Electron Microscopical Morphology of Cytoplasmic Granules from Horse Eosinophil Leucocytes

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The structure of specific granules from horse eosinophil leucocytes is still largely unknown. In this work, electron microscopical studies of horse eosinophils reveal that the large cytoplasmic granules contain an external membrane, a matrix of less density, and a dense (non crystalline) core. Round vacuolar inclusions of matrix materials were often observed within the cores. Horse eosinophil granules showed a considerable heterogeneity, and three morphological types could be identified according to structural features of the core and matrix.

The specific granule of mammalian eosinophil leucocytes is a special type of cytoplasmic organelle possessing the nature of both lysosomes and peroxisomes [1]. Horse eosinophils contain very large granules, their size being considerably greater than those of other organisms [2, 3]. As occurs in other mammals, the presence of highly basic proteins in horse eosinophil granules [4, 5] accounts for their well known acidophilia [6, 7]. These big granules can be easily recognized as individual elements, and thus, they are very suitable for light microscopic observations [2, 8] and cytochemical studies [7, 9–13].

Under the electron microscope, the specific granules of mammalian eosinophils show a compact (generally crystalline) core or "internum" surrounded by a less dense matrix or "externum" [14–16]. However, no clear morphological differentiation has been reported to occur in granules of horse eosinophils [17, 18], possibly due to their high electron density and/or the use of an inadequate methodology. The aim of this work is to describe the occurrence of characteristic core and matrix components in horse eosinophil granules.

Smears of horse peripheral blood were made on glass slides. After fixation in methanol for 2 min and air drying, they were stained with May-Grünwald-Giemsa as usual or with 0.1% indigocarmine (Serva) in distilled water for 5 min [11]. Observations were performed in a Zeiss photomicroscope III under bright field illumination. To visualize the granular component by scanning electron microscopy (SEM), air dried and unstained smears (not subjected to critical-point drying) were coated with Au/Pd in a JFC 1100 sputtering device (Jeol) for 3 min, and observed in a Hitachi S-2500 scanning EM operating at 15 kV.

Small samples of horse blood clots were fixed in 2.5% glutaraldehyde in Sörensen's buffer at pH 7 for 2 h at 4 °C, and postfixed in 1% osmium tetroxide in the same buffer for 2 h at 4 °C. After washing in the buffer, samples were dehydrated in acetone and embedded in Epon 812. Thin sections were cut with an LKB Ultrotome III, mounted on copper grids with Formvar, contrasted for 30 min with uranyl acetate (3.75% in 70% methanol) and lead citrate [19], and observed by transmission electron microscopy (TEM) in a Zeiss 109 EM operating at 60 kV.

After May-Grünwald-Giemsa or indigocarmine staining, horse eosinophils showed large and acidophilic granules of about 1–2 μm in diameter (Fig. 1 a). In some indigocarmine stained granules a darker central region could be also observed. When examined by SEM, large and homogeneous cytoplasmic granules were easily identified (Fig. 1 b). As procedures to preserve the cell topography were not used, SEM images did not represent the actual volume and surface of eosinophil leucocytes, but instead they showed cytoplasmic components which were very similar to those commonly seen in light microscopy.

When observed by TEM, horse eosinophil granules showed a limiting membrane, a dense and often eccentric core, and a less dense matrix (Fig. 1 c and 2). Cores appeared generally round, although straight or angular outlines could be also observed. Ordered or crystalline patterns of core

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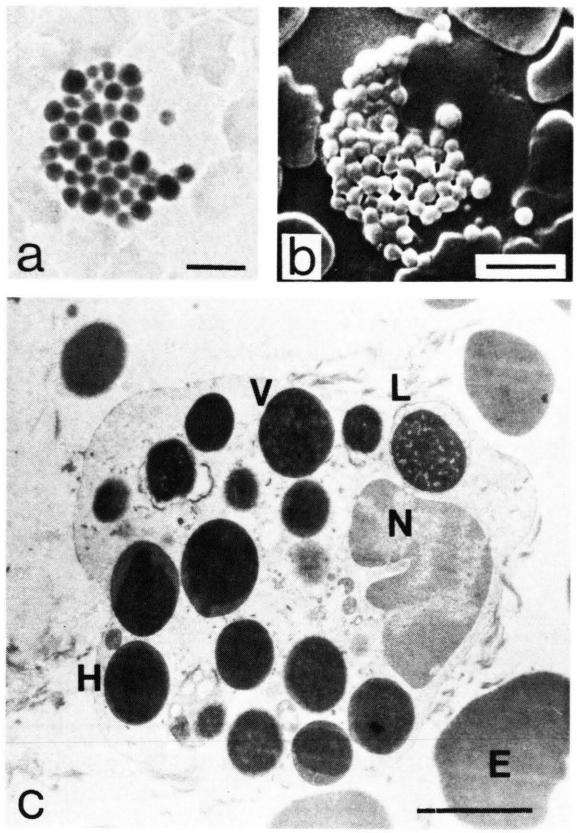

Fig. 1. a: Photomicrograph of a horse eosinophil showing the selective staining of specific granules by indigocarmine. b: SEM image of a horse eosinophil; large granules and the nuclear outline can be seen. c: Ultrathin section of a horse eosinophil showing the cytoplasmic granules as observed by TEM. Morphological types H, V, and L are labelled. N: nucleus; E: erythrocyte. Bars represent 5 μm (a, b) and 2 μm (c).

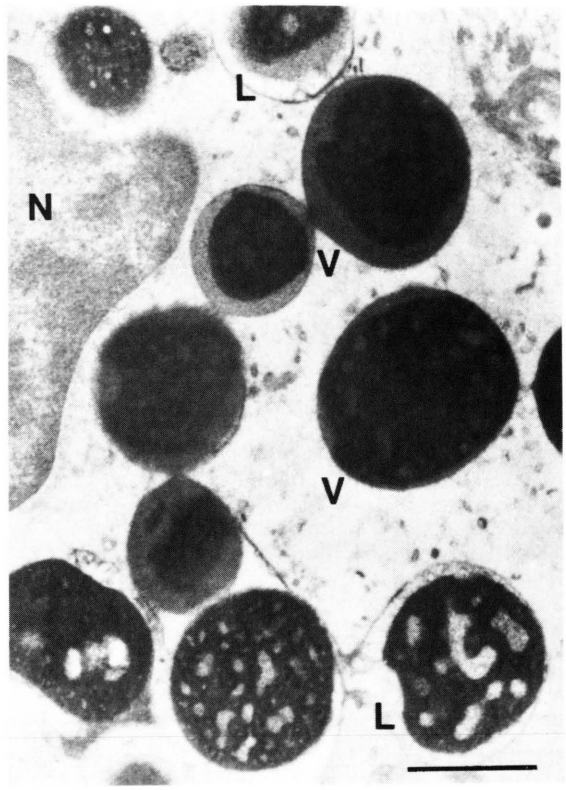

Fig. 2. Electron micrograph of cytoplasmic granules from a horse eosinophil at greater magnification, showing details of some granular types (labelled). N: Nucleus. The bar represents 1 μm.

materials were not found. Considerable heterogeneity in the structure of granules was evident, and at least three morphological types could be recognized. Granules H showed Homogeneous cores and a finely granular matrix (in some cases a narrow outer band). In granules V (the most abundant) the matrix was similar to that of type H, but cores presented a Vacuolate aspect, with round inclusions of matrix materials. Granules L (sometimes with an irregular shape) showed a Light, poorly structured or hyaline matrix and a vacuolate core with finely granular or hyaline inclusions; some portions of the membrane appeared with increased thickness and density, and membranous profiles were also found within the matrix. Several transition images between these granular types could be often observed.

The polymorphism of horse eosinophil granules is intriguing and its significance deserves further investigation. It seems logical to assume that different morphological types could be related to the maturation or functionality of this organelle. The occurrence of core and matrix components in the large granules of horse eosinophils confirms previous light microscopic observations, in which a specific ring-shaped reaction was found in granules stained by the Timm sulphide-silver method for metal cations [12].

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