THE EFFECTS OF STAINING WITH BUFFERED AZURE A EOSIN B AFTER FIXATIVES CONTAINING PICRIC ACID

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ABSTRACT. — In tissues fixed in fixatives containing picric acid and stained with the azure A eosin B technic, the nuclei were stained either with acid dye or with basic dye depending upon the pH of the staining solution.

During routine staining with the buffered azure A eosin B method (Lillie 1965), it was observed that the nuclei of tissue fixed in modified Bouin's fluid (Lillie 1965) stained pink with eosin B (acid dye) at pH 4.0 and stained blue with azure A (basic dye) at pH 6.5.

An investigation of this problem was conducted, and the results are presented in this paper.

METHODS AND MATERIALS

Tissue from a human skin tumor and from a guinea pig spinal cord were fixed in each of the following fixatives: modified Bouin, picro-formalin (10% formalin in saturated aqueous picric acid), picro-acetic acid (5% glacial acetic acid in saturated aqueous picric acid), and in saturated aqueous picric acid. The control tissues were fixed in 10% formalin.

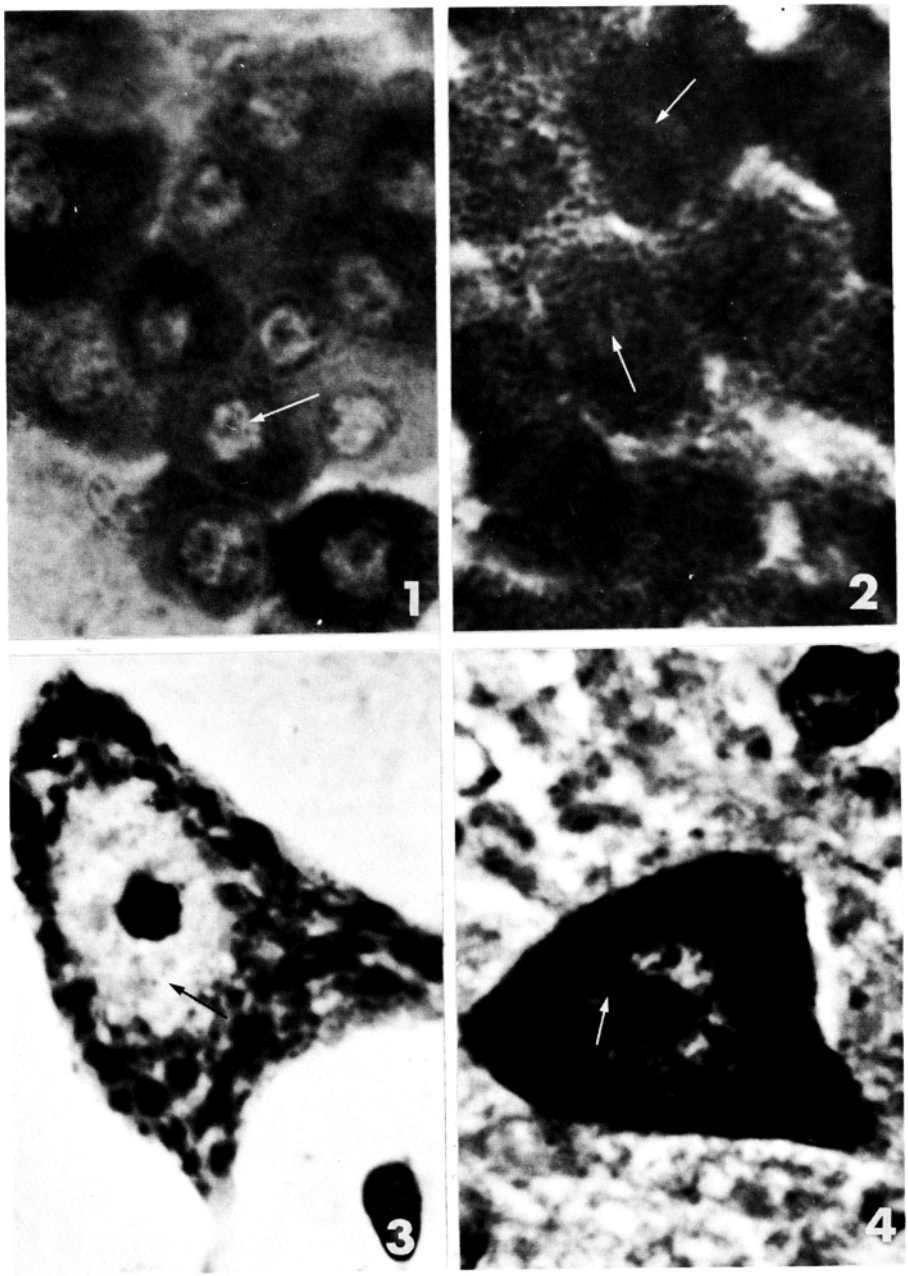
All tissues were then embedded in paraffin by the standard procedure and sectioned at 6μ.

Staining with azure A eosin B was done in a stepwise series of staining mixtures graduated by pH 0.5 from pH 4.0 to pH 7.5. Staining solutions were buffered with Walpole's acetate buffer from pH 4.0 to pH 5.5 and with Sorensen's phosphate buffer from pH 6.0 to pH 7.5 (Lillie 1965). The final dilutions were 0.01 M for acetate buffers and 0.0125 M for phosphate buffers. As a control, two series of staining mixtures comparable to that above were set up in which eosin B was replaced with the same amount of Fast green FSH or Biebrich scarlet.

RESULTS

The staining results were similar for tissues fixed in fixatives containing picric acid. The morphological details were best preserved in tissue fixed in modified Bouin's fluid. At pH 4.5 and below, the nuclei were stained with the acid dye (Figures 1 and 3). At pH 6.0 and above, the nuclei were stained with the basic dye (Figure 4). Between these limits, the nuclei were stained with both dyes. Nuclei of the control tissue fixed in 10% formalin were stained with the basic dye at all pH's used (Figure 2).

The type of acid dye used (eosin B, Fast green FSH, or Biebrich scarlet) did not influence the results. The cytoplasmic basophilia and nucleoli were stained with the basic dye regardless of the fixative or pH used (Figures 1, 2, 3, and 4).



FIGURES 1-4. All preparations were stained with the buffered azure A eosin B technic. X 1,800.

1. — Cells of the human skin tumor, fixed in modified Bouin, and stained at pH 4.0. Arrow points to the nucleus.

2. — Comparable tissue, fixed in 10% Formalin, and stained at pH 4.0. Arrows point to nuclei. Compare with figure 1.

3. — Neuron from guinea pig spinal cord, fixed in modified Bouin, and stained at pH 4.5. Arrow points to the nucleus.

4. — Same tissue but stained at pH 6.5. Arrow points to the nucleus. Compare with figure 3.

Discussion

Some fixatives will permanently increase the affinity of tissue proteins for either basic or acid dyes. Tolstoouhov (1928) showed that this phenomenon was due to a permanent alternation of the isoelectric point of these proteins, toward the lower or higher pH value, caused by the particular fixative fluid. Fixatives containing picric acid render the cytoplasmic proteins acidophilic and produce unusual staining results with nucleic acids. Spicer (1961) and Lhotka and Davenport (1947) showed that fixation with Bouin's fluid produced aldehyde groups in DNA (deoxyribonucleic acid). Lillie (1965) reported that picric acid rendered RNA (ribonucleic acid) insoluble and more resistant to digestion with ribonuclease. Baker (1958) stated that picric acid separated protein of the chromatin from DNA, which was then dissolved.

Our results are in agreement with the last two statements. The dissolved DNA did not stain in tissues fixed in fixatives containing picric acid. The coagulated protein of the chromatin stained either with acid dye (pink) or basic dye (blue) or both (purple) according to the pH of the staining fluid (Baker 1958, Singer 1952). Our results may be explained in a different way. The reactive groups of DNA were either masked with picric acid or firmly bound to the protein of the chromatin. The staining reaction of RNA with basic dye was not affected by the pH of the staining solution or by fixation. Lillie's buffered azure A-eosin B technic used at pH 4.0 or 4.5 for tissue treated with a fixative containing picric acid offers striking staining contrast in cells with strongly basophilic cytoplasm.

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