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THE HAEMOCYTOLOGY AND HISTOLOGY OF THE HAEMOPOIETIC ORGANS OF SOUTH AFRICAN FRESHWATER FISH. IV. ULTRASTRUCTURE OF SOME CELLS OF *CLARIAS GARIEPINUS* AND *SAROTHERODON MOSSAMBICUS**

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ABSTRACT

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This paper describes the ultrastructure of several cells found in the blood and haemopoietic tissues of the catfish (*Clarias gariepinus*) and the Mozambique bream (*Sarotherodon mossambicus*). The cells are haemocytoblasts, small lymphoid haemoblasts, thrombocytes, monocytes, lymphocytes, developing and mature neutrophilic granulocytes, plasma cells and macrophages. The various organelles normally found in mammalian haemocytes, plasma cells and macrophages were observed in those of fish. With the exception of the mature erythrocytes, which resemble the normoblasts of mammals, the various cells show distinct similarities to those of other fish species as well as of mammals.

Résumé

L'HAEMOCYTOLOGIE ET L'HISTOLOGIE DES ORGANES HAEMOPOIETIQUES DU POISSON D'EAU DOUCE D'AFRIQUE DU SUD. IV. ULTRASTRUCTURE DE CERTAINES CELLULES DU CLARIAS GARIEPINUS *ET DU* SAROTHERODON MOSSAMBICUS

Cet exposé décrit l'ultrastructure de plusieurs cellules trouvées dans le sang et les tissues haemopoietiques du poisson-chat (Clarias gariepinus *et de la brème du Mozambique* (Sarotherodon mossambicus).

Ces cellules sont des haemocytoblastes, des petites haemoblastes lymphoides, des thrombocytes, des monocytes, des lymphocytes, des granulocytes neutrophiliques en develóppement et mûres, des plasmocytes et des macrophages. Les organites variées normalement trouvés dans les haemocytes mammifères, les plasmocytes et les macrophages ont été observés dans celles du poisson. A l'exception des erythrocytes mûres, qui ressemblent aux normoblastes des mammifères, les différentes cellules montrent des similarités distinctes à celles d'autres espèces de poissons aussi bien que de mammifères.

INTRODUCTION

Little has been published on the ultrastructure of the various blood cells of fishes. The earliest record is that of Meves (1904), who described an internal cytoskeleton in erythrocytes, a finding that has since been confirmed by several authors (Davis, 1961; Weinreb, 1963; Fawcett & Witebsky, 1964).

Shepro, Belamarich & Branson (1966) found that thrombocytes contain large numbers of microtubules, and the developing and mature lymphocyte has been illustrated by Grey & Biesele (1955), Pease (1956), Fey (1966a & b), Sandborn (1970) and Ferguson (1976). The plasma cells have been described by Smith, Wivel & Potter (1970) and the various granulocytes by Fey (1966 a and b).

This paper deals with the ultrastructure of some of the haemocytes as well as of the plasma cells and macrophages of the catfish (*Clarias gariepinus*) and the Mozambique bream (*Sarotherodon mossambicus*).

MATERIALS AND METHODS

Blood from the caudal artery of the fish was collected in heparinized microhaematocrit tubes (internal diameter 1,1 mm) which had previously been half-filled with cooled 4% glutaraldehyde buffered with Millonig's buffer and stored at 4°C. The tubes were filled with the blood, sealed and inverted a few times to ensure proper mixing of the blood and glutaraldehyde, and then centrifuged at 3 000 rpm for 3 min. The resulting pellets of sediment were carefully removed from the tubes and transferred to fresh glutaraldehyde in which they were fixed for another 2 h (Potgieter, 1977). Thereafter they were divided into 1 mm^3 cubes.

In addition, 1 mm^3 cubes were removed from the various haemopoietic organs and fixed in 4% buffered glutaraldehyde at 4°C.

Both blood and tissue blocks were post-fixed in 2% osmium tetroxide in phosphate buffer. They were then dehydrated through graded ethyl alcohol, transferred to propylene oxide and embedded in Epon 218 (Pease, 1964).

For orientation purposes, 1,5 μm thick sections were cut and stained with Giemsa's stain, toluidine blue and haematoxylin and eosin (HE). Thin sections were cut at 50–60 nm thickness and mounted on copper grids. They were stained with 1% aqueous uranyl acetate solution for 10 min at 60°C, and with Reynold's lead citrate at room temperature (20°C) for 3 min (Reynolds, 1963). Scanning and photography were done with a Siemens Elmiskop 102.

RESULTS

Haemocytoblasts

The haemocytoblasts are large cells with prominent nuclei. They have all the components seen in mammalian cells, viz., a trilaminar unit membrane that encloses the cytoplasm, free ribosomes, mitochondria, a Golgi apparatus, a granular endoplasmic reticulum (ER) and a centrosome (Fig. 1 & 15).

The cytoplasmic matrix is finely granular, and contains the various organelles. Lysosomes are seen as small, electron dense, membrane-bound vesicles. Mitochondria are round to elongate and their internal cristae are usually long and slender. The ER is poorly developed and is of the granular type. Pinocytotic vesicles are occasionally seen and occur mostly along the edges of the cell.

The nucleus contains finely granular nucleoplasm in which the chromatin is arranged in diffuse condensation. The nucleolus is prominent.

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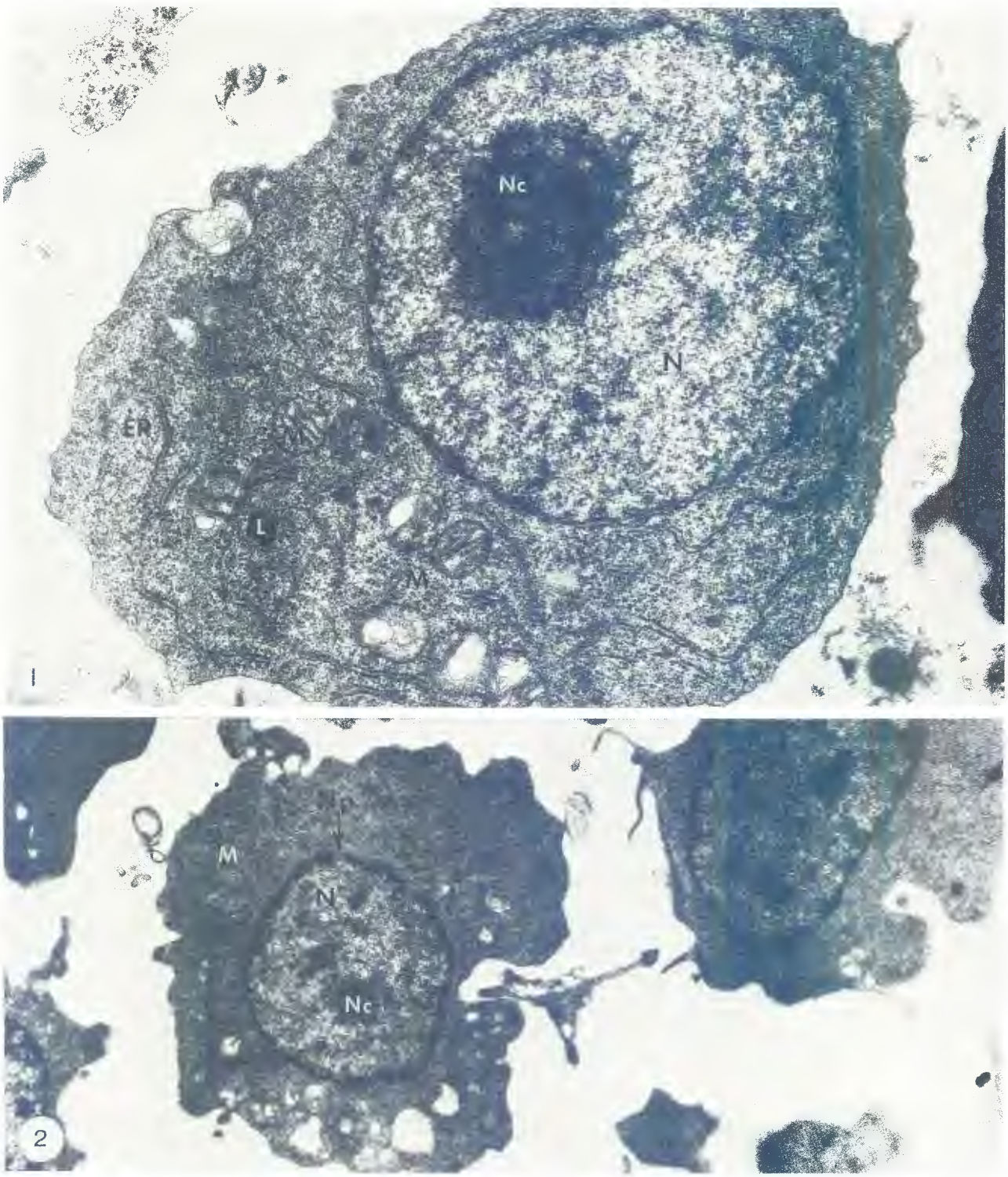


FIG. 1 A haemocytoblast in the pronephros of the catfish; ×23 000
FIG. 2 Two small lymphoid haemoblasts in the mesonephros of the bream; ×16 800

Small lymphoid haemoblasts

These cells are similar to the haemocytoblasts, but are smaller, contain more mitochondria and have a more distinct granular ER.

When compared with the more diffuse nature of the nucleus of the haemocytoblast, that of the small lymphoid haemoblast is a more advanced type and the chromatin is visible as distinct condensations. The nucleolus is clearly visible (Fig. 2).

Thrombocytes

Round and elongate thrombocytes are seen in the catfish and the bream (Fig. 3). The latter thrombocytes are also known as spindle cells (Fig. 4 & 5). In both forms the cytoplasm is finely granular and contains a few mitochondria and lysosomes. Numerous electron-dense granules that occur singly or in groups are spread throughout the cytoplasm. These are of uniform size, larger and more electron-dense than the ribosomes, and are believed to be glycogen granules.

Microtubules are numerous and are concentrated at the poles of the cells as well as in the indentation in the nucleus of the spindle cells.

The nucleus of both forms is bilobed and the chromatin appears as large blocks. A single nucleolus is present.

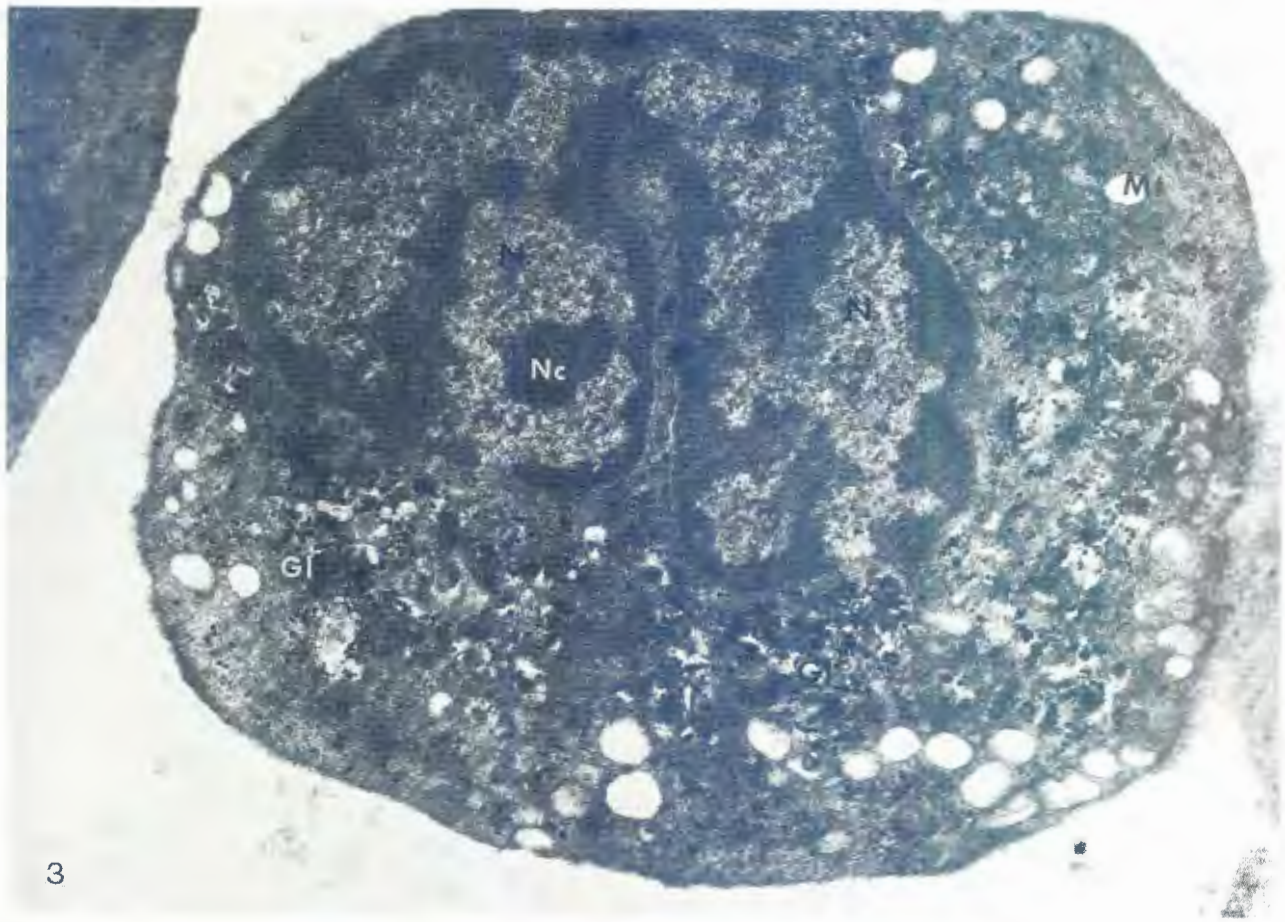
Monocytes

A typical monocyte has granular cytoplasm in which numerous ribosomes are scattered. Many round or slightly elongated mitochondria with distinct cristae are present (Fig. 6). The ER is reasonably well developed and is of the granular type. A few membrane-bound vesicles are present. The Golgi apparatus is well developed.

The nucleus is horseshoe-shaped, and the chromatin is dense and arranged peripherally. From 1–3 nucleoli are present.

Lymphocytes

These are irregularly shaped cells with a distinctly granular cytoplasm throughout which numerous



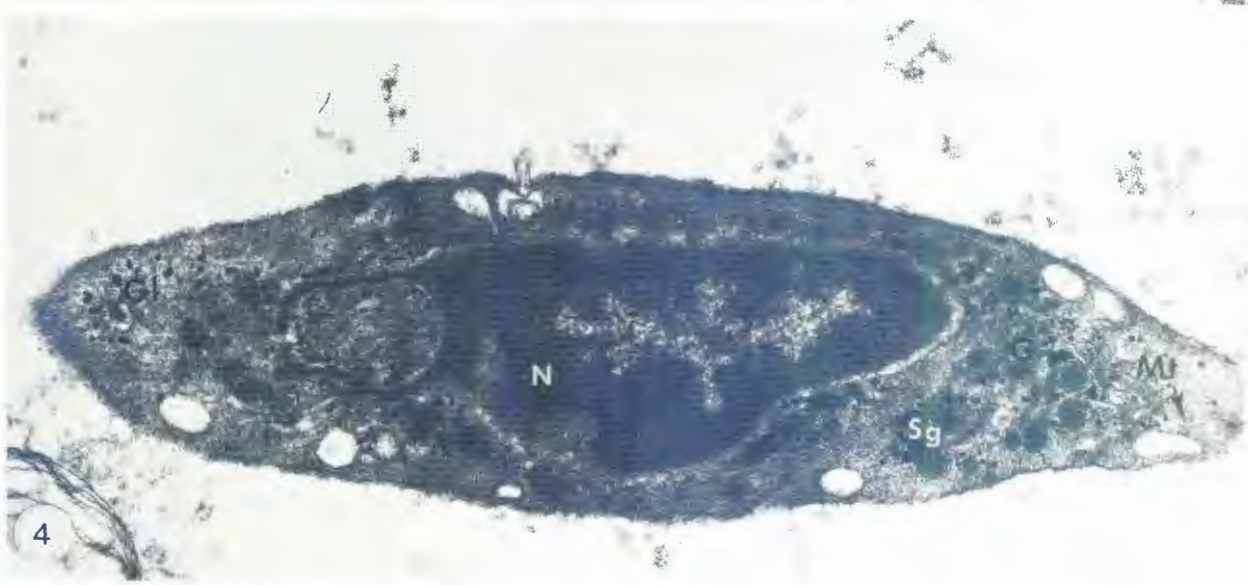


FIG. 3 A round thrombocyte in the circulating blood of the catfish; ×43 000
FIG. 4 An elongate thrombocyte (spindle cell) in the circulating blood of the catfish; ×30 000

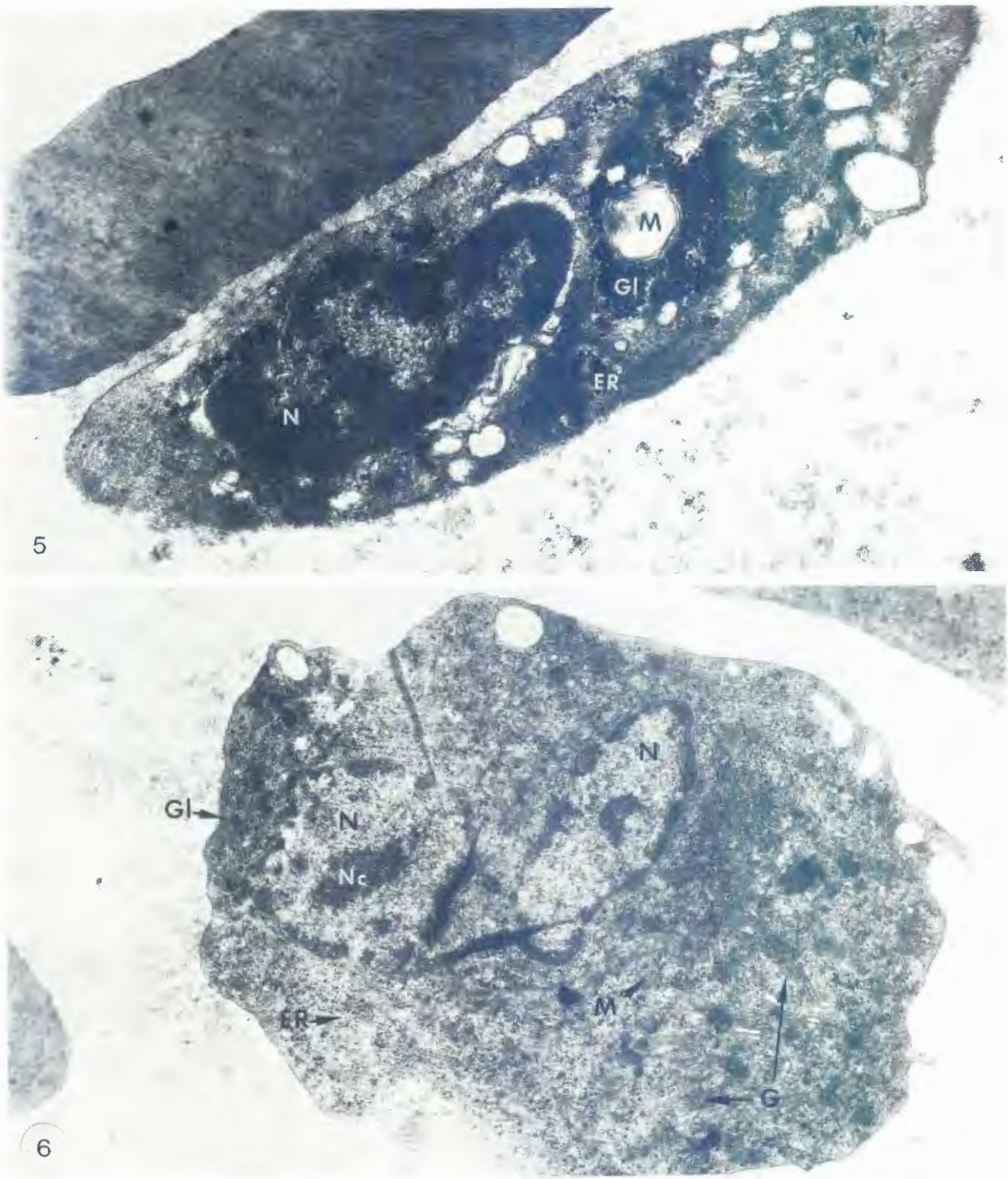


FIG. 5 Spindle cell in the circulating blood of the catfish; ×32 500
FIG. 6 A cell that is believed to be a monocyte. Note the well developed Golgi apparatus and large number of small mitochondria; mesonephros of the bream, ×26 000

ribosomes are scattered (Fig. 7 & 8). Only a few round to oval mitochondria are present. The ER is of the granular type and is poorly developed. A very distinct centriole was seen in one case (Fig. 8).

The nucleus is large, with a well-developed, radially arranged chromatin network. The nucleolus is distinct.

Erythrocytes

The finely granular cytoplasm in these cells appears almost homogeneous. Small mitochondria with long cristae are occasionally present. Microtubules are concentrated at the poles of the cells, but are indistinct (Fig. 10).

The nucleus is round or oval in catfish and polymorphic in bream. The chromatin is concentrated peripherally, forming a dense network in which the nucleolus occurs.

Developing neutrophilic granulocytes

A cell that is thought to be a neutrophilic metagranulocyte is illustrated in Fig. 11. The cytoplasm is moderately dense and contains a few very small

mitochondria. A poorly developed granular ER is present.

The nucleus has already assumed the band-shape and appears as 2 separate entities in Fig. 11. The chromatin is dense and arranged peripherally. A distinct nucleolus is present.

Mature neutrophilic granulocytes

These cells can be recognized by the presence of numerous large, electron-dense granules in the cytoplasm (Fig. 9). Mitochondria are scarce and only a few strands of the granular ER remain.

The chromatin is arranged peripherally, and there is a distinct nucleolus.

Plasma cells

A cell that appears to be either a developing plasma or plasmacytoid cell is illustrated in Fig. 12. This cell already shows characteristics of the plasma cell in that it has an extensive granular ER.

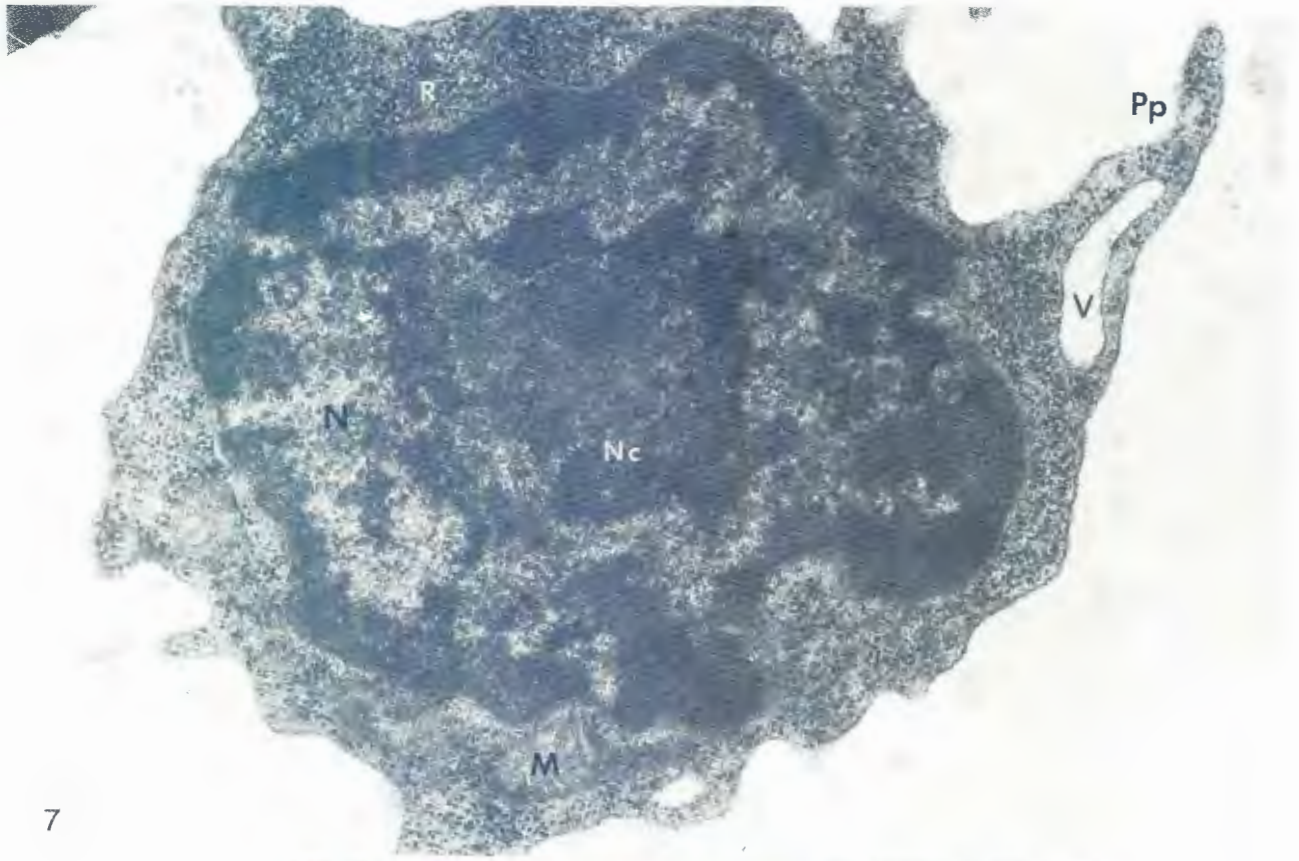


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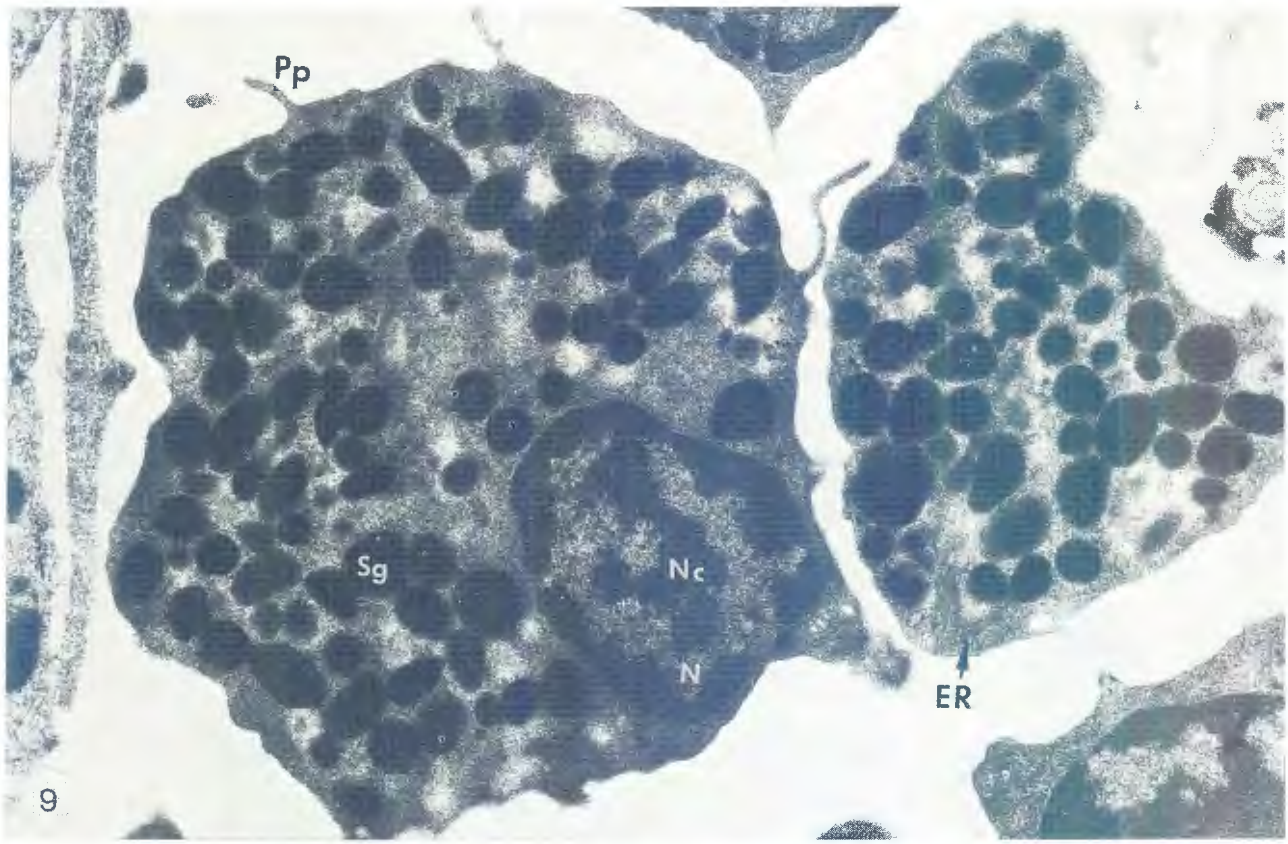
FIG. 7 A lymphocyte in the circulating blood of the catfish; ×21 600
FIG. 8 Lymphocyte in the circulating blood of the catfish, showing a distinct centriole (S); ×22 000



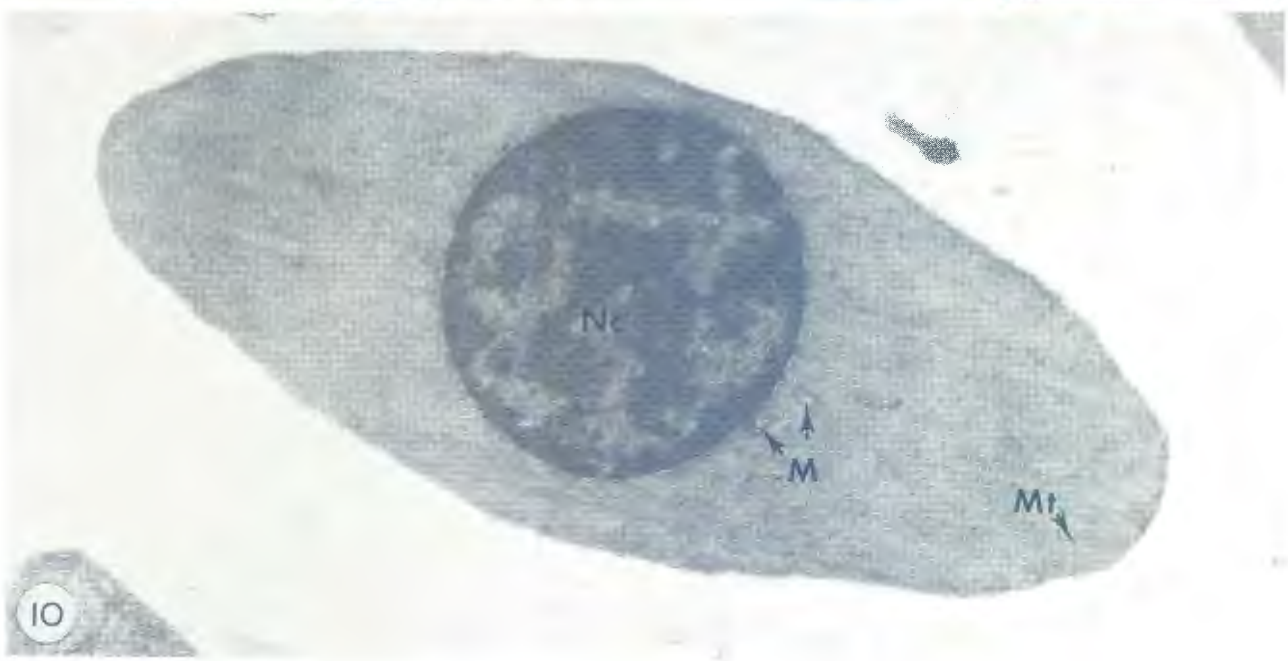


FIG. 9 A neutrophil in the mesonephros of the catfish; ×12 600
FIG. 10 An erythrocyte of the bream, transverse section; ×22 400

In the mature plasma cell (Fig. 13 & 14) the greater part of the cytoplasm is occupied by the extensive and well-developed cisternae of the granular ER. Ribosomes are plentiful, and a well-developed Golgi apparatus as well as a few small mitochondria are present.

The nucleus is large in comparison with the nuclei of other cells, and the chromatin arrangement is similar to that of the lymphocyte.

A plasma cell containing 2 Russell bodies (marked X) is illustrated in Fig. 13. The Russell bodies appear as homogenous electron-dense structures within the dilated ER, and are not membrane-bound.

Macrophages

The macrophages are easily recognized by their cytoplasmic inclusions. These inclusions are of varying sizes and densities and constitute cellular debris (Fig. 15). Only a few mitochondria and a poorly developed ER are present, but lysosomes are numerous.

The nucleus is fairly large, eccentrically situated and contains chromatin that is less dense than that of the lymphocyte.

Discussion

No descriptions of the ultrastructure of the haemocytoblasts and small lymphoid haemoblasts of fish could be found in the literature, so comparisons could not be made with those in this study. Smith *et al.* (1970) illustrated a cell from the pronephros of *Cyprinus carpio,* which they assumed to be a haemocytoblast, while Pease (1956) illustrated the haemocytoblasts of mice and guinea-pigs. In this study it was found that there are distinct similarities between the haemocytoblasts found in the catfish and the bream and those seen by Pease (1956) and Smith *et al.* (1970).

There are very few morphological differences between the erythroblasts from the fishes examined in this study and those of mammals (Pease, 1956). In addition, the erythroblasts of both the bream and

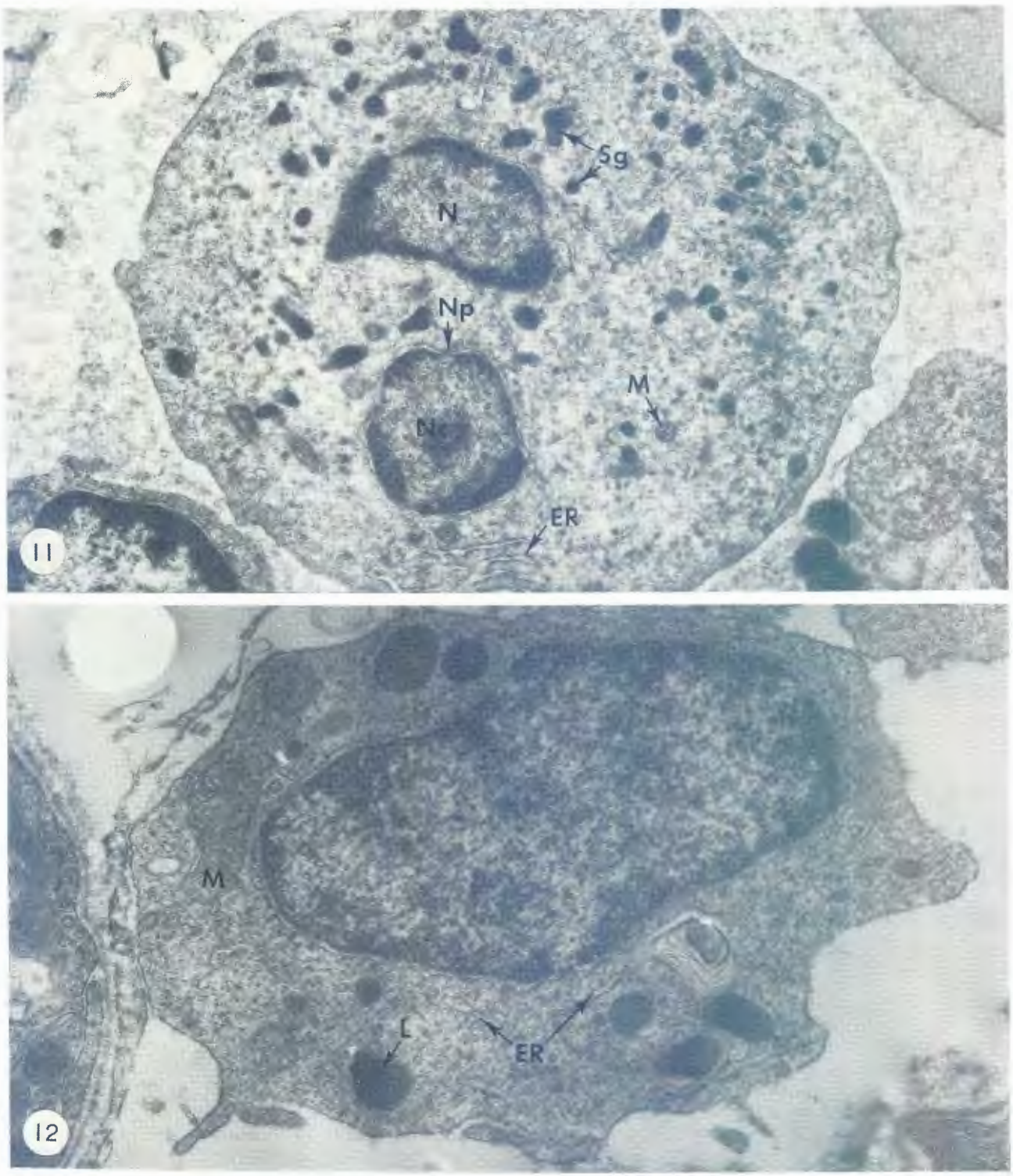


FIG. 11 A cell presumed to be a neutrophilic metagranulocyte in the mesonephros of the bream: ×20 900
FIG. 12 A plasmacytoid cell in the pronphros of the bream; ×22 400

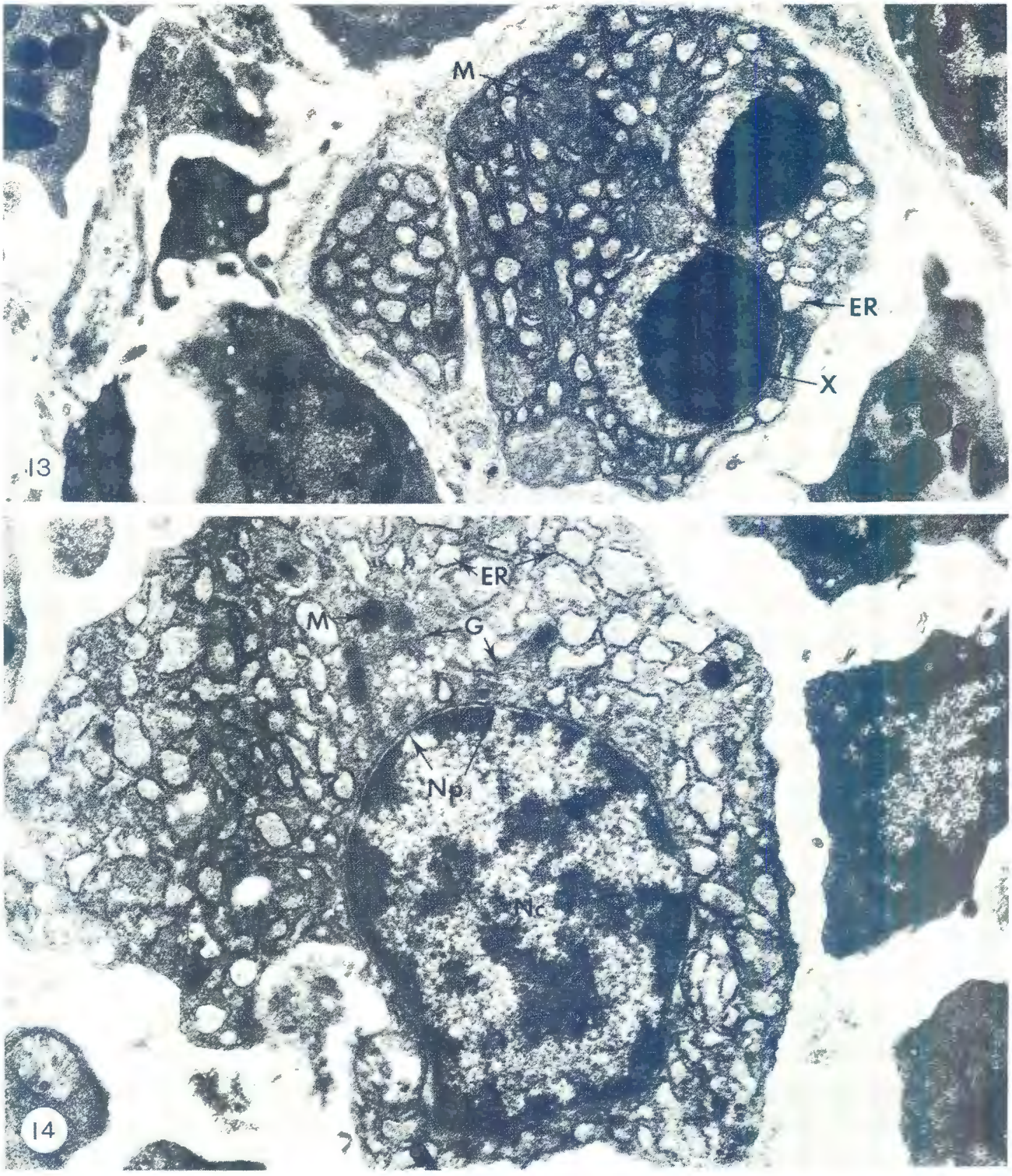


FIG. 13 A Russell body plasma cell in the spleen of the catfish; ×20 000
FIG. 14 Plasma cell in the spleen of the catfish; ×28 000

the catfish contain the characteristic ferritin molecules also seen in mammalian erythroblasts.

The polychromatophilic erythrocytes, which are not illustrated here, may be distinguished from the mature erythrocytes in that the former contain more free ribosomes and cytoplasmic organelles. The polychromatophilic erythrocytes also have a more vesicular nucleus and a more granular cytoplasm. Although the various developmental stages of the polychromatophilic erythrocytes in the 2 fish species studied (Boomker, 1980) show certain similarities with the developmental stages of the erythrocytes of mice and guinea-pigs described by Pease (1956), they cannot be regarded as homologues. The primitive type of erythrocytic development which results in nucleated erythrocytes is seen in fish, whereas those in mammals pass rapidly through the primitive developmental stages to form the specialized, non-nucleated mature erythrocytes. Moreover, the mature erythroblast (normoblast) of mammals resembles the mature erythrocytes of fish (Arey, 1966).

Meves (1904) found an "internal skeleton" in the erythrocytes of amphibians. Davis (1961) substituted the term "marginal bands" for this "internal skeleton" and showed that it consists of subunits. Maser (1963), Weinreb (1963) and Maser & Philpott (1964) showed that the subunits were microtubules which were mainly concentrated at the poles of the cells. Fawcett & Witebsky (1964) provided a detailed description of the microtubules in the erythrocytes and thrombocytes of a number of lower vertebrates. They postulated that the microtubules are responsible for the motility and elasticity of the erythrocytes, and that they prevent rupture of these cells when sudden changes of shape occur. Weinreb & Weinreb (1965) extended the observations of Fawcett & Witebsky (1964) and proposed a dual function for the marginal bands, viz., support for the erythrocyte by hydro-elastic characteristics of both the tubules and the fluid they contain and, secondly, the transport of nutrients within the erythrocytes.

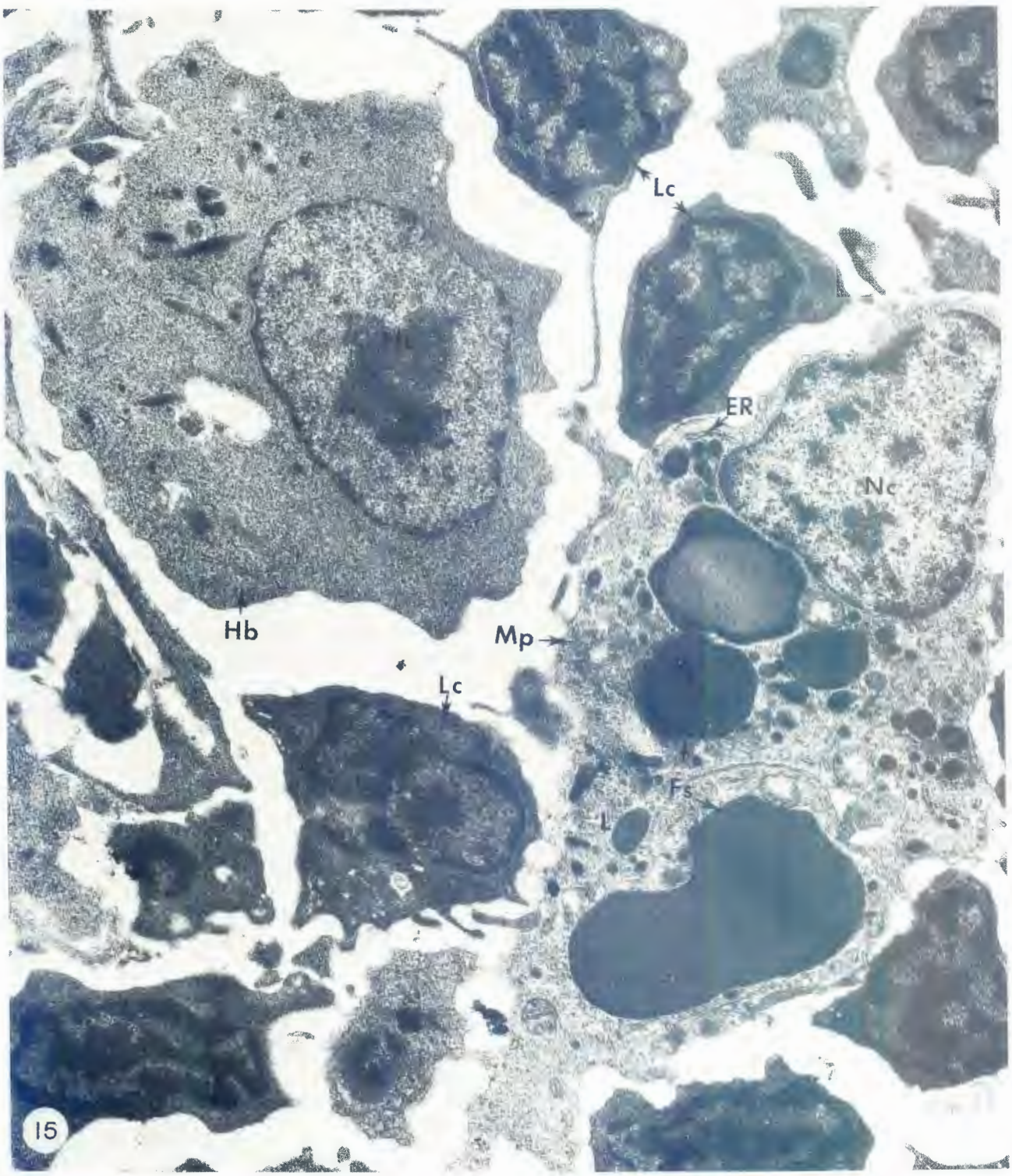


FIG. 15 A few cells in the mesonephros of the catfish. The macrophage (Mp) is recognized by its cytoplasmic inclusions. A haemocytoblast (Hb) is seen in the top left, and lymphocytes (Lc) occur throughout. Note the relative sizes of the various cells; ×11 400

The ultrastructure of the mature erythrocytes of both species of fish examined is similar and corresponds to that seen in other fish species and lower vertebrates (Davis, 1961; Fawcett & Witebsky, 1964; Weinreb, 1963). As can be seen from Fig. 10, microtubules, although few in the erythrocytes, are present both in these cells and the thrombocytes.

Fawcett & Witebsky (1964) also found that the nuclei of the erythrocytes contained the same haemoglobin found in the cytoplasm. They state that this is an indication that the pores in the nuclear membrane are large enough to allow large molecules to pass through freely. In this study it was found that ferritin molecules were present not only in the cytoplasm but also in the nucleus.

The structure of the thrombocyte of both the catfish and the bream are similar to that of the thrombocytes described by Fawcett & Witebsky (1964), Shepro *et al.* (1966) and Ferguson (1976). The large numbers of microtubules found by Fawcett & Witebsky (1964) in thrombocytes of the dogfish, *Mustelus canis,* were confirmed by Shepro *et al.* (1966) for inactivated thrombocytes of the plaice, *Pleuronectes platessa.* These cells were more or less the same size as lymphocytes and contained bilobed nuclei, little cytoplasm and few cytoplasmic organelles.

According to Ferguson (1976), the main characteristic of thrombocytes is the large number of peripheral vesicles in the cytoplasm. He regards the vesicles as analogues of the surface-connecting system of the blood platelets of mammals. The nuclei of the thrombocytes illustrated in Fig. 3 clearly show the typical bilobed shape and arrangement of the chromatin described by Ferguson (1976). It is thought that the vesicles in the cytoplasm start forming from the microtubles once the thrombocyte is activated.

The lymphocytes of the 2 fish species examined in this study show close similarities to those of other fish species (Fey, 1966 b; Ferguson, 1976) as well as those of mammals (Grey & Biesele, 1955; Pease, 1956; Sandborn, 1970).

Carr (1973) set certain criteria to which a monocyte should conform in order to be classified as such. It was found that the monocytes of the 2 fish species studied, as well as those described by Fey (1966b) and Ferguson (1976), fulfilled these requirements. Fey (1966 b) regards the monocytes of fish and those of mammals as homologues.

Ultramicroscopically, the neutrophilic granulocytes are recognizable because of their intracytoplasmic granules. Those found in the catfish are similar to those found in the bream, and both are similar to those of mammals (Grey & Biesele, 1955; Pease, 1956). Ferguson (1976) found that the granules of neutrophilic granulocytes of *P. platessa* have a lamellated appearance, a characteristic seen in neither of the species examined in this study. In addition, Ferguson (1976) found in *P. platessa* what he named a "neutrophil type" that contained granules with a dense fibrillar structure. They are unlike the azurophil granules or the neutrophilic granules found in mammals, but show a resemblance to the Type 3 granules found in human neutrophils (Daems, 1968). This type of neutrophilic granulocyte was not seen in either catfish or bream.

Fey (1966 a & b) distinguishes both neutrophils and heterophils in the species he examined. He regards the neutrophils of carp as being heterophils and states that they can be differentiated from neutrophils by the structure and distribution of the specific granules.

Neither eosinophilic and basophilic granulocytes nor their precursors could be found in the catfish. They are present in the bream, however (Boomker, 1981), and have been described from many fish species (Fey, 1966 b). Ferguson (1976) could not find eosinophils in *P. platessa,* but they are the dominant granulocyte in carp and contain granules with 2 crystalline structures (Smith *et al.,* 1970). The eosinophils of *Lampetra* sp., however, have granules without the crystalline structure (Fey, 1966 b).

The granules of the basophils of *C. carpio* are oval and have a distinct limiting membrane which encloses a granular and finely lamellated matrix (Fey, 1966 b). Ferguson (1976) failed to find basophils in *P. platessa.* Small numbers of basophilic granulocytes have been found in the bream (Boomker, 1981), but neither basophilic nor eosinophilic granulocytes could be identified with the electron microscope.

Ultrastructurally, the plasma cells are easily recognizable by their dilated and well-developed granular ER. The plasma cells found in catfish and bream are identical with those found in carp (Smith *et al.,* 1970), and also with those in mammals (Sandborn, 1970).

A cell intermediate between a lymphocyte and a plasma cell is illustrated in Fig. 12. Smith *et al.* (1970), found similar cells in the pronephroi of carp. According to Smith *et al.* (1970), the intermediate cells show characteristics of both lymphocytes and plasma cells, a finding confirmed in this study especially in the bream. The occurrence of Russell bodies in human plasma cells has been described by Welsh (1960), and a Russell body (X) in a plasma cell of the catfish is illustrated in Fig. 13. It is identical with that illustrated by Welsh (1960).

The macrophages seen in the 2 fish species studied are identical with those of mammals and have the same ultrastructural characteristics.

Explanation of symbols used in the illustrations:

ER—Endoplasmic reticulum
Fs—Phagosome
G—Golgi apparatus
Gl—Glycogen
Hb—Haemocytoblast
L—Lysosome
Lc—Lymphocyte
M—Mitochondrion
Mp—Macrophage
Mt—Microtubules
N—Nucleus
Nc—Nucleolus
Np—Pore in nuclear membrane
Pp—Pseudopod
R—Ribosome
S—Centriole
Sg—Specific granule
V—Vacuole
X—Russell body

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