

Electronmicroscopical Contrast by Palladium Chloride

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Thin sections of glutaraldehyde-fixed, epoxy resin-embedded bone marrow from rats were treated with 2% palladium chloride in 2% concentrated HCl. This procedure was found to induce high electron density in chromatin from all cell types and in cytoplasmic granules of neutrophils and eosinophils. In the latter, the crystalline body showed more contrast than the matrix.

Contrast enhancement in electron microscopy (EM) usually involves “staining” with salts of heavy metals, the most frequently employed elements being U and Pb [1–3]. The use of other heavy metals as general or selective electron dense stains is also known, *e.g.* Fe, Cr, Co, Ru, In, Bi, Tl, Hg, Au, Ag, Os, Mo, W [2, 4–10]. However, elements from the platinum group (Pt, Pd) have scarcely been explored as contrasting agents for EM. Platinum was used as PtBr₄ [11], *cis*-dichloro-diammine-Pt(II) [12] and Pt-pyrimidine complexes [13], but no references were found in the case of palladium. We report here preliminary results on the use of palladium chloride as an electron stain.

Bone marrow from normal rats were fixed in 4% glutaraldehyde in 1/15 M Sörensen’s buffer at pH 7.2 for 2 h and then rinsed in the same buffer for 2–3 h. Samples were dehydrated in acetone and embedded in Epon (Epikote 812) as usual. Thin sections were obtained with a Jum-7 (Jeol) ultramicrotome and transferred to gold grids without formvar. A 2% solution of palladium chloride (PdCl₂, Merck) in 2% concentrated hydrochloric acid was prepared in distilled water. This solution, which corresponds to chloropalladous acid (H₂PdCl₄) in a small excess of HCl [14], was directly used for contrasting of sections. Treatments were made either at room temperature or at 35–40 °C for 2–3 h, after which sections were rinsed in distilled water and observed in a Jeol 100-S electron microscope operating at 60 KV.

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Chromatin, specific granules of eosinophils and small round granules in neutrophils showed the most intense electron opacity after PdCl₂ treatment (Fig. 1). High density was observed in chromatin

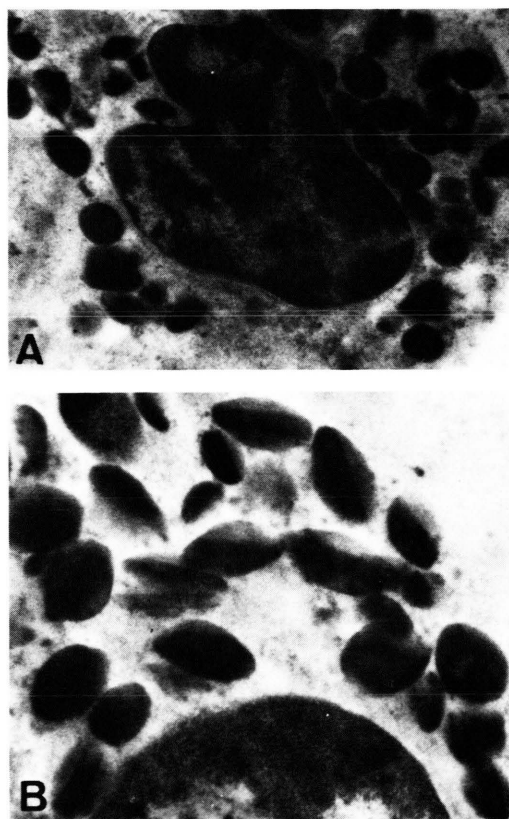


Fig. 1, A and B. Electron micrographs of eosinophil leucocytes showing the contrasting reaction of chromatin and cytoplasmic granules induced by PdCl₂ treatment at 35 °C for 2 h. A: ×12300; B: ×24200.



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masses from all cell types present in bone marrow. The crystalline inclusion in eosinophil granules appeared more contrasted than the matrix, and a highly opaque boundary was often observed between them. Control sections (lacking PdCl₂ treatment) did not reveal electron density in these structures, and only diffuse outlines of cellular components were observed. Slight differences in the contrast degree were found in dependence on the temperature and duration of PdCl₂ treatment, the most intense contrast appearing after 3 h at 40 °C.

At present, it is difficult to explain the precise mechanism of this reaction. The reduction of Pd from oxidation state 2+ to zero by olefinic double bonds from lipids [14] does not seem to occur as no granular deposits of the metal were observed in EM. Pd(II) easily coordinate to nitrogen, oxygen and sulphur ligands [15], and chelate complexes of Pd with amino acids, their derivatives, and peptides [16–18], as well as pyrimidine and purine bases are known

[19]. It is also reasonable to expect that the chloropalladate anion (PdCl₄)²⁻ would bind to positively charged groups in tissues [14]. Reactions of PdCl₂ with nitrogen ligands from DNA, histones, and other basic proteins could account for the electron opacity of chromatin and cytoplasmic granules. In addition, these structures are compact and poorly infiltrated by epoxy resins, which allows an easier penetration of aqueous reagents [20]. Although further investigations are required to determine more precisely the contrasting mechanism and selectivity by PdCl₂, these results show that Pd (atomic weight 106.4) can be applied as an interesting metal to enhance contrast in EM.

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