CHAPTER 2:

DEMONSTRATE KNOWLEDGE IN HUMAN ANATOMY

2.1 Introduction of the Unit of Learning / Unit of Competency

This unit specifies the competencies required to understand human anatomy. It involves analyzing the scope of anatomy, identifying anatomical terminologies, demonstrating the knowledge of cell and cell division, identifying histological and cytological methods and demonstrating knowledge of types of tissues and their location.

2.2 Performance Standard

By the end of this unit of learning/competency, the trainee should be able to analyse the scope of anatomy based on resource materials, identify and define anatomical terminologies as per relative position, demonstrate knowledge of the cell and cell division based on the cell physiology, identify histological and cytological methods and demonstrate knowledge of types of tissues and their location as per the scope.

2.3 Learning Outcomes

2.3.1 List of the Learning Outcomes

i) Identify anatomical terminologies

ii) Demonstrate the knowledge of cell and cell division

iii) Identify histological and cytological methods

iv) Demonstrate knowledge of types of tissues and their location

2.3.2 Learning Outcome 1: Identify Anatomical Terminologies

2.3.2.1 Learning Activities

Learning activity	Special instructions
i) Demonstrate understanding of anatomical and physiological terminologies	Define common anatomical terminologies and terms of relative position
ii) Apply relevant anatomical and physiological terminology when undertaking daily tasks	Illustrate relative position of anatomical terminologies in the human body

2.3.2.2 Information Sheet

Meaning of Human Anatomy

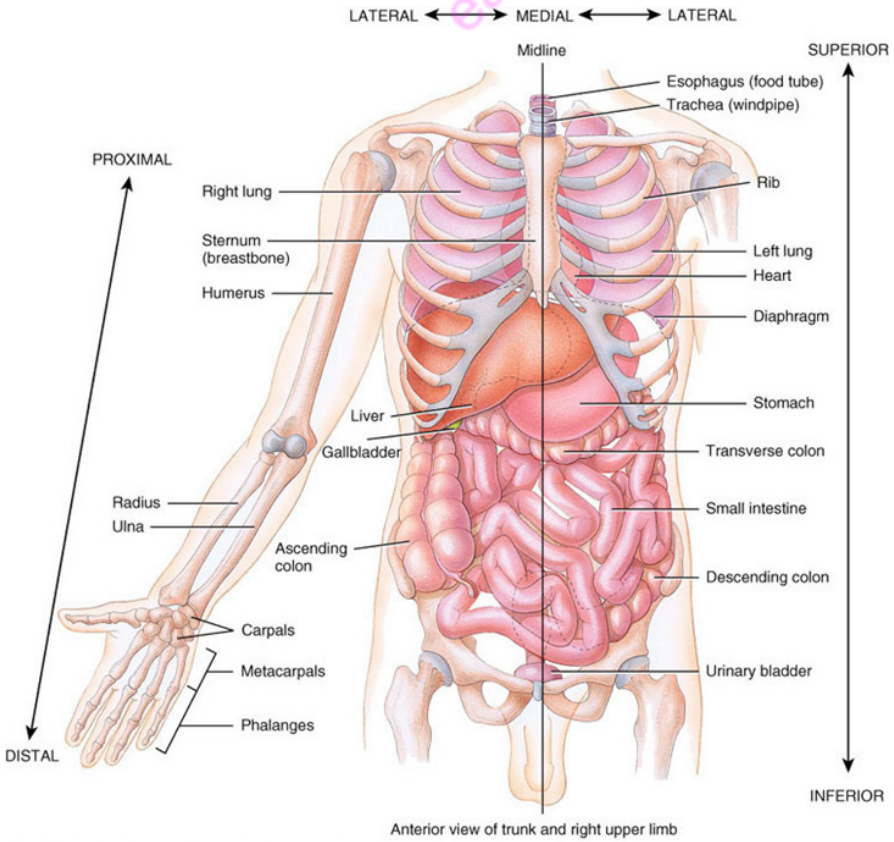
- Anatomy – Scientific study of human body structure
- Physiology – Study of human body functions
- "Structure dictates function."

Relevant Anatomical and Physiological Terminology

1. Anatomical Position – standing erect, facing forward, upper limbs at the sides, palms facing forward and thumbs out.

Orientation and Directional Terms

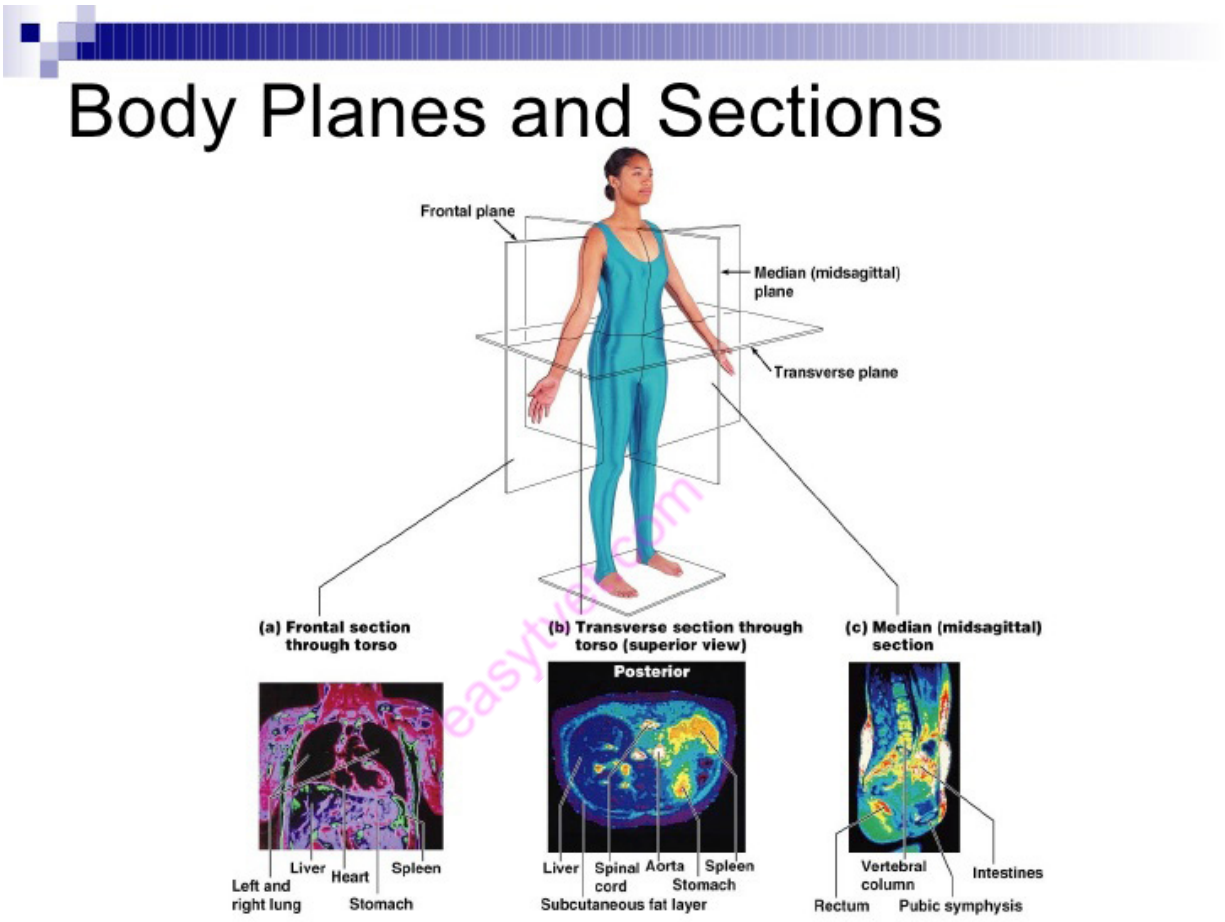
1. Terms of Relative Position (based on anatomical position):
    - Superior versus Inferior
    - Anterior versus Posterior
    - Medial versus Lateral
    - Proximal versus Distal (only in the extremities)
    - Superficial versus Deep
    - Internal versus External

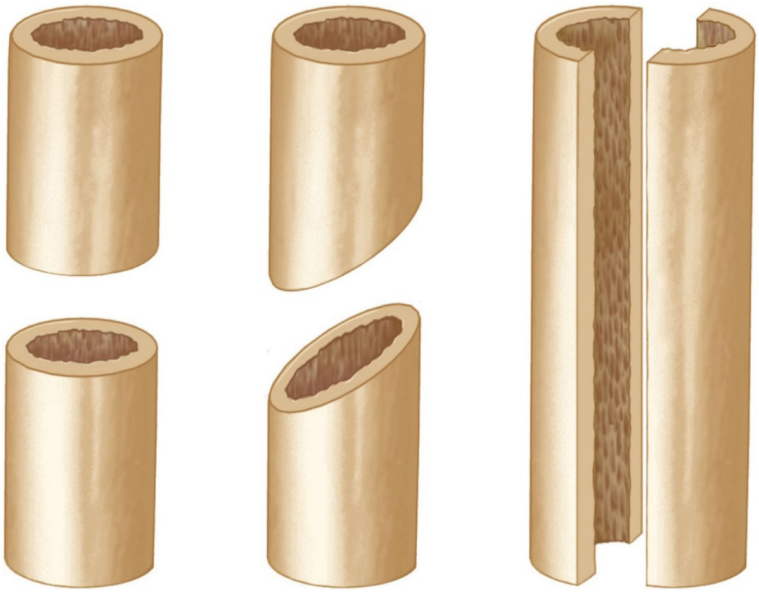


Anterior view of trunk and right upper limb

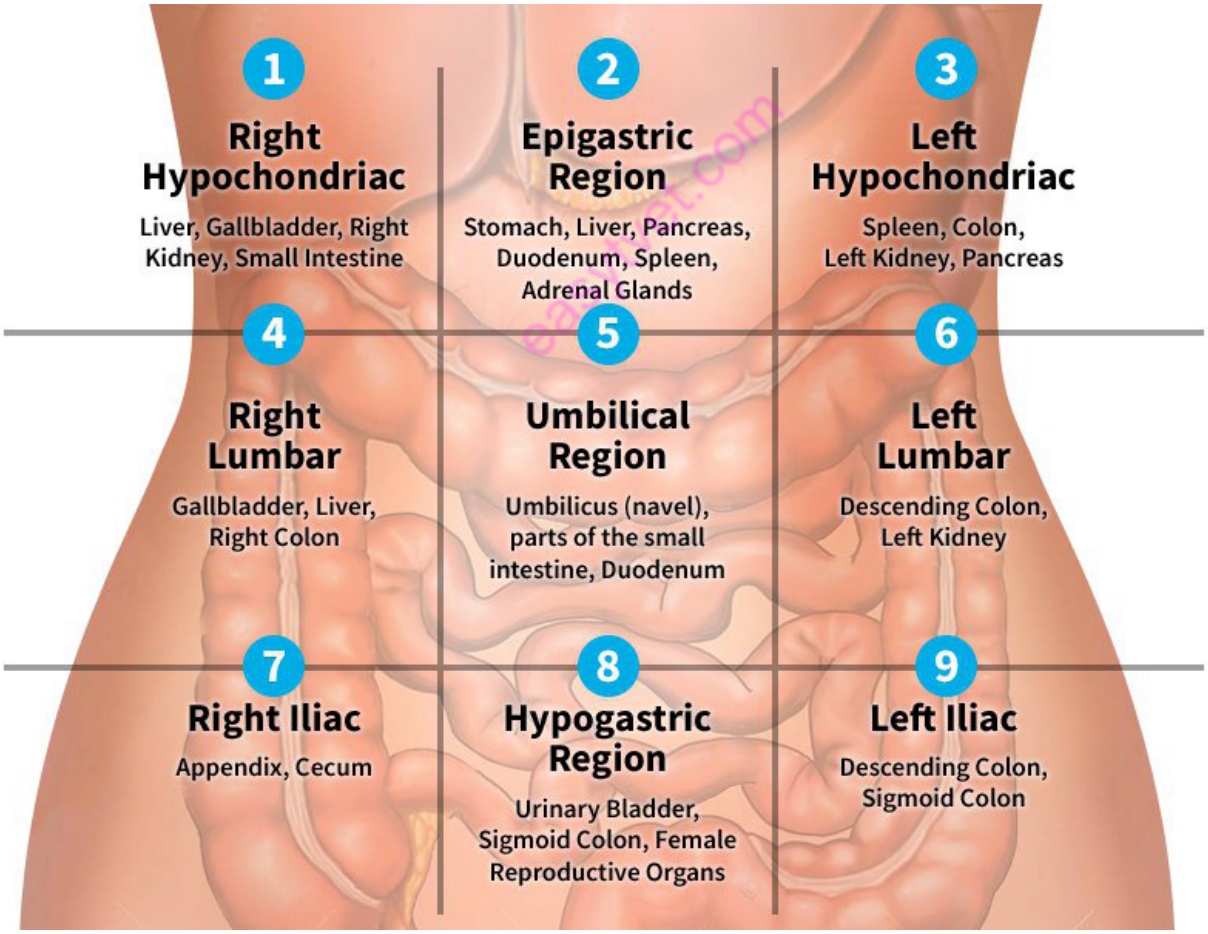
Body Sections or Planes (3)

- Sagittal or Median – divides body into left and right portions
  - Mid-sagittal – divides body into equal left and right portions
- Transverse or Horizontal – divides body into superior and inferior portions
- Coronal or Frontal – divides body into anterior and posterior portions





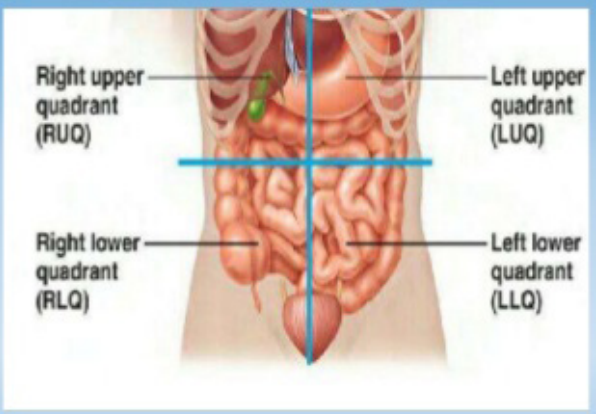
Abdominal Subdivisions (2)

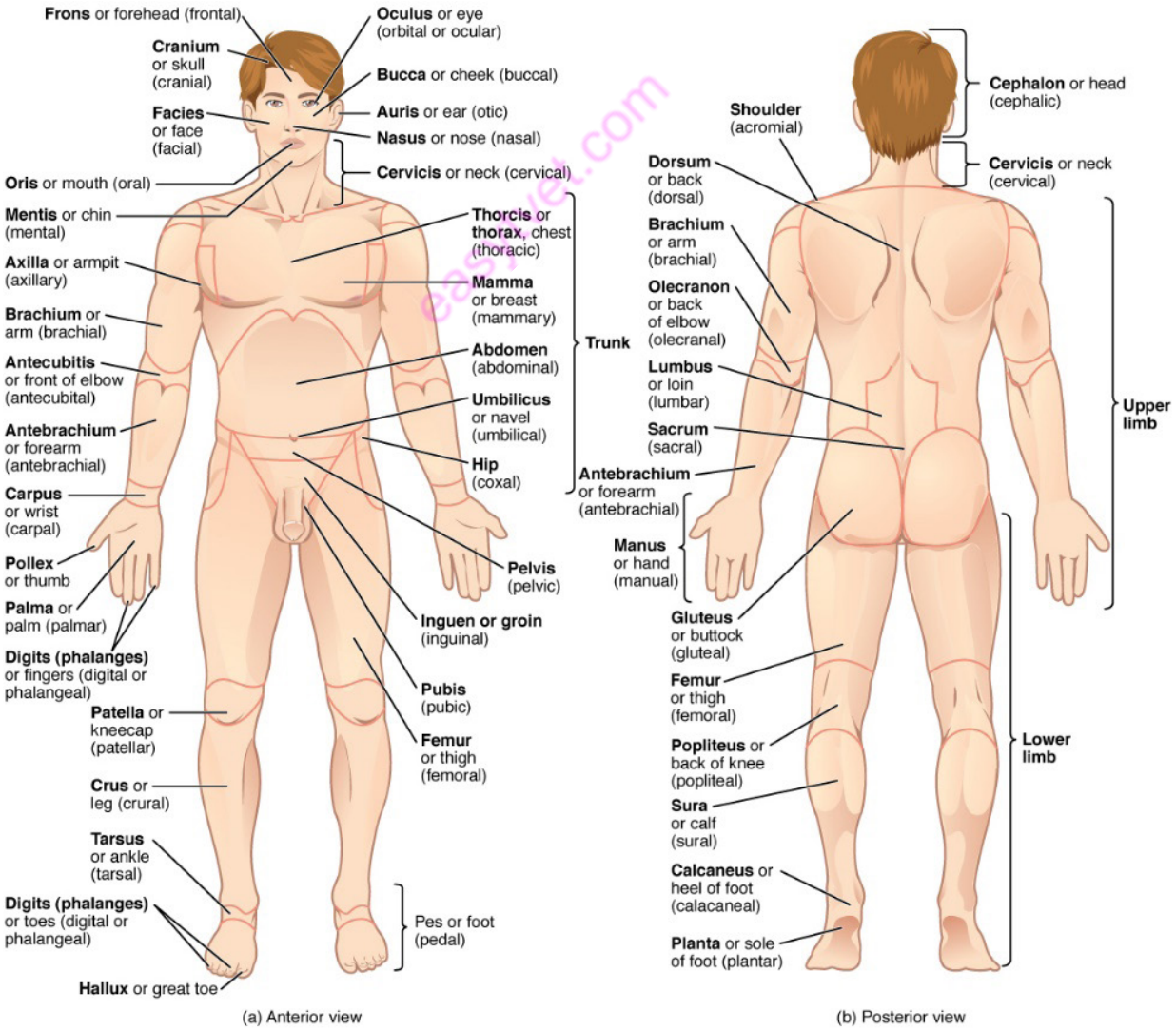


Quadrants of Abdomen

- Dividing the abdomen into various sections will help doctors determine what the cause of the illness is. The abdomen can also be divided into four quadrants:

Abdominopelvic Quadrants





Application of Relevant Anatomical and Physiological Terminology

Anatomical and physiological terminologies are applied in surgery and other diagnostic procedures in the hospital set up. They have enabled in the diagnosis of many medical and surgical conditions that affect human beings. These terminologies are the mainstay means of communication in the health care sector.

2.3.2.3 Self-Assessment

1. Define anatomy and physiology
2. Use a well labeled diagram to explain the nine regions of the abdomen.
3. The following organs are in the epigastric region of human abdomen which one is not
   A. Stomach
   B. Small intestine
   C. Spleen
   D. Liver
4. State and explain five terms of relative position as applied in anatomy
5. Define anatomical position
6. State whether the following statements is TRUE or FALSE
   A. Sagittal or Median body plane divides body into equal left and right portions
   B. Mid-sagittal body plane divides body into left and right portions
   C. Transverse or Horizontal body plane divides body into superior and inferior portions
   D. Coronal or Frontal body plane divides body into anterior and posterior portions

2.3.2.4 Tools, Equipment, Supplies and Materials

Dummy human body, dummy internal organs, microscope, slides, cadaver, anatomy text books, white board

2.3.2.5 References

Waugh A. & Grant A. (2016) Ross and Wilson Anatomy & Physiology 12th Edition; Churchill Livingstone

Bartholomew E.F. & Martini F.H. (2019) Essentials of Anatomy and Physiology; 8th Edition

Elaine N. & Katja H., 2016: Humana Anatomy and Physiology, 5th Edition

2.3.3 Learning Outcome 2: Demonstrate the knowledge of cell and cell division

2.3.3.1 Learning Activities

Learning activity		Special instructions
i)	Identify cell types	
ii)	Identify components of a human cell	Mount a pre-collected human cell on a light microscope and observe the parts Draw your observation and label the components
iii)	Outline processes of cell division	Only outline cell division in eukaryotic cells
iv)	Describe the composition of cytoplasm	
v)	Identify type of cell division	

2.3.3.2 Information Sheet

Definition of terms

Cells are the smallest living structure

Cell = functional unit of the body

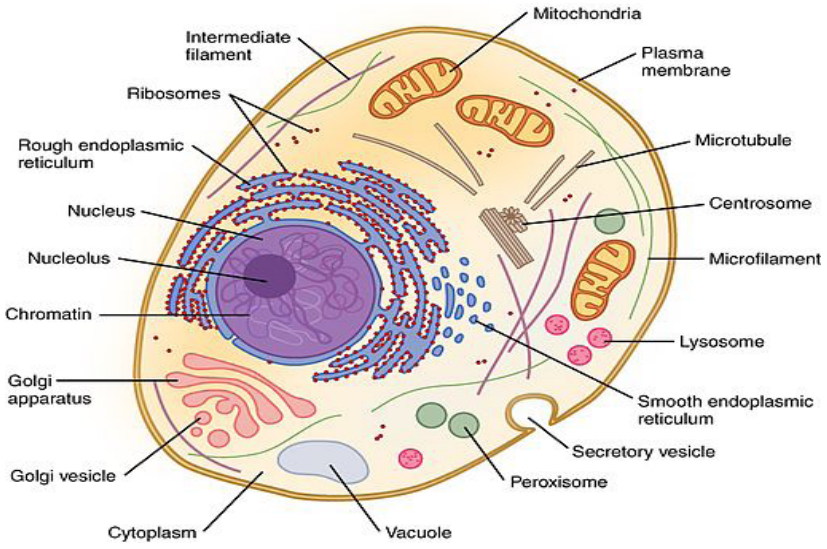
Cytology = The Study of Cells

Ultrastructural Cytology = Cytology at the Electron Microscopic level

Histology = the study of tissues

The basic organizational structure of the human body is the cell. There are 50-100 trillion cells in the human body. Differentiation is when cells specialize. As a result of differentiation, cells vary in size and shape due to their unique function.

The cell structure



- Also called a 'typical' cell
- Major parts include:
    - Nucleus: contains DNA
    - Cytoplasm: cellular contents between plasma membrane & nucleus
    - Cell membrane: selective barrier

Cell division

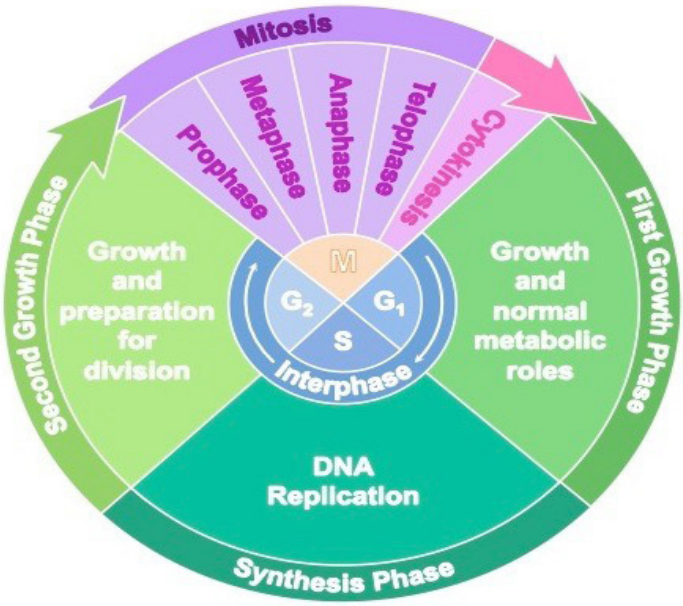
- Divides by mitosis-produces 2 new genetically identical daughter cells.
- Gametes (sex cells); division takes place by meiosis-produces four haploid cells.
- Interphase: period between cell division
- At the end of interphase the chromatin replicates and becomes tightly coiled forming a double chromosome i.e. chromatids for cell division.
- Chromatids are joined at the center by a centromere.
- Chromatid is one copy of a newly copied chromosome which is still joined to the original chromosome by a single centromere.

The Cell Cycle

Series of changes a cell undergoes from the time it forms until the time it divide.

Stages:

1. Interphase
2. Mitosis
3. Cytokinesis



Interphase

- Very active period

- Cell grows
- Cell maintains routine functions
- Cell replicates genetic material to prepare for nuclear division
- Cell synthesizes new organelles to prepare for cytoplasmic division

Phases:

1. G phases – cell grows and synthesizes structures other than DNA
2. S phase – cell replicates DNA

Mitosis

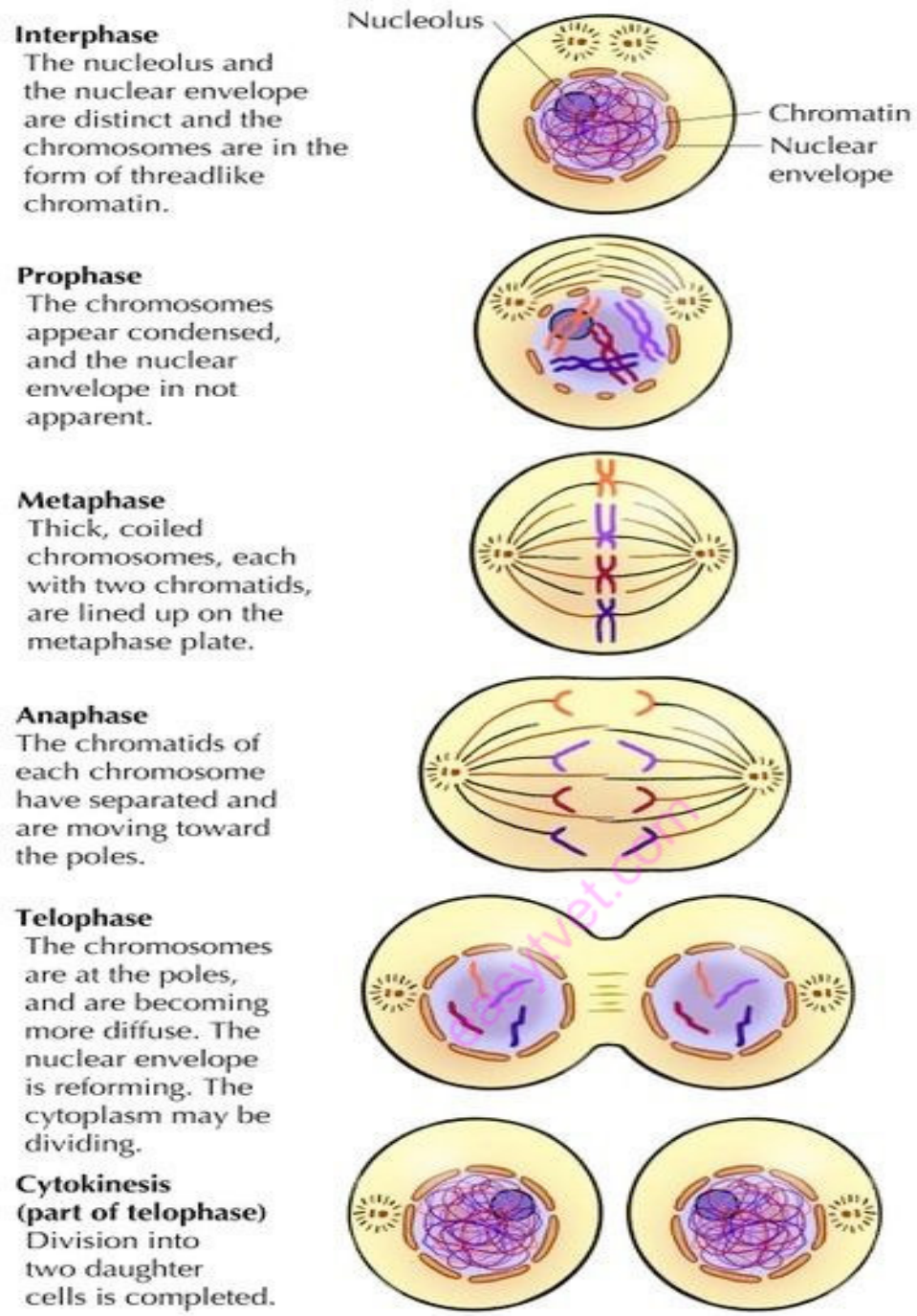
- Produces two identical daughter cells from an original somatic cell.
- Nucleus divides – karyokinesis
- Cytoplasm divides – cytokinesis
- Phases of Nuclear Division:
    1. Prophase – chromosomes form; nuclear envelope disappears
    2. Metaphase – chromosomes align midway between centrioles
    3. Anaphase – chromosomes separate and move to the opposite poles of the cell.
    4. Telophase – chromatin forms; nuclear envelope forms

Cytoplasmic Division

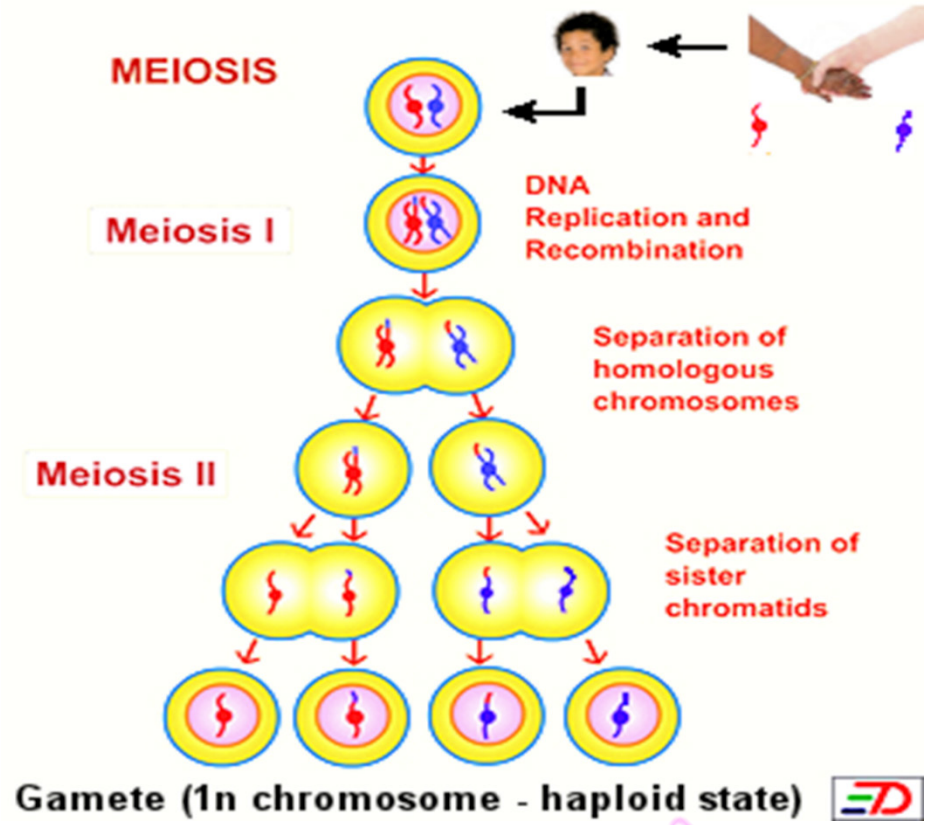
- Also known as cytokinesis
- Begins during anaphase
- Continues through telophase
- Contractile ring pinches cytoplasm in half.

Meiosis

- Is a specialized type of cell division that reduces the chromosome number by half, creating four haploid cells, each genetically distinct from the parent cell that gave rise to them.
- This type of cell division is only used to form gametes cells i.e. sperm and ovum.
- In meiosis, the chromosomes duplicate (during interphase) and homologous chromosomes exchange genetic information (chromosomal crossover) during the first division, called meiosis I. The daughter cells divide again in meiosis II, splitting up sister chromatids to form haploid gametes.
- Two gametes fuse during fertilization, creating a diploid cell with a complete set of paired chromosomes.



Differences Between Mitosis and Meiosis	
Mitosis	**Meiosis**
This type of division takes place in somatic cells	This type of division takes place in gamete cells
Two daughter cells are formed	Four daughter cells are formed
Number of chromosomes remains diploid in daughter cells	Number of chromosomes becomes haploid in daughter cells
Mitosis is necessary for growth and repair	Meiosis is necessary for sexual reproduction
Crossing over does not take place	Crossing over takes place



Control of Cell Division

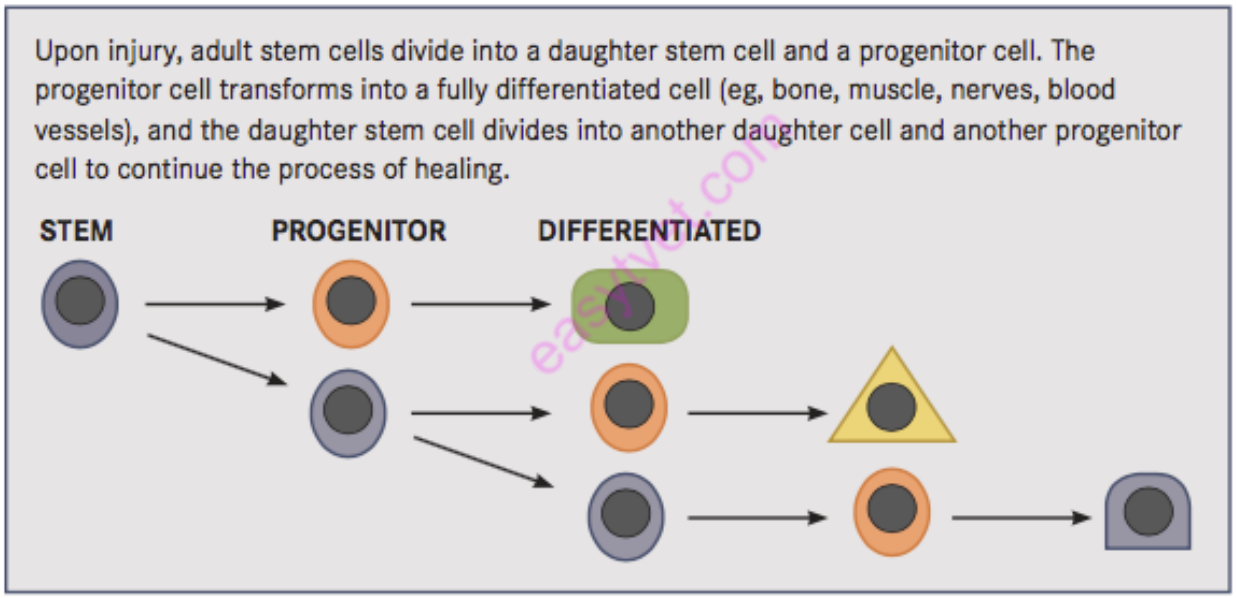
- Cell division capacities vary greatly among cell types
    - Skin and blood cells divide often and continually
    - Neuron cells divide a specific number of times then cease
  - Cells divide to provide a more favorable surface area to volume relationship
  - Growth factors and hormones stimulate cell division
      - Hormones stimulate mitosis of smooth muscle cells in uterus
      - Epidermal growth factor stimulates growth of new skin
  - Tumors are the consequence of a loss of cell cycle control.

Tumors

- Two types of tumors:
    1. Benign – usually remains localized
    2. Malignant – invasive and can metastasize; cancerous
- Two major types of genes cause cancer:
    1. Oncogenes – activate other genes that increase cell division
    2. Tumor suppressor genes – normally regulate mitosis; if inactivated they are unable to regulate mitosis
        - Cells are now known as “immortal”

Stem and Progenitor Cells

- Stem cell: an undifferentiated cell of a multicellular organism which is capable of giving rise to indefinitely more cells of the same type, and from which certain other kinds of cell arise by differentiation.
    - Can divide to form two new stem cells
        - Self-renewal
    - Can divide to form a stem cell and a progenitor cell
    - Totipotent – can give rise to every cell type
    - Pluripotent – can give rise to a restricted number of cell types
- Progenitor cell:
    - Committed cell that can divide into restricted specific cells.
    - Can divide to become any of a restricted number of cells
    - Pluripotent

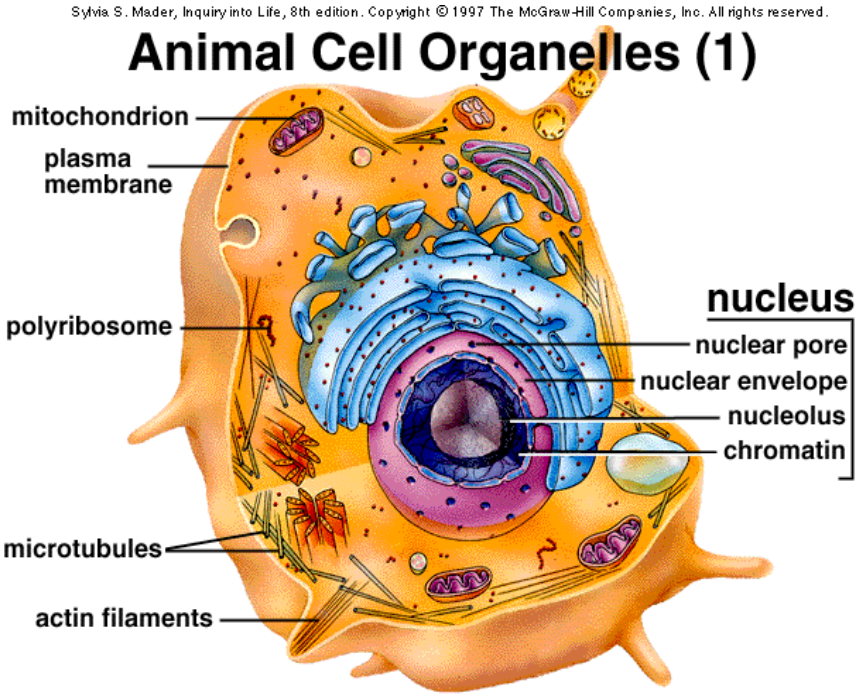


Cell Death

Apoptosis:

- Programmed cell death
- Acts as a protective mechanism
- Is a continuous process

Components of a human cell



- The composition of cytoplasm
    - Cytoplasm is the Cellular content between plasma membrane & nucleus Cytoplasm=cytosol + organelles
    - Cytosol = watery fluid
    - Organelles = solids small organs; highly specialized functions

- Roles and functions of cell components

  Organelles

  - Structures INSIDE a cell that have specific functions.

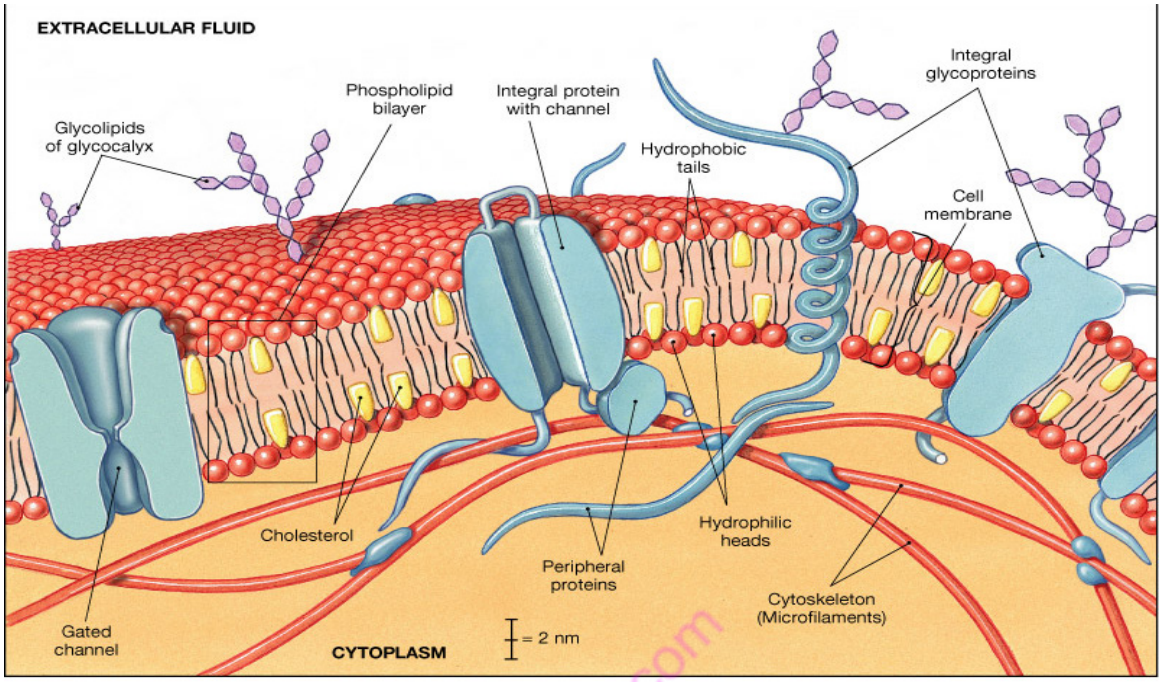
  Membranous

    - Nucleus
    - Golgi apparatus
    - Endoplasmic reticulum
    - Mitochondria
    - Vesicles and lysosomes

  Non-membranous

    - Ribosomes
    - Microtubules (cytoskeleton)
    - Actin/Myosin in muscle cells

1. Cell membrane
   - It consists of a lipid bilayer with embedded proteins.
   - Outer limit of the cell
   - Controls what moves in and out of the cell



2. Endoplasmic Reticulum (ER)
   - Connected, membrane-bound sacs, canals, and vesicles
   - Transport system
   - Rough ER--Studded with ribosomes (protein synthesis)
   - Smooth ER--Lipid synthesis
3. Ribosomes
   - Free floating or connected to ER
   - protein synthesis
4. Golgi apparatus
   - Stack of flattened, membranous sacs
   - Modifies, packages and delivers proteins
5. Vesicles
   - Membranous sacs
   - Store substances

6. Mitochondria
    - Membranous sacs with inner partitions
    - Generate energy
7. Lysosomes
    - Enzyme-containing sacs
    - Digest worn out cell parts or unwanted substances
8. Centrosome
    - Directs organisation of microtubules
    - Two rod-like centrioles (cell division.)
    - Used to produce cilia and flagella
    - Distributes chromosomes during cell division
9. Peroxisomes
    - Enzyme-containing sacs
    - Break down organic molecules
10. Cilia
    - Short hair-like projections
    - Propel substances on cell surface
11. Flagellum
    - Long tail-like projection
    - Provides motility to sperm
12. Microfilaments and microtubules
    - Thin rods and tubules
    - Support cytoplasm
    - Allows for movement of Organelles
13. Cell nucleus
    - It is found in the center of the cell. It contains the genetic material that is DNA and RNA
    - It controls all the activities of the cell.

2.3.3.3 Self-Assessment

1. Draw a well labeled diagram of a typical cell showing the various cell organelles
2. State five cell organelles and their functions
3. Differentiate between mitosis and meiosis
4. State whether the following statement in cell division is TRUE or FALSE
    a) Skin and blood cells divide often and then cease
    b) Neuron cells divide a specific number of times then cease
5. State whether the following statement on cell division is TRUE or FALSE
    a) In meosis cell division, the cell division takes place in the somatic cells
    b) In mitosis cell division, crossing over takes place
    c) In meosis cell division, cell division four daughter cell are formed
    d) The Mitosis cell division is necessary for growth and repair
6. Outline the steps in mitosis cell division.
7. Differentiate between a stem cell and a progenitor cell.

2.3.3.4 Tools, Equipment, Supplies and Materials

- Microscope

2.3.3.5 References

Waugh A. & Grant A. (2016) Ross and Wilson Anatomy & Physiology 12th Edition; Churchill Livingstone

Bartholomew E.F. & Martini F.H. (2019) Essentials of Anatomy and Physiology; 8th Edition

Elaine N. & Katja H., 2016: Humana Anatomy and Physiology, 5th Edition

2.3.4 Learning Outcome 3: Identify histological and cytological methods

2.3.4.1 Learning Activities

Learning activity	Special instructions
i) Perform direct observation	➢ Demonstrate knowledge of observation method as a means of getting anatomical information
ii) Identify histochemical methods	➢ Illustrate knowledge of histochemical methods
iii) Identify chemical methods	➢ Demonstrate the ability to identify the different chemical methods
iv) Identify physical methods	➢ Demonstrate the knowledge of physical methods based on the available materials
v) Identify staining methods	➢ Illustrate knowledge of different staining methods as per the workplace procedures
vi) Identify immunohistochemical methods	➢ Describe the different immunohistochemical methods based on the available materials
vii) Perform X-ray diffraction	➢ Demonstrate the ability to perform X-ray diffraction as per the workplace procedures

2.3.4.2 Information Sheet

Direct observation process

Direct observation, also known as observational study, is a method of collecting evaluative information in which the evaluator watches the subject in his or her usual environment without altering that environment. This method help the student observe events as they happen in their natural settings without interfering with their natural habitat. This process enable the students to capture first-hand information that act as baseline information for gathering useful anatomical information. A direct observation usually refers to observing a behaviour and knowing exactly what is happening. For example, if I see a pigeon peck at some bread, I have observed it pecking

Histochemical methods

3 Histochemical Techniques

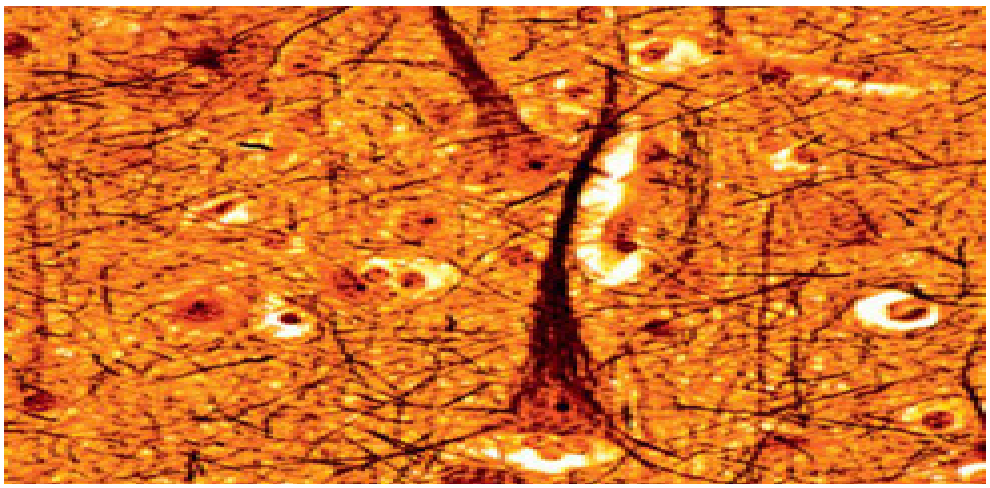
Since most cell structures are transparent, very little detail of the structure can be seen, unless the cells are stained. The same is true of components of the extracellular matrix. Because different parts of the cell are biochemically different, they take up specific stains to varying degrees. For example, haematoxylin binds strongly to acids and consequently binds to nuclear DNA and stains nuclei blue. Histologists have developed many stains which are suited to particular purposes, allowing cell structures to be differentiated. It is important to remember

that the colours of stains are not the real colour of a particular tissue, and that a structure that appears as one colour using one stain, may be a quite different colour using another stain.

The great majority of routine histology is done with haematoxylin and eosin (H&E) staining, because it is quick, cheap and informative. Staining with H&E is very reliable although it does show some variation depending on the exact formulation of the stain, and the stain density is considerably affected by the thickness of the sections - thicker sections take up more stain. It is also generally done before any additional staining techniques, because histology with H&E can confirm the basic tissue type and help to localise the lesion. (The term lesion is used by pathologists to indicate any area of damage, infection, inflammation, tumour, necrosis or otherwise abnormal tissue.) For example, to diagnose a lymphoma within a lymph node, one would initially carry out H&E staining to confirm the basic diagnosis and localise the affected cells, before doing immunohistochemistry to identify exactly which type of lymphocyte was present.

A wide variety of other histochemical stains are also available, each of which can help identify particular structures. Some are relatively simple to perform, merely requiring that the section is dipped in the stain for a set time. Others require a number of sequential steps, and in some cases the results can be surprisingly variable or unpredictable. For example, the silver staining technique originally developed by Camillo Golgi (he of the Golgi apparatus) is notably temperamental (See Figure below). Some of the more commonly used techniques are outlined below:

1. Giemsa stain consists of a mixture of methylene-blue and eosin. It is mostly used on methanol-fixed blood films, where it stains erythrocytes pink and the different types of leukocyte, allowing their identification according to size and shape of their nucleii. It also binds to some pathogens, including spirochaetes (syphilis), trypanosomes (sleeping sickness and Chagas disease) and plasmodium (malarial parasites). In addition it can also be used to stain some bacteria in tissue sections pink, and it is therefore particularly useful if infection is suspected.
2. Gram stain is used to identify and differentiate bacteria. For example, staphylococci, streptococci and pneumocci are Gram-positive and stain a deep blue, whereas coliforms and neisseria are Gram-negative and stain pink.
3. Ziehl Nielsen stain is used to identify ‹acid-fast› bacilli, including mycobacteria (tuberculosis and leprosy), which appear as black rods.



Axons and dendrites of a neuron - Bielchowsky silver stain, a development of the Golgi silver staining method.

Note that identification of the bacteria requires that the shape of the bacteria can be distinguished as well as their ability to take up stain, so sections have to be observed using the highest magnification possible.

Many stains are useful because they can differentiate elements in the tissue. They include:

- Luxol fast blue/Cresyl violet is used to identify myelin which stains blue, while other elements of the nervous system stain pink or violet.
- Oil red O is a dye that is more soluble in fat than in water or alcohols, hence it is used as a stain for neutral lipids. For example when myelin is broken down in the CNS, in diseases such as multiple sclerosis, macrophages take up the lipid-rich debris and stain strongly with this dye.
- Masson's trichrome stain, covers a variety of different techniques that developed from Masson›s original formulation, each of which uses three dyes to stain different structures. It is valuable for distinguishing elements of connective tissue. Typically the cell cytoplasm, muscle and keratin are stained red, nucleii are black and collagen is blue. This stain benefits from having tissue fixed using Bouin›s fixative, although formalin-fixation is still workable.
- Periodic acid Schiff (PAS) stains carbohydrates magenta, including components of the basal lamina, surface glycoproteins on cells and intracellular carbohydrates such as glycogen in hepatocytes. Cells that secrete mucus are also strongly stained.
- Alcian blue is often combined with PAS, as it stains acidic mucins blue, whereas PAS stains neutral mucins red, hence it can be used to distinguish elements of the extracellular matrix. It also stains some fungi and parasites.
- Congo red is used to identify deposits of protein in tissue called amyloid.
- Silver staining methods have a long history; they deposit silver, which appears black onto structures that reduce silver nitrate. They can be particularly valuable for identifying individual cells, such as a single nerve cell within a group of cells, because the methods do not uniformly stain every cell of a type within the tissue. The image of a single cell within a complex tissue can be very informative, but getting the precise conditions to produce this partial staining can be difficult.
- Toluidine blue is a particularly versatile dye that stains nuclei blue, and can be used to differentiate different types of granules (e.g. within mast cells). Because it can permeate the resins that are used to embed sections for electron microscopy, it is often used as a preliminary stain, to identify sections that will later be examined by EM.
- Van Gieson stain binds to collagen in the extracellular matrix, staining it pink. Often it is combined with a stain for elastic fibres (elastic van Gieson) which stain black, allowing the two major elements of connective tissue to be differentiated.

The methods described above are only a small proportion of those that are available. As can be seen in the examples of trichrome stains, dyes may be used in combination to obtain additional information from the sections. The precise methodology and timings for the staining procedure may also vary slightly between laboratories. Some laboratories regularly use high temperatures in their staining procedures (microwaving) during particular steps, either to enhance the staining or reduce the time taken. Some stains are also more labile than others, and need to be remade regularly to maintain consistent results.

Chemical methods

- Samples must be properly prepared for microscopy. This may involve staining, fixation, and/or cutting thin sections.
- A variety of staining techniques can be used with light microscopy, including Gram staining, acid-fast staining, capsule staining, endospore staining, and flagella staining.

Gram Staining

The Gram stain procedure is a differential staining procedure that involves multiple steps. It was developed by Danish microbiologist Hans Christian Gram in 1884 as an effective method to distinguish between bacteria with different types of cell walls, and even today it remains one of the most frequently used staining techniques. Gram-staining is a differential staining technique that uses a primary stain and a secondary counterstain to distinguish between gram-positive and gram-negative bacteria.

Acid-Fast Stains

Acid-fast staining is another commonly used, differential staining technique that can be an important diagnostic tool. An acid-fast stain is able to differentiate two types of gram-positive cells: those that have waxy mycolic acids in their cell walls, and those that do not. Two different methods for acid-fast staining are the Ziehl-Neelsen technique and the Kinyoun technique. Both use carbolfuchsin as the primary stain. The waxy, acid-fast cells retain the carbolfuchsin even after a decolorizing agent (an acid-alcohol solution) is applied. A secondary counterstain, methylene blue, is then applied, which renders non–acid-fast cells blue.

Capsule Staining

Certain bacteria and yeasts have a protective outer structure called a capsule. Since the presence of a capsule is directly related to a microbe's virulence (its ability to cause disease), the ability to determine whether cells in a sample have capsules is an important diagnostic tool. Capsules do not absorb most basic dyes; therefore, a negative staining technique (staining around the cells) is typically used for capsule staining. The dye stains the background but does not penetrate the capsules, which appear like halos around the borders of the cell. The specimen does not need to be heat-fixed prior to negative staining.

Endospore Staining

Endospores are structures produced within certain bacterial cells that allow them to survive harsh conditions. Gram staining alone cannot be used to visualize endospores, which appear clear when Gram-stained cells are viewed. Endospore staining uses two stains to differentiate endospores from the rest of the cell. The Schaeffer-Fulton method (the most commonly used endospore-staining technique) uses heat to push the primary stain (malachite green) into the endospore. Washing with water decolorizes the cell, but the endospore retains the green stain. The cell is then counterstained pink with safranin. The resulting image reveals the shape and location of endospores, if they are present. The green endospores will appear either within the pink vegetative cells or as separate from the pink cells altogether. If no endospores are present, then only the pink vegetative cells will be visible

Flagella Staining

Flagella (singular: flagellum) are tail-like cellular structures used for locomotion by some bacteria, archaea, and eukaryotes. Because they are so thin, flagella typically cannot be seen under a light microscope without a specialized flagella staining technique. Flagella staining

thickens the flagella by first applying mordant (generally tannic acid, but sometimes potassium alum), which coats the flagella; then the specimen is stained with pararosaniline (most commonly) or basic fuchsin

Physical methods

Fixation is the essential first step in preserving cellular structures with the goal of keeping them as “lifelike” as possible. ... Physical fixation can include microwaving and cryopreserving samples to rapidly inactivate cellular activity.

Staining methods

A variety of staining techniques can be used with light microscopy, including Gram staining, acid-fast staining, capsule staining, endospore staining, and flagella staining.

Immunohistochemical methods

The Enzymatic method for immunohistochemistry uses reagents like Calcium Chloride, Sodium Hydroxide, Hydrochloric Acid solutions, Xylenes for dewaxing, and Methanol. Immunohistochemistry use different staining procedures such as one step direct method, ABC methods, two-step indirect method and Tyramide signal amplification.

Direct Method: Direct method is one step staining method, and involves a labeled antibody (i.e. FITC conjugated antiserum) reacting directly with the antigen in tissue sections. This technique utilizes only one antibody and the procedure is short and quick. However, it is insensitive due to little signal amplification and rarely used since the introduction of indirect method.

Indirect Method: Indirect method involves an unlabeled primary antibody (first layer) which react with tissue antigen, and a labeled secondary antibody (second layer) react with primary antibody (Note: The secondary antibody must be against the IgG of the animal species in which the primary antibody has been raised). This method is more sensitive due to signal amplification through several secondary antibody reactions with different antigenic sites on the primary antibody. In addition, it is also economy since one labeled second layer antibody can be used with many first layer antibodies (raised from the same animal species) to different antigens. The second layer antibody can be labeled with a fluorescent dye such as FITC, rhodamine or Texas red, and this is called indirect immunofluorescence method. The second layer antibody may be labeled with an enzyme such as peroxidase, alkaline phosphatase or glucose oxidase, and this is called indirect immunoenzyme method.

PAP Method (peroxidase anti-peroxidase method): PAP method is a further development of the indirect technique and it involves a third layer which is a rabbit antibody to peroxidase, coupled with peroxidase to make a very stable peroxidase anti-peroxidase complex. The complex, composed of rabbit gaba-globulin and peroxidase, acts as a third layer antigen and becomes bound to the unconjugated goat anti-rabbit gaba-globulin of the second layer. The sensitivity is about 100 to 1000 times higher since the peroxidase molecule is not chemically conjugated to the anti IgG but immunologically bound, and loses none of its enzyme activity. It also allows for much higher dilution of the primary antibody, thus eliminating many of the unwanted antibodies and reducing non-specific background staining.

Avidin-Biotin Complex (ABC) Method: ABC method is standard IHC method and one of widely used technique for immunhistochemical staining. Avidin, a large glycoprotein, can be labeled with peroxidase or fluorescein and has a very high affinity for biotin. Biotin, a low molecular weight vitamin, can be conjugated to a variety of biological molecules such as antibodies. The technique involves three layers. The first layer is unlabeled primary antibody. The second layer is biotinylated secondary antibody. The third layer is a complex of avidin-biotin peroxidase. The peroxidase is then developed by the DAB or other substrate to produce different colorimetric end products.

Labeled StreptAvidin Biotin (LSAB) Method: Streptavidin, derived from streptococcus avidini, is a recent innovation for substitution of avidin. The streptavidin molecule is uncharged relative to animal tissue, unlike avidin which has an isoelectric point of 10, and therefore electrostatic binding to tissue is eliminated. In addition, streptavidin does not contain carbohydrate groups which might bind to tissue lectins, resulting in some background staining. LSAB is technically similar to standard ABC method. The first layer is unlabeled primary antibody. The second layer is biotinylated secondary antibody. The third layer is Enzyme-Streptavidin conjugates (HRP-Streptavidin or AP-Streptavidin) to replace the complex of avidin-biotin peroxidase. The enzyme is then visualized by application of the substrate chromogen solutions to produce different colorimetric end products. The third layer can also be Fluorescent dye-Streptavidin such as FITC-Streptavidin if fluorescence labeling is preferred. A recent report suggests that LSAB method is about 5 to 10 times more sensitive than standard ABC method.

- X-ray diffraction

  X-Ray Diffraction (XRD) is a laboratory-based technique commonly used for identification of crystalline materials and analysis of unit cell dimensions.

2.3.4.3 Self-Assessment

1. Briefly explain three histochemical techniques
2. The following staining techniques can be used with light microscopy which one is not
   A. Gram staining
   B. Acid-fast staining
   C. Capsule staining
   D. Plasma staining
3. Briefly explain five immunohistochemical methods

2.3.4.4 Tools, Equipment, Supplies and Materials

- Microscope
- Carbolfuchsin stain
- Heating apparatus

2.3.4.5 References

Suvarna K.S., Layton C., Bancroft J.D. (2013) Bancroft's theory and practice of histological techniques, 7th edn. Churchill Livingstone, London

Karahara I., Kang B.H. (2014) High-pressure freezing and low-temperature processing of plant tissue samples for electron microscopy. Meth Mol Biol 1080:147–157 Cross Ref Google Scholar

Dandekar (1 January 2004). Practicals And Viva In Medical Biochemistry. Elsevier India. p. 28. ISBN 978-81-8147-025-6.

2.4.4 Learning outcome 4: Demonstrate knowledge of types of tissues and their location

2.4.4.1 Learning Activities

Learning activity	Special instructions
i) Identify and describe the various types of tissue	Name the tissues of the body
ii) Identify and describe the location of different tissues within the human body	Demonstrate knowledge in the location of different tissues within the human body
iii) Identify and describe the functions of tissues	

2.4.4.2 Information Sheet

Types of tissues

- Epithelial tissues
- Connective tissues
- Nervous tissue
- Muscular tissue

Intercellular Junctions

Tight junctions

- Close the space between cells
- Located among cells that form linings

Desmosomes

- Form “spot welds” between cells
- Located among outer skin cells

Gap junctions

- Tubular channels between cells
- Links cytoplasm of 2 cells
- Located in cardiac muscle cells

Location of tissues

2.Epithelial Tissue

General Characteristics

- Cover organs and the body
- Line body cavities
- Line hollow organs
- Have a free surface (apical)
- Have a basement membraneAre avascular
- Cells readily divide
- Cells tightly packed
- Cells often have desmosomes
- Function in protection, secretion, absorption, and excretion
- Classified according to cell shape and number of cell layers

Simple Epithelium

- Single layer of identical cell
- Found on the absorptive and secretory surfaces
- Named according to the shapes of the cells
- The more active the tissue the taller the cells.

Stratified Epithelia

- Several layers of cells of various shapes
- Superficial layers grow up from below
- Basement membrane absent
- Protects the underlying structures from mechanical wear and tear

Simple Squamous:

- Single layer of flat cells; tightly packed, forms smooth & thin membrane
- Substances pass easily through
- Line air sacs, heart
- Line blood vessels
- Line lymphatic vessels-endothelium

Simple Cuboidal:

- Single layer of cube-shaped cells
- Line kidney tubules
- Cover ovaries
- Line ducts of some glands
- Involved in secretion, absorption and excretion

Simple Columnar:

- Single layer of elongated cells
- Nuclei usually near the basement membrane at same level
- Sometimes possess cilia
- Sometimes possess microvilli
    - Often have goblet cells
    - Line uterus, stomach, intestines

Pseudostratified Columnar:

- Single layer of elongated cells
- Nuclei at two or more levels
- Appear striated
- Often have cilia
- Often have goblet cells
- Line respiratory passageways

Stratified Squamous:

- Many cell layers
- Top cells are flat (shed), deepest layers columnar
- Outer layer of skin
- Line oral cavity, vagina, and anal canal

Stratified Cuboidal:

- 2-3 layers
- Cube-shaped cells
- Line ducts of mammary glands, sweat glands, salivary glands, and the pancreas

Stratified Columnar:

- Top layer of elongated cells
- Cube-shaped cells in deeper layers
- Line part of male urethra and part of pharynx
- Keratinised:-wear & tear, dry -skin
- Non-keratinised:-wear & tear, wet surfaces-mouth

Transitional Epithelium:

- Many cell layers
- Cube-shaped and elongated cells.
- Stretches--Change shape

- Line urinary bladder, ureters, and part of urethra

GLANDULAR EPITHELIUM

- Composed of cells that are specialized to produce and secrete substances
- There are two (2) types:
  - Endocrine glands are ductless (key word: hormone)
  - Exocrine glands have ducts.

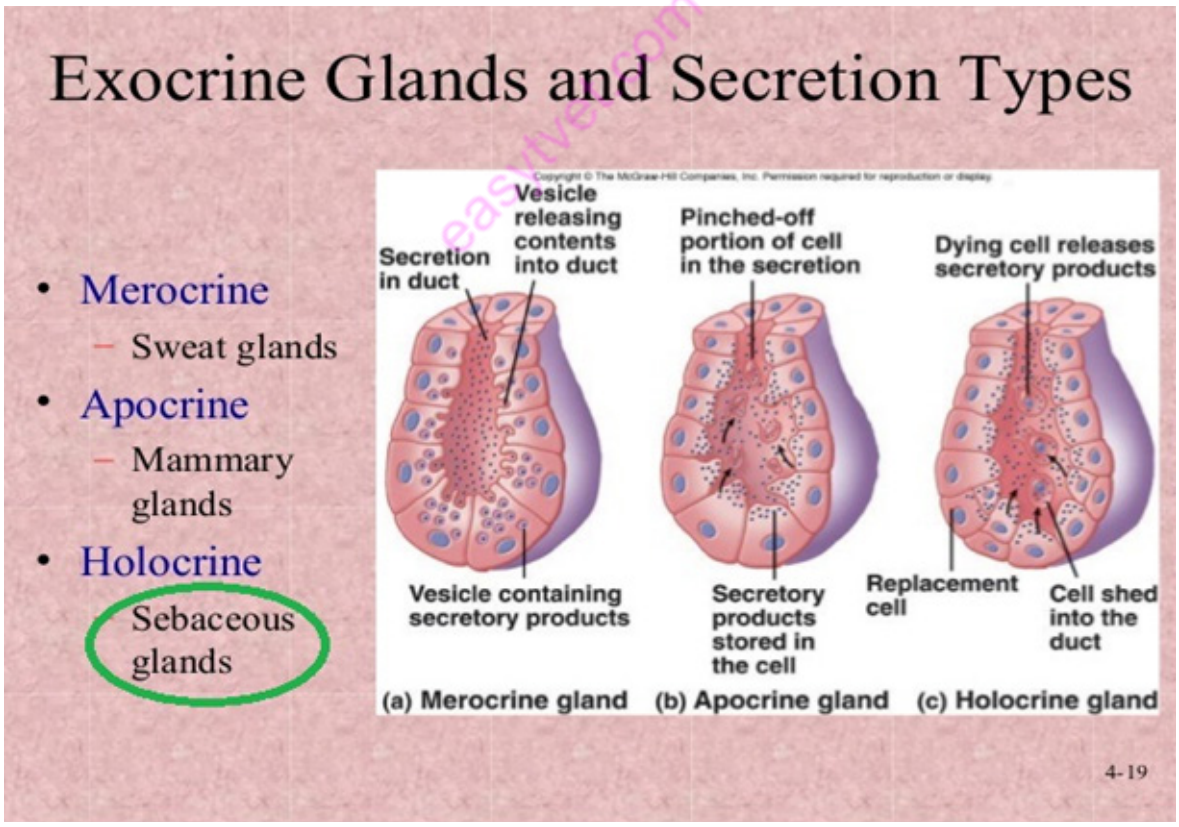
Unicellular exocrine gland:

- Composed of one cell
- Goblet cell

Multicellular exocrine gland:

- Composed of many cells
- Sweat glands, salivary glands, etc.
- Simple and compound

Types of Glandular Secretions



Merocrine Glands

- Fluid product
- Salivary glands

- Pancreas gland
- Sweat glands

Apocrine Glands

- Portions of cells
- Mammary glands
- Ceruminous glands

Holocrine Glands

- Whole cells
- Sebaceous glands

3. Connective Tissues

General Characteristics:

- Most abundant tissue type
- Many functions:
- Bind structures
- Provide support and protection
- Serve as frameworks
- Fill spaces
- Store fat
- Produce blood cells
- Protect against infections
- Help repair tissue damage
- Have a matrix (intercellular substance)-
- Connective fibres are present in the matrix; semi solid, dense and rigid
- Have varying degrees of vascularity
- Have cells that usually divide

Major Cell Types Present in Connective Tissue

1. Fibroblasts

- Fixed cell
- Large, star-shaped
- Most common cell
- Produce fibers

2. Mast cells

- Fixed cell
- Release heparin
- Release histamine

3. Macrophages

- Wandering cell
- Phagocytic
- Important in injury or infection

4. Fat Cells

- Form the adipose tissue

Types of Fibers in Connective Tissue

- There are three types of fibers in connective tissue

1. Collagenous fibers

- Thick
- Great tensile strength
- Hold structures together
- Composed of collagen
- Abundant in dense CT
- Tendons, ligaments

2. Reticular fibers

- Very thin collagenous fibers
- Highly branched
- Form supportive networks

3. Elastic fibers

- Bundles of microfibrils embedded in elastin
- Fibers branch
- Elastic
- Vocal cords, air passages

Types of Connective Tissues

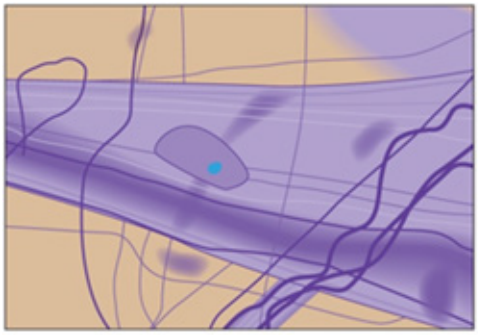
Connective Tissue Proper:

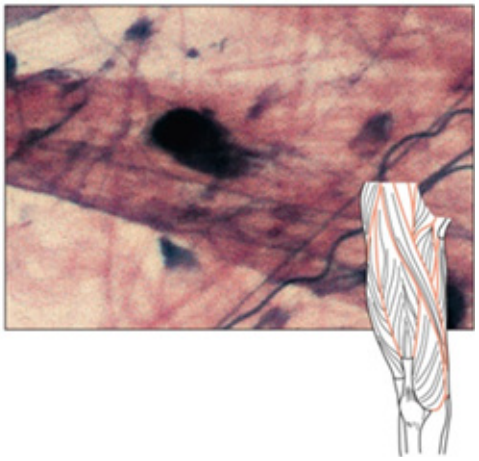
1. Loose connective tissue
2. Adipose tissue
3. Reticular connective tissue
4. Dense connective tissue
5. Elastic connective tissue

Specialized Connective Tissue:

1. Cartilage
2. Bone
3. Blood

1. Loose Connective Tissue





Mainly fibroblasts

- Fluid to gel-like matrix
- Collagenous fibers
- Elastic fibers
- Bind skin to structures
- Beneath most epithelia
- Blood vessels nourish nearby epithelial cells
- Between muscles.

2. Adipose Tissue

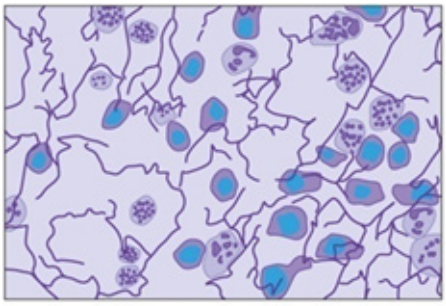
- Adipocytes
- Insulates
- Beneath skin
- Around kidneys and heart
- Cushions
- Store fats
- Behind eyeballs

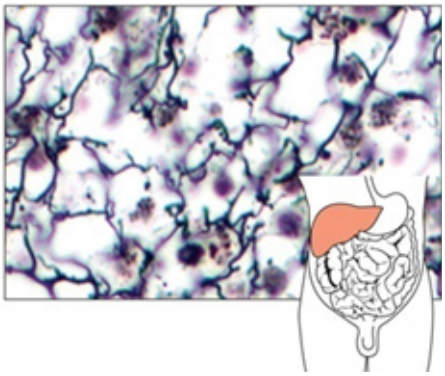
3. Reticular Connective Tissue

a. Composed of reticular fibers

b. Supports internal organ walls

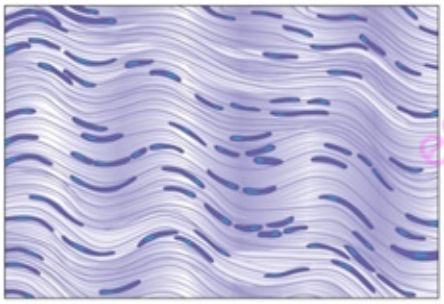
c. Walls of liver, spleen, lymphatic organs.

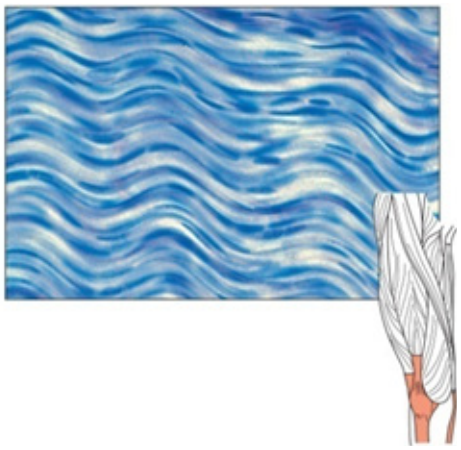




4. Dense Connective Tissue

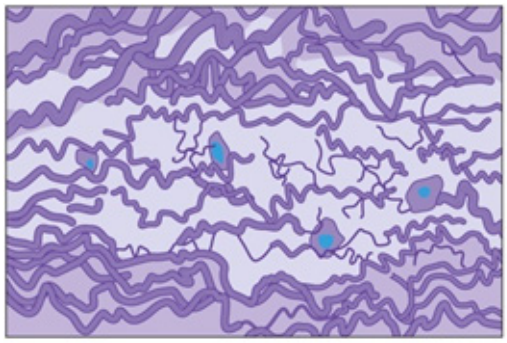
- Packed collagenous fibers
- Few fibroblasts
- Tendons, ligaments, dermis
- Elastic fibers
- Bind body parts together
- Poor blood supply

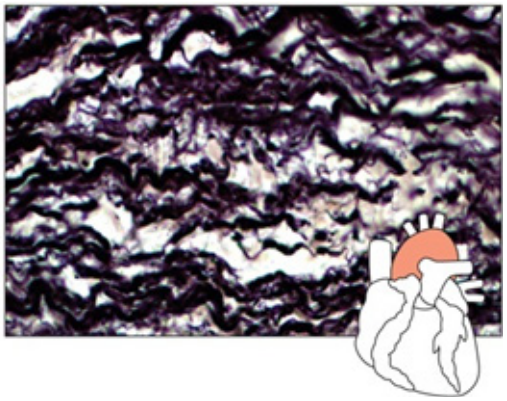




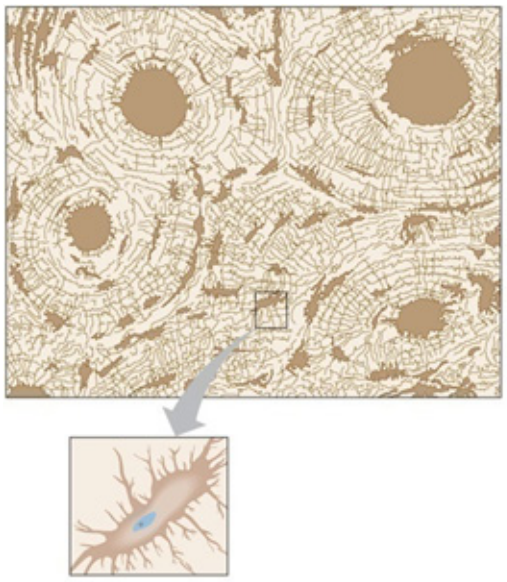
5. Elastic Connective Tissue

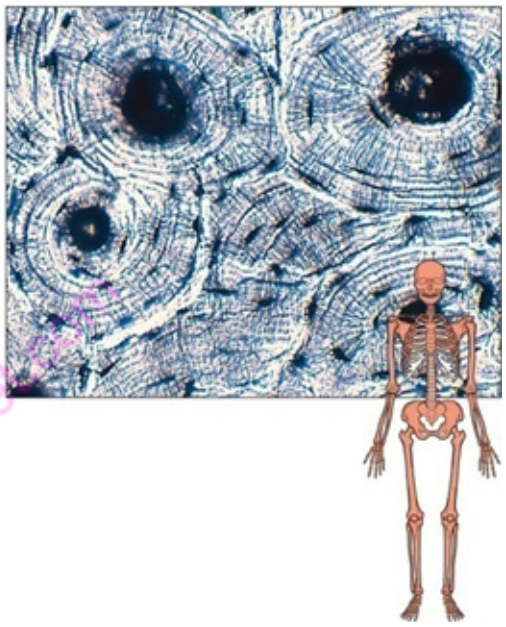
- Abundant in elastic fibers
- Some collagenous fibers
- Fibroblasts
- Attachments between bones
- Walls of large arteries, airways, heart





6. Bone (Osseous Tissue)





- Solid matrix
- Protects
- Attachment for muscles
- Osteocytes in lacunae
- Supports
- Forms blood cells
- Skeleton

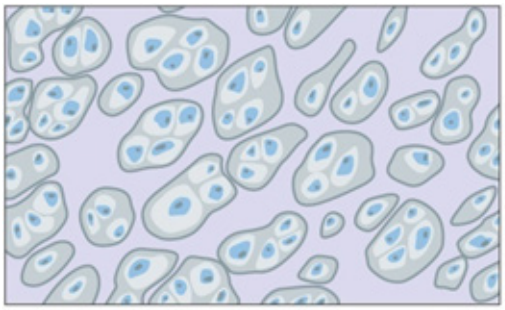
7. Cartilage

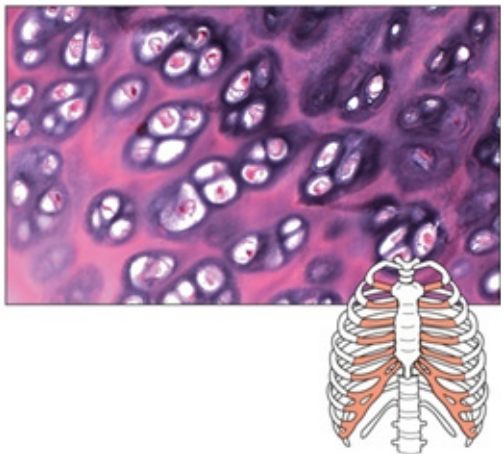
- Rigid matrix
- Chondrocytes in lacunae
- Poor blood supply

Three (3) types:

- 1) Hyaline Cartilage
- 2) Elastic Cartilage
- 3) Fibrocartilage

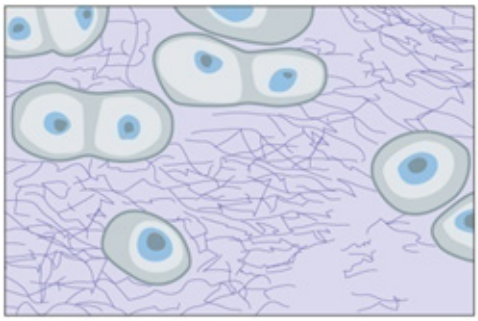
Hyaline cartilage

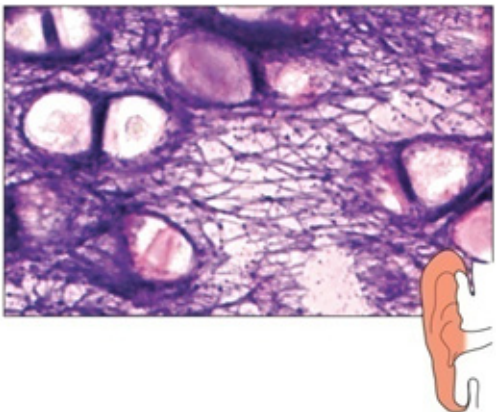




- Most abundant
- Ends of bones
- Nose, respiratory passages
- Embryonic skeleton

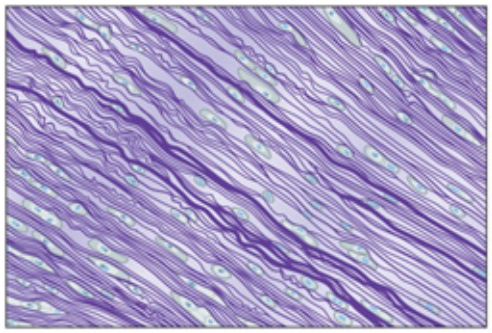
Elastic cartilage

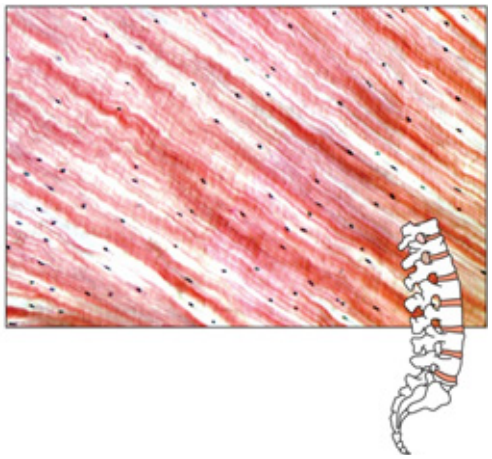




- Flexible
- External ear, larynx

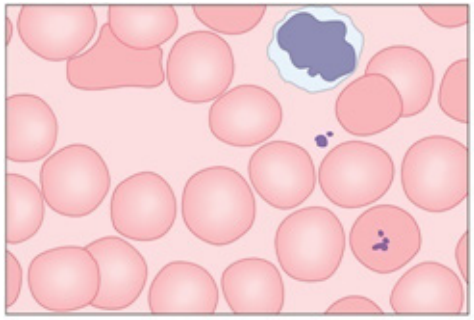
Fibrocartilage

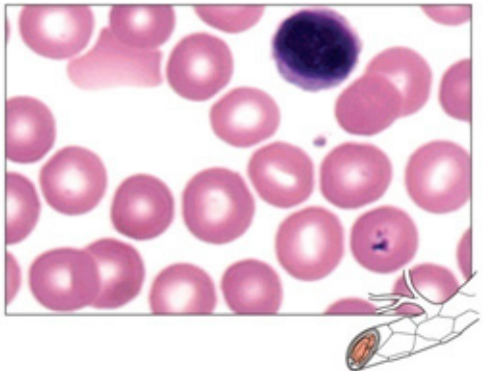




- Very tough
- Shock absorber
- Intervertebral discs
- Pads of knee and pelvic girdle

8. Blood





- Fluid matrix called plasma
- White blood cells
- Transports
- Involved in clotting
- Heart

- Red blood cells
- Platelets
- Defends
- Throughout body in blood vessels

Types of Membranes

There are four (4) types of epithelial membranes:

1. Serous Membranes

- Line body cavities that do not open to the outside
- Reduce friction
- Inner lining of thorax and abdomen
- Cover organs of thorax and abdomen
- Secrete serous fluid

2. Mucous Membranes

- Line tubes and organs that open to outside world
- Lining of mouth, nose, throat, etc.
- Secrete mucus

3. Cutaneous Membranes

- Covers body
- Skin

4. Synovial Membranes

- Composed entirely of connective tissue
- Lines joints

3. Muscle Tissues

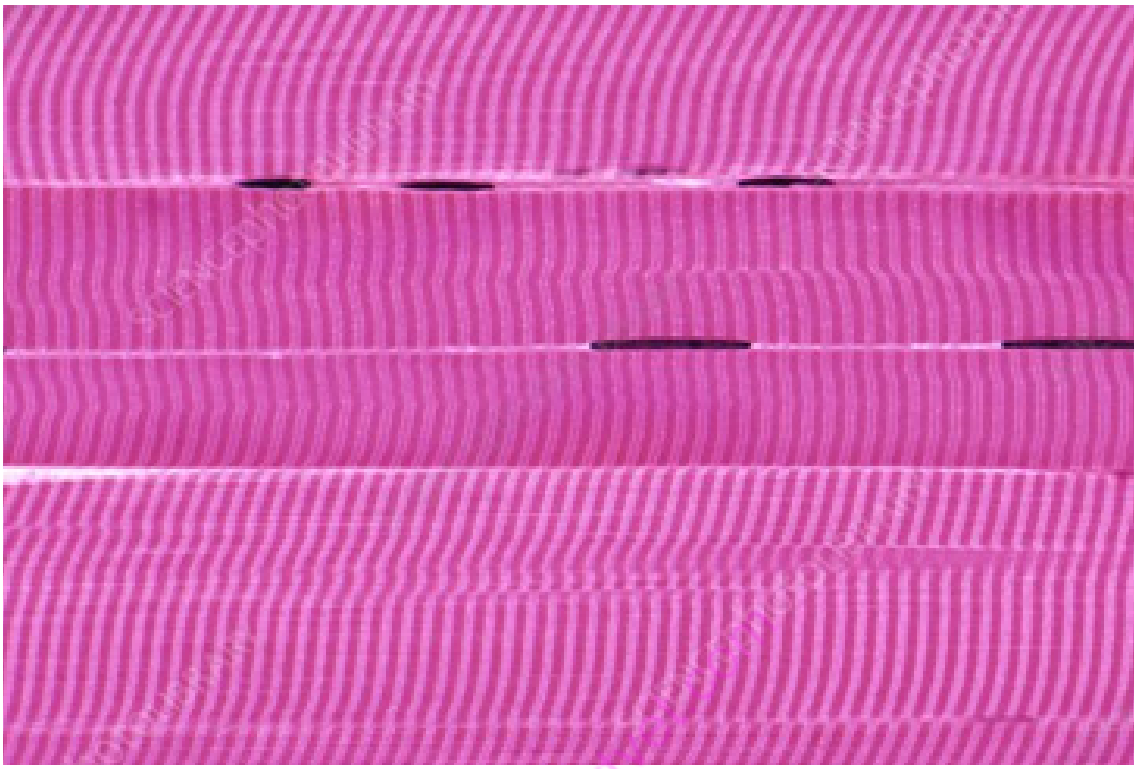
- General characteristics:
- Muscle cells also called muscle fibers
- Contractile

Three (3) types:

1. Skeletal muscle
2. Smooth muscle
3. Cardiac muscle

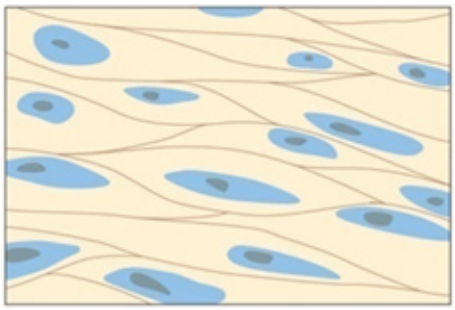
Skeletal muscle

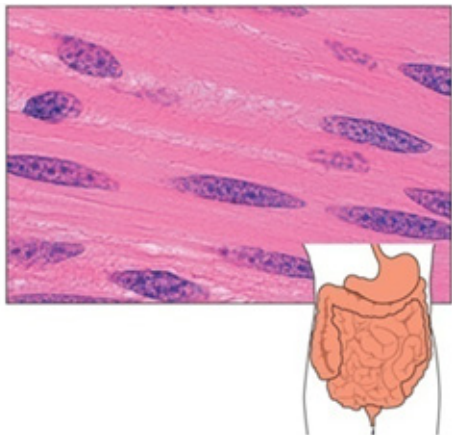
- Attached to bones
- Striated
- Voluntary



Smooth muscle

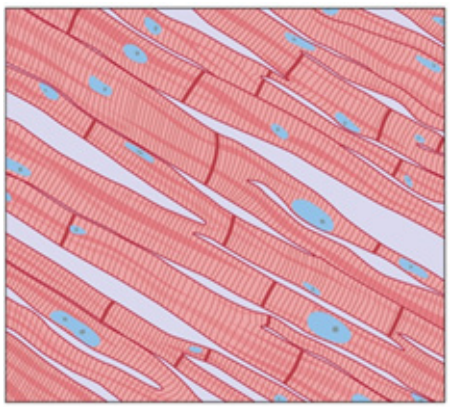
- Walls of organs
- Walls of blood vessels
- Non-striated
- Skin
- Involuntary

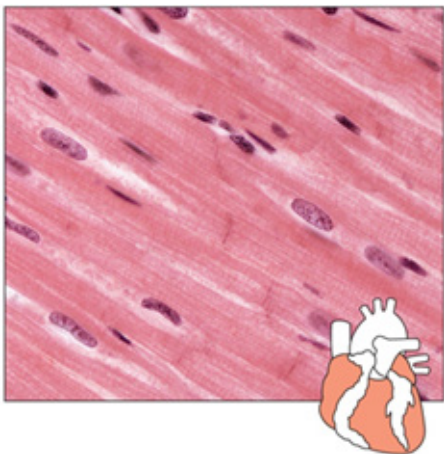




Cardiac muscle

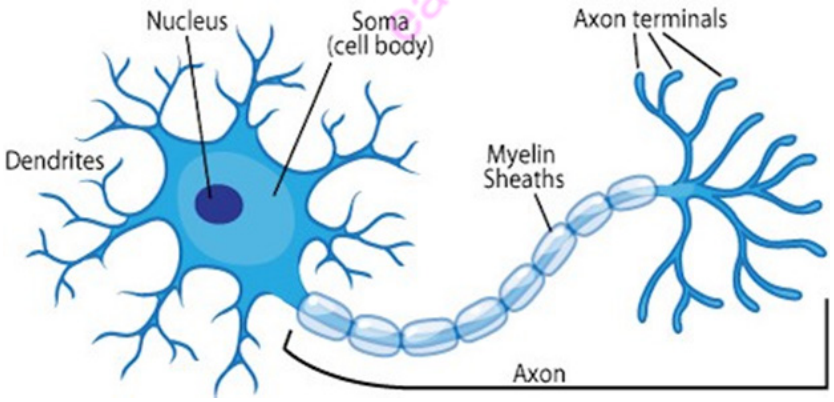
- Heart wall
- Striated
- Involuntary
- Intercalated discs





9. Nervous Tissue

- Found in brain, spinal cord, and peripheral nerves
- Functional cells are neurons
- Neuroglial cells support and bind nervous tissue components
- Sensory reception
- Conduction of nerve impulses



THE PROCESS OF ORGANOGENESIS

Early Embryonic Development

Fertilization is the process in which gametes (an egg and sperm) fuse to form a zygote (Figure 13.8). To ensure that the offspring has only one complete diploid set of chromosomes, only one sperm must fuse with one egg. In mammals, a layer called the zona pellucida protects the egg. At the tip of the head of a sperm cell is a structure like a lysosome called the acrosome, which contains enzymes. When a sperm binds to the zona pellucida, a series of events, called the acrosomal reactions, take place. These reactions, involving enzymes from the acrosome, allow the sperm plasma membrane to fuse with the egg plasma membrane and permit the sperm nucleus to transfer into the ovum. The nuclear membranes of the egg and sperm break down and the two haploid nuclei fuse to form a diploid nucleus or genome.

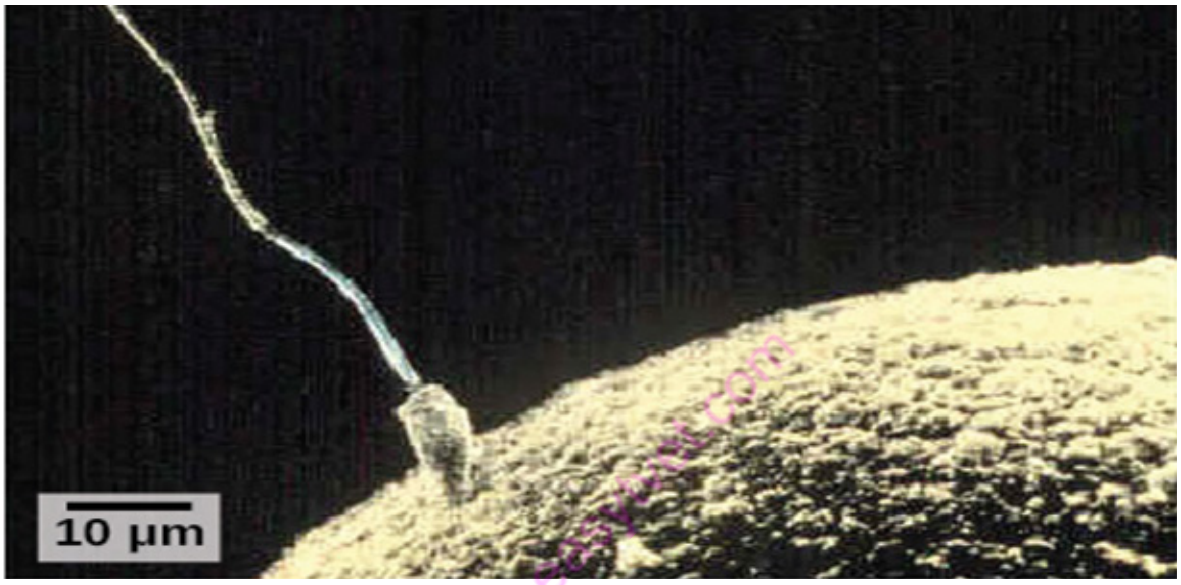
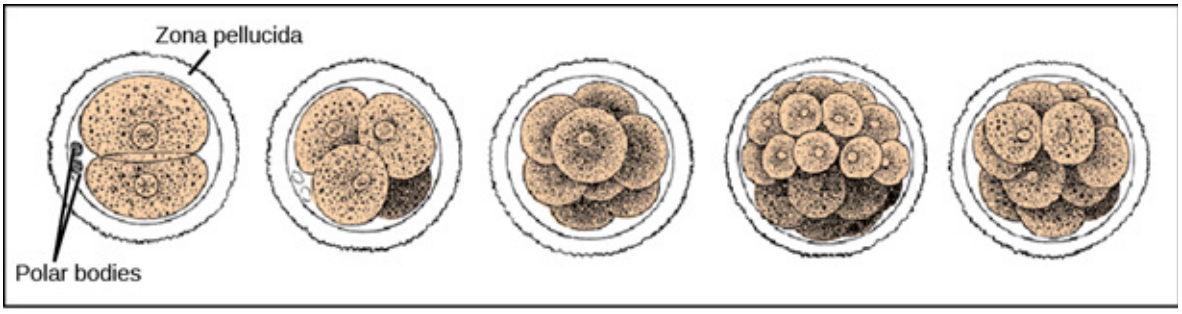


Figure 13.8 Fertilization is the process in which sperm and egg fuse to form a zygote. (credit: scale-bar data from Matt Russell)

To ensure that no more than one sperm fertilizes the egg, once the acrosomal reactions take place at one location of the egg membrane, the egg releases proteins in other locations to prevent other sperm from fusing with the egg.

The development of multi-cellular organisms begins from this single-celled zygote, which undergoes rapid cell division, called cleavage (Figure 13.9 a), to form a hollow ball of cells called a blastula (Figure 13.9 b).



(a)

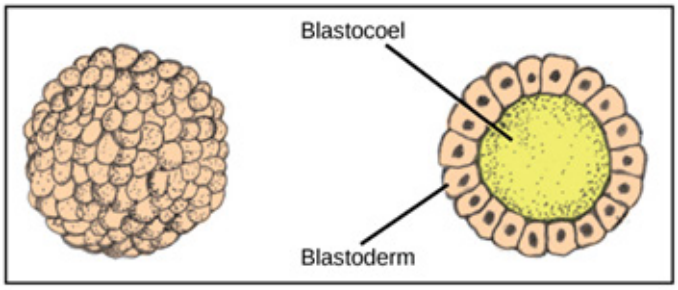


Figure 13.9 (a) During cleavage, the zygote rapidly divides into multiple cells. (b) The cells rearrange themselves to form a hollow ball called the blastula. (credit a: modification of work by Gray's Anatomy; credit b: modification of work by Pearson Scott Foresman; donated to the Wikimedia Foundation)

In mammals, the blastula forms the blastocyst in the next stage of development. Here the cells in the blastula arrange themselves in two layers: the inner cell mass, and an outer layer called the trophoblast. The inner cell mass will go on to form the embryo. The trophoblast secretes enzymes that allow implantation of the blastocyst into the endometrium of the uterus. The trophoblast will contribute to the placenta and nourish the embryo.

Concept in Action

Visit the Virtual Human Embryo project at the Endowment for Human Development site to click through an interactive of the stages of embryo development, including micrographs and rotating 3-D images.

The cells in the blastula then rearrange themselves spatially to form three layers of cells. This process is called gastrulation. During gastrulation, the blastula folds in on itself and cells migrate to form the three layers of cells (Figure 13.10) in a structure, the gastrula, with a hollow space that will become the digestive tract. Each of the layers of cells is called a germ layer and will differentiate into different organ systems.

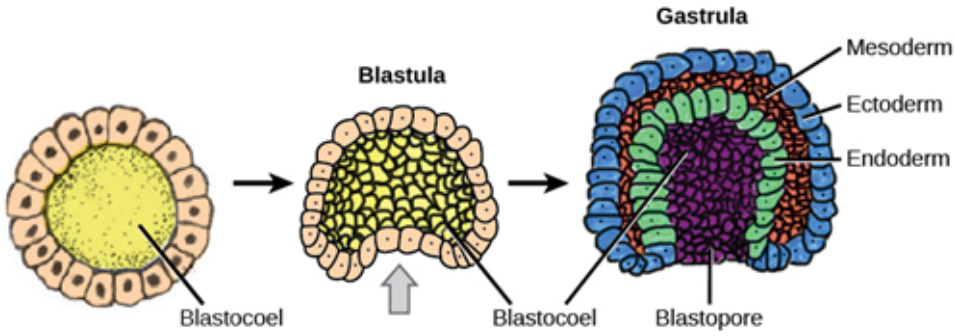


Figure 13.10 Gastrulation is the process wherein the cells in the blastula rearrange themselves to form the germ layers. (credit: modification of work by Abigail Pyne).

The three germ layers are the endoderm, the ectoderm, and the mesoderm. Cells in each germ layer differentiate into tissues and embryonic organs. The ectoderm gives rise to the nervous system and the epidermis, among other tissues. The mesoderm gives rise to the muscle cells and connective tissue in the body. The endoderm gives rise to the gut and many internal organs.

Organogenesis

Gastrulation leads to the formation of the three germ layers that give rise during further development to the different organs in the animal body. This process is called **organogenesis**.

Organs develop from the germ layers through the process of differentiation. During differentiation, the embryonic stem cells express specific sets of genes that will determine their ultimate cell type. For example, some cells in the ectoderm will express the genes specific to skin cells. As a result, these cells will take on the shape and characteristics of epidermal cells. The process of differentiation is regulated by location-specific chemical signals from the cell's embryonic environment that sets in play a cascade of events that regulates gene expression.

Vertebrate Axis Formation

Through the expression patterns of different genes, the three axes of the body are established, aiding in tissue and organ development.

Key Points

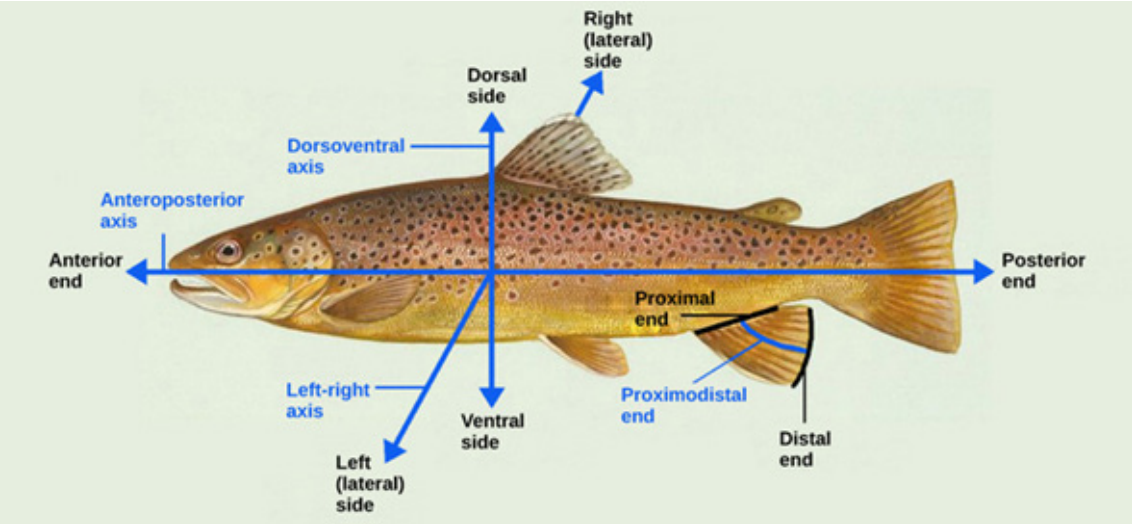
- As an animal develops, it must organize its internal and external structures such that the anterior/posterior (forward/backward), dorsal / ventral (back/belly), and lateral/medial (side/middle) axes are correctly determined.
- Proteins that are part of the Wnt signaling pathway help determine the anterior/posterior axis by guiding the axons of the spinal cord in an anterior/posterior direction.
- Together with the sonic hedgehog (Shh) protein, Wnt determines the dorsal/ventral axis; Wnt levels are highest in the dorsal region and lessen toward the ventral region, while Shh levels are highest in the ventral region and lessen toward the dorsal region.

Key Terms

- **dorsal**: with respect to, or concerning the side in which the backbone is located, or the analogous side of an invertebrate
- **ventral**: on the front side of the human body, or the corresponding surface of an animal, usually the lower surface
- **notochord:** a flexible rodlike structure that forms the main support of the body in the lowest chordates; a primitive spine
- **Wnt signaling pathway**: a group of signal transduction pathways made of proteins that pass signals from outside of a cell through cell surface receptors to the inside of the cell.

Vertebrate Axis Formation

Even as the germ layers form, the ball of cells still retains its spherical shape. However, animal bodies have lateral-medial (toward the side-toward the midline), dorsal-ventral (toward the back-toward the belly), and anterior-posterior (toward the front-toward the back) axes. As the body forms, it must develop in such a way that cells, tissues, and organs are organized correctly along these axes.



Body axes: *Animal bodies have three axes for symmetry: anterior/posterior (front/behind), dorsal/ventral (back/belly), and lateral/medial (side/middle).*

How are these established? In one of the most seminal experiments ever to be carried out in developmental biology, Spemann and Mangold took dorsal cells from one embryo and transplanted them into the belly region of another embryo. They found that the transplanted embryo now had two notochords: one at the dorsal site from the original cells and another at the transplanted site. This suggested that the dorsal cells were genetically programmed to form the notochord and define the dorsal-ventral axis. Since then, researchers have identified many genes that are responsible for axis formation. Mutations in these genes leads to the loss of symmetry required for organism development. Many of these genes are involved in the Wnt signaling pathway.

In early embryonic development, the formation of the primary body axes is a crucial step in establishing the overall body plan of each particular organism. Wnt signaling can be implicated in the formation of the anteroposterior and dorsoventral axes. Wnt signaling activity in anterior-posterior development can be seen in several organisms including mammals, fish, and frogs. Wnt signaling is also involved in the axis formation of specific body parts and organ systems that are a part of later development. In vertebrates, sonic hedgehog (Shh) and Wnt morphogenetic signaling gradients establish the dorsoventral axis of the central nervous system during neural tube axial patterning. High Wnt signaling establishes the dorsal region while high Shh signaling indicates in the ventral region. Wnt is also involved in the dorsal-ventral formation of the central nervous system through its involvement in axon guidance. Wnt proteins guide the axons of the spinal cord in an anterior-posterior direction. Wnt is also involved in the formation of the limb dorsal-ventral axis. Specifically, Wnt7a helps produce the dorsal patterning of the developing limb.

Section Summary

The early stages of embryonic development begin with fertilization. The process of fertilization is tightly controlled to ensure that only one sperm fuses with one egg. After fertilization, the zygote undergoes cleavage to form the blastula. The blastula, which in some species is a hollow ball of cells, undergoes a process called gastrulation, during which the three germ layers form. The ectoderm gives rise to the nervous system and the epidermal skin cells, the mesoderm gives rise to the muscle cells and connective tissue in the body, and the endoderm gives rise to the digestive system and other internal organs. Organogenesis is the formation of organs from the germ layers. Each germ layer gives rise to specific tissue types.

2.4.4.3 Self-Assessment

1. The process of gastrulation forms the _____.
   A. Blastula B. Zygote
   C. Organs D. Germ layers
2. Which of the following gives rise to the skin cells?
   A. Ectoderm B. Endoderm
   C. Mesoderm D. None of the above
3. Which one of the following characteristic does not describe collagenous fibers
   A. Thick
   B. Composed of collagen
   C. Great tensile strength
   D. Very thin collagenous fibers
4. What do you think would happen if multiple sperm fused with one egg?

2.4.4.4 Tools, Equipment, Supplies and Materials

Dummy human body, microscope, slides, cadaver, anatomy text books, white board, mark pen, skills laboratory

2.4.4.5 References

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