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Course : Histochemistry
Course code: Ana 304

Assignment

Questions

1. What is the name of the staining technique used to identify DNA /RNA in the peripheral neuron?
2. Explain the process for the demonstration of the demyelination in the PNS?

Answers

1. Staining Techniques

- Acridine Orange (AO)

Acridine orange is a stain that intercalates or binds with the nucleic acid (either DNA or RNA) present in organisms and fluoresce to emit various colors that help in differentiation of cellular organelles. This binding is the result the electrostatic interactions of acridine molecule between the nucleic acid base pairs. Acridine orange is a cell-permeable,nucleic acid selective stain that emits green fluorescence when bound to dsDNA(at 520) and red fluorescence when bound to ssDNA or RNA(at 650 nm). Since it is a cationic stain, it also enter acidic compartments such as lysosomes which in low pH conditions, will emit orange light. This technique ensures that the red emission reflected RNA in intact cells, a human oligodendrocyte cell line is fixed and stained with AO. Consistent with RNA-rich cytoplasm and nuclear bodies, when stained with AO, these cells exhibited diffuse red staining throughout the cytoplasm and a distinctly green nucleus containing red punctate structures.

Collectively, common tissue-processing techniques adequately preserve cellular RNA, which can be measured along with DNA changes based on the AO spectrum.

Requirements needed for Acridine Orange(AO)

Materials: Acridine orange,Glacial Acetic acid, Distilled water

- Preparation of reagent: 50 mg acridine orange is dissolved in 10 ml of distilled water to prepare stock solution and stored in the refrigerator.1 ml of Acridine orange stock solution and 0.5 ml of glacial acetic acid is added to 50 ml of distilled water to prepare a working solution.
- Staining procedure

1. Prepare a smear in a clean grease free slide and allow it to air dry.
2. The slide is then fixed with methanol and dried again.
3. It is then put in trough with acridine orange staining working solution (i.e 0.01 per cent).
4. After 2 minutes of staining, the slides are washed gently with water and dried and then examined in a fluorescent microscope.

Observance: Bacteria stain orange against a green to yellow background of human cells and debris.

Another stain that can be used to express RNA and DNA

- METHYL GREEN PYRONIN

  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.

RESULTS

- RNA - Pink to Red
- DNA - Blue-Green to Green

THE PROCESS OF 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.

Although 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. Bi-col staining (degenerating myelin sheaths appears orange)

2. Swank Davenport's Marchi Method for degenerating myelin

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

<u>SWANK AND DAVENPORT'S MARCHI METHOD FOR DEGENERATING MYELIN:</u>

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

The staining solution 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.