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Original Research Article

Longevity of Paraffin Sections in Neurohistology

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ABSTRACT

In Histology and histopathology laboratories, the normal practice is to cut the required sections from the paraffin blocks and the blocks are preserved for further use. Cut paraffin sections are not usually stored. In our laboratory soon after the blocks are made few sections are cut and stained for observation of proper structures and after approval around 100-150 sections are cut and the slides are stored in a slide cabinet. Since our students are provided with individual slide boxes for study, the required number of slides is stained. Rest of the paraffin sections are stored, and as and when required, the slides are stained for routine study and special staining for demonstration purposes. We have kept the paraffin sections for years together and haven't come across any deterioration of structures in any of the systems. The blocks are preserved for further use. With regard to neurohistology, we wanted to observe the longevity of the paraffin sections and any changes in structures. Well fixed and processed human central nervous system, Paraffin blocks were cut and slides were stored in slide cabinet in October 1967. In September 2012 after a period of 45 years, slides were stained by various neurohistological stains i.e., Kluver Barrera, cresyl fast violet, neutral red and newly identified Methyl Violet 10B stain for nerve cells and fibers, for observance of any changes and we have found the structures are intact and well stained. As such sections for neurohistology can be cut and stored for many years without any problem and stained as and when required, including special staining procedures.
With this method sections can be cut only once at a stretch, slides can be stored, avoiding later section cutting problems.

Key words: Central nervous system, paraffin sections, section cutting, staining.

INTRODUCTION

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 best method of fixing is after embalming, the brain is removed carefully and suspended in neutral buffered formalin by string attached to the vessels of the circle of willis. The spinal cord is to be suspended in a large volumetric cylinder by a string inserted into a small cut in the dura. The dura is to be opened with scissors along the back of the spinal cord to allow penetration of the fixative and should be kept hanging freely. It should be allowed to fix for a week, after which the selected pieces are cut and placed in individual

containers of fixative for a week. This should be transferred to 80% alcohol for storage and processing. [1]

Tissue from the central nervous system is usually most unsatisfactory when prepared by the ordinary routine paraffin wax technique. The disadvantages are:

i. Considerable breaking of the grey matter
ii. Excessive shrinkage
iii. The tissue has a great tendency to break up during the process of staining.

Hence the impregnation with the molten paraffin is to be done in a vacuum chamber at 10-12 inches of mercury negative pressure ensuring complete impregnation. When the sections have been cut and floated out, it should be kept at 37 ºC incubator overnight. With immediate fixation, proper processing and cutting the paraffin sections of the central nervous system can be stored for a very long period without any harm to the structures.

MATERIAL AND METHOD

Human central nervous system tissue was fixed in 10% buffered neutral formalin. Tissues were processed and impregnation with molten paraffin was done in a vacuum chamber. Sections were cut at 6 microns in a Minot rotary microtome, floated out and kept at 37 ºC incubator overnight. Slides were stored in a slide cabinet. Sections were cut and stored in October 1967. After a period of 45 years in September 2012 slides were stained by the following methods:

i. Kluver Barrera [2]
ii. Cresyl fast violet [3]
iii. Neutral red [4]
iv. MV10B stain [5]

RESULTS

Nerve cells and fibres were well stained. Structures were intact.

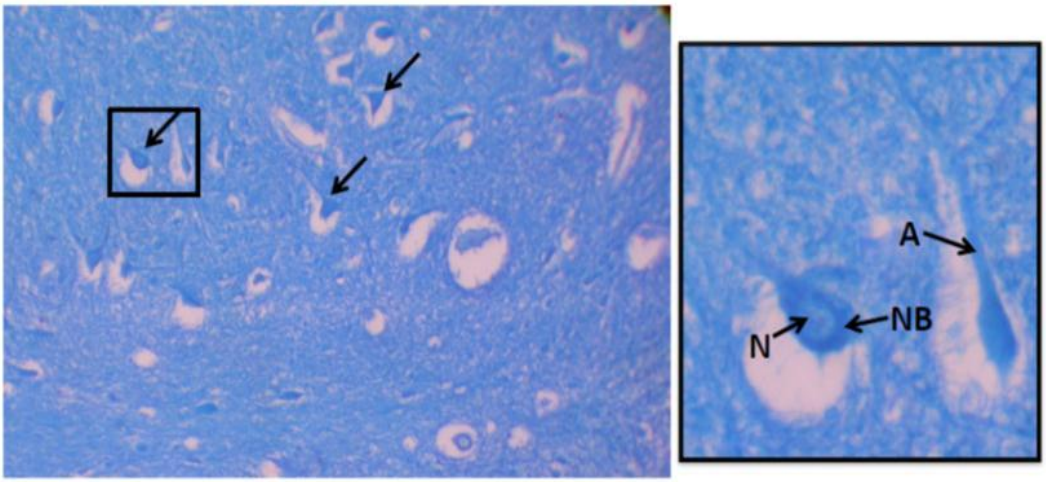

Figure 1: Kluver Barrera stain- Spinal cord(x100): Arrows - Multipolar neurons. Inset (x400): NB – Nissl Bodies, N – Nucleus, A – Axon.

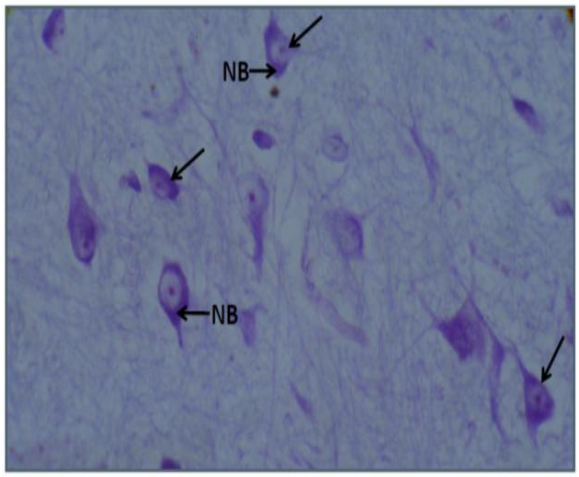

Figure 2: Cresyl fast violet stain- Spinal cord (x400): Arrows - Multipolar neurons. NB – Nissl Bodies

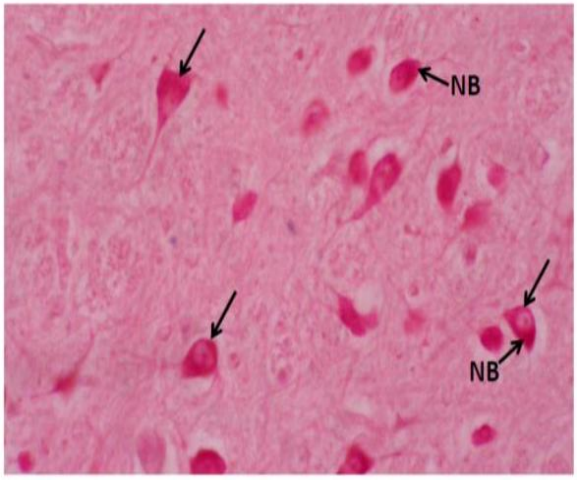

Figure 3: Neutreal red stain- Spinal cord (x400): Arrows – Multipolar neurons. NB – Nissl Bodies

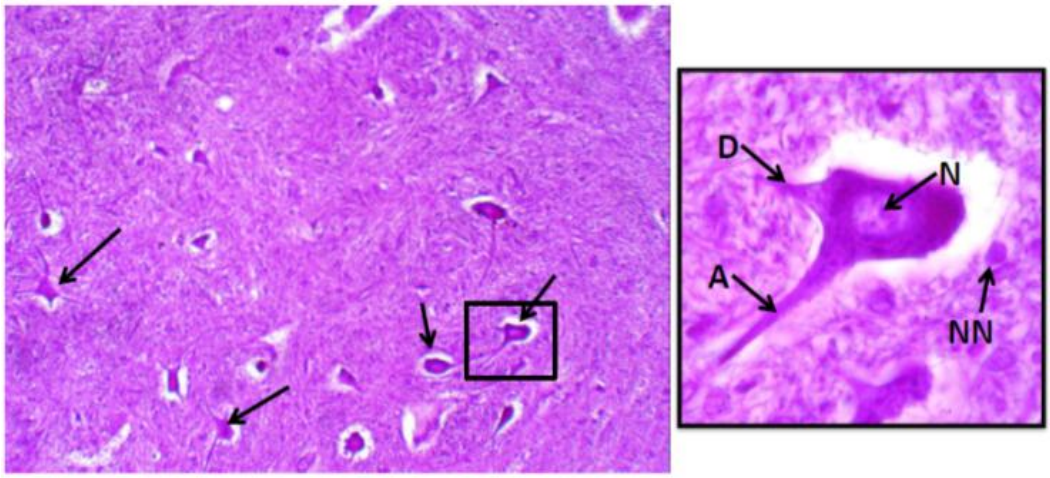

Figure 4: MV 10B stain - Spinal Cord (x100): Arrows - Multipolar Motor neurons. Inset (x400): N - Nucleus, D - Dendrite, A - Axon, NN - Nuclei of Neuroglial cell

DISCUSSION

Immediate fixation, proper processing and cutting is necessary to ensure good sections of the central nervous system. Successful sections require-

a) Properly prepared material with the supporting medium matching the specimen.

b) A sharp knife: Poorly prepared material can sometimes be cut by a good knife, but a well prepared block may be ruined by a poor knife.

c) A proper microtome; the choice of microtome should depend on the application. Unless very old, misused, damaged or of poor quality a microtomeis rarely the cause of poor sections.

d) A skilled operator; practice is the word and the acquiring of skills. A skilled operator must be able to recognize and correct difficulties as they arise.

To ensure the longevity of paraffin sections in neurohistology the four points must be taken into account. Good sections can be stored for many years without any problems and stained as and when necessary. Sections can be cut from freshly prepared block at a stretch and preserved in a slide cabinet assuring dust free sections.

By this, frequent cutting of sections is avoided and considerable time is saved, without compromising the quality.

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