NAME: NWACHUKWU CHNAZA

DEPARTMENT: ANATOMY

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 1a.) **METHYL GREEN PYRONIN STAIN (MGP)**

It is a classical histological staining technique that uses two basic dyes for the demonstration and differentiation of DNA and RNA.

 Methyl green is specific for phosphate radicals in the DNA double helix staining it blue.

PRINCIPLE: This stain demonstrates RNA and DNA

SPECIMEN: Any well fixed paraffin embedded tissue cut at 5microns

PROCEEDURE

-Deparaffinize slide using Xylene or Xylene Substitute and hydrate through alcohols

-Rinse slide with tap water

-Rinse slide thoroughly in distilled water

-Place slide in room temperature METHYL GREEN PYRONIN STAIN for 2-7 minutes

-Dip slide 1 to 2 times each through 2 changes of room temperature distilled water

-Dehydrate slide through 3 changes of fresh reagent alcohol

-Clear slide through 3 changes of fresh Xylene (Do not use a Xylene substitute)

-Cover slip using a permanent mounting medium

RESULTS:

DNA: It appears blue in color

RNA: It appears pink in color

1b**.) FUELGEN REACTION**

Fuelgen reaction is the development of a brilliant purple color by DNA in a microscopic preparation stained with a modified Schiff’s reagent

**FUELGIN STAIN**: is a staining technique which was discovered by ROBERT FUELGEN and used in histology to identify chromosomal material or DNA in a cell specimen.

It is darkly stained and depends on acid hydrolysis of DNA therefore fixating agents using strong acids should be avoided

 PREPARATION

Schiff’s reagent is prepared by pouring 200mL of boiling distilled water over 1-g basic Fuchsin, Shake thoroughly and cool to 50 degrees, filter and add 30ml 1NHCL to filtrate then cool to room temperature and add 1g potassium met bisulphate ( K2S2O5).

RESULT

It gives DNA a purple coloration

2.) Luxol Fast Bue Stain is commonly used to detect demyelination in the Central Nervous System, but cannot be used to discern myelination in the Peripheral Nervous System.

**DEMYELINATION IN THE PERIPHERAL NERVOUS SYSTEM**

Demyelination is a process where myelin which is the protective coating of nerve cells,experiences damage. When this happens, neurological problems can occur. It could be as a result of various medical conditions such as multiple sclerosis.

Histochemistry makes us to know that a normal myelin sheath contains acidic polysaccharide and this compound is liberated during demyelination.

--SWANK AND DAVENPORT’S MARCHI METHOD FOR DEGENERATING MYELIN

 This technique incorporates improvements that minimize artifacts such as sporadic staining of normal myelinated fibers.

The collected specimens are fixed for 2- days in phosphate buffered dealdehyde depending on the size, as a greater amount would be required for a larger brain. After which the specimen is then cut into slices no thicker than 3mm.

The needed solutions which are used only once are as follows:

 **Water (200ml),**

**Osmium tetroxide (0.5g),**

 **Formalin (37-40%) 0.5ml,**

**Potassium Chlorate (15g),**

**Glacial acetic acid (2.5ml)**

**The end result shows that a normal myelin appears to be brownish-orange in colour while degenerating myelin is black in colour.**

Other methods for demonstrating a degenerating myelin include the following:

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

b) Bi-coli staining (degenerating myelin sheaths appears to be orange in colour)