CONTRAST ACCENTUATING TECHNIQUES SPECIFIC FOR DNA-CONTAINING STRUCTURES IN ULTRATHIN SECTIONS

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When tissues fixed with aldehydes are stained with uranyl acetate (UA), staining takes place mainly on account of binding of uranyl cations with the phosphate groups of DNA [4]. The routine technique used in electron miscroscopy, of postfixation with osmium, staining with UA during dehydration, followed by additional staining of the sections with lead salts, converts a type of staining preferentially for DNA into a nonspecific type. However, the omission of these stages does not always lead to satisfactory contrast of chromatin in material embedded in plastic.

In light cytochemistry, in order to increase the basophilia of DNA, a method of introducing additional anionic groups into it has recently been suggested [3, 1, 2]. This same principle was adopted during development of the method suggested in this paper. It consists of depurinizing acid hydrolysis followed by addition of an aldehyde blocker – sodium bisulfite – to the aldehyde groups of deoxyribose thus formed. The bisulfite anions introduced into the DNA, as the investigation described below showed, can bind additional uranyl cations.

EXPERIMENTAL METHOD

Zajdela ascites hepatoma cells from rats were used. The material was fixed with 4% formaldehyde, freshly prepared from paraformaldehyde in 0.1 M phosphate buffer, pH 7.35, with 0.2 M sucrose at 4°C for 1 h. The cells, washed twice for 30 min each time with cold buffer and quickly with water (only freshly prepared bidistilled water was used), were dehydrated in ethanol and acetone and were embedded in Epon the same day. Ultrathin sections placed on copper grids (from LKB), coated with formvar, were hydrolyzed by flotation in 5 N HCl at room temperature for 1 h and were rinsed for 1 h in 3 portions of water, for 2 min each time. The grids with the sections were then placed on the bottom of a closed bottle containing a 10% aqueous solution of sodium bisulfite at 60°C for 90 min. They were then washed in 3 portions of water and stained in a drop of freshly prepared and filtered 2% aqueous solutions of UA at 37°C for 30 min. The stained sections were immersed for a second in water, dried in air, and examined in the EVM-100L electron microscope with an accelerating voltage of 75 kV. Control sections were studied with omission of the sodium bisulfite treatment or of that treatment and hydrolysis also.

To remove DNA, cells fixed for 20 min with cold formaldehyde were treated with 0.1% DNase (from Serva) in fixation buffer at 37°C for 3 h, after which they were washed with cold trichloracetic acid.

EXPERIMENTAL RESULTS

Fixation with formaldehyde promotes selective staining of nuclear structures by UA in Epon sections, mainly of chromatin (Fig. 1a), which acquires moderate contrast. Acid hydrolysis on the sections causes virtually no change in the composition of the nuclei or in the ultrastructure of the chromatin, but increases its contrast a little (Fig. 1b). Addition of sodium bisulfite significantly enhanced the contrast of chromatin (Fig. 1c). Bisulfite was ineffective if acid hydrolysis was omitted. Cells treated with DNase after fixation had greatly reduced contrast of their nuclei after hydrolysis and staining of the sections and addition of bisulfite did not alter the results of staining (Fig. 1d).

The experiments thus showed that UA can give increased contrast of chromatin after treatment of hydrolyzed Epon sections with sodium bisulfite. The results of the DNase control showed that the substrate of

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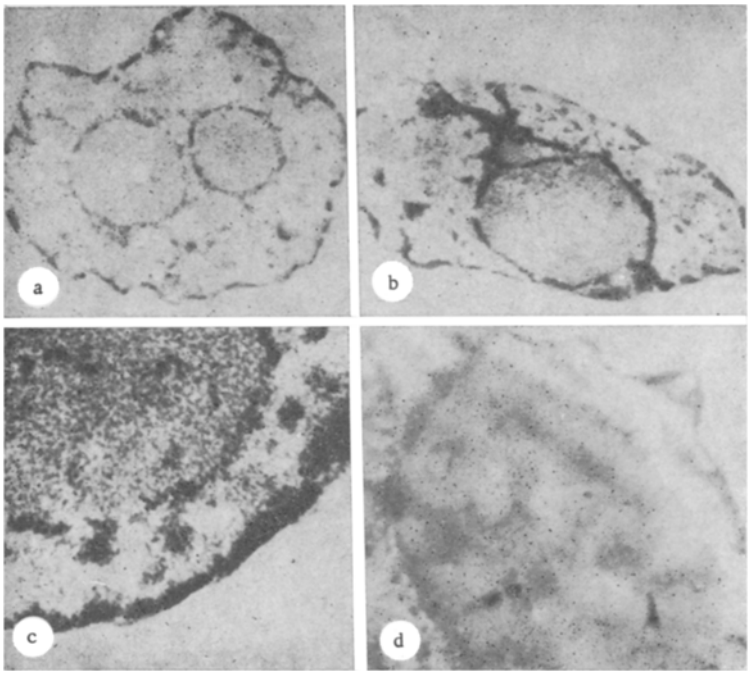

Fig. 1. Electron-micrographs of Zajdela ascites hepatoma cells fixed with formaldehyde and stained in sections with 2% UA. a) Without hydrolysis. 11,000×; b) with hydrolysis. 11,000×; c) with hydrolysis and subsequent treatment with sodium bisulfite. 20,000×; d) after treatment of fixed cells with DNase, remaining procedures as in c. 20,000×.

the reaction is DNA, and the dependence of the bisulfite effect on hydrolysis indicates the importance of depurinization as a condition for manifestations of the action of bisulfite. It will be evident that, just as when other cationic dyes are used [1], staining of DNA under these conditions is a combination of preferential binding of uranyl cations with DNA phosphate groups and binding of uranyl cations with bisulfite anions specifically attached to deoxyribose.

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