Topic Introduction

Anionic Counterstains

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Adapted from “Histological Staining in One or Two Colours,” Chapter 6, in *Histological and Histochemical Methods*, 4th edition, by John Alan Kiernan. Scion Publishing Ltd., Oxfordshire, UK, 2008.

INTRODUCTION

For the study of microscopic anatomy and of pathological material, it is usual to stain sections of tissue in such a way as to impart a dark color to the nuclei of cells and a lighter, contrasting color to the cytoplasm and extracellular structures. In most of the general “oversight” methods used in histology, a blue, purple, or black nuclear stain is followed by a paler, usually pink, counterstain, which colors all the other components of the tissue. Simple counterstaining procedures using anionic dyes are considered in this article. The rationales of the techniques are discussed, but methods of higher chemical specificity (e.g., for nucleic acids, carbohydrates, and functional groups of proteins) are not covered.

RELATED INFORMATION

Articles describing **Nuclear Stains** (Kiernan 2008) and **Hematoxylin and Eosin Staining of Tissue and Cell Sections** (Fischer et al. 2008) are also available.

ANIONIC COUNTERSTAINS

Eosin Y is suitable for counterstaining tissue after nuclear staining. It is an anionic dye, so it is bound principally by ionized cationic groups of protein molecules. The most numerous of these are the β -amino group of the side-chain of lysine and the guanidino group of arginine. Nearly all proteins contain these two amino acids, so eosin and other anionic dyes are bound by almost all the structures present in any tissue. A few objects are, however, missed by anionic counterstains. The extracellular matrix surrounding the collagen fibers of connective tissue is composed largely of proteoglycans, which have negatively charged molecules and therefore cannot bind anionic dyes. The same is true of many glycoprotein secretory products and of the matrix of cartilage. Glycogen, a neutral polysaccharide, is unable to bind anionic or cationic dyes. The granules of mast cells contain a basic protein, but this is already neutralized by heparin, a strongly acid proteoglycan also present in the granules. These are therefore stained by cationic dyes but not by dye-metal combinations such as hemalum or by the usual anionic counterstains. Usually it is not possible to see very fine fibers (e.g., reticulin, nerve fibers) or cytoplasmic organelles in sections colored by a single anionic counterstain. Although these delicate structures bind the dye, the degree of contrast is insufficient to permit their resolution in sections more than 0.5–1.0 μm thick.

Anionic dyes other than eosin may also be used as counterstains, but those with strong tinctorial power (e.g., methyl blue, acid fuchsin) are usually avoided because they might obscure the primary staining of the nuclei. When a red nuclear stain has been used, metanil yellow (Quintero-Hunter et al. 1991) or fast green FCF is suitable.

The composition of a solution of an anionic counterstain is not critical. The following usually work well if applied for ~1 min. The lowest indicated concentration is sufficient for most specimens, but

stronger solutions may be needed for very thin sections, for osmicated material, or for specimens that have spent months in formaldehyde.

Anionic counterstaining solutions:

<R>Eosin Y counterstaining solution

<R>Fast green FCF counterstaining solution

<R>Metanil yellow counterstaining solution

For a longer list of suitable counterstains, see the text by Presnell and Schreibman (1997).

If the maximum amount of anionic dye is to be retained in the tissue, the wash after staining should be in 0.5% acetic acid, not H₂O. There is usually some loss of dye into 70% ethanol. This may be desirable, but for maximum retention of color, blot the slides after washing, and take directly to the first of three changes of absolute ethanol.

REFERENCES

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