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Staining with Ziehl's Fuchsin of Semithin Sections Mounted on Slides

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Abstract

The authors describe a simple method for the staining of semithin sections mounted on slides, using Ziehl's fuchsin, that improves the results obtained with toluidine blue.

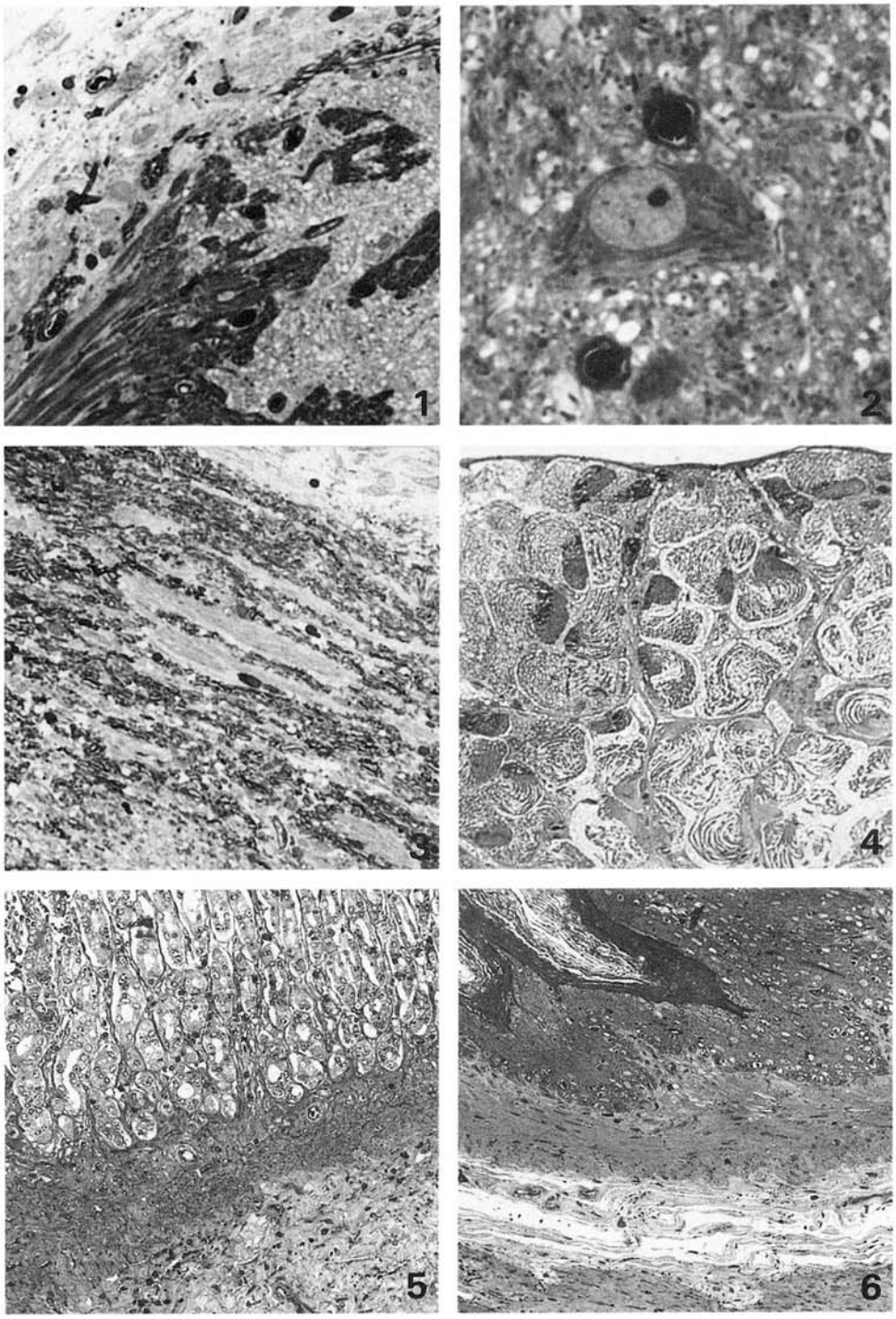
Introduction

Different techniques have been employed for the staining of semithin sections; some involve previous deplastification (MUNGER 1961; MAYOR et al. 1961; YENSEN 1968; CASANOVA 1974) while other do not. Of the latter, the classic and most frequently used method is that of TRUMP et al. (1961) which employs 0.1% toluidine blue at *p*H 11.1. GRIMLEY et al. (1965) developed a stain with toluidine blue and malachite green in ethanol and, after the use of potassium permanganate as mordant, the stain with azur B and PTAH. More recently, BÖCK (1984) describe different techniques using basic fuchsin as counterstain for semithin sections primarily stained with toluidine blue, methylene blue-azur B, hematoxylin, crystal violet and, also as contrast dye, for the Berlin's blue reaction. TOLIVIA and TOLIVIA (1985) developed two techniques for staining semithin sections using dyes of the fuchsin type as unique staining. These techniques offer certain advantages: the risk of precipitates almost disappears; their stability is comparatively greater than in sections stained with routine techniques, and it is possible to use, both during light microscope observations and in photography, a green filter with which owing to the reddish colour of the stain, the contrast is considerably enhanced (TOLIVIA and TOLIVIA 1985). However, the method proposed by these authors has the drawback that the sections must be handled individually, using wells with dyes heated uniformly at 60 °C and illuminated from below since the sections sink in the wells. This procedure involves a lengthy and tedious handling of a small number of sections and while being carried out the sections are often damaged.

We propose a modification that permits one to work on semithin sections dried on a slide such that at the same time as maintaining the advantages of the fuchsin technique, the procedure is rapid and easy to perform. Moreover, the serial nature of the sections and their integrity are preserved and the stain is uniform and provides excellent contrast.

Method

We used 5 specimens of the rainbow trout *Salmo gairdneri,* 5 specimens of the mediterranean barbel *Barbus bocagei,* 2 specimens of the newt *Triturus marmoratus* and 2 specimens of *Rattus norvegicus*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perfused with 2% glutaraldehyde-2% paraformaldehyde in 0.12 M phosphate buffer (pH = 7.2). Different organs were extracted, postfixed in 2% osmium tetroxide and embedded in Spurr resin (SPURR 1969). Semithin sections between 0.4 and 1.3 μm, were subjected to the following procedure:

1. The sections are placed on a drop of distilled water on a slide and dried in an oven or a heating plate at 60 °C (all steps involving a drying procedure should be long with a minimum of 30 min, since for uniformity of the stain the section must be completely dry).
2. One drop, larger than that of the distilled water, of concentrated photographic fixative (e.g. FYVAL from Valca) is added to where the sections have been placed and the slide is heated on a heating plate at 60 °C for 2 min (the exact time is when the fixative begins to crystallize on the edge of the drop). This step also improves the results obtained with other dyes.
3. The sections are washed abundantly in distilled water for several minutes and then dried on a heating plate.
4. The sections are stained for 30–60 secs. on a heating plate with one drop of a fuchsin solution obtained in the following way;
   a) Preparation of the stock solution of Ziehl's fuchsin:
      - basic fuchsin 1 g
      - phenol 5 g
      - 95% ethanol 10 ml

   This is ground in a pestle and 90 ml of distilled water is added dropwise. The mixture is left to sediment and then filtered (it can be kept for unlimited periods).
   b) Preparation of working solution:
      - stock solution of Ziehl's fuchsin 30 ml
      - distilled water 70 ml

   This is left in an open container at room temperature for 7 days. At the moment of use it is shaken and carefully filtered.
5. Wash, dry, and mount.

Results

In the nervous tissue, cell bodies, both neuronal and glial, are stained perfectly, as are the myelinated and unmyelinated fascicles (in tones rangeing from pale pink to strong red). In other tissues, the proposed stain offers a high contrast and easy differentiation of the different cell types (Figs. 1–6).

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Fig. 1. Glomerular zone of the olfactory bulb of *Salmo gairdneri*. ×290.

Fig. 2. Mitral cell. *Barbus bocagei*. ×1,900.

Fig. 3. Myelinic and amyelinic bundles in the caudal zone of the olfactory bulb. *Barbus bocagei*. ×110.

Fig. 4. Testis. *Triturus marmoratus*. ×92.

Fig. 5. Stomach. *Rattus norvegicus*. ×130.

Fig. 6. Esophagus. *Rattus norvegicus*. ×130.

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