Lab # (1)……

GENERAL LABORATORY PRACTICES and MICROSCOPE

This laboratory manual has been developed to accompany the Medical Biology course. The coursework, lecture and lab, are designed to provide the student with a wide range of information about living organisms and systems. **Safety** in the lab demands that lab directions be followed carefully.

GENERAL LABORATROY PRACTICES

1. No food or drinks in the Lab.

2. No smoking.

3. Wear all safety equipment as required by the lab procedure or your instructor. Learn the location of all lab safety equipment. Wear safety glasses or goggles at all times. DO NOT WEAR CONTACT LENS AT ANY TIME.

4. Tie long hair back to keep it out of flames or harmful liquids.

5. Do not work in the laboratory in the absence of your instructor or his authorized representative.

MICROSCOPY

Microscopy Materials List

- Light or compound microscope
- Prepared slides:
- Colored threads
- Clean microscope slides
- Cover slips
- Knife
- Toothpicks
- Iodine solution

INTRODUCTION

The microscope is a precise piece of equipment that should be handled with special care. A microscope may be seriously damaged if dropped or bumped against a hard object. The student should report immediately to the instructor any defects that might occur to his or her microscope. The microscope should always be carried with both hands, one under the base and the other on the arm.

LIGHT MICROSCOPE

The following parts are the parts of the microscope that the student should be familiar with. Refer to Figure 1 to aid in locating these parts on your microscope.

1. The OCULAR (eyepiece) contains the upper most lenses of the microscope. Its function is to magnify. The part may be loose, but it should never be removed from the microscope as such practice will allow dust to get inside the instrument. As you look through the ocular, you may notice a solid line; this is a POINTER. Never attempt to clean the inner part of the ocular for you will remove the pointer.

2. The BODY TUBE connects the ocular to the nosepiece. This is a tube through which light rays pass between the upper and lower lenses.

3. The NOSEPIECE is a rotating disc on which the objectives are mounted. When moving the nosepiece, the fingers should be placed on the disc and not the objectives.

4. There may be three or four **OBJECTIVES** of different lengths and magnifying powers attached to the nosepiece of your microscope. These objectives, together with the ocular, magnify the size of the objects that you are observing. Remember, the shorter the objective the lower the power of magnification.

5. The ARM supports the above parts. This is one of two structures that should be held when carrying the microscope.

6. The STAGE is the platform with a mechanical stage for holding the slides in place. Note the circular opening in the center of the stage which allows light to pass through. The object which is to be viewed should be centered over this opening.

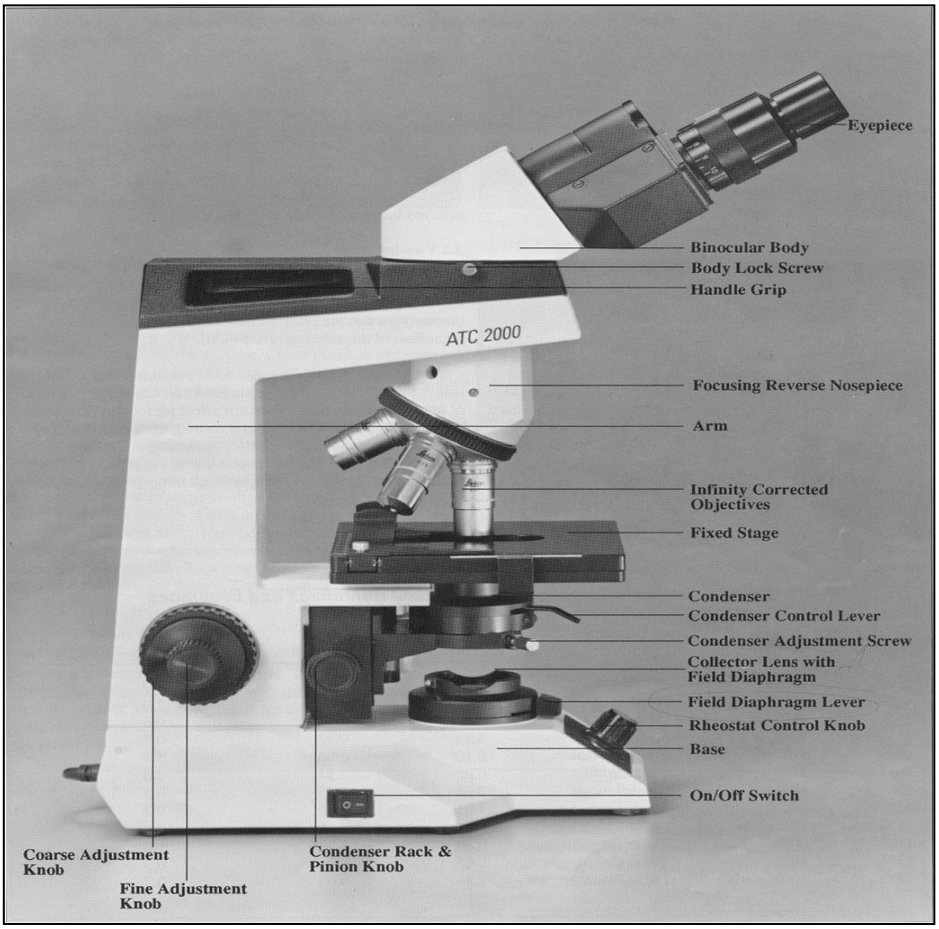
7. The ILLUMNATOR is a small lamp located directly beneath the stage. Electrical outlets are located on the tables.

8. The DIAPHRAGM may be an iris or rotating disc, depending on the kind of microscope. It is located below the stage. The diameter of the diaphragm may be controlled by either a lever on the iris or by rotation of the disc. Various objects can be seen better under certain light conditions. When using the highest magnification, more light is needed than when using lower magnifications.

9. The CONDENSER is a group of lenses beneath the stage. The condenser causes light rays from the illuminator to converge on the surface of the microscope slide. For most microscopic work, it is best to keep the condenser at its highest level. Only rarely is it desirable to lower it slightly. When the condenser is used at a lowered position, the resolving power is greatly reduced. There is a small milled wheel just under the stage that is used to control the position of the condenser.

10. The BASE is the heavy, horseshoe-shaped structure upon which the microscope rests. This is the other part of the microscope that is held when the microscope is being carried.
11. The COARSE ADJUSTMENT is the large milled wheel on the microscope, which is used in focusing the lenses.

12. The FINE ADJUSTMENT is the smaller milled wheel on the microscope. The wheel may be separate from the coarse adjustment wheel on some microscopes, where on others it is the smaller, outermost portion of a dual wheel assembly.



Resolution and Magnification

The resolution limit or resolving power of a microscope lens is a function of the **wavelength of light**, the design of the condenser, and the use of immersion oil with the **100X** objective. The shortest wavelengths of visible light provide the maximum resolution. It is for this reason that all microscopes use blue filters over the light source. The best compound microscope lenses have a resolving power of approximately 0.2 microns. This means that two small objects that are 0.2 microns apart will be seen as separate entities under the oil immersion lens. If they are closer than this, a single object is seen due to the fusion of the images.

Magnification of an object seen through the microscope is a function of the power of the ocular and objective. If the 10X objective is used with a 10X ocular, the magnification is 100X. When the oil immersion lens is used, the magnification is 10X x 100X, of 1000X.

THE SCANNING ELECTRON MICROSCOPE

Scanning electron microscopy provides a three-dimensional view of surface features. With a **scanning electron microscope** (**SEM**), a narrow electron beam is played back and forth across a specimen's surface, which is either conductive itself or has been coated with a thin metal layer.

Electron energy triggers the emission of secondary electrons in the metal. Equipment similar to a television camera detects the emission patterns, and an image is formed.

In **contrast** to the light microscope, the scanning electron microscope has a depth of field at all magnifications up to 500 times greater than the light microscope.

PROCEDURE (1): Using the Microscope

When properly used, the microscope should cause no eye strain. Try to keep both eyes open when working the monocular microscope and use the dominant eye to look through the ocular. If you wear glasses, it will not be necessary to use them with the microscope, since the microscope automatically corrects for this.

1. Obtain the microscope to which you were assigned from the microscope cabinet. When carrying the microscope, remember to place one hand under the base and the other on the arm.

2. Before the microscope is place on the desk, ample space must be provided for it. All books, purses and other unneeded paraphernalia should be put away.

3. Place your microscope in front of you in a comfortable working position about one inch from the table's edge. The nosepiece should have the low-power (**4X**) objective in position over the opening in the stage. Make sure the switch is in the off position and plug the power cord into a suitable grounded electrical outlet. Turn on the illuminator.

4. Obtain a prepared microscope slide. Notice the label which describes the material mounted on the center of the slide. Make a gross examination of the slide. (If the slide is dirty, clean it by rubbing lightly with a soft cloth or paper towel; do not use expensive lens paper for this purpose.)

5. Progress from the **4X** to the **10X** objective. Rotate the nosepiece until the **10X** objective clicks into place. To focus, ONLY the fine adjustment wheel needs to be moved.

Lab # (2)……

Preparation of direct wet mount Slide

PROCEDURE (2): Preparing Wet Mounts

Wet mounts specimens are those that you make fresh in lab. The specimen is placed in a drop of water or stain on a microscope slide and then covered by a thin piece of glass or plastic called a cover slip. Wet mounts are useful for speed and easy preparation, but they do not usually allow great detail to be observed.

1. Make a wet mount of some onion cells using iodine stain.

a. Obtain a clean slide and cover slip.

b. Place a drop of iodine on the center of the slide, and then place a single layer of onion skin (skin between the onion layers) in the iodine drop. Be sure to spread it out evenly so there is no overlap or double layering.

c. Touch the cover slip to one edge of the drop, and gently lower it. (If you drop the cover slip too quickly, air bubbles will be trapped. You cannot see through an air bubble).

d. Observe these cells first at **4X**, then **10X** and make a sketch of a few cells in the space provided on the data sheet. Be careful when working with the stain!

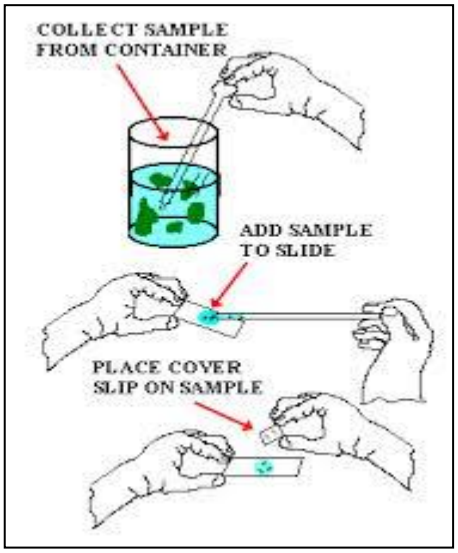
2. Make a wet mount of some cheek cells using iodine stain.

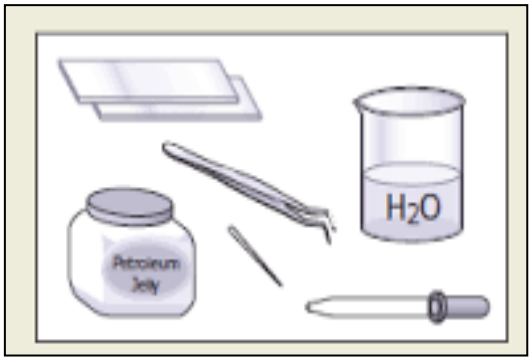
a. Obtain a clean slide and cover slip.

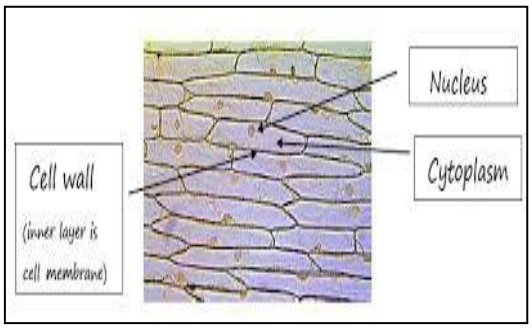
b. Place a drop of iodine stain on the center of the slide.

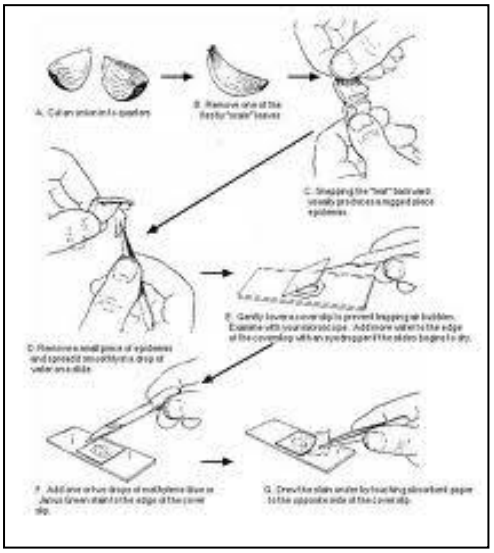
c. Using a clean unused toothpick, gently scrape the inside of your cheek and mix it into iodine drop.

d. Observe these cells first at 4X, 10X, and then **40X** and make a sketch of a few cells in the space provided on the lab report.


COLLECT SAMPLE
FROM CONTAINER
ADD SAMPLE
TO SLIDE
PLACE COVER
SLIP ON SAMPLE


H_2O
Petroleum
Jelly


Nucleus
Cytoplasm
Cell wall
(inner layer is
cell membrane)



Lab # (3)……

Human Cell Types

INTRODUCTION: All organisms, including humans, are composed of cells. This is not apparent until you compare unicellular organisms with the tissues of multicellular ones under the microscope. The cell theory, one of the fundamental principles of modern biology, was not formulated until after the invention of the microscope in the seventeenth century. Most cells are small and can be seen only under a microscope. The small size of cells means that they are measured using the smaller units of the metric system, such as the *micrometer* (µm). Most human cells are about 100 µm in diameter, about the width of a human hair. The internal contents of a cell are even smaller and, in most cases, may only be viewed using powerful microscopes.

Biologists classify cells into two broad categories-the **prokaryotes** and **eukaryotes**. The prokaryotic group includes the bacteria; the eukaryotic group consists of animals, plants, fungi, and some single-celled organisms. Despite their differences, both types of cells have a **plasma membrane,** an outer membrane that regulates what enters and exits a cell. Eukaryotic cells have many different types of organelles.

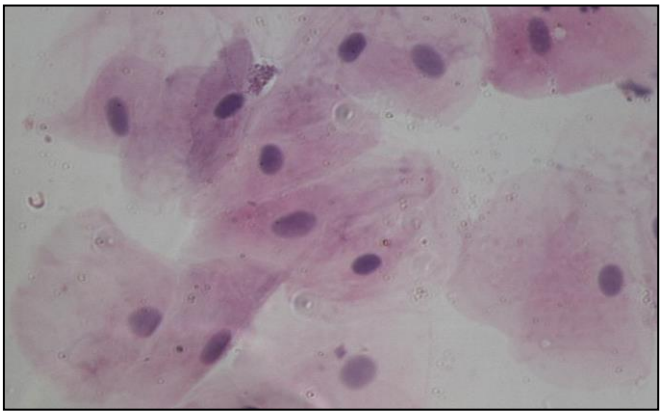
There are different types of animal cells, the most important cells including the followings:

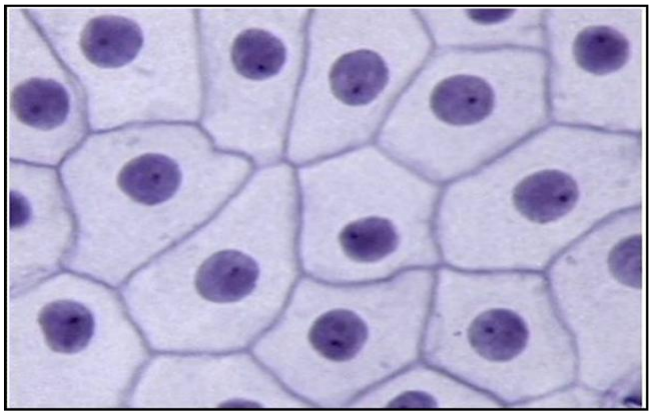
1- SQUAMOUS CELL:

These cells are large but thin, and have a prominent nucleus. It has flattened shape in profile. The function of this cell is covering and lining membranes (protection of outer or inner surfaces.

The location of this cell in the:

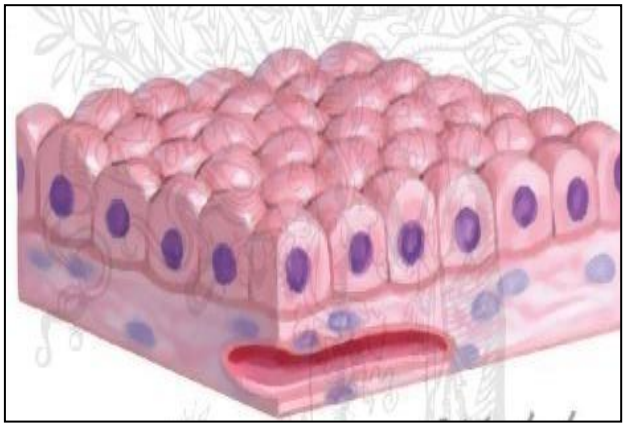
- Epidermis of skin
- Endothelial wall of bowmans capsule of kidney
- Wall of capillaries
- Aleveoli of lungs

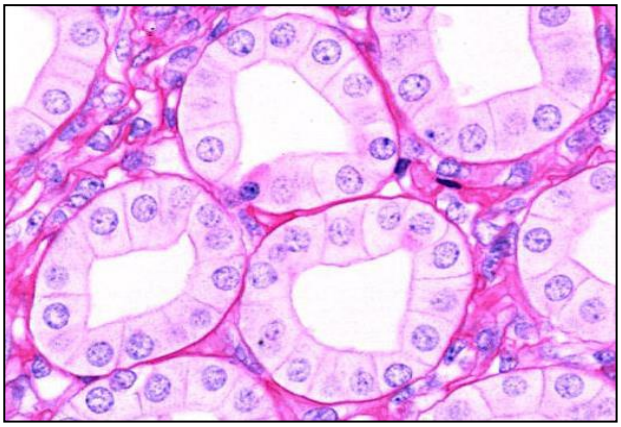




2- CUBOIDAL CELL:

This cell has cubic or square shape in cross section with large spherical central nucleus, these cells always arranged as tubules or ring. Cuboidal cells lining the wall of sweat gland and tubules of kidney.

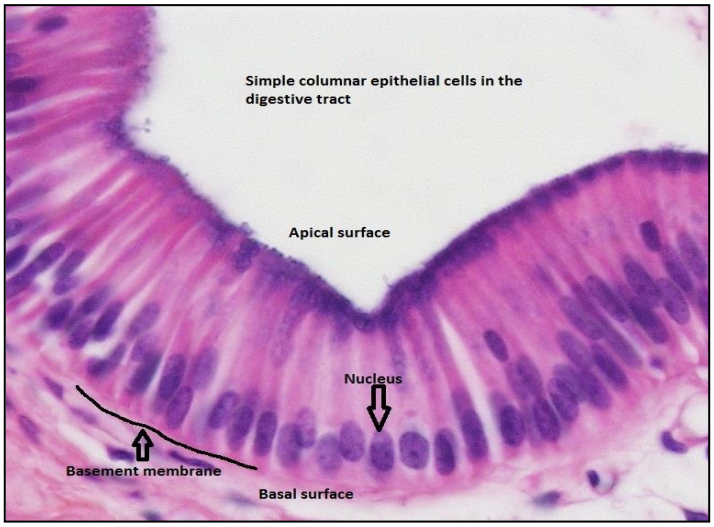




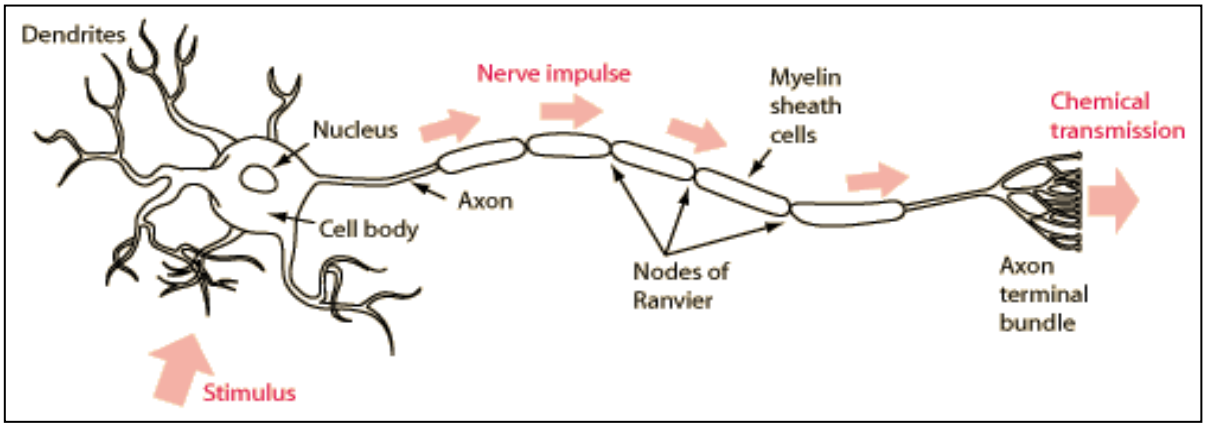
3- COLUMNAR CELL:

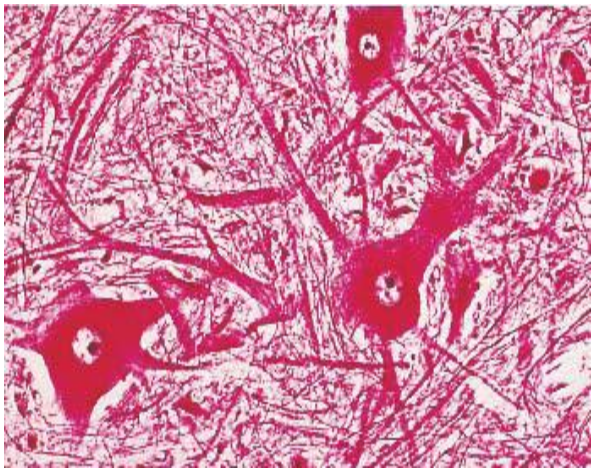
This cell has tall, vertical shape, and oval nucleus located near the basement membrane. These cells make up simple columnar epithelial tissue. The location of this cell as covering and lining membranes like in:

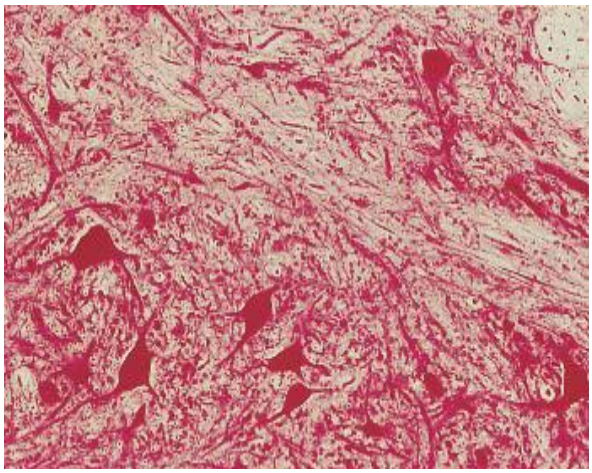
- Small intestine
- Gastrointestinal tract from esophagus to cecum
- Columnar cell play important role in the secretion & absorption especially in stomach and small intestine.



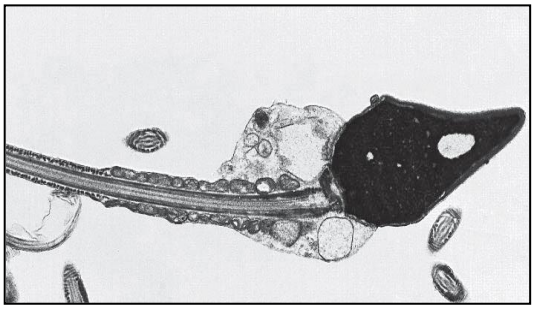
4- **NERVE CELL:** ***Neurons*** are the cells that receive and transmit signals. Each neuron contains a ***nerve cell body*** with a nucleus and **organelles** such as **mitochondria**, **endoplasmic reticulum**, and **Golgi apparatus**. Branching off the nerve cell body are the ***dendrites***, which act like tiny antennae picking up signals from other cells. At the opposite end of the nerve cell body is the ***axon***, which is a long, thin fiber with branches at the end that sends signals. The axon is insulated by a myelin sheath made up of segments called **Schwann cells**. Nerve impulses are received by the dendrites, travel down the branches of the dendrites to the nerve cell body, and are carried along the axon.

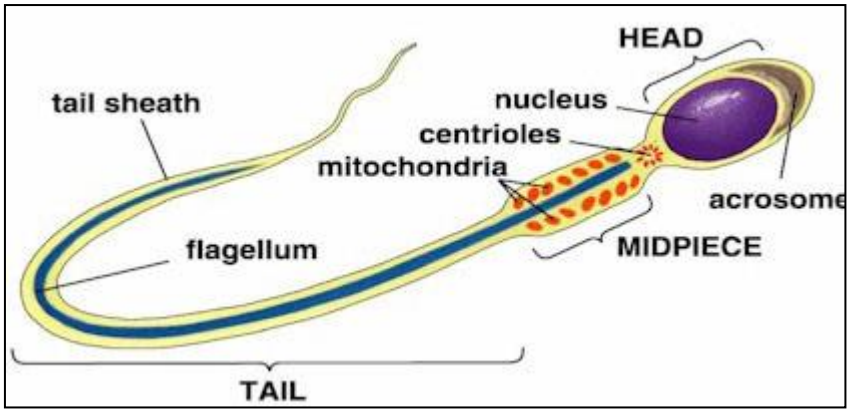




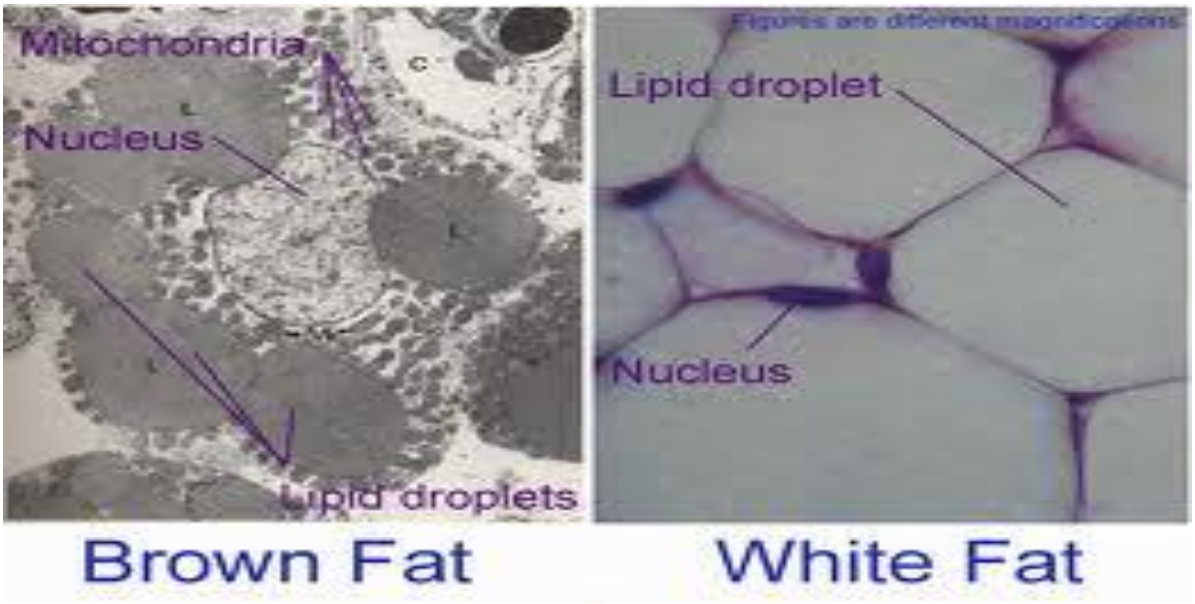


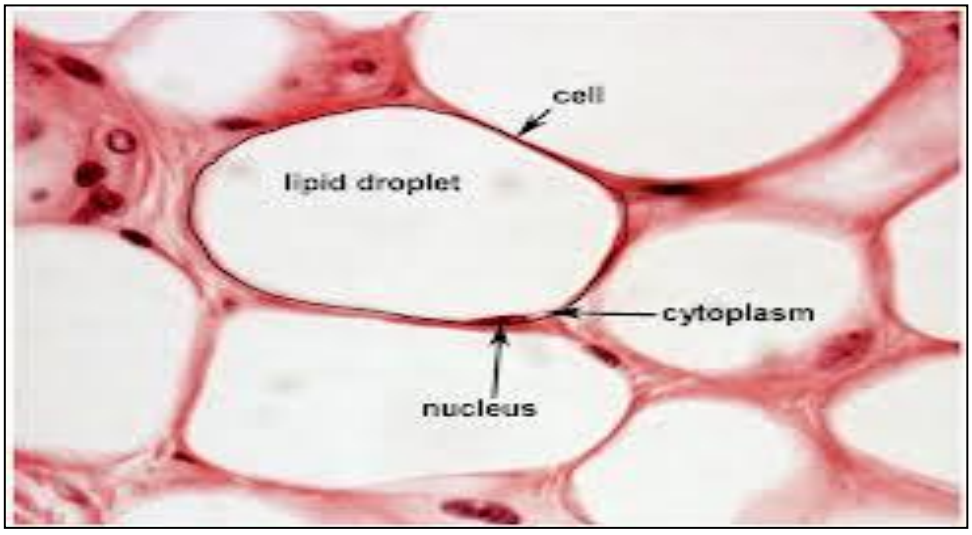
5- SPERM CELLS: Sperm were first observed in 1677 by Antonie van Leeuwenhoek using a microscope, the sperm cell consists of a **head**, a **midpiece** and a **tail**. The head contains the **nucleus** with densely coiled chromatin fibres, surrounded anteriorly by an acrosome, which contains enzymes used for penetrating the female egg. The midpiece has a central filamentous core with many mitochondria spiralled around it, used for ATP production for the journey through the female cervix, uterus and uterine tubes. The tail or "**flagellum**" executes the lashing movements that propel the spermatocyte.





6- ADIPOSE CELLS: Adipose cell, also called **adipocyte** or **fat cell**, connective-tissue cell specialized to synthesize and contain large globules of fat. There are two types of adipose cells: **white adipose cells** contain large fat droplets, only a small amount of **cytoplasm**, and flattened, noncentrally located **nuclei**; and **brown adipose cells** contain fat droplets of differing size, a large amount of cytoplasm, numerous mitochondria, and round, centrally located nuclei. The chief chemical constituents of adipose cell fat are **triglycerides**, which are **esters** made up of a **glycerol** and one or more **fatty acids**.

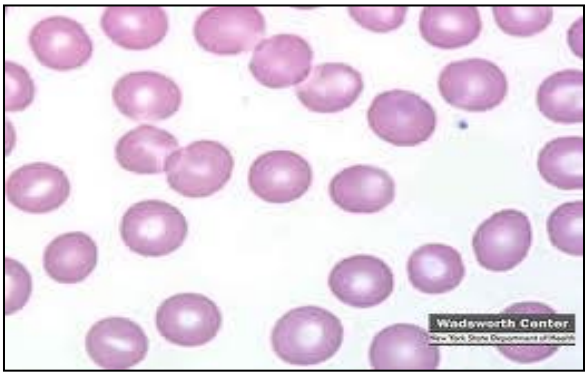


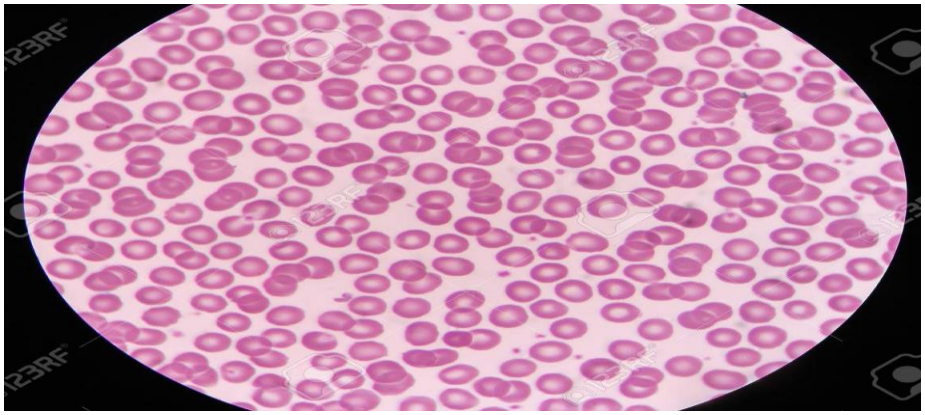


Lab # (4)……

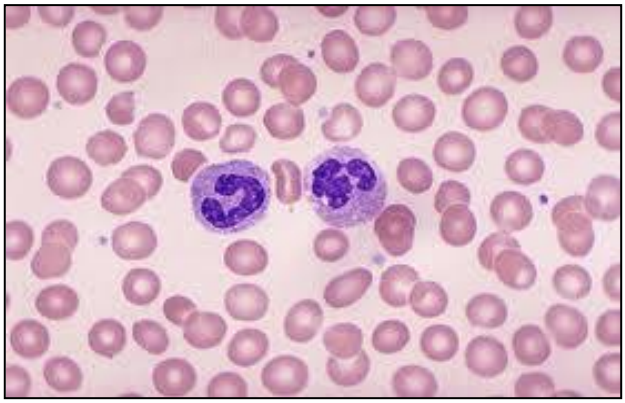
Human Cell Types

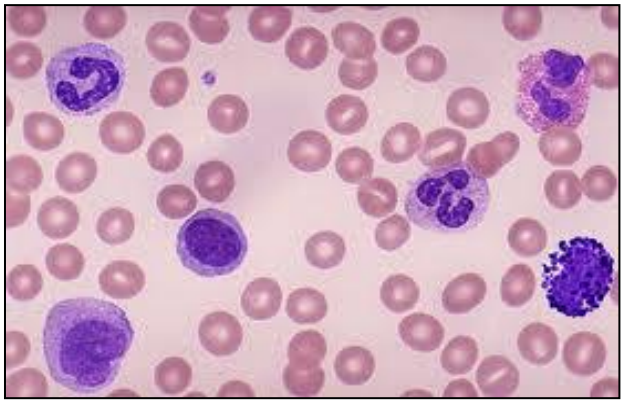
7- HUMAN RED BLOOD CELLS: Red blood cell, also called **erythrocyte**, cellular component of **blood**, millions of which in the **circulation** of vertebrates give the **blood** its characteristic colour and carry **oxygen** from the lungs to the tissues. The mature human red blood cell is small, round, and biconcave; it appears dumbbell-shaped in profile. The cell is flexible and assumes a bell shape as it passes through extremely small blood vessels. It is covered with a membrane composed of lipids and proteins, lacks a **nucleus**, and contains **hemoglobin** a red, iron-rich protein that binds **oxygen**.

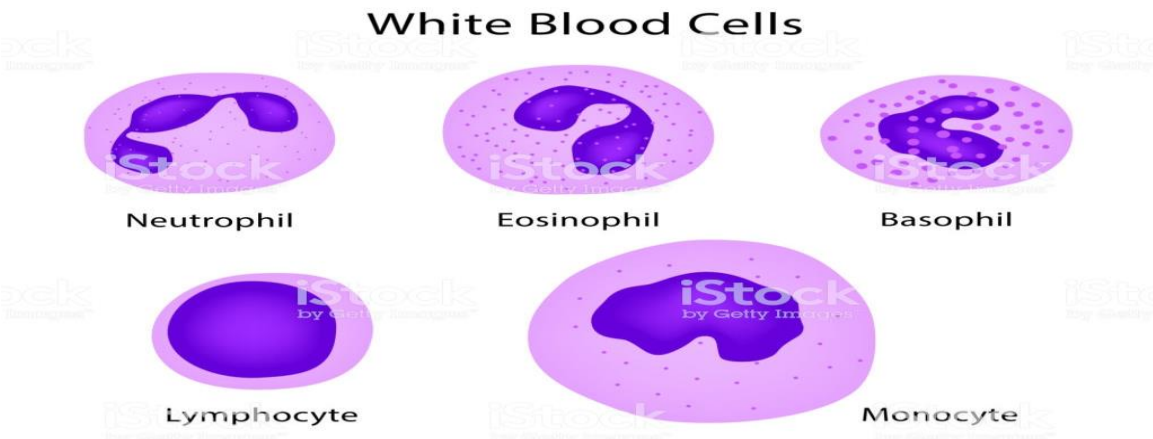




8- HUMAN WHITE BLOOD CELLS: White blood cells are **larger** than red blood cells, making up less than 1% of the total blood volume, WBC's like red cells are mostly formed from stem cells in the bone marrow. They have a defensive role in destroying invading organisms and also assist the removal of dead or damaged tissue cells. White cells consist of **lymphocytes** and **monocytes**, with relatively clear cytoplasm, and 3 types of **granulocyte - neutrophils**, **eosinophils** and **basophils**, whose cytoplasm is filled with granules. Unlike RBC's, WBC's contain **nuclei**, they are also much larger and colourless. They use the blood to reach tissues and cells, where they migrate to perform specific functions.

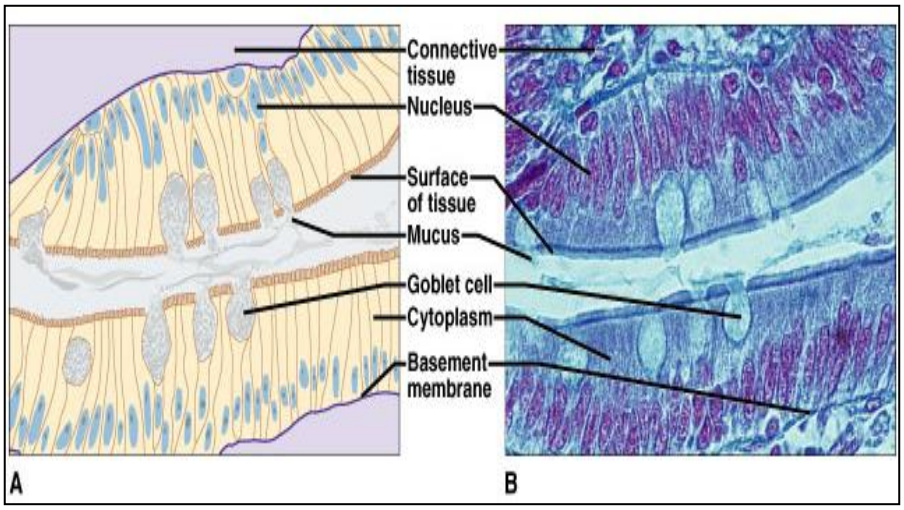


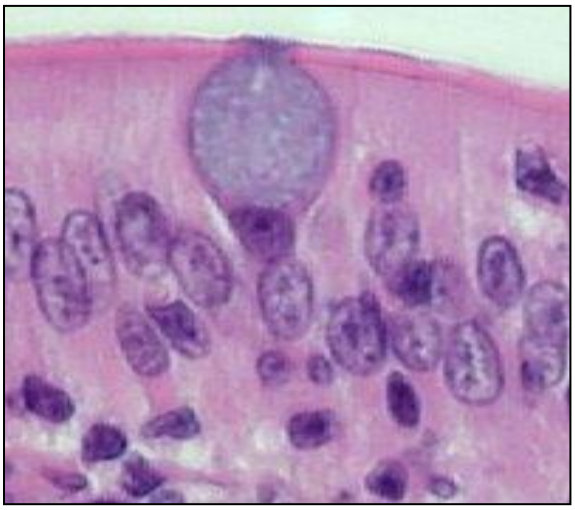




9- GOBLET CELL:

The name of this cell derived from their characteristic shape in tissues, which is a narrow base with expanded apical portion that sometimes extends into lumen shape. Goblet cells are found scattered among other cells in the epithelium of many organs, especially in intestinal and respiratory tract. These cells secret mucous, a viscous fluid composed of proteins called (mucin). The mucous functions include protection and aids in digestion of food.

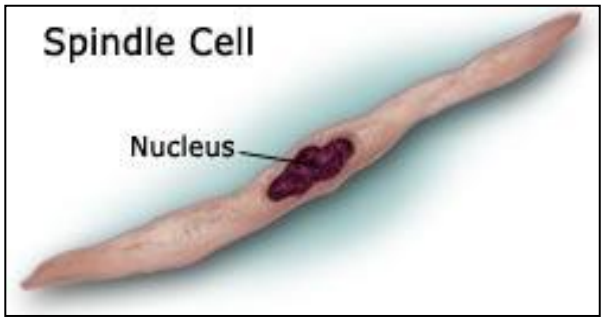




10- SPINDLE CELL:

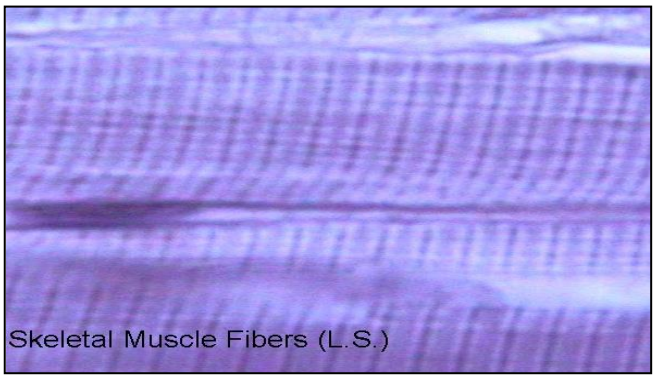
It is type of animal cell has spindle, long shape with two tapered ends and contain large spherical nucleus. It is called fibers in muscle structure, their cytoplasm is called (sarcoplasm) filled with bundles of myofibrils, this cell surrounded by membrane named (sarcolemma).

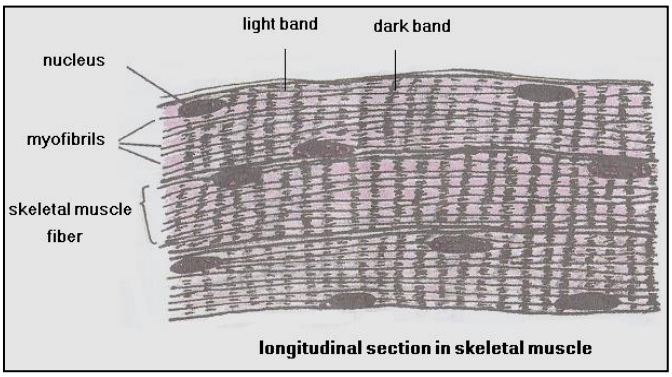
Spindle cells are components of smooth muscle which form the structure in digestive system, blood vessels and others.



11- STRIATED CELL:

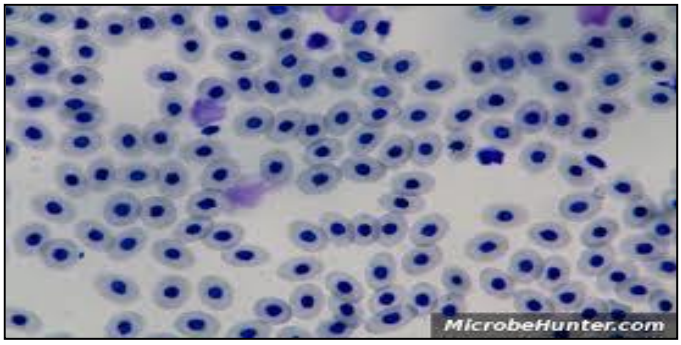
This cell has cylindrical shape, don't branch and multinucleated with peripheral location. This cell surrounded by thin sarcolemma and their sacroplasm contain myofibrils consist of contractile proteins that give appearance of striation (dark and light bands)…. The example of this cell is skeletal muscle.

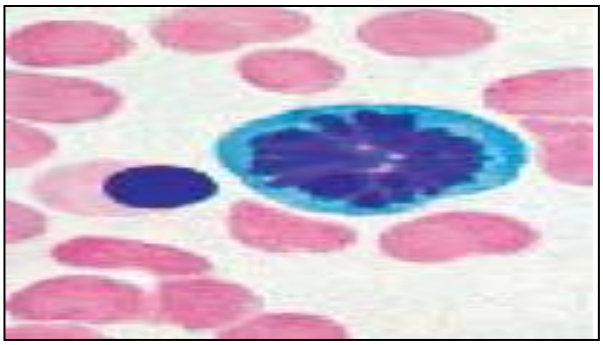


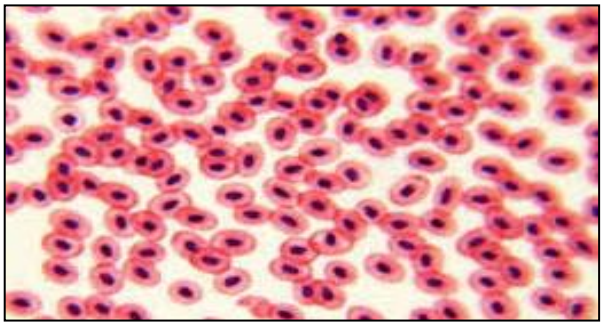


12- FROG RED BLOOD CELL

Has oval shape, nucleated & containing a respiratory pigment called haemoglobin.







Dr. Muhannad

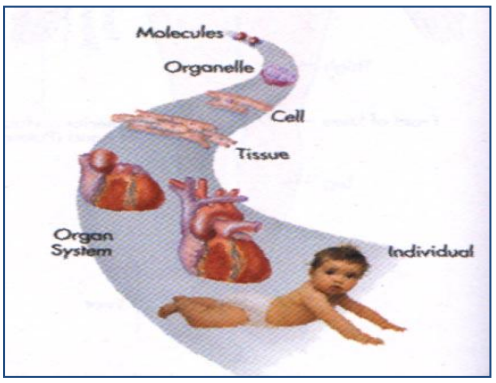
Practical Human Biology
1st stage / College of Pharmacy

Lab # (5)……

Cells to Tissues

As human body develops from single to multicellular, cells specialize.

Body is interdependent system. Cells specialize into types of tissues, then interspersed into organs.



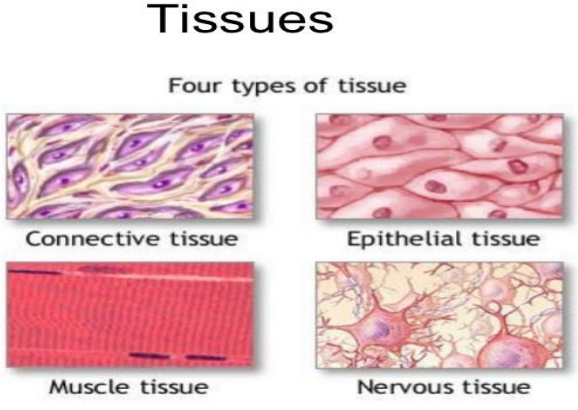
How it all is connected?

- Cells = basic unit of life
- Cells come together to form TISSUES
- TISSUES come together to form ORGANS
- ORGANS come together to form SYSTEMS
- SYSTEMS come together to form individual.

So, what is TISSUE?

- "Layers or groups of **SIMILAR** cells with a **COMMON** function."
- Tissues are distinguished from each other because of differences in size, organization, and function

Tissues are groups of cells of **similar** structure and origin that function together. There are four kinds of tissues: **epithelial, connective,**



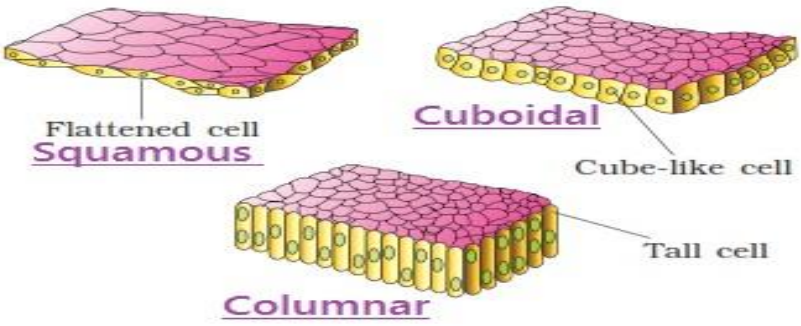
muscular, and **nervous**. In this lab section, you will study epithelial tissue and connective as they occur in human being.

4 TYPES OF TISSUES:

- **Epithelial Tissues**:
- Covers the body's surface and organs.
- **Connective Tissues**:
- Tendons, ligaments, cartilage, blood, fat, bone
- **Muscle Tissues**:
- Skeletal Muscles, heart, smooth muscle
- **Nervous Tissues**:
- Brain, spinal cord, nerves

EPITHELIAL TISSUES: So if Epithelial tissues are on the body surface and surround the organs, then what do you think this type of tissue's function is?

- Protection
- Secretion
- Absorption
- Filtration



Where are they located?

- Surface of the body
- Cover the internal organs
- Compose the glands

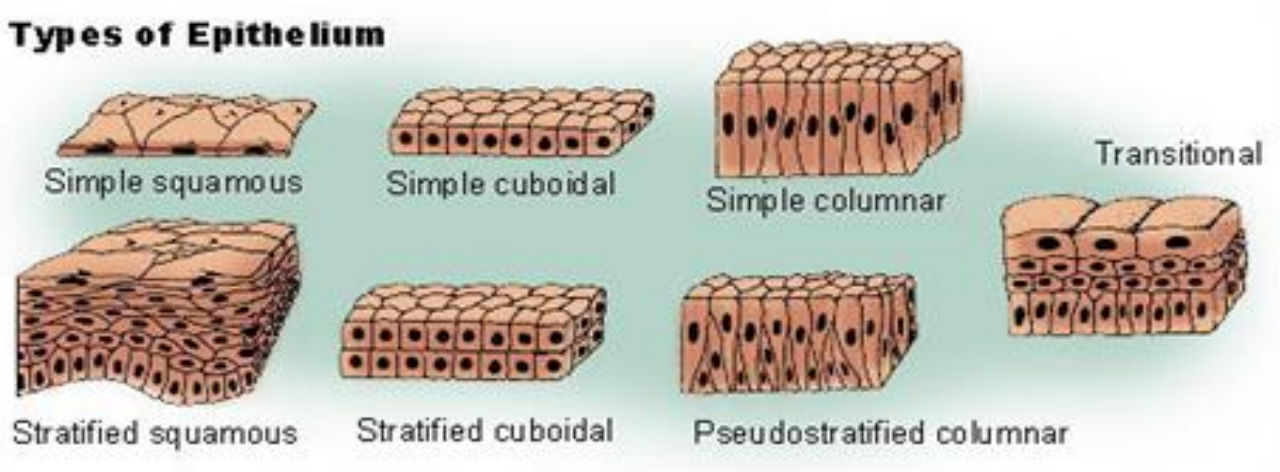
Distinguishing Characteristics?

- No blood vessels. Why do you think?
- Cells divide rapidly. Why is this good?
- Cells are tightly packed. How does this help us?

Epithelial layers contain **no blood vessels**, so they must receive nourishment via diffusion of substances from the underlying **connective tissue**, through the basement membrane. Cell junctions are well employed in **epithelial tissues**.

Since **epithelial cells** are **avascular**, they have the ability of reproducing themselves without causing harm to our bodies. **Avascular** simply means it doesn't have a blood flow. This is an advantage, because we can constantly shed and reproduce new **epithelial** tissue without it hurting

Epithelial Tissue



our bodies.

Epithelial Tissue: Tightly packed cells. Cell Junctions – form continuous sheets held together by **cell junctions**. Tight junctions – Nothing passes through. Surfaces – apical, lateral and basal.

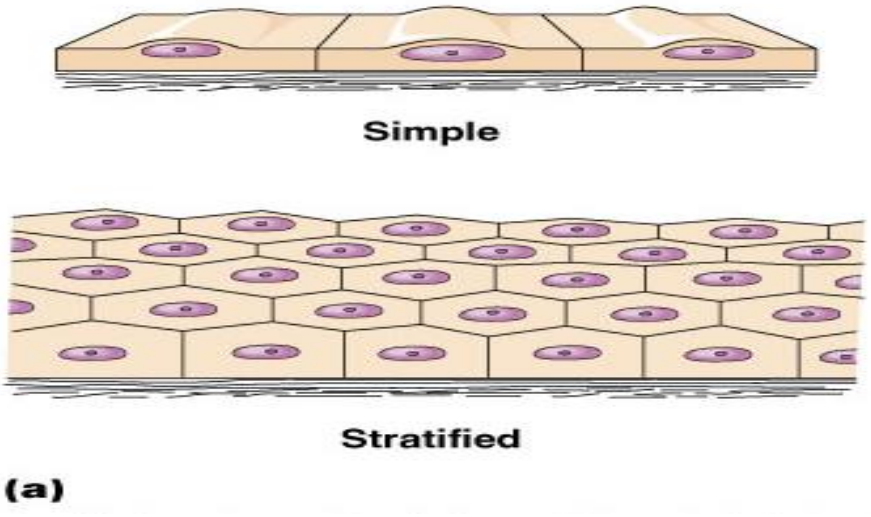
- **Found in different areas**
  - Body coverings
  - Body linings
  - Glandular tissue
- **Functions**
  - Protection – Skin, lining of internal organs
  - Absorption – intestines
  - Filtration – Kidney
  - Secretion – Hormones, mucus, sweat, etc.

- **Two types:**
  1. **Covering and lining epithelium**
     - Outer covering of skin, and internal organs
     - Body cavities
     - Blood vessels and ducts
     - Interior of respiratory, digestive, urinary and reproductive organs
     - Parts of sense organs
  2. **Glandular epithelium**
     - Secreting portion of glands

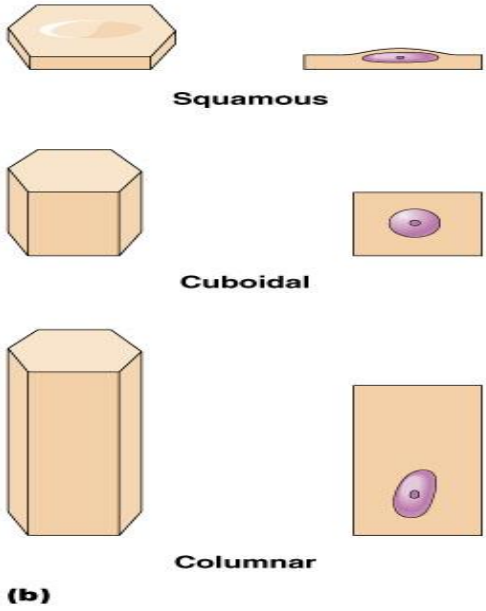
- **Epithelium Characteristics**
- Cells fit closely together
- Tissue layer always has one exposed surface (Apical surface)
- The lower surface (basal surface) is bound by a **basement membrane** – Fibers
- The side surface (lateral surface) is bound to other epithelial cells.
- Avascular (have no blood supply)
- Nerve supply
- Regenerate easily if well nourished

Classification of Epithelium

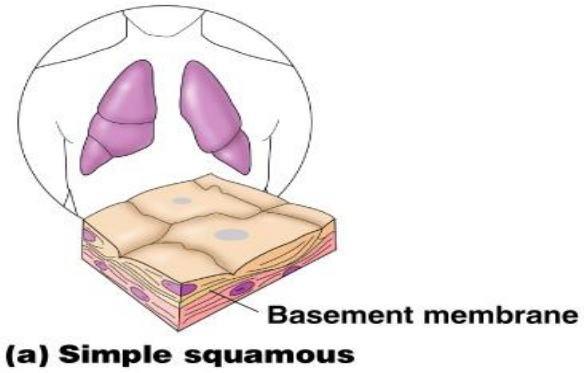
- **Number of cell layers**
  - **Simple** – one layer: diffusion (lungs), osmosis, filtration (kidneys), secretion (glands), absorption (intestines)
  - **Stratified** – more than one layer: protection, secretion



- **Shape of cells**
  - Squamous – flattened
  - Cuboidal – cube-shaped
  - Columnar – column-like
- **Cilia**
  1. Nonciliated – absorptive cells (microvilli) and goblet cells (secrete mucus)
  2. Ciliated – to move substances (Ex. Ovaries)



Simple Epithelium



- **Simple squamous**

Single layer of flat cells

Usually forms membranes

Lines body cavities

Lines lungs and capillaries

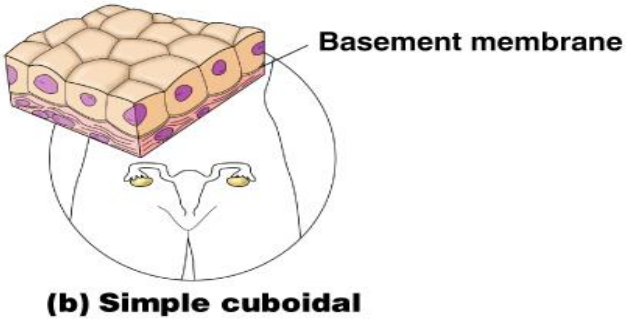
- **Simple cuboidal**

Single layer of cube-like cells

Common in glands and their ducts

Forms walls
of kidney tubules

Covers the ovaries

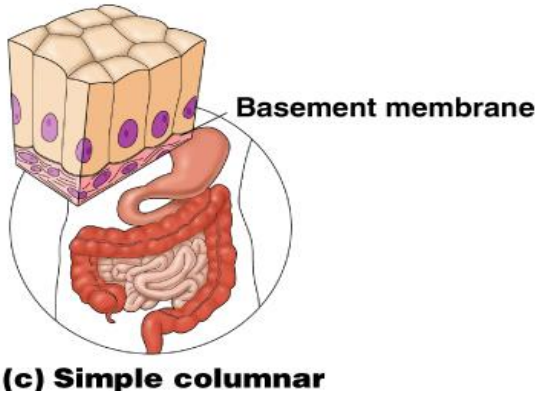


- **Simple columnar**

Single layer of tall cells

Often includes goblet cells -- produce mucus

Lines digestive tract – absorption of nutrients.



- **Stratified squamous**

Cells at the free edge are flattened

Cells below can have other shapes

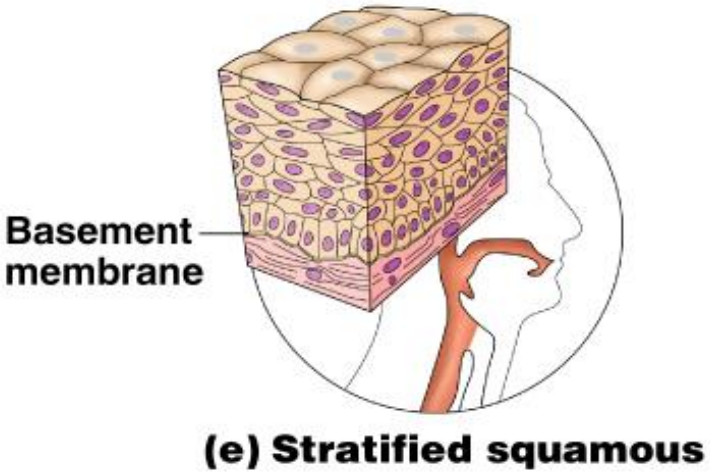
Found as a protective covering where friction is common

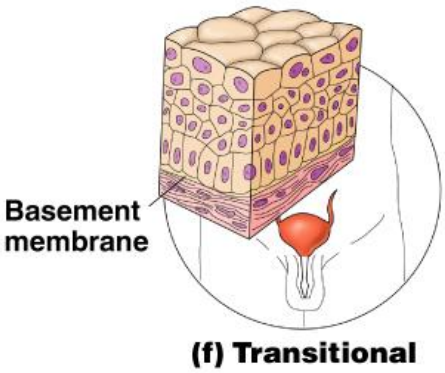
<u>Locations</u>

Skin

Mouth

Esophagus





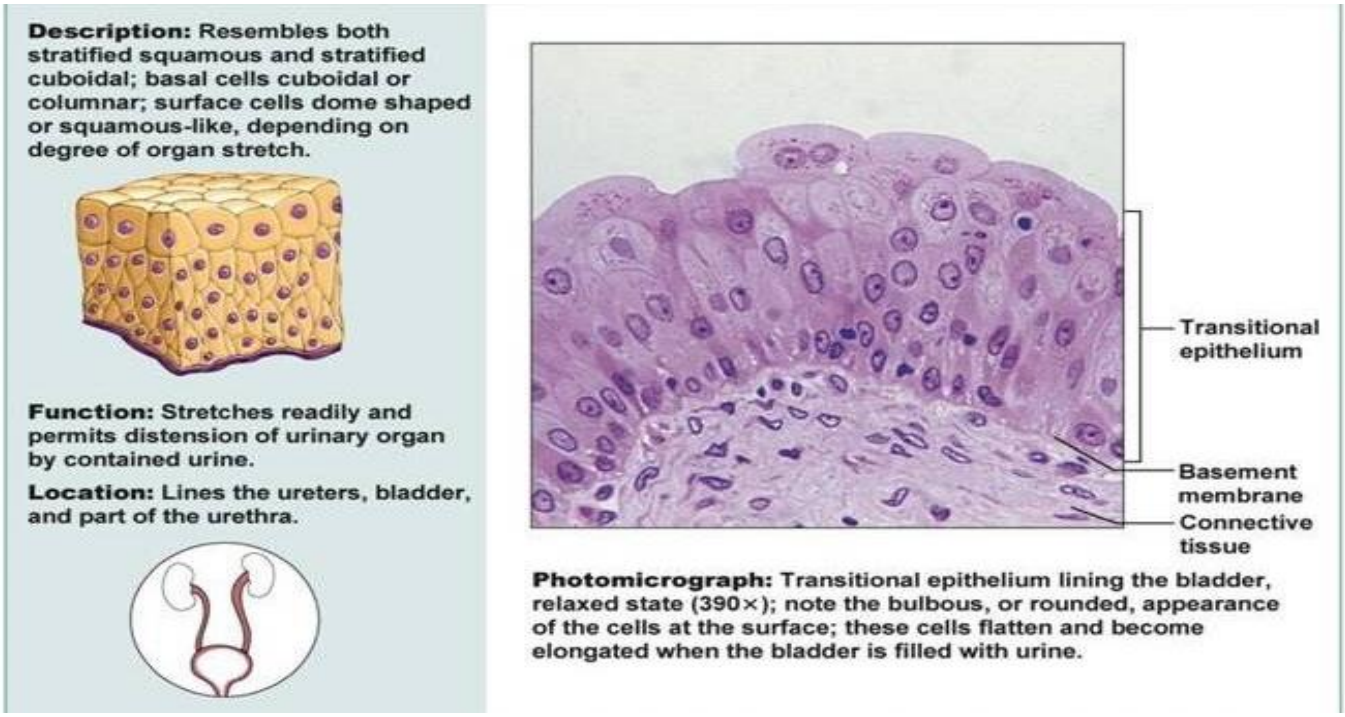
- **Transitional epithelium**

Elastic

Shape of cells depends upon the amount of stretching

As the cells stretch, they become flattened

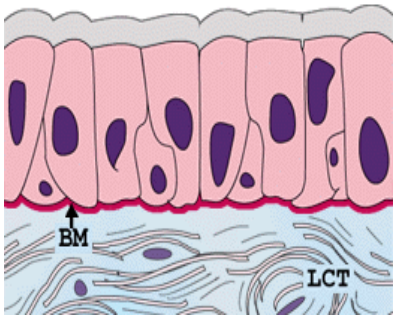
Lines organs of the urinary system

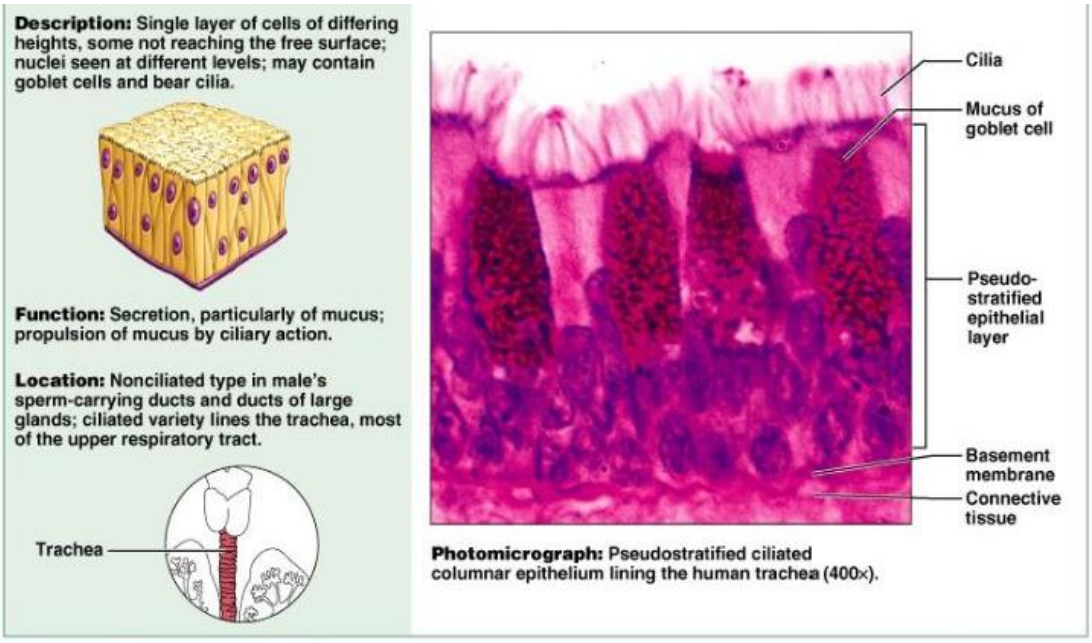


Pseudostratified Columnar Epithelium

- Not a true stratified tissue.
- All cells are attached to the basement membrane but not all reach the apical surface.

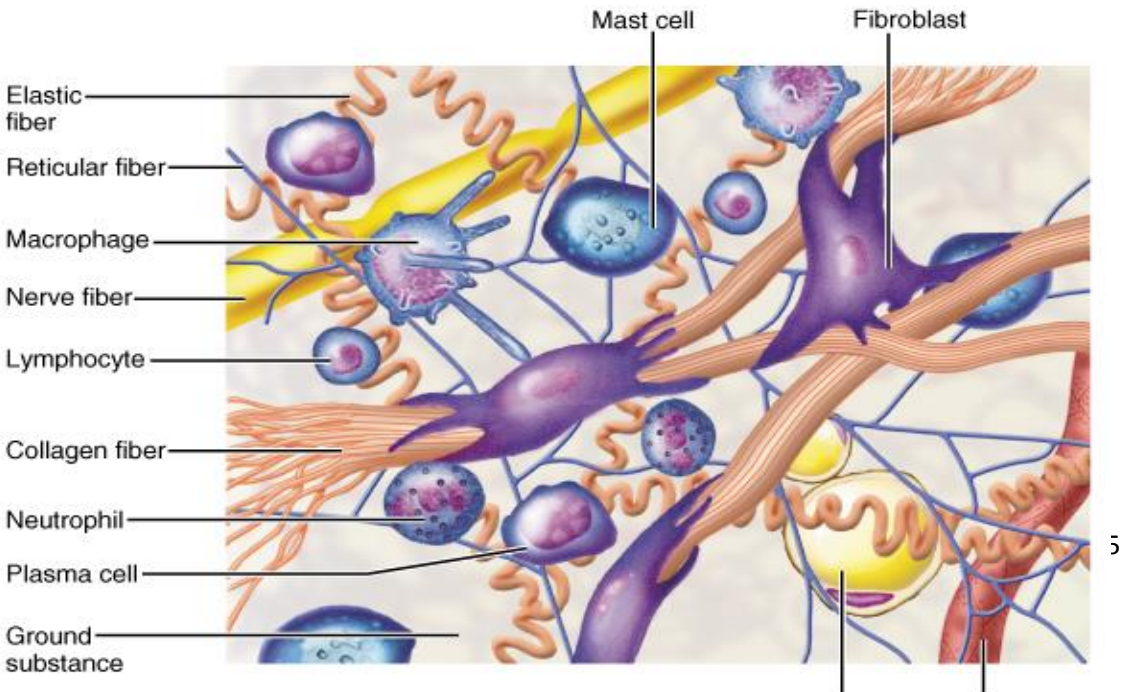
- When viewed from the side, it appears that they have several layers





CONNECTIVE TISSUE

Connective tissue: Is diverse in structure and function. Even so, all types have three components: **specialized cells**, **ground substance**, and **protein fibers**. These components are shown in Figure below, a diagrammatic representation of loose fibrous connective tissue. The ground substance is a noncellular material that separates the cells. It varies in consistency from solid (bone) to semifluid (cartilage) to fluid (blood).

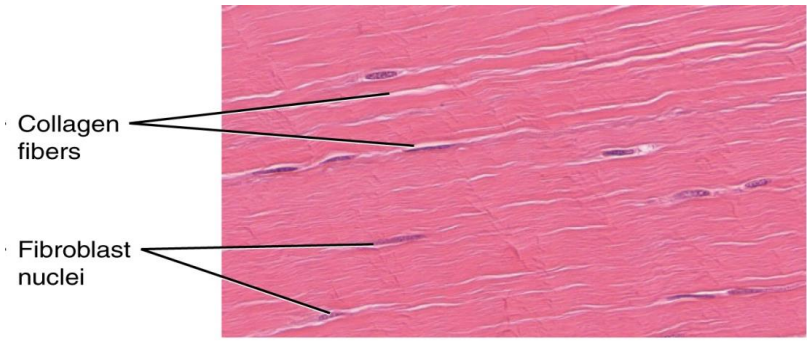


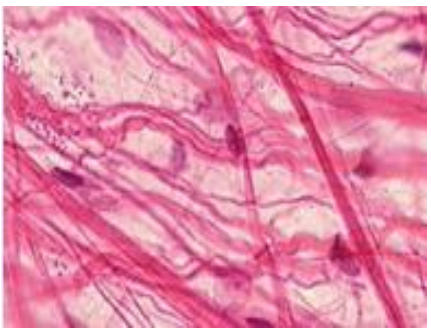
Connective tissue functions:

1. Bind structures
2. Provide support and protection
3. Serve as a framework
4. Fill spaces
5. Store fat
6. Produce blood cells
7. Protect against infection
8. Help repair tissue damage.

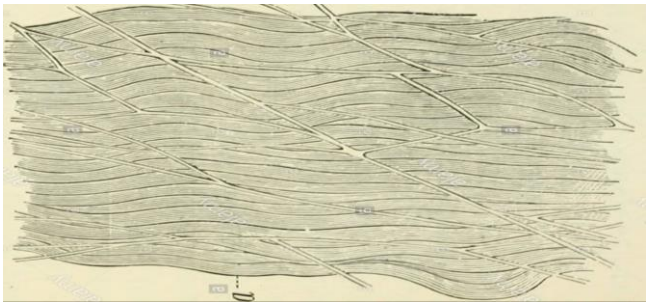
Matrix Fibers in connective tissue:

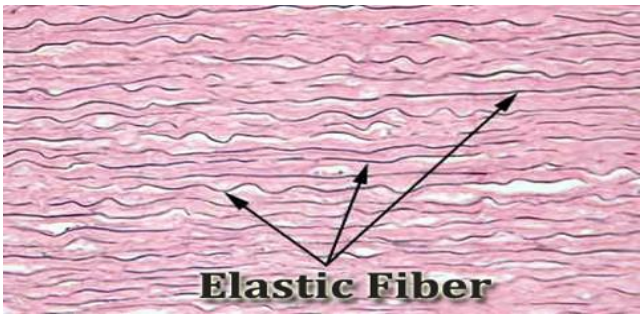
1-Collagen Fibers: Large fibers made of the protein called **collagen** and are typically the most abundant fibers arranged as bundles as in (**tendons**). Promote tissue **flexibility,** not branching and non-elastic nature.



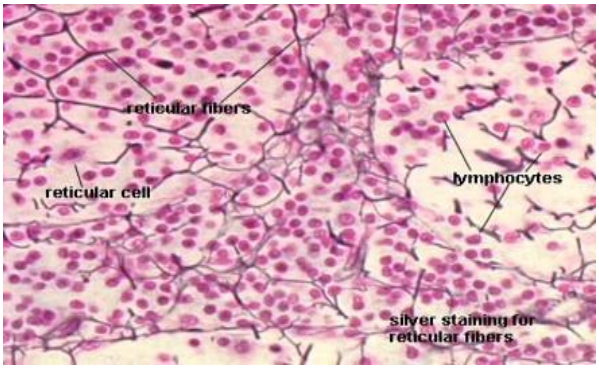


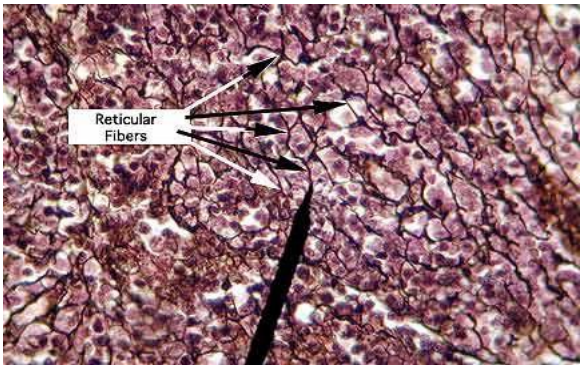
2-Elastic Fibers: Intermediate fibers made of the protein known with **elastin**. Branching fibers that allow for stretch and recoil in response to tension, appears yellow fibers, fine and not arranged as bundles.





3-Reticular Fibers: Small delicate, branched fibers that have same chemical composition of collagen. They are highly branched and fine therefore forms structural framework for organs such as spleen, Liver, lymph nodes and bone marrow.





Types of connective tissue

1. True Connective Tissue:

a. Loose Connective Tissue

b. Dense Connective Tissue

2. Supportive Connective Tissue:

a. Cartilage

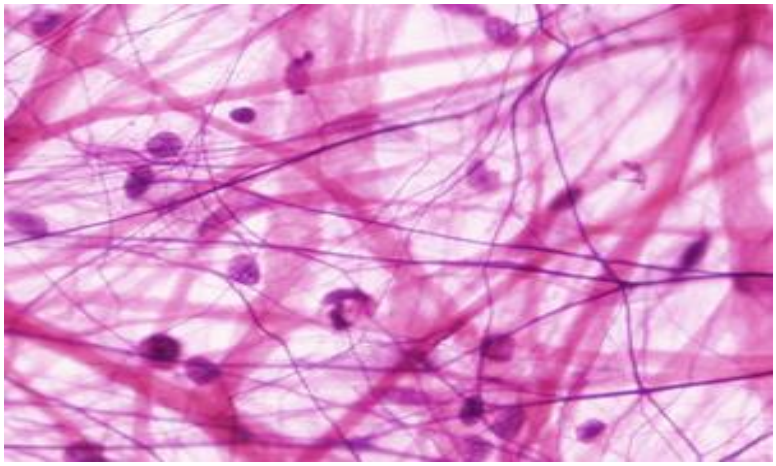
b. Bone

5. Liquid Connective Tissue:

a. Blood

A- Loose (areolar) Connective tissue:

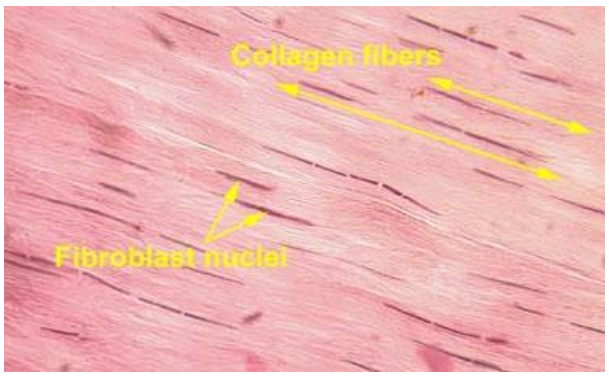
This tissue connects the skin with underlying structures and fills the spaces between organs. It is kind of generalized connective tissue.

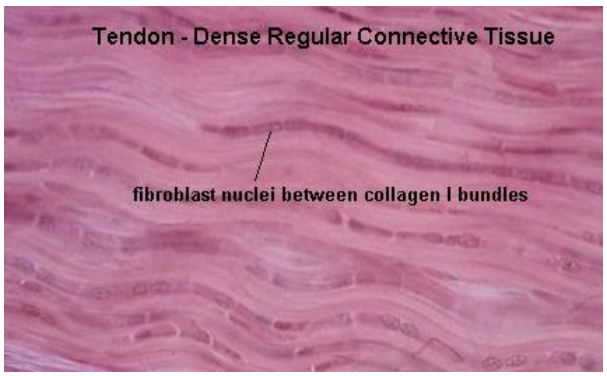


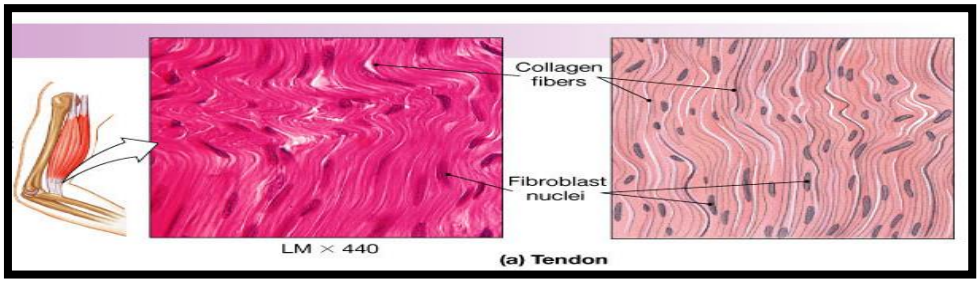
B- Dense connective tissue: include two types:

1- Regular C.T. Include 2 types:

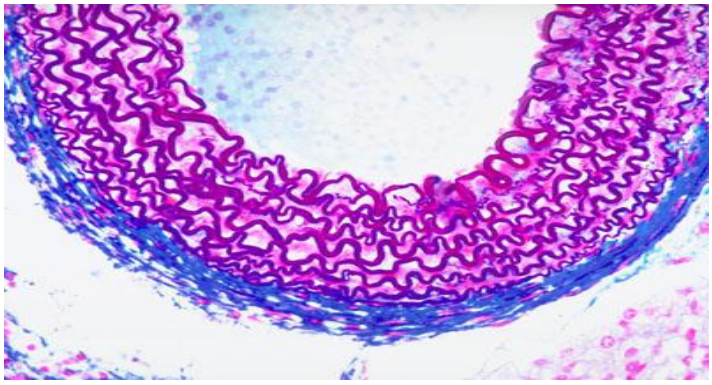
a- Collagen (white) C.T. Composed of closely-packed bundles of collagen fibers arranged in parallel (wavy or straight), with relatively few cells. Fibroblast is the most common type. Such as in **tendons** which connect skeletal muscles to bone, and ligaments that connect bone to bone.

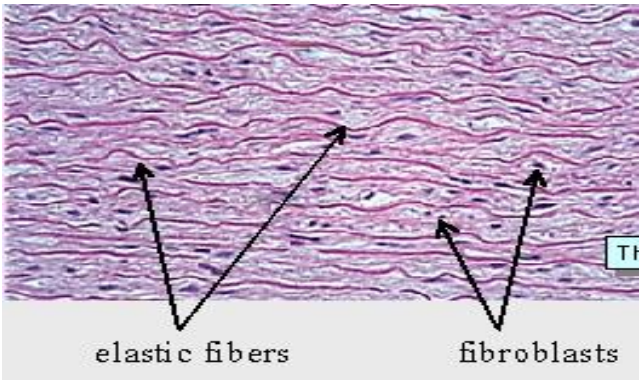


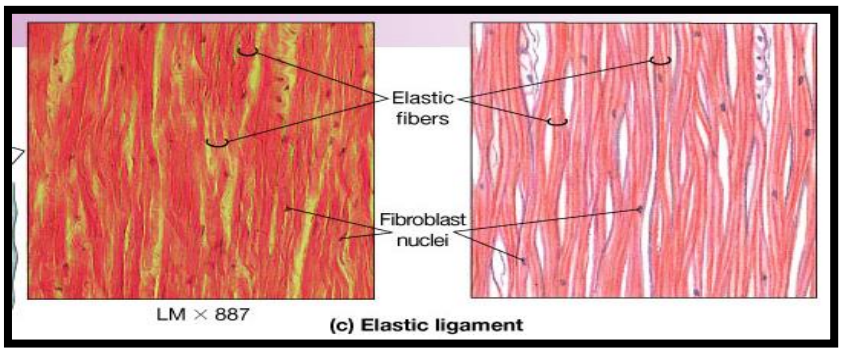




b- Elastic (Yellow) C.T. Composed of elastic fibers arranged singly, branched, but not in bundles with fibroblasts, such as in **large arteries**.

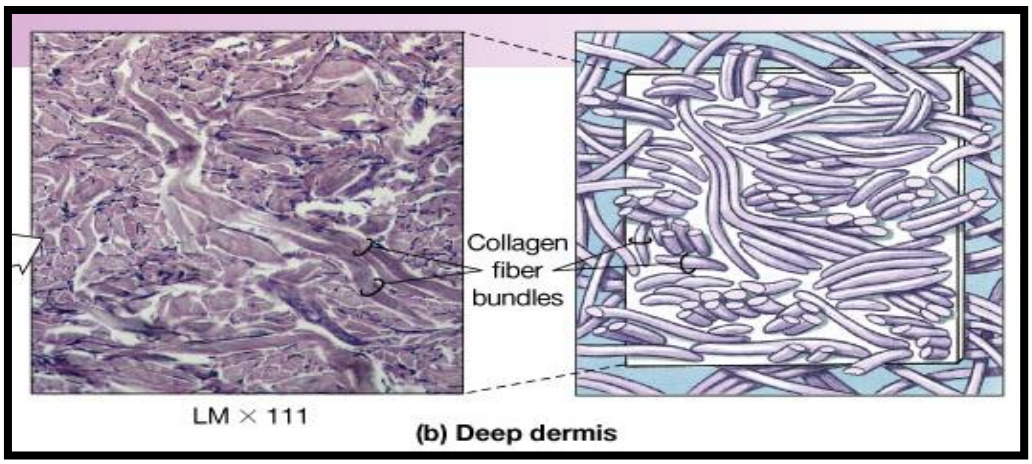






2- Irregular C.T.

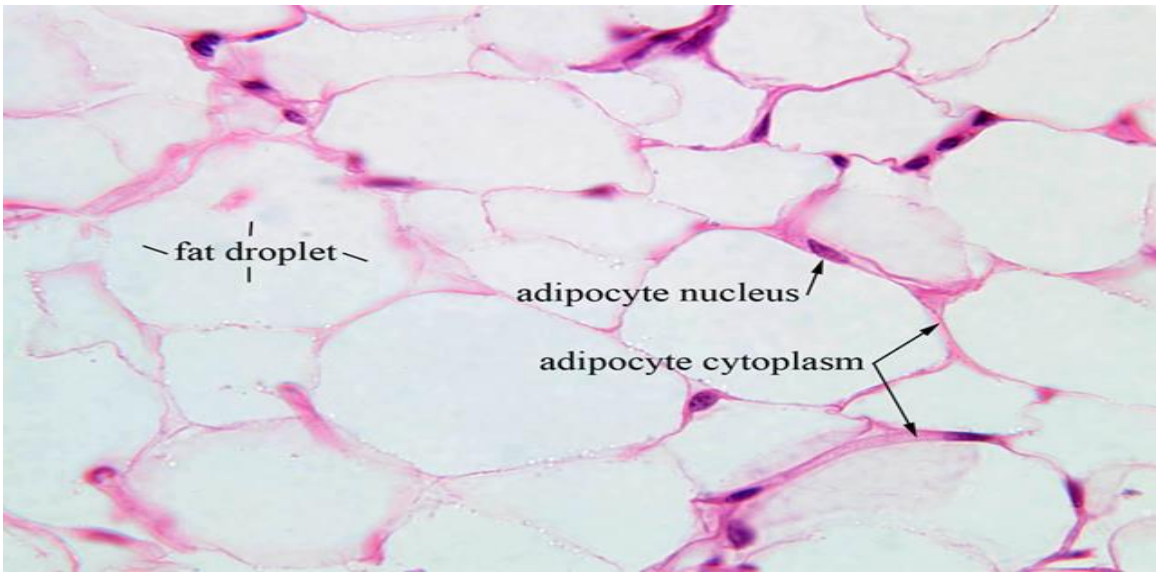
Composed mainly bundles of collagen fibers arranged randomly in addition to fibroblasts, as in dermis of skin and sub-mucosa of digestive tract



a- <u>Adipose connective tissue</u>:

Basically composed of fat cells (adipocytes) and thin layers of loose connective tissue. Its main role is to storage of fats, cushioning, and thermal insulation.

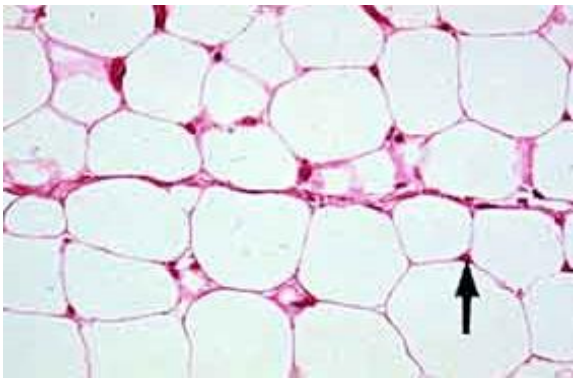
- These cells accumulate fat, they enlarge and their nuclei is pushed to one side
- When they become too abundant they crowd out other cell types and form "adipose tissue"



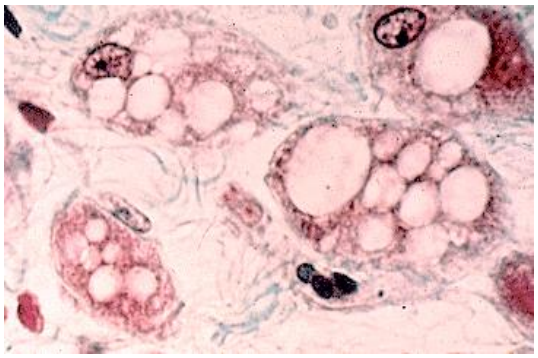
- Found: between muscles, around kidneys, behind eyeballs, surface of the heart, and around joints

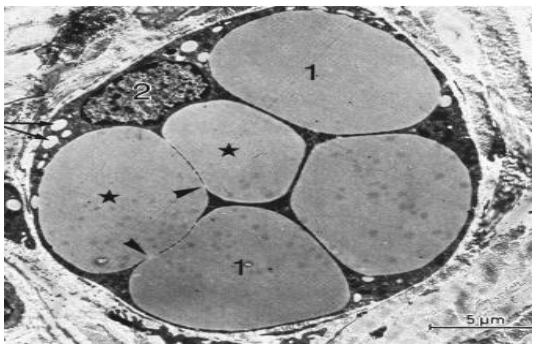
There are two types of this tissue:

- **Unilocular adipose tissue:** contain one large lipid droplet, white color, found in adult.



- **Multilocular adipose tissue:** contain many lipid droplets, brown color, found in embryo.





b- <u>Reticular connective tissue</u>:

This tissue consists of **reticular cells and network of reticular fibers** formed by them, in addition to few other fibers and cells like macrophages, mast cells, plasma cells, fat cells. It provides a soft structural framework for organs as in liver, spleen and red bone marrow.