An Azure and Eosin Rapid Staining Technique

R. F. Cross and P. D. Moorhead*

The azure and eosin technique has been used for routine tissue staining in this laboratory for approximately two years. Azure A has several advantages over hematoxylin as a routine basophilic stain. It dyes certain structures (Nissl bodies, bacteria, certain protozoa) more intensely than hematoxylin and, because it is a metachromatic stain, will demonstrate mast cells and more sharply define cartilage and goblet cells. Lille (1) gives an extensive list of the staining properties of various tissues and micro-organisms when stained with azure and eosin. The main disadvantages of the technique are that the staining solution should be freshly made each day and the staining time is somewhat prolonged (usually one hour).

In an attempt to eliminate these disadvantages, numerous variations of the standard technique (1) were tried. Slides stained with the experimental techniques were compared with duplicates stained in the standard manner until a satisfactory experimental method was found. After a sufficient number of comparisons (between 50 and 100), the staining of duplicate slides was discontinued.

The following technique has been used to stain approximately 600 tissue sections. The tissues originated from cattle, sheep, pigs, dogs, cats, chickens, and turkeys. All tissues were fixed in neutral formalin, embedded in paraffin, and sectioned at six to eight microns using standard techniques (2). Bone specimens were decalcified with formic acid and sodium citrate solutions (2). All tissue types were included in the series.

The procedure is as follows:

1. Remove paraffin and hydrate the sections in the usual manner.
2. Stain with 0.1% aqueous Azure A for 30 seconds.
3. Dip in water.
4. Differentiate by dipping 15 times in McIlvaine buffer at pH 4.3.
5. Dip in water.
6. Stain with 0.1% aqueous eosin (B or Y) for 30 seconds.
7. Dip in water.
8. Dehydrate in acetone (two changes of two minutes each).
9. Clear in equal parts of acetone and xylene for two minutes.
10. Two changes of xylene before mounting.

Buffer solution:

0.1 M citric acid	115 ml
0.2 M sodium phosphate, dibasic	85 ml

Contact with water (steps 3, 5, and 7) serves only to rinse away excess reagent between steps and may be omitted without affecting staining quality. If the water dips are used, they should be as brief as possible. The staining times given (steps 2 and 6) are minimal and may be extended to one minute if desired.

The pH of the buffer used for differentiation is critical. It has been stated that any desired depth of staining may be obtained with thionine, azure A, B, or C, and methylene blue merely by dissolving the dye in a buffered solvent of the proper pH (3). In this procedure, the tissue is initially overstained with azure A and then selectively destained with the buffer. A pH of 4.3 seems best to the authors but it can be varied to suit individual tastes. Increasing acidity will increase eosin staining and decreasing acidity will increase azure staining but the pH selected should be between 4.0 and 4.5. Stained slides must be dehydrated in acetone because alcohol will extract azure A.

The use of other fixatives or embedding procedures would probably require alteration of the technique but, when used as described, it has been quite satisfactory as an alternate to the standard staining method and has the advantage of utilizing stable dye solutions and requiring a minimum of time.

REFERENCES

1. LILLIE, R. D. Histopathologic technic and practical histochemistry. New York and Toronto: The Blakiston Co. 1954.
2. PREECE, ANN. A manual for histologic technicians. 2nd Ed. Boston: Little, Brown and Co. 1965.
3. THOMPSON, S. W. and R. D. HUNT. Selected histochemical and histopathological methods. Springfield, Illinois: Charles C. Thomass. 1966.

* Ohio Agricultural Research and Development Center, Wooster, Ohio 44691. This project was supported by U.S.D.A. Grant number 668-15-1 and Ohio Special Grant 146.