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Original Research Article

Longevity of DPX Mounted Slides in Neurohistology

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ABSTRACT

This study was undertaken to review the following aspect of the mounting medium DPX. The longevity of the medium, preservation of the Neurohistological stains, to observe for formation of air space under the cover glass.

Two sets of slides stained in 1967 and 1987 were selected from the collection of histology laboratory. These slides were well fixed and processed human central nervous system paraffin blocks of six micron thickness.

The first set of slides were stained with various histological stains i.e. Margolis, Kluver Berrera, Luxol fast blue, Cresyl fast violet, Mallory Azan and mounted in DPX mountant in 1967.

The second set of slides was stained with Catechu stain, which was developed and mounted in the DPX mountant in 1987 by one of the authors.

These set of slides have been carefully monitored from time to time for any deterioration of structures, preservation, fading of stains and development of air spaces for the last 45 years. Till recent years these slides were used as demonstration slides during neurohistology practicals.

On observation it has been found that the DPX mountant has not caused any deterioration what so ever and the structures are well appreciated, as though the staining has been performed recently. It appears that the DPX mountant will preserve the stained slides for a few more decades without deterioration.

KEY WORDS: central nervous system, paraffin sections, staining, DPX mountant

INTRODUCTION

Numerous experiments have been made with resins and plastics in search of a more acceptable mounting medium. Mounting media which have refractive index similar to that of glass slide (1.51-1.52) are generally used as their refractive indices must match that of the tissue component for maximum transparency. [1]

The best preservation of basic aniline dye stains is generally observed in mounting media with low acid numbers or in those containing no free acid. It is to be recalled that the natural resins consist of resin acids and their esters and that these compounds are often unsaturated and may bleach certain basic dyes by their reducing action as well as by their essential acidity. The natural resins are composed of unsaturated resin

acid and their esters, dissolved in natural solvents such as turpentine. Since they are composed largely of acids, any attempt of alkali or alkaline with carbonol have been largely futile. Usually the neutral solvents are dissolved off and the plastic solid resins are dissolved in aromatic hydrocarbons for use sometimes in chlorinated hydrocarbons or in alcohols. [2]

Polystyrenes have little or no residual unsaturation and no free acid, have rather limited solubility in aromatic hydrocarbons and neither high refractive indices.

One of the most commonly used mountant, DPX is a colourless, neutral medium in which most standard stains are well preserved. It is prepared by dissolving the common plastic, polystyrene in a suitable hydrocarbon solvent, usually xylene. Major disadvantages of polystyrene medium however is that they set quickly and in doing so often retract from the edge of the cover slip. This can be prevented by adding a plasticizer which is thought to resist effectively forming a mesh with the polyunsaturated plastics.

The reagents required for the preparation of DPX mountant are as follows:

1. Polystyrene (Distrene 80).....18g
2. Dibutyl Phthalate .....7.5 ml
3. Xylene .....52.5 ml

The refractive Index of the solution is 1.523. [3]

MATERIALS & METHODS

Human central nervous system tissues were fixed in 10% buffered neutral formalin. Tissues were processed and impregnated with molten paraffin, in a vacuum chamber. Sections were cut at 6 microns in Minot Rotary Microtome. First set of slides were stained by the following methods – Margolis, [4] Kluver Berrera, [5] Cresyl fast violet, [6] Mallory Azan and mounted with DPX mountant in 1967. The second sets of slides were stained in Catechu stain which was developed in 1987 by one of the authors and mounted in DPX mountant in the same year. The stained slides were observed over the years.

RESULTS

Nerve cells and fibres remain well stained. Structures continue to be well preserved even after forty five years as depicted in the micrographs.

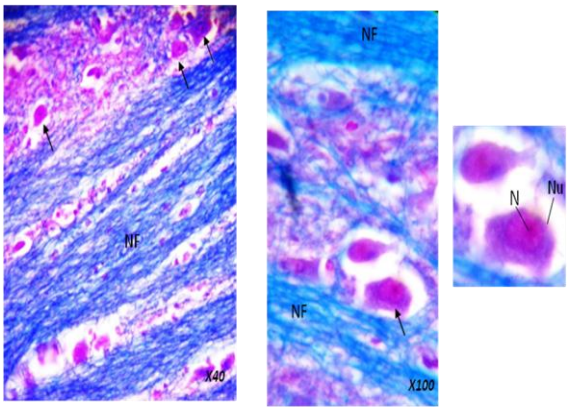

Figure 1: Photomicrograph of DPX mounted Kluver Barrera stained cross section of the Pons. The nerve fibres (NF) appear blue, nerve cell bodies (arrows) with nucleus appear red, background colourless.

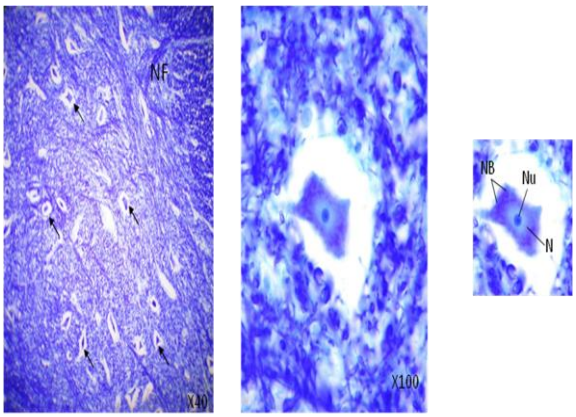

Figure : 2 Photo micrograph of DPX mounted Luxol fast Blue stained cross section of the ventral horn of human Spinal cord, nerve fibres (NF) appear - blue, Nucleus (N) light blue, Nucleolus (Nu) – dark blue, Nissl bodies (NB)- violet

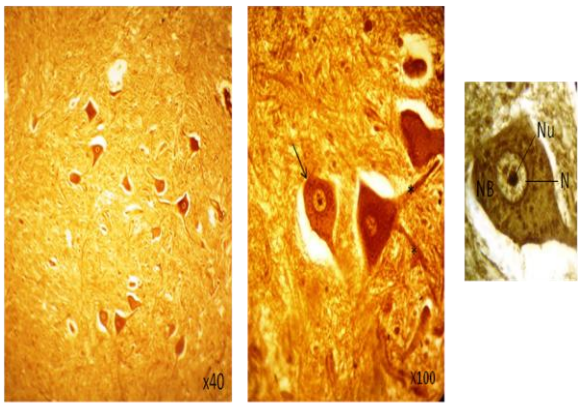

Figure :3 Photomicrograph of the DPX mounted Catechu stained cross section of the spinal cord. Cell body (arrows) filled with Nissil boddies (NB), nucleus(N), nucleoli(Nu) along with processes (*)

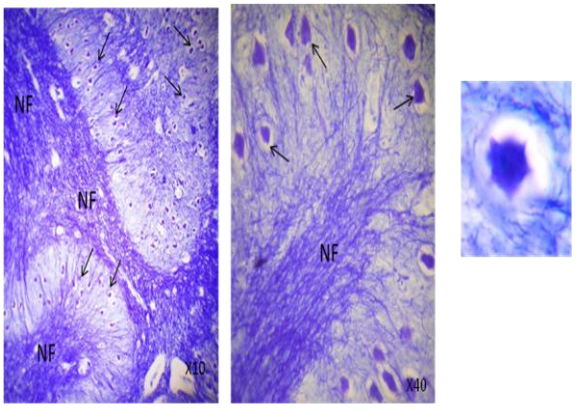

Figure:4 Photomicrograph of DPX Mounted Luxol Fast Blue stained cross section of the Medulla oblonga at th level of inferior olivary nucleus showing myelinated nerve fibres (NF) with neurons (arrows).

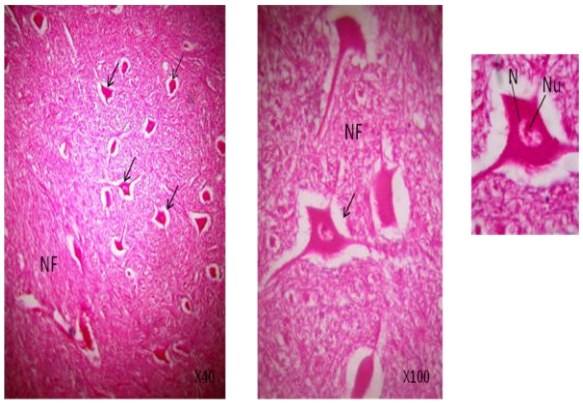

Figure:5 Photomicrograph of the DPX mounted Mallory Azan stained cross section of the Spinal cord. Nerve fibre (NF),

DISCUSSION

The ideal mounting medium must be the one that preserves the colour of all the stains. Unfortunately at present there is no such mountant, as some stains remain best in acid and others in a neutral or alkaline medium. Considering the above fact one should always consider the following criteria before selecting a mountant. The mountant should harden fairly quickly, should not become acid, such that aniline dyes fade, should not crack or develop granules.

Numerous works have been done with regard to the mountants and cover slip free mountants by various authors. Vroman has used an acrylic spray as a substitute for coverslips. [7] Donald used a plastic seal method for mounting sections of ground bone. [8] One of the authors of the present study in the past have reported a cover slip free mounting medium [9] and acrylic paste medium for ground bone.[10] In our laboratory we have also made use of quick fix, a resinous product as a mountant. [11-13] Considering the above factors the authors find DPX to be a satisfactory mountant.

CONCLUSION

We have reported the longevity of DPX mountant. This preserves the various neurohistological stains very well. The first set of slides stained and mounted in DPX mountant in 1967 and the second set of slides stained with catechu stain and mounted in 1987 showed that the mountant is excellent and can be depended upon. Thus DPX mounted neurohistology slides can be kept preserved for decades.

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