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Original Research Article

A Simple Cost Effective Rapid Differential Staining Technique for Myelinated Fibres and Nerve Cells

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ABSTRACT

Nerve cell bodies and nerve fibres are commonly stained by Kluver Barrera method and Margolis method. However few studies have performed for cost effective staining. A simple, cost effective, single stain, rapid method for the differential staining of myelinated nerve fibres and nerve cell bodies applicable to paraffin sections of central nervous system was described. Neutral buffered formalin fixed human cadaver brains and cord were used. Sections of 8 micron thickness of spinal cord, Cerebellum, Pons, Medulla and Mid brain were attached to egg albumin coated slides. Slides were dewaxed and hydrated and brought to water. Slides were stained for two minutes in the following staining solution. 0.25gms of MV10B stain dissolved in 100 ml of autoclaved distilled water and filtered. Stained slides were decolorized in 95% alcohol, dehydrated in absolute alcohol, cleared in xylene and mounted in DPX. Myelinated fibres and nerve cell bodies were stained in varying shades of violet color, which gives an adequate contrast for observation and photography. The present technique was very simple highly cost effective and rapid stain which doesn't require any differentiation. The whole process of staining will be completed within 15-20 mins with adequate results.

Keywords: Central nervous system, Kluver Barrera and Margolis method, MV10B stain, Paraffin sections

INTRODUCTION

Techniques for staining nerve cell bodies and fibres are frequently used in basic and experimental Neuro anatomy. In spite of the many procedures used a routine technique to stain nerve cells and fibres in a simple cost effective, rapid and with uniform results is not available. [1] Kluver barrera, [2] Margolis, [3] Catechu stain [4] are generally used in neuro histology for routine and research work. Although these methods provide good results, it cannot be considered as routine techniques of simple application because the staining process requires over coloration followed by critical

differentiation in both myelin and Nissl staining ensuring good results. Apart from this, these techniques are not cost effective, require higher temperature for staining & time consuming.

A new technique for block staining with catechu stain was published for differential and simultaneous staining of nerve cells and fibres that avoids these disadvantages and giving uniform results. [5]

In this paper we describe a procedure to stain both myelinated fibres and nerve cells in paraffin embedded material. We have used MV10B stain, a methyl derivative of Para rosaniline. [6]

The technique described in the present work does not require any differentiation, highly cost effective, low dye concentration, simple, easy to perform, rapid, gives consistent results with adequate contrast between fibres and cells.

MATERIALS AND METHODS

To ensure procurement of fresh material, the operation was well planned in advance and things were kept ready for immediate embalming and removal of the material.

When dealing with material from central nervous system it is essential that it should be fresh. Even a delay of few hours is sufficient to render certain elements undemonstrable. The body was embalmed with neutral buffered formalin (AR grade) within two hours of death. After embalming the brain was removed carefully and suspended in neutral buffered formalin by string attached to the vessels of the circle of Willis. The spinal cord was suspended in a large volumetric cylinder by a string inserted into a small cut in the dura. The dura was opened with scissors along the back of the spinal cord to allow penetration of the fixative and was kept hanging freely. [7] It was kept for a week. After which blocks were cut and placed again in individual containers of fixative for one more week. Then the blocks were transferred to 80% alcohol for storage and processing. The tissues were processed and the impregnated with the molten paraffin wax was done in a vaccum chamber at 10-12 inches of mercury negative pressure ensuring complete impregnation. After blocking sections of medulla, midbrain, pons, spinal cord, cerebellum were cut at 8 microns and attached to egg albumin coated slides and kept at 37 degree incubator overnight.

The following stain was prepared and kept in a brown bottle at room temperature:

0.25gm MV10BN stain dissolved in 100 ml of autoclaved distilled water and filtered with whatt man filter paper.

The slides were dewaxed and hydrated by successive alcohols (100%, 90%, 70%) for 4 mins each.

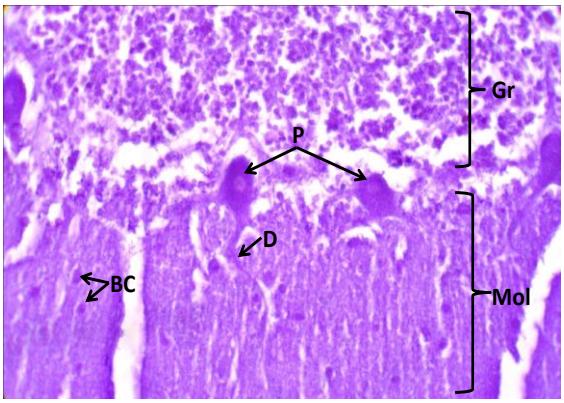
After hydration, the slides were placed in distilled water for few minutes.

- Stained in MV10B stain for 2 mins
- Decolorized in 95% alcohol until the alcohol is clear (appox. 10 -15 secs)
- Dehydrated in 100% alcohol
- Cleared in xylol
- Mounted in DPX

RESULTS

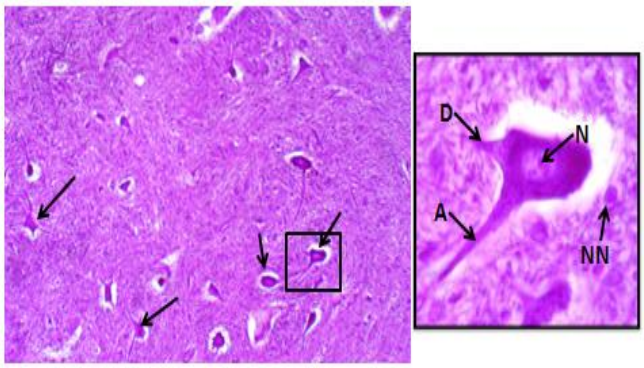
The application of this procedure gave a good demonstration of fibers with varying colors of violet (as shown in fig.1) and nerve cell distribution(as shown in fig.2), with similar results obtained with other methods.

Fig. 1



Cerebellum (x400): Gr - Granular layer, Mol - Molecular layer, P - Purkinje cells, D - Dendrite, BC - Basket cells

Fig. 2



Spinal Cord - Ventral horn (x100): Arrows - Multipolar Motor neurons.
Inset (x400): N - Nucleus, D - Dendrite, A - Axon, NN - Nuclei of Neuroglial cell

DISCUSSION

Several techniques for simultaneous and differential staining of nerve cells and fibres of central nervous system have been published. The kluver barrera method and Margolis method requires crictical differentiation steps in both Nissl and myelin staining , a fact that constitutes a serious problem in obtaining uniform results. The kluver barrera method is a double staining procedure which involves Luxol fast blue for myelin staining and Cresyl echt violet for Nissl substances. The Margolis method is a triple stain of Luxol fast blue, PAS and Harris Haematoxylin. Both the stains are not cost effective and staining has to be done at a temperature of 60 degrees overnight. The Catechu stain though gives good results; it has to be performed at 60 degrees overnight. All these procedures are time consuming. The present technique is very simple highly cost effective and rapid stain which doesn't require any differentiation. The whole process of staining will be completed within 15-20 mins with adequate results. This can be used as a routine stain in neurohistology for routine and research work.

Classical neuropathology has relied heavily on the use of a large array of empirical staining techniques to demonstrate the specialized structure encountered in the central nervous system. [8]

Methyl violet is a family of organic compounds that are mainly used as dyes. Depending on the amount of attached methyl groups, the color of the dye can be altered. Its main use is as a purple dye for textiles and to give deep violet colors 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) and is the active ingredient in a 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, turning 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 cell viability assays in biochemistry. However, this binding to DNA will cause replication errors in living tissue, possibly leading to mutations and cancer. [9] Crystal violet stain has been used in neurohistology to demonstrate glia fibres. [10] A diluted solution of crystal violet stain was used by Lillie to demonstrate Amyloid. [11]

In an unpublished work, the second author has used MV10B stain for sperm morphology. It gives consistent results to identify the normal and abnormal sperms. This can be used in andrology laboratories for sperm morphology.

We have identified MV10B stain for neuro histology after trying various stains. The technique described here is very simple, highly cost effective, low dye concentration, rapid, giving consistent results, requires no differentiation. For all these reasons this technique can be used routinely to stain nerve cells and fibres in routine and research work. We have used human material. This can be used for other species also.

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