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**Matric Number: 17/MHS01/253**

**Department: Anatomy**

**Course Code: ANA 304**

**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?**

**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.**

**Answers**

1. Method 6: Sudan Black B The “Sudan” dyes (including oil red O) are nonionic azo dyes with hydrophobic molecules that fit into hydrophobic domains of cells and tissues, notably lipids. These dyes are used as saturated or supersaturated solutions in polar solvents such as 70% ethanol, propylene glycol, or ethylene glycol. Hydrogen bonding between hydroxy groups of the solvent molecules results in the formation of clusters, with the dye molecules occupying the spaces between the clusters. When the dye solution is in contact with a more hydrophobic domain, such as a structure composed of lipid, the dye diffuses from the solution into the hydrophobic structure (52). A simple way of expressing this mechanism is to say that the dye is more soluble in the lipid than in the solvent from which it is applied . The simpler statement implies that the lipids in a section of fixed tissue are in a liquid state. In living nervous tissue, the myelin sheath grows with the addition of newly synthesized lipid and protein molecules to the surface of the Schwann cell or oligodendrocyte. The added components flow in the plane of the membrane, which behaves as a film of liquid (53). In fixed myelin, however, the protein components have been chemically cross-linked, and the lipids may not retain their liquid properties. Liquidity is not a prerequisite for uptake of hydrophobic dyes. Epoxy resins used as embedding media for electron microscopy are decidedly solid; they can be stained with “Sudan” dyes, which reveal the extent of infiltration of the tissue .

The red “Sudan” dyes (Sudan IV and oil red O) stain myelin rather weakly because they are effective stains only for hydrophobic lipids such as fats (glycerol esters of unsaturated fatty acids) and cholesterol esters of unsaturated fatty acids (49), which do not occur in normal myelin. Sudan black B is less hydrophobic than the red dyes, and it will stain most types of lipid , including the phospho- and sphingolipids of normal myelin. Cholesterol is a major hydrophobic component of normal myelin (see Table 1) but it is not stained by any of the Sudan dyes , probably because it is solid (melts at 148°C). When viewed with crossed polars, myelin stained with Sudan black B appears orange-red on a dark background . Sudan black B is suitable for staining frozen sections of tissue fixed in calcium-formaldehyde or neutral buffered formaldehyde. Solution Needed Sudan Black B in 70% Alcohol. This may be used repeatedly, but it deteriorates after about 4 weeks. Addition of 600 mg of Sudan Black B (CI 26,150) to 200 mL of 70% Ethanol. Place on a magnetic stirrer for 2 h, then pour into a screw-capped bottle and leave overnight before using.

Procedure

1. Filter the solution of Sudan black B into a Coplin jar. 2. Briefly rinse slides with mounted frozen sections in 70% ethanol, and then transfer them to the stain and place a lid on the jar. 3. After 10 min, transfer the slides to 70% ethanol, 5–10 s with gentle agitation, then move the slides into water. At this stage a suitable counterstain may be applied. Nuclear fast red (CI 60,760), 0.1% in 5% aluminum sulfate, for 3 min, is recommended (56). 4. Apply a coverslip, using an aqueous mounting medium.

Result

Myelin Dark Gray to Blue–Black. If the preparation is examined with crossed polars, the natural birefringence of the myelinated fibers is enhanced and they appear orange to red. Scattered large birefringent crystals are commonly seen, randomLy scattered in normal tracts of myelinated fibers in the brain; they are not stained by Sudan dyes and are thought to be artifacts that form during storage in formaldehyde . Modified Sudan black B methods are available for staining the peripheral nervous system in whole-mounts of a variety of tissues and animals . The ganglia and tracts of the myenteric plexus of the intestine are stained in such preparations even though they contain few or no myelinated axons. The lipids responsible for staining must, in this tissue, reside in the cell membranes of the numerous thin unmyelinated axons and their associated glial cells.

2 No.

Demyelination in the PNS

Method 10: Swank & Davenport’s Marchi Method for Degenerating Myelin The following technique (100) incorporates improvements (101,106) that minimize artifacts such as sporadic staining of normal myelinated fibers. Specimens are fixed for 2–4 d in phosphate-buffered formaldehyde (2–3 weeks for human or other large brains) and then cut into slices no more than 3 mm thick.

Solution Needed

Staining Solution. This can be kept for a few months in a suitable bottle. See Method 1, Table 3 for instructions on working safely with osmium tetroxide. The solution is used only once. Potassium chlorate (KClO3): 1.5 g Water: 200 mL Osmium tetroxide: 0.5 g Formalin (37–40% HCHO): 0.5 mL Glacial acetic acid: 2.5 mL Alternatively, use 175 mL of water and 25 mL of a stock 2% aqueous solution (see Box 2) of osmium tetroxide.

Procedure

1. Transfer slices of tissue (without washing) from the fixative into about 15 time their volume of the staining solution in a screw-capped jar. Agitate daily to expose all surfaces of the specimens to the solution. (Alternatively place the container on a gently rotating platform.) 2. After 7 d, remove the specimens and wash them in running tap water for 24 h. 3. Either cut frozen sections or process into paraffin before sectioning. Sections should be 20 m or thicker for appreciation of degenerating tracts of fibers in the brain. 4. Dehydrate and clear frozen sections, or dewax paraffin sections and transfer to clean xylene. Coverslip with a resinous mounting medium.

Result

 Degenerating Myelin (Early and Late Products) Black Background: colorless to pale brown. Occasional normal fibers may be black, and there may be some fine black granular material on the surface, at the edges of the sections. In some species Marchi-positive material is normally present in nerve roots, at the junction between the central and peripheral nervous systems (107).