Feulgen positive nucleoli in Epon semithin sections: Fact or artifact?[1]

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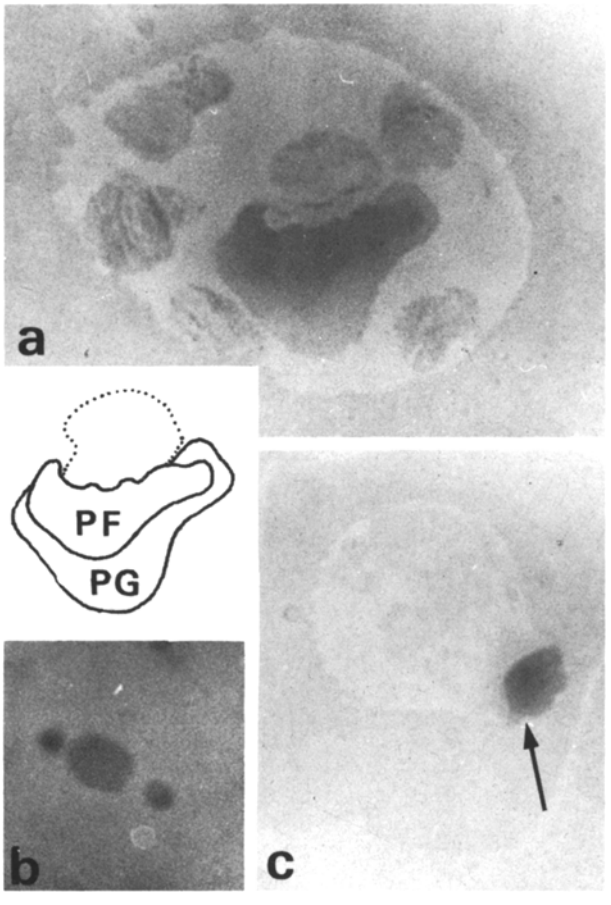
Summary. Epon semithin sections from glutaraldehyde fixed tissues show stained nucleoli after treatment with the Schiff's reagent. The possibility that aldehyde groups already present could account for this positive staining reaction is briefly discussed.

Owing to the high morphological preservation of the cell structures, the technique of semithin sections from plastic-embedded tissues is gradually finding wider applications in light microscopy work. Today, several selective and cytochemical staining methods have been successfully used on Epon semithin sections[2,3] including the Feulgen reaction. Recently, glutaraldehyde-fixed, Epon-embedded *Acetabularia* nucleoli were found to be Feulgen positive[4]. Interestingly, a close correspondence between the location of the positive nucleolar region and that of the pars fibrosa can easily be observed in *Acetabularia*[4]. Since the material was fixed in glutaraldehyde, and bearing in mind that the pars fibrosa shows the highest protein concentration in nucleoli[5], it seemed logical to test whether the nucleolar staining by the Feulgen reaction can actually be taken as cytochemical evidence under the conditions mentioned above.



Photomicrographs from glutaraldehyde/Epon sections, treated with Schiff's reagent and observed under bright field illumination. *a* Salivary gland nucleus showing some portions of chromosomes and a segregated nucleolus induced by a heat shock. Note the more intense staining in the central region (PF). *b* Nucleolar complex in a Sertoli cell nucleus. Both the nucleolus and 2 associated chromocenters are stained. *c* Spermatocyte nucleus from a *D. hydei* testis. The polarized nucleolus (long arrow) and some granules of the clubs show the highest Schiff reactivity.

Salivary glands of *Chironomus pallidivittatus* larvae, testes and fat bodies of *Drosophila hydei* larvae, root tips of *Allium cepa*, and mouse testes were fixed for 2 h in 6.5% glutaraldehyde in phosphate buffer, washed overnight in 6.5% sucrose solution, dehydrated in ethanol and embedded in Epon as usual. Semithin sections (1–2 μm) were mounted in slides and stained directly with Schiff's reagent for 1–2 h in darkness and at room temperature. They were briefly washed in distilled water and treated for 5 min with a 2% sodium bisulphite solution.

After this procedure, nucleoli from normal *Chironomus* larvae show a definite pink colour. In secregated nucleoli induced by a heat-shock treatment[6,7], the most intense staining appears in the central region (composed of pars fibrosa) while the peripheral region (pars granulosa) stains less (figure, a). Chromosome bands, cytoplasm and the secretion content (but not the special secretion) also show a positive staining reaction. Sections which are not treated by Schiff's reagent do not present any coloration; those subjected to 5N HCl hydrolysis for 1 h at room temperature followed by the Schiff's solution show highly stained chromatin, though the nucleolus remains as positive as in nonhydrolyzed sections. On the other hand, semithin sections treated for 1 h with a saturated solution of sodium bisulphite before the Schiff's reagent do not appear stained, which indicates that tissue aldehyde or ketone groups susceptible to bisulphite blocking[8] could account for the Schiff reactivity in unhydrolyzed materials.

In Sertoli nuclei from mouse testes, a clear example of Schiff staining of nucleoli can also be observed. Although the presence of DNA in the 2 nucleolus-associated chromatin masses is recognized after the Feulgen reaction, both the nucleolus and the associated chromatin are definitely stained in control sections without hydrolysis (figure, b). Spermatogoniae, spermatocytes and nutritive cells from *Drosophila* testes show a nucleolar staining pattern like that of *Chironomus* cells (figure, c). The same reaction occurs in nucleoli of *Allium* roots as well as in

1 This work was partially supported by a grant from the Comisión Asesora de Investigación Científica y Técnica, Spain. The author is greatly indebted to O.D. Colman and O. Partearroyo for valuable collaboration.

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nucleoli and round cytoplasmic inclusions of *Drosophila* fat bodies. In addition, the dense granules of clubs (one of the Y-chromosome loops in *D. hydei* spermatocytes) also appear stained by Schiff's reagent. Interestingly, all these stained structures are very compact and highly positive after staining techniques selective for proteins in Epon semithin sections[9,10].

Today it is widely accepted that ribosomal DNA probably embedded within the fibrillar region is a principal component of the nucleolar body[11]. In such a situation, the concentration of DNA seems to be simply too low to give a positive Feulgen reaction, as revealed in nucleoli from squashes and paraffin sections[4,12]. After acidic toluidine blue has been applied to Epon sections, the typical orthochromatic staining of DNA is also lacking from *Allium* and *Chironomus* nucleoli[13]. Other organisms in which nucleoli show conspicuous positive reactions for DNA are clearly different, since they are surrounded or permeated by large masses of condensated chromatin[14]. It is already known that some plant nuclei (particularly algae and bryophites) show very weak Feulgen staining[15]. Unmistakable Feulgen reactions are obviously expected to occur in cells with a great amount of condensed chromatin (e.g. polytene bands, chromocenters, mitotic chromosomes). However, the nonspecific staining on non-hydrolyzed sections could be caused by Schiff-reactive groups already present. As a consequence of incomplete cross-linking reactions with tissue elements, glutaraldehyde fixation may introduce free aldehyde groups[16–19], specially in protein-rich cell structures.

Since glutaraldehyde-fixed, Epon-embedded sections are capable of giving perceptible staining directly after the Schiff's reagent, careful control seems to be necessary to decide whether or not a given structure contains DNA. The present results suggest that by using the Feulgen reaction on semithin sections, not only false negatives but also false positives are possible.

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CONGRESSUS

Italy

3rd international symposium of cytopharmacology

in Venice, 10–14 July 1978

This symposium will be dedicated to 'Neurotoxins and their use as tools in neurobiology'. For further information write to B. Ceccarelli, C. N. R. Center of Cytopharmacology, Institute of Pharmacology of Milano, via Vanvitelli 32, I–20129 Milano, Italy.

9th international symposium on chromatography and electrophoresis

Riva del Garda (Italy), 15–17 May 1978

The symposium is organized by the Belgian Society for Pharmaceutical Sciences, the Italian Group for Mass Spectrometry in Biochemistry and Medicine and the Italian Society for Pharmaceutical Sciences. Communications should be submitted before 4 February 1978.

Further information by the chairman: Dr Alberto Frigerio, Istituto de Ricerche Farmacologiche 'Mario Negri', via Eritrea 62, I–20157 Milano, Italy.

5th international symposium on mass spectrometry in biochemistry and medicine

at Rimini, 19–21 June 1978

This symposium by the Italian Group for Mass Spectrometry in Biochemistry and Medicine will discuss all the latest aspects of mass spectrometry and their areas of application and consist of presentations of invited speakers and free communications. Deadline 28 February 1978.

Further information by the president, Dr Alberto Frigerio, Istituto di Ricerche Farmacologiche 'Mario Negri', via Eritrea 62, I–20157 Milano, Italy.

Belgium

Satellite symposium of the 7th international congress of pharmacology

in Brussels, 24 July 1978

Topic of the symposium: The inflammatory process. For information contact: Dr J. P. Famaey, Service de Rhumatologie et Physiothérapie, Hop. Univ. Saint-Pierre, 322, rue Haute, B–1000 Bruxelles.

France

Satellite symposium of the 7th international congress of pharmacology

in Paris, 22 July 1978

Topic of the symposium: Antiinflammatory and antirheumatic drugs. For information contact Prof. J. P. Giroud, Department of Pharmacology, 27, rue du Faubourg Saint-Jacques, C. H. U. Cochin, Paris XIV, France.

Switzerland

EUCHEM Conference on Stereochemistry

at the Bürgenstock, near Lucerne, 30 April–6 May 1978

The number of participants will be limited. Inquiries and applications (no special forms are required) should be addressed before January 15, 1978 to the Chairman: Prof. H. A. Staab, Max-Planck-Institut, Organ.-chem. Abteilung, Jahnstrasse 29, D–69 Heidelberg.