Electron Microscopic Demonstration of Acid Phosphatase Activity in the Developing and Mature Heterophils of the Chicken

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Summary. As shown by electron microscopic histochemistry using a modified Gomori lead salt technique, acid phosphatase is present in large dense granules and the Golgi apparatus—but not the light granules—in both immature and mature heterophils in the chicken. The large dense granules appear to form by budding from the Golgi cisternae while the light granules appear to be unassociated with the Golgi apparatus. The findings indicate that the large, dense granules are the lysosomes of the heterophils in the chicken.

Introduction

By light microscopy, acid phosphatase activity could not be demonstrated histochemically in the mature heterophils of the chicken (Merkal and Mora, 1962) or the Japanese quail (Atwal and McFarland, 1966) and on this basis it was suggested that the heterophil granules in these cells should not be considered as lysosomes (Atwal and McFarland, 1966). However, in more recent studies, Nair (1973) presented histochemical evidence for the occurrence of lysosomes both in immature and mature heterophils in the chicken (since by light microscopy the final product was found to be present in granular sites in these cells).

In some species of mammals, for instance the rabbit, the existence of two types of granules in neutrophils is well established, the azurophil granules being considered as primary lysosomes because of their content of acid hydrolases, while the mature so called specific granules appear to represent an entirely different “secretion product” which contains alkaline phosphatase and a so far unidentified phosphatase active at low pH (Bainton and Farquhar, 1966, 1968a, b; Spicer and Hardin, 1969). Thus, Bainton and Farquhar (1968a, b) and Farquhar et al. (1972) demonstrated presence of acid phosphatase in the azurophil granules in immature heterophils in rabbits; furthermore, they reported latency of enzyme with metal salt technique in mature azurophil granules in rabbits and postulated that during granule maturation some alteration occurs in the permeability of the granule membrane or in the form of its contents.

Although azurophil granules are present in the heterophils in the chicken, these granules do not appear to be equivalent to those in mammals. In the chicken there appear to exist three types of granules in the heterophils (Campbell, 1967; Dhingra et al., 1969; Enbergs and Kriesten, 1968; Maxwell and Trejo, 1970), viz. “large, dense granules” “small, dense granules”, and “light granules” (Nair, 1973).
The aim of the present investigation was to try to elucidate which of these granules contain acid phosphatase and thus may represent lysosomes.

Materials and Methods

Tissues from inflammatory lesions in 3 chickens, induced by the injection of 2 % dextran sulphate into the wing web, as well as bone marrow from the femurs of 3 normal chickens, were fixed at +4°C in 2 % purified glutaraldehyde in 0.1 M cacodylate buffer containing 3.42 % sucrose (pH 7.2) "overnight" (~16 hours) (Brunk and Ericsson, 1972). Following washing in 0.1 M cacodylate buffer containing 3.42 % sucrose and 10 % dimethylsulfoxide (DMSO) at +4°C for 24 hours (Helminen and Ericsson, 1970), approximately 50 µ thick frozen sections were prepared on a regular freezing microtome and were subsequently incubated in a modified Gomori medium (Barka and Anderson, 1962) containing 10 % DMSO and 3.42 % sucrose (Brunk and Ericsson, 1972; Helminen and Ericsson, 1970). Incubation times varied between 30 and 90 minutes. Controls were incubated (for 90 minutes) in a medium lacking the substrate (β-glycerophosphate) or a complete medium to which 0.01 M NaF had been added. After incubation was completed the sections were briefly washed in 0.1 M cacodylate buffer with sucrose and were then "postfixed" in s-collidine buffered 2 % OsO4 (pH 7.4) for 2 hours. Dehydration, embedding, and cutting was performed as described previously (Brunk and Ericsson, 1972). The thin sections were studied and photographed unstained in a Siemens Elmiskop I electron microscope.

Tissues directly fixed in 1 % OsO4 (Millonig, 1961) and in glutaraldehyde (see above) followed by OsO4 and processed routinely for electron microscopy were also studied. The thin sections were stained with uranyl acetate in aqueous solution.

Results

I. Fine Structure of the Heterophil Granules

Three types of granules—"the large dense granules", "the small dense granules", and "the light granules"—were observed in the chicken heterophils after conventional direct fixation in phosphate buffered OsO4 (Fig. 1).

In the promyelocytes and myelocytes the large, dense granules—which were up to 3.5 µ long and 1.6 µ broad—were rounded or oval; as the cells matured, these granules tended to assume a more fusiform appearance. Many of the granules presented an internal electron dense core, and quite often the matrix appeared extracted leaving irregular pale spaces behind (Fig. 1). However, the appearance of the granule matrix varied with different modes of fixation and section staining. Thus, the granule matrix was homogeneously electron dense when the cells were fixed in glutaraldehyde followed by OsO4 and the sections were stained with uranyl acetate. In sections of glutaraldehyde-OsO4-fixed material the granules were less dense than the stained ones and sometimes had a pale core (Fig. 3).

The small, dense granules were rounded or oval and 0.1 to 0.2 µ in largest dimension. They were located in and around the Golgi zone in immature cells, while they were scattered over the entire cytoplasm in the mature heterophils. These granules had an internal dense core surrounded by an electron-lucent periphery. The appearance was about the same irrespective of the mode of fixation.

The light granules were intermediate in size between the other two types of granules. The matrix had a fibrillar appearance in OsO4-fixed cells and was not as electron-dense as in the large dense granules. Following fixation in glutaral-