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Original Research Article

A Newly Identified Stain for Mast Cells in Human Mesentery

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ABSTRACT

For invitro studies in mesentery toluidine blue and thioninare generally used. We have identified a new stain and found it to be good as it showed good contrast between Mast cells and the background tissue.
A simple, cost effective and rapid method for staining mast cells in human mesentery is described. Mesentery was removed from embalmed cadaver and cut into pieces, spread on glass slides, tied with the thread on both sides and fixed in 10% formalin overnight and stored in 70% alcohol. Small pieces of convenient size were cut and transferred into a petri dish, washed thoroughly and was spread on a glass slide. The tissue was stained with Methyl Violet 10B (MV10B) stain for 1 minute, washed, dehydrated, cleared and mounted in DPX. Mast cells were seen against light connective tissue background.
Key words: Stain, Mast Cells, Human Mesentery

INTRODUCTION

Mast cell function has been attributed to wound healing, immune allergic reaction and regeneration among other functions. They are widely distributed in the connective tissue. The abundant granules stain metachromatically with thiazide dies such as toluidine blue. They also stain with copper phthalocyanine dyes such as alcian blue and astra blue. [1]
Astra blue has been used to stain differentially mast cells in the intestine. However the procedure has not been widely used because of difficulty in preparing and using the dye solution. [2]

In the present study the contrast between the deeply stained granules and the lightly stained background facilitates detection and counting of mast cells. This can be used to advantage for routine study of mast cells.

MATERIALS AND METHODS

Mesentery was removed from embalmed cadaver during the routine dissection of the abdomen. The sheet of mesentery was cut into small pieces to accommodate in the slides. The cut pieces were spread on a slide and tied with thread from both sides. The slides were fixed in 10 % formalin overnight and storedin 70% alcohol. Small pieces were cut, transferred into a petri dish and were washed thoroughly. The bits were taken on to the slides and stained with MV10 stain for 1 minute, washed, dehydrated cleared and mounted in DPX.

Preparation of the MV10B stain:

-MV10B stain 0.25 gms,

-Autoclave distilled water : 100ml

-Dissolved and filtered with whatman filter paper and stored in a brown bottle at room temperature.

Staining procedure:

1.Stain in MV10B stain for one minute

2.Wash in water until the color ceases.

3.Dehydrate in absolute alcohol

4.Clear in xylene

5.Mount in DPX

RESULTS

Darkly stained granules with light staining nucleus of mast cells are seen against connective tissue background.

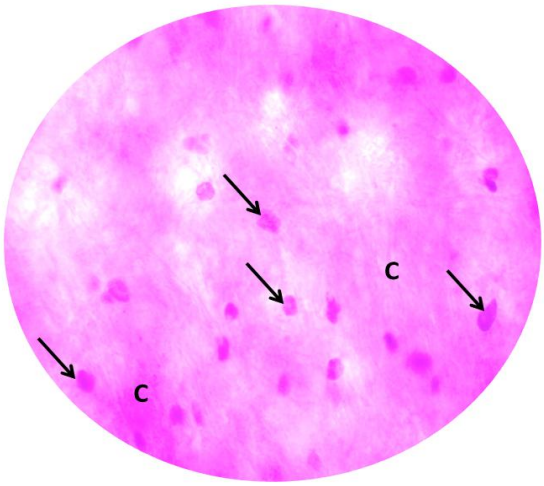

Fig. 1. Mesentery (x400): Arrows- Mast cells, C- Connective tissue background

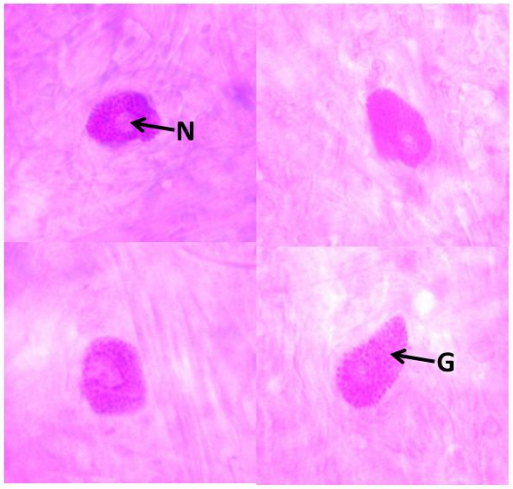

Fig. 2. Mast Cells in the mesentery (x1000): G- Granules, N- Nucleus

DISCUSSION

Thioninhas been used to stain mast cells in mesentery. [3] The methods involves staining with a diluted solution of thionin for 30 minutes, differentiated in 0.2 % acetic acid controlled microscopically and counter stained with saturated tartrazine in cello solve. [4]

Previously we had reported a modified thioninacridine orange stain for mast cells in rat mesentery which can be completed within 10 minutes and doesn't require any differentiation. [5]

The present study involves a new single staining technique using which the darkly stained granules stand out in better contrast on a fairly stained background. The simplicity and reproducibility of the procedure combined with the compatibility with a variety of mast tissue fixatives makes it useful for routine use in histopathological laboratories.

Methyl violet is the family of organic compound that are mainly used as dyes. Depending on the amount of attached methyl groups the colour of the dye can be altered. Its main use is as the purple dye for textiles and to give deep violet colours in paint and ink. Methyl violet 10B is also known as crystal violet (and many other names) and has medical uses.

Methyl violet 10B has six methyl groups. It is known in medicine as gentian violet (or crystal violet or pyoctanin(e) & is the active ingredient in gram stain used to classify bacteria. It is used as a pH indicator with a range between 0 and 1.6. The protonated form (found in acidic conditions)

is yellow terming blue violet above pH levels of 1.6. Gentian violet destroys cells and can be used as a disinfectant. Compounds related to methyl violet are potential carcinogens.

Methyl violet 10B also inhibits the growth of many gram positive bacteria except streptococci, when used in conjunction with nalidixic acid (which destroys gram negative bacteria). It can be used to isolate the streptococci bacteria for the diagnosis of an infection.

Methyl violet 10B also binds to DNA. This means it can be used in all viability assays in biochemistry. However this binding to DNA will cause replication errors in living tissue, possibly leading to mutations and cancer. [6]

CONCLUSION

The advantages of the newly identified MV10B staining techniques are - easy to prepare, low dye concentration, no differentiation, consistent results and easy to perform the technique.

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