mature forms. No nucleoli were observed in their nuclei. All types of metamyelocytes give positive reactions with all cytochemical tests applied, except for the basophilic metamyelocyte which gives negative result with peroxidase reagent.

The nucleus in the neutrophilic metamyelocyte consists of two oval parts joined by a broad band. Later, the two lobes become connected by a thin filament of chromatin (Fig. 15,

Nt). This stage is called the filamented stage. Polymorphonuclear neutrophil with nucleus of four lobes exists in Clarias (Fig. 16). Chromatin clumps of the nucleus are large and easily recognized. The cytoplasm is filled with fine granules. In some cells, small vacuoles may be seen in the cytoplasm.

Neutrophils are positive to PAS reagent. The cell cytoplasm reacts weakly, but the granules are strongly positive. Neutrophils are also positive to peroxidase and Sudan Black B.

The eosinophilis are smaller than the neutrophils and are irregular or oval in shape (Fig. 4 Fs). The mature cells contain a large quantity of cytoplasm. The development of the nucleus resembles that of the neutrophil with the fine chromatin network becoming progressively coarser. Although most cells contain a single eccentric round or oval nucleus (Fig. 4), they sometimes may have a bilobed one. The cytoplasm appears to be acidophilic due to the accumulation of the coarse eosinophilic granules. Mature eosinophilis give positive reactions to all the cytochemical tests applied.

Mature basophils are the smallest and rarest cells in the blood of Clarias. They may be irregular or oval in shape. The basophilic cytoplasm is filled with scattered highly refractile granules (Fig. 17). The basophilic granules are larger than the eosinophilic ones. Certain basophils may contain from 8 to 10 large granules (Fig. 17 Bs), but the majority contains even more than these numbers. The nucleus may be round or oval in shape and stains blue to purple and eccentrically located in the cytoplasm. No polymorphonuclear basophils is observed in the blood of Clarias. Basophils give positive results with both Sudan Blake B and PAS but negative reaction with peroxidase.

When the cells of granulocytic series reach the end of their physiological activity, some disintegrate in the blood circulation as seen in blood smears (Figs. 8 & 18). Others degenerate in the lymphomyeloid tissue of the haemopoietic organs, especially those of the spleen and the kidney (Fig. 6, GC). They appear as dark stained masses of irregular shapes. The term ghost cells is given to these degenerating granulocytes.

II. Agranulocytic leucocytes

The agranulocytic type of Clarias leucocytes includes the lymphocytes which are the prevailing white cells in circulating blood and the macrophage, whose presence is restricted only to the lymphoid tissue of the haemopoietic organs. In tissue smears, macrophages are often seen containing several vacuoles in their cytoplasm (Fig. 6 Mc).

Lymphooytes in Clarias are derived from prolymphocytes (Fig. 11, PrL) whose mother cell is the Lymphoblast, which in turn originates from the stem cell, the haemocytoblast.

The lymphoblasts (Lbs), occur mainly in the haemopoietic tissue, sometimes in the peripheral circulation (Fig. 10). and possess nuclei made up of coarse reticular chromatin network with the one or more rounded nucleoli. Some nuclei are deeply indented (Fig. 12, DL)). Senile lymphocytes (Fig. 10, SL) are characterized by the disappearance of normal nuclear details.

The lymphocytes in the circulating blood of Clarias lazera are predominatly small (Figs. 7 & 9, SL) and occassionlly of medium size (Fig. 9, ML). The nucleus with its clumped chromatin occupies the entire volume of the cell, and is surrounded by thin cytoplasm. The cytostome may be rounded or have an amaeboid shape due to numerous pseudopodia (Figs. 9, 10 & 11). Both lymphoblasts and lymphocytes are weakly positive to the PAS reagent and are negative to both peroxidase and Sudan Black B reactions.

Macrophages are giant cells with a large amount of cytoplasm, a loosely reticular, often of vacuolated (Fig. 6 Mc). It was noticed that the nucleus of the macrophage resembles that of the primitive reticular cell of the lymphoid tissue in the haemopoietic organs. However, it is larger and contains more chromatin granules and two prominent nucleoli.

Cytochemically, macrophages give weakly positive reaction with Sudan Black B (Fig. 5) and negative reactions for both Peroxidase and Periodic Acid Schiff.

Thrombocytes

In the peripheral blood of Clarias lazera, thrombocytes occur in various stages of development. These thrombocytes are represented by prothrombocytes, various intermediate forms and mature thrombocytes. In blood smears the thrombocytes appear either round or oval (Figs. 10 & 11, Th) and contain an oval nucleus and a small amount of cytoplasm.

Thrombocytes are derived from their mother cell, the thrombocyte, which in turn comes from the haemopoietic stem cell; the haemocytoblast, frequently seen in the lymphomyeloid tissue of haemopoietic organs (Fig. 12 PrTh). The size of prothrombocyte is relatively smaller than those of small lymphocytes. The nucleus is kidney-shaped with fine reticular network of chromatin, and has one or more nucleoli. However, the amount of cytoplasm is considerably large than of the thrombocyte, and is moderately basophilic in reaction.

The mature thrombocytes are spheroid or oval in shape (Fig. 19), sometimes oblong (Fig. 20 ObTh). The cytoplasm has a fine reticular flaky appearance. The nucleus is large compared to the amount of cytoplasm. In some cases, thrombocytes contain one polar granule or a single vacuole. Occasionally the vacuole is located in the concavity of the nucleus (Figs. 19 and 20 STh & Oth). Mature thrombocytes are able to radiate cytoplasmic pseudopodia.

Figure 18 shows two thrombocytes projecting several thin pseudopodia, with one pseudopodium elongating to link two thrombocytes. It appears that these thrombocytic networks may serve in blood clotting of Clarias.

DISCUSSION

Blood smears of Clarias lazera showed that erythrocytes, lymphocytes and granulocytes were present. Typical monocytes were absent. Mature and immature cell types at various transitional stages of development were also noticed, a state similar to that reported by Weinreb (1963).

The results show that all different types of blood cells originate from a stem cell, the haemocytoblast, found in the lymphomyeloid tissue of haemopoietic organs, particularly the kidney and spleen. It arises from a primitive reticular cell. This is in agreement with Catton (1951) and Watson et al. (1963). In Clarias lazera, haemocytoblasts vary in size. The large haemocytoblasts develop to erythrocytes and granulocytic leucocytes, while medium or small-sized ones form lymphocytes and thrombocytes. These observations are in accordance with data presented on the perch blood (Yokoyama, 1960) and on Carassius auratus (Watson et al., 1963 and Weinreb and Weinreb, 1969).

During erythropoiesis, the haemocytoblast undergoes marked transformation in nucleus and cytoplasm to give developmental stages (pro-, basophilic, polychromatophil and orthochromatophil normoblasts) until it reaches the mature erythrocyte. Fish haematologists applied various nomenclatures to the erythrocyte developmental stages. However, the terms used in the present work are more or less identical to those given by Yokoyama (1960) in the perch. The polychromatophil of the present work appears to be similar to the proerythrocyte described by Ellis (1976) in the plaice with the exception that the nucleus of the latter is sometimes eccentric. Furthermore, the orthochromatophil normoblast of Clarias lazera was not recorded by the majority of workers, although Ellis (1976) described a similar stage in the plaice which he called young erythrocyte . Catton (1951) and El- Feky (1982) described a similar developmental stage in a number of teleosts, which they called reticulocyte.

All erythrocytic stages gave negative results with peroxidase reaction. This conclusion confirms the observations of many authors such as Yuki (1957); Ellis (1976); and El-Feky (1982), but contradicts the data of Caxton-Martins (1978) and Cannon et al. (1980). Only mature cells showed positive Sudan Black B granules, whereas developmental stages gave negative results. Similar results were reported for the plaice erythroblasts (Ellis, 1976).

Mature erythrocytes of Clarias lazera showed negative reaction with PAS reagent, similar to the findings of Hayhoe et al. (1960); Caxton-Martins (1977, 1978 & 1979) and El-Feky (1982).

During development of lymphocytes in Clarias lazera, the haemocytoblast transforms into a lymphoblast which in turn gives rise to the prolymphocyte. From the latter stage, a mature lymphocyte is derived. Similar reports have been given for lymphocyte development in teleost fish (Catton, 1951; Watson et al., 1963; and Weinreb and Weinreb, 1969). However, McKnight (1966) was unable to distinguish lymphoblasts in imprints of haemopoietic organs in the mountain whitefish.

Both lymphoblasts and lymphocytes are weakly positive to PAS reagent, similar to the lymphocytes of the plaice (Ellis, 1976) and Schilbe mystis (El-Feky, 1982). As to peroxidase reaction, lymphocytes are negative. This result agrees with those reported by Ellis (1976) and El-Feky (1982).

The lymphocytes of Clarias lazera were found to give negative results with Sudan Black B reaction similar to the finding of Baillif and Kimbrough (1946), and Blaxhall and Daisley (1973).

Macrophages were noticed to be confined only to haemopoietic organs and were not found in the peripheral circulation. Such observation resembles these reported by Yokoyama (1960); Van Furth et al. (1972); Ellis and De Sousa (1974); Ellis (1976 & 1977) for other fish species. However, Watson et al. (1963) reported the presence of macrophages in the blood circulation as well as in the haemopoietic tissues of the goldfish.

As regards the ontogeny of macrophages in Clarias lazera, they appear to be derived directly from primitive reticular cells. This view finds support by Jordan and Speidel (1924). Cytochemically, macrophages give weakly positive reaction with Sudan Black B and negative reactions for both peroxidase and PAS tests.

Cytochemically, promyelocytes, myelocytes and metamyelocytes give positive reaction with PAS in both granules and cytoplasm. This result confirms those of Ellis (1976) and Barber and Westermann (1978). As to the Sudan Black B reaction, all immature stages of leucocytes described in Clarias lazera give moderate positive results. This observation is contrary to that reported by Ellis (1976) in the plaice, where the only Sudan Black positive cells were the mature leucocytes.

The promyelocyte, neutrophilic and eosinophilic myelocytes along with metamyelocytes give positive results with peroxidase reaction, while basophilic myelo- and metamyelocytes reacted negatively. However, Ellis (1976) reported that immature stages of all leucocyte types have a negative peroxidase reaction.

The neutrophils of Clarias lazera, the cytoplasm is [illegible] while the granules show strong PAS reaction, [illegible] results of El-Feky (1982), but contradicts the observation of Roubal (1986). Granules also give positive reaction with Sudan Black B, in agreement with Baillif and Kimbrough (1946). They give positive results with peroxidase. This confirms the results of Yuki (1957), Ellis (1976), Cannon et al. (1980) and El-Feky (1982) in various teleosts. On the other hand, the present observations are contradictory to those of Kelenyi and Nemeth (1969) who stated that neutrophilic granules in teleosts were peroxidase negative.

Eosinophils of Clarias lazera give positive reaction to PAS, Sudan Black B and peroxidase. This agrees with results of El-Feky (1982); Baillif and Kimbrough (1946); and Hattori (1958).

Basophils give positive results with PAS, similar to Schilbe mystis (El-Feky, 1982). They reacted positively with Sudan Black B, contrary to the results of Baillif and Kimbrough (1946). As regards the peroxidase reaction, the basophilic granules in Clarias gave negative results, this agrees with the results of Hattori (1958), and El-Feky (1982).

Cytochemically, it was noticed that both prothrombocytes and thrombocytes were negative for PAS reaction. This result confirms these of Duthie (1939) and Caxton-Martins (1979) on some teleosts. Contrary to the present observation is that of Ellis (1976) on the thrombocytes of the plaice. As to Sudan Black B reaction, thrombocytes of Clarias gave negative results to peroxidase reaction. This observation supports the work of Duthie (1939) on teleost fish.

Finally, it is proposed that blood cell formation in Clarias lazera is monophyletic according to the following scheme:

Proposed scheme showing the origin and development of the blood cells of **Clarias lazera.**

Haemocytoblast

Erythrocyte	Granulocyte series			Agranulocyte series		Thrombocyte series
1. Pronormoblast	1. Promyelocyte					1. Prothrombocyte
2. Basophilic Normoblast				Macrophages	Lymphocyte series	2. Mature Thrombocyte
3. Polychromat Ophil Norm	2. Neutrophilic Myelocyte	2. Eosinophilic Myelocyte	2. Basophilic Myelocyte		1. Lymphoblast	
4. Orthrochromat Ophil Norm	3. Nt. Meta-Myelocyte	3. Eos. Meta-Myelocyte	3. Bas. Meta-Myelocyte		2. Prolymphocyte	
5. Mature Erythrocyte	4. Mature-non-filamented	4. Mature Eosinophil	4. Mature Basophil		3. Mature lymphocyte	
	5. Bilobed				Large	Small
	6. Polymorphnuclear					

REFERENCES

Baillif, R.N and C. Kimbrough, 1946. Studies on leucocyte granules after stainig with Sudan Black B and May-Grunwald Giemsa. **I. Lab and Clin., Med.**, 32 (2) : 155-166.

Barber, D.L and J.E.M. Westerman, 1978. Occurrence of the Periodic Acid-Schiff positive granular leucocyte (PAS-GL) in some fishes and its significance. **J. fish Biol .**, 12: 35-43.

Barber, D.L.; J.E.M. Westerman and M.G. White, 1981. The blood cells of Antarctic icefish **Chaenocephalus aceratus** lonnberg : Light and electron Microscopic observations. **J. Fish. Biol.**, 19: 11-28.

Bergeron, T. and B. Woodward, 1983. Ultrastructure of the granule cells in the small intestine of the rainbow trout **Salmo gairdneri** before and after stratum granulosum formation. **Can. J. Zoo.**, 1, 61: 133-138.

Blaxhall, P.C and K.W. Daisley, 1973. Routine Haematological methods for use with fish blood. **Fish Biol.**, 5: 771-781.

Cannon, M.S.; M.M. Mollenhauer; T.E. Eurell; D.H. Lewis; A.M. Cannon and C. Tompkins, 1980. An ultrastructural study of leucocytes of the channel catfish, Ictalurus punctatus. **J. Morph.** 164: 1-23.

Catton, W.T., 1951. Blood cell formation in certain teleost fishes. **Blood**, 6: 39-60.

Caxton-Martins, E.A., 1977. Cytochemistry of blood cells in peripheral smears of some West African reptiles. **J. Anat.**, 124 (2): 393-400.

Caxton-Martins, E.A., 1978. Cytochemistry of blood cells in two West African amphibians. **J. Anat.**, 125 (2): 231-235.

Caxton-Martins, E.A., 1979. Cytochemical stydies of cell population in peripheral blood smears of two West African teleeosts. **J. Anat.**, 128 (2): 269-276.

Duthie, E.S., 1939. The origin, development and function of the blood cells in certain marine teleosts. Part I-Morphology. **J. Anat.**, 73: 396-412.

El-Feky, M.K., 1982. **Comparative studies on the blood picture and haematopoietic organs in some poikilothermic animals.** M. Sc. Thesis. Faculty of Science, Tanta University, Egypt.

Ellis, A.E., 1976. Leucocytes and related cells in the plaice **Pleuronectes platessa**. J. Fish Biol., 8: 143-156.

Ellis, A.E., 1977. The leucocytes of fish: A review **J. Fish Biol.**, 11: 453-491.

Ellis, A.E. and M.A.B. De sousa, 1974. Phylogeny of lymphoid system. I. A study of the fate of circulating lymphocytes in plaice Eur. **J. Immun.**, 4: 338-343.

Gardner, G.R. and P.P. Yevich, 1969. Studies on the blood morphology of three estuarine Cyprinodontiforme fishes. **J. Fish. Res. Bd. Can.**, 2: 433-447.

Hattori, K., 1958. An improved method of peroxidase reaction combined with Giemsa's stain for blood cells. **J. Labo. & Clin. Medi.**, 51, 829.

Hayhoe, J.C.F.; D. Quaglino and J.R. Flemans, 1960. Consecutive use of Romanowsky and Periodic- Acid-Schiff techniques in the study of blood and bone-marrow cells. **Brit. J. Haemat.**, 6: 23-25.

Hightower, J.A.; L.J. McCumber, M.G. Welsh; D.S. Whatly, R.E. Hartivgsen and M.S. Sigel, 1984. Blood cells of fundulus heteroclitus (L.). J. Fish. Biol., 24: 587-298.

Hoole, D. and C. Arme, 1982. Ultrastructural studies on the cellular response of roach, Rutilus rutilus. J. Fish Dis., 5: 131-144.

Jordan, H.E., and C.C. Speidel, 1924. Studies on lymphocytes. II. The origin, function, and fate of the lymphocytes in fishes. J. Morpho., 38 (4): 529-549.

Kelenyi, G. and A. Nemeth, 1969. Comparative histochemistry and electron microscopy of the eosinophil leucocytes of vertebrates. I. A study of the avain, reptile, amphibian and fish. (c.f. El-Feky, N.K., 1982).

Mahajan, C.L. and J.S. Dheer, 1979. Cell types in the peripheral blood of an air-breathing fish Channa punctatus. J. Fish Biol., 14: 481-487.

McKnight, L.M, 1966. A hematological study on the mountain whitefish Prosopium williamsoni. J. fish. Res. Bd. Canada, 23: 45-64.

Miller, N.W.; A. Deuter, and L.W. Clem, 1986. Phylogeny of lymphocyte heterogeneity the cellular requirements for mixed leucocyte reaction with channel catfish Ictalurus punctatus. Immunology, 59 (1): 123-128.

Pearse, A.G.E., 1972. Histochemistry, theoretical and applied. 3rd edn., Vol. II. London : Churchill Livingstone.

Roubal, F.R, 1986. Blood and other possible inflammatory cells in the Sparid Acanthopagrus australis (Gunther). J. Fish Biol., 28: 573-593.

Scott, A.L.; W.A. Rogers and PH Klesius, 1985. Chemiluminescence by peripheral blood phagocytes from channel catfish Ictalurus punctatus function of opsonin and temperature. DEV comp. Imunol, 9 (2): 24-250.

Van Furth, R.; Z.A Cohn; J.G Hirsch; J.H. Humphrey; W.g. Spector and H.L. Langevoort, 1972. The mononuclear phagocytic system : A new classification of macrophages, monocytes and their precursor cells. W.H.O., 46: 845-952.

Watson, M.F.; K.W. Guenther, and R.D. Royce, 1963. Haematology of healthy and virus diseased Sockeye salmon, Oncorhynchus nerka. Zoologica. N.Y., 41: 27-37.

Weinreb, E.L., 1963. Studies on the fine structure of teleost blood cells. Anat. Rec., 147: 219-238.

Weinreb. E.L. and S. Weinreb, 1969. A study of experimentally induced Endocytosis in a teleost. I. Light microscopy of peripheral blood cell respnses. Zoologica, 54: 25-37.

Yokoyama, H.O., 1960. Studies on the origin, development, and seasonal variations in the blood cells of the perch, Perca flavescens. Wildl. Disease, 6: 1-103.

Yuki, R., 1957. Peroxidase staining of the leucocytes in rainbow trout. Bull. Fac. Fich. Hokkaido Univ., 8: 36-44.