

## Ruthenium Red Staining of Chromatin in Epon Sections

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### Ruthenium Red, Chromatin Staining

After staining with a 50% ethanol solution of ruthenium red, chromatin from onion and mouse cells showed red color and high contrast in the light and electron microscope, respectively. When applied to glutaraldehyde-fixed, Epon-embedded tissues, this selective staining technique proved to be dependent on the DNA content of chromatin.

For a long time, the ammoniated ruthenium oxychloride "ruthenium red" (today formulated [1, 2] as  $[(\text{NH}_3)_5\text{Ru} \cdot \text{O} \cdot \text{Ru}(\text{NH}_3)_4 \cdot \text{O} \cdot \text{Ru}(\text{NH}_3)_5]^{6+} \cdot \text{Cl}_6^-$ ), has been used as a microscopic test for pectins [2, 3]. Several applications of this contrasting agent for electron microscopic detection of acid polysaccharides and proteoglycans have been studied [4–10]. Its use as a Schiff-like reagent was reviewed recently [11]. However, it seems to have been overlooked that ruthenium red (RR) could also stain nuclei [12], and only scarce observations on the contrast enhancement of chromatin and DNA by RR have been reported [4, 11, 13].

The strong binding of RR to polyanionic macromolecules [10–13] brought to our attention the possibility that it could serve as a staining and contrasting agent for chromatin in Epon sections. A drawback of this approach, however, is the poor penetrability of plastic-embedded tissues by aqueous solutions of RR [4]. In order to overcome this difficulty, staining of semithin sections was attempted under several different conditions (7% acetic acid, 50% ethanol, increasing RR concentrations or staining time, heating, etc.). Among these, acceptable results were only achieved by using RR in 50% ethanol. A fresh 0.5% solution of RR (Scharlau) in distilled water was diluted 1:1 (v/v) with absolute ethanol. After 24 h a dark brown, flocculent precipitate is separated and the remaining bright red solution can be used after filtration or centrifugation.

Spleen, kidney, testis and tongue from normal mice, as well as onion root-tips, were fixed for 2 h in 3% glutaraldehyde in Sörensen's phosphate buffer at pH 7. Tissues were rinsed in 6.5% sucrose, dehydrated in ethanol and embedded in Epon. Some roots were fixed for only 5 min, exhaustively washed in tap water and treated with RNase (Serva, 1 mg/ml in distilled water at pH 6 for 3 h at room temperature). Other roots were subjected either to 5% perchloric acid (PCA) at 4 °C for 18 h, 5 N HCl at room temperature for 1 h, or 5% trichloroacetic acid (TCA) at 90 °C for 15 min. Semithin sections (1 µm) and thin sections were stained with the 50% alcoholic RR solution at room temperature for 1 h and then washed with 50% ethanol. Some semithin sections were also treated with 1% ammonium aluminium sulfate (Al) for 1 h before RR staining.

When examined by light microscopy, all the semithin tissue sections show deeply red colored chromatin after RR staining (Fig. 1a). Nucleoli also appear moderately stained, while the basophilic cytoplasm stains very pale or not at all. Only few structures (acrosomes, elastic fibers, the crystalloid of eosinophil granules, and mast cell granules) were noticed to stain after application of RR on semithin

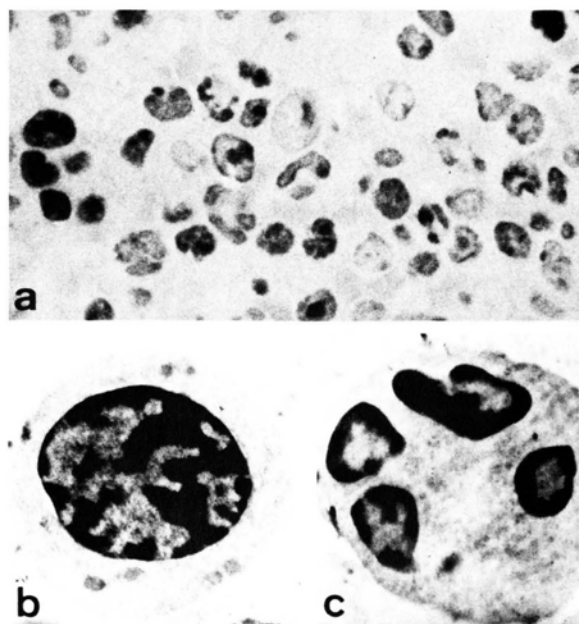


Fig. 1. Epon sections of mouse spleen cells stained by ruthenium red as described in the text. a: semithin section showing the chromatin staining in several cell types. b and c: electron micrographs of a lymphocyte (b), and a neutrophil leukocyte (c). a =  $\times 840$ ; b, c, =  $\times 5100$ .

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Table I. Light microscopic RR staining on Epon semithin sections from onion root-tips. Effect of several pretreatments on the staining reaction of meristematic cells. G: glutaraldehyde; Ep: Epon. ++ and - indicate red and no staining, respectively.

Staining experiment	Chromatin	Nucleolus	Cytoplasm
G, Ep, RR	++	+	±
G, PCA, Ep, RR	++	±	-
G, RNase, Ep, RR	++	-	-
G, HCl, Ep, RR	±	±	-
G, TCA, Ep, RR	-	±	-
G, Ep, Al, RR	-	-	-

or thin sections. Observation of stained sections by electron microscopy reveals a contrast pattern which closely resembles that found in the light microscope: the definition and electron opacity of chromatin are considerably enhanced by RR in all the cell types examined (Fig. 1 b and c). The results of extraction procedures are summarized in Table I. Removal of RNA does not affect the affinity of chromatin for RR, while extraction of DNA or severe modifications in its secondary structure abolishes, or greatly decreases, the RR binding capacity.

The large number of phosphate groups from nucleic acids (which appear blocked after treatment with aluminium ions or increasing electrolyte concentration [4]), constitute obvious sites for interaction of chromatin with RR. The high affinity of this heavy cationic complex for acid groups and the possible binding mechanisms were discussed by several authors [2, 3, 10, 13]. However, staining conditions, which led to a suitable contrast in chromatin were scarcely explored (see results on glycol methacrylate-embedded tissues [4]). The method presented here gives an adequate and preferential contrast for chromatin in both semithin and thin Epon sections. Further investigations to analyze the staining interaction between RR and nucleic acids more precisely are under way.

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