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QUESTIONS

1. what staining technique is used to identify DNA/RNA in peripheral neurons

2. Demonstration of demylination in the Peripheral Nervous System

STAINING TECHNIQUE USED TO IDENTIFY DNA AND RNA IN PERIPHERAL NERVOUS SYSTEM

1. Feulgen stain

Feulgen stain is a staining technique discovered by Robert Feulgen and used in histology to identify chromosomal material or DNA in cell specimens. It is darkly stained. It depends on acid hydrolysis of DNA, therefore fixating agents using strong acid should be avoided.

 Principle

This is a relatively new technique that is used for demonstrating DNA in tissue sections. It is a sensitive means of detecting aldehydes, which makes it the ideal method for detecting the presence of DNA. Here, the section is treated with dilute hydrochloric acid in order to remove the bases. The sugar part that remains reacts as an aldehyde ultimately forming a visible color. Therefore, this method can be said to be divided in to two main parts:

Procedure1: The first part of the procedure is the hydrolysis phase that involves the use of 5N HCl, ambient temperature for 40 minutes. This step is aimed at separately selecting 2 purine bases (adenine and guanine) which are removed from the DNA molecule.

Procedure 2: The second step is the staining phase. The reagent used is preferred because it is highly selective for DNA rather than RNA. Here, RNA does not react because of the presence of hydroxyl on carbon 2 of ribose, which prevents the acid (HCl) from hydrolyzing sugar. The reaction is also precise for the localization of DNA given that deoxyribose radicals are bound to phosphoric acid of the apurinic acid molecule following the removal of purine bases.

Reagents

Hydrochloric acid solution

Schiff reagent

Fixative solution

Counterstain

STAINING PROCEDURE :

1. Prepare Hydrochloric Acid working solution; combine and mix well.

 Hydrochloric Acid, 20% aqueous 16ml

 Distilled water. 24ml

 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 100% and 95% alcohols, 10 dips each. Wash well with distilled water.

3. Hydrolyze sections in hydrochloric acid working solution at 60.c for 10 minutes .

4. Place slides directly in schiff reagent, McManus for 45 minutes.

5. Wash in running tap water for 5 minutes, rinse in distilled water.

6. Counterstain in light Green SF Yellowish stain 0.2%, Aqueous for 1 minute.

7. Dehydrate in two changes each of 95% and 100% ethyl alcohol. Clear in three changes of xylene, 10 dips each.

8. Coverslip with compatible mounting medium.

RESULT :

DNA Red-purple

Nuclei Red-purple

Background Green

PRECAUTION:

1. Do not allow sections to dry out at any point during staining procedure.

2. Drain staining rack/slides after each step to prevent solution carry over

3. Bouin fixed tissue is unsatisfactory for use with FUELGENreaction.

4. Prolonged exposure to hydrochloric acid may over hydrolyze sections with poor staining result

Some other stains used for both DNA/RNA include ;

1. Methyl green pyronin stain

2. Acridine orange

Methy green pyronin stain

Methy green pyronin is a classical histological staining technique using to basic(cationic) dyes for the demonstration and differentiation of DNA and RNA. Methyl green is specific for phosphats radicals in DNA double helix staining it green blue. Pyronin does not possess this affinity and binds to the remaining negatively charged RNA staining it red.

 This method is useful in identifying the distribution of nissl substances in neuronal cell bodies.

QUESTION 2 DEMONSTRATION OF DEMYELINATION IN THE PNS :

Peripheral nerves are complex histological structures whose main components are neuron axons, myelin sheaths synthesized by Schwann cells and a collagen-rich extracellular matrix (ECM).

For most researchers, the gold standard in peripheral nerve histology is toluidine blue staining of resin-embedded semi thin sections, which allows the accurate identification of most myelinated fibers.

In the central nervous system the breakdown of myelin sheath after atonal degeneration occurs at a much slower pace. This temporal difference suggests that oligodendrocytes, which form the myelin sheaths of central nerve fibers, do not behave like Schwann cells, but that 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 tissue. Living myelinated fibers can be seen in thin tissue cultures derived from sensory or autonomic ganglia or from immature brain or spinal cord . The nervous tissue is fixed, sectioned and stained to provide permanent preparations.

When a neuron dies, or if it’s 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 by phagocytosis cells. This process is known as wallerian degeneration .

There are several methods for demonstrating a degenerating myelin;

1. Swank and Davenport’s Marchi Method for degenerating myelin

2. Adams’s OTAN Method for Normal and Degenerating Myelin

3. Polarized light method for degenerating myelin(applicable to central nervous tissue ).

4. Toluidine blue staining of nerve cross sections obtained from resin embedded nerve sections is a reproducible method for qualitative and quantitative assessments of peripheral nerves, enabling visualization of morphology number of axons and degree of myelination.

SWANK AND DAVENPORT’s MARCHI METHOD FOR DEGENERATING MYELIN:

The technique incorporates improvements that minimize artifacts such as sporadic staining of normal myelinated fibers. Specimens are fixed for 2-4 days in phosphate buffered formaldehyde (2-3 weeks for human or other large brain) 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 includes ;

1. Potassium chlorate 15g

2. Water 200ml

3. Osmium tetroxide 0.5g (used only once)

4. Formalin (37-40%) 0.5ml

5. Glacial acetic acid 2.5ml

 Procedures

1. Transfer slices of tissue (without washing) from the fixative into about 15 time their volume of the staining solution in a screw-capped jar. Agitate daily to expose all surfaces of the specimens to the solution. (Alternatively place the container on a gently rotating platform.)

2. After 7 day, remove the specimens and wash them in running tap water for 24 h.

3. Either cut frozen sections or process into paraffin before sectioning. Sections should be 20 \_m or thicker for appreciation of degenerating tracts of fibers in the brain.

4. Dehydrate and clear frozen sections, or dewax paraffin sections and transfer to clean xylene. Coverslip with a resinous mounting medium.

Result :

Degenerating Myelin (Early and Late Products) Black

Background: colorless to pale brown. Occasional normal fibers may be black, and there may be some fine black granular material on the surface, at the edges of the sections.

In some species Marchi-positive material is normally present

in nerve roots, at the junction between the central and

peripheral nervous systems

ADAMS’S OTAN METHOD FOR NORMAL AND DEGENERATING MYELIN

The abbreviation is for osmium tetroxide and alpha-naphthylamine. The technique is applied to cryostat sections, ideally after calcium-formaldehyde fixation.

Solutions Needed

A. Osmium Tetroxide-Potassium Chlorate. This is made up as required and used only once.

Osmium tetroxide, 2% stock solution in water: 5 mL

Potassium chlorate (KClO3), 1% stock solution in water:

30 mL

Water: 5 mL

B. Saturated \_-Naphthylamine Solution. Dissolve a

few crystal of \_-naphthylamine in 40 mL of water at 40°C filter. This solution is used at 37.C.

Procedure

1. Treat the sections with osmium tetroxide-potassium chlorate (Solution A) overnight at room temperature, in a tightly closed glass container.

2. Wash the sections for 10 min in water (3 changes with occasional agitation).

3. Treat the sections with saturated \_-naphthylamine solution (B) for 20 min at 37°C.

4. Wash the sections for 5 min in water (3 changes with occasional agitation5. Apply 5.coverslips, using an aqueous mounting medium.

Result

Normal myelin is brownish–orange.

Degenerating myelin (late products only) is black. Fat, if present in the tissue, is also blackened.