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MATRIC NO : 17/MHS03/ 003

DEPARTMENT: ANATOMY

COLLEGE: MEDICINE AND HEALTH SCIENCES

COURSE CODE : ANA 304

COURSE TITLE : HISTOCHEMISTRY

QUESTION

1. If Nissl stain is used to demonstrate RNA/DNA in the neurons of the CNS , what staining

technique is used for identifying the same in peripheral neurons .

1. Is luxol fast blue stain also used to detect demyelination in the PNS ? whatever your answer is Explain the procedure involved in the demonstration of demyelination in the PNS

ANSWER

1. The staining technique used in to identify RNA/DNA in the peripheral nervous system is called the fuelgen stain

 This technique is used to demonstrate DNA in tissue sections. It **Fuelgen stain** is a staining technique discovered by Robert Feulgen and used in histology to identify chromosomal material or DNA in cell specimens. It is sensitive in detecting aldehydes, which makes it the right method for detecting the presence of DNA. The specimen is subjected to warm (60 °C) hydrochloric acid in other to remove the bases .The sugar part that remains reacts as an aldehyde ultimately forming a visible color.

STAINING PROCEDURE

1. Prepare Hydrochloric Acid Working Solution; combine and mix well.
	1. *Hydrochloric Acid, 20% Aqueous            16 ml*
	2. *Distilled Water                                          24 ml*
	3. *Preheat and maintain Hydrochloric Acid Working Solution at 60°C prior to use.*
2. Deparaffinize sections thoroughly in three changes of xylene, 3 minutes each.  Hydrate through two changes each of 100% and 95% ethyl alcohols, 10 dips each.  Wash well with distilled water.
	1. *See Procedure Notes #2 and #3.*
3. Hydrolyze sections in Hydrochloric Acid Working Solution at 60°C for 10 minutes.
	1. *See Procedure Notes #4 and #5.*
4. Place slides directly in Schiff Reagent, McManus for 45 minutes.
	1. *See Procedure Note #6.*
5. Wash in running tap water for 5 minutes; rinse in distilled water.
6. Counterstain in Light Green SF Yellowish Stain 0.2%, Aqueous (Part 12202) for 1 minute.
7. Dehydrate in two changes each of 95% and 100% ethyl alcohol. Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.

RESULTS

|  |  |
| --- | --- |
| DNA | Red-purple |
| Nuclei | Red-purple |
| Background | Green |

PRECAUTIONS

1. Avoid prolonged exposure to hydrochloric acid it may over-hydrolyze sections with poor staining results.
2. Do not allow sections to dry out at any point during staining procedure.
3. Drain staining rack/slides after each step to prevent solution carry over.
4. Bouin fixed tissue is unsatisfactory for use with Feulgen reaction.
5. Luxol fast blue is used to detect demyelination in the central nervous system but not In the peripheral nervous system .

The disintegration of myelin sheaths of degenerating peripheral nerves occurs in two stages . The early stage is characterized by a physical disruption of myelin sheaths without chemical alteration . The last stage , beginning about one week after axonal section ,consists of a chemical degradation of myelin lipids . In degenerating peripheral nerves these changes develop within schwann cells , indicating that these cells are capable not only of forming but also of digesting myelin sheaths .

 In the central nervous system the breakdown of myelin sheaths after axonal degeneration occurs at a much slower pace . This temporal difference suggest that the oligodendrocytes which form , the myelin sheaths of central nerve fibers , do not behave like schwann cells , but that the myelin digestion must await the arrival of macrophages .

Myelinated axons can be observed in the fresh state by phase-contest microscopy of teased nerves or of squash or touch preparations of central nervous tissues . Living myelinated fibers can be seen in thin tissues cultures derived from sensory or autonomic ganglia or from immature brain or spinal cord. The nervous tissue is fixed Swank and Davenport’s Marchi method for degenerating myelin ,sectioned and stained to provide permanent preparations . When neurons die , or if the axon has been severed , the part that has been cut off from the neuronal cell body becomes beaded and then breaks into a string of fragments which are eventually taken up y phagocytosis cells.

 METHODS OF DEMONSTRATING A DEGENERATING MYELIN

1. Bi-col staining (degenerating myelin sheaths appears orange )
2. Swank and Davenport’s Marchi method for degenerating myelin
3. Polarized light method for degenerating myelin

SWANK AND DAVENPORT’S MARCHI METHOD FOR DEGENERATING MYELIN

The specimens are fixed for2-4 days in phosphate buffered formaldehyde (2-3 weeks) and then cut into slices no more than 3mm thick .

SOLUTION NEEDED

Staining solution this can be kept for a few months in a suitable bottle . It is used only once. They include;

1. Potassium chlorate 15g
2. Water 200ml
3. Osmium tetroxide 0.5g
4. Formalin (37-40) 0.5ml
5. Glacial acetic acid 2.5ml

PROCEDURE

1. Fix tissue in 10% formaldehyde
2. Without washing place in the following solutions ;

Potassium chlorate ,1%

Osmic acid 1%

Glacial acetic acid

Formaldehyde, concentrated

Place block of tissue on top of a small piece of glass wool in the bottom jar . Turn block over and agitate solution daily .

1. After 7 to 10 days remove from staining solution and wash in running water overnight.
2. Embed
3. Cut section mount on slides and dry
4. Deparaffinize sections through several changes of xyol

RESULTS

Degenerated myelin – black

Background – tan to yellow

 Normal myelin-brownish –orange