

Oxidized *p*-Phenylenediamine Staining of Epoxy Resin Sections

J. C. Stockert, R. Armas-Portela, O. D. Colman, J. M. Ferrer, and A. Tato

Departamento de Citología e Histología, Facultad de Ciencias, Universidad Autónoma de Madrid, Canto Blanco, Madrid-34, Spain, and Cátedra de Citología e Histología, Facultad de Biología, Universidad de Valencia, Spain

Z. Naturforsch. **39 c**, 835–836 (1984);
received December 19, 1983/March 5, 1984

p-Phenylenediamine, Epoxy Resin Sections, Light Microscopy, Electron Microscopy, Staining and Contrasting Methods

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 polyanionic 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 μ m) and thin sections were obtained with an Ultracut Reichert-Jung and transferred 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-

Reprint requests to Prof. Dr. Juan C. Stockert.

0341-0382/84/0700-0835 \$ 01.30/0



Dieses Werk wurde im Jahr 2013 vom Verlag Zeitschrift für Naturforschung in Zusammenarbeit mit der Max-Planck-Gesellschaft zur Förderung der Wissenschaften e.V. digitalisiert und unter folgender Lizenz veröffentlicht: Creative Commons Namensnennung-Keine Bearbeitung 3.0 Deutschland Lizenz.

Zum 01.01.2015 ist eine Anpassung der Lizenzbedingungen (Entfall der Creative Commons Lizenzbedingung „Keine Bearbeitung“) beabsichtigt, um eine Nachnutzung auch im Rahmen zukünftiger wissenschaftlicher Nutzungsformen zu ermöglichen.

This work has been digitalized and published in 2013 by Verlag Zeitschrift für Naturforschung in cooperation with the Max Planck Society for the Advancement of Science under a Creative Commons Attribution-NoDerivs 3.0 Germany License.

On 01.01.2015 it is planned to change the License Conditions (the removal of the Creative Commons License condition "no derivative works"). This is to allow reuse in the area of future scientific usage.

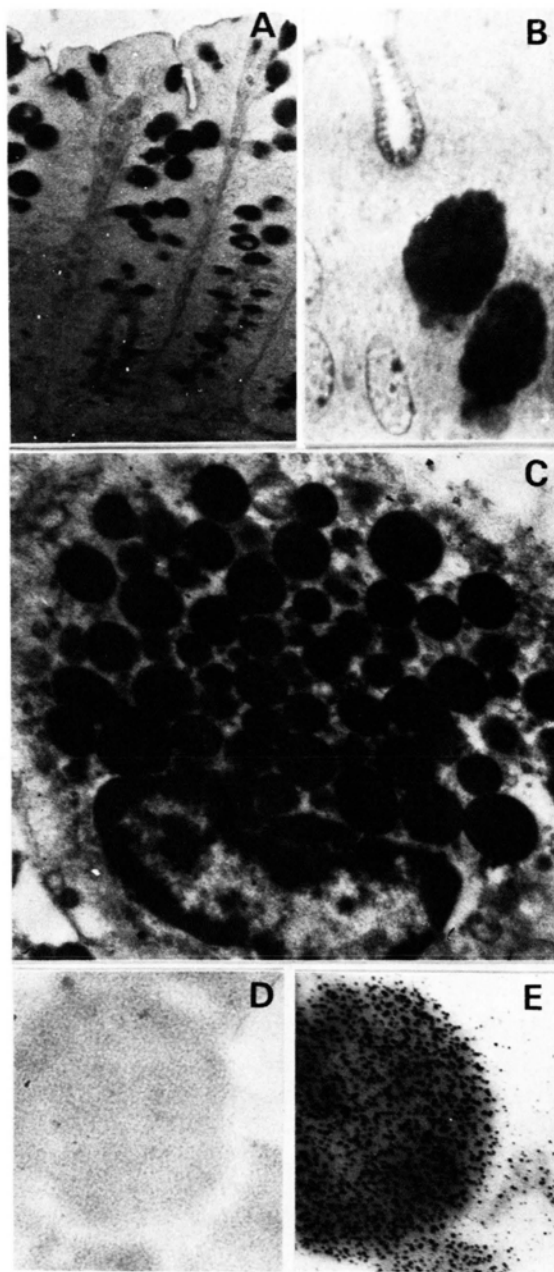


Fig. 1. A and B. Staining pattern of semithin sections from the mouse large intestine after 20 min treatment with oxidized PPD (7 days aged, 0.5% solution in distilled water). Staining reactions of goblet cell mucin, nuclei and microvilli are clearly observed. Bright field illumination. A: $\times 330$; B: $\times 1700$. 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. $\times 10\,500$. D. Cytoplasmic granule in a mast cell from a gold treated section lacking the previous oxidized PPD step. $\times 61\,000$. E. Mast cell granule after treatments with oxidized PPD and gold chloride (as in C). $\times 61\,000$.

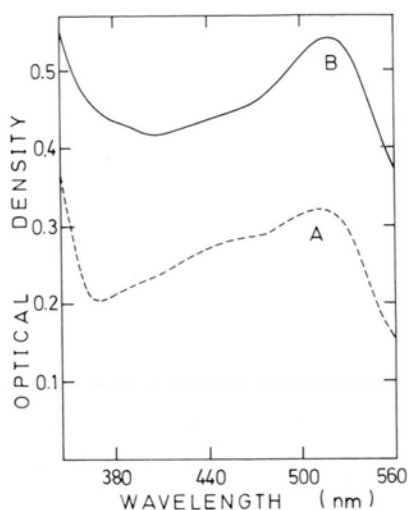


Fig. 2. Absorption spectra of oxidized PPD (10 days aged, 0.025% solution in distilled water) (A), and the same solution in the presence of 1.25% heparin (B).

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

Acknowledgements

This work was partially supported by a grant from the Comisión Asesora de Investigación Científica y Técnica, Spain.

- [1] F. Davie, *Science Tools* **17**, 59 (1970).
- [2] M. A. Hayat, *Positive staining for electron microscopy*, Van Nostrand Reinhold Co., New York 1975.
- [3] M. Cañete and J. C. Stockert, *J. Microscopy* **122**, 321 (1981).
- [4] J. F. Estable-Puig, W. C. Bauer, and J. M. Blumberg, *J. Neuropathol. Exp. Neurol.* **24**, 531 (1965).
- [5] J. M. Ledingham and F. O. Simpson, *Stain Technol.* **45**, 255 (1970).
- [6] J. C. Stockert, *Histochemistry* **53**, 43 (1977).
- [7] P. Esponda and J. C. Stockert, *Chromosoma* **68**, 83 (1978).
- [8] O. D. Colman and J. C. Stockert, *Acta Histochem.* **72**, 123 (1983).
- [9] M. Gabe, *Techniques histologiques*. Masson et Cie., Eds. Paris 1968.
- [10] L. Lison, *Histochemie et cytochemie animales*. 3e. Ed., Gauthier-Villars, Paris 1960.
- [11] J. C. Stockert, O. D. Colman, and A. Juarranz, *Acta histochem.* **25** (suppl.), 129 (1982).