Neurohistology Techniques

Formalin-fixed paraffin-embedded (FFPE): This is the standard preparation for most of the histology images you will see. Tissues are fixed in 10% formalin (3.7% formaldehyde), embedded in paraffin and sectioned at 4-10 micrometers (microns). Usually the slides are stained with hematoxylin and eosin (H&E). Hematoxylin stains nuclei and nucleic acids blue and eosin stains cytoplasm pink/red. Masson trichrome stain is useful for examination of peripheral nerve. It stains connective tissue (collagen) blue and myelin red.

Resin-embedded toluidine blue-stained: This preparation is commonly used for examination of peripheral nerve. It is also a step that is preliminary to electron microscopic (ultrastructural) examination. Tissues are fixed in 4% glutaraldehyde which enables better preservation of membranes and organelles than formalin. They are treated with osmium tetroxide, which both fixes and stains tissue components. It also adds “electron density” to the organelles and thus enables them to be visible in the electron microscope. The tissue is embedded in a plastic resin and sectioned at one micron. Finally, it is stained with toluidine blue which stains both nuclei and cytoplasm different shades of blue.

Electron microscopy: Tissues that are resin embedded as described above are sectioned at 40 to 80 nanometers. The sections are put onto a tiny metal grid and stained with a heavy metal solution such as lead citrate or uranyl acetate, which makes the structures even more electron dense. They are viewed in an electron microscope. The electron beam will destroy the thin sections if viewed too long in the microscope, so photographs are taken and analyzed. This is the highest resolution image that is commonly used in histology and pathology. (There are other variations of electron microscopy, such as scanning EM, negative staining of isolated organelles, and immunogold staining of ultrathin frozen sections, that you should be aware of but will not be discussed in this seminar.)

Flash frozen sections: This preparation is commonly used for the examination of skeletal muscle. Unfixed muscle tissue is oriented so that the muscle is viewed in cross section and frozen very rapidly in isopentane that is cooled to liquid nitrogen temperature (-160C). The tissue is warmed to –20C and cut using a refrigerated microtome (cryostat). Section thickness is usually 10-15 microns, and sections are placed onto glass slides. This preparation can be stained with any histological stain, but is most useful for enzyme histochemistry.

Enzyme histochemistry: The rapid freezing of tissue preserves enzyme activity. The sections are warmed to room temperature and a soluble substrate is applied. The enzymes in the tissue convert the substrate into a solid colored reaction product that is precipitated onto the tissue in the vicinity of the enzyme. This results in an “in situ” localization of the enzyme.

Teased fibers: This is a specialized technique that is used in the examination of peripheral nerve. A segment of nerve (usually 1 cm in length) is fixed in glutaraldehyde and post fixed in osmium tetroxide. The specimen is viewed in a dissecting microscope, and the individual nerve fibers (axons) are gently teased apart on a glass slide. This permits the microscopic examination of individual myelin internodes and nodes of Ranvier. This preparation is tedious and difficult to perform well, but it provides the best demonstration of axonal degeneration, demyelination and remyelination.