Name: Saliu Halima

Matric Number: 17/Mhs01/294

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Ana 304 Histochemistry Assignment

**Question 1**

If nissl stain is used to demonstrate RNA/DNA in the neurons of CNS, what staining technique is used for identifying the same in peripheral neurons?

**Answer**

 There are two staining techniques that could be used to identify DNA and RNA in neurons of the peripheral nervous system. They are:

* Fuelgen reaction,
* Methyl green pyronin stain,
* Acridine orange stain.

 **Feulgen Reaction,** is a relatively new technique that is used for demonstrating DNA in tissue sections. It is ideal for detecting the presence of DNA because it is a sensitive method for the detection of aldehydes. Using this method, the section is treated with dilute hydrochloric acid to remove the bases. After this, the remaining sugar part reacts as an aldehyde thereby forming a visible color. Usually this method is divided into two main parts:

Part one (1): this is the hydrolysis phase that involves the use of 5N HCL at an ambient temperature for 40 minutes. The sole purpose of this step is to separately select two purine bases which are; adenine and guanine which are removed from the DNA molecule.

Part two (2): this is the staining phase. The reagent used here is usually preferred is highly selective for DNA instead of RNA. In this phase, RNA does not react because of the presence of hydroxyl on carbon 2 of ribose bases.

Method for Feulgen Reaction

1. The tissue is fixed with 10% formalin.
2. Paraffin section sections are cut at 5 microns.

Staining Procedure for Feulgen Reaction

1. The hydrochloric acid working solution is prepared with hydrochloric acid (20% aqueous 16ml) and distilled water (24ml). preheat and maintain hydrochloric acid working solution at 60 prior to use.
2. Sections are deparaffinized in three changes of xylene for three minutes each. Sections are hydrated through two changes each 100% and 95% alcohol, 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 a few minutes.
5. Slides are washed in running tap water for five minutes and rinsed in distilled water
6. Counterstaining is carried out in light green SF Yellowish stain 0.2%, aqueous for one minute.
7. Dehydrate in two changes each of 95% and 100% ethyl alcohol. Clear in three changes of xylene, 10 dips each.
8. Slides are cover slipped.

Results for Feulgen Reaction

1. DNA shows a red to purple coloration
2. Nuclei show a red-to purple coloration
3. Background shows a green coloration

Precautions to Be Taking During the Process

1. Tissues fixed with bouin’s solution are unsatisfactory for use with Feulgen reaction.
2. Sections are not to be allowed to dry out at any point in the staining procedure.
3. Staining rack should be drained after each step to prevent solution carry over.

 **Methyl green pyronin stain**, the stain demonstrates RNA and DNA in the PNS. The specimen used must be a well fixed paraffin embedded tissue cut at 5 microns

Procedure for Methyl Green Pyronin Stain

1. Slide is deparaffinised using xylene and hydrated through alcohols.
2. Slide is rinsed in running tap water.
3. Slide is rinsed thoroughly in distilled water.
4. slide is placed in room temperature methyl green pyronin stain for two to seven minutes.
5. Slide is dipped in two changes of room temperature distilled water for one to two minutes.
6. Slide is dehydrated through three changes of fresh reagent alcohol
7. Slide is cleared through three changes of fresh xylene.
8. Slide is cover slipped using a permanent mounting medium.

Results for Methyl Green Pyronin Stain

1. DNA shows a blue-green to green coloration
2. RNA shows a pink to red coloration
3. Mast cell granules show a pink coloration

**Question 2**

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**

 Luxol fast blue stain (LFB stain) is a commonly used stain to observed myelin under light microscopy. LFB is commonly used to detect demyelination in the central nervous system (CNS) but cannot detect demyelination in the peripheral nervous system (PNS).

 The disintegration of myelin sheaths in degenerating peripheral nerves occurs in two stages. The beginning stage consists of a physical disruption of myelin sheaths with chemical alteration. The later stage consists of chemical degradation of myelin lipids. In degenerating peripheral nerves, these changes develop within schwann call, indicating that these cells are capable of both forming and disintegrating myelin sheaths. In the CNS the breakdown of myelin sheath after atonal degeneration occurs at a slow pace. This tells us that oligodendrocytes which are responsible for forming the myelin sheaths of the CNS do not behave like schwann cells but that myelin digestion must await the arrival of macrophages.

There are several methods for demonstrating a degenerating myelin in the peripheral nervous system:

* Swank and Davenport’s marchi method for degenerating myelin
* Polarized light method for degenerating myelin
* 23`Bi-col staining

**Swank and Davenport’s Marchi Method for Degenerating Myelin**

 The specimens used in this techniques are fixed for two to four days in phosphate buffered formaldehyde and then cut into slices about 2-3mm thick. Larger brains are fixed for two to three weeks. This technique incorporates improvements that minimize artifacts such as sporadic staining of normal myelinated fibbers.

Materials Needed

1. Staining solution: it consists of
* Potassium chlorate 15g
* Glacial acetic acid 2.5ml
* 37-40% formalin 0.5ml
* Water 200ml
* Osmium teroxide 0.5g

This solution can only be used once and can be kept for a few months in a suitable bottle.

Results

1. Normal myelin shows a brownish-orange coloration.
2. Degeneration shows a black coloration