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An Improved Method of Staining Paraffin-Embedded Bone Marrow Sections

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Cytological detail is well demonstrated in an improved method of staining aspirated bone marrow sections embedded in paraffin. The tinctorial patterns of the stain resemble some of its predecessors; thus, cell counting and identification involve little difficulty. The method has proven reliable, reproducible and a valuable addition in the diagnosis of bone marrow disorders. It is a useful tool in research problems in which objectives are the study of the bone marrow.

In this paper we report an improved stain intended for paraffin-embedded tissue sections of aspirated bone marrow specimens. The method has differential staining properties useful both in the histologic evaluation of bone marrow disorders and in a variety of other problems involving the cytological study of bone marrow. The method gives not only differential staining of paraffin-embedded bone marrows, but is also a general staining method for smears of bone marrow and peripheral blood, malarial parasites in blood, tissue impression preparations, chromosomes and decalcified sections of bone.

Materials and Methods

For best results bone marrow specimens should be fixed in B-5 or in Zenker's fluid containing acetic acid. Fixation in 10% formalin is also permissible without resultant loss of important details.

Cut routinely processed paraffin section at 4 micra.

I. *Solutions Required*

A) Buffer mixture: Acetate-acetic acid (Walpole), pH 5.4-5.6
B) 5% sodium thiosulfate
C) Lugol's iodine or 0.5% iodine in 70% alcohol
D) Differentiating solution
Glacial acetic acid .....0.5-1.0 ml
Distilled water, enough to make .....100.0 ml

II. *Stock Villanueva Blood Stain**

Villanueva stain powder ....0.75 g
Methanol, 100% (A. R.) ..200.00 ml
Dissolve the powder thoroughly for at least a half hour with a mechanical stirrer or shaker. Cover the solution tightly and allow to stand 24 hours. Filter and store in a stoppered brown bottle. Solution is ready to use.

III. *Working Staining Mixture*

Stock Villanueva blood stain 5.0 ml
Buffer mixture .....5.0 ml
Tap water .....30.0 ml

Mix thoroughly.

Staining Procedure

1) Deparaffinize section as usual and run through water. Remove mercury precipitate

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*Available in either dry powder form or solution from Lipshaw Manufacturing Co., Detroit, Michigan

with iodine for 5 minutes, followed by rinsing in distilled water. Transfer to 5% sodium thiosulfate for another 5 minutes, then wash in running water for 5 minutes.

2) Rinse in distilled water.

3) Stain in the working staining mixture for 25 minutes or longer. (Minimum staining time is 25 minutes but may be longer to suit individual requirements). Note: *Carry one slide at a time in the next remaining steps.*

4) Differentiate in the differentiating solution: Immerse slide in distilled water then check differentiation under a microscope to ascertain the depth of staining but take care that the preparation is not allowed to dry. If the desired degree of staining has not been attained, return the slide to the differentiating solution and rinse again with distilled water before further examination. This process may be repeated several times until differentiation is complete. (The landmark is sharp, brilliant purple nuclei).

5) Dehydrate and clear in:

95% alcohol .....5 to 10 dips
absolute alcohol .....5 to 10 dips
equal parts: xylene and absolute alcohol .....10 to 20 dips
xylene .....10 to 20 dips

This time check the slide again under a microscope for the final color differentiation. If overdifferentiated, nuclei and red cells are pale blue and pale pink respectively. Return the slide into the alcohols to distilled water, then restain for another 25 minutes. If underdifferentiated, eosinophil granules are dark red. Slide can be carried back with quick dips to alcohol and back again to xylene. Repeat this procedure until the granules are brilliant pink.

xylene .....1 minute
xylene .....5 minutes

6) Mount in any neutral synthetic resin.

Results

A correctly-stained slide should show grossly bright pink and dark blue. Erythrocytes and eosinophilic granules are brilliant pink. The cytoplasm of most cells is pale pink, their nuclei bluish purple. Granulocytic elements are easily distinguished from normoblasts, lymphocytes and plasma cells. Megakaryocytes are easily identified by their large size and characteristic nuclei. Their cytoplasm is stained pink and is usually fine, homogeneous and granular or it may be coarse, granular and bluish color. Normoblasts, together with all forms of early developmental cell lines, are sharply delineated.

Mast cell granules are stained dark violet to reddish purple; basophil granules are also stained reddish purple but less densely than the mast cell granules. Inclusion bodies and bacteria are blue. Bone matrix is stained pink and cartilage is stained a dark blue to violet color.

Discussion

This stain brings out prominently the nuclear, cytoplasmic and granular properties of sectioned bone marrow cells, especially in specimens that are subjected to B-5 and Zenker acetic fixations prior to staining. In specimens fixed in formalin, very little variation in staining occurs. Colors are not as brilliant but the cellular features are clear and adequately stained.

The tinctorial patterns of this stain are similar to other Romanowsky-type stains[1-4] and identification of cell types presents little difficulty. Although basic similarity exists in the staining, some obvious differences of this stain will occur to the pathologist or hematologist who is experienced in interpreting bone marrow sections. Conspicuous among them are the sharply outlined features of the normoblasts together with all forms of early developmental cell lines, brilliant staining of eosinophilic elements, delicately stained nuclear chromatin and generally improved cytologic detail (Fig 1). We have found

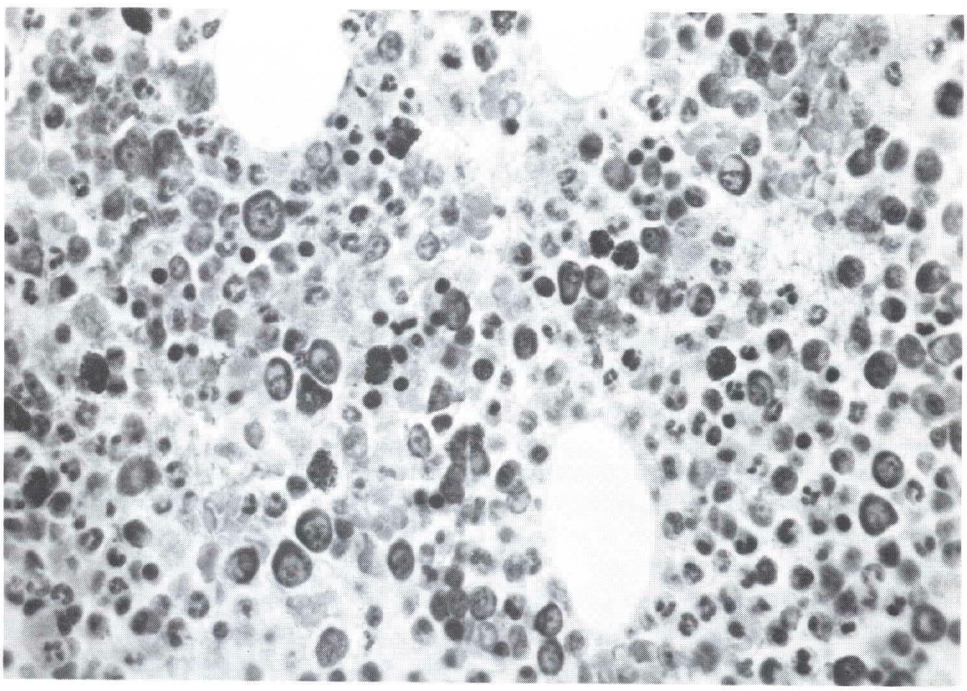

Figure 1

Photomicrograph of paraffin-embedded bone marrow section in plasma cell myeloma. Most of the plasma cells pictured are obvious tumor cells. Note that, characteristically, the myeloma cells show distinct perinuclear zones. Note also the sharply outlined features of the normoblasts, the dark stained granules of few eosinophils and early developmental cell lines. *Villanueva Blood Stain 700X.*

the method useful for various inclusion bodies, frozen sections and staining of tissue impression preparations and decalcified sections of bone.[5] Furthermore, it is useful in routine staining of peripheral blood and bone marrow smears, malarial parasites in blood, and in staining of chromosomes.[6]

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