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Questions :

1. What is the staining technique used to identify the RNA/DNA in the peripheral neuron .
2. Explain the procedure involved in the demonstration of demyelination in the PNS.

ANSWERS

1. STAINING TECHNIQUE:

Nucleic acids and DNA of the Peripheral nervous system are mostly stained using **FUELGEN REACTION**.

❖ FUELGEN REACTION:

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 divided into two main parts.

1. The first part of the procedure is the hydrolysis phase that involves the use of 5N HCL, ambient temperature for 40minutes. This step is aimed at separately selecting two purine bases (adenine and guanine)which are removed from the DNA molecule .
2. The second step is the staining phase. The reagent used is preferred because it is highly selective for DNA rather than RNA.
3. Here, RNA does not react because of the presence of hydroxyl on carbon 2 of ribose , which bases.

METHOD:

1. Fix the tissue with 10% formalin.
2. Paraffin sections cut at 5 microns

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. 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 for 10 minutes .

4. Place slides directly in shift reagent, McManus for 4t 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 FUELGEN reaction

Another stain includes :

❖ **METHYL GREEN PYRONIN STAIN :**

The stain demonstrates RNA and DNA. The specimen must be a well fixed paraffin embedded tissue cut at 5microns.

PROCEDURE:

1. Deparaffinize slide using xylene or xylene substitutes and hydrate through alcohols.
2. Rinse slide in running tap water
3. Rinse slide thoroughly in distilled water
4. Place slide in room temperature methyl green pyronin stain for 2 to 7 minutes. [increasing stain time intensifies pyronin(RED)]
5. Dip slide 1 to 2 times each through 2 changes of room temperature distilled water
6. Dehydrate slide through 3 changes of fresh reagent alcohol
7. Clear slide through 3 changes of fresh xylene . (do not use xylene substitutes)
8. Coverslip using a permanent mounting medium.

RESULT:

DNA	Blue-Green to Green
RNA	Pink to Red
MAST CELL GRANULES	Pink

Other stain used for both DNA/RNA include ;

1. Acridine orange

● **2 DEMONSTRATION OF DEMYELINATION IN THE PNS :**

Luxol blue is used to detect demyelination in the central nervous system but not in the peripheral nervous system .

On the basis of histochemistry studies, it has been known that normal myelin sheath contains acidic polysaccharide and this compound is liberated during demyelination. 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 late stage, beginning about 1 week after atonal 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 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-contrast 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. Polarized light method for degenerating myelin (applicable to central nervous tissue) .
3. Bi-col staining (degenerating myelin sheaths appears orange)

❖ **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 is used only once. It includes ;

- Potassium chlorate 15g
- Water 200ml
- Osmium tetroxide 0.5g
- Formalin (37-40%) 0.5ml
- Glacial acetic acid 2.5ml

Result :

Normal myelin is brownish-orange.

Degenerating myelin is black.

