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# THE HORSE EOSINOPHIL AS A MODEL LEUCOCYTE FOR MORPHOLOGICAL AND CYTOCHEMICAL STUDIES*

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## ABSTRACT

Mammalian eosinophil leucocytes contain specific lysosome- and peroxisome-like cytoplasmic granules that have important implications in inflammation, allergy and the bactericidal response to microorganisms. Several highly cationic proteins are responsible for the intense acidophilia of eosinophil granules, which also have a characteristic ultrastructure consisting of a dense core and an external matrix. The granules of horse eosinophils are large in size and are easily recognizable as individual elements by light microscopy. These characteristics make these cells an adequate model for cytochemical analyses and precise light-electron microscopical correlations. In this work, the selective and cytochemical staining and fluorescence reactions applied to horse blood smears are reviewed, and show that eosinophil granules are very suitable structures for testing new light microscopical methods. Transmission electron microscopy has shown that horse eosinophil granules have an electron-dense, non-crystalline core surrounded by a less dense external matrix, although there is considerable heterogeneity in their ultrastructural morphology. Cytochemical results show a ring-like pattern for some staining and fluorescence reactions (glutaraldehyde-oxidized hematoxylin, fast green FCF, 1-hydroxy-3,6,8-pyrene-trisulfonate at pH 12, Timm sulfide-silver method), indicating that the external matrix of horse eosinophil granules contains metal cations and cationic proteins with high isoelectric points.

**Key words:** Cytochemistry, fluorescence microscopy, horse eosinophils, leucocyte morphology, staining reactions

## INTRODUCTION

The specific granules of mammalian eosinophil leucocytes contain typical lysosomal acid hydrolases [4,9,67], and are now considered as a characteristic organelle with the features of lysosomes and peroxisomes [69]. The acidophilic nature of eosinophil granules (isoelectric point of about pH 11; see Kelenyi *et al.* [32]) originates from their content of strongly cationic proteins [5,9,26], with their name corresponding to the intense stainability with eosin.

In the case of human eosinophil leucocytes, arginine-rich major basic protein (MBP, isoelectric point at pH 10), arginine-rich eosinophil cationic protein (ECP, isoelectric point at pH 11), eosinophil-derived neurotoxin (EDN), and strongly basic eosinophil peroxidase (EPO) are the most cationic proteins of the granule [7,20,30,33,65]. The occurrence of these (or very similar) basic proteins in granules from other mammalian eosinophils (e.g. horse [5,8,43,]) also accounts for their well known acidophilia.

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*Dedicated to Prof. Dr. Benedicto de Campos Vidal on his 75th birthday.

Eosinophils can secrete their granule contents, including the bactericidal EPO, and release hydrogen peroxide ($H_2O_2$) extracellularly. The EPO-$H_2O_2$-halide (iodide, bromide, or chloride) system exerts a potent cytotoxic effect upon a variety of target cells [30], and also initiates mast cell degranulation [14]. Interestingly, eosinophils and mast cells are closely associated in areas of allergic reactions. Indeed, some of the chemical mediators released by mast cells after stimulation attract eosinophils and may account for the local eosinophilia that occurs at the site of mast cell degranulation [6]. In skin mastocytosis, mast cells, together with a large number of eosinophils, accumulate in the dermal connective tissue around blood vessels, and the use of selective fluorochromes allows easy recognition of both cell types in this pathological condition [46].

The specific granules of eosinophil leucocytes have a characteristic appearance in electron microscopy, with a limiting membrane enclosing a matrix of medium electron density (*externum*) and a core of higher electron density. This basic pattern was first observed in human eosinophils [2] and then repeatedly found in a number of mammalian species [7,9,29,38]. The central dense mass has been termed

the *internum* or, because it often shows a crystalline appearance, the crystalloid or crystalline inclusion. Interestingly, no morphological differentiation has been reported to the granules of horse eosinophils [11,27], possibly because of their high electron density and/or the use of an inadequate methodology. However, more detailed studies have now revealed that horse granules show both the matrix and a core component [56].

Compared with eosinophil granules from other mammalian species, horse eosinophils have only occasionally been used in microscopic studies [4,27,56]. These cells have the advantage that they contain extremely large granules [4,5,43,56,63]. These cells are easily recognizable as individual elements, thereby allowing a very detailed observation of their structure and cytochemical reactivity by light microscopy.

## METHODOLOGICAL ASPECTS

Because of their availability and easy preparation, blood cell smears are a suitable microscopical test system for designing, developing and applying new staining and fluorescence reactions. Several methods were used here to analyze the morphology and staining affinity of horse eosinophils, with some comparisons being made with human and rat eosinophils, as well as with acidophilic chicken heterophils. Direct observation of blood cells using phase contrast microscopy was done with methanol-fixed smears. Selective staining techniques using several dyes, fluorochromes and reagents have been applied to examine the morphology and affinity of eosinophils. The synthesis, chemistry and properties of compounds (mainly dyes and fluorochromes) that are commonly used in research on blood cells are described in detail in several books and reviews [1,22-25,28,35,42,68].

### *General staining and fluorescence methods*

May-Grünwald-Giemsa (MGG) staining was applied to smears as described elsewhere [21], in some cases after prior fixation with absolute methanol for 2 min followed by treatment with 2% sodium citrate or 0.2 M EDTA for 2 - 8 h to remove metal cations. Anionic dyes and fluorochromes were used after fixation with absolute methanol for 2 min and air-drying. Eosin Y, indigocarmine, acid fuchsine, nuclear fast red, and fast green FCF were applied as 0.5 mg/ml solutions in distilled water for 5 min at room temperature (RT), and the slides observed under immersion oil [48,59]. In some cases, anionic dyes were applied in the presence of NaCl (from 0.062 to 2 M) [59]. Fluorochromes such as saffron (used as a saturated alkaline solution) [62], mercurochrome (2,7-dibromo-4-hydroxymercuri-fluorescein) [18], phenyl fluorone (9-phenyl-2,3,7-trihydroxy-6-fluorone) [10], oxidized hematoxylin [50], morin (3,5,7,2',4'-pentahydroxyflavonol, used as a saturated aqueous solution) [47], 1-hydroxy-3,6,8-pyrene-trisulfonate (HPTS, 10 μg/ml for 5 min) [60], thioflavine S, and primuline [31] were also applied to methanol-fixed horse blood smears. The preparations were observed directly under immersion oil or after mounting in DePeX.

### *Fluorogenic reactions*

Selective fluorogenic reactions for eosinophil granules were done using fluorescamine and 2-methoxy-2,4-diphenyl-3(2H)-furanone (MDPF) (0.5 ml of 0.5 mg/ml solution of fluorescamine or MDPF in acetone added to methanol-fixed blood smears previously covered with 0.5 ml of 0.05 M borate buffer at pH 9) [51,55], isatin (0.1% in absolute ethanol for 30 min at RT) [61], 3-amino-1,8-naphthalic anhydride (ANA, 0.25% in dimethylformamide for 30 min at RT) [53], coupled tetrazonium method using fast blue B salt and 1-naphthol, [16], N-bromosuccinimide (NBS, used as a fresh 10 mM aqueous solution for 10 min at RT) [37], 9,10-phenanthrenequinone (PQ, 0.1% in alkaline ethanolic solution for 30 min at RT) [36], and conversion of indigocarmine to the fluorescent leuco derivative by alkaline reduction with 0.5 mg of sodium borohydride/ml at pH 10 for 30 min [50].

### *Glutaraldehyde-oxidized hematoxylin*

After fixation in 2% glutaraldehyde in phosphate-buffered saline (PBS), horse blood smears were stained with a spontaneously oxidized 0.1% solution of hematoxylin in distilled water for 30 min, washed in tap water, air dried and observed under immersion oil or after mounting in DePeX. This simple and original staining technique allowed an easy and detailed visualization of the morphology of granules.

### *Timm sulfide-silver method*

The Timm sulfide-silver method [15] was applied to horse blood smears to demonstrate the presence and localization of metal cations (e.g. $Zn^{2+}$) in eosinophil

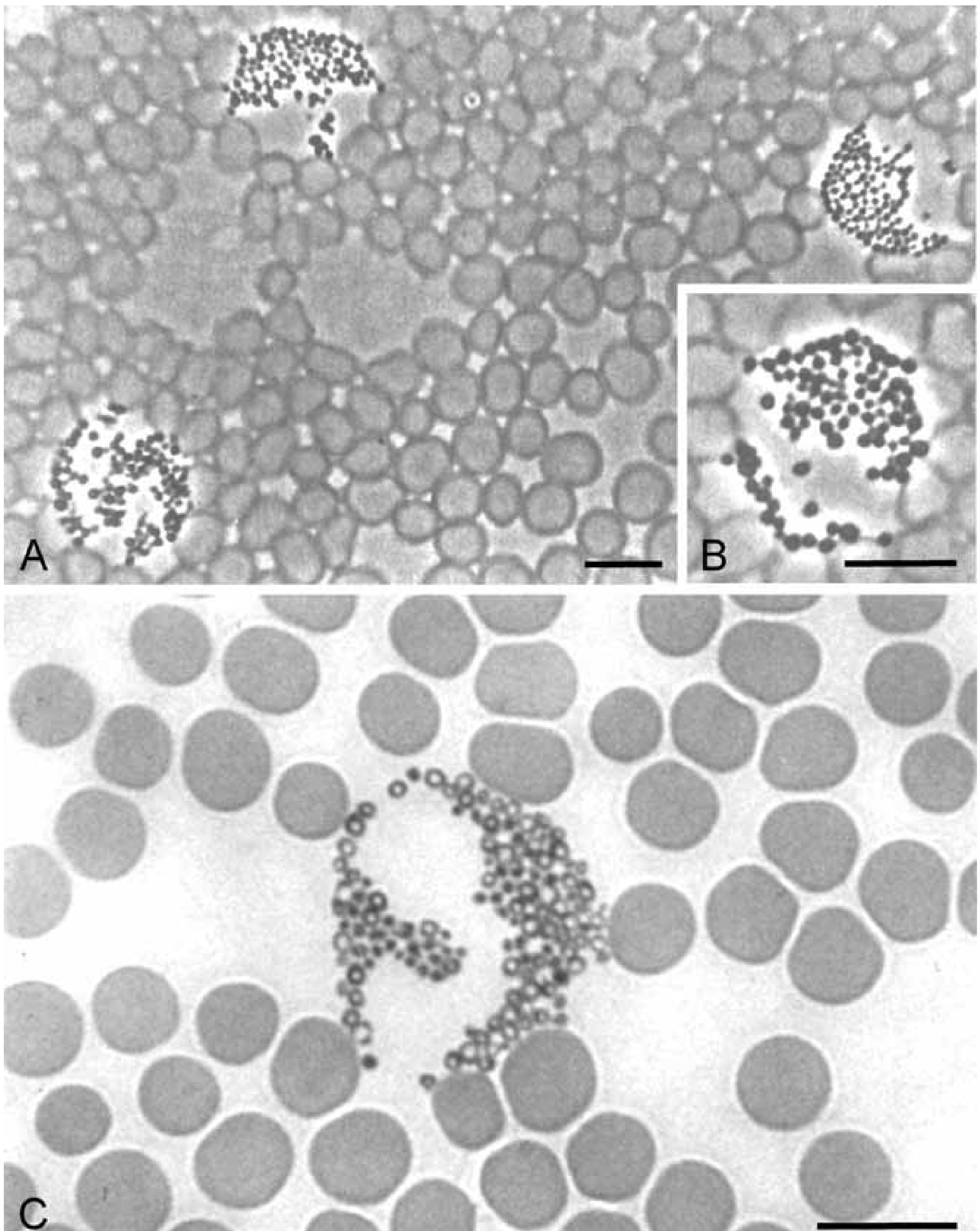


**Figure 1.** (**A**) Panoramic view of a horse blood smear observed by phase contrast microscopy under immersion oil after fixation in methanol. The three conspicuous granulocytes are eosinophils. (**B**) Eosinophil leucocyte at higher magnification (phase contrast). (**C**) Horse blood smear fixed with glutaraldehyde followed by staining with oxidized hematoxylin (bright field illumination). Scale bars: 10 µm.

granules [63]. After fixation in 5% glutaraldehyde containing 1% potassium sulfide ($K_2S$) for 2 h at RT, the smears were post-treated for 1 h with 1% $K_2S$, washed and incubated with a solution of silver nitrate (containing gum arabic as protecting colloid) for 30 min at 40ºC. After treatment with a photographic fixing solution, the smears were examined by light and scanning electron microscopy.

*Scanning and transmission electron microscopy*

Horse eosinophils were also studied by scanning (SEM) and transmission (TEM) electron microscopy. Although the detection of secondary electrons (SEs) is the most common imaging mode to examine the surface structure by SEM, detectors of backscattered electrons (BSEs) are now expanding the biological use of SEM because of the high atomic number (Z) contrast provided by BSE detection [39,64]. After applying the ammoniacal silver method for lysine- and arginine-rich basic proteins [45] to glutaraldehyde-fixed blood smears, silver deposits in eosinophil granules were visualized by BSE imaging using a Robinson detector and identified by energy-dispersive X-ray microanalysis (XRMA). Since procedures to preserve the cell morphology (e.g. critical-point drying) were not used, the SEM images did not represent the actual volume and surface properties of the blood cells, but instead they showed a morphology very similar to that commonly seen in light microscopy [54,63]. For TEM studies, small samples of horse blood clots were fixed in 2.5% glutaraldehyde in phosphate buffer at pH 7 for 2 h at 4ºC, followed by 1% osmium tetroxide for 2 h at 4ºC. After dehydration in acetone, samples were embedded in Epon 812, and thin sections were contrasted with uranyl acetate and lead citrate as usual.

## MORPHOLOGICAL STUDIES

The large size and high refringence of granules that almost completely fill the cytoplasm of horse eosinophils are easily seen in unstained smears observed under phase contrast (Fig. 1A,B). A pale greenish yellow autofluorescence has been reported for eosinophil granules from horse and other mammalian species under violet-blue excitation [19,49]. Therefore, when eosinophils are expected to be present, careful controls are necessary in order to adequately evaluate immunofluorescence results. The presence of unsaturated fatty acids and Schiff base reaction products derived from the interaction of lysosomotropic amines with aldehydes formed by lipid oxidation could contribute to the autofluorescence of eosinophil granules.

Numerous anionic dyes have been used in morphological analyses of eosinophils, most of them belonging to the hydroxy-xanthene, indigo, anthraquinone, azo, and triarylmethane groups. Since the studies of Romanowsky [68], the combination of several cationic thiazines with eosin Y has been a common practice in hematological staining (e.g. May-Grünwald, Giemsa), and is responsible for the red-orange color of eosinophil granules. The extraction of metal cations with chelating agents (sodium citrate, EDTA) before staining with MGG or anionic dyes alone, results in a strikingly enhanced acidophilia of eosinophils because of the unmasking of metal chelating amine and imidazole groups of lysine and histidine, respectively [21,47,59]. This feature should be taken into account for the correct evaluation of the degree of acidophilia when staining reactions are done on blood smears.

The staining sequence glutaraldehyde-oxidized hematoxylin produces a strong color in large equine eosinophil granules which, under bright field illumination, appear as characteristic ring structures (a dark blue-stained annular region surrounding an unstained core) (Fig. 1C). Erythrocytes show moderate staining whereas chromatin is unstained. The mechanism of this staining reaction is not well known, but it is possible that hematoxylin either reveals heavy metal cations or react through its *o*-hydroxy-quinone group with the guanidine group of arginine [35]. Interestingly, a similar ring-like pattern is also seen after staining with the anionic dye fast green FCF at alkaline pH (unpublished observations).

The high sensitivity of fluorescence techniques had led to increased interest in the development of new fluorescence reactions [25,28,46]. In this context, eosinophil leucocytes can be used as suitable models to test the design and performance of selective fluorochromes, such as the natural dyes morin [47] and saffron [62].

The weakly acid natural dye morin induces a yellow (metachromatic) fluorescence in acidophilic leucocytes under violet-blue excitation. The dense core of avian heterophil leucocytes shows a higher emission than the fusiform matrix [47], whereas avian and mammalian eosinophils present a rather

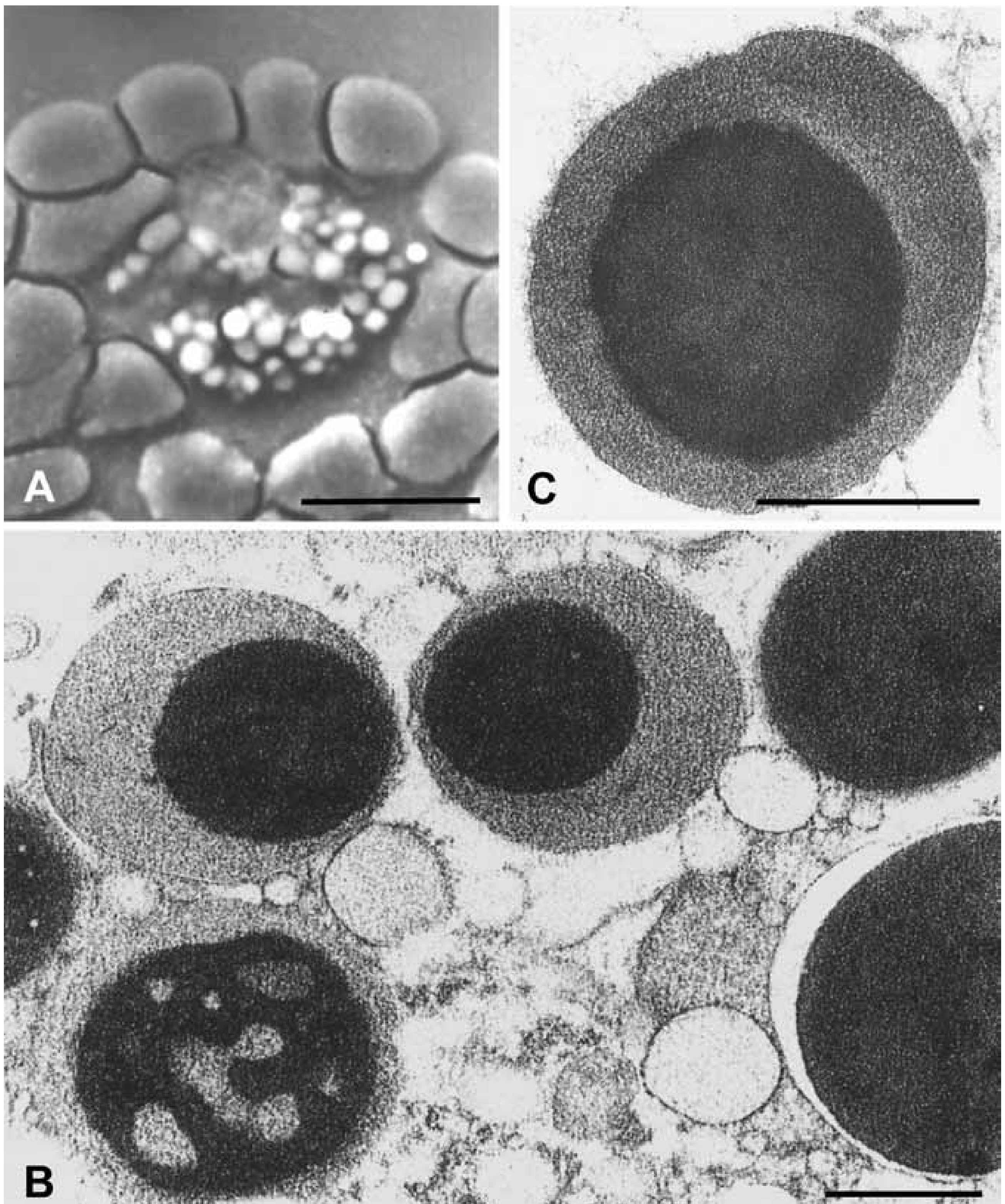


**Figure 2.** (**A**) Scanning electron micrograph showing a backscattered electron image of a horse eosinophil stained with ammoniacal silver for the visualization of basic proteins. Cytoplasmic granules appear bright because of the high atomic number contrast provided by silver deposits. Scale bar: 10 µm. (**B**) Electron micrograph of a horse eosinophil showing the cytoplasmic granules. Some morphologically different types of granules can be seen. (**C**) Electron micrograph of a typical horse eosinophil granule at greater magnification, showing the core and the external matrix. Scale bars in (B) and (C): 0.5 µm.

uniform fluorescence. Likewise, the polyene dye saffron also induces a bright yellow-green emission in eosinophil and heterophil granules under violet-blue excitation [62]. Although saffron is a neutral ester, it is easily hydrolyzed in alkaline media to yield the polyene-dicarboxylate derivative [23,35].

SEM imaging by the detection of SEs shows mainly surface features, whereas the detection of BSEs provides information on the atomic number (Z contrast; the higher the atomic number the greater the backscattering of electrons). The BSE signal allows the visualization of structural details that are often missing in SE imaging. Since the Robinson detector simultaneously provides topography and Z contrast, it is very suitable for SEM studies of biological samples [39,54,64].

Light microscopical observation of horse eosinophils stained with ammoniacal silver for basic proteins reveals a strong positive reaction (dark brown) in eosinophil granules. When observed by SEM using the Robinson detector (Fig. 2A), the large granules clearly show the increased brightness (high Z contrast) induced by the selective deposition of silver [54].

TEM of horse eosinophils after glutaraldehyde-osmium fixation and uranyl-lead contrasting reveals that the granules (about 1-2 µm in diameter) have a limiting membrane, an electron-dense and often eccentric core, and a less dense matrix (Fig. 2B,C). There is considerable heterogeneity in the structure of the granules, with the core appearing either compact or vacuolated, and the matrix showing moderate or very low electron density [56]. This polymorphism could be related to the maturation or functionality of horse eosinophils.

There have been few attempts to identify the ultrastructural distribution of basic proteins within the eosinophil granule by analyzing their affinity for anionic electron stains. This is an interesting aspect to be studied because (a) the acidophilic character of these granules in light microscopy is well known [5,21,43,59], (b) in electron microscopy, eosinophil granules show two well-differentiated regions, namely the core and the external matrix [9,29,38], (c) granules from most mammalian eosinophils are too small to permit a clear identification of these two regions by light microscopy (although some pictures of MGG-stained human eosinophils show ring-shaped granules [9]), and (d) most electron contrasting agents are heavy cations, and no electron dense anions are routinely used to contrast samples for examination by electron microscopy [57].

In glutaraldehyde-fixed rat eosinophils, ethanolic -phosphotungstic acid (E-PTA) selectively contrasts the external matrix of granules, leaving the core uncontrasted [58]. The highly basic proteins ECP, EDN and EPO, and other components such as phospholipids and metal cations (e.g. $Zn^{2+}$) are located in the granule matrix [7,12,38,40,41,63], whereas the crystalline core contains the cationic MBP [40]. Therefore, components of the polycationic matrix could be responsible for the selective E-PTA contrast, and the occurrence of an uncontrasted core may be explained by the removal of some basic proteins (e.g. the labile MBP [13]) under the strongly acid conditions imposed by E-PTA treatment [29,58].

## SELECTIVE AND CYTOCHEMICAL STAINING

The basic proteins of eosinophil granules (responsible for their acidophilia [9,12,26,32]) represent a rigid macromolecular matrix in which anionic dyes and fluorochromes bind predominantly by ionic attraction to cationic amino acid residues [59], although hydrophobic, van der Waals, and hydrogen bonds may also be involved in the binding mechanisms. The concept of critical electrolyte concentration (CEC), in which salts (NaCl, $MgCl_2$) are used as competing agents to quantify the intensity of ionic binding, has been widely applied to staining reactions involving cationic dyes [44]. The same principle can be applied in the staining of eosinophil granules by some anionic dyes in the presence of increasing NaCl concentrations [59]. Dye binding is mainly ionic, but since staining was not abolished by 2 M NaCl, residual non-ionic binding mechanisms can not be excluded.

In addition to dyes, several anionic fluorochromes have been used in selective cytochemical studies of eosinophil granules, examples being the benzothiazole fluorochromes thioflavine S and primuline [31], which under UV light induce a bright white-blue emission in horse granules. Eosin Y is a fluorescent dye because of its hydroxy-xanthene ring [3,17], which is also responsible for the fluorescence of other similar dyes such as mercurochrome (2,7-dibromo-4-hydroxymercurifluorescein) [18] and phenyl fluorone (9-phenyl-2,3,7-trihydroxy-6-fluorone) [10]. Eosinophil granules stained by all of these fluorescent dyes show a yel-

low-green or red fluorescence under blue or green excitation, respectively [10,18,46].

The staining sequence hematoxylin-eosin (H-E) is the procedure most widely used to stain paraffin sections, and confers different colors to basophilic chromatin and acidophilic cytoplasm. In this method, oxidized hematoxylin, a weak acid, forms a coordination complex (lake) with a metal ion (e.g. $Al^{3+}$, $Fe^{3+}$), that has high affinity for phosphate polymers such as nucleic acids. However, oxidized hematoxylin alone (lacking metal) also has staining and fluorescence properties [50], that are very similar to those of eosin Y since both dyes contain the same fluorophore (a rigid hydroxy-diarylmethane structure) (Fig. 3). After fixation in methanol, oxidized hematoxylin shows a bright yellow-green or red emission (under blue or green exciting light, respectively) in eosinophil granules (Fig. 4A), but appears red under bright field illumination. These observations agree with those on the hematoxylin-induced fluorescence of chicken heterophil granules [50].

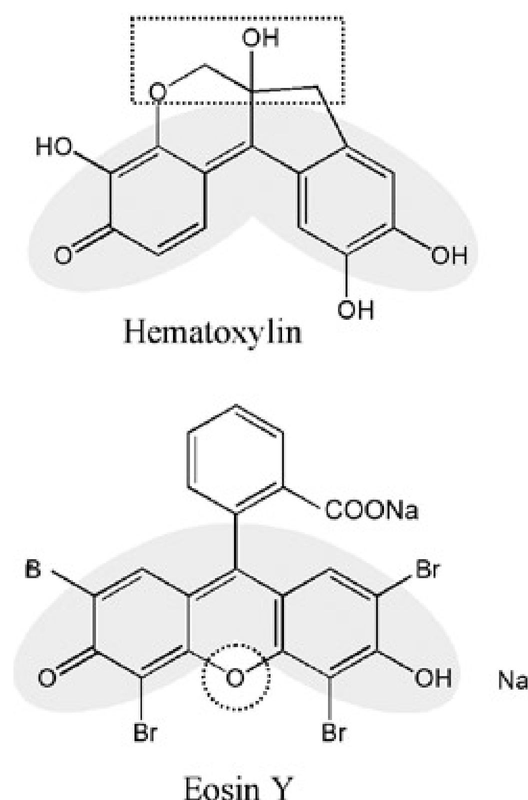


**Figure 3.** Chemical structure of oxidized hematoxylin and eosin Y, showing the hydroxy-diarylmethane structure (**labeled in grey**) common to both dyes, and the bridge atoms (**dotted circle and rectangle**) that make the whole chromophore rigid.

The fluorescence of triarylmethane and azo dyes has been largely overlooked in microscopy, although a suitable emission from acidophilic cell and tissue structures is observed after staining with some anionic dyes from these chemical groups [3,16,42]. The anionic triarylmethane fast green FCF (commonly used to detect basic proteins in bright field microscopy) gives a strong fluorescent reaction in horse eosinophil granules that depends on a rigid binding site on cationic proteins [48].

1-Hydroxy-3,6,8-pyrene-trisulfonate (HPTS) is a sensitive fluorescent pH indicator with industrial and microscopical applications. When applied to methanol-fixed blood smears, HPTS induces a bright green emission of eosinophil granules under violet-blue excitation (Fig. 4B), that is dependent on the pH of the staining solution. At pH 10, the fluorescence of horse granules by HPTS is rather homogeneous, but at pH 12 only a peripheral annular region shows strong fluorescence. At pH 12, only those proteins with the highest isoelectric points remain ionized (positively charged) and therefore, the annular pattern of HPTS fluorescence at this pH value reflects the location of these proteins in horse eosinophil granules [60].

Following treatment with strong reducing agents in alkaline media, the dye indigo is reduced to its yellow, water soluble and fluorescent leuco derivative (indigo white), which has industrial use for dyeing cellulose fibers; when exposed to air indigo white is oxidized again to insoluble indigo [1,22]. Likewise, the anionic dye indigocarmine (indigo-5,5'-disulfonate), commonly used in microscopical techniques [23,24,28,35], can be reduced to leuco indigocarmine. Methanol-fixed blood smears stained with indigocarmine show an intense blue staining of eosinophil granules that bleaches after treatment with reducing agent at alkaline pH, whereas a strong green emission can be observed under violet-blue exciting light [52] (Fig. 4C). This is an interesting cytochemical feature since the conversion of a non-fluorescent dye to a leuco dye fluorochrome is an infrequent phenomenon in microscopical work.

Isatin (indole-2,3-dione) binds easily to compounds with a reactive methylene group such as position 2 of the tryptophan indole ring [61]. When isatin is used in organic solvents, a specific fluorogenic reaction occurs with tryptophan and tryptophan-containing proteins [61]. At the microscopical level, the application of ethanolic isatin to methanol-fixed horse blood smears yields eosinophil granules with

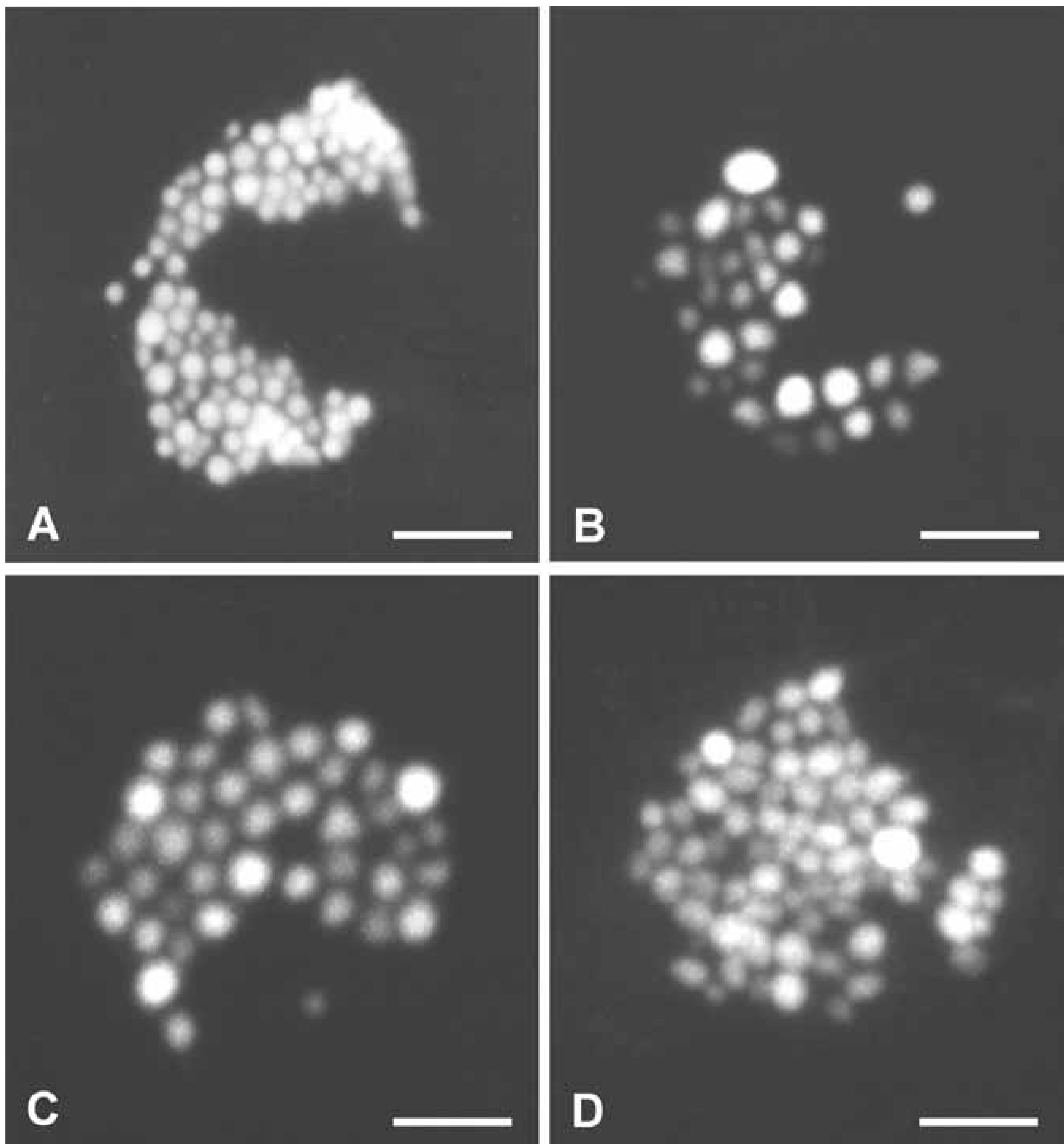


**Figure 4.** Fluorescence micrographs of horse eosinophils after treatment with the following fluorochromes or staining methods: (**A**) oxidized hematoxylin, (**B**) HPTS at pH 10, (**C**) indigocarmine followed by alkaline reduction with sodium borohydride, and (**D**) ethanolic isatin. Scale bars: 5 μm.

a strong green fluorescence under violet-blue excitation (Fig. 4D). Although the chemical structure of the fluorescent product is not yet known, some possibilities are indicated in Figure 5.

The highly reactive protein reagent N-bromosuccinimide (NBS) modifies tryptophan and histidine residues to produce a visible fluorescence under UV light. When applied on methanol-fixed blood smears, NBS induces a white-blue emission in eosinophil and heterophil granules, the intensity of which increases 1.7 times after 1 min of UV excitation [37]. Considering that cationic proteins

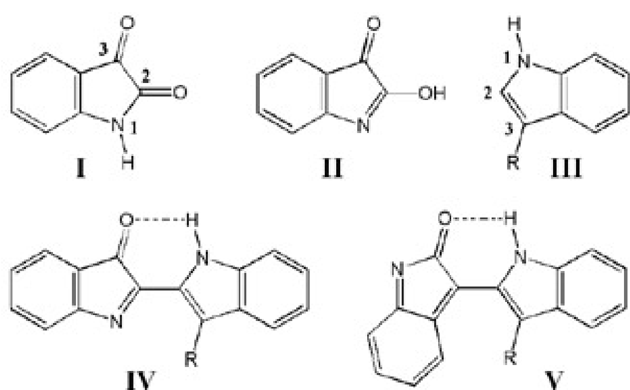


**Figure 5.** Proposed reaction mechanism between the reactants isatin (shown as the keto (**I**) and enol (**II**) tautomers) and tryptophan (**III**, R: lateral chain of the amino acid), giving the indigoid fluorophores (**IV**) or (**V**) as the reaction products between the position 2 of tryptophan indole group and the C2 or C3 atoms of isatin. The configuration of (**IV**) and (**V**) is similar to that of indigo and indirubin derivatives, respectively.

of eosinophil granules have a large amount of tryptophan and histidine residues [12], it is logical to assume that they are responsible for the fluorogenic reaction induced by NBS.

Other selective methods have been used to reveal aromatic amino acids in eosinophil granules. The coupled tetrazonium method for proteins, which identifies tryptophan, histidine and tyrosine, produces a violet bisazo dye product in horse eosinophil granules that also shows deep red fluorescence after excitation with green light [16]. The Ehrlich reagent dimethylamino-benzaldehyde (DMAB), used to demonstrate the indole group, induces ring-shaped staining of tryptophan-containing proteins in eosinophil granules [34].

The amino groups of proteins can be detected by several fluorochromes and reagents, examples being reactive dyes (e.g. FITC, dansyl chloride),

**Figure 6.** Horse eosinophils stained with (**A**) fluorescamine, (**B**) ANA, and (**C**) the Timm sulfide-silver method. Scale bars: 5 μm.

and furanone derivatives such as fluorescamine and MDPF. Furanone compounds are almost specific reagents for the fluorogenic demonstration of primary amines [66], and have been used for the microscopical detection of protein amino groups [55]. Fluorescamine induces a strong, selective, white-blue emission of eosinophil granules under UV excitation (Fig. 6A). Identical results are obtained using MDPF [51].

Methods for synthesizing naphthalimides from 1,8-naphthalic anhydride and aliphatic amines *in vitro* also allow the cytochemical demonstration of protein amino groups *in situ* [53]. The treatment of methanol-fixed horse blood smears with ANA, results in an intense yellow-green emission produced by the resulting naphthalimide fluorophore in eosinophil granules under violet-blue excitation (Fig. 6B).

The guanidine group of arginine can also be detected cytochemically by means a fluorogenic reaction with 9,10-phenanthrenequinone (PQ) [36]. In methanol-fixed horse blood smears, PQ induces a white-blue emission of eosinophil granules under UV light, with the fluorescence becoming higher after continuous irradiation for 3-5 min.

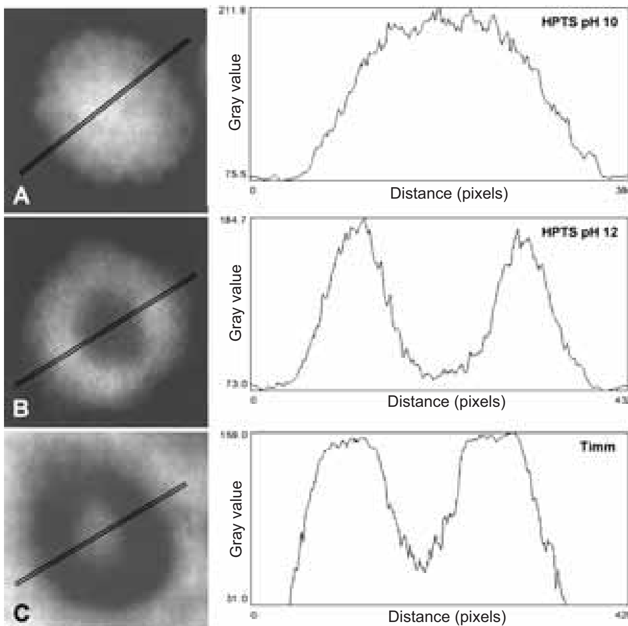


**Figure 7.** On the left, images of individual horse eosinophil granules after staining with HPTS at pH 10 (**A**), HPTS at pH 12 (**B**), and the Timm sulfide-silver method (**C**). On the right, the corresponding densitometric profiles (using the public domain Scion Image Beta 4.02 software [http://rsb.info.nih.gov/nih-image/]) obtained by scanning along the double lines. The densitometric plot of (C) corresponds to a reverse image.

The occurrence of metal cations in eosinophil granules is well known [26,41]. The use of the Timm sulfide-silver method to detect heavy metals [15] yields a positive reaction, in the form of black rings that surround a less stained area at the periphery of horse eosinophil granules (Fig. 6C). This annular region also shows a higher Z contrast when observed by SEM with the Robinson detector, as well as considerable zinc and calcium signals following XRMA [63]. A zinc-containing cationic protein has been found in horse eosinophil granules [8], and the matrix-located human ECP contains 2.5 moles of zinc per mole of protein [65].

Topographic relationships are evident when the results of fluorescence and staining reactions are subjected to image processing and analysis. Densitometric profiles obtained from eosinophil granules clearly indicate that metal cations and proteins with the highest isoelectric points are localized in the peripheral annular region (matrix) of horse granules (Fig. 7).

## CONCLUDING REMARKS

On account of their large size and easy visualization, the cytoplasmic granules of horse eosinophils are a very suitable cytological model for the design and development of new fluorescent probes and fluorogenic reactions. Several selective staining methods induce a characteristic annular staining pattern in horse granules. Using TEM, these granules show a dense, non-crystalline core and a less dense external matrix. This ultrastructural morphology agrees well with light microscopy results showing a ring-shaped reaction after staining with glutaraldehyde-oxidized hematoxylin, fast green FCF, HPTS at pH 12, DMBA, and the Timm method. Together, these findings indicate that the external matrix of horse granules contains metal cations and cationic proteins with very high isoelectric points.

The advantages of using horse eosinophils as a model acidophilic leucocyte for testing selective staining and cytochemical reactions are obvious. The size and structure of horse granules allow a rapid identification and detailed observation under light microscopy, as well as the easy application of new staining and fluorescence techniques that may result in more elaborate cytochemical approaches. The well-defined morphological features of horse eosinophil granules may also be useful in

establishing a topographic correlation between the findings of light and electron microscopy.


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