Introduction to Microanatomy

Microanatomy Versus Histology

DEFINITION

Histology is derived from the Greek words *Histos* (tissue) and *Logos* (study of [science of]). In the combined form, it means the study of tissues.

APPLICATION

(1) It is concerned with how the three germ layers of the embryo develop and how they become fitted together during development according to fairly common patterns, to form complicated structures such as organs.

(2) It is concerned with the relation of structure to function at the level of the light microscope and how structure changes in relation to different states of function.

(3) It has been a very important partner in interdisciplinary studies, which have provided a vast amount of new information about structure, its relation to function and the chemistry of function at the sub-cellular level (cell biology).

Microanatomy may not involve itself with aspects of function. It primarily focuses on structure while Histology incorporates both structure and function. Because the study of histology requires a firm foundation in cell biology, BASIC HISTOLOGY begins with an accurate, up-to-date description of the structure and function of cells and their products and a brief introduction into the molecular biology of the cell.

TEACHING AND LEARNING TOOLS

The effectiveness of teaching and learning microanatomy depends on numerous factors: The **microscopes** (microscopy: Light microscopy, phase contrast microcopy polarizing microscopy, electron microscopy

[Transmission & Scanning] and Fluorescent microscopy), **Atlases or text atlases, projectors, micrographs** and **sections**. Others that the student ought to have readily in the laboratory or histology practical class are: **notebook** (drawing book), **pencil** and **eraser**. Of these, the availability to the student of diverse histological preparations is a *sine qua non*. This is where sections are very important. Also important is a concise compilation of diagrams, micrographs, pictures, picture-sketches and line drawings. These are provided by the Atlases or text-atlases, which the student must strive to possess. Microscopes, projectors, audio-visual equipments, etc are often difficult to own by students and are thus the responsibility of institutions. These materials are all laboratory-based, and thus emphasize the laboratory basis of the study of histology. It is also important to stress the importance of three-dimensional concept of illustrations in understanding the morphologic and functional features of cells, tissues and organs.

ORGANIZATION OF TISSUES

Histology helps to build a good concept of tissues beginning from cellular level. Then, it is followed by study of different organs and organ systems. In doing this, a knowledge and description of the four basic tissues of the body is necessary emphasizing how cells become specialized to perform the specific functions of the tissues.

Thus, histological structure (of tissues and organs) provides the basis for critical appraisal of normal functioning of the various organs; thus enabling pathology to be properly recognized and understood, diagnosed and interpreted.

UNITS OF MEASUREMENT

1) The Angstrom unit $A^0 = 10^{-10}$ meter. This is however, no longer recognized in the international system of units.

2) The nanometer nm = 10^{-9} meter. This is used instead of the Angstrom unit.
3) The micrometer (μm) has replaced the micron (μ) but has the same value of 10^{-6} meter.

SECTIONS

These are extremely thin, transparent shavings cut from a little piece of body tissue, laid flat on a glass slide, stained, covered first with a small amount of mounting fluid of the proper refractive index and finally with a thin glass coverslip which is pressed down firmly on the flat shaving of stained tissue.

Although the term section is commonly used to designate the complete preparation (glass slide, stained shaving, mounting medium and coverslip) it is used also for the shaving itself.

Preparation of Sections

(1) Coagulation of soft tissue (with a fixing solution).
(2) Infiltrating such fixed tissue with a fluid embedding material that subsequently hardens after which it can be sliced thinly. **This is the paraffin technique**.

STEPS IN THE PARAFFIN TECHNIQUE

(1) Obtaining the tissue

Little pieces of tissue obtained are termed blocks of tissue. Blocks are removed with a sharp knife with very little pressure. Blocks obtained should be quickly transferred to a fixative and should not be more than a few mm thick, so that the fixative can penetrate quickly and thoroughly to avoid postmortem degeneration.

(2) Fixing the tissue

Fixatives harden tissue by coagulating tissue protein jelly-like sols. e.g. 4% solution of buffered formaldehyde

Others: Potassium dichromate
Acetic acid
Picric acid
Osmic acid
Glutaraldehyde
Ethanol
Bouin's fluid
OsO_4 (Osmium tetroxide).

Action of fixatives

- Keep from washing away of components (hardening?)
- Good antiseptics that kill bacteria, etc
- Affect tissue to the extent that reaction to other stains is favoured.

3. Dehydration – in graded series of alcohol.

4. Clearing – clearing is to remove alcohol from tissue. A good clearing agent should be miscible in both alcohol and wax. Xylene is a common clearing agent.

5. Embedding – usually in molten paraffin. This is maintained until all the xylene is replaced by molten paraffin.

<u>Embedding materials</u>

- Paraffin Wax (commonest)
- Polyester wax
- Nitrocellulose
- Synthetic resins.

6. Sectioning

Slices of a few microns thick are difficult to cut. 1μ (one micron) is 1/1000mm. Special machines are widely employed for this purpose. These machines are called **MICROTOMES**.

7. Staining and mounting.

Paraffin has to be removed and replaced by water. Thus, the section will have to be dipped in xylene, and then taken through decreasing strengths of alcohol (rehydration) and finally in water.

Freezing

This is an option for making tissues firm for cutting, if such tissues should avoid treatment of chemicals especially those meant for sensitive work. The machine used is called the **CRYOSTAT**. It is a microtome built into a transparent freezer, so to say, or freezer through whose top the inside can be seen without opening it.

STAINS AND STAINING

Acid and Basic Stains

Most common combination of stain is **Haematoxylin and Eosin (H&E)**. Haematoxylin imparts a blue-to-purple colour to the tissue constituents with which it combines i.e. basophilic constituents. It is thus, a basic dye.

Eosin imparts a pink-to-red colour to the tissue constituents with which it combines i.e. acidophilic constituents. It is thus, an acidic dye.

From the above, it can be understood that the staining of some tissue ingredient – say with basic stain for instance – does not depend on the tissue ingredient diffusely absorbing the stain, but instead, on the fact that the tissue ingredient contains negatively charged radicals that

combine firmly with the positively charged, basic-colour-imparting radicals of the stain. The reverse is true for an acid stain.

Stains in general are dependent on the mechanism stated above. However there may be variations in respect of some special stains which are utilized in selectively displaying some organelles, inclusions or deposits or enzymes in tissues.

Some of the Stains

Routine (Nucleus and Cytoplasm)	-	**H &E**
DNA	-	**Fuelgen**
Connective Tissue Fibres	-	**Masson's Trichrome**
	-	**Silver impregnation**
Fat	-	**Sudan III**
Carbohydrate	-	**Periodic-Acid-Shiff (PAS)**
Mucopolysaccharide	-	**Toluidine blue**
		Alcian blue
		Hale's Colloidal Iron
Elastic fibers	-	**Mallory Azan**

Reaction of Proteins to Dyes

Proteins are amphoteric i.e. can act either as acids or bases. This is because the amino-acid of which they are composed have side chains, some of which dissociate as acids and others as bases.

Depending on the pH of the body which usually is on the neutral, when the acid dye is added for instance, the acid tends to suppress the dissociation of side chains that act as acids, but not those that act as bases. So, the basic side chains dissociate and act to neutralize the extra-acid. At this point, the proteins are slightly basic because they

have a preponderance of basic side chains. So they readily stain with acid dyes. They are then colored red-pink.

Nucleic Acids and Nucleoproteins

These are predominant in the Nucleus as DNA and in cytoplasm as various types of RNA clumps. They are acid in nature. Therefore, they react to basic dyes. Thus, with H & E, they will react with haematoxylin and so, will stain blue. Thus, the blue-staining components in cytoplasm (granules) represent spots where protein synthesis is taking place. Otherwise, cytoplasm is generally pink.

Inorganic materials and other substances of low Molecular Weight

These are usually washed away during tissue preparation. Those that are trapped may need special means to demonstrate them in a slide.

Lipids

These are mostly stored fat, housed in the cytoplasm as large droplets. In routine preparations, fat is usually washed away and so the spaces they occupy are vacuoles, which will not stain.

Osmium Tetraoxide (OsO_4) is a special fixative reacting with fat, rendering it sufficiently insoluble to resist washing away or dissolution by clearing agents. Fat appears black by OsO_4, so tissues fixed with it make fat look black in section. Frozen sections help to retain fat. So when this kind of sectioning is involved, then a dye that stains fat needs to be used e.g. Sudan IV. It colors fat red.

Carbohydrates

There are four (4) categories:

(1) Simple sugars e.g. glucose

(2) Polymers of simple sugars e.g. glycogen

(3) Glycoproteins i.e. carbohydrate + protein

(4) Mucopolysaccharides

A. Simple sugars: These are washed out of tissue during processing.

B. Polymers: Usually found in liver and muscle cells. They are insoluble in water and not readily dissolved away during tissue preparation. They are also not stained by either H or E, so they are translucent. Special staining called Periodic-Acid Schiff (PAS) is used to demonstrate them in tissues. When present they show a purple-magenta colour.

C. Glycoproteins and Mucopolysaccharides: These are in a way related because they contain macromolecules of carbohydrates as well as amino acids/proteins.

However, glycoprotein is different from mucopolysaccharide because the latter (mucopolysaccharide) has a longer chain of low molecular weight carbohydrate.

Glycoproteins are PAS positive which mucopolysaccharides are not, or at best weekly so.

Mucopolysaccharides are produced in, and are very important components of connective tissues. Glycoproteins are produced chiefly by epithelial cells such as those lining the intestines that produce mucus.

The carbohydrate chains of mucopolycaccharide also contain amino sugars, hence Nitrogen. They have prominent acidic side chains and so are called acid-mucopolysaccharides. The acidic side chains may be organic acid groups (- COOH -) or sulfuric acid groups (sulfated mucopolysaccharides).

When not sulfated, they are commonly called hyaluronic acid found in joint cavities, interstices between protein fibers, intercellular space in connective tissue – where they are called ground substance. Example of sulfated mucopolysaccharide is chondroitin sulphate (intercellular substance of cartilage and to

some extent, connective tissue). Another example is Heparin, observed in form of granules in mast cells.

Staining of mucopolysaccharides.

(1) Metachromatic methods e.g. Toluidine blue
(2) Alcian blue
(3) Hale's colloidal iron method.

By metachromatic method, it is meant that when stains are used, they impart a different colour from their own colour. The chief dye involved is toluidine blue. When used, it stains Red, Pink or Purple.

- Alcian blue colours acid mucopolysaccharide blue
- Hale's colloidal iron technique has the advantage that at pH 2.5, the acetic group of the mucopolysaccharide binds colloidal iron, which can then be stained by a method specific for iron.

OTHER WAYS OF PREPARING SECTION

(1) The frozen section techniques
(2) Bone tissue section
(3) Blood smear
(4) Paraffin infiltration by the freeze-drying method
(5) Celloidin method

INTERPRETATION OF TISSUE SECTION

The microscope is utilized in the study and interpretation of stained tissue sections.

The observed product is the end-result of a series of processes that considerably distort the image leading to a considerable departure from the living tissue structure. The major culprit here is **SHRINKAGE**.

Shrinkage results from the action of the fixative, and other chemicals involved in the tissue processing. As a consequence, some of the spaces seen between cells and other tissue components are **ARTIFACTS**.

A. ARTIFACTS

Each of the steps involved in processing a section provides an opportunity for something to happen that will make the final product less than perfect. The various imperfections that can exist in the final product are termed artifacts; because they occur during manipulation of the tissue.

Causes of artifacts

1. Shrinkage
2. Folds and wrinkles
3. Nicks in microtone knife
4. Rough handling of fresh tissue
5. Postmortem degeneration
6. Dirty stains leading to precipitates.

B. THREE DIMENSIONS

There is a tendency to think in terms of two dimensions when examining thin sections even though the structures from which the sections are made actively have three dimensions. In order to understand the architecture of an organ or organelle, it is therefore necessary to study sections made in different planes and interpret accordingly. Such planes include the following:

- Longitudinal
- Transverse or cross

- Oblique
- Tangential

and the various combinations of the above.

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