Int. J. Dev. Biol. 35: 341-344 (1991)

Differentiation of eosinophilic granulocytes of carp (*Cyprinus carpio* L.)

NADA KRALJ-KLOBUČAR

Department of Zoology, Faculty of Natural Sciences, Zagreb, Republic of Croatia, Yugoslavia

ABSTRACT Electron-microscopic studies were conducted to observe ultrastructural changes during differentiation of eosinophilic granulocytes in carp (*Cyprinus carpio* L.). Differentiation at the myelocyte stage was found to relate to specific granules made of dense and light fields. By maturation they assume a mosaic-like texture and in each granule of mature granulocytes, a light, central «internum» and a peripheral dense wrapper can be distinguished. The activity of peroxidase and acid phosphatase is located in the internum and of peroxidase in the wrapper of the granules.

KEY WORDS: *eosinophilic granulocyte, carp, differentiation*

Introduction

Electron-microscopic studies and histochemical techniques have shown that the granules of eosinophilic granulocytes contain the enzymes acid phosphatase and peroxidase (Seeman and Palade, 1967; Miller and Herzog, 1969; Bainton and Farquhar, 1970; Presentey *et al.*, 1980). In the eosinophils of various animals the enzyme content varies, as does the form of the granules (Fey, 1966; Miller *et al.*, 1966; Scott and Horn, 1970; Cannon *et al.*, 1980). In fishes the presence of eosinophilic granulocytes was confirmed by recent electron microscopic investigations (Turdyev, 1976; Bielek, 1981). In our studies we observed the differentiation of the carp's eosinophilic granulocytes, the ultrastructural composition of their granules, and related cytochemical reactions.

Results

In the kidney of the carp (*Cyprinus carpio* L.), among secretory tubules, the located tissue constitutes various developing stages of blood cells. Among a great number of heterogeneous cells we determined the cells corresponding to the developmental series of eosinophilic granulocytes. Special attention was given to transitory stages when in the cell there are granules of different differentiation degrees incorporating the characteristics of neighboring stages in the process of maturation.

The analysis of the ultrastructural morphology of the granules of eosinophilic granulocytes suggests a characteristic sequence in their differentiation.

Dense, homogeneous granules which differentiate at the promyelocyte stage (Fig. 1) and are also numerous in early myelocytes correspond to primary granules. Their number in later stages, probably due to the cell division, is considerably reduced.

The second type of granule that starts forming in early myelocytes corresponds to specific granules. In early myelocytes the granules are large, of a fluffy content, and only partially condensed (Fig. 2). At the myelocyte stage the cytoplasm is filled with specific granules of various condensation degrees. In the Golgi zone there are initial developing forms of specific granules built of a dense and separate light zone. Towards the cell periphery the granules grow in size, their dense and light content intermingles, and they assume a mosaic-like texture (Figs. 3 and 4). In metamyelocytes, along with the granules of a mosic-like texture, specific granules with a round central fluffy zone surrounded with a dense wrapper are formed (Fig. 5). In mature eosinophilic granulocytes all granules have a central zone, the «internum», surrounded by a dark wrapper which is often elongated at one end, giving the granules an elliptic form (Fig. 6).

The activity of peroxidase is present in immature and mature specific granules. In mature granules it is located in the peripheral wrapper but also in the central «internum» (Fig. 7).

Acid phosphatase is found in the Golgi lamellae, in the extragranular cytoplasm, and in the «internum». The reaction is particularly pronounced in the border-line zone of the «internum», in the direction of the wrapper (Fig. 8). Since, along with the formed granules, the granules of the mosaic-like texture are also present in the cell, it is concluded that the cell is a late myelocyte, with possibly incompletely condensed granules.

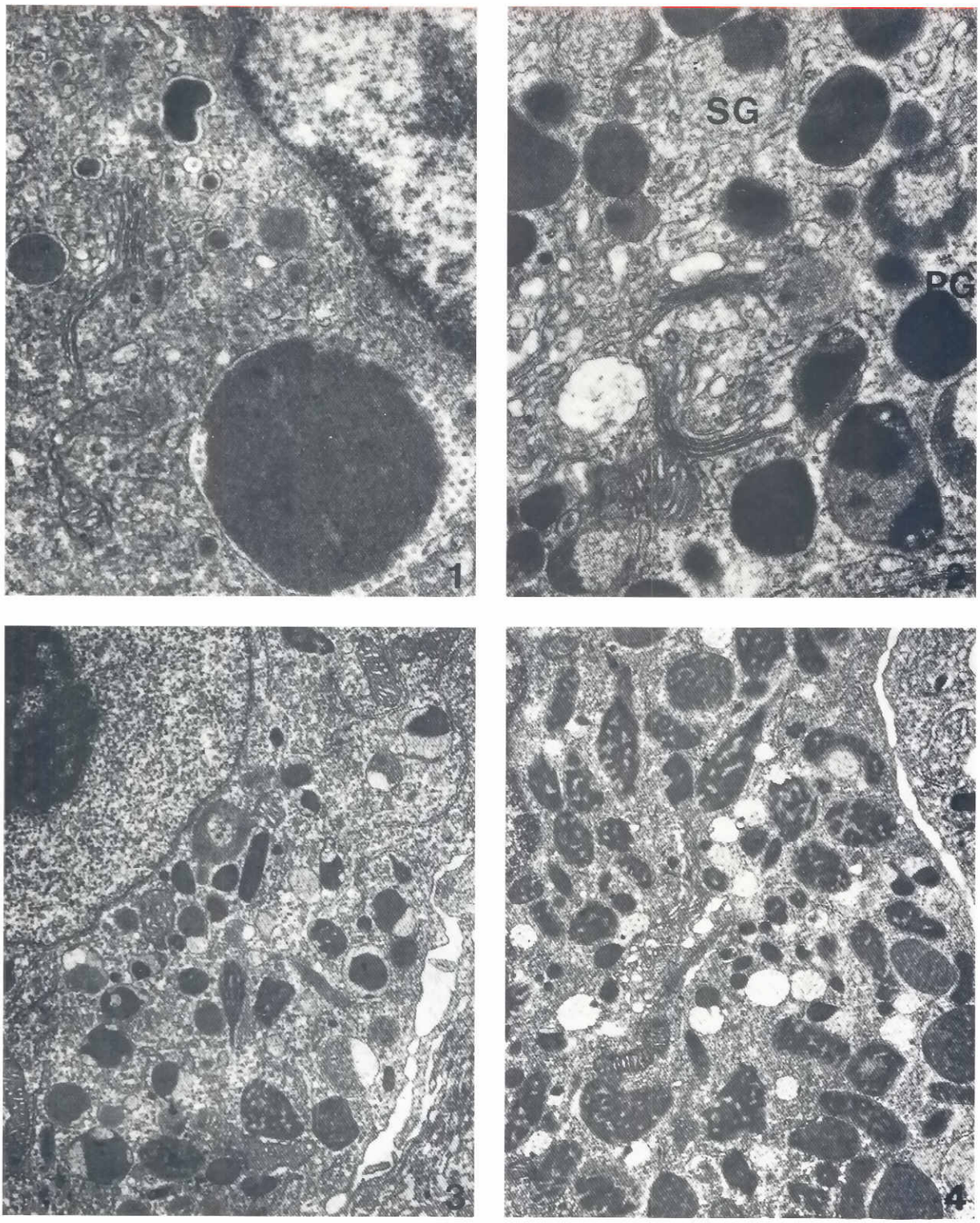
Discussion

Changes were observed in the ultrastructural composition of granules during the maturation of eosinophilic granulocytes of carp

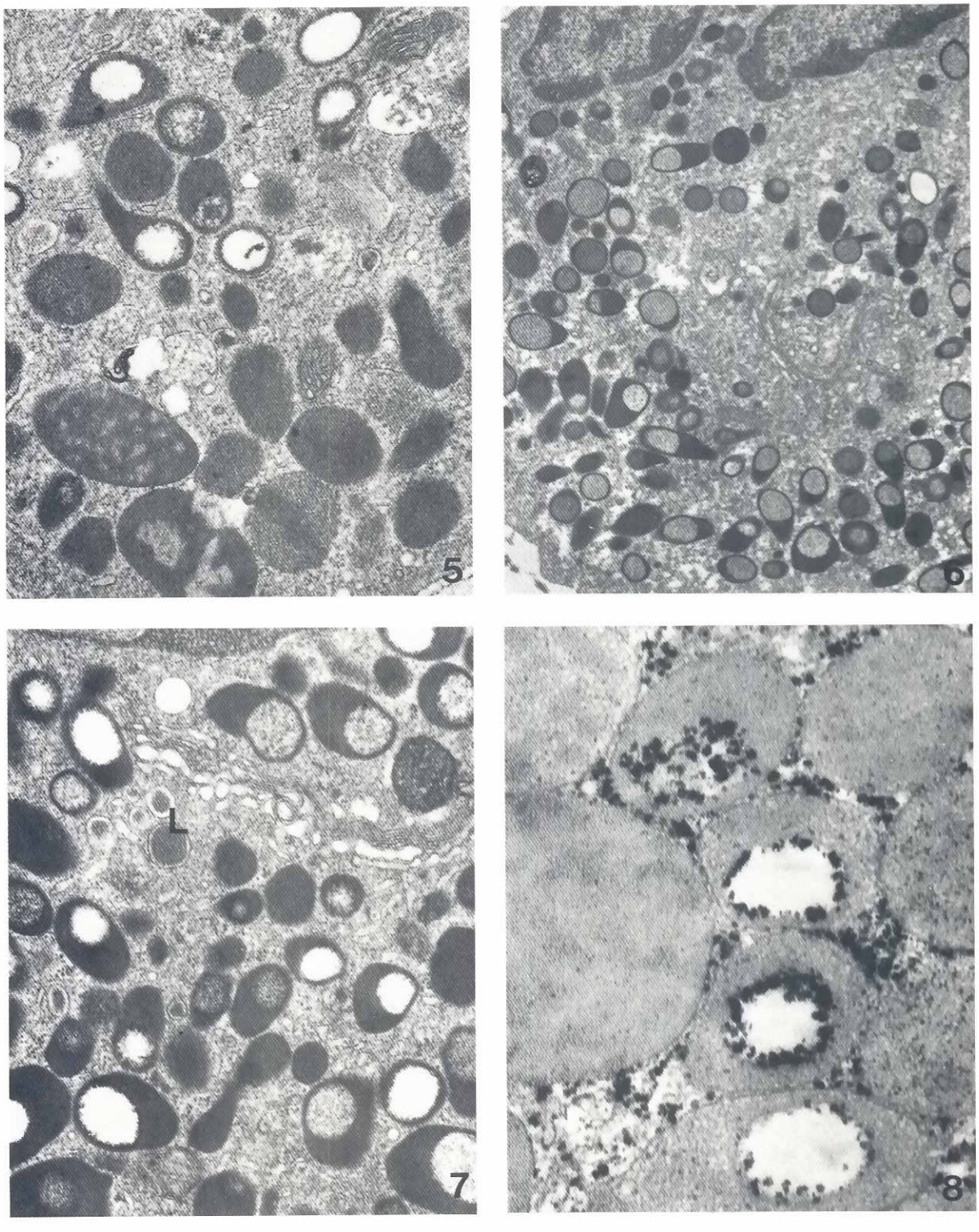
Abbreviations used in this paper: PG, primary granules; SG, secondary granules; L, lysosomes.

***Address for reprints**: Department of Zoology, Faculty of Natural Sciences, Rooseveltov trg 6, 41000 Zagreb, Croatia, Yugoslavia. FAX: 38-41-432.526

0214-6282/91/$03.00



Figs. 1-4. Electron micrographs represent stages of eosinophil granulocyte maturation. (1) *Progranulocyte, probably eosinophilic. A large nucleus and numerous granules in the vicinity of the Golgi zone. Tiny vesicles are mixed with the matrix of large primary granules. x30,000.* **(2)** *Promyelocyte. Along with the primary granules (PG) with a dense, homogeneous content, there are also larger granules with a delicate, fluffy content which is only partly condensed (SG, secondary granules). x28,500.* **(3)** *Myelocyte. Secondary granules with content condensed of dark and light areas, and also the granules of a fine mosaic-like structure. x9200.* **(4)** *Myelocyte. In the central part, along with the Golgi zone, there are polar built granules composed of a dense and a light half. In the cell periphery the granules are of a mosaic-like texture, with intermixed dense and light areas. x14,400.*



Figs. 5-8. Electron micrographs represent stages of eosinophil granulocyte maturation. (1) *Metamyelocyte. The cell contains the granules of a mosaic-like texture, but also granules with a round, vacuolar «internum». The central «internum» is clearly divided from the peripheral wrapper, its content filled with minute grains which in some granules is extracted, making the «internum» appear empty. x18,500.* **(6)** *Mature eosinophilic granulocyte. All the granules contain a central «internum». x9200.* **(7)** *Mature eosinophilic granulocyte. The activity of peroxidase is located in the wrapper and internum of mature specific granules. Near the Golgi zone there are smaller, peroxidase negative, granules which probably correspond to lysosomes (L). x18,500.* **(8)** *Metamyelocyte. The activity of acid phosphatase is located in the intergranular endoplasmatic reticulum and the «internum» zone of mature specific granules. The activity is particularly pronounced in the border-line zone, in the direction of the wrapper. x81,000.*

(*Cyprinus carpio* L.). Due to the lack of ultrastructural information on the mechanism of granulocyte formation in fishes, our description is similar to that observed in higher vertebrates. Promyelocytic, myelocytic, and metamyelocytic stages for the different granulocytic lines have been reported. The differentiation includes the formation of characteristic granules of each type (Bainton and Farquhar, 1970). At the progranulocyte stage the primary granules are formed and at the myelocyte stage the secondary or specific granules are formed. Mature specific granules of eosinophilic granulocytes of the carp contain a characteristic «internum».

For specific granules a certain enzymatic composition is characteristic. During the differentiation of eosinophilic granulocytes in rabbits and rats, the activity of peroxidase is recorded in immature and mature specific granules, while the activity of acid phosphatase was found only in immature specific granules (Bainton and Farquhar, 1970).

The enzymatic activity of the granules of the carp's eosinophilic granulocytes corresponds that activity of specific granules. Acid phosphatase is present in the central «internum» and the peripheral wrapper of the granules. A crucial role in proving the presence of peroxidase is played by the medium pH (Kelenyi and Nemeth, 1969), the positive activity having been found at pH 9, while the lower values showed no activity. The results of Bielek (1981) are in agreement with this, indicating a significant activity of the peroxidase reaction at pH 9 in the eosinophilic granulocytes of the three kinds of fishes, the carp included. Our results also conform with this.

The «internum» zone of carp's specific granules contains the activity of both acid phosphatase and peroxidase, thus showing its proteinic nature. The «internum» cannot be compared with the crystal-like (Bainton and Farquhar, 1970) or the myelin-like cylindrical structure (Presentey *et al.*, 1980) in the core of the animal's eosinophilic specific granules, since in them neither the activity of acid phosphatase nor the activity of peroxidase was determined. The «internum» corresponds to the structure described by Kelenyi and Nemeth (1969) as a «hole». These authors suggest that it may represent the sites of the interaction of the granules and the cytoplasm. Our studies suggest that the internum is neither a «hole» nor a part of the cytoplasm but the integral part of specific granules.

Materials and Methods

Observations were made on hemopoietic tissue of the carp (*Cyprinus carpio* L.) located between the kidney tubules. Tissue was fixed in 5% glutaraldehyde in 0.2 M Na-cacodylate buffer pH 7.2 at 4°C for 3-4 hours. Cryostat sections 30 micra thick were incubated 30 min at 25°C in medium for peroxidase (Graham and Karnovsky, 1966) containing 2% solution of 3.3'-diaminobenzidine tetrahydrochloride (DAB) and 1% H_2O_2. Incubations were carried out at pH 7.6 and pH 8.9. Controls consisted of adding 0.01 M KCN to the medium or of omitting the substrate.

Acid phosphatase was demonstrated on sections incubated in a medium (Barka, 1964) with ß-glycerophosphate and lead nitrate at pH 5.2, for 60 min at 37°C. Controls were carried out by adding 0.01 M NaF to the medium.

After incubation, the sections were embedded in EPON 812 and cut by ultramicrotom Reichert Om-U/2 at 60-90 nm. They were stained with 4% uranil acetate and 1.3% alkaline lead citrate (Reynolds, 1963). The control sections were observed unstained. The observations were carried out by a Zeiss EM 9 electron microscope.

References

BAINTON, D.F. and FARQUHAR, M.G. (1970). Segregation and packaging of granule enzymes in eosinophilic leukocytes. *J. Cell Biol. 45:* 54-73

BARKA, T. (1964). Electron histochemical localization of acid phosphatase activity in the small intestine of mouse. *J. Histochem. Cytochem. 12:* 229-239.

BIELEK, E. (1981). Developmental stages and localization of peroxidatic activity in the leucocytes of three teleost species *(Cyprinus carpio* L.; *Tinca tinca* L.; *Salmo gairdneri* Richardson). *Cell Tissue Res. 220:* 163-180.

CANNON, M.S., MOLENHAUER, H.H., EURELL, T.E., LEVIS, D. H., CANNON, A.M. and TOMPKINS, C. (1980). An ultrastructural study of the leukocytes of the channel catfish, *Ictalurus punctatus. J. Morphol. 164:* 1-23.

FEY, F. (1966). Vergleichende Hamocytologie niederer Vertebraten. III. Heterofilen, Eosinofilen und Basofilen Granulociten. *Folia Haematol. 86:* 1-20.

GRAHAM, R.C. Jr. and KARNOVSKY, M.J. (1966). The early stages of absorption of injected horseradish peroxidase in the proximal tubules of mouse kidney: ultrastructural cytochemistry by a new technique. *J. Histochem. Cytochem. 14:* 291-302.

KELENYI, G. and NEMETH, A. (1969). Comparative histochemistry and electron microscopy of the eosinophil leucocytes of vertebrates. I. A study of the avian, reptile, amphibian and fish leucocytes. *Acta Biol. Acad. Sci. Hung. 20:* 405-422.

MILLER, F., De HARVEN, E. and PALADE, G.E. (1966). The structure of eosinophil leukocyte granules in rodents and in man. *J. Cell Biol. 31:* 349-362.

MILLER, F. and HERZOG, V. (1969). Die Lokalisation von Peroxidase und saurer Phosphatase in eosinophilen Leukocyten während der Reifung. Elektronenmikroskopisch-citochemische Untersuchungen am Knochenmark von Ratte und Kaninchen. *Z. Zellforsch. Mik. Ana. 97:* 84-110.

PRESENTEY, B., JERUSHALMY, Z., BEN-BASSAT, M. and PERK, K. (1980). Genesis, ultrastructure and cytochemical study of the cat eosinophil. *Anat. Rec. 196:* 119-127.

REYNOLDS, E.S. (1963). The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol. 17:* 208-212.

SCOTT, R. and HORN, R.G. (1970). Fine structural features of eosinophil granulocyte development in human bone marrow. Evidence for granule secretion. *J. Ultrastruct. Res. 33:* 16-28.

SEEMAN, P.M. and PALADE, G.E. (1967). Acid phosphatase localization in rabbit eosinophils. *J. Cell Biol. 34:* 745-756.

TURDYEV, A.A. (1976). The ultrastructure of the blood granulocytes of the carp fishes. *Arhiv Anat. Gistol. Embryol. 58:* 15-21.