Oxidized *p*-Phenylenediamine Staining of Epoxy Resin Sections

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Semithin and thin sections of glutaraldehyde-fixed, epoxy resin-embedded animal tissues were treated with solutions of oxidized *p*-phenylenediamine (PPD). This method is suitable to reveal the general morphology of tissues in light microscopy, showing a high staining degree in some polyanion containing components. Posttreatments of thin sections with gold chloride solutions give considerable electron opacity in PPD positive structures.

Numerous stains and staining combinations have been applied to semithin sections of epoxy resin-embedded tissues, either to obtain an overall picture or to achieve selective staining of some components [1–3]. *p*-Phenylenediamine (PPD) has been found very useful in light and electron microscopical methods to enhance the contrast degree of certain structures after osmium fixation [4–8]. During the course of studies on the application of PPD on semithin sections, it was noticed that several tissue components showed an intensive staining reaction after treatment with aged solutions of this compound. We report here simple staining and contrasting methods by using solutions of oxidized PPD.

Large intestine, tongue, uterus, bone marrow and testes from mice and rats were fixed in 3% glutaraldehyde in Sörensen's buffer at pH 6.8 for 24 h at room temperature, and then rinsed in the same buffer for 2–3 h. Samples were dehydrated in ethanol and embedded in Epon or Durcupan as usual. Semithin (1–2 µm) and thin sections were obtained with an Ultracut Reichert-Jung and transfered to slides or copper grids, respectively. 0.5–1% solutions of PPD (Merck) either in distilled water or in 50% ethanol were allowed to age for variable times at room temperature. On account of the spontaneous oxidation process [9, 10], the colour of solutions increases rapidly and becomes ochre-brown in a few days. Sections were treated with filtered solutions of oxidized PPD, washed in distilled water and observed in a Zeiss photomicroscope III or in the Jeol 100-S electron microscope operating at 60 KV. Spectral studies were performed in a Perkin-Elmer spectrophotometer 551-S.

Different aging times and staining conditions were examined to achieve the optimal contrast degree. The best results were obtained by using 7–10 days aged 0.5% aqueous PPD solutions for 20–30 min at room temperature. Good results were also achieved by staining with a 10 days aged 1% PPD solution in 50% ethanol for 20–30 min at room temperature.

Under the light microscope, microvilli, mucin granules from goblet cells, acrosomes, mast cell granules, chromatin, elastic fibers, collagen and basal membranes showed an intensive staining reaction (ochre-brown or yellow-brown). Photomicrographs of typical preparations are shown in Fig. 1 (A and B), in which some of these structures appear deeply stained. After oxidized PPD treatments, thin sections did not present electron density, but a high contrast was obtained by using an additional treatment with gold chloride (Fig. 1, C), which stabilize the colour of oxidized PPD [9]. PPD reactive structures appeared with massive gold deposits at high magnification (50–80 Å in diameter), which were absent in sections lacking previous PPD treatment (Fig. 1, compare D and E).

Spectrophotometric studies were made to substantiate some of these staining reactions. High absorption in the ultraviolet, a peak at 510 nm and a shoulder at about 440 nm were found for oxidized PPD solutions (Fig. 2). In the presence of heparin, the absorption spectrum of the dye showed a hyperchromic shift, which suggests strong interactions with the polyanionic substrate. Although PPD is not a stain on its own, it is easy and spontaneously oxidized to yield brown coloured products [9, 10], which stain acidic substrates such as the DNA component of chromatin from cell smears [11]. The procedure described here can be used as a simple staining method for epoxy sections of glutaral-

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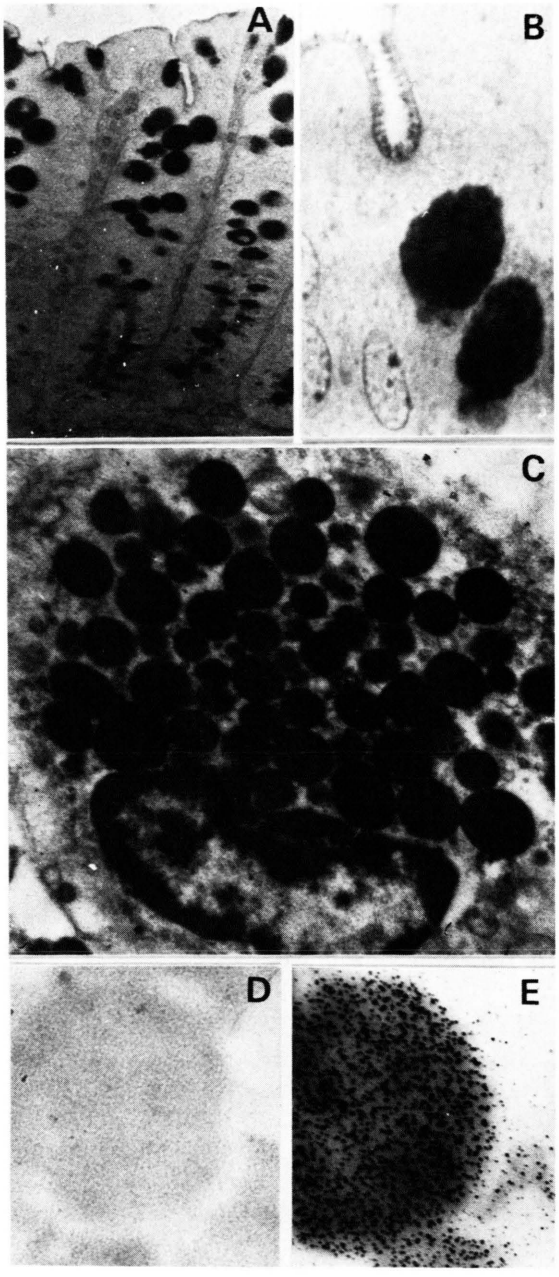

Fig. 1, A and B. Staining pattern of semithin sections from the mouse large intestine after 20 min treatment with oxidized PPD (7 days aged, 0.5% solution in distilled water). Staining reactions of goblet cell mucin, nuclei and microvilli are clearly observed. Bright field illumination. A: ×330; B: ×1700. C. Electron micrograph of a rat mast cell showing the contrast of cytoplasmic granules and chromatin after 3 h staining with oxidized PPD (7 days aged, 0.5% solution in distilled water), followed by a treatment with 0.25% $AuCl_3$ in distilled water for 2 min. ×10 500. D. Cytoplasmic granule in a mast cell from a gold treated section lacking the previous oxidized PPD step. ×61 000. E. Mast cell granule after treatments with oxidized PPD and gold chloride (as in C). ×61 000.

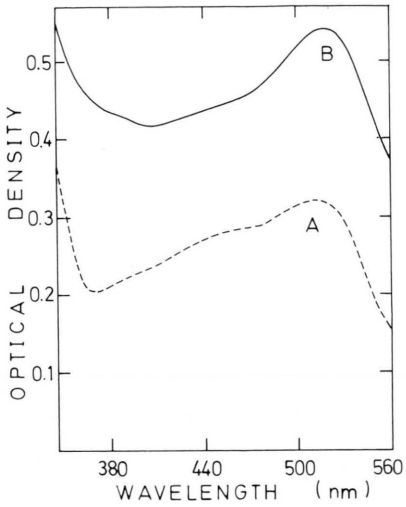

Fig. 2. Absorption spectra of oxidized PPD (10 days aged, 0.025% solution in distilled water) (A), and the same solution in the presence of 1.25% heparin (B).

dehyde fixed materials, giving a suitable contrast for some tissue and cell components in light and electron microscopy.

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