MODIFICATION OF THE METHOD OF STAINING FOR DNA WITH BASIC DYES

E. A. Érenpreisa

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The mechanism of the technique of bisulfite treatment followed by staining with toluidine and methylene blue, after hydrolysis by Feulgen's method was studied and its suitability for histochemistry assessed. Bisulfite treatment was found to increase the intensity of the reaction by 1.5 times, and binding of the dye takes place stoichiometrically. Toluidine blue gives metachromatic and anisotropic staining of the cell nuclei. The method is recommended as a sensitive test for DNA in cytochemical investigations and for studying dichroism of DNA-containing structures.

KEY WORDS: *Feulgen hydrolysis; sodium bisulfite; basic dyes.*

In recent years the reaction for DNA with basic dyes after performance of hydrolysis of the Feulgen type has been used in histochemistry. The mechanism of the reaction depends on binding of the phosphate groups of DNA with the cations of the dye [7]. The selectivity of staining is due to the fact that RNA is extracted in the course of hydrolysis, but the carboxyl groups of the proteins are not yet dissociated at pH values of the staining solution of 3.0 to 4.5. Whereas in native nucleohistone only some of the phosphate groups are liberated from their bond with histones [4], as a result of Feulgen hydrolysis practically all phosphate groups are liberated.

A method based on blocking the aldehyde groups of DNA, when apurinized by Feulgen hydrolysis, with sodium bisulfite ($NaHSO_3$) in order to intensify the subsequent reaction with toluidine blue is described in the literature [3].

The object of this investigation was to study the mechanism of this reaction and to assess its suitability for histochemical purposes.

EXPERIMENTAL METHOD

Normal liver and Walker's carcinosarcoma of rats were used. The technique of the reaction is described in the writer's modification. Dried, unfixed squash preparations were hydrolyzed in 5 N HCl at room temperature for 20 min, rinsed with distilled water, and stained with 0.05% toluidine blue O (Hartmann-Leddon Co.) or methylene blue (Fisher Scientific Co.) at pH 4.0 for 5 min. The preparations were rinsed with distilled water, dehydrated in tertiary butyl alcohol, taken through xylol, and embedded in polystyrene. Before staining, the experimental preparations were kept for 15 min in a mixture of 50% sodium bisulfite with 96% ethanol (4:1) [1]. The control and experimental preparations also were stained with Schiff's reagent.

Thirty diploid and 30 tetraploid hepatocytes were examined cytophotometrically with the CITO-2 discrete scanning integrating cytospectrophotometer.

EXPERIMENTAL RESULTS

Preparations treated with bisulfite, unlike the controls, did not stain with Schiff's reagent, but their chromatin stained more intensely and clearly with both thiazine dyes with

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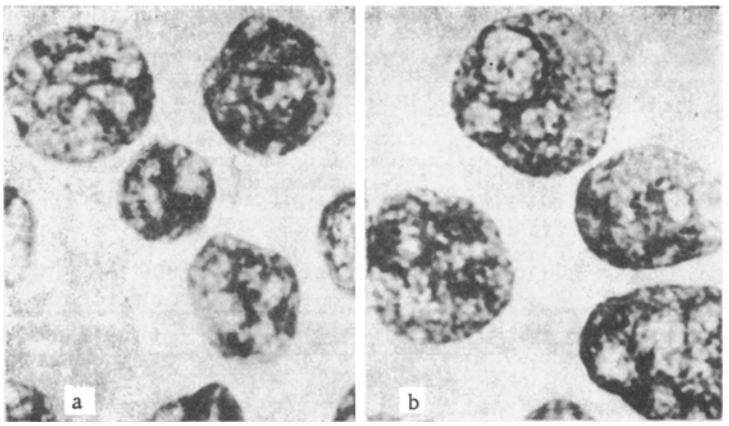

Fig. 1. Detection of DNA in cell nuclei of Walker's tumor after treatment with sodium bisulfite: a) stained with methylene blue; b) stained with toluidine blue. 900×.

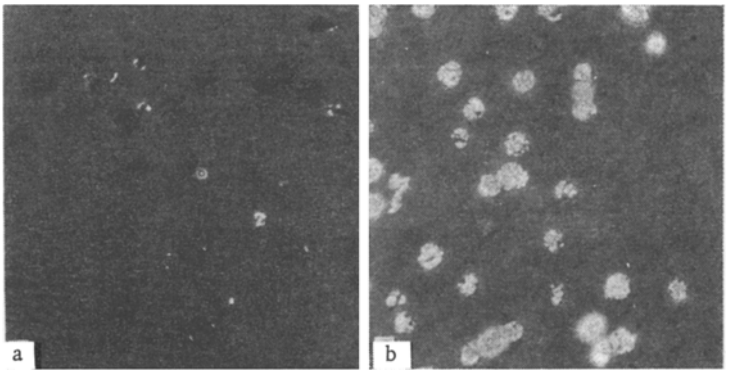

Fig. 2. Dichroism of hepatocyte nuclei in polarized light. Stained with toluidine blue; a) without, b) with sodium bisulfite. 200×.

TABLE 1. Mean Optical Density of Nucleus of Hepatocyte Stained with Toluidine Blue, Expressed in Conventional Units ($\lambda = 550$ nm)

	Tetraploid 4c	Diploid 2c	Ratio 4c/2c
With bisulfite (*a*)	4407±61,42	2228±36,18	1,978
Without bisulfite (b)	2998±40,14	1506±21,33	1,997
Ratio *a*/b	1,47	1,48	

increased metachromasia: On staining with methylene blue, mitotic chromosomes and individual chromocenters in interphase nuclei became metachromatic, and on staining with toluidine blue the nuclei acquired a purplish-violet color (simply violet in the control). The structure of the nuclei (Fig. 1) was similar to that in the control. On investigation of the preparations in polarized light, a birefringence effect was observed, and its intensity correlated with the metachromasia (Fig. 2). In no case did the coefficient of variation exceed 8%.

The results show that bisulfite interacts with aldehyde groups, for Schiff's reagent did not stain these preparations.

According to cytophotometry (Table 1), binding of the dye after bisulfite treatment was increased by 1.47-1.48 times. These results are close to the calculated values: Since

the conditions of hydrolysis were apurinizing and the bisulfite ion could be incorporated only into purine nucleotides, amounting to half of all the nucleotides, and since a reacting phosphate group was present in each nucleotide, binding of the dye after bisulfite treatment should have increased by 1.5 times. We know from the literature that bisulfite is also used for the modification of nitrogenous bases, for it reacts with uracil and with a small percentage of thymine bases [6]. Since the reaction with thymine would have given a very small increase in the intensity of staining, it is difficult to judge the presence of such binding on the basis of the cytophotometric data.

It also follows from Table 1 that both in the absence and in the presence of bisulfite the 4c/2c ratio was close to 2, i.e., the reaction proceeds stoichiometrically and can be recommended for cytophotometry. The high optical density of these preparations also reduces background errors.

The appearance of marked metachromatic and anisotropic staining after treatment with bisulfite is evidence of an oriented arrangement of the molecule of the dye relative to the DNA fibrils. The use of bisulfite in order to obtain metachromatic [2] and anisotropic [5] staining of carbohydrates in the modified PAS reaction is known from the literature.

The results of these experiments can be summed up by saying that bisulfite ions, by incorporation into the molecule of apurinized DNA at the site of the aldehyde groups of deoxyribose, facilitate additional quantitative binding of marker molecules of a cationic dye to phosphate groups. The reaction can be used as a sensitive test for DNA in cytochemistry, including cytophotometry, and also to study the dichroism of DNA-containing structures.

LITERATURE CITED

1. A. G. E. Pearse, Histochemistry: Theoretical and Applied, Little Brown, Boston (1960). [Russian translation, Moscow (1962), p. 791].
2. G. I. Malinin, J. Histochem. Cytochem., 18, 834 (1970).
3. G. I. Malinin, J. Histochem. Cytochem., 23, 974 (1975).
4. A. Miura and Y. Ohba, Biochim. Biophys. Acta, 145, 436 (1967).
5. G. Romanyi and L. Molnar, Nature, 249, 486 (1974).
6. R. Shapiro, M. Welcher, V. Nelson, et al., Biochim. Biophys. Acta, 425, 115 (1976).
7. K. Smetana, J. Lejnar, and M. Potměsil, Folia Haematol., 88, 305 (1967).