Chapter 1
Cellular Organization and Microtechniqus

1.1. Relationship Between Cells, Tissues, and Organs

1..1. 1. The Cell

Cytology is the study of the cell, the basic unit of life. In unicellular organisms the cell must be able to perform all of the functions essential for life. In multicellular organisms cells can cooperate allowing for a division of labor. Different cells will have functional differences since they will perform different tasks and, as a result, will often display morphological differences (ex; flagella). When functionally different and structurally similar cells form groups within an organism those groups of cells are known as Tissues. Through specialization cells have lost the multifunctional capacity found in unicellular organisms in favor of fewer, emphasized properties. Their organization into tissues and organs allows the multicellular organism to perform biological functions with a greater economy.

1.1. 2. The Tissues

Three primordial germ layers will develop in the embryos of vertebrates. They are the: Ectoderm - the outer layer, Mesoderm - the middle layer and Endoderm - the inner layer. The three germ layers will give rise to the four classes of tissues. Epithelium - arise form all three of the germ layers. The endoderm gives rise to the epithelium lining most of the G.I. tract and it's glands, The epithelium lining the respiratory tract and it's glands, the epithelium lining the bladder and certain portions of the urogenital systems. And the epithelium lining the lumenal surfaces of the blood vessels and heart called endothelium. The mesoderm gives rise to:The epithelium lining the serous membranes of the body called mesothelium, the epithelium lining many portions of the urogenital systems. The ectoderm gives rise to the epithelium covering the body's surface (e.g.; the skin). The epithelium lining select portions of the G.I. tract such as the the anal canal, the extrinsic glands of the oral cavity, the taste buds, and the enamel of teeth, the epithelium lining portions of the eye, ear, and nose, the neuroepithelial cells. Connective Tissue - arise form primarily the mesoderm. The exception are certain of the neuroglia. Muscle Tissue - arise from primarily the mesoderm. The exceptions are the smooth muscles of the sweat glands and of the pupil. Nervous Tissue - arises only from the ectoderm. Each of the four classes of tissues can be further subdivided based on functional and morphological variations due to their different specializations. For example there are three classes of muscle tissue. This follows an old adage that "form follows function". The study of the four classes of tissues and their specializations is called Histology.

1.1. 3. The Organs

The four tissues classes will be further organized into organs which, in turn, will be organized into systems.An organ is defined as a group of functionally similar tissues working together to perform a certain task.

1.2. Microtechniques

1.2.1. The Light Microscope

The light microscope allows the researcher to observe a specimen that is too small to study by the unaided eye. The light microscope projects a beam of light through the specimen. To facilitate this the specimen must be thin enough for light to pass through it. The beam of light will pass through the specimen and then through a series of magnifying lenses and a prism which directs the light towards the researcher's eyes. The basic light microscope has three lenses arranged in the following sequence:

a. Condenser Lens - focuses the light from the source into a cone which will illuminate the specimen.
b. Objective Lens - collects the light which has passe through the specimen. It magnifies the specimen's image and directs it towards the ocular lens. Often a light microscope will have several objective lenses with varying degrees of magnifying ability.
c. Ocular Lens -receives the image from the objective lens and transmits it towards the eye (or camera). The ocular lens will often also magnify the image. The arrangement of lenses will also allow for resolution of the image.
d. Resolution - the smallest distance that can be seem between two visibly separate objects.

1.2.2. Histological Preparation for Light Microscopy

The specimen can be prepared in a number of ways but generally histological preparation will follow these steps:

a] Fixation

Fixation must occur either before or soon after the removal of the tissue specimen to prevent degradation. One draw back to fixation is that fixative agents kill cells and so will prevent the observation of cellular processes. In cases where the full enzymatic activity of the tissue must be preserved the specimen can be frozen in liquid nitrogen or liquid helium. One of the most commonly used fixatives is Formalin, a concentrated formaldehyde solution. It does not denature proteins or alter tissue structure as severely as does other fixatives. Other fixatives include: picric acid, paraformaldehyde, and gluteraldehyde.

b] Dehydration

Dehydration will often be a prerequisite for subsequent steps in histo prep. Many embedding media require water to be removed from the specimen. Many staining procedures also require water to be removed from the specimen. Dehydration is most often accomplished by immersing the tissue in a series of increasingly more concentrated solvents such as ethyl alcohol. By gradually increasing the strength of the solvent you reduce the chance and/or degree of damage to the tissue. Even so, dehydration will often cause Artifacts to occur such as tissue shrinkage or the false separation of cells/tissue layers.

c] Clearing

The specimen may be treated by a variety of chemicals to prepare it for staining. This process is called Clearing.

d] Embedding and Sectioning

Sectioning is the cutting of the tissue into sections thin enough to transmit light (~1 to 20

um). Sectioning requires the tissue to be supported and rigid enough to slice uniformly. This is the purpose to embedding. The specimen is embedded prior to sectioning. A variety of embedding media are used. The two most commonly used are: Paraffin, a wax, Epoxy, a harder substance used to make thinner sections (i.e.; 1 - 5 um). By dehydrating the tissue prior to embedding the embedding media can enter the tissue to give it the required support. Sectioning of the embedded specimen is accomplished by means of a Microtome. Typically the microtome will have a very fine edged metal blade but for thinner sections sometimes glass and even diamond blades are used. The sectioned specimen is then placed on a slide, often on a drop of water, to await staining.

e] Staining

To improve the visibility and clarity of cytological structures the specimen will often be stained. There are a wide variety of histological stains. Many stains attach to structures by electrostatic forces of attraction (i.e.; opposites attract). So, cationic stains will be attracted to anionic structures and visa versa. Many times a variety of dyes are used that will impart different colors. Often a basophilic (cationic) dye is used in combination with an acidophilic (anionic) dye. The basophilic dye will attach to negatively charged structures (ex; the nucleus). The acidophilic dye will attach to positively charged structures (ex; the mitochondria). The most frequently used combination is the H & E (hematoxylin and eosin) stain. Hematoxylin (a cationic dye) will stain structures blue to purple. Eosin (an anionic dye) will stain structures red to orange. In some case a Metachromatic Stain is used. A metachromatic dye will stain differently charged structures differently. (Ex; toluidine blue)

1.3. Other Types of Light Microscopes

1.3. 1. Fluorescence Microscopy

Unlike the typical light microscope, the fluorescent microscope uses ultraviolet light. The u.v. light will be of a wavelength so as to excite a known fluorescent marker within the tissue. The fluorescent marker responds by emitting a wavelength of visible light (i.e.; fluorescence). Fluorescent microscopy is used quite frequently today. Ex; fluorescent tagged antibodies can be made which are specific for any protein.

1.3.2. Phase Contrast Microscopy

Phase contrast microscopy uses a special pair of condenser and objective lenses to differentiate between structures based on their different reflective properties. This makes staining unnecessary. It is used, for example, where staining procedures may disturb or obscure cellular processes.

1.3.3. Electron Microscopy

a) Transmission Electron Microscopy (TEM)

TEM uses a beam of electrons, instead of light, to illuminate the specimen. This improves the resolution and thus the degree of magnification which can be achieved. Also, instead of using a series of magnifying lenses, in TEM the electron beam is shaped by electromagnets to provide magnification. Staining is accomplished by the use of heavy metals (such as uranyl and osmium) which will obstruct the passage of electrons through the tissue. Different portions of the cells will have different affinities for these metal ions. Due to the different resulting contrasts we see a two dimensional image of varying electron density which will be photographed. Since electrons on a conventional TEM do not have high penetration the tissue sections must be thinner than those used in light

microscopy.
b) Scanning Electron Microscopy (SEM)
SEM differs from TEM in that it makes visible only the surface of the specimen. An electron beam is bounced off of the specimen producing a three dimensional image which will be photographed. To accomplish this the specimen must be coated in precious metal such as gold, silver, or copper.

1.3.4. The Freeze Fracture Technique

Certain cellular structures can be best studied by freezing the cell in liquid nitrogen, breaking it with a knife, and then looking at the components under a microscope. (Ex; the cell membrane)