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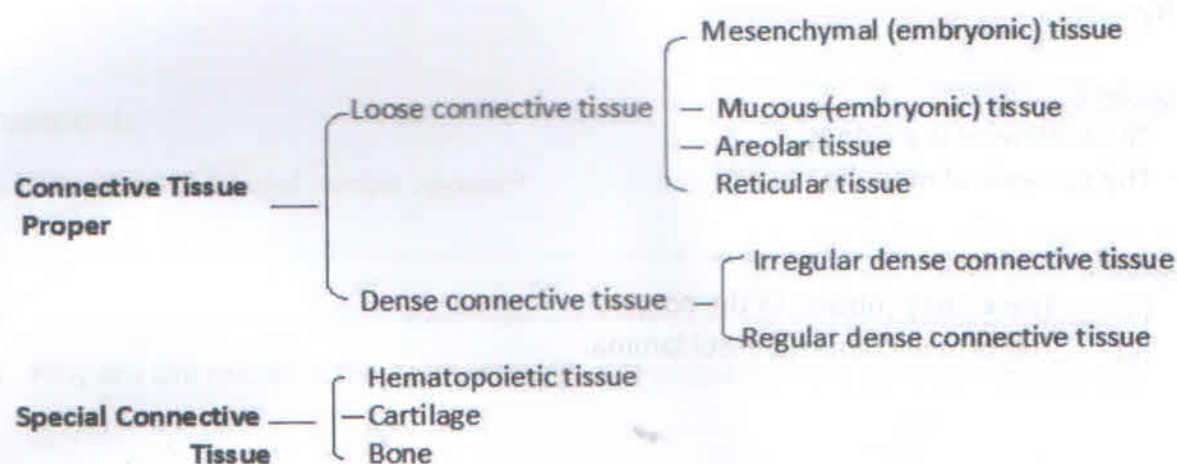
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CHAPTER THREE

CONNECTIVE TISSUES

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Connective tissues provide the matrix that connects and binds the cells and organs. They are of different types, and are functionally responsible for providing and maintaining form in the body. Connective tissue comprises a number of three main components (Cells, Fibres and Amorphous ground substance), whose arrangements and abundance determine the type of connective tissue. The connective tissues will be studied according to the following scheme:



At the end of this section, students should be able to classify any connective tissue. Hence, students should do the following:

- Identify the different types of connective tissues and where they can be found
- Identify the various components of the connective tissues and their locations within the specimen
- Observe and take note of the staining reaction of the amorphous ground substance, cells and fibres.
- Observe and take note of the relative ratio of the amorphous ground substance, cells and fibres to one another.
- Observe and take note of the distribution of the cells in relation to the fibres.

- Take note of the relative ratio of cells and fibres to one another.
- Observe and take note of the relative ratio of the different types of fibres.
- Identify sections of tissues i.e. either transverse or longitudinal.
- Make a table for each type of connective tissue, describing and comparing each of the three components.

3.1 Connective Tissue Proper

3.1.1. Basal Lamina

Slide 17: This specimen of the kidney is stained with silver to demonstrate the basal lamina. The purpose of this practical session is to illustrate the basement membrane and general connective tissue.

Identify:

Macroscopic

- The outline of the kidney.
- The cortex and medulla regions.

Microscopic

- The kidney tubules in the cortex.
- The brown coloured basal lamina.

Draw and annotate:

A few kidney tubules with surrounding basal lamina.

Questions:

1. How does the basal lamina appear?

.....

.....

2. Why are the nuclei of the epithelial cells not visible but the basal lamina can be seen?

.....

.....

3. What is the difference between the basal lamina and basement membrane?

.....

.....

3.1.2. *Connective Tissue Cells*

Slide 18: This specimen is a section through the colon and is a good example of different types of connective tissue cells.

Identify:

Macroscopic

- (i) A thin dark pink coloured line, the mucosa.
- (ii) A central light pink coloured area fine pink coloured structures, submucosa.
- (iii) An outer pink coloured irregular area, the out muscle layer.

Microscopic

In the lamina propria of the mucosa, which comprises an epithelial layer and a loose connective tissue layer, the lamina propria, the following cells must be identified:

- | | |
|-------------------|----------------------------|
| (i) Fibroblasts | (v) Eosinophils |
| (ii) Plasma cells | (vi) Lymphocytes |
| (iii) Macrophages | (vii) Smooth muscle fibres |
| (iv) Mast cells | |

Draw and annotate:

The different cell types. List with each cell type, their distinguishing characteristics

Diagram of the different cell types (continued)

3.1.3. Mucous or Gelatinous Connective Tissue

Slide 19: This specimen is a cross section through the umbilical cord.

Identify:

Macroscopic

The three large blood vessels of the umbilical cord.

Microscopic

In the tissue and between the blood vessels the following:

- (i) Fibroblasts
- (ii) Collagen fibres
- (iii) Amorphous ground substance

Draw and annotate:

The three components of gelatinous connective tissue in the correct proportions. Draw an additional fibroblast showing its microscopic detail (at 400x magnification).

Questions:

1. What is the shape of the fibroblasts and their nuclei? Compare this to the smooth muscle present in the blood vessel.

.....

.....

2. How is the collagen arranged?

.....

.....

3. How do the collagen fibres appear?

.....

4. How does the amorphous substance appear and how does it stain?

.....

.....

3.1.4. Loose Connective Tissue (Collagen Fibres)

Slide 20: This specimen is a spread of the mesenterium, stained to show collagen fibers and connective tissue nuclei.

Identify:

Macroscopic

- (i) Spread of the mesenterium

Microscopic

- (i) Nuclei of the connective tissue cells
- (ii) Collagen fibers

Draw and annotate:

The collagen fibers and the different connective tissue nuclei. Draw the different thicknesses, structures and course of the fibers.

Questions:

1. What characteristics are used to distinguish between different types of collagen fibres?

.....

2. How are the different connective tissue cells distinguished by their nuclei?

.....

.....

.....

3. Are the large, lightly stained nuclei also connective tissue cells?

.....

4. How is the amorphous substance distinguished and how does it stain?

.....

.....

3.1.5. Loose Connective Tissue (Elastic Fibres)

Slide 21: This specimen is a spread of the mesenterium that is stained for elastic fibres.

Identify:

Microscopic

- (i) Elastic fibres
- (ii) Mast cell granules

Draw and annotate:

Elastic fibres and mast cell granules. Indicate clearly the thicknesses, colour and branching of the elastic fibres.

Questions:

1. What characteristics are used to identify elastic fibres?

.....

.....

2. How do elastic fibres differ from collagen fibres?

.....

.....

3.1.6. Reticular Fibres

Slide 22: This is a section through the liver to demonstrate reticular fibres.

Identify:

Macroscopic

- (i) The mass of liver tissue

Microscopic

- (i) Reticular fibres
- (ii) Outlines of liver cells

Draw and annotate:

Draw the general structural outline of the liver cells.

Questions:

1. What characteristics are used to distinguish reticular fibres?

.....

.....

2. How do reticular fibres differ from collagen and elastic fibres?

.....

.....

3.1.7. Loose Connective Tissue (Sectioned)

Slide 23: This is a section of thin skin to demonstrate sectioned loose connective tissue.

Identify:

Macroscopic

- (i) A fine dark area, the epidermis
- (ii) A thick lighter eosinophilic area, the dermis.
- (iii) A very thick light area with a fine eosinophilic line running through, the hypodermis.

Microscopic

The dermis is that part of the skin just beneath the epidermis (epithelium). It is divided into two parts, namely the papillary and reticular layers. The papillary layer is situated directly beneath the epidermis and consists of loose connective tissue. The reticular part is composed of dense irregular connective tissue.

- (i) Collagen fibres sectioned in different planes
- (ii) Nuclei of the connective tissue cells

Draw and annotate:

Collagen fibres sectioned in different directions also indicate the different connective tissue nuclei.

Questions:

1. How do the sectioned collagen fibres differ from those of the mesenteric as spread out [compare Slides 2, 20 and 21]?
.....
.....

2. What is the relative composition of this tissue, with regard to percentage of collagen fibres, cells and amorphous ground substance?
.....
.....

3.1.8. Dense Irregular Connective Tissue

Slide 24: This specimen is a section through the thin skin of the thigh to demonstrate dense irregular connective tissue in the dermis.

Identify:

Macroscopic

- (i) The different staining layers of thin skin.

Microscopic

- (i) Dense irregular connective tissue [is present in the reticular part i.e. the deeper part of the dermis]
- (ii) Fibroblast nuclei.

Draw and annotate:

Dense irregular connective tissue. Indicate the collagen fibres sectioned in different planes.

Questions:

1. How do the fibres of dense irregular connective tissue differ from connective tissue in a tissue section?

.....
.....

2. Are there more or less cells in dense irregular connective tissue than loose connective tissue?

.....
.....

3.1.9. Dense Regular Connective Tissue

Slide 25: This specimen demonstrates a cross and longitudinal section of a tendon.

Identify:

Macroscopic

- (i) The cross and longitudinal sections of the tendon.

Microscopic

- (i) Dense regular connective tissue.
- (ii) Tendon cell nuclei.
- (iii) Loose connective tissue.

Draw and annotate:

Dense regular connective tissue in cross as well as longitudinal section.

1. Are the cells of connective tissue close to or far away from other connective tissue cells?

2. What is characteristic in the course of the collagen fibres?

3. Where are the tendon cell nuclei found and what is their shape?

4. How does dense regular connective tissue differ from dense irregular connective tissue and loose connective tissue?

5. What are connective tissue diseases? Give examples.

Adipose tissue is a special type of connective tissue in which adipose or fat cells (adipocytes) predominate. Fat cells can either be found scattered in small groups within the connective tissue itself, adipose tissue and other structures (common, or yellow) or multilocular (or brown) adipose tissue. The two known types differ with respect to their different locations, structures, colors, and pathological characteristics. Unilocular adipose tissue consists of cells which when completely developed, contain one large central droplet of yellow fat in their cytoplasm, while multilocular adipose tissue consists of cells that contain numerous lipid droplets and abundant brown mitochondria.

Questions:

1. How are the functions of connective tissues adapted to their features?

.....
.....

2. Are the cells of connective tissue close to or far away from other connective tissue cells?

.....

3. What is characteristic in the course of the collagen fibres?

.....

4. Where are the tendon cell nuclei found and what is their shape?

.....

5. How does dense regular connective tissue differ from dense irregular connective tissue and loose connective tissue?

.....
.....

6. What are connective tissue diseases? Give examples.

.....
.....

3.1.10. Adipose Tissue

Adipose tissue is a special type of connective tissue in which adipose or fat cells (**adipocytes**) predominate. Fat cells can either be found isolated or in small groups within the connective tissue itself. Adipose tissues are either **Unilocular** (common, or yellow) or **Multilocular** (or brown) **adipose tissue**. The two known types differ with respect to their different locations, structures, colors, and pathological characteristics. **Unilocular adipose tissue** comprises of cells which when completely developed, contain one large central droplet of yellow fat in their cytoplasm, while **Multilocular adipose tissue** comprises of cells that contain numerous lipid droplets and abundant brown mitochondria.

Slide 26: This is a specimen of the mesenterium stained to demonstrate fat cells and their nuclei.

Identify:

Macroscopic

- (i) Spread of the mesenterium

Microscopic

- (i) Fat cell nuclei
- (ii) Lipid filled cytoplasm
- (iii) Connective tissue septa

Draw and annotate:

A few fat cells to demonstrate their characteristic structure.

Questions:

1. How does the appearance of these fat cells differ from those that were stained with Sudan IV?

.....

.....

Slide 27: This is a section through the hypodermis of thick skin to demonstrate connective tissue.

Identify:

Macroscopic

- (i) The different textures and intensity of stained layers of thick skin.

Microscopic

- (i) Fat cells
- (ii) Connective tissue septa.

Draw and annotate:

A line diagram of a few lobules of fatty tissue. In one of the lobules, cells as well as the surrounding connective tissue septa.

Questions:

1. How do the fat cells appear?

.....

.....

2. How are the nuclei, cytoplasm and organelles of fat cells arranged?

.....

4.

3.2 Cartilage

The purpose of this practical session is to demonstrate cartilage as a specialized connective tissue.

3.2.1. Hyaline Cartilage

Slide 28: This is a cross section through the trachea to demonstrate hyaline cartilage.

Identify:

Macroscopic

(i) The C-shape cartilage rings in the centre of the trachea

Microscopic

(i) Lacunae

(ii) Chondrocytes

(iii) Matrix

(iv) Perichondrium

Draw and annotate:

A portion of the cartilage and perichondrium in fine detail.

Questions:

1. How are the functions of cartilages adapted to their features?

.....

.....

2. What is characteristic of the shape, size and arrangement of the lacunae?

.....

.....

3. What is the appearance of chondrocytes?

.....

.....

4. What is the appearance of the matrix?

.....

5. What type of growth is found in hyaline cartilage?

.....

3.2.2. Elastic Cartilage

Slide 29: This is a section through the pinna of the ear to demonstrate elastic cartilage.

Identify:

Macroscopic

- (i) The dark purple stained elastic cartilage in the centre of the tissue.

Microscopic

- (i) Lacunae
- (ii) Chondrocytes
- (iii) Matrix
- (iv) Perichondrium

Draw and annotate:

A portion of the cartilage and perichondrium in fine detail

Questions:

1. How do the lacunae appear with the respect to their shape, size and arrangement?

.....

.....

2. What is the characteristic of matrix?

.....

3.2.3. Fibrocartilage (Fibrous)

Slide 30: This is a cross and longitudinal section of tendon just prior to its attachment to bone.

Identify:

- (i) Chondrocytes
- (ii) Collagen fibres
- (iii) Matrix

Draw and annotate:

A portion of fibrocartilage in detail.

Questions:

1. What is characteristic of the shape and arrangement of the chondrocytes?
.....
.....
2. How does the amorphous ground substance appear?
.....
.....
3. How are the collagen fibres arranged?
.....
.....
4. How does this tissue differ from dense regular connective tissue?
.....
5. Complete the following table relating to the three types of cartilage.

	Hyaline cartilage	Elastic cartilage	Fibrocartilage
Cells			
Fibers and collagen			
Amorphous ground substance			
Matrix			
Location and function			

Revision Notes on Connective Tissues:

1. Name the 5 main classes of connective tissues.

.....

.....

.....

.....

.....

2. Name the 5 major components of the basement membrane.

.....

.....

.....

.....

.....

3. Name the 3 main functions of the basement membrane.

.....

.....

.....

4. Name the 3 types of cartilage.

.....

.....

.....

5. Tabulate the histological differences between chondroblasts and chondrocytes.

Chondroblasts	Chondrocytes

6. Name the 2 types of growth of cartilage.

.....

.....

7. Name the 4 functions of adipose tissue.

.....

.....

.....

.....

8. How is a fibrous scar formed?

.....

.....

.....

3.3. Bone

The purpose of this practical session is to demonstrate bone as a specialized connective tissue.

3.3.1 Bone

Slide 31: This is a cross section through decalcified bone. There are two ways to study bone histology. Dry bone is cut and polished before mounting on a slide. **Decalcification** is the removal of the **mineral** component using an acid, leaving bone soft and easy to cut. In dry ground bone, the organic material is removed. For contrast, in decalcified slides the relationship between the living components of bone is retained.

Identify:

Macroscopic

- (i) Compact decalcified stained bone

Microscopic

- (i) Haversian and Volkmann's canals
- (ii) Lacunae
- (iii) Bone matrix
- (iv) Periosteum
- (v) Fat cells of the bone marrow

Draw and annotate:

A section of bone in which Haversian systems and periosteum are indicated.

Questions:

1. What is present in the Haversian canals? What is their function?

.....

.....

2. How are the lacunae arranged and what is found in them? What is their function?

.....

.....

3.3.2 Ground Bone (Longitudinal Section)

Slide 32: This is a longitudinal ground section of macerated bone.

Identify:

Macroscopic

- (i) The unstained white-yellow compact bone.

Microscopic

- (i) Haversian and Volkmann's canals
(ii) Lamellae, lacunae and canaliculi

Draw and annotate:

A section of longitudinal ground bone to show Haversian and Volkmann's canals, lamellae, lacunae and canaliculi.

Questions:

1. Explain why the lacunae all lie parallel to one another in the specimen?

.....

.....

2. How does the course of the Haversian canal differ from the Volkmann's canal?

.....

.....

3.3.3. Ground Bone (Transverse Section)

Slide 33: This is a cross ground section of macerated bone.

Identify:

Macroscopic

- (i) Haversian system
- (ii) Haversian and Volkmann's canals
- (iii) Lacunae and Lamellae
- (iv) Canaliculi
- (v) Interstitial lamellar systems
- (vi) Circumferential lamellar systems

Draw and annotate:

A section of bone in which all three types of lamellae and their components are indicated.

Questions:

1. How are the lacunae and lamellae arranged in the three systems respectively?
.....
.....

2. How are the lacunae and lamellae arranged with respect to one another?
.....
.....

3. What course do the canaliculi take?
.....
.....

3.3.4. Bone Development

Slide 34: This specimen is a longitudinal section through the head of a rabbit's femur.

Identify:

Macroscopic

- (i) The orientation of the head of the femur, the epiphyseal plate and the shaft of the femur.

Microscopic

- (i) The different zones of endochondral bone development
- (ii) Osteoblasts and osteoclasts
- (ii) Bone marrow

Draw and annotate:

Each of the different zones of bone development.

Questions:

1. What is characteristic of each of the zones?

.....

.....

.....

2. What is characteristic of osteoblasts and osteoclasts and in which are they found?

.....

.....

.....

3. Does your specimen have a secondary ossification centrum?

.....

Revision Notes on Musculoskeletal System – Bone:

1. How are the functions of bones adapted to their features?

.....

.....

2. Name the 4 main functions of bone.

.....

.....

.....

.....

3. What is bone composed of?

.....

.....

4. What does a Haversian system consist of?

.....

.....

.....

5. In a longitudinal section of long bone, what is the orientation of Haversian and Volkmaan's canals?

.....

.....

.....

6. What are the differences between Haversian system and Volkmaan's canals?

.....

.....

.....

7. Where is an interstitial system present?

.....

8. Where are the inner and outer circumferential lamellae located?

.....

.....

.....

.....

9. Name the 4 main types of bone cells

.....

.....

.....

.....

10. Which cells are affected in osteosarcoma?

.....

.....

3.4. Blood Cells

The purpose of this practical session is to demonstrate the different blood cells and to do a differential white blood cell count.

3.4.1 Blood Smear

Slide 35: This is a blood smear to demonstrate red and white blood cells.

Identify:

Microscopic

- (i) Erythrocytes
- (ii) Neutrophils
- (iii) Eosinophils
- (iv) Basophils
- (v) Lymphocytes
- (vi) Monocytes

Draw and annotate:

The different blood cells and their distinguishing characteristics in detail.

Questions:

1. How are the different white blood cells distinguished from one another? Explain.

.....

.....

.....

.....

3.4.2. Differential White Blood Cell Count

To do a differential white blood cell count a blood smear must be made and stained as follows:

Procedure:

1. Sterilize a fingertip with alcohol and let dry completely since alcohol haemolises blood.
2. Prick the fingertip with a blood lancet deep enough to produce a drop of blood.
3. Carefully touch the drop of blood with one side of a clean glass slide.
4. Take a second glass slide and place the one side against the drop of blood so that the blood spreads along the edge.
5. With this second slide kept at an angle of 45° push the slide against the other slide to produce a blood smear. Do not use too large a drop of blood and do not make the angle of the slide too large as this will produce a thick unusable blood smear.
6. Let the smear dry.
7. Place the slide with the smear on a rack over a basin.
8. Draw 1ml Wrights staining fluid into a pipette and cover the blood smear carefully with the staining fluid. Be careful that the fluid does not run over the sides of the slide.
9. Stain the slide for 45 seconds.
10. Draw 1ml buffer into a pipette and add very carefully to the staining fluid on the slide.
11. Leave the slide to stain for 7 minutes.

12. Rinse quickly with tap water and carefully blot the slide dry. Do not wipe the slide as this will rub the blood smear off.
13. Let the slide dry well.

Results

Nuclei of leucocytes	-	dark and blue to purple
Eosinophilic granules	-	red to orange
Basophilic granules	-	dark purple to black
Lymphocytes	-	dark purple nuclei with light blue cytoplasm
Platelets	-	Violet to purple

3.4.3. Differential Counting

Counting takes place systematically, field after field, from left to right. If 200 cells have not been counted the first time across the slide, move the slide one field down and work across the slide this time from right to left. Continue in this way until 200 leucocytes have been counted.

All the different types of leucocytes must be counted and written down until 200 have been counted. The results are then given as a percentage of the total leucocytes counted.

Table I: Normal Differential Count in Adults

Type of cell	Total/mm	% of total leucocytes
Neutrophils	2500 – 7500	40 – 75
Eosinophils	40 – 440	1 – 6
Basophils	0 – 100	0 – 1
Lymphocytes	1500 – 3500	20 – 45
Monocytes	200 – 800	

Table II: Own Count

Type of cell	Total/mm	% of total leucocytes
Neutrophils		
Eosinophils		
Basophils		
Lymphocytes		
Monocytes		

3.4.4. Bone Marrow

Slide 36: This is a section through bone to demonstrate bone marrow.

Identify:

Microscopic

- (i) Bone
- (ii) Fat Cells
- (iii) Developing red and white blood cells
- (iv) Megakaryocytes, mature red blood cells, mature white blood cells

Draw and annotate:

Each of the different blood cells mentioned.

White Type	Nucleus	Cytoplasm	Granules
Eosinophil			
Neutrophil			
Lymphocyte			
Monocyte			

Questions:

1. What are the characteristics of each of the blood cells?

.....

.....

.....

Revision Notes on Blood Cell:

1. How are the functions of blood cells adapted to their features?

.....

.....

2. How are the functions of the bone marrow adapted to its features?

.....

.....

3. Name the 4 major elements of blood.

.....

.....

.....

4. Name the 5 types of white blood cells and tabulate their histological differences.

WBC Type	Nucleus	Cytoplasm	Granules
Neutrophils			
Eosinophils			
Basophils			
Lymphocytes			
Monocytes			

5. Name the precursor cells of platelets.

.....

6. What is the thrombocytopenia?

.....

7. What is the main site of haemopoiesis?

.....

8. Which blood disorder is the result of either impaired red cell formation or excessive red cell destruction?

.....