NAME: OSIBANJO IYANUOLUWA A.

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1. If Nissl stain is used to demonstrate RNA/DNA in the neurons of CNS, what is used for identifying the same in peripheral neurons

Answer: Toluidine blue stain

1. Is Luxol fast blue stain (LFBS) 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: No, Luxol fast blue stain is not used to detect demyelination in PNS but used to detect demyelination in CNS alone.

**Neurofilament stain** shows myelination in PNS but Adam’s often method shows for degenerating myelin

**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 fixa- tion.

Solutions Needed

1. **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

**Preparing and using osmium tetroxide solutions**

* Osmium tetroxide is supplied in a sealed glass capsule containing 0.25–1.0 g of solid OsO4. This must be dissolved in water without exposing the worker to OsO4 vapor, and the solution must be stored in a manner that minimizes evaporative loss of this toxic and very expensive compound. The container of the solution must be an extremely clean glass bottle with a black rubber stopper (Gabe,1976). Ground glass stoppers allow the escape of vapor, which can blacken nearby paper, plastic and wood. Greases used to lubricate ground glass junctions dissolve OsO4 and may also reduce it. Osmium tetroxide solutions are not light-sensitive (Griffith, 1967), and the common practice of enclosing bottles in aluminum foil derives from ignorant superstition. Storage in a refrigerator may slightly retard evaporative losses from inadequately stoppered bottles but escaped vapor blackens plastic. Storage in a fume hood makes more sense.
* Remove the label from the glass capsule and wash off any residual glue. Put the capsule in a very clean bottle and add enough of your purest available water (double-distilled, deionized, etc) to make a 2% solution. Bash the capsule with a very clean glass rod, and swirl to disperse the fragments. Insert a black rubber stopper and wait for complete dissolution of the OsO4. With occasional agitation this may take a few hours. Your stoppered 2% aqueous OsO4 stock solution should be OK for at least a year.
* To make a working solution for blackening myelin (usually 0.5% or 1% OsO4), use a disposable Pasteur pipette to withdraw 1–2 mL of the 2% solution. Add this to an equal or greater volume of a suitable diluent. For unfixed tissue, the diluent should be phosphate-buffered saline, pH 7.2–7.6. For tissue that has been well fixed in formaldehyde or glutaraldehyde, water is a suitable diluent. The concentration of osmium tetroxide is not critical, and there is no need to measure the volumes accurately.
* Used osmium tetroxide solutions should be collected into a glass bottle containing an excess of ethyl or methyl alcohol to reduce OsO4 to OsO2, which is insoluble and harmLess. OsO2 is not considered an environmental hazard (Smith *et al.*, 1978). Methods are available for reclamation of osmium tetroxide in the laboratory (Jacobs *et* *al.,*1971; Kiernan, 1978); they require careful use of potentially hazardous reagents.

1. **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 so- lution (B) for 20 min at 37°C.

4. Wash the sections for 5 min in water (3 changes with occasional agitation).

5. Apply coverslips, using an aqueous mounting me- dium.

**Result**

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