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THE HAEMOCYTOLOGY AND HISTOLOGY OF THE HAEMOPOIETIC ORGANS OF SOUTH AFRICAN FRESH WATER FISH. I. THE HAEMOPOIETIC ORGANS OF *CLARIAS GARIEPINUS* AND *SAROTHERODON MOSSAMBICUS**

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ABSTRACT

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The haemopoietic organs of the catfish, *Clarias gariepinus*, and the Mozambique bream, *Sarotherodon mossambicus*, were studied. In both species the primary haemopoietic organs are the pronephros, the mesonephros and the spleen. The peritoneum and, particularly in catfish, the omentum are of secondary importance in haemopoiesis.

Résumé

HÉMOCYTOLOGIE ET HISTOLOGIE DES ORGANES HÉMOPOIÉTIQUES DES POISSONS D'EAU DOUCE SUD-AFRICAINS. I. LES ORGANES HÉMOPOIÉTIQUES DE CLARIAS GARIEPINUS *ET* SAROTHERODON MOSSAMBICUS

On a étudié les organes hémopoiétiques du poisson-chat, Clarias gariepinus, *et de la brème du Mozambique*, Sarotherodon mossambicus. *Chez les deux espèces, les organes hémopoiétiques primaires sont le pronéphros, le mésonéphros et la rate. Le péritoine et, particulièrement chez le poisson-chat, l'épiploon ne jouent qu'un rôle secondaire dans l'hémopoièse.*

INTRODUCTION

Extensive studies have been made on the haemocytology and haemopoietic organs of cold water fish, notably those of North America, Europe and Russia, but there has been little investigation on warm water fish. Sirvastava (1968) studied the haemotology and haemocytology of *Clarias batrachus* in India, and Banerjee & Banerjee (1966) described the variation in size of the erythrocytes of a number of lower vertebrates, including *C. batrachus*. The haemocytology and some haematological findings of the marine fish *Scomberomorus maculatus* have been described by Pitombeira & Martins (1970). Srivastava & Griffith (1974) described the morphology of the mature erythrocyte of 20 species and subspecies and 1 hybrid of *Fundulus*. Hattingh (1972, 1973) and Hattingh & Du Toit (1973) studied *Barbus holubi, Sarotherodon mossambicus* and *Clarias gariepinus,* but limited their investigation to haematology. The haemocytology and histology of the haemopoietic organs have not yet been studied.

Jubb (1967) described the morphology and some aspects of the ecology of the catfish, *C. gariepinus,* and the Mozambique bream, *S. mossambicus,* and these were chosen for this study because both species may be suitable for fish production in mixed ponds.

In the present paper, the histology of the most important haemopoietic organs is described.

MATERIALS AND METHODS

Fifteen catfish, varying from 29–70 cm in length, were collected from 2 irrigation dams, and 12 Mozambique bream, 10–30 cm in length, were obtained from the Provincial Administration's fisheries, Hartbeespoort, Transvaal.

The fish were acclimatized for 1 week in glass aquaria at 20–22 °C. The bream were fed once daily with a commercial fish food, and the catfish on alternate days with chopped sheep's liver and intestines. All the fish were healthy and, except for one which died as a result of injuries sustained when it was caught, no fish died during the acclimatization period.

All the fish were fully anaesthetized in water containing 0,5 g/ℓ tricaine methanesulphonate* before they were killed. The various haemopoietic organs were removed, cut into 1 cm cubes, and fixed in 10% neutral buffered formalin. After being embedded in paraffin wax, sections were cut 5 μm thick and routinely stained with haematoxylin and eosin (HE).

The different staining techniques used to demonstrate various components of the haemopoietic organs included Berlin blue (BB) for trivalent iron (Perl's method, Pearse, 1961), Von Giesson (VG) for collagen (Lillie, 1954), Schmorl's method for lipofuscin (Pearse, 1961), modified Jones' method for basal membranes (Luna, 1968), periodic acid-Schiff for glycogen (McManus method, Pearse, 1961), Wohlbach's modified Giemsa's stain for developing blood elements (Thompson, 1970) and Gomori's silver impregnation for reticular fibres (Mallory, 1938).

Neither the limnological parameters of the water nor the size, sex and age differences were taken into account in this study.

RESULTS

In both the catfish (Fig. 1) and the bream (Fig. 2), the pronephros, the mesonephros and the spleen were found to be the primary haemopoietic organs. The peritoneum and, especially in the catfish, the omentum play a secondary role in haemopoiesis.

The pronephros

The pronephros of catfish is a bilaterally symmetrical organ, separated from the mesonephros by a thin fibrous transverse septum (Fig. 4). Situated caudally to the heart but cranially to the septum, it consists of 2 triangular lobes which are connected on their caudal extremities only. The organ is greyish-pink in colour and is covered by a thin semi-translucent fibrous capsule. Dorsally and caudally, the capsule is continuous with the peritoneum, thereby forming a suspensory apparatus for the pronephros.

The pronephros of bream has the same apperance as that of the catfish but the 2 lobes are joined throughout their caudal parts.

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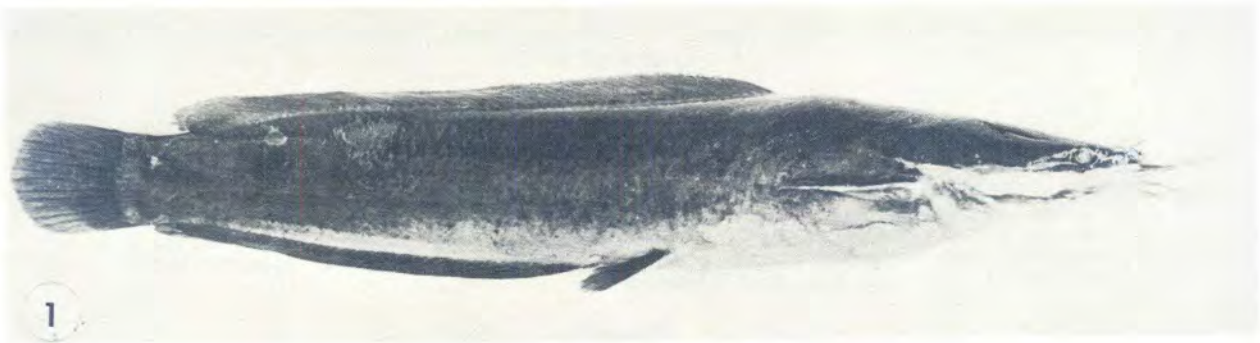
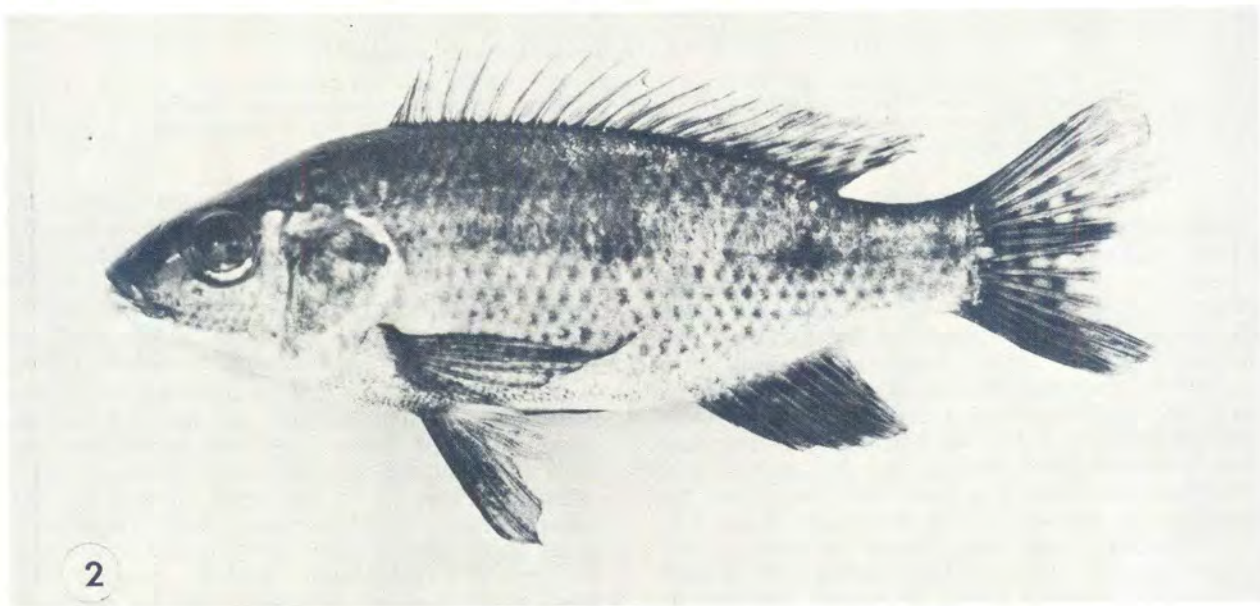




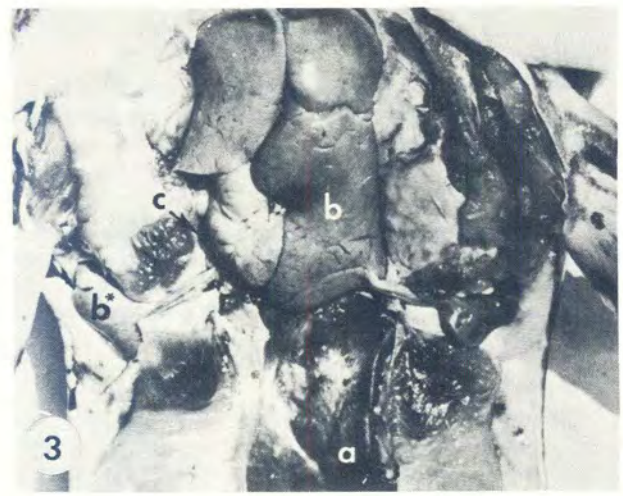


FIG. 1 The catfish, *Clarias gariepinus*, lateral view; $\times\frac{1}{3}$
FIG. 2 The Mozambique bream, *Sarotherodon mossambicus*, lateral view; $\times 1$
FIG. 3 Ventral view of the organs of *C. gariepinus in situ*: (a) mesonephros (b) liver (b*) caudal lobe of liver (c) edge of the spleen, just visible underneath the stomach; $\times\frac{1}{2}$
FIG. 4 Ventral view of the organs of *C. gariepinus* after removal of the liver and alimentary canal; (a) mesonephros (b) pronephros (c) transverse septum; $\times\frac{1}{2}$

Microscopically, the pronephroi of both species are densely cellular organs which contain many blood vessels and sinusoids (Fig. 5 & 6). The afferent vessels originate from the dorsal aorta and are recognized by the presence of a tunica media, which consists of concentric layers of smooth muscle. They terminate in a number of sinusoids which are lined by endothelium. The sinusoids are generally free of cells in fish which have been bled, and it is often possible to observe their connection with venules.

The haemopoietic component, which forms the parenchyma of the pronephros, is found between the sinusoids and is arranged in cords and plates. Except for the occasional clumping of lymphocytes, there is no definite pattern of cell distribution. Cells which could be identified include lymphocytes, monocytes, erythroblasts, developing and mature erythrocytes, thrombocytes and plasma cells. In catfish there are also developing and mature neutrophils, and in bream a few developmental stages of eosinophils and basophils.

The macrophages, found between the cells of the parenchyma and occurring singly or in groups, have a yellow-brown granular cytoplasm, and a pyknotic, crescent-shaped peripheral nucleus. Staining with BB and Schmorl's indicates that the yellow-brown granules are made up of iron containing haemosiderin and iron-free lipofuscin (Fig. 7 & 8).

The mesonephros

In both bream and catfish the mesonephros is an elongated, triangular organ, situated directly ventral to the spinal column. In catfish it is a firm organ, bluish-red in colour (Fig. 3 & 4). There are a number of irregular white areas about 1 mm in diameter on the sides of the organ which are known as the corpuscles of Stannius.

In bream the mesonephros, which partially surrounds the vertebral column, is soft and ruptures easily. It is bluish-red in colour, with a number of irregular black spots, the white corpuscles of Stannius occurring along its edges.

In both bream and catfish the mesonephros is covered by a thin fibrous capsule which is attached to the transverse septum cranially and is continuous with the peritoneum. The blood is supplied by lateral branches of the dorsal aorta which penetrate the mesonephors.

Microscopically, 3 functional units can be distinguished in the mesonephros: the excretory, secretory and haemopoietic.

The excretory component consists of the capsules of Bowman, glomeruli, convoluted tubules and collecting tubules (Fig. 9 & 10).

The secretory component is represented by the corpuscles of Stannius, which consist of spherical groups of cells arranged around a central capillary and surrounded by a thin fibrous capsule (Fig. 12 & 13). Delicate trabeculae, which contain blood vessels, arise from the capsule and penetrate in between the cells. The cells form cords around either the central capillary or a sinusoid (Fig. 13). Their cytoplasm stains blue with HE and has either a finely granular or foamy apperance. The nucleus varies in size and the chromatin is compact.

The haemopoietic component consists of the intertubular tissue (Fig. 10) which is supported by a network of reticular fibres (Fig. 9 & 11). The reticular network is more strongly developed in catfish than in bream. The cell elements are arranged in plates and cords and, except for clumping of lymphocytes and macrophages, there is no specific arrangement of the various types of cells. The vascular network consists of arterioles, venules and sinusoids.

Precursors of the erythrocytic and granulocytic series are found mainly in the mesonephros, whereas the thrombocytic series and mononuclear leukocytes are present mainly in the pronephros and spleen.

The spleen

In catfish, the spleen is situated dorsally to the stomach and may be displaced to the left (Fig. 3); in bream, it is caudal and to the left of the stomach and is closely associated with the intestines.

In both species the spleen is purplish-red in colour and is round to oval, or triangular in shape. In catfish, the spleen is covered by a thin fibrous capsule continuous with the serosa of the stomach and, in bream, continuous with the mesenterium.

The spleen consists of lymphoid tissue and pulp which are not macroscopically distinguishable. Microscopically, the spleen consists of a trabecular network which originates from the surrounding capsule. The cords of Billroth, which contain the arteries and veins, consist of spongy reticular tissue which divides the spleen into "compartments" of lymphoid nodules (Fig. 14). The lymphoid nodules consist of developing and mature mononuclear cells and a few developing and mature granulocytes. Large numbers of macrophages occur singly or in groups throughout the spleen.

Blood enters the spleen through the hilus and the trabecular arteries. The trabecular arteries become small arterioles, each of which forms a number of capillary vessels. The capillaries leave the trabeculae and enter the lymphoid nodules more or less centrally to form the central arteries which are surrounded by a prominent layer of smooth muscle fibres (Fig. 17). Erythrocytes pass through the wall of the central arteries and return to the central circulation by way of the sinusoids and capillaries and hence to the efferent veins in the junction of a number of cords of Billroth.

The structure of the spleen of bream is the same as that of the catfish except that the reticular network is not as pronounced and the trabeculae and cords of Billroth are not as strongly developed (Fig. 15 & 16).

Discussion

In this investigation it was found that plasma cells, mononuclear leucocytes and thrombocytes as well as their precursors are present in greater numbers in the pronephros than in the other haemopoietic organs. It is thus assumed that they are formed mainly in the pronephros. There is also some granulo- and erythropoiesis, but this to a lesser degree. According to Chiller, Hodgins, Chambers & Weiser (1969), the immunocompetent cells, especially the plasma cells, but also lymphocytes, monocytes and macrophages are formed in the pronephros. Smith, Potter & Merchant (1967) and Smith, Wivel & Potter (1970) confirmed these observations in *Lepomis macrochirus* and *Cyprinus carpio* respectively. As the main lymphoid tissues in catfish consist of the pronephros, the spleen, and a few small lymph nodes in the tail, the pronephros is probably the centre for the production of antibody-forming cells.

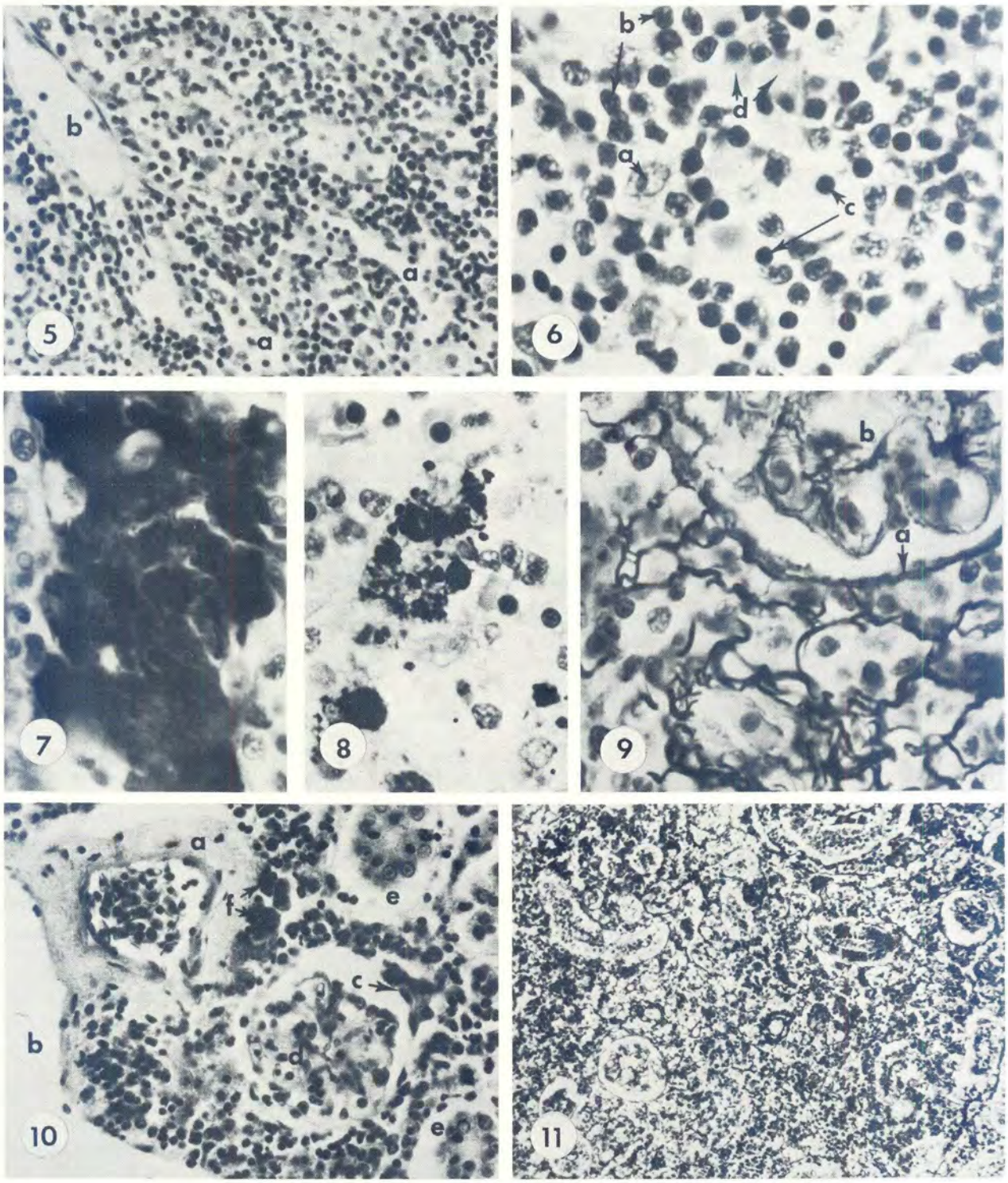
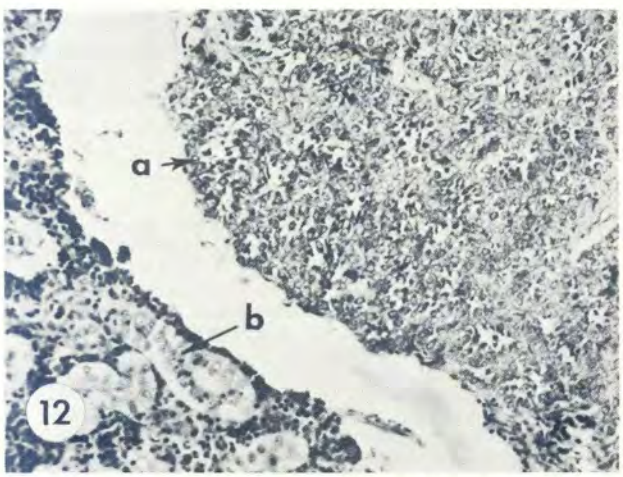
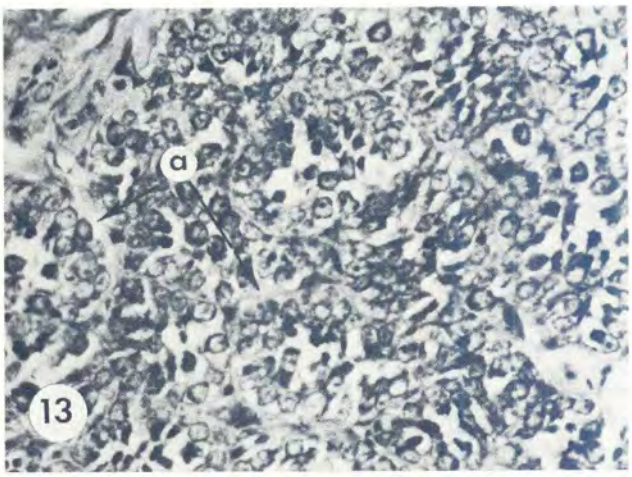
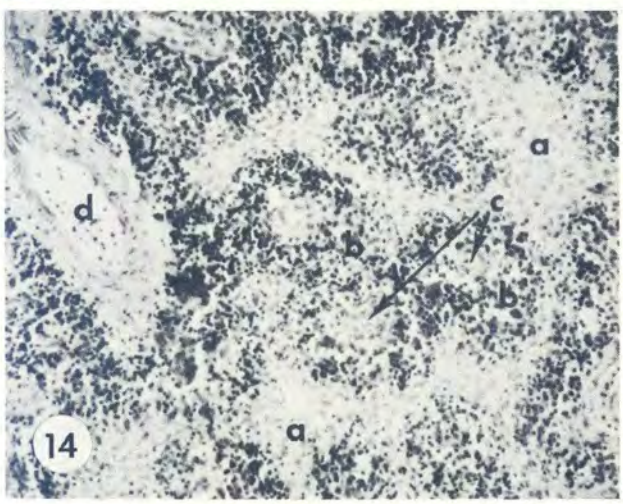
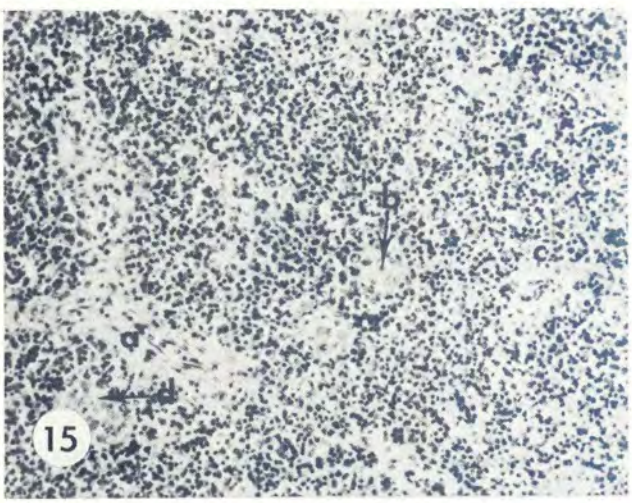


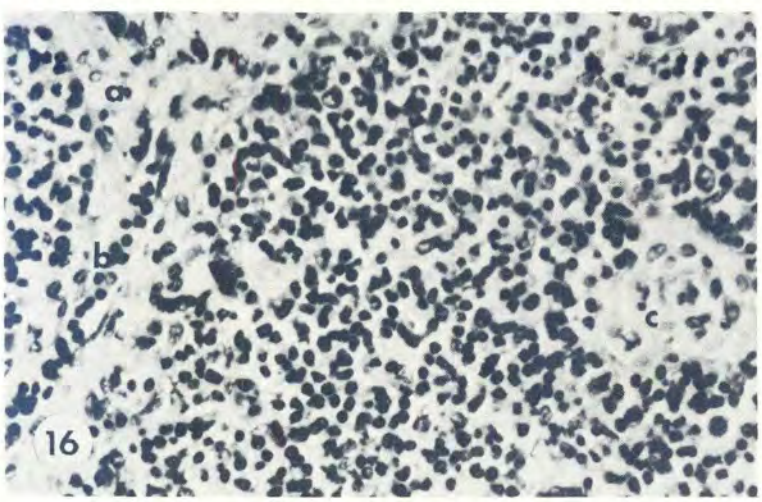
FIG. 5 The pronephros of *S. mossambicus*, showing the sinusoids (a) and blood vessel (b) HE, ×500
FIG. 6 Higher magnification of Fig. 5, showing (a) haemocytoblasts (b) lymphocytes (c) erythrocyte nuclei and (d) neutrophils; HE, ×1 200
FIG. 7 A group of macrophages in the spleen of a catfish, stained with Schmorl's to illustrate the iron-free lipofuscin; ×1 200
FIG. 8 A group of macrophages in the kidney of a bream containing iron-positive haemosiderin; BB, ×1 200
FIG. 9 The kidney of a catfish at high magnification. Bowman's capsule (a) and glomerulus (b) are easily distinguishable; Reticulum stain, ×1 200
FIG. 10 The kidney of catfish: (a) the arteriole (b) the venule (c) Bowman's capsule (d) the glomerulus (e) the proximal convoluted tubules and (f) a group of macrophages; HE, ×500
FIG. 11 The kidney of a catfish, showing the extensive reticular network; Reticulum stain, ×200











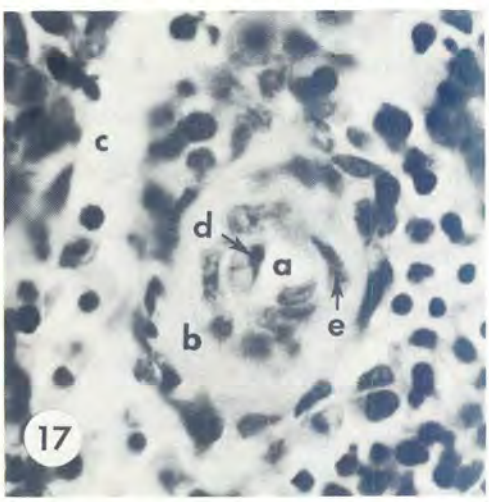


FIG. 12 The corpuscle of Stannius (a) of catfish showing its close proximity to the mesonephros (b); HE, ×200
FIG. 13 A corpuscle of Stannius of a catfish, showing the acinar structure. Thin connective tissue capsules (a) separate the acini; Giemsa's, ×500
FIG. 14 The spleen of catfish: (a) cords of Billroth (b) lymphoid nodules (c) central arteries and (d) afferent bloodvessel; HE, ×200
FIG. 15 The spleen of bream: cords of Billroth are less numerous; (a) cord of Billroth (b) central artery (c) lymphoid nodules (d) group of macrophages; HE ×200
FIG. 16 Higher magnification of spleen of bream: (a) the cords of Billroth, containing the efferent vessel (b) The central artery (c) is surrounded by tunica media; HE, ×500
FIG. 17 The spleen of a catfish: the central artery (a) surrounded by a prominent smooth muscle layer (b); sinusoid (c) drains blood back to the cords of Billroth. A few erythrocytes (d) are visible, and (e) is an endothelial cell; HE, ×1 200

The corpuscles of Stannius produce steroid hormones and have an osmoregulatory function (Heyl, 1970). That the corpuscles are mainly responsible for osmoregulation is shown by their greater activity in *Mugil* species in salt water than those in fresh water (Johnson, 1972). Krishnamurthy (1967) found that the corpuscles originate from the mesonephric tubules and believes that the corpuscles have the same function in fish as the juxtaglomerular apparatus and adrenal cortex in mammals.

The excretory part of the mesonephros consists of the same subunits as that of mammals. In *Gasterosteus aculeatus* it was found that the tubules could be differentiated microscopically in proximal and distal convoluted tubules, a connecting piece and a collecting tubule (Häckert-Korde, 1975). In this study, the same structures could be differentiated in bream and catfish. In catfish there are a few glomeruli in the caudal mesonephros, but the majority of glomeruli are in the cranial region. The majority of the tubules, however, are in the caudal mesonephros. No explanation can be offered for the distribution of these structures.

The haemopoietic tissue forms the greater part of the mesonephros and is limited to the intertubular tissue. This is the main centre for erythro- and granulopoiesis. The presence of large numbers of macrophages indicates that the haemopoietic part also acts as a filter for the blood. The structure of the cells and the cell populations found in the haemopoietic organs of different fish species (Chiller *et al.*, 1969; Häckert-Korde, 1975; Smith *et al.*, 1967; Yoffey, 1929) differ only in the relative numbers of the various types of cells. This is presumably due to the physiological condition of the fish, their immediate environment, and the season of the year (Ezzat, Shabana & Farghaly, 1974).

In both bream and catfish the spleen does not differ from that of *Scylliorhinus canicola* as described by Yoffey (1929). However, in bream, the reticular system is not as well developed as that in catfish.

The haemopoietic activity of the spleen is mostly concerned with mononuclear leucocytes, although there is some evidence of erythro- and granulopoiesis. Plasma cells are present in the spleen but in much smaller numbers than in the pronephros.

In both species studied, the general apperance of the spleen shows a distinct similarity to that of mammals. The finer branching of the central artery has not been studied, but it appears that penicilli, as found in mammals, are lacking in fish.

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References

BANERJEE, V. & BANERJEE, M., 1966. Size variation in some vertebrate erythrocytes. *Naturwissenschaften*, 53, 562.

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

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.

HÄCKERT-KORDE, KRISTA, 1975. Histologische und Histochemische Untersuchungen an der Niere des Stichlings *Gasterosteus aculeatus L. Zeitschrift für mikroskopisch-anatomische Forschung*, 89, 373–385.

HATTING, J., 1972. Observations on the blood physiology of five South African fresh water fish. *Journal of Fish Biology*, 4, 555–563.

HATTINGH, J., 1973. Some blood parameters of the yellow fish (*Barbus holubi*) and the barbel (*Clarias gariepinus*). *Zoologica Africana*, 8, 35–39.

HATTINGH, J. & DU TOIT, P. J., 1973. Haemoglobins of the yellow fish and barbel. *South African Journal of Medical Science*, 38, 37–41.

HEYL, H. L., 1970. Changes in the corpuscle of Stannius during the spawning journey of Atlantic salmon (*Salmo salar*). *General and Comparative Endocrinology*, 14, 43–52.

JOHNSON, D. W., 1972. Variations in the interrenal and corpuscle of Stannius of *Mugil cephalus* from the Colorado river and its estuary. *General and Comparative Endocrinology*, 19, 7–25.

JUBB, R. A., 1967. Fresh water fishes of southern Africa. Cape Town, Amsterdam: A. A. Balkema.

KRISHNAMURTHY, V. G., 1967. Development of the corpuscles of Stannius in the anabantid teleost (*Corisa lalia*). *Journal of Morphology*, 123, 109–120.

LILLIE, R. D., 1954. Histopathologic technic and practical histochemistry. New York, Toronto: The Blakeston Co.

LUNA, L. G., Ed., 1968. Manual of histologic staining methods of the Armed Forces Institute of Pathology, 3rd ed. New York, Toronto, London, Sydney: Mc Graw-Hill Book Co.

MALLORY, F. B., 1938. Pathological technique. Philadelphia: W. B. Saunders Co.

PEARSE, A. G. E., 1961. Histochemistry, theoretical and applied. Second ed. London: J. & A. Churchill Ltd.

PITOMBEIRA, MARIA DA SILVA & MARTINS, J. M., 1970. Hematology of the Spanish mackerel, *Scomberomorus maculatus. Copeia*, 1, 182–186.

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 pronephros of the carp (*Cyprinus carpio*). *Anatomical Record*, 167, 351–370.

SRIVASTAVA, A. K., 1968. Studies on the hematology of certain fresh water teleosts I. Erythrocytes. *Anatomischer Anzeiger*, 123, 233–249.

SRIVASTAVA, A. K. & GRIFFITH, R. W., 1974. Erythrocyte morphology and the ecology of species of *Fundulus. Copeia*, 1, 136–141.

THOMPSON, S. W., 1970. Selected histochemical and histopathological methods. Springfield, Ill., U.S.A.: Charles C. Thomas.

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.

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