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MATRIC NO: 18/MHS02/208

COURSE: CELLULAR PATHOLOGY

ASSIGNMENT

Write explicitly on 5 diagnosis techniques use in pathology, relevant illustrations and examples required.

Diagnostic Pathology

Diagnostic pathology identifies the cause of disease based on morphologic and/or clinical pathology findings, as well as history, clinical signs, and ancillary test results. It is important in all areas of pathology, both in spontaneous and in experimentally-induced disease. In experimental studies, it is important to separate out the effects of spontaneous disease and those induced by the experimental agent/test article.

Diagnostic pathology is essential to investigate unexpected disease or death in laboratory animal colonies or prior to the termination of a study.

TECHNIQUES OF PATHOLOGY

1. **Light Microscopy**- the structure of tissues & cells in health & disease. Microscopy is the technique used to view objects that cannot be seen by the naked eye. The range can be anything between mm and nm. There are 3 main microscopic techniques that are used; Optical microscopy, Scanning probe microscopy and Electron microscopy.

Optical microscopy: Otherwise known as light microscopy, it involves the use-age of visible light and one or more lens to produce an enlarged image of an object that is placed in the focal plane of the lens. This can either branch off into transmission, where the beam of light passes through the sample or reflection where the beam reflects off the sample surface, i.e reflected light microscope. There are many applications to Optical microscopy such as in nanophysics and biotechnology but in medicine it is mostly known as being used in diagnosis when we are dealing with tissues or tests on free cells known as a smear test.

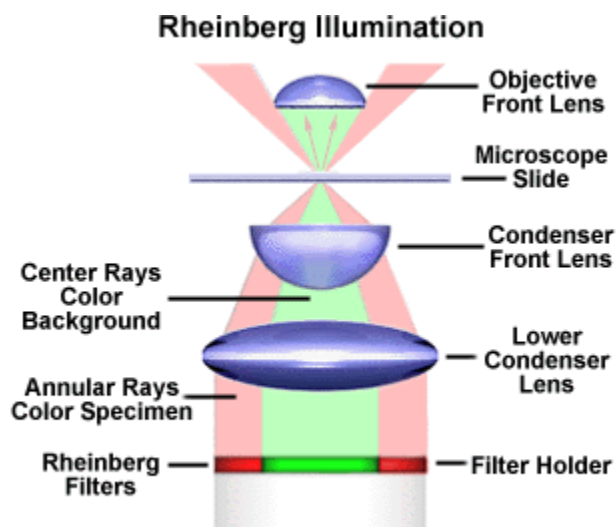


Figure 1

2. **Histochemistry**- is the study of the chemistry of tissues. Histochemistry is an important technique that is used for the visualization of biological structures. As such, it is concerned with the identification and distribution of various chemical components of tissues through the use of stains, indicators as well as microscopy.

essentially, identification and distribution of chemical constituents of tissues is achieved through the exploitation of unique chemical environments in cells, heterologous expression techniques as well as enzymatic activities.

Histochemical Techniques/Methods

Perls's Reaction

This method is particularly important for the detection of iron levels (ferric ions). Because it can help detect the presence of ferric ions, this technique is used to determine the level of these ions in such organs as the spleen and bone marrow. It can be used to tell whether there are excessive amounts of the ion as observed in hemochromatosis (excessive levels of ferric ions with deposits in the liver and pancreas) or hemosiderosis where deposits can be found in the liver, spleen and the lymph nodes.

Von Kossa Technique

This is also an ion based technique that is used in histochemistry. It is a more sensitive technique that can be used to identify the presence of calcium deposits on cyst fluids, ductal ectasia and papillomatosis. However, excessive amount

of calcium may be found in any given part of the body and can be demonstrated using the Von Kossa technique.

Although this technique is used to demonstrate the presence of calcium, it demonstrates an anion rather than the calcium ion itself.

Lipids Staining

This technique is dependent on dyes that are soluble in lipids. Some of the most common dyes used include:

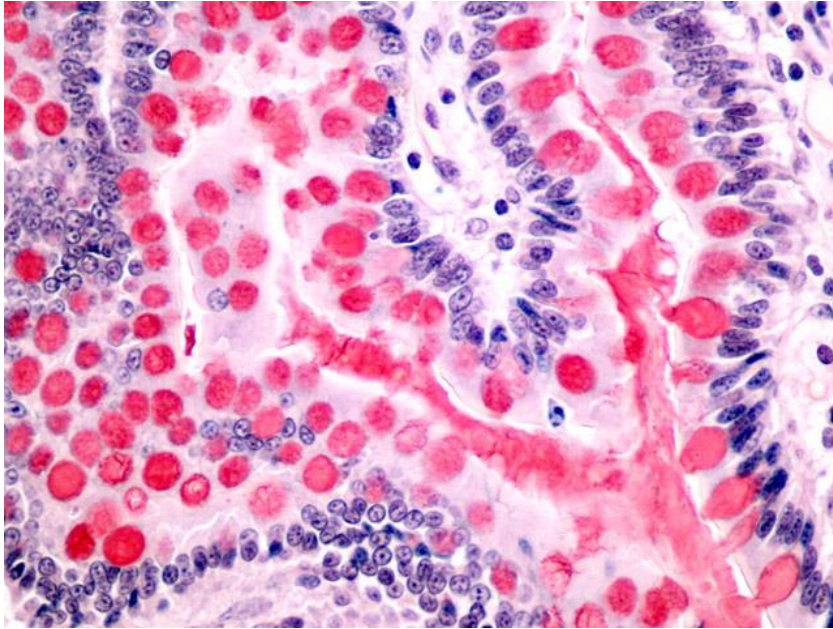
Sudan VI

Sudan black

Oil Red O

Nile blue

Lipid staining is a useful technique that is used for demonstrating intracellular lipids in various tissue sections.

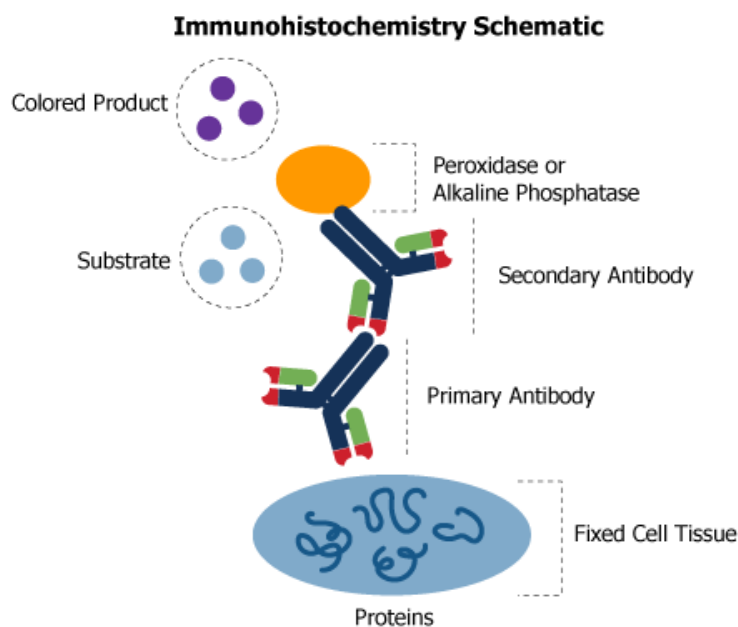


3. **IMMUNOHISTOCHEMISTRY**- employ antibodies (immunoglobulins with antigen

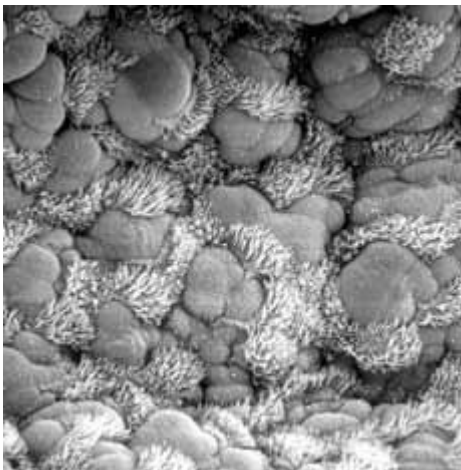
immunohistochemistry Techniques uses antibodies, reagents and stains for the diagnosis and research of cancer. The common nuclear counterstains are: Hematoxylin, Light Green, Fast Red, Toliudine Blue and Methylene Blue. Also an Alum-Mordant base on Hematoxylin is used such as Harris's Hematoxylin (now is offered without mercury). Mayer's Hematoxylin is one of the most popular mordant used in immunohistochemistry as well as Gill's Hematoxylins that are classified as 1,2,3. Immunohistochemistry Techniques uses different methods and approaches. The specimen needs to be well fixed. One of the most popular fixatives is 10% Neutral Formalin and Zinc Formalin. Also in immunohistochemistry, a transport solution is needed to transport the specimen. The

most popular is Michel's Immunofluorescence Working. Immunohistochemical techniques detect antigens in tissue sections by means of immunological and chemical reactions. This technique is highly sensitive and specific and can detect a wide variety of antigens in multiple animal species. This chapter reviews common immunohistochemical methods used in the characterization of normal and pathologic tissue and the reagents used. Pretreatments such as blocking steps for endogenous activities and antigen retrieval are included. Standard procedures on formalin-fixed, paraffin-embedded tissues as well as method standardization for new antibodies and troubleshooting are emphasized.

specificity) to visualize substances in tissues sections or cell preparations



4. **ELECTRON MICROSCOPY**- study of disorders at an organelle level, & to the demonstration of viruses in tissue samples. Electron microscopy (EM) is a technique for obtaining high resolution images of biological and non-biological specimens. It is used in biomedical research to investigate the detailed structure of tissues, cells, organelles and macromolecular complexes. The high resolution of EM images results from the use of electrons (which have very short wavelengths) as the source of illuminating radiation. Electron microscopy is used in conjunction with a variety of ancillary techniques (e.g. thin sectioning, immuno-labeling, negative staining) to answer specific questions. EM images provide key information on the structural basis of cell function and of cell disease.



5. **BIOCHEMICAL TECHNIQUES**-fluid & electrolyte homeostasis, serum enzyme assay e.g. raised

levels of cardiac enzymes in the blood. Biochemistry techniques are Protein Purification, perfusion, Homogenization, Differential Centrifugation, Purification of LDH, Purification of LDH , LDH Enzyme assays, Protein assays, Characterization of LDH, Western blotting, Gel filtration chromatography, Protein crystallography, PCR, Ligation and transformation, Selection and screening and Enzyme Kinetics.

2

CELLULAR ADAPTATION

Cellular Adaptations

For the sake of survival on exposure to stress, the cells make adjustments with the changes in their environment or adapt

- to the physiologic needs (physiologic adaptation) and
- to non-lethal pathologic injury (pathologic adaptation)

Example of Cellular Adaptations

Plasia Means : Number

Trophy means: size

Hyperplasia, Dysplasia, Metaplasia, Atrophy, Hypertrophy

Atrophy

Atrophy: Reduction of the number and size of parenchymal cells of an organ

- **Physiologic atrophy.**

Atrophy of lymphoid tissue in lymph nodes, appendix and thymus.

ii) Atrophy of gonads after menopause.

iii) Atrophy of brain with aging.

- **Pathologic atrophy**

Starvation atrophy.

Ischemic atrophy.

Hypertrophy

Hypertrophy: Increased tissue size via enlargement of cells caused by an increase in organelles, and structural proteins

- **Physiologic Hypertrophy:**

Increased muscle mass through sport

Uterus enlargement during the pregnancy

- **Pathologic hypertrophy**

Hypertrophy of cardiac muscle

Hyperplasia

Hyperplasia: Hyperplasia is an increase in the number of parenchymal cells resulting in enlargement of the organ or tissue

- **Physiologic Hyperplasia**

Hyperplasia of female breast at puberty, during pregnancy and lactation.

- **Pathologic hyperplasia**

Endometrial hyperplasia following estrogen excess

Metaplasia

Metaplasia: defined as a reversible change of one type of epithelial or mesenchymal adult cells to another type of adult epithelial or mesenchymal cells.

- **Physiological metaplasia:**

Metaplasia of endocervix: columnar epithelium in to squamous epithelium

- **Pathological metaplasia**

Respiratory epithelium in smokers Ciliated columnar epithelium to squamous epithelium

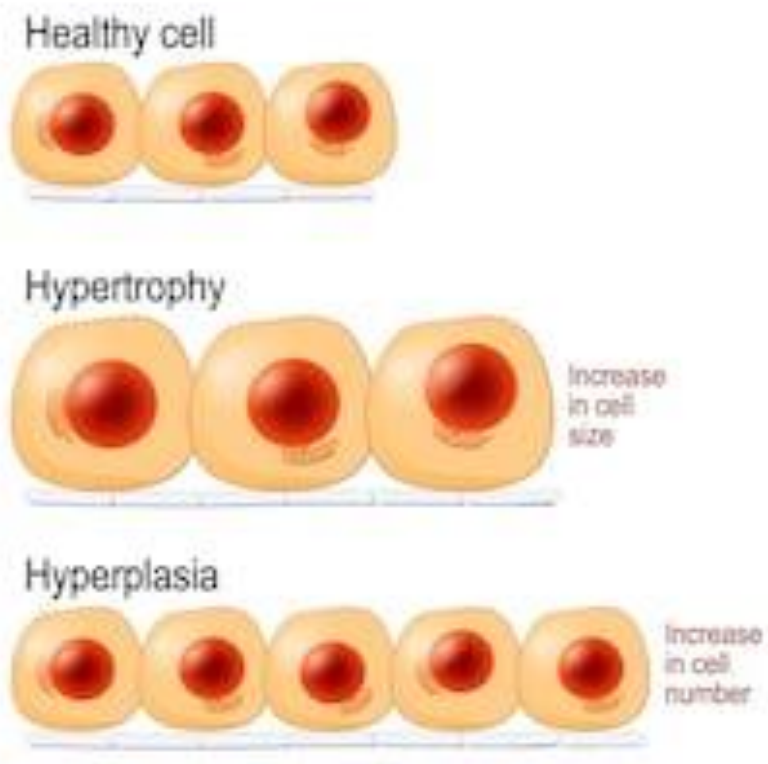
Dysplasia

Dysplasia: Dysplasia means ‘disordered cellular development’, often accompanied with metaplasia and hyperplasia; It is therefore also referred to as atypical hyperplasia.

Dysplasia occurs most often in epithelial cells

Epithelial dysplasia is characterized by cellular proliferation and cytological changes

1. Increased number of layers of epithelial cells
 2. Disorderly arrangement of cells from basal layer to the surface layer
- Dysplastic changes often occur due to chronic irritation or prolonged inflammation



- **Metaplasia:** the exchange of normal epithelium for another type of epithelium. Metaplasia is reversible when the stimulus for it is taken away.

