# Reading Material for
# Medical Lab. Technician
# (Elementary Anatomy & Micro techniques)



## Compiled By:

# Punjab Medical Faculty

**Specialized Healthcare & Medical Education Department**

**Government of the Punjab**

# PREFACE

A two years post matric teaching program of Medical Laboratory Technician for the students of Allied Health Sciences. The purpose of this reading material is to provide basic education to the paramedics about Elementary Anatomy & Micro techniques. This reading material attempts to cover almost all the basic theoretical knowledge required by students about Elementary Anatomy & Micro techniques so that they can perform their work better in Pathology laboratory and blood bank.

# Contents

# Section I Elementary Anatomy

# Chapter 1
# Introduction to Anatomy

## 1. Introduction

Human anatomy is the study and organization of the structures which make up the human body. Anatomy refers to the internal and external structures of the body and their physical relationships, whereas physiology refers to the study of the functions of those structures.

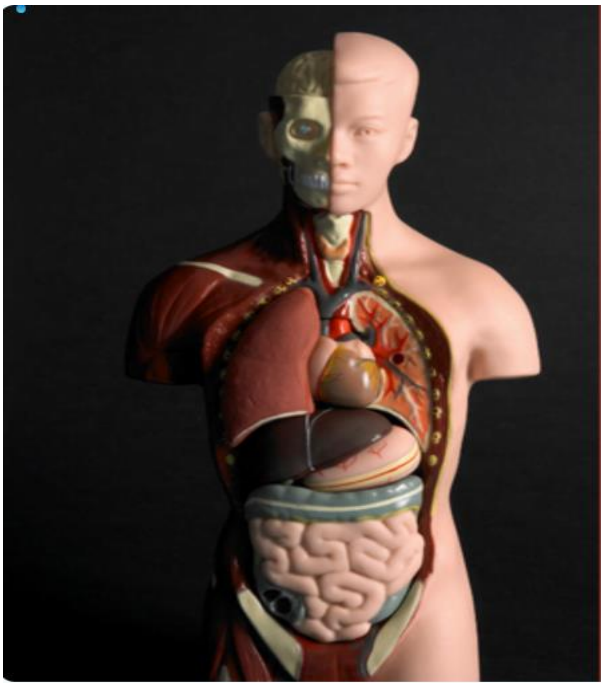

Figure 1.1: Human body anatomy

There are many ways to study Anatomy.

- **Regional anatomy** considers the body as organized into segments or parts.
- **Systemic anatomy** sees the body as organized into organ systems.
- **Surface anatomy** provides information about structures that may be observed or palpated beneath the skin.
- **Radiographic**, sectional and endoscopic anatomy allows appreciation of structures in the living, as they are affected by muscle tone, body fluids and pressures, and gravity.
- **Clinical anatomy** emphasizes application of anatomical knowledge to the practice of medicine.

Anatomy consists of various levels of complexity, from the microscopic level of cells to the macroscopic level of organs and organ systems.

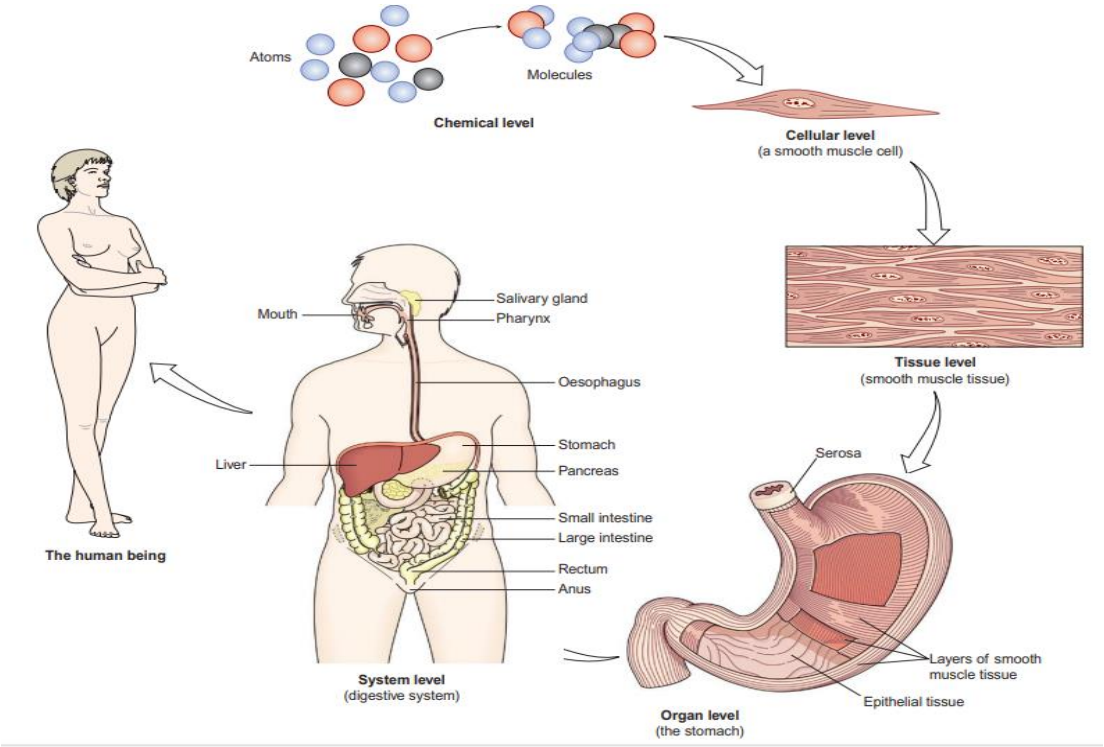


Figure 1.2: Anatomy of human body parts

## 1.1. Anatomical Terminology

The anatomical position refers to the body position as if the person were standing upright with the:

- head, gaze (eyes) and toes directed anteriorly (forward),
- arms adjacent to the sides with the palms facing anteriorly and
- lower limbs close together with the feet parallel.

## 1.2. Anatomical Planes

Anatomical descriptions are based on four imaginary planes (median, sagittal, frontal, and transverse) that intersect the body in the anatomical position as shown in figure 1.3:

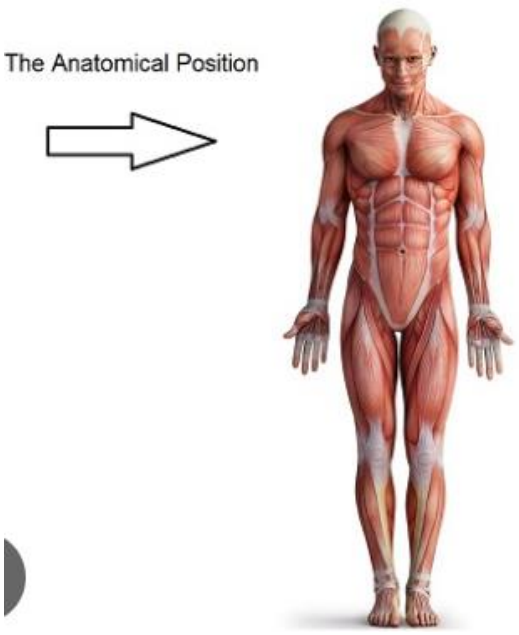


Figure 1.3: Anatomical Position

- The median plane, the vertical plane passing longitudinally through the body, divides the body into right and left halves as shown in figure 1.4.
- Sagittal planes are vertical planes passing through the body parallel to the median plane. However, a plane parallel and near to the median plane may be referred to as a paramedian plane.
- Frontal (coronal) planes are vertical planes passing through the body at right angles to the median plane, dividing the body into anterior (front) and posterior (back) parts.
- Transverse planes are horizontal planes passing through the body at right angles to the median and frontal planes, dividing the body into superior (upper) and inferior (lower) parts.
- Radiologists refer to transverse planes as transaxial, which is commonly shortened to axial planes.

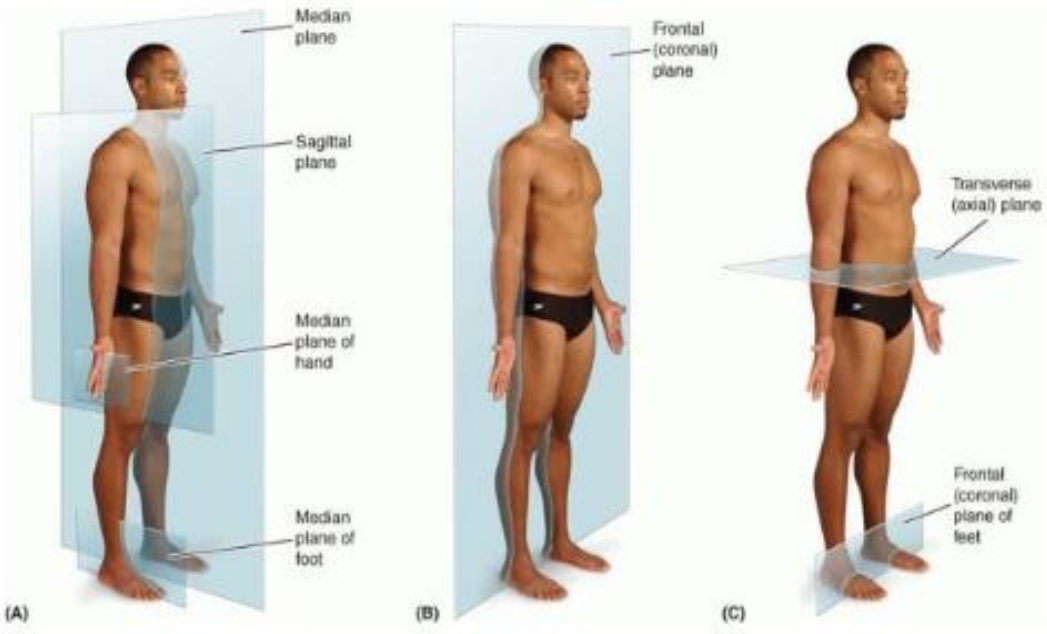


Figure 1.4: Anatomical planes: the main planes of the body

## 1.3. Anatomical terms of movement

These terms are used to describe the actions of muscles upon the skeleton. Muscles contract to produce movement at joints, and the subsequent movements can be precisely described using this terminology.
The terms used assume that the body begins in the anatomical position. Most movements have an opposite movement – also known as an antagonistic movement.

### 1.3.1. Flexion and Extension

Flexion and extension are movements that occur in the sagittal plane as shown in figure 1.5. They refer to increasing and decreasing the angle between two body parts:
**Flexion** refers to a movement that decreases the angle between two body parts. Flexion at the elbow is decreasing the angle between the ulna and the humerus. When the knee flexes, the ankle moves closer to the buttock, and the angle between the femur and tibia gets smaller.
**Extension** refers to a movement that increases the angle between two body parts. Extension at the elbow is increasing the angle between the ulna and the humerus. Extension of the knee straightens the lower limb.

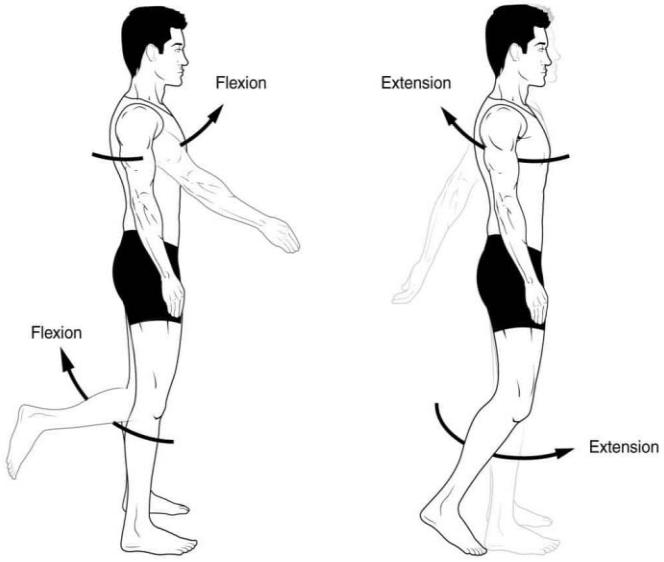


Figure 1.5: Flexion and extension movements

### 1.3.2. Abduction and Adduction

Abduction and adduction are two terms that are used to describe movements towards or away from the midline of the body as shown in figure 1.6.

**Abduction** is a movement away from the midline – just as abducting someone is to take them away. For example, abduction of the shoulder raises the arms out to the sides of the body.

**Adduction** is a movement towards the midline. Adduction of the hip squeezes the legs together.

In fingers and toes, the midline used is not the midline of the body, but of the hand and foot respectively. Therefore, abducting the fingers spreads them out.

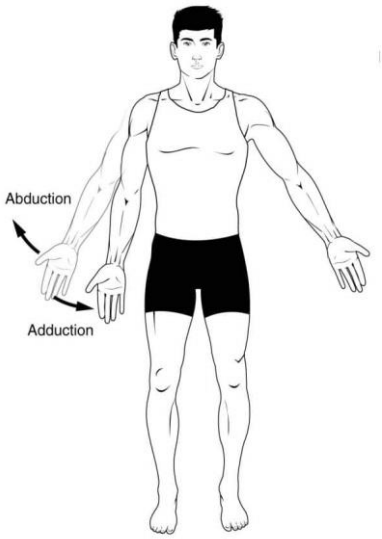


Figure 1.6: Abduction and adduction movements

### 1.3.3. Medial and Lateral Rotation

Medial and lateral rotation describe movement of the limbs around their long axis as shown in figure 1.7:

**Medial rotation** is a rotational movement towards the midline. It is sometimes referred to as internal rotation. To understand this, we have two scenarios to imagine. Firstly, with a straight leg, rotate it to point the toes inward. This is medial rotation of the hip. Secondly, imagine you are carrying a tea tray in front of you, with elbow at 90 degrees. Now rotate the arm, bringing your hand towards your opposite hip (elbow still at 90 degrees). This is internal rotation of the shoulder.

**Lateral rotation** is a rotating movement away from the midline. This is in the opposite direction to the movements described above.

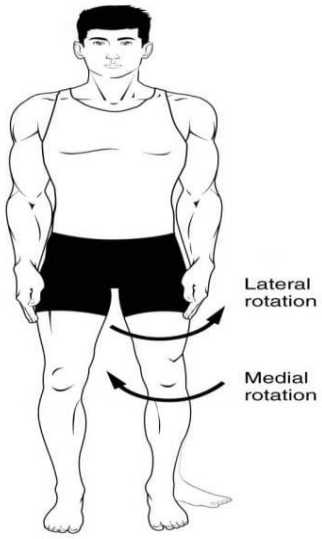


Figure 1.7: Medial and lateral movements

### 1.3.4. Elevation and Depression

Elevation refers to movement in a superior direction (e.g. shoulder shrug), depression refers to movement in an inferior direction.

### 1.3.5. Pronation and Supination

This is easily confused with medial and lateral rotation, but the difference is subtle. With your hand resting on a table in front of you, and keeping your shoulder and elbow still, turn your hand onto its back, palm up. This is the supine position, and so this movement is supination.

Again, keeping the elbow and shoulder still, flip your hand onto its front, palm down. This is the prone position, and so this movement is named pronation.

These terms also apply to the whole body – when lying flat on the back, the body is supine. When lying flat on the front, the body is prone.

### 1.3.6. Dorsiflexion and Plantarflexion

Dorsiflexion and plantarflexion are terms used to describe movements at the ankle. They refer to the two surfaces of the foot; the dorsum (superior surface) and the plantar surface (the sole) as shown in the figure 1.8.

**Dorsiflexion** refers to flexion at the ankle, so that the foot points more superiorly. Dorsiflexion of the hand is a confusing term, and so is rarely used. The dorsum of the hand is the posterior surface, and so movement in that direction is extension. Therefore, we can say that dorsiflexion of the wrist is the same as extension.

**Plantarflexion** refers extension at the ankle, so that the foot points inferiorly. Similarly, there is a term for the hand, which is palmar flexion.

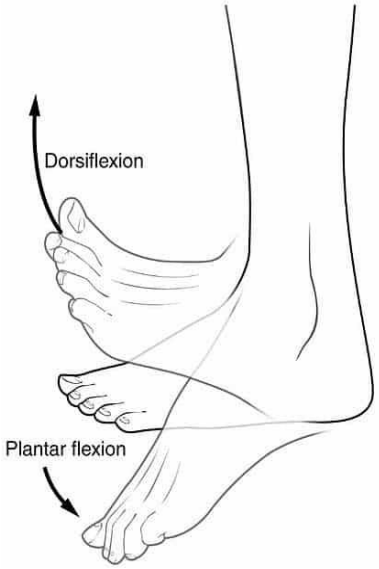


Figure 1.8: Dorsiflexion and plantarflexion movements

### 1.3.7. Inversion and Eversion

Inversion and eversion are movements which occur at the ankle joint, referring to the rotation of the foot around its long axis.

**Inversion** involves the movement of the sole towards the median plane – so that the sole faces in a medial direction.

**Eversion** involves the movement of the sole away from the median plane – so that the sole faces in a lateral direction.

### 1.3.8. Opposition and Reposition

A pair of movements that are limited to humans and some great apes, these terms apply to the additional movements that the hand and thumb can perform in these species.

Opposition brings the thumb and little finger together.
Reposition is a movement that moves the thumb and the little finger away from each other, effectively reversing opposition.

### 1.3.9. Circumduction

Circumduction can be defined as a conical movement of a limb extending from the joint at which the movement is controlled.
It is sometimes talked about as a circular motion, but is more accurately conical due to the ‘cone’ formed by the moving limb.

### 1.3.10. Protraction and Retraction

Protraction describes the anterolateral movement of the scapula on the thoracic wall that allows the shoulder to move anteriorly. In practice, this is the movement of ‘reaching out’ to something.
Retraction refers to the posteromedial movement of the scapula on the thoracic wall, which causes the shoulder region to move posteriorly i.e. picking something up.

## 1.4. Tissue

A human tissue is a group of cells with similar structure and specialized function. Tissues combine to form organs, and organs work together in organ systems. For instance, muscle tissue is composed of muscle cells, and the heart is an organ made up of various tissues, including muscle tissue, connective tissue, and nerve tissue.
The human body is composed of only four basic types of tissue: epithelial, connective, muscular, and nervous. These tissues, which are formed by cells and molecules of the extracellular matrix, exist not as isolated units but rather in association with one another and in variable proportions, forming different organs and systems of the body. The main characteristics of these basic types of tissue are shown in Table 1.1.

Table 1.1: Main characteristics of the four basic types of tissues

| Tissue | Cells | Extracellular Matrix | Main Functions |
|---|---|---|---|
| Nervous | Elongated cells with extremely fine processes | Very small amount | Transmission of nerve impulses |
| Epithelial | Aggregated polyhedral cells | Small amount | Lining of surface or body cavities; glandular secretion |
| Muscle | Elongated contractile cells | Moderate amount | Strong contraction; body movements |

| | | | |
|---|---|---|---|
| Connective | Several types of fixed and wandering cells | Abundant amount | Support and protection of tissues/ organs |

## 1.5. Organ System

Organs are structures composed of two or more tissue types, working together to perform specific functions.

Organ systems are groups of organs that collaborate to carry out particular physiological functions. Examples include the cardiovascular, respiratory, and digestive systems, excretory system, nervous system etc.

Major Organ Systems:

**Cardiovascular System:** Heart, blood vessels, and blood for circulation.

**Respiratory System:** Lungs and airways for breathing.

**Digestive System:** Organs like stomach, intestines, and liver for processing and absorbing nutrients.

**Nervous System:** Brain, spinal cord, and nerves for communication and control.

**Muscular System:** Muscles for movement and support.

**Skeletal System:** Bones and joints for support and protection.

**Endocrine System:** Glands like the thyroid and pancreas for hormone regulation.

### 1.6. Systemic Anatomy

Systemic anatomy is the study of the body's organ systems that work together to carry out complex functions.

i. The integumentary system (dermatology) consists of the skin and its appendages—hair, nails, and sweat glands, for example—and the subcutaneous tissue just beneath it.
ii. The skeletal system (osteology) consists of bones and cartilage; it provides our basic shape and support for the body and is what the muscular system acts on to produce movement. It also protects vital organs such as the heart, lungs, and pelvic organs.
iii. The articular system (arthrology) consists of joints and their associated ligaments, connecting the bony parts of the skeletal system and providing the sites at which movements occur.
iv. The muscular system (myology) consists of skeletal muscles that act (contract) to move or position parts of the body (e.g., the bones that articulate at joints), or smooth and cardiac muscle that propels, expels, or controls the flow of fluids and contained substance.

v. The nervous system (neurology) consists of the central nervous system (brain and spinal cord) and the peripheral nervous system (nerves and ganglia, together with their motor and sensory endings). The nervous system controls and coordinates the functions of the organ systems, enabling the body's responses to and activities within its environment. The sense organs, including the olfactory organ (sense of smell), eye or visual system (ophthalmology), ear (sense of hearing and balance—otology), and gustatory organ (sense of taste), are often considered with the nervous system in systemic anatomy.

vi. The circulatory system (angiology) consists of the cardiovascular and lymphatic systems, which function in parallel to transport the body's fluids.

   a. The cardiovascular system (cardiology) consists of the heart and blood vessels that propel and conduct blood through the body, delivering oxygen, nutrients, and hormones to cells and removing their waste products.
   b. The lymphatic system is a network of lymphatic vessels that withdraws excess tissue fluid (lymph) from the body's interstitial (intercellular) fluid compartment, filters it through lymph nodes, and returns it to the bloodstream.

vii. The alimentary or digestive system (gastroenterology) consists of the digestive tract from the mouth to the anus, with all its associated organs and glands that function in ingestion, mastication (chewing), deglutition (swallowing), digestion, and absorption of food and the elimination of the solid waste (feces) remaining after the nutrients have been absorbed.

viii. The respiratory system (pulmonology) consists of the air passages and lungs that supply oxygen to the blood for cellular respiration and eliminate carbon dioxide from it. The diaphragm and larynx control the flow of air through the system, which may also produce tone in the larynx that is further modified by the tongue, teeth, and lips into speech.

ix. The urinary system (urology) consists of the kidneys, ureters, urinary bladder, and urethra, which filter blood and subsequently produce, transport, store, and intermittently excrete urine (liquid waste).

x. The genital (reproductive) system (gynecology for females; andrology for males) consists of the gonads (ovaries and testes) that produce oocytes (eggs) and sperms, the ducts that transport them, and the genitalia that enable their union. After conception, the female reproductive tract nourishes and delivers the fetus.

xi. The endocrine system (endocrinology) consists of specialized structures that secrete hormones, including discrete ductless endocrine glands (such as the thyroid gland), isolated and clustered cells of the gut and blood vessel walls, and specialized nerve endings.

# Chapter 2
# Respiratory System

## 2. The Respiratory System

The respiratory system plays a vital role in gas exchange, providing oxygen to the body's cells and removing carbon dioxide. The respiratory system maintains homeostasis by adjusting the rate and depth of breathing to meet the body's oxygen demands. Disorders like asthma, chronic obstructive pulmonary disease (COPD), and pneumonia can affect respiratory function. The respiratory system is shown in figure 2.1.

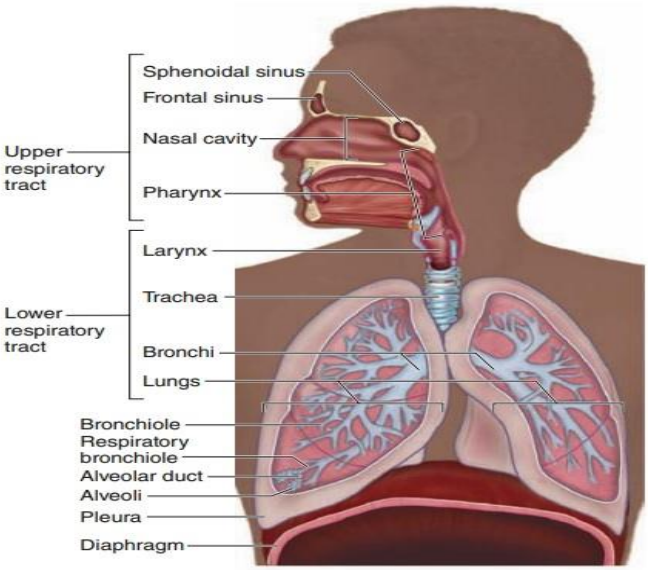


Figure 2.1: Human Respiratory System

## 2.1. Upper Respiratory Tract

The upper respiratory tract (URT) comprises the structures involved in the initial stages of breathing and the filtration of air before it reaches the lower respiratory tract. Key components of the URT include:

### 2.1.1. Nose

*Structure:* External nostrils lead to nasal cavities.
*Function:* Filters, humidifies, and warms incoming air; contains sensory receptors for the sense of smell.

### 2.1.2. Nasal Cavities:

*Structure:* Divided by the nasal septum; lined with mucous membranes and cilia.
*Function:* Filters, moistens, and warms inspired air; traps dust and pathogens.

### 2.1.3. Paranasal Sinuses:

*Structure:* Air-filled cavities in the bones surrounding the nasal cavities.
*Function:* Lighten the skull, produce mucus, and contribute to voice resonance.

### 2.1.4. Pharynx (Throat):

*Structure:* Connects nasal cavities and mouth to the larynx and esophagus.
*Function:* Common pathway for air and food; serves as a resonating chamber for speech.

### 2.1.5. Larynx (Voice Box):

*Structure:* Located below the pharynx; contains vocal cords.
*Function:* Houses vocal cords, allowing for the production of sound during speech.

The upper respiratory tract serves as the first line of defense against pathogens and particulate matter. The mucous membranes and cilia in the nasal cavities trap and move foreign particles, preventing them from entering the lower respiratory tract. Additionally, the nasal secretions contain enzymes and antibodies that contribute to immune defense. Common ailments affecting the upper respiratory tract include the common cold, sinusitis, and allergic rhinitis. These conditions often involve inflammation of the nasal passages, sinuses, or throat, leading to symptoms such as congestion, sneezing, and sore throat. Proper care and hygiene, including handwashing and avoiding exposure to respiratory viruses, help maintain the health of the upper respiratory tract.

## 2.2. Lower Respiratory Tract

The lower respiratory tract consists of several key components:

### 2.2.1. Trachea (Wind pipe)

A tubular structure that connects the larynx to the bronchi, allowing the passage of air to and from the lungs.

### 2.2.2. Bronchi

The trachea branches into two bronchi (singular: bronchus), one leading to each lung. These bronchi further divide into smaller branches called bronchioles.

### 2.2.3. Bronchioles

Smaller air passages that arise from the bronchi and extend into the lungs. They continue to divide into even smaller tubes, eventually leading to the alveoli.

### 2.2.4. Alveoli

Tiny air sacs located at the end of the bronchioles. This is where the exchange of oxygen and carbon dioxide takes place between the air and the bloodstream.

### 2.2.5. Lungs

The primary organs of the lower respiratory tract, consisting of lobes filled with bronchi, bronchioles, and alveoli. The right lung has three lobes, while the left lung has two.

### 2.2.6. Pleura

A double-layered membrane surrounding the lungs and lining the chest cavity. The space between these layers contains a small amount of fluid, which helps reduce friction during breathing.

The lower respiratory tract is crucial for the exchange of gases, allowing oxygen to be absorbed into the bloodstream and carbon dioxide to be expelled from the body. This process is essential for cellular respiration and maintaining proper oxygen levels in the body.

# Chapter 3
# Digestive System

# 3. The Gastrointestinal System

The gastrointestinal (GI) system, also known as the digestive system, is a long, twisting tube that starts at the mouth and goes through the oesophagus, stomach, small intestine, large intestine and ends at the anus.
The digestive system breaks down food into simple nutrients such as carbohydrates, fats and proteins. It is a complex network of organs responsible for processing food and extracting nutrients essential for the body's functioning.

## 3.1. Digestive Organs

The main digestive organs are shown in the figure 3.1.

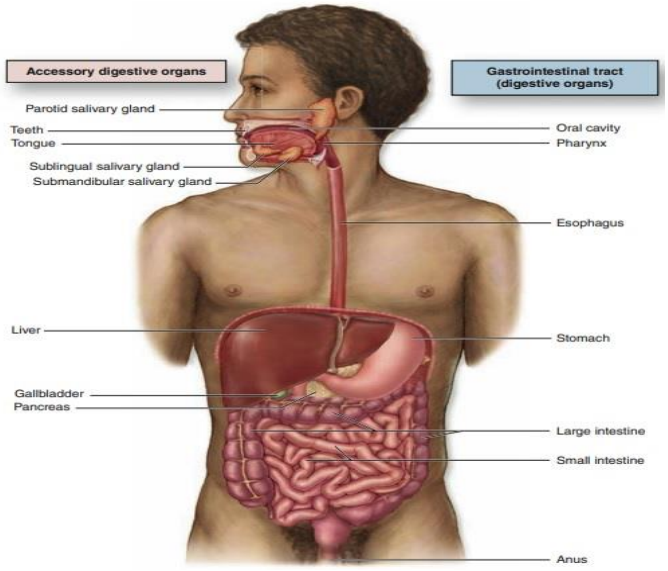


Figure 3.1: Digestive System of a human body

### 3.1.1. Mouth

*Function:* Mechanical and chemical breakdown of food begins with chewing and the action of saliva containing enzymes like amylase. The food is converted to semisolid Bolus.

### 3.1.2. Salivary Glands

Produce saliva to aid in digestion.

### 3.1.3. Esophagus

*Function:* Transports chewed food from the mouth to the stomach through coordinated muscle contractions called peristalsis.

### 3.1.4. Stomach

*Function:* Further digestion and mixing of food with gastric juices containing hydrochloric acid and enzymes. This forms Chyme.

### 3.1.5. Gastric Mucosa

Contains specialized cells secreting digestive enzymes and mucus.

### 3.1.6. Small Intestine

*Function:* Primary site for nutrient absorption. Divided into three parts: duodenum, jejunum, and ileum.

### 3.1.7. Villi and Microvilli

Increase surface area for absorption of nutrients into the bloodstream.

### 3.1.8. Liver

Function: Produces bile, which emulsifies fats for easier digestion. Detoxifies harmful substances and stores nutrients.

### 3.1.9. Gallbladder

Stores and releases bile into the small intestine.

### 3.1.10. Pancreas

Function: Produces digestive enzymes (lipases, amylases, proteases) and releases them into the small intestine.

### 3.1.11. Large Intestine (Colon)

Consists of Ascending colon, transverse colon, descending colon, sigmoid colon

*Function:* Absorbs water and electrolytes, forming feces. Houses beneficial bacteria that aid in fermentation of undigested food.

### 3.1.12. Rectum and Anus

Store and expel feces during the process of defecation.

### 3.1.13. Peritoneum:

Function: Thin membrane lining the abdominal cavity, providing support and protection to the digestive organs.

### 3.1.14. Hormones:

Gastrin, Insulin, Glucagon: Regulate digestive processes and nutrient absorption.
Secretin, Cholecystokinin (CCK): Control the release of digestive juices.

## 3.2. Nervous Control:

### 3.2.1. Enteric Nervous System:

Intrinsic nerve network regulating gut function.

### 3.2.2. Autonomic Nervous System:

Sympathetic and parasympathetic branches influence digestive activity.

Maintaining a healthy GI system is crucial for overall well-being, as it ensures proper nutrient absorption and waste elimination. Disorders like gastroenteritis, ulcers, and inflammatory bowel diseases can impact the functioning of the gastrointestinal system.

# Chapter 4
# The Urinary System

# 4. Urinary System

The human kidney has a distinct anatomy that includes various structures responsible for its vital functions as following

- Regulation of the balance between water and electrolytes (inorganic ions) and the acid-base balance.
- Excretion of metabolic wastes along with excess water and electrolytes in urine, the kidneys' excretory product which passes through the ureters for temporary storage in the bladder before its release to the exterior by the urethra.

## 4.1. Anatomy of Urinary System

The brief view of anatomy of urinary system is as follow

4.1.1. **Renal Cortex**

The outer region of the kidney, containing the renal corpuscles and convoluted tubules.

4.1.2. **Renal Medulla**

The inner region, composed of renal pyramids. The medulla houses structures like the loops of Henle and collecting ducts.

4.1.3. **Renal Pelvis**

A funnel-shaped structure at the center of the kidney that collects urine from the nephrons.

4.1.4. **Nephron**

The functional unit of the kidney, consisting of a renal corpuscle (Bowman's capsule and glomerulus) and renal tubule (proximal and distal convoluted tubules, loop of Henle).

4.1.5. **Renal Artery and Vein**

The renal artery brings blood to the kidney for filtration, while the renal vein carries purified blood away.

4.1.6. **Ureter**

A tube that connects the renal pelvis to the bladder, allowing the flow of urine from the kidneys to the bladder.

4.1.7. **Urinary Bladder**

A muscular organ that stores urine until it is released through the urethra.

4.1.8. **Urethra**

A tube through which urine is expelled from the bladder out of the body.

## 4.2. Location of Kidneys

The cross section of a kidney is shown in figure 4.1.

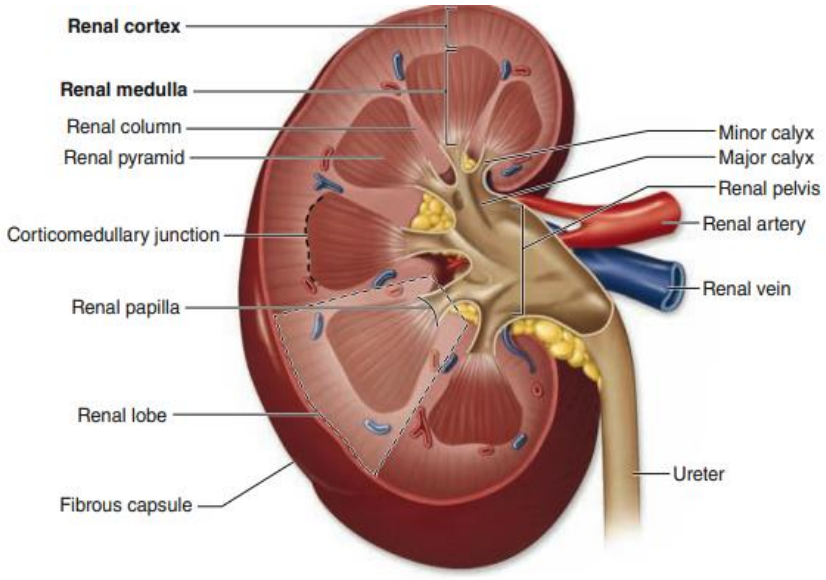


Figure 4.1: Human kidney

The Kidneys lie behind the peritoneum in the abdomen, either side of vertebral column. They extend from T12 to L3, right kidney is slightly lower than the left due to the liver's position. Both kidneys function similarly, filtering blood and producing urine.

# Chapter 5
# The Circulatory System

## 5. The Circulatory System

The circulatory system, also known as the cardiovascular system as shown in figure 5.1, is a complex network of organs and vessels that transport blood, nutrients, oxygen, and waste products throughout the body. Comprising the heart, blood vessels, and blood, this system plays a crucial role in maintaining homeostasis.

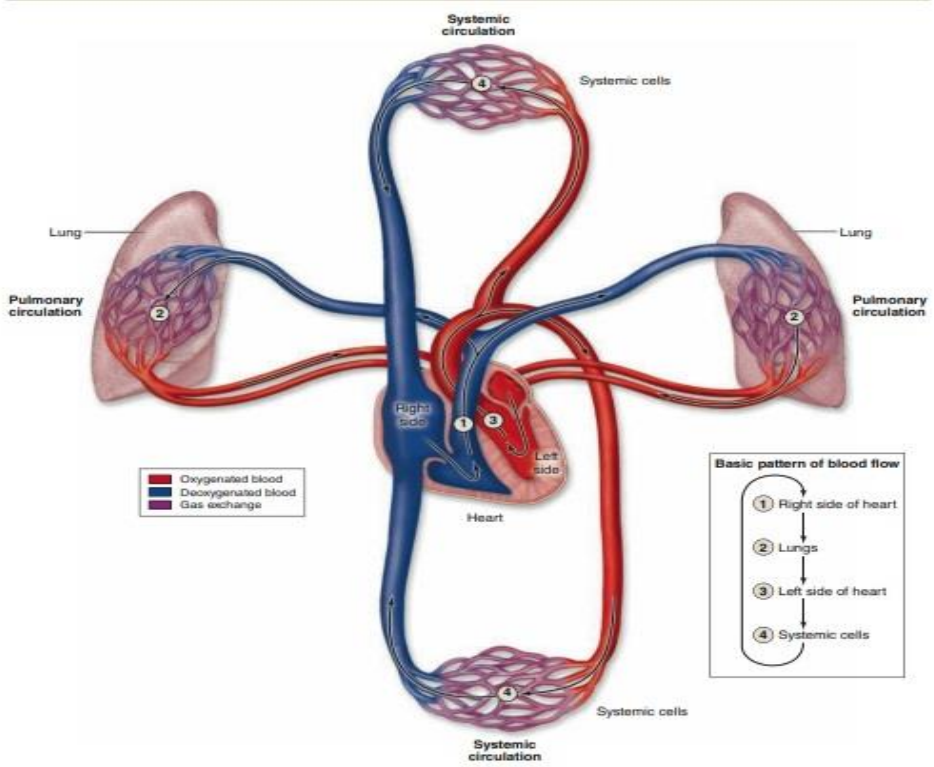


Figure 5.1: Cardiovascular system

### 5.1. Heart

The heart, a muscular organ, serves as the central pump of the circulatory system.
It is divided into four chambers: two atria (upper chambers) and two ventricles (lower chambers). Deoxygenated blood from the body returns to the right atrium and is pumped to the lungs for oxygenation by the right ventricle. Oxygenated blood from the lungs enters the left atrium and is pumped to the rest of the body by the left ventricle.

### 5.2. Blood Vessels:

There are three types of blood vessels

5.2.1. **Arteries**
Carry oxygenated blood away from the heart to the body's tissues, except the pulmonary arteries which carry deoxygenated blood from heart to lungs. The largest artery is the aorta.
5.2.2. **Veins**
Transport deoxygenated blood back to the heart, except the pulmonary veins which carry oxygenated blood from lungs to heart. The superior and inferior vena cava are major veins.
5.2.3. **Capillaries**
Microscopic vessels connecting arteries and veins, facilitating the exchange of nutrients and waste products at the tissue level.

## 5.3. Blood:

Composed of red blood cells (carry oxygen), white blood cells (immune defense), platelets (blood clotting), and plasma (fluid medium).
The blood's main function is to transport oxygen, nutrients, hormones, and waste products throughout the body.

## 5.4. Circulation:

5.4.1. **Systemic Circulation**
The left side of the heart pumps oxygenated blood to the body through the aorta, and the deoxygenated blood returns via veins.
5.4.2. **Pulmonary Circulation**: The right side of the heart pumps deoxygenated blood to the lungs through the pulmonary artery, and oxygenated blood returns via pulmonary veins.

### 5.4.3. Regulation:

The circulatory system is regulated by the autonomic nervous system and hormones.
Hormones such as adrenaline can increase heart rate and blood pressure during stress or exercise.

### 5.4.4. Homeostasis:

The circulatory system contributes to maintaining a stable internal environment by regulating temperature, pH, and fluid balance.
5.4.5. **Diseases and Disorders**
Conditions like atherosclerosis, hypertension, and heart failure can adversely affect the circulatory system.

# Chapter 6
# Nervous System

## 6. General Design of Nervous System:

The nervous system is a complex network of cells that enables communication and coordination throughout the body. The nervous system functions to receive, process, and respond to stimuli, allowing for coordinated movement, sensory perception, and maintenance of homeostasis as shown in figure 6.1. Its intricate design ensures efficient communication and adaptability to changing environmental conditions.

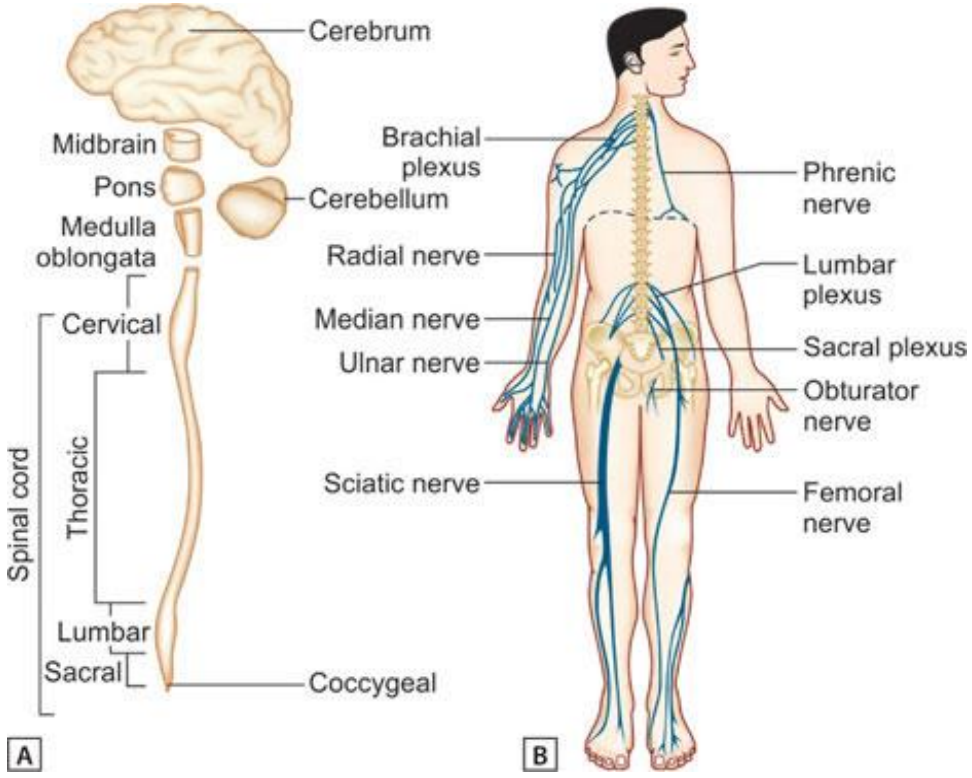


Figure 6.1: (A) The main division of the central nervous system, (B) The part of the peripheral nervous system

## 6.1. Types of Nervous System

Its general design involves two main components: the central nervous system (CNS) and the peripheral nervous system (PNS).

### 6.1.1. Central Nervous System (CNS)

#### 6.1.1.1. *Brain and Spinal Cord*

The CNS consists of the brain and spinal cord, which are protected by bone (skull and vertebral column) and three layers of meninges. The brain is housed within the skull, while the spinal cord is encased in the vertebral column.

#### 6.1.1.2. *Integration and Processing*

The CNS is responsible for integrating and processing information. The brain interprets sensory input, initiates motor responses, and is the center for higher functions such as learning, memory, and emotions.

#### 6.1.1.3. *Command Center*

It serves as the command center that controls and coordinates activities throughout the body.

### 6.1.2. Peripheral Nervous System (PNS)

#### 6.1.2.1. *Nerves and Ganglia*

The PNS includes nerves and ganglia outside the CNS. Nerves are bundles of nerve fibers (axons) that transmit signals between the CNS and various parts of the body. Ganglia are clusters of nerve cell bodies.

#### 6.1.2.2. *Somatic and Autonomic Divisions*

The PNS is divided into the somatic nervous system (SNS), responsible for voluntary movements and sensory perception, and the autonomic nervous system (ANS), which regulates involuntary functions like heart rate, digestion, and respiratory rate.

#### 6.1.2.3. *Cranial and Spinal Nerves*

The PNS includes cranial nerves arising from the brain and spinal nerves arising from the spinal cord. These nerves connect the CNS to muscles, organs, and sensory receptors.

## 6.2. Neurons:

Neurons are the fundamental building blocks of the nervous system. They are the basic Structural and Functional Units. They receive, process, and transmit information in the form of electrical impulses.

6.2.1. **Structure of Neurons**: Neurons typically consist of a cell body (soma), dendrites that receive signals, and an axon that transmits signals to other neurons or target cells.

### 6.2.2. Glial Cells:

Glial cells provide support, protection, and nourishment to neurons.

#### 6.2.2.1. *Types of Glial Cells*

Glial cells include astrocytes (support and nutrient transfer), oligodendrocytes and Schwann cells (produce myelin, which insulates axons), microglia (immune defense), and ependymal cells (produce cerebrospinal fluid).

### 6.2.3. Synapses

Neurons communicate with each other at synapses. The axon terminal of one neuron releases neurotransmitters into the synapse, which bind to receptors on the dendrites or cell body of the next neuron, transmitting the signal.

### 6.3. Cerebrospinal Fluid (CSF)

CSF surrounds the brain and spinal cord, providing buoyancy and protection against mechanical shocks.

## 6.4. Functions of Brain

The brain, as the central organ of the nervous system, performs a multitude of complex functions that are essential for the overall functioning of the body. Here are some key functions of the brain:

#### 6.4.1. Cognition

The brain is the seat of cognitive functions, including thinking, reasoning, problem-solving, and decision-making.

#### 6.4.2. Memory

It plays a crucial role in the formation, storage, and retrieval of memories, allowing for learning and adaptation based on past experiences.

#### 6.4.3. Motor Control

The brain is responsible for initiating and coordinating voluntary movements and motor functions. Motor areas in the cerebral cortex plan and execute movements.

#### 6.4.4. Sensory Processing

The brain processes sensory information received from the environment, allowing individuals to perceive and interpret various stimuli, including sight, sound, touch, taste, and smell.

#### 6.4.5. Emotion Regulation

It regulates emotions, influencing mood, motivation, and responses to emotional stimuli. Limbic system structures, such as the amygdala and hippocampus, play key roles in emotional processing.

#### 6.4.6. Homeostasis

The brain maintains internal balance and stability through the regulation of physiological processes such as body temperature, blood pressure, and hormonal levels.

#### 6.4.7. Autonomic Functions

It controls involuntary processes such as heart rate, respiratory rate, digestion, and other autonomic functions through the autonomic nervous system.

#### 6.4.8. Language Processing

The brain's language centers, including Broca's area and Wernicke's area, are involved in language comprehension, production, and communication.

#### 6.4.9. Attention and Concentration

The brain enables the ability to focus attention, sustain concentration, and shift attention as needed.

**6.4.10. Sleep Regulation:** It regulates sleep-wake cycles and various stages of sleep, impacting overall restorative functions and memory consolidation.

**6.4.11. Learning and Adaptation**

The brain is vital for learning new information, skills, and behaviors, facilitating adaptation to changing environments.

**6.4.12. Social and Interpersonal Skills**

It plays a role in social cognition, allowing individuals to understand and navigate social interactions, interpret others' emotions, and form social bonds.

**6.4.13. Higher Cognitive Functions**

The prefrontal cortex is involved in executive functions such as planning, organizing, problem-solving, and self-control.

**6.4.14. Creativity and Innovation**

The brain supports creative thinking and innovation by forming

## 6.5. Functions of Spinal Cord

The spinal cord is a vital part of the central nervous system, connecting the brain to the peripheral nervous system and serving essential functions in communication and coordination. Here are detailed functions of the spinal cord:

**6.5.1. Conduction of Nerve Signals**

The primary function of the spinal cord is to conduct nerve signals between the brain and the rest of the body. Nerve impulses travel along the spinal cord's neural pathways, transmitting sensory information from the periphery to the brain and motor commands from the brain to muscles and glands.

**6.5.2. Reflex Integration**

The spinal cord plays a crucial role in reflex actions. Reflexes are rapid, involuntary responses to stimuli that do not require conscious thought.

**6.5.3. Motor Function**

The spinal cord controls voluntary movements and motor functions. Motor neurons in the spinal cord transmit signals from the brain to muscles, enabling coordinated movements of the limbs and other body parts.

**6.5.4. Sensory Function**

Sensory neurons convey information from various body parts to the spinal cord. These sensory signals, such as pain, temperature, touch, and proprioception, are then transmitted to the brain for interpretation and response.

**6.5.5. Coordination of Reflex Arcs**

The spinal cord coordinates reflex arcs, which involve sensory neurons, interneurons in the spinal cord, and motor neurons. This coordination allows for rapid, automatic responses to stimuli, such as withdrawing from a painful stimulus.

**6.5.6. Ascending and Descending Tracts**

Ascending tracts carry sensory information from the periphery to the brain, while descending tracts transmit motor commands from the brain to peripheral effectors (muscles or glands).

#### 6.5.7. Transmission of Pain Signals

The spinal cord is a key player in the transmission of pain signals. Nociceptive information (pain) is conveyed through specific pathways to the brain for perception and response.

#### 6.5.8. Integration of Sensory Information

The spinal cord integrates sensory information received from various parts of the body, contributing to a coherent perception of the external environment and the body's internal state.

#### 6.5.9. Autonomic Nervous System Control

The spinal cord is involved in the control of the autonomic nervous system (ANS), which regulates involuntary functions such as heart rate, digestion, and respiratory rate.

#### 6.5.10. Initiation of Simple Movements

The spinal cord can initiate simple movements without direct input from the brain, such as walking or reflexive responses.

#### 6.5.11. Transmission of Sympathetic and Parasympathetic Signals

Autonomic signals for the sympathetic and parasympathetic branches of the ANS pass through the spinal cord, influencing various physiological processes.

While the brain is responsible for higher cognitive functions, the spinal cord focuses on the rapid transmission of signals and reflex activities, playing a pivotal role in basic sensory-motor coordination and response to environmental stimuli.

## 6.6. Functions of Cranial Nerves

The cranial nerves are a set of twelve pairs of nerves that emerge directly from the brain and primarily serve functions related to the head and neck. Each cranial nerve has specific roles, involving sensory, motor, or both functions. Here are the functions of the cranial nerves in detail:

#### 6.6.1. Olfactory Nerve

*Function:* Responsible for the sense of smell.

*Pathway:* Olfactory receptors in the nasal cavity transmit signals to the olfactory bulb and then to higher olfactory centers in the brain.

#### 6.6.2. Optic Nerve

*Function:* Carries visual information from the retina to the brain.

*Pathway:* Axons of the retinal ganglion cells form the optic nerve, which transmits signals to the optic chiasm and then to the visual centers in the brain.

#### 6.6.3. Oculomotor Nerve

*Motor:* Controls most eye movements, including raising the eyelid, directing the eyeball, and constricting the pupil.

*Parasympathetic:* Regulates the size of the pupil and shape of the lens for near vision.

### 6.6.4. Trochlear Nerve

*Function:* Primarily involved in the movement of the eyeball.

*Pathway:* Controls the superior oblique muscle, aiding in downward and inward eye movements.

### 6.6.5. Trigeminal Nerve

*Sensory:* Responsible for sensation in the face (ophthalmic, maxillary, and mandibular divisions).

*Motor:* Controls muscles involved in chewing (mastication).

### 6.6.6. Abducent Nerve

*Function:* Primarily involved in the lateral movement of the eyeball.

*Pathway:* Controls the lateral rectus muscle, aiding in outward eye movement.

### 6.6.7. Facial Nerve

*Sensory:* Involved in taste sensation from the anterior two-thirds of the tongue.

*Motor:* Controls facial muscles, expression, and secretion of saliva and tears.

*Parasympathetic:* Regulates salivary and lacrimal glands.

### 6.6.8. Vestibulocochlear Nerve

*Vestibular Branch:* Involved in balance and spatial orientation.

*Cochlear Branch:* Responsible for hearing.

### 6.6.9. Glossopharyngeal Nerve

*Sensory:* Involved in taste sensation from the posterior one-third of the tongue.

*Motor:* Controls muscles involved in swallowing.

Parasympathetic: Regulates salivary glands.

### 6.6.10. Vagus Nerve

*Sensory:* Involved in visceral sensations (e.g., from organs in the thoracic and abdominal cavities).

*Motor:* Controls muscles involved in speech and swallowing.

*Parasympathetic:* Regulates many organs in the thoracic and abdominal cavities.

### 6.6.11. Accessory Nerve

*Cranial Part:* Controls muscles involved in head movement.

*Spinal Part:* Controls muscles of the neck and shoulder.

### 11.6.12. Hypoglossal Nerve

*Function:* Controls muscles of the tongue, primarily involved in speech and swallowing.

These cranial nerves are crucial for various sensory and motor functions in the head and neck, contributing to sensory perception, facial expressions, hearing, balance, taste, and many other essential activities.

# Chapter 7
# Reproductive System

# 7. Reproductive System

The major function of the reproductive system is to ensure survival of the species. Other systems in the body, such as the endocrine and urinary systems, work continuously to maintain homeostasis for survival of the individual. An individual may live a long, healthy, and happy life without producing offspring, but if the species is to continue, at least some individuals must produce offspring.

## 7.1. The Male Reproductive System

The male reproductive system consists of several organs that work together for the production and delivery of sperm as shown in the figure 7.1.

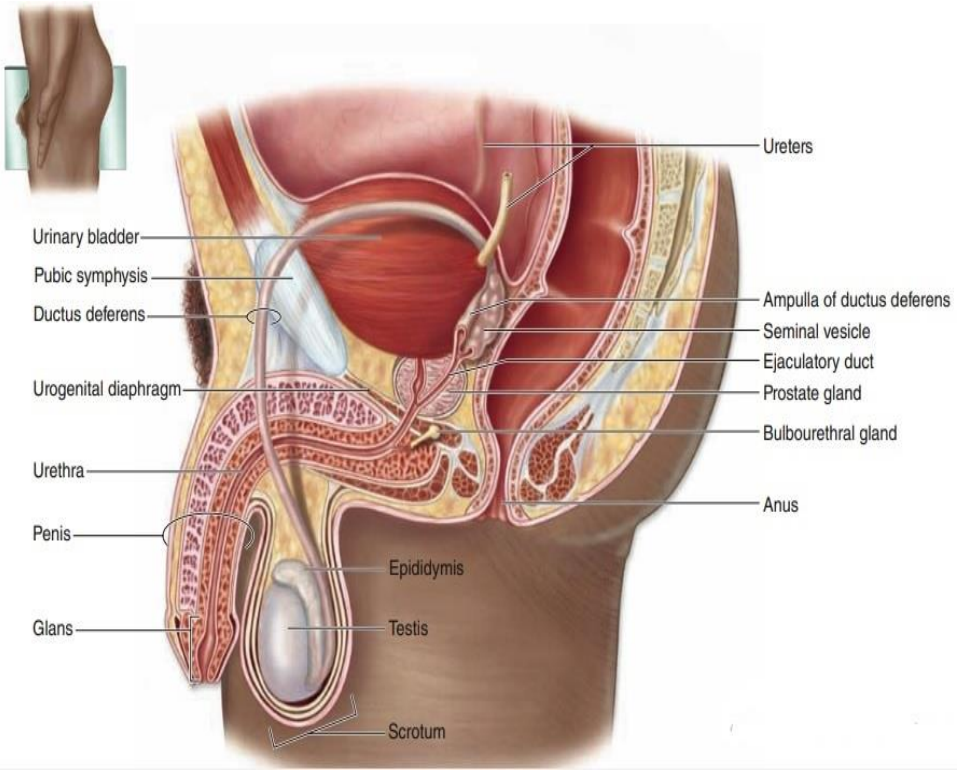


Figure 7.1: The male reproductive system

### 7.1.1. Testes

*Anatomy:* Paired organs located in the scrotum, responsible for sperm and testosterone production.
*Physiology:* Sperm production (spermatogenesis) occurs in the seminiferous tubules, while Leydig cells produce testosterone.

### 7.1.2. Epididymis

*Anatomy:* Coiled tube attached to each testis.

*Physiology:* Sperm mature and gain motility while stored in the epididymis.

### 7.1.3. Vas Deferens

*Anatomy:* Duct that transports mature sperm from the epididymis to the urethra.
*Physiology:* Sperm travel through the vas deferens during ejaculation.

### 7.1.4. Seminal Vesicles

*Anatomy:* Glandular structures near the base of the bladder.
*Physiology:* Produce seminal fluid, contributing to semen volume and providing nutrients for sperm.

### 7.1.5. Prostate Gland

The prostate gland is a part of the male reproductive system, situated just below the bladder and surrounding the urethra. It plays a crucial role in the production of seminal fluid. Here's a detailed overview:

#### 7.1.5.1. Location

The prostate is located in the pelvis, beneath the bladder and in front of the rectum.

#### *7.1.5.2.* Structure

The prostate is a walnut-sized gland with several lobes. It is composed of glandular and fibromuscular tissue.

#### 7.1.5.3. Function

The primary function of the prostate is to produce a fluid that, along with sperm from the testes and seminal vesicle fluids, makes up semen. This fluid helps nourish and transport sperm during ejaculation.

#### 7.1.5.4. Structure of Prostate

*Peripheral Zone:* The outer part of the prostate, where most prostate cancers originate.
Central Zone: Surrounds the ejaculatory ducts and makes up a smaller portion of the gland.
*Transitional Zone:* Located near the urethra, this zone is where benign prostatic hyperplasia (BPH) typically occurs.

#### 7.1.5.5. Ducts and Glands

The prostate contains numerous ducts and glands that produce the prostatic fluid. This fluid is released into the urethra during ejaculation.
Muscles: The fibromuscular tissue of the prostate helps propel semen during ejaculation.

#### 7.1.5.6. Nerve Supply

The prostate receives its nerve supply from the autonomic nervous system, which plays a role in controlling its functions.

#### 7.1.5.7. Prostate-Specific Antigen (PSA)

A blood test measuring PSA levels is often used as a screening tool for prostate conditions, including prostate cancer.
Problems: The prostate can be affected by various conditions, such as benign prostatic hyperplasia (BPH), prostatitis (inflammation), and prostate cancer.

#### 7.1.5.8. Bulbourethral Glands (Cowper's Glands)

Anatomy: Small glands near the base of the penis.
Physiology: Release a clear, lubricating fluid before ejaculation, preparing the urethra for sperm passage.

### 7.1.6. Penis

*Anatomy:* External organ with erectile tissues.

*Physiology:* During sexual arousal, blood flow increases to erectile tissues, causing an erection. The urethra passes through the penis, allowing the passage of semen during ejaculation.

### 7.1.7. Scrotum

A pouch of skin and muscle that houses and protects the testes. The temperature of the scrotum is lower than the body temperature, which is crucial for sperm production.

### 7.1.8. Sperm

The male reproductive cells. Sperm carry the genetic material needed for fertilization. The coordinated function of these organs ensures the production, maturation, and delivery of sperm, contributing to the male's role in reproduction.

### 7.1.9. Testosterone and other Male Sex Hormones

Testosterone is the primary male sex hormone and belongs to a class of hormones called androgens. Here's an overview of testosterone and other important male sex hormones:

#### *7.1.9.1. Testosterone*

Produced mainly by the testes, with a small amount also produced by the adrenal glands.
*Functions:* Development of Male Reproductive Organs: Testosterone is crucial for the development and maintenance of the male reproductive system, including the testes, epididymis, vas deferens, and penis.
*Secondary Sexual Characteristics:* It promotes the development of secondary sexual characteristics such as facial and body hair, deepening of the voice, and increased muscle mass.
*Sperm Production:* Testosterone stimulates the production of sperm in the testes.
*Libido (Sex Drive):* It plays a role in regulating sexual desire and libido.

#### *7.1.9.2. Dihydrotestosterone (DHT)*

Derived from testosterone through the action of the enzyme 5-alpha reductase.
*Functions:* Development of Male External Genitalia: DHT is essential for the development of the penis and the growth of the prostate gland during fetal development.

## 7.2. Female Reproductive System

The female reproductive system is responsible for producing, nurturing, and delivering ova (eggs) and supporting the development of a fertilized egg into a fetus. Female reproductive system is shown in figure 7.2. The female reproductive system is complex and undergoes significant changes throughout a woman's life, from puberty to menopause. Its primary function is to support reproduction and the continuation of the species.

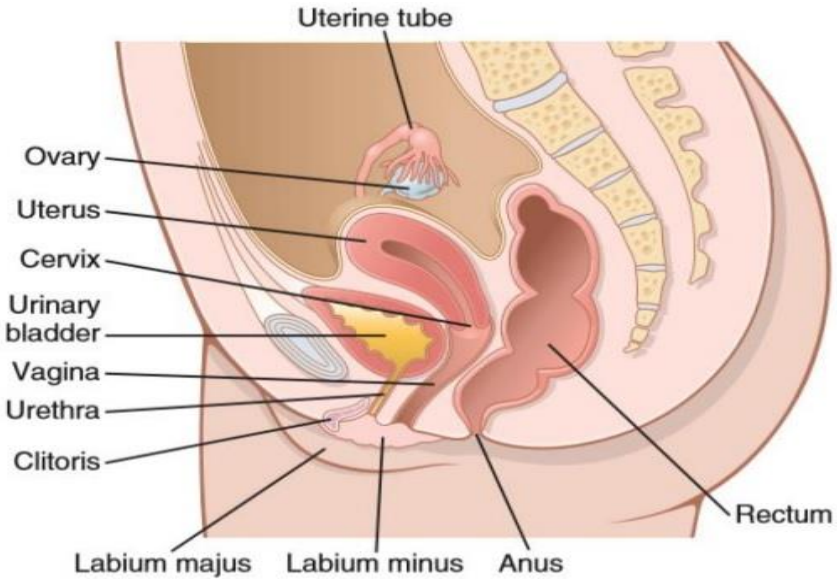


Figure 7.2: The female reproductive system

### 7.2.1. Ovaries

The primary female reproductive organs.

*Egg Production:* Ovaries produce and release eggs (ova) during the menstrual cycle.

*Hormone Production:* Ovaries secrete estrogen and progesterone, which regulate the menstrual cycle and play a crucial role in pregnancy.

### 7.2.2. Fallopian Tubes

Serve as pathways for eggs to travel from the ovaries to the uterus.

*Site of Fertilization*: Fertilization typically occurs in the fallopian tubes when a sperm meets an egg.

### 7.2.3. Uterus

A muscular organ where a fertilized egg implants and grows during pregnancy. The lining of the uterus thickens during the menstrual cycle, preparing for a potential pregnancy. If no pregnancy occurs, the lining is shed during menstruation.

### 7.2.4. Cervix

The lower part of the uterus that connects to the vagina.
*Mucus Production*: The cervix produces mucus that changes in consistency throughout the menstrual cycle to facilitate or inhibit the passage of sperm**.**

**7.2.5. Vagina**

The birth canal and the site for sexual intercourse. The vagina serves as the exit for menstrual blood during menstruation.

**7.2.6. External Genitalia (Vulva)**

Includes the labia, clitoris, and other structures that protect and enclose the openings of the urethra and vagina.

**7.2.7. Placenta (During Pregnancy)**

An organ that develops during pregnancy and provides nutrients and oxygen to the fetus, removes waste products, and produces hormones.

**7.2.8. Hormonal Regulation**

Function: Hormones such as estrogen and progesterone play key roles in regulating the menstrual cycle, pregnancy, and various physiological processes.

7.2.8.1. *Estrogen*

Produced primarily by the ovaries, especially during the follicular phase of the menstrual cycle. Estrogen plays a key role in the development of female secondary sexual characteristics, regulation of the menstrual cycle, and support of reproductive tissues.

7.2.8.2. *Progesterone*

Produced by the ovaries, particularly during the luteal phase of the menstrual cycle. Progesterone prepares the uterine lining for potential embryo implantation and supports early pregnancy.

**7.2.9. Menstrual Cycle**

*Function:* A regular cycle involving hormonal changes that prepare the body for potential pregnancy.
*Ovulation:* Release of an egg from the ovary, typically occurring around the midpoint of the menstrual cycle.
*Follicular Phase:* Initiated by increased levels of estrogen, leading to the development and maturation of ovarian follicles. This phase culminates in ovulation.
*Luteal Phase:* Triggered by the rise in progesterone after ovulation. Progesterone prepares the uterine lining for a potential pregnancy.

**7.2.10. Fertilization:**

If an egg is fertilized by sperm during ovulation, it forms a zygote that travels down the fallopian tube.

**7.2.11. Implantation:**

The fertilized egg implants itself into the thickened uterine lining.

### 7.2.12. Pregnancy changes

The developing placenta produces human chorionic gonadotropin (hCG), signaling the corpus luteum to continue producing progesterone and estrogen, sustaining the early pregnancy.

### 7.2.13. Pregnancy Hormones

Throughout pregnancy, the placenta produces increasing amounts of estrogen, progesterone, and other hormones to support fetal development and maintain the pregnancy.

### 7.2.14. Lactational changes

#### 7.2.14.1. *Prolactin*:

Produced by the pituitary gland, prolactin stimulates milk production in the mammary glands.

#### 7.2.14.2. *Oxytocin*

Also produced by the pituitary gland, oxytocin triggers the letdown reflex, causing the release of milk from the mammary glands.

#### 7.2.14.3. *Colostrum*

The initial milk produced in the first few days after childbirth, rich in nutrients and antibodies.

#### 7.2.14.4. *Mature Milk*

As lactation continues, mature milk is produced, providing complete nutrition for the infant.

### 7.2.15. Postpartum Changes:

Hormonal Shifts: After childbirth, hormonal changes occur, with a decrease in estrogen and progesterone levels.

### 7.2.16. Hormonal Regulation:

The hypothalamus releases gonadotropin-releasing hormone (GnRH), stimulating the pituitary gland to release luteinizing hormone (LH) and follicle-stimulating hormone (FSH). LH stimulates testosterone production in the testes, and FSH stimulates spermatogenesis. The coordinated function of these structures allows for the production, maturation, and delivery of sperm, contributing to the male reproductive system's overall anatomy and physiology.

# Chapter 8
# Human Skeletal System

# 8. Bone

Bone is a mineralized dense connective tissue that is made up of few cells in mineralized matrix Consisting of 30-40 % of our body weight.

## 8.1. Functions of Bones

Following are the main functions of the bone.

- Support, Protection & Movement:
- Gives shape to the body.
- Supports body weight.
- Protects sensitive parts of the body.
- Blood Cell Formation:
- The red bone marrow found in the connective tissue of certain bones is the site of blood cell production.

## 8.2. Human skeleton

There are total 206 bones which make up the human skeleton. The skeletal is divided into two main parts, the axial skeleton and the appendicular skeleton.

### 8.2.1. The Axial Skeleton

The axial skeleton consists of 80 bones includes the skull, vertebral column, and rib cage as shown in the figure 8.1.

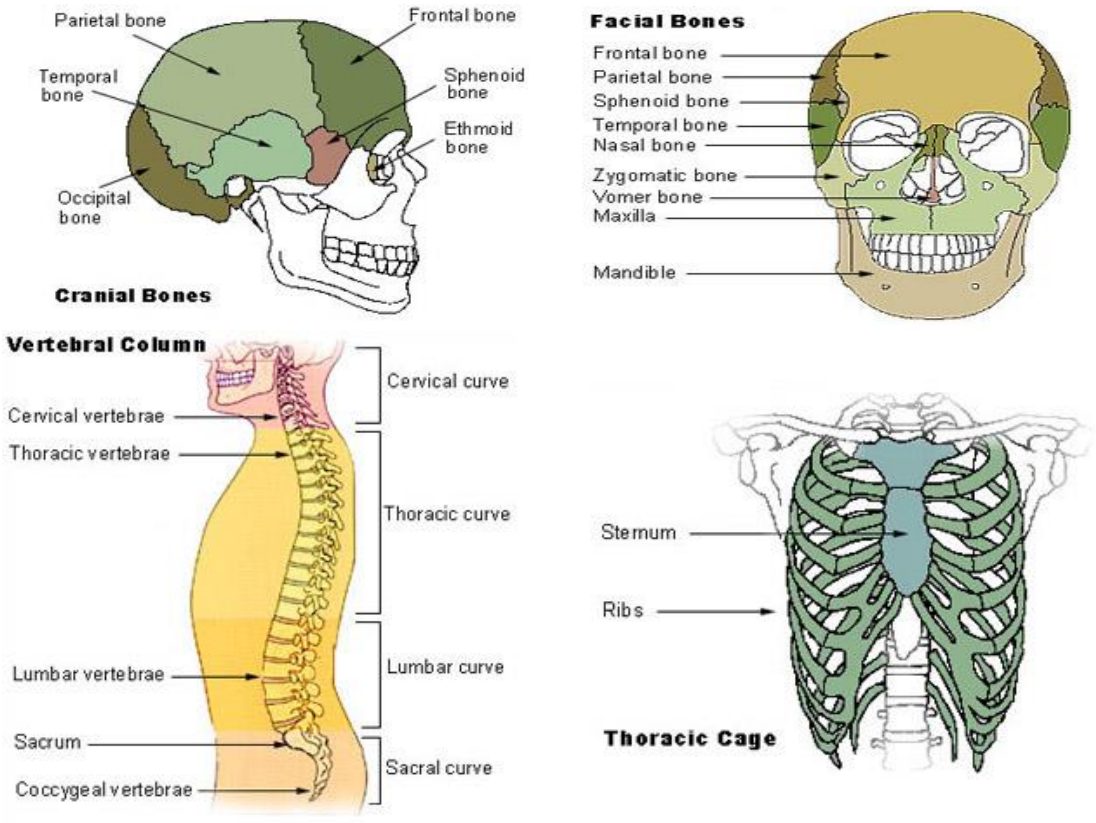


Figure 8.1: Axial Skeleton bones

The axial skeleton forms the vertical axis of the body. The skull consists of 8 cranial bones and 14 facial bones. The vertebral column consists of 26 bones, rib cage consists of 25 bones and other 7 bones.

### 8.2.2. The Appendicular Skeleton

The appendicular skeleton consists of 126 bones comprises limbs, shoulders, and hips as shown in the figure 8.2.

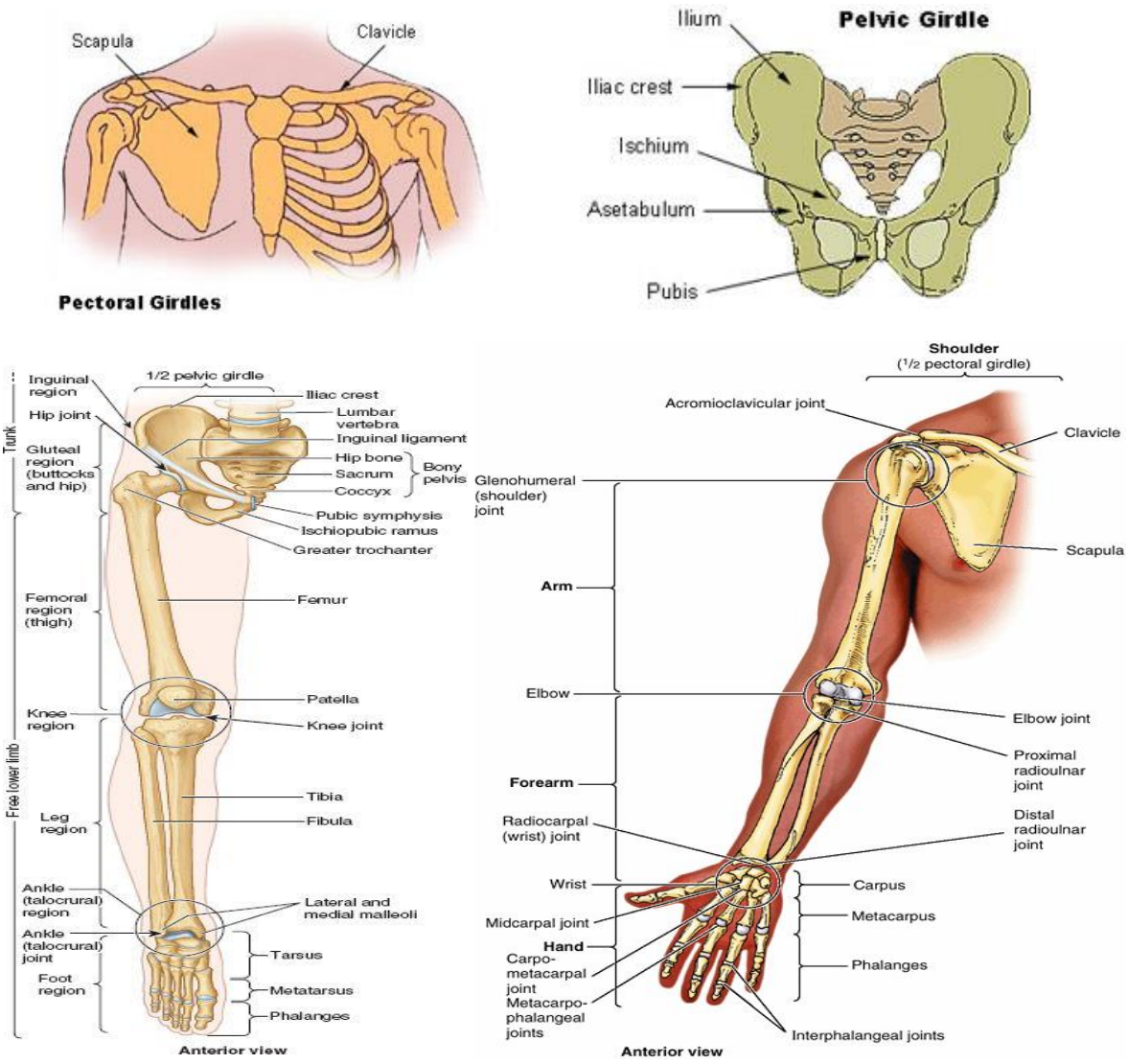


Figure 8.2: The Appendicular Skeleton

The limbs consist of upper extremity (arm, hand and fingers) and lower extremity (leg, ankle and foot) and both are comprised of 60 bones each. The shoulders (pectoral girdels) have 4 bones and hips (pelvic girdles) have 2bones.

Bones serve as attachment points for muscles, aiding movement, and store minerals like calcium. The skull safeguards the brain, ribs protect vital organs, and the spine offers spinal cord protection. Understanding this framework is crucial for comprehending human anatomy and physiology.

The complete details of bones in axial and appendicular skeleton are shown in table 1 and 2 respectively.

Table 8.1: Details of Axial Skeleton Bones

| Main Component | Types of bones present | Function |
|---|---|---|
| Cranial Bones | Parietal (2)<br>Temporal (2)<br>Frontal (1)<br>Occipital (1)<br>Ethmoid (1)<br>Sphenoid (1) | Protects the brain and houses sensory organs<br>Protects the brain and houses sensory organs |
| Facial Bones | Maxilla (2)<br>Zygomatic (2)<br>Mandible (1)<br>Nasal (2)<br>Platine (2)<br>Inferior nasal concha (2)<br>Lacrimal (2)<br>Vomer (1) | |
| Auditory Ossicles | Malleus (2)<br>Incus (2)<br>Stapes (2) | transfer and amplify air vibrations into the inner ear to be processed as sound |
| Hyoid | Hyoid (1) | supports the tongue and plays a key role in speaking and swallowing |
| Vertebral Column | Cervical vertebrae (7)<br>Thoracic vertebrae (12)<br>Lumbar vertebrae (5)<br>Sacrum (1)<br>Coccyx (1) | Supports the body, protects spinal cord, allows movement. |
| Thoracic Cage | Sternum (1)<br>Ribs (24) | Protects thoracic organs. Consists of true ribs, false ribs, and floating ribs. |

Table 8.2: Details of Appendicular Skeleton Bones

| Main Component | Types of bones present | Function |
|---|---|---|
| Pectoral girdles | Clavicle (2)<br>Scapula (2) | Connects the upper limbs to the axial skeleton. |
| Upper Extremity | Humerus (2)<br>Radius (2)<br>Ulna (2)<br>Carpals (16)<br>Metacarpals (10)<br>Phalanges (28) | Arm bones (humerus, radius, ulna). Hand bones (carpals, metacarpals, phalanges). |
| Pelvic Girdle | Coxal, innominate, or hip bones (2) | Supports the trunk and protects pelvic organs. |
| Lower Extremity | Femur (2)<br>Tibia (2)<br>Fibula (2)<br>Patella (2)<br>Tarsals (14)<br>Metatarsals (10)<br>Phalanges (28) | Thigh bone (femur), leg bones (tibia, fibula). Foot bones (tarsals, metatarsals, phalanges).<br>The lower limbs (extremities) are extensions from the trunk specialized to support body weight, for locomotion (the ability to move from one place to another), and to maintain balance. |

## 8.3. Gross Anatomy of a Long Bone

A long bone has two main regions: the diaphysis and the epiphysis as shown in the figure 8.3.

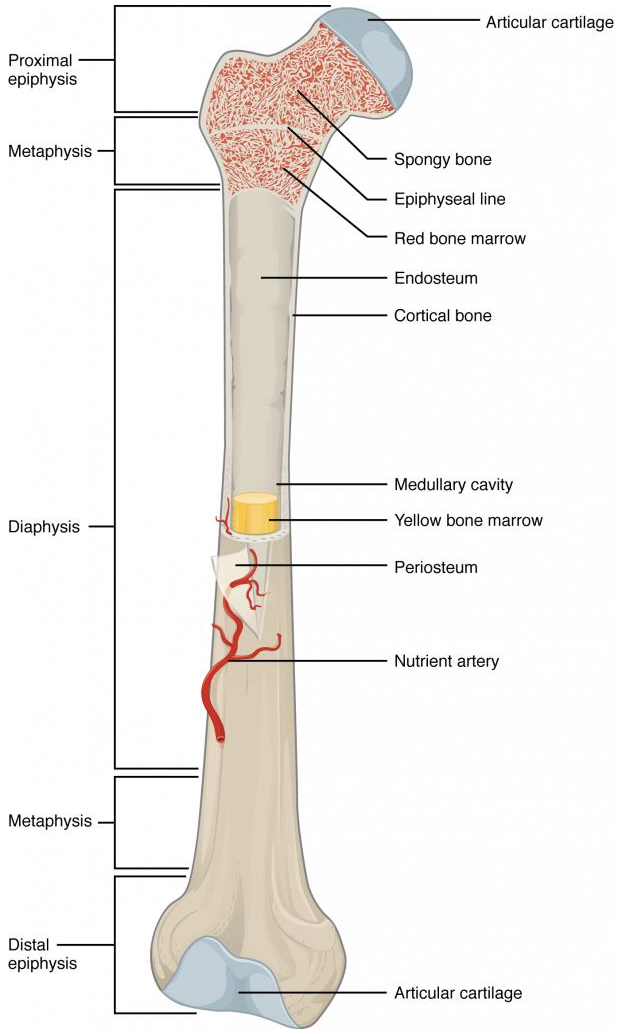


Figure 8.3: long bone showing anatomical features

### 8.3.1. The diaphysis

The diaphysis is the hollow, tubular shaft that runs between the proximal and distal ends of the bone.

### 8.3.2. Medullary cavity

Inside the diaphysis is the medullary cavity, which is filled with yellow bone marrow in an adult. The outer walls of the diaphysis (cortex, cortical bone) are composed of dense and hard compact bone, a form of osseous tissue.

### 8.3.3. The epiphysis

The wider section at each end of the bone is called the epiphysis, which is filled internally with spongy bone, another type of osseous tissue. Red bone marrow fills the spaces between the spongy bone in some long bones. Each epiphysis meets the diaphysis at the metaphysis.

## 8.4. Chest Bones:

The clavicle, sternum, and ribs are components of the human thoracic cage, providing structural support and protection for vital organs in the chest.

### 8.4.1. Clavicle (Collarbone)

The clavicle is a long, S-shaped bone located horizontally at the base of the neck. It connects the sternum to the scapula, forming part of the shoulder girdle. The lateral end of the clavicle articulates with the acromion process of the scapula, while the medial end connects to the sternum at the sternoclavicular joint.

### 8.4.2. Sternum (Breastbone)

The sternum is a flat, elongated bone located in the center of the anterior chest. It consists of three parts:

*Manubrium:* The broad, upper part that articulates with the clavicles.

*Body:* The midsection, forming the bulk of the sternum.

*Xiphoid process:* The smallest and most inferior part, often cartilaginous in structure.

### 8.4.3. Ribs

There are 12 pairs of ribs in the human body, and they are categorized into three types:

*True ribs (1-7):* Directly attach to the sternum via costal cartilage.

*False ribs (8-12):* Attach to the sternum indirectly or not at all.

*Floating ribs (11-12):* Have no anterior attachment to the sternum.

The ribs in the human body are shown in figure 8.4.

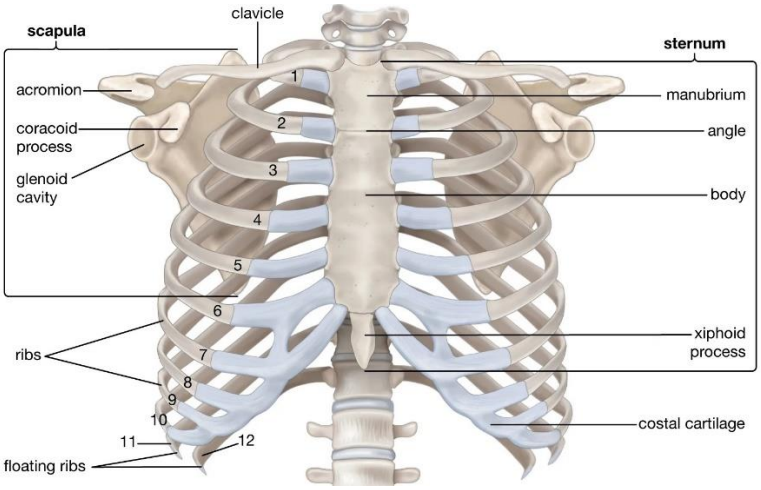


Figure 8.4: ribs in the human body

Together, the clavicle, sternum, and ribs create a protective cage around the thoracic organs such as the heart and lungs. They also play a role in respiration and support the upper limbs.

## 8.5. Vertebrae

There are 33 vertebrae in the human spine, grouped into different regions and shown in figure 8.5:
*Cervical Vertebrae (C1-C7):* Located in the neck region. The first cervical vertebra is called the atlas, and the second is the axis.
*Thoracic Vertebrae (T1-T12):* These are in the upper and mid-back, attached to the ribs.
*Lumbar Vertebrae (L1-L5):* Found in the lower back, supporting more body weight.
*Sacral Vertebrae (S1-S5):* Initially separate, these fuse into the sacrum, forming the back of the pelvis.
*Coccygeal Vertebrae (Co1-Co4):* The coccyx or tailbone, consisting of fused vertebrae.

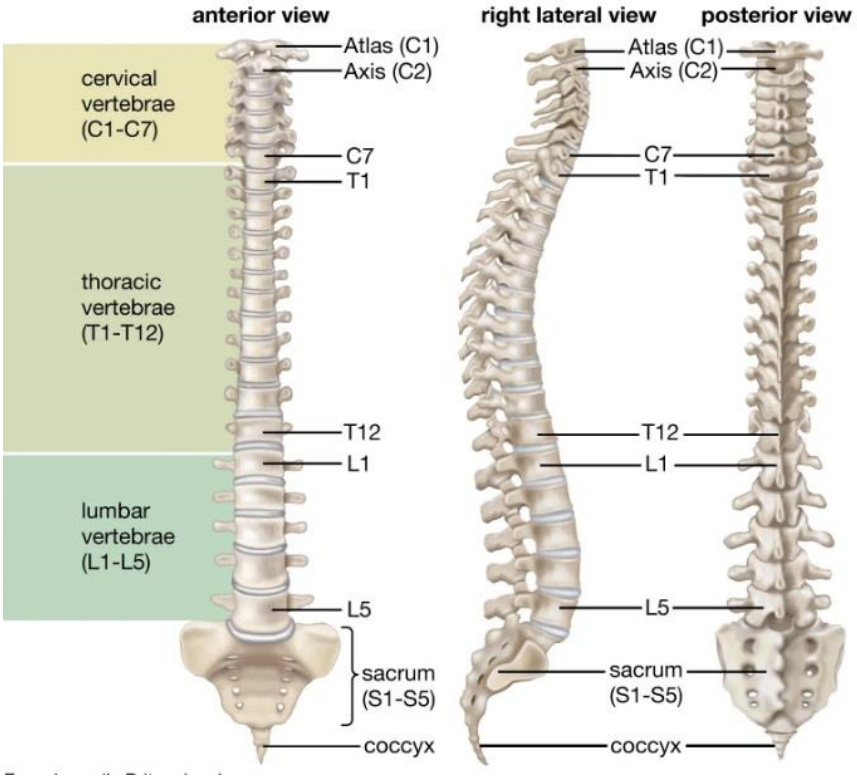

Figure 8.5: Human Vertebrae

## 8.6. Sacrum

The sacrum is a triangular bone formed by the fusion of five sacral vertebrae (S1-S5) as shown in the figure 8.6. It articulates with the last lumbar vertebra above and the coccyx

below. The sacrum is part of the pelvic girdle, contributing to the formation of the pelvic cavity and supporting the weight of the upper body.

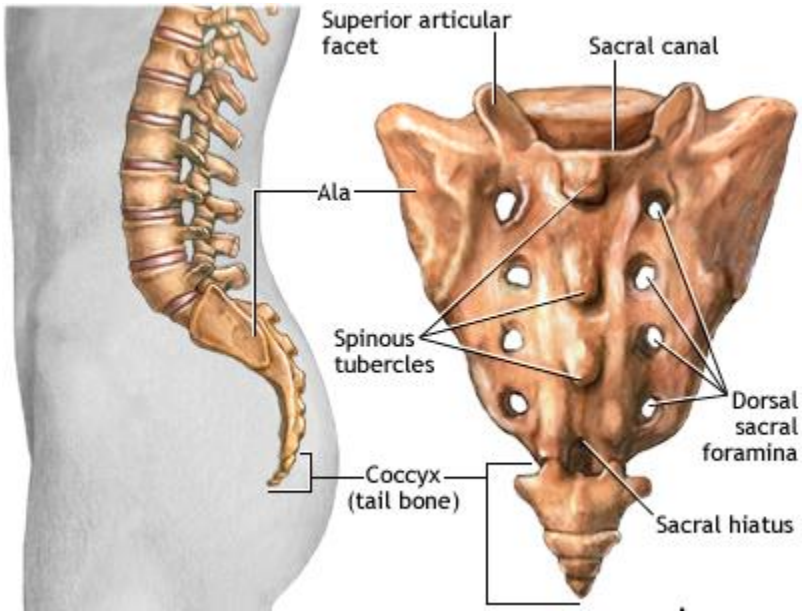


Figure 8.6: Sacrum

Together, the vertebral column and sacrum provide structural support, protect the spinal cord, and facilitate various body movements.

## 8.7. Joints

The point at which two or more bones meet is called a **joint**, or **articulation**. Joints hold the skeletal bones together. Allow the skeleton some flexibility so gross movement can occur and make bone growth possible.

### 8.7.1. Structural Classification of Joints

The structural classification of joints is tabulated in table 8.3.

Table 8.3: Structural classification of joints

| Joint Type | Tissue | Movement | Examples |
|---|---|---|---|
| Fibrous | Dense Connective tissue | Immovable | Skull, Teeth |
| Cartilaginous | Bones united by cartilage | Slightly moveable | Ribs, vertebrae |

| | | | |
|---|---|---|---|
| Synovial | Bones enclosed within a capsule lined with synovial fluid | Freely moveable (most joints in the body are in this category) | Subtypes: hinge, pivot, gliding, condyloid, saddle, ball and socket |

Different Types of Synovial Joints are shown in figure 8.7 and tabulated in table 8.4

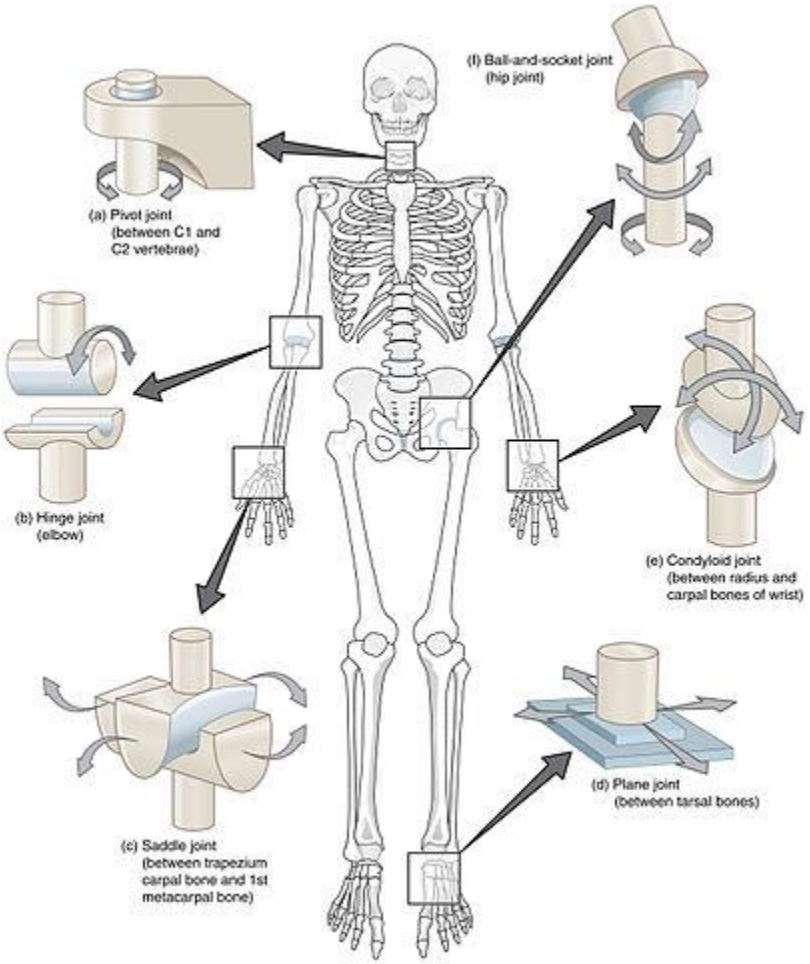


Figure 8.7: Types of Synovial Joints

Table 8.4: Synovial Joint types with movement and examples

| Joint Type | Movement | Example |
|---|---|---|
| Hinge Joint | Flexion and extension | Elbow joint |
| Ball-and-Socket Joint | Flexion, extension, abduction, adduction, rotation | Shoulder joint, hip joint |
| Pivot Joint | Rotation | Radioulnar joint in the forearm |
| Ellipsoid Joint: | Flexion, extension, abduction, adduction | Wrist joint |
| Condylar Joint | permits movements in two directions | Temporomandibular joint (TMJ) |
| Saddle Joint | Flexion, extension, abduction, adduction, circumduction | Carpometacarpal joint of the thumb |

### 8.7.2. Classification of Joints on the Basis of Function

The functional classification divides joints into three categories:

#### *8.7.2.1. Synarthrosis*

A synarthrosis is a joint that is immovable. This includes sutures, gomphoses, and synchondroses.

#### *8.7.2.2. Amphiarthroses*

Amphiarthroses are joints that allow slight movement, including syndesmoses and symphyses.

#### *8.7.2.3. Diarthroses*

Diarthroses are joints that allow for free movement of the joint, as in synovial joints.

### 8.7.3. Bone Marrow:

The soft, spongy tissue that has many blood vessels and is found in the center of most bones. There are two types of bone marrow.

#### *8.7.3.1. Red bone marrow*

Red bone marrow contains blood stem cells that can become red blood cells, white blood cells, or platelets.

#### *8.7.3.2. Yellow bone marrow*

Yellow bone marrow is made mostly of fat and contains stem cells that can become cartilage, fat, or bone cells.

### 8.7.4. Bone Tissue:

Tissue that gives strength and structure to bones are known as bone tissues. Bone is made up of compact tissue (the hard, outer layer) and cancellous tissue (the spongy, inner layer that contains red marrow). Bone tissue is maintained by bone-forming cells called osteoblasts and cells that break down bone called osteoclasts. Bones also contain blood vessels, nerves, proteins, vitamins, and minerals. Also called osseous tissue.
Bone anatomy including bone marrow and bone tissues is shown in figure 8.8.

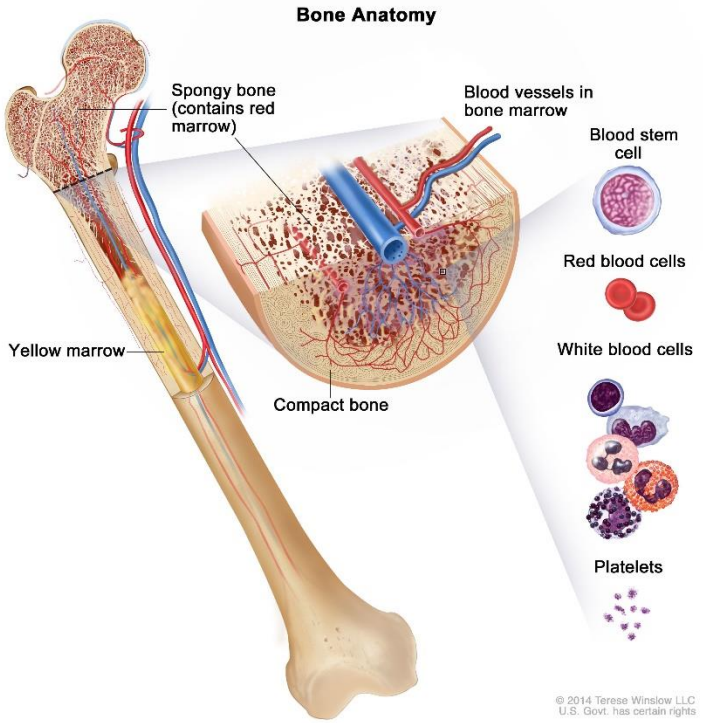


Figure 8.8: Bone Anatomy

# Chapter 9
# The Endocrine System

# 9. The Endocrine System

## 9.1. The Endocrine System

The endocrine system is a complex network of glands that produce and release hormones, chemical messengers that regulate various physiological processes in the body as shown in the figure 9.1.

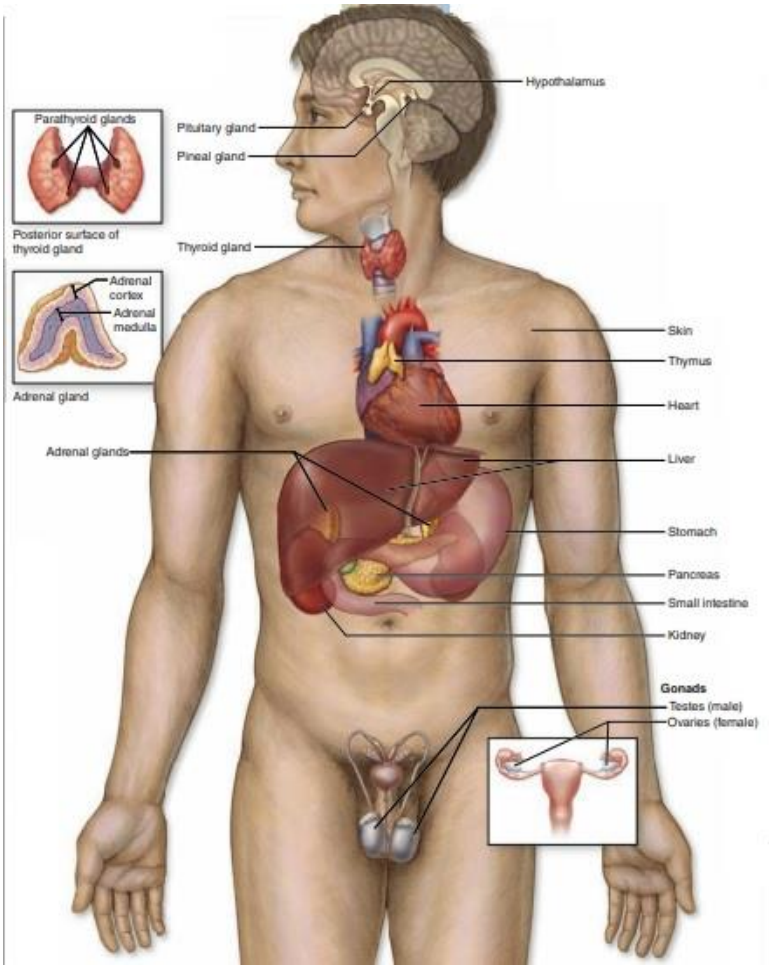


Figure 9.1: Endocrine System

Major glands include the hypothalamus, pituitary, thyroid, parathyroids, adrenal glands, pancreas, ovaries (in females), and testes (in males).

### 9.1.1. Hypothalamus

Located in the brain, it regulates the pituitary gland and connects the nervous and endocrine systems.

### 9.1.2. Pituitary Gland

Often called the "master gland," it controls other glands and produces hormones like growth hormone and thyroid-stimulating hormone. Pituitary gland is located at the base of the brain below the hypothalamus.

### 9.1.3. Thyroid Gland

Produces hormones that regulate metabolism, growth, and development. It is located in the lower and front area of the neck. It's has left and right lobes and an isthmus in between the two lobes. Thyroid cells are arranged in different size of thyroid follicles. Colloid is the proteinaceous material present in the thyroid follicles and contain the thyroid hormones.

### 9.1.4. Parathyroid Glands

Regulate calcium levels in the blood, influencing bone health and nerve function. Parathyroid glands are small glands located behind the thyroid gland.

### 9.1.5. Adrenal Glands

Situated on top of each kidney, they produce hormones such as cortisol and adrenaline, involved in stress response and metabolism. The adrenal cortex produce aldosterone (a mineralocorticoid), cortisol (a glucocorticoid), and androgens and estrogen (sex hormones).

### 9.1.6. Pancreas

Secretes insulin and glucagon, regulating blood sugar levels. It is located near the gall bladder and liver.

### 9.1.7. Ovaries (Females)

Produce estrogen and progesterone, influencing menstrual cycles and pregnancy. Two ovaries are present in a female. Ovaries produce eggs which form a new born after meeting with the sperm.

### 9.1.8. Testes (Males)

Generate testosterone, controlling male reproductive functions and secondary sexual characteristics. Testes are present behind the penis in skin covered sacs called scrotum. Scrotal sacs lie outside of the abdomen and pelvis.

# Section II Microtechniques

# Chapter 10

# Introduction to Microtechniques

# 10. Introduction

## 10.1. Background and overall preparation of tissues for microscopic examination

Histology is the branch of anatomy that focuses on the study of tissues of animals and plants. The term tissue refers typically to a collection of cells. In humans, organs comprise two or more tissue types, including epithelial, connective tissue, nervous, and muscular. The word “histology” stems from the Greek word “histos,” meaning web or tissue, and “logia,” meaning branch of learning. In brief, histological processing involves obtaining fresh tissue, preserving it (i.e., fixing it) in order to allow it to remain in as life-like a state as possible, cutting it into very thin sections (3–8 microns), mounting it on glass microscopic slides, and then staining the sections so that they can be observed under a microscope to identify different histological components within the tissue.

## 10.2. Techniques

### 10.2.1. Preparing the tissue

For tissue removal, it is necessary to gather first the informed consent of the patient, as tissue taken from a live individual for diagnosis or treatment requires his/her consent. In other words, the patient must know at the time he/she consents, the purpose of tissue removal (e.g., diagnosis, research purposes, etc.).

### 10.2.2. Tissue acquisition

An important first step in the histological process is tissue acquisition. This step can be achieved by means of traditional tissue dissection or endoscopic ultrasound (EUS)-guided fine needle aspiration it is important to ensure that sharp dissecting tools are used to minimize crushing the tissue while cutting for removal fibers and collagen.

### 10.2.3. Fixation

Surgical specimens after removal should be placed in an adequate quantity of fixative (10% formal saline) as soon as possible. For optimal fixation a piece of tissue should be immersed in at least 10 times its own volume of fixative as illustrated in fig 10.1.

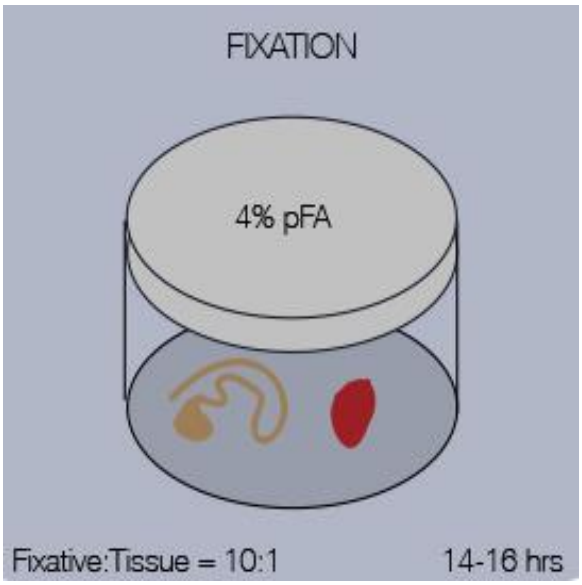


**Figure 10.1: Showing Size of container, volume of fixatives and time of fixation**

## 10.3. Why Fixatives are used?

It is critical that fixation be carried out as soon as possible after removal of the tissues to

a) Prevent autolysis and putrefaction
b) to prevent the tissue from undergoing osmotic shock
c) Distortion, and shrinkage of the tissue

Unfortunately, fixatives may, unintentionally, introduce artifacts which can interfere with interpretation of cellular ultrastructure.

## 10.4. Principles of fixatives

The fixative acts to denature proteins by

a) Coagulation (of secondary and tertiary protein structures to form insoluble gels)
b) Forming additive compounds (cross-linking end-groups of amino acids)
c) A combination of coagulative and additive processes.
d) Promote the attachment of dyes to particular cell components by opening up protein side groups to which dyes may attach,
e) Remove bound water to increase tissue refractive index to improve optical differentiation
f) Alter the refractive index of tissues to improve contrast for viewing without staining.
g) Prolonged fixation may result in the chemical masking of specific protein targets and prevention of antibody binding during immunohistochemistry protocols.

h) In such cases, alternative fixation methods may be incorporated depending on the biological material. Therefore, there is no universal fixative which will serve all requirements.
i) When tissue is fixed, it is important to keep the sample size small, if possible (i.e., 2–3 $mm^3$), as increased thickness will retard fixative penetration.
j) The volume of the fixative should be 2 Histology 20–25 times the volume of the tissue.
k) The peritoneum or capsule around the tissue should be removed or pierced. The blood and mucus should be rinsed off with saline.
l) The tissues should be cut with a new, sharp razor blade/scalpel, rather than scissors, as the latter could result in squeezing of the tissue, causing damage.
m) Some tissues/organs (e.g., lung, eye, etc.) will require special handling to ensure that the fixative reaches all internal components.
n) Care should be given to ensure that the specimen has one or more cut sides to guarantee good penetration of the fixative.

### 10.5. Fixation Containers

Jars or bottles with screw tops and of suitable capacity should be used as shown in figure 10.2.

**Figure 10.2: Tightly screwed top jars**

### **10.5.1.** Large specimens

Large specimens should not be squeezed into a smaller container. This will result in inadequate fixation and will allow autolysis to proceed unchecked. If a specimen is too large to fit easily into the largest size of container it should be brought as such to the laboratory without delay in a bucket or other suitable container. Amputated limbs may be wrapped in a rubber sheet.

### **10.5.2.** Bulky specimens

Bulky specimens are like Large tumors, spleen, etc. should be bisected cleanly with a large sharp knife before being placed in fixative.

### **10.5.3.** Solid organs

Cut slices as big as necessary but not thicker than 5 mm as shown in figure 10.3.

**Figure 10.3: Bread loafing and painting of solid organs**

### **10.5.4. Brain**

For fixing the uncut brain, pass a thick thread under the vessels at the base of the brain. The organ is gently lowered into a bucket containing the solution and allowed to float with the help of thread as shown in figure 10.4.

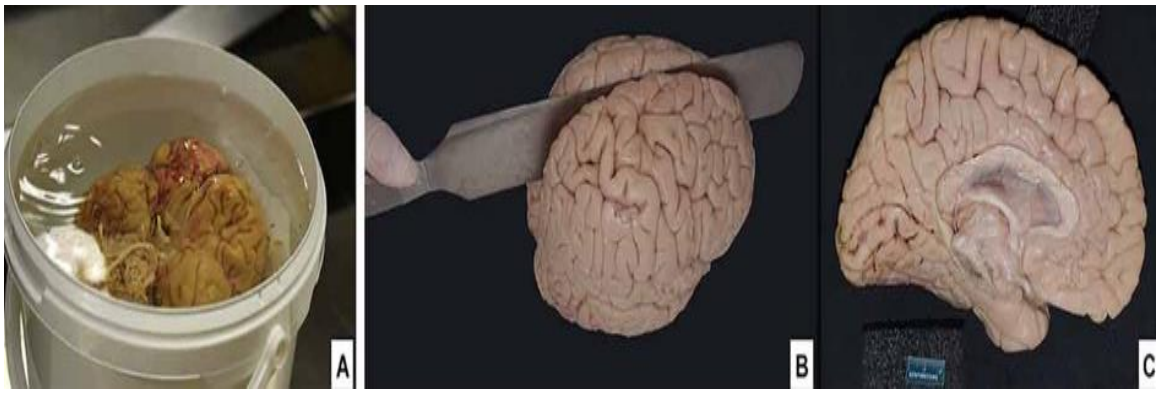


**Figure 10.4: Steps of brain fixation**

### 10.5.5. Hollow viscera

such as portions of stomach and intestine should be opened at both ends or cut open along their length (stomach should be opened along the greater curvature) as shown in figure 10.5.

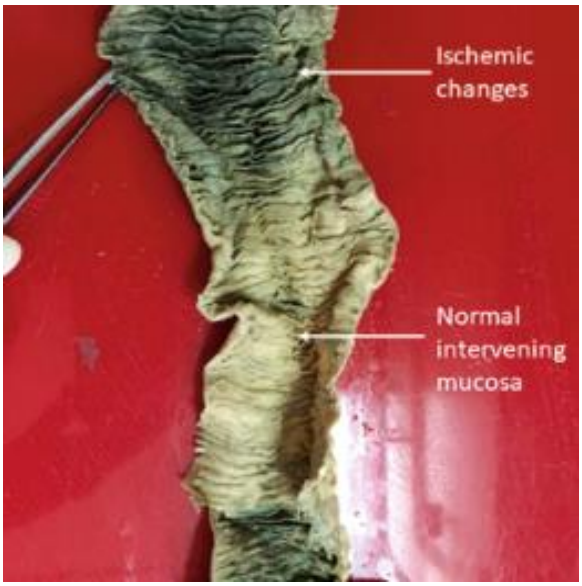


**Figure 10. 5: showing longitudinal opening of colon**

### 10.5.6. Small biopsies

In order to preserve the tissue in original orientation, it is better to place it first on a piece of filter paper and then put in the solution (Figure 10.6)

**Figure 10.6: Properly labelled, tightly closed small jars for tiny biopsies**

**Note:** the specimen should be taken fresh to the laboratory or wrapped in moist cotton wool and put in the refrigerator overnight. The specimen should never be put into water or normal saline because this will hasten autolysis.

## 10.6. Types of fixatives

There are three main types of fixatives:

### 10.6.1. Primary fixatives

Consist of a single fixative in solution (e.g., may be in absolute form, such as absolute ethanol or 10% formalin).

### 10.6.2. Compound fixatives

Consist of two or more fixatives in solution, such as Zenker's, Helly's, and Bouin's fixatives.

### 10.6.3. Cytological fixatives

Cytological fixatives preserve cellular structures or inclusions (e.g., mitochondria), often at the expense of even penetration and allow the tissues to be cut relatively easily.

## 10.7. Properties of an ideal fixative

a) Prevents autolysis and bacterial decomposition.
b) Preserves tissues in their natural state and fixes all components (protein, carbohydrates, fats).
c) Makes the cellular components insoluble to reagents used in tissue processing.
d) Preserves tissue volume.
e) Avoids excessive hardness of fixed tissue.
f) Allows enhanced staining of tissues.

g) Is be non-toxic and non-allergic for user.
h) Is not be very expensive.

### 10.8. Amount of fixing fluid

This should be approximately 10-20 times the volume of the specimen.

### 10.9. Classification of fixatives

Fixatives can be classified on the basis of three main criteria:

a) action on proteins;
b) Types of fixative solution; and
c) Use

Action on proteins Fixatives can have two main actions on proteins. They can be coagulant or non-coagulant fixatives.

#### 10.9.1. Tissue fixatives

a) Buffered formal saline
b) Buffered glutaraldehyde
c) Zenker's formal saline
d) Bouin's fluid

#### 10.9.2. Cytological fixatives

a) Ethanol
b) Methanol
c) Ether

#### 10.9.3. Histochemical fixatives

a) Formal saline
b) Cold acetone
c) Absolute alcohol

### 10.10. Common Fixatives

*Routine Formalin*
Formalin is sold as 40% W/W solution of formaldehyde gas in water. It is used as 10% or better 15% solution (V/V) in normal saline, or calcium chloride solution. It does not precipitate protein but combines with $NH_2$ group to form an insoluble gel. It preserves practically all elements including fats and keeps phospholipid insoluble in fat solvents. It is the cheapest and most popular fixative.

*Buffered formalin*

Routine (10%) formal saline has an acidic pH, which results in formation of haemitin crystals in the tissues. These crystals also interfere with staining. It is recommended that any fixative used must have a neutral ph. For this purpose, phosphate buffers are added to the fixative. To prepare 10% buffered formal saline mix the following:

Pure formalin 10 ml
Sodium dihydrogen phosphate 0.4 g
Disodium hydrogen phosphate 0.65 g
Normal saline up to 100 ml

## 10.11. Advantages of buffered formalin

Buffered formalin has the following advantages:

a) Tissues can be left in fixative for long period of time e.g., one year.
b) There is no damage or hardening of tissue.
c) Sectioning is easy.
d) No haematin crystals are formed.
e) A number of staining procedures can be used.

## 10.12. Chemicals Used during Fixation

### 10.12.1. Ethyl alcohol

It is used in 90-100% strength. It precipitates albumin and globulin but not nucleoproteins. It causes shrinkage and hardening of tissues. It destroys mitochondria. It is a reducing agent and, therefore, cannot be used with chromic acid, chromates and osmium tetra oxide. It preserves glycogen and is useful for histochemistry (glycogen, uric acid and iron) etc.

### 10.12.2. Mercuric chloride

It is used as a saturate (70%) or half saturated aqueous solution. It penetrates rapidly, precipitates proteins, fixes chromatin well and enhances its subsequent staining capability. It is rarely used alone but it is valuable for nuclear fixation.

### 10.12.3. Picric acid

It is used as a saturated aqueous solution (1%). Its penetration is poor and causes shrinkage but does not harden. It preserves glycogen and nearly all other elements. It does not affect the staining. It is not used alone.

### 10.12.4. Chromic acid

It is used either as a pure chemical or as a mixture of dichrome and acetic acid (e.g., in Zenker's solution). It is an oxidizing agent and therefore incompatible with formalin or alcohol. It preserves most elements. It tends to weaken nuclear staining by dissolving nucleoproteins.

### 10.12.5. Potassium dichromate

It is used as 2-3% aqueous solution. It is a weak oxidizing agent and tends to dissolve chromatin. It is a good cytoplasmic but bad nuclear fixative. It gives chromaffin reaction.

### 10.12.6. Osmium tetra oxide (Osmic acid)

It is used as 2% aqueous solution. It is expensive and unstable. It is rapidly converted to vapors, which are irritating. It is a powerful oxidizing agent. It penetrates very badly. It preserves fat and gives a black precipitate of osmium dioxide with unsaturated fats. Also preserves very fine cell details e.g., Golgi apparatus etc.

### 10.12.7. B-5 Fixative

It is used for fixation of lymph nodes.

### 10.12.8. Zenker's Solution

It is used for bone marrow trephine biopsy and Negri bodies.

## 10.13. Factors affecting fixation

a) Size and thickness: Increase in size and thickness of tissue will inversely affect fixation of the piece of tissue
b) Tissues covered by large amounts of mucus or blood, or organs containing very large amount of blood fix slowly.
c) Fatty and lipomatous tissues fix slowly.
d) Agitation: Fixation is accelerated by agitation.
e) Temperature: Fixation is accelerated by maintaining temperature around 60°C.
f) Time: Smaller biopsies are fixed in few hours and larger biopsies must be cut open and then put into fixatives for 8-12 hrs.
g) Volume: Volume of fixative must be 10-20 times the volume of specimen.
h) Cost: Expensive chemical must be used in low concentration.
i) Penetration of fixatives: Different fixatives have variable penetration

Formaldehyde: 0.78mm/h

Mercuric Chloride: 0.5 mm/h

Ethanol: 1.0 mm/h

Potassium Dichromate:1.33mm/h

# Chapter 11

# Gross room and specimen handling

# 11. Introduction

The initial dissection and preparation of any specimen for histological/microscopic analysis involves more than simply the transcribed macroscopic description and sampling of the specimen. Whilst the dissection and laboratory area are often perceived as the two key elements of the department, it must be clearly understood that there are many steps which follow specimen receipt, interfacing with the dissection room, that directly affect case handling.

## 11.1. Safety first and last

The histopathology department is rich in hazards e.g. infection/biological, chemical and radiation. There are also various risks reflecting the range of materials used to store, process and analyze tissues. These may be toxic, flammable, allergenic, carcinogenic and electrical. The presence of sharp cutting implements, complex machinery and the movement of the specimens around the laboratory heightens all of these risks. Staff need to be fully trained to be aware of all of these potential hazards, must wear PPE and capable of operating safely in this environment as shown in figure 11.1. Every laboratory should have accessible and clear standard operating procedures (SOPs) Ongoing safety education as part of continuing professional development is required.

**Figure 11. 1: Personal protective equipment, gloves, goggles and lab coat**

## 11.2. Specimen reception

A separate room is required for specimen reception which acts as the interface between non-laboratory hospital staff, other visitors and the pathological laboratory.

The area must be equipped with appropriate easily cleaned benching, adequate lighting, good ventilation, safety equipment, disinfectants, absorption granules and protective clothing. In the event of specimen spillage, e.g. body fluids, fixative leakage or other mishap, the immediate response by staff in this area will limit any potential local health risk and prevent risk to other laboratory personnel. The key point of this room is to receive samples safely and securely.

Important Points:

1. Confirm identity
2. Assigned a unique laboratory
3. Clinical history
4. Hospital registration number
5. Full name and date of birth
6. CNIC,
7. Contact number for correspondence

The usual numerical method of specimen identification is simply the year, expressed in two digits, with a sequential numbering system starting with one (1) and proceeding up to the final specimen of each year.

**Case 1**: case 2345L/17 is the two thousand, three hundred and forty-fifth sample of the year 2017.

**Case 2:** multiple specimens from a single patient may be received on the same day for analysis. Some laboratories prefer to annotate each sample with a separate number. However, a single laboratory number may suffice, but with sub-parts of the specimen being separately designated, e.g. sample A, sample B, etc. Within this framework, if multiple blocks are taken from a sub-part of the specimen then these can be designated with individual numbers or letters in a similar ascending fashion. Thus, a gastrectomy sample with two separate lymph node groups and the spleen could have one case number, 2345L/17, multiple sub-part specimens (A, B, C) and multiple blocks (1, 2, 3, etc.) which can be correlated against the surgeon's operative dissection. Using the number described above, e.g. the spleen in this case could be designated 2345L/17. C.2 (C. indicating the sub-part of the third sample = spleen and the block number = 2).

At this stage the sample may now confidently be passed into the dissection room.

### 11.3. Dissection room

Dissection room well lit, ventilated and have a grossing table as shown in figure 11.2

Figure 11.2: Shows grossing table

**Figure 11.2: working on a surgical specimen on a ventilated top**

It is imperative that the dissection area must have good

a) Electrical or natural lighting as shown in figure 11.3.
b) Good ventilation and non-absorbent wipe-clean surfaces.
c) Within the area there must also be appropriate protective clothing for the laboratory personnel including gloves and other equipment,
d) Photography instruments
e) Tissue macerators and disposal bins.

f) The dissection room should be a comfortable environment permitting undisturbed work by the pathologist and support technical staff
g) Protecting all the staff from formalin vapor and hazardous fluids. All tools and materials should be ergonomically accessible.

## 11.4. Important points to consider before dissection

### 11.4.1. Preserving tissue for other studies

Prior to fixation it may be relevant to reserve some fresh tissue from the specimens for microbiology assessment by placing into appropriate culture media and/or electron microscopy which requires glutaraldehyde fixation. Fresh tissue may be taken for frozen section immediate analysis. Unfixed tissue can also be taken for DNA extraction, cytogenetics and molecular pathology techniques. The latter is becoming increasingly common and important in the arena of personalized therapy by reflecting the tumor genotype and characteristics. Other specialized tests, e.g. enzyme assay and mass spectroscopy may also require tissue retention before standard formalin fixation.

### 11.4.2. Macroscopic Examination:

Possibly with photography and other physical techniques should be done

### 11.4.3. Other Materials:

Examples include various mechanical/prosthetic implants, foreign bodies, bullets, gallstones and medical devices must be noted and documented.

### 11.4.4. Storage Capacity:

This dissection/blocking/grossing/cut-up facility must have an appropriate storage area immediately to hand allowing clearance of already-examined samples promptly, preventing the dissecting area becoming cluttered as shown in figure 11.4.

**Figure 11.3: showing Biopsy Specimen Storage cabinet**

A storage area for cases following dissection and sampling. There is sturdy shelving with non-crowded samples.

### 11.5. Dissecting room and table maintenance:

a) Wide range of sharp cutting blades along with forceps, long knives for large specimen as shown in figure 11.5.
b) Ventilated bench
c) Clear dissection zone
d) Absorbent cloths should be available

**Figure 11.4: Shows wide range of cutting blades, scissors, measuring scale and tissue**

### 11.5.1. Cassettes

The blocks of tissue taken into standard tissue cassettes which are usually made of plastic. (20 × 20 × 3 mm thick tissue) as shown in figure 11.6

**Figure 11.5: Showing standard tissue cassettes**

Tissue blocks are placed into cassettes. Note the samples should not fill the cassette, and must permit room for processing fluid circulation.

**Note:** The specimens should be analyzed with only one pot open at any one time. The request and specimen identity should be checked, ideally by two persons, the dissector and their assistant. The sample should be described in terms of the nature, shape, size and also any defining characteristics.
Small Biopsies: This means that small biopsies, e.g. endoscopic mucosal samples, may simply be afforded a simple descriptor in the form of the number of pieces and the size (SI units, usually mm) of the largest piece of tissue. An example could be ‘three pieces of brown tissue, the largest 3 mm diameter.

### 11.5.2. Margin Painting

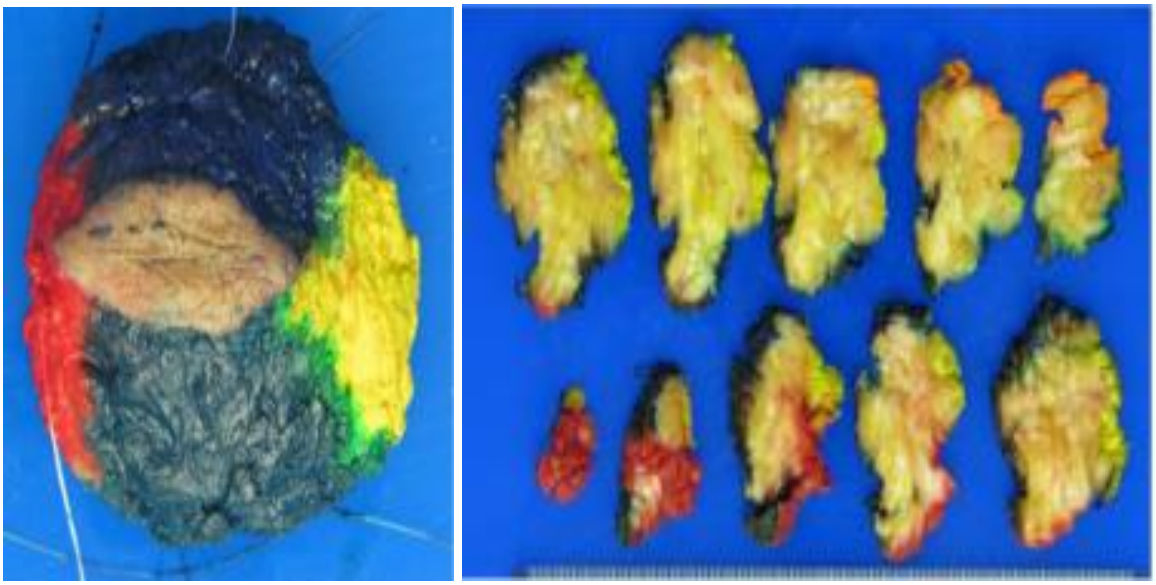

**Figure 11.6: Shows inking of margins with different colors**

The samples have been marked with different colored inks to permit designation of the sidedness of the samples and the resection margins as shown in figure 11.7.

### 11.5.3. Photography

Photographing the macroscopic specimen, whole or during the dissection, is particularly important in cases of complex surgical excision, e.g. Wertheim's hysterectomy, pneumonectomy and localization samples. It may also be of use in later analysis/case discussion (figure 11.8).

**Figure 11.7: Photography of very tiny sample**

After placing the sample in cassettes, whole batch is transported to the automated tissue processor as shown in figure 11.9.

**Figure 11.8: shows transportation of whole batch to the automated tissue processor**

# Chapter 12
# Tissue Processing

# 12. Introduction

## 12.1. Tissue Processing

In order to cut thin sections of the tissue, the tissues must have a suitable hardness and consistency when presented to the knife-edge. These properties can be imparted by infiltrating and surrounding the tissues with paraffin wax, celloidin or low viscosity nitrocellulose (LVN), various types of resins or by freezing. The process is called tissue processing.

### 12.1.1. Labelling of samples

It is important that all specimens are properly labelled before processing is started. For labelling, pen containing ordinary ink should not be used. Printed, graphite pencil written, type written or India ink written labels are satisfactory.

**Note**: Tissues that are fixed in osmium tetra oxide should be labelled on jar, as osmium tetra oxide will turn the label black. The label should be clearly written and must contain, in block letters, all necessary information.

### 12.1.2. Transportation of samples:

A system of transportation is required to carry the tissue through various steps in processing. The representative sections or entire biopsy specimen, when of small size are put in muslin cloth together with their label and are then transported from reagent to reagent in metal containers that have perforated walls, so that the reagent enters into the tissues. Alternatively labelled plastic cassettes with perforated walls are used to carry the sections.

## 12.2. Sample Processing

Tissue processing is a long procedure and requires 24 hours. Alternatively labelled plastic cassettes with perforated walls are used to carry each section.

Processing of tissue can be done:

1. *Manually*: In which the tissue is moved from one container of reagent to another by hand. Agitation is also done manually.
2. *Automatically*: In which the same steps are completed automatically by a mechanical device. Now automatic tissue processors are available. In these processors there are different jars containing reagents.

### 12.2.1. Automatic tissue processor

These are arranged in a sequence. A mechanical device moves the tissue from one jar to another as sown in figure 12.1. Agitation is also done mechanically. Timings are

controlled by a timer, which can be adjusted in respect of hours and min. Temperature is maintained around 60°C in jars containing paraffin wax.


**Figure 12. 1: Automated Tissue Processor**

### 12.2.2. Steps of tissue processing:

It is done in stages. It can be sub-divided into

a) dehydration,
b) clearing,
c) impregnation and embedding.

The steps involved in processing, whether done manually or mechanically, remain the same and are as under:

***Dehydration***: Using increasing strengths of alcohol e.g., 70%, 90% and absolute alcohol, dehydrates tissues. The duration for which tissues are kept in each strength of alcohol depends upon the size of tissue, fixative used and type of tissue. After fixation in aqueous fixatives delicate tissues need to be dehydrated slowly starting in 50% ethyl alcohol directly whereas most tissue specimens may be put into 70% alcohol. Delicate tissues will shrink too much when exposed to a high concentration of alcohol. For routine, sections no thicker than 7 µm, the following scheme may be followed:

a) 70% alcohol - Methylated spirit for 1 hour
b) 90% alcohol - Rectified spirit 2 changes for 2 hours each
c) 100% alcohol - Absolute alcohol 2 changes for 2 hours each

In the above process dehydration is helped by agitation of the tissues hence duration is 2 hours. If not agitated, it may take much longer for the procedure. In the absolute alcohol chamber 1/2-1-inch-thick layer of anhydrous copper sulphate separated by filter paper

may be used. It takes away the water derived from the tissues. The volume of alcohol should be 50- 100 times that of tissues. If this is not possible then frequent changes may be used. **Clearing (To remove alcohol)**: During dehydration the water in the tissue has been replaced by alcohol. In the next step alcohol is to be replaced by wax. As wax is not alcohol soluble, we replace alcohol with a substance in which wax is soluble. This step is called clearing. Clearing of tissues is achieved by immersing the tissue in any of the following substances.

a) Xylene
b) Chloroform
c) Benzene
d) Carbon tetrachloride
e) Toluene

Xylene is commonly used. Small pieces of tissue are cleared in 1/2-1 hour, whereas large (5 mm or more thick) are cleared in 2-4 hours. Cedar wood oil can also be used. It is an excellent clearing agent and tissues may be kept for months in it without hardening. However, it is slow in action and extra time is required in molten wax.

*Impregnation with Wax*: This is allowed to occur at melting temperature of wax, which is 54-60°C. Volume of wax should be about 25-30 times the volume of tissues. For better results, impregnation is done serially in 3-4 jars, However, 2 jars are sufficient. The duration of impregnation depends on the size and type of tissue and the clearing agent employed. Longer periods are required for larger pieces and also for harder tissues like bones and skin as compared to liver, kidney, spleen, lung etc. Xylene is easiest to remove and 1-2 changes of wax are sufficient. Total duration of 4 hours is sufficient in all the jars for routine processing.

## 12.3. Types of waxes employed for impregnation

### 12.3.1. Paraffin Wax

It is used routinely. It has hard consistency, so sections of 3-4micron thickness can be cut.

### 12.3.2. Water-soluble Wax

It has the advantage that the tissue can be directly placed in it, without dehydration and clearing. However, the disadvantage is that fragmentation of the section takes place in the floating bath.

## 12.4. Other materials used for impregnation

### 12.4.1. Celloidin

The consistency of celloidin is rubbery so it can be used for hard tissues like bone. High temperature is not required during processing so tissue shrinkage does not take place.

### 12.4.2. Gelatin

This is used for embedding friable tissue. It has the advantage that creases can be removed easily.

### 12.4.3. Paraplast

This material is the combination of paraffin wax and several plastic polymers. Its consistency is softer than paraffin and its sections are free from any wrinkles. Its melting point is 56°C. Another substance called paraplast plus is superior because its penetration is more, and this reduces the processing time.

### 12.4.4. Casting or Blocking:

Embedded tissues are placed in a mould, which may be metal or plastic with their label and then fresh molten wax is poured in it and allowed to settle and solidify as shown in figure 12.2-12.4. Care is taken not to allow any bubbles to form. Once the block has cooled sufficiently to form a surface skin it should be immersed in cold water to cool it rapidly. Failure to do this will often cause crystallization of wax. After the block has completely cooled it is cut into individual blocks and each is trimmed. The labels are made to adhere to the surface of the block by melting the wax with a metal strip sufficiently warmed.

**Figure 12.2: Metal Molds**

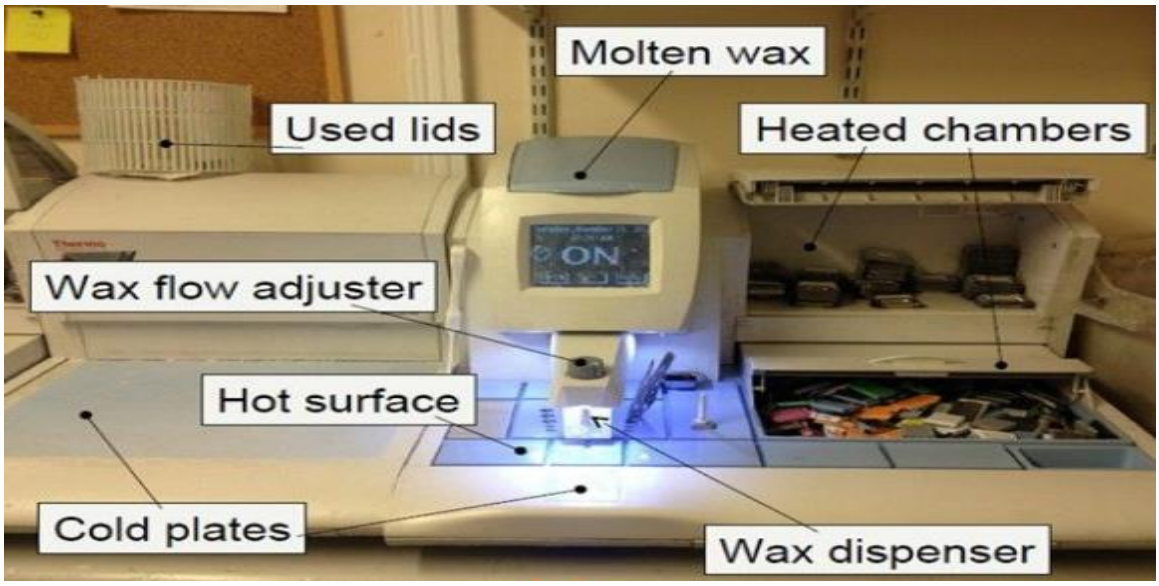


**Figure 12.3 showing different parts of tissue blocking surface**

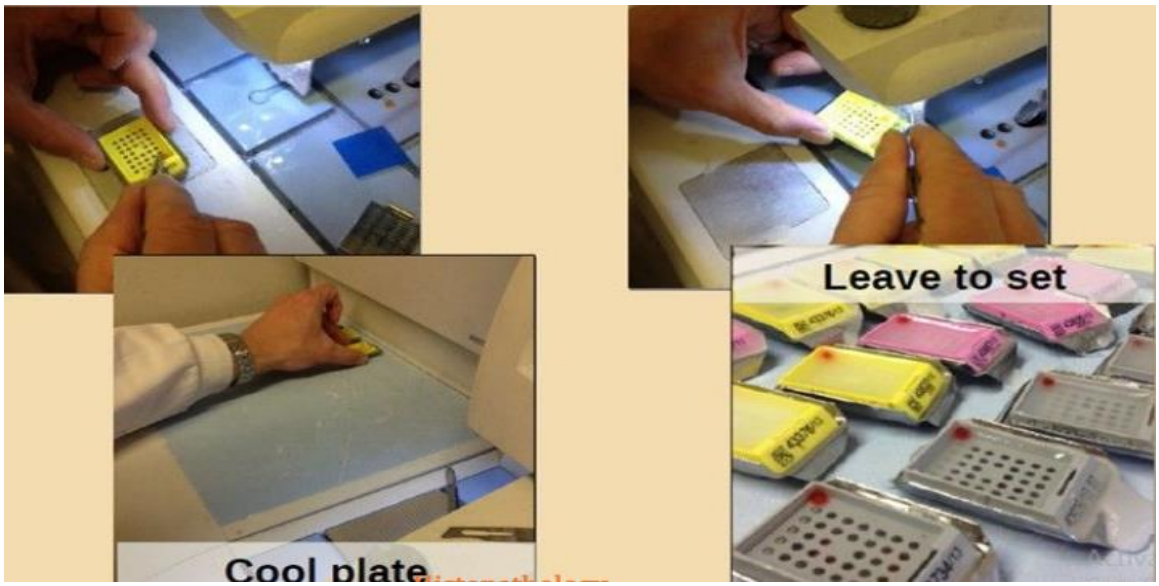


**Figure 12.4 showing casting of biopsy specimen in metal molds with liquid paraffin wax**

**Summary of Paraffin Wax embedding**:
**Dehydration Long time routine Short time routine**
70% Ethanol 1 hour 15 min
90% Ethanol I 2-hour 20 min
90% Ethanol II 2 hours 20 min
100% Ethanol I 2-hour 20 min

100% Ethanol II 2 hours 20 min

**Clearing**

Xylene I 1 hours 20 min

Xylene II 1 hours 20 min

**Wax impregnation**

Paraffin wax I 2-hour 40 min

Paraffin wax II 2-hour 40 min

# Chapter 13

# Microtomy for paraffin and frozen sections

# 13. Introduction

Microtomy is the means by which tissue is sectioned and attached to the surface of a glass slide for further microscopic examination.

## 13.1. Principle of Microtomy

The basic principles are applicable to both paraffin and frozen sections although most is performed on paraffin wax-embedded tissue blocks. The instrument used to cut sections is the microtome. This has an advancing mechanism which moves the object, the paraffin block, for a predetermined distance until it is in contact with the cutting tool, the knife or blade. The specimen moves vertically past this cutting surface and a tissue section is produced.

## 13.2. Types of microtome

There are several types of microtome, each designed for a specific purpose, although many have multifunctional roles

a) Rotary microtome
b) Base sledge microtome
c) Rotary rocking microtome
d) Sliding microtome

### 13.2.1. Rotary Microtomy

Rotary microtome may be manual, i.e. completely manipulated by the operator; semi-automated with one motor to advance either the fine or coarse hand wheel; or fully automated, when two motors drive the fine and the coarse advance hand-wheel. The mechanism for block advancement may be retracting or non-retracting.

### 13.2.2. Hand wheel microtome:

Traditionally hand wheel microtome is used in laboratories as shown below in figure 13.1.

**Figure 13.1: Showing traditional hand wheel microtome**

Advantages of hand wheel:

a) Turns in one direction
b) Advances the block towards knife
c) It has safety lock

### 13.2.3. Semi-Automated microtome:

Technological advances in the automation of Microtomy have improved section quality, increased productivity and improved occupational safety for the technologist (Figure 13.2).


**Figure 13.2: Semi automated microtome**

#### 13.2.3.1. Base Sludge microtome

Here the specimen is held stationary and the knife slides across the top of it during sectioning. Its uses are

a) primarily for large blocks,
b) hard tissues or
c) Whole mounts, it is especially useful in neurological and ophthalmic pathology but 3 µm sections are difficult to produce.

#### *13.2.3.2.* Rotary Rocking Microtome*:*

Commonly used in cryostats, the retracting action moves the tissue block away from the knife on the upstroke, producing a flat face to the tissue block

#### *13.2.3.3. Sliding Microtome:*

The knife or blade is stationary and the specimen slides under it during sectioning. This microtome was developed for use with celloidin-embedded tissue blocks used primarily for research.

### 13.3. Microtomy Knives

Following Microtomy knives are used:

Disposable blades are used for routine microtomy and cryotome, providing a sharp cutting edge which can produce almost flawless 2–4 µm sections. Reliability of a constant sharp edge, ease of use, low or high profiles adaptable to a variety of tissue and paraffin types, and low cost relative to steel knife sharpening, make these blades the mainstay in most laboratories. Glass and diamond knives are used in electron microscopy and with plastic resin-embedded blocks

### 13.4. Paraffin section cutting

#### 13.4.1. Equipment required

- Flotation (water) bath.
- Slide drying oven or hot plate.
- Fine pointed or curved forceps.
- Sable or camel haired brush.
- Scalpel.
- Slide rack.
- Clean slides.
- Teasing needle.
- Ice tray or cooling platform.
- Chemical-resistant pencil or pen.
- Electronic slide labeling instrument.

### 13.4.2. Flotation (water) bath

A thermostatically controlled water bath is used for floating out tissue ribbons after sectioning. The temperature of the water in the bath should be 10°C below the melting point of the paraffin wax to be sectioned. Care should be taken to prevent water bubbles from being trapped under the section and this can be accomplished by using distilled water in the bath. Alcohol or a small drop of detergent may be added to the water to reduce the surface tension allowing the section to flatten out with ease.

### 13.4.3. Drying oven or hot plate

Drying ovens incorporate fans which keep the warm air circulating around the slides. The temperature setting should be approximately that of the melting point of the paraffin wax. If the oven is too hot there may be distortion to the cells causing dark pyknotic nuclei, nuclear bubbling and loss of nuclear detail.

Drying times vary depending on

- the type of tissue,
- the number of slides to be dried and
- size of the drying device.

Many automated stainers have drying ovens as part of the instrument and the time and temperature is easily regulated. Special care should be taken when drying delicate or central nervous system tissue, a lower temperature is required to prevent splitting and cracking of the section and 37°C for 24 hours is recommended. Time for drying slides should be monitored as many Immunohistochemical or special stains are heat sensitive.

### 13.4.4. Brush and forceps

These or teasing needles are helpful in removing folds, creases and bubbles which may form during floating out of the section on the water bath. They are also helpful for manipulating the section as it passes across the edge of the blade.

### 13.4.5. Slides

For normal routine work, 76 × 25 mm slides are universally used as shown in figure 13.3. Although slides are available in a variety of thicknesses, 1.0–1.2 mm thickness are preferred because they do not break easily.


**Figure 13.3: Dimensions of universal slide**

Most slide racks are made to accommodate this slide size as shown in figure 13.4

**Figure 13.4 Slide rack**

Larger slides are available for use with specialty tissues such as eyes or brains. Unique identification numbers or codes, patient name or other information should be etched, embossed or written on each slide. Automated instruments which imprint the patient's information on the glass slide are available. Chemical-resistant pens and pencils are routinely used to label the slide. Slides which are positively charged or pre-treated with an adhesive resist detachment of the tissue from the slide during staining. Colored, frost-ended slides may be used for specialized techniques.

## 13.5. Section adhesives

Providing clean slides are used and sections are adequately dried, the problem of sections detaching from the slide during staining should not occur. Occasions when sections may detach from the slide are:

- Exposure to strong alkali solutions during staining.
- Cryostat sections for immunofluorescence, immunohistochemistry or intraoperative consultation.
- Central nervous system (CNS) tissues.
- Sections which are submitted to extreme temperatures.
- Tissues containing blood and mucus.
- Decalcified tissues.

Adhesives may alleviate the problem of tissue loss. Protein adhesives such as albumen, gelatin and starch may be prone to bacterial growth or heavy staining but close monitoring will prevent these problems. Other adhesives which may be used are:

**Poly-L-lysine (PLL)**

This is bought as a 0.1% solution which is further diluted for use 1:10 with distilled water. Slides are coated with the diluted solution and allowed to dry. The effectiveness of the coating to adhere the tissue to the slide will diminish within a few days.

**3-aminopropyltriethoxysilane (APES)**

Slides are dipped in a 2% solution of APES in acetone, drained, dipped in acetone and drained again. The process is complete when the slides are dipped in distilled water and placed upright in a rack to dry. These slides are useful for cytology and specimens which may be bloody or contain proteinaceous material.

**Charged or plus slides**

Laboratories often use slides which have been manufactured with a permanent positive charge. Placing a positive charge on the slides is accomplished by coating the slide with a basic polymer in which a chemical reaction occurs, leaving the amino groups linked by covalent bonds to the silicon atoms of the glass. These slides are superior in their resistance to cell and tissue loss during staining or pre-treatments such as enzyme and antigen retrieval.

**Practical microtomy**

Practical experience under the guidance of a skilled tutor is the best way to gain the confidence and coordination necessary to manipulate the microtome and the sections produced.

**Setup of the microtome**

Maintenance of the microtome is important to the production of quality slides for diagnosis. The manufacturer’s recommendation regarding the proper care of the instrument should be closely followed.

A departmental policy should be implemented, *Practical microtomy* outlining daily, weekly, quarterly and yearly preventive maintenance procedures.

**Position of apparatus:**
The water bath and the microtome should be ergonomically positioned to reduce stress and tension on the employee's neck and shoulders.
**Temperature of water bath**:
The water bath may be filled with distilled or tap water and adjusted to the correct temperature for the paraffin wax. The temperature of the water bath should be recorded and monitored throughout the day for quality purpose. Care should be taken to reduce air bubbles which may distort the tissue section.

### 13.6. Blades:
The blade should be sharp and defect free. The blade or knife holder should be adjusted to optimize the clearance angle, the distance between the lower facet angle and the surface of the block face. The recommended angle varies from 2–4° for paraffin and 5–7° for frozen sections. The correct angle reduces friction as the blade passes through the block, preventing compression of the section.
If a disposable blade is to be used, care should be taken to ensure enough pressure is being exerted on the blade to provide support, but it should not be over-tightened, as this causes thick and thin sectioning.

### 13.7. Sectioning

#### 13.7.1. Trimming the tissue blocks
The paraffin block may be faced or "rough cut" by setting the micrometer at 15–30 µm or by advancing the block using the coarse feed mechanism. Aggressive trimming will cause "moth hole" artifacts.
Care must be taken to ensure that the block clamped in the chuck has been retracted so that there is no contact with the blade on the initial down stroke. It is possible to damage the tissue by gouging or scoring when trimming the block.

#### 13.7.2. Cutting sections
Blocks should be arranged in numerical order on an ice tray or cooling mechanism, cooling both the tissue and the paraffin wax to a consistent temperature.
A small amount of water is absorbed into the tissue causing slight swelling and making sectioning easier. Over-soaking may cause expansion and distortion of the tissue section. Proper processing greatly reduces or eliminates the need to pre-soak blocks. Routine surgical material should be cut at 3–4 µm. The micrometer setting does not guarantee that each section will be that exact thickness. Thickness depends on many factors including temperature, knife angle and cutting speed. Experience will determine the speed of the stroke but in general, one should use a smooth, slow stroke. If there is difficulty cutting a smooth flat section, warming the block face with warm water, or gently

exhaling breath onto the block surface during sectioning may help. This has the effect of expanding the block, giving a slightly thicker section.
Ideally, successive sections will stick edge to edge, forming a ribbon. If the entire block is to be sectioned and retained, the ribbons are stored in a receptacle for future use. Ribbons are the most convenient way of handling sections. When a ribbon of several sections has been cut, the first section is held by forceps or teasing needle and the last section eased from the knife edge with a small brush.

### 13.8. Floating out sections

The floating out of the ribbon must be smooth, the railing end of the ribbon making contact with the water first. The slight drag produced when the rest of the ribbon is laid on the water is sufficient to remove most, if not all of the folds which occur. Sections are floated on the water bath shiny side down. Folds in the section may be removed by simply teasing with the forceps. Approximately 30 seconds should be long enough for a ribbon to flatten, longer on the water causes excessive expansion distorting the tissue. Individual sections or ribbons may be floated onto the slide. Circular structures such as eyes may be difficult to flatten. Various techniques are useful in these situations, e.g. placing the section on a slide which has been pre-flooded with 50% alcohol. The slide is gently immersed in the water bath and the section of eye will float on the surface. The presence of the alcohol will set up diffusion currents which help flatten the tissue section. The water bath should be cleaned after each block is cut, removing debris and tissue fragments by dragging tissue paper across the surface.

### 13.9. Drying sections

The small amount of water held under the section will allow further flattening to occur when heat is applied to dry the section. The temperature should be at the melting point of the paraffin wax.
It is important to eliminate over-heating during the drying stage as cellular details may be compromised. Hot plates may cause localized overheating of the drying delicate tissues; less distortion will occur if the temperature is reduced and the time prolonged. Overnight drying at 37°C or room temperature is recommended for many tissues.

### 13.10. Cutting hard tissues

This has become less problematic since the introduction of disposable blades. Cutting difficulties are more likely due to poor fixation or over-processing. Prolonged soaking of the block or exposing the block surface to running tap water for 30 minutes overcomes many of the problems associated with cutting hard tissues.

## 13.11. Surface decalcification

When small foci of calcium are present in the tissue section, cutting a quality section may be difficult. The block may be removed from the chuck after rough cutting the tissue and placed face down in a dish which contains a small amount of decalcifying solution. The exposure time will vary depending on the tissue and requires close monitoring. The block is rinsed well, blotted dry, chilled and returned to the microtome. An immediate section should be taken since the decalcification achieved will be limited. Diagnostic materials may be compromised if over-decalcification occurs

## 13.12. Problems and solutions

Table 1 addresses the most common problems encountered during microtomy and their possible solutions.

Table 1: Common problems encountered during microtomy and its solutions

| Causes | Solutions |
|---|---|
| **Ribbon of consecutive sections are curved** | |
| Block edges not parallel | Trim block until parallel |
| Dull blade edge | Replace blade or move to a different area |
| Excessive paraffin wax | Trim away excess paraffin wax |
| Tissue varying in consistency | Re-orient block |
| **Thick and thin sections** | |
| Paraffin wax too soft for tissue or conditions | Cool block with ice or re-embed in higher melting point wax |
| Insufficient clearance angle | Increase clearance angle |
| Faulty microtome mechanisms | Maintain microtome – lubricate and calibrate. Check for obvious faults with microtome, parts may be worn |
| Blade or block loose in holders | Tighten block and blade |
| **Chatter – thick and thin zones parallel to blade edge** | |
| Blade or block loose in their holders | Tighten blade and block holders |
| Excessively steep clearance angle or knife tilt | Reduce angle |
| Tissue or paraffin wax too hard for sectioning | Use softening fluid |
| Calcified areas in tissue | Rehydrate and surface decalcify |
| Over-dehydration of the tissue | Re-embed in fresh paraffin wax |
| Dull blade | Replace or use new area of blade, clean blade edge to remove excess paraffin wax |
| **Splitting of sections at right angles to knife edge** | |
| Nicks in blade | Use different part of blade or replace |
| Hard particles in tissue | Surface decalcify if calcium deposit |
| Hard particles in paraffin wax | Remove with fine sharp pointed scalpel if mineral or other particle |
| **Sections will not form ribbons** | |
| Paraffin wax too hard for sectioning conditions | Re-embed in lower melting point paraffin wax |
| Debris on knife edge | Clean with xylene moist cloth |
| Clearance angle incorrect | Adjust to optimal angle |

## 13.13. Frozen and related sections

This is a discussion of the methods used to produce sections which preserve cellular morphology without the use of dehydrating and clearing solutions and heat. Frozen sections have important clinical and research applications.

Clinically, frozen sections are used for

- Rapid intraoperative diagnoses,
- Moh's procedures for surgical margins and
- Sentinel node evaluation.

All of which have great significance in patient management. The technical skills needed to produce quality, interpretable slides require an understanding of microtomy and the anatomy of the tissue being sectioned. Good hand-eye coordination, attention to detail and the ability to work under pressure are essential traits needed to perform this task correctly.

### 13.13.1. Uses of frozen sections

Frozen sections have many applications in histology laboratories including:

- Intraoperative diagnosis.
- Diagnostic and research enzyme histochemistry for labile enzymes.
- Immunofluorescence.
- Sentinel node evaluation.

- Immunohistochemistry techniques when heat and fixation may inactivate or destroy the antigens.
- Diagnostic and research non-enzyme histochemistry, e.g. lipids and some carbohydrates.
- Silver stains, particularly in neuropathology.

### 13.13.2. Theoretical considerations

The principle of cutting frozen sections is simple: when the tissue is frozen, the interstitial water in the tissue turns to ice and in this state the tissue is firm, the ice acting as the embedding medium. The consistency of the frozen block may be altered by varying the temperature of the tissue. Reducing the temperature will produce a harder block; raising the temperature makes the tissue block softer. The majority of non-fatty unfixed tissues section well at −20°C. The sectioning of fixed tissue requires a block.

### 13.13.3. The cryostat

This is a refrigerated cabinet in which a modified microtome is housed. All the controls for the microtome are operated outside the cabinet. The first cryostats were introduced in

1954 and the following developments in design have improved both sectioning and laboratory safety:

- Electronic temperature control.
- Electronically controlled advance and retraction of the block.
- Specimen orientation facility.
- Digital visualization of chuck and cabinet temperature.
- Mechanical control of cutting speed and section thickness.
- Automatic defrost mechanism.
- Automated decontamination and sterilization.
- Freezing shelf / bar or a “Peltier” device.

### 13.13.4. Freezing of fresh unfixed tissue

The fresh tissue should be frozen as rapidly as possible without creating freeze artifacts. Suitable techniques include:

- Liquefied nitrogen (−190°C).
- Isopentane (2-methylbutane) cooled by liquid nitrogen (−150°C).
- Dry ice (−70°C).
- Carbon dioxide gas (−70°C).
- Aerosol sprays (−50°C).
- Internal freezing shelf or bar.

Freeze artifact occurs when the water in the tissue freezes and forms ice crystals; the size and quantity of crystals is proportional to the speed at which the tissue is frozen. The tissue is cut and the sections placed on slides at room temperature; at this point the tissue section is thawed. The thawing of the ice crystals produces freeze artifact which appears as holes, or a discontinuation of the tissue architecture when viewed microscopically.

The best frozen sections are obtained when the tissue is frozen quickly. The method of choice is isopentane cooled by liquid nitrogen. The problem with using liquid nitrogen alone is the formation of nitrogen vapor bubbles around the tissue which act as an insulator and inhibit rapid, even cooling of the tissue. This can produce freeze artifact in the tissue making diagnostic interpretation difficult, especially in muscle biopsies. This problem can be overcome by snap freezing the tissue in an agent with a high thermal conductivity which has been cooled to approximately −160°C by immersion of the isopentane in liquid nitrogen.

### 13.13.5. Frozen and related sections

The block compromising the sample and causing sectioning problems. The tissue may be rolled in talc prior to snap freezing to reduce freezing artifact. Solid carbon dioxide (dry ice) may be used for freezing tissue blocks.

Carbon dioxide gas from a $CO_2$ cylinder has been successful in the past. Tissue blocks are frozen by adapting a conventional freezing microtome with a gas supply or by using a special adaptor for the $CO_2$ tank which holds the tissue chuck.

Aerosol sprays have gained popularity as a means of freezing small tissue blocks. These sprays have the advantage of being readily available and easily stored. A major problem is the environmental issues of aerosol emissions. Additionally, there is a risk to the scientist of both aerosol inhalation and possible contamination by microbial exposure released from the tissue by the spray.
The tissue is frozen simultaneously from the block face and the block holder. Freeze artifact may be reduced if all objects are kept cold and ready for use. This method is quick and often used for intraoperative frozen sections.

### 13.13.6. Fixed tissue and the cryostat

For most diagnostic purposes in a routine laboratory, cryostat sections of unfixed tissue are suitable. However, freezing unfixed tissue causes the diffusion of labile substances. This is enhanced when the section is cut in the cryostat, producing heat which causes slight thawing of the cut section.

### 13.13.7. Cryostat sectioning

**Cabinet temperature**

The temperature of the microtome and the cryostat chamber should be monitored, many cryostats having digital displays of the block and cabinet temperature. Most unfixed material will section well between −15 and −23°C. Tissues containing large amounts of water will section best at the warmer temperature, and harder tissues and those which contain fat require a colder temperature. Microtomy for paraffin and frozen sections are shattered with chatter lines this is an indication that the block is too cold. Most fixed tissues will section best within the range of −7 to −12°C, depending on the hardness of the tissue. Small blocks of undecalcified cancellous bone can be sectioned but care must be taken to remove any cortical bone fragments prior to freezing. Most cryostats have a defrost cycle which occurs daily, care must be taken to avoid leaving a specimen in the cabinet overnight. Thawing and refreezing of the sample during the defrost stage may alter the specimen and make it unsuitable for future studies.

### 13.13.8. Microtome

This should be defrosted, cleaned and oiled according to manufacturer's recommendations if cutting problems are encountered. A policy should be in place which outlines a routine maintenance schedule for each cryostat, including a section on decontamination of the instrument.

## 13.14. Cryo-embedding medium

There are many different cryo-embedding media commercially available. The properties of each should be carefully considered before use, including the temperature, freezing mode and type of tissue being frozen.

### 13.14.1. Blade or knife

Disposable blades have become routine in most clinical laboratories. They produce a perfect, sharp edge, are instantly available and can be rapidly cooled because of their size, but tissues which are extremely hard or dense may be troublesome. Stainless steel knives may be necessary in research and animal pathology laboratories as the type of tissue and the procedures to be performed may dictate their use. If a knife is used, sharpening techniques should be discussed in the procedure manual. A sharp edge is paramount in obtaining a quality frozen section. The microtome blade angle and block face angle should be closely monitored.

### 13.14.2. Anti-roll plate

This is attached to the front of the microtome blade adaptor and is intended to stop the natural tendency of frozen sections to curl upwards on sectioning.

Anti-roll adjustments include:

- Correct height of blade edge.
- Correct angle of blade.
- Edge of plate should not be nicked or damaged.
- Cabinet temperature.

If the anti-roll plate is not working correctly, a sable hair brush can be used to manipulate the section.

## 13.15. Sectioning technique

Frozen sectioning requires practice to master the technique. Speed, tissue type and temperature of the block and cabinet play important roles in frozen sectioning. The cut section rests on the surface of the blade holder, a room temperature slide is held above it and electrostatic attraction causes the tissue to adhere to the slide. If tissues are being cut which require harsh or lengthy staining procedures, positively charged or coated slides should be used.

### 13.15.1. Decontamination

It is extremely important for the safety of individuals using the cryostat that decontamination policies be written and followed. Many newer cryostats have disinfecting apparatus built into the cabinet using either a disinfectant spray system, ozone spray, formaldehyde vapors or a disinfectant spray and UV light combination. Safety should always be foremost in the minds of those handling unfixed, fresh tissue samples.

Frozen sections provide a valuable tool in the rapid diagnosis of tissues during surgery. The pathologist selects a piece of tissue and this is frozen using one of the techniques previously discussed. The correct orientation of the tissue cannot be over emphasized. The slide is immediately submerged in cold acetone or 95% alcohol and the sections are stained immediately by a rapid hematoxylin and eosin (H&E), methylene blue or polychrome stain. With properly cut and stained slides a rapid diagnosis can be made for

the surgeon. Tissue samples or the area of interest may be small and care should be taken to provide a slide of diagnostic quality.

## 13.16. Ultra-cryotomy

This is used primarily in research laboratories. It involves rapid freezing of fixed or unfixed tissue by using isopentane and liquid nitrogen and cutting Microtomy for paraffin and frozen sections at 50-150 nm.

### 13.16.1. Frozen section substitution

The technique of frozen section substitution involves the rapid freezing of the tissue to −160°C in isopentane super cooled by liquid nitrogen. Cryostat sections are cut at 8–10 μm and placed in a cold container maintained at cryostat temperature. The sections are transferred to water-free acetone and cooled to −70°C for 12 hours. The sections are floated onto slides, allowed to dry and the required histochemical method applied.

# Chapter 14
# Staining

# 14. Introduction

Staining is a process by which a color is imparted to sectioned tissue. Specially manufactured dyes are used for this purpose. These dyes are prepared by adding an auxochrome to a chromophore. An auxochrome is a compound which when added to a chromophore forms a dye.

This may be acidic or basic. A **chromophore** is a compound, which although colored, does not have the properties of a dye or stain. The dye stains the tissues by binding with specific sites. Compounds called **mordants** help in achieving this binding.

## 14.1. Classification of stains:

All stains are composed of an acid and a basic component. Generally, the stains are classified as:

- Acid stains
- Basic stains
- Neutral stains

### 14.1.1. Acid stains

In an acid stain the acidic component is coloured and the basic component is colorless e.g., in acid fuchsin, which is composed of sodium and rosaniline Tri sulphonic acid, the sodium is colorless and rosaniline Tri sulphonic acid is coloured. Acid dyes stain basic components of tissue e.g., cytoplasmic proteins. The colours imparted are shades of red. Most commonly used acid dye is eosin and is shown in figure 5.1 and 5.2.

### 14.1.2. Basic stains

In the basic dyes the basic component is coloured and the acidic component is colorless. The example is basic fuchsin. Basic dyes stain acidic components of tissue e.g., nucleic acids. The colors imparted are shades of blue. Most commonly used basic dye is hematoxylin as shown in figure 14.1 and 14.2.

**Figure 14.1: Showing Eosin and hematoxylin stains**

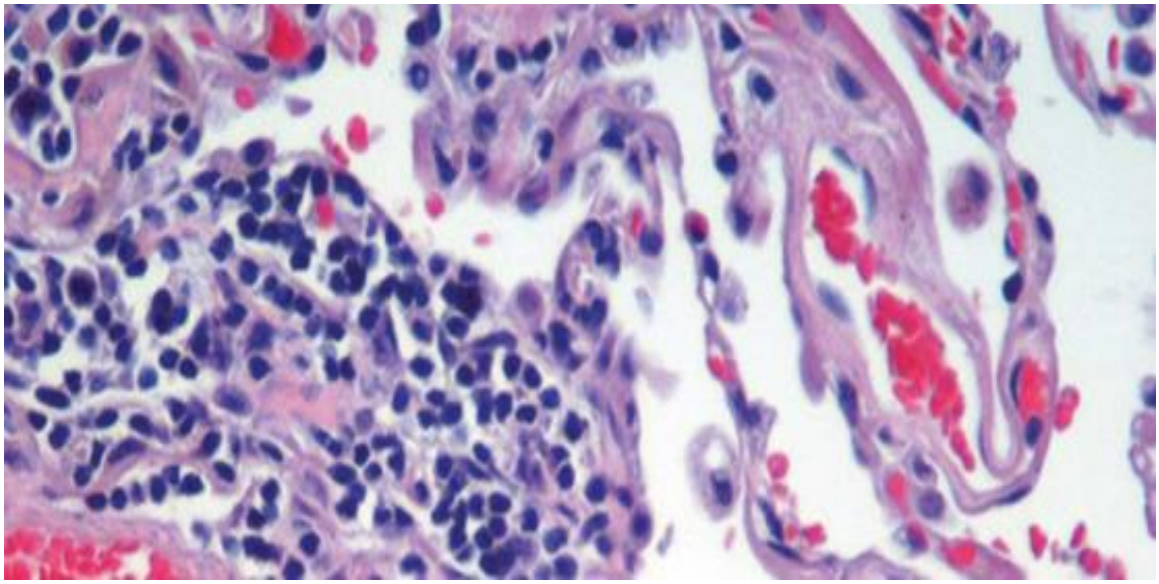

**Figure 14.2: Showing Hematoxylin and eosin stained tissue**

### 14.1.3. Neutral stains

When an acidic dye is combined with a basic dye a neutral dye is formed. As it contains both colouring components it stains all components of tissue but with different colours. This is the basis of Romanowsky stains (e.g., Leishman stain).

## 14.2. Procedure of Staining

Like processing, staining can also be performed manually or mechanically.

### 14.2.1. Manual staining

In a small laboratory where only, a few slides are stained this is the method of choice. It is time consuming, but economical. Reagent containers are placed in a sequence (figure 14.3). Slides are placed in a carrier and are then moved from one container to other at specified intervals till the process is complete as shown figure 14.4.

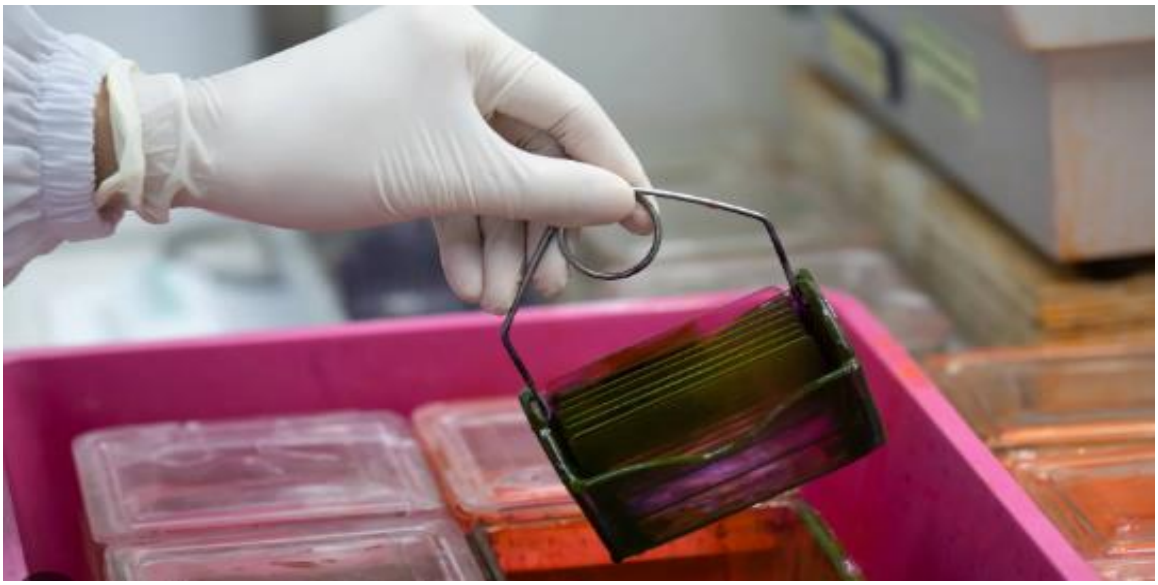

**Figure 14.3: Staining process**

**Figure 14.4: Showing staining**

### 14.2.2. Automated staining

The above procedure is performed with the help of a mechanical device similar to one described for processing. Automated stainers of various kinds are now freely available (figure 14.5) In these the reagent jars are arranged according to a desired sequence. The carrier containing slides is rotated through these at intervals, which are set by the operator. These are usually microprocessor controlled and are programmable.

**Figure 14.5**: Automatic stainer

The advantages are:

- Reduce manpower requirements
- Precise control the timing
- Large number of slides stained simultaneously
- Less reagent consumed

### 14.3. Hematoxylin and Eosin Staining

It is commonly used for routine histopathology and in diagnostic cytology. Its particular value lies in its ability of imparting proper differentiation to distinguish between different types of connective tissue fibers and matrices, by staining them different shades of red and pink.

**Principle**: First the tissue is cleared of all wax and then rehydrated to facilitate the entry of dyes. The tissue sections are then sequentially exposed to a basic dye e.g., Harris's Hematoxylin and an acid dye e.g., eosin. This stains both basic and acid components of the tissue.

**Reagents**:

Harris's Hematoxylin:
Hematoxylin crystals 5.0 g
Alcohol 95% 50 ml
Ammonium or Potassium Alum 100 g
Mercuric oxide 2.5 g
Distilled water 1 liter
Glacial acetic acid 40 ml

Dissolve separately by heating, hematoxylin in alcohol and alum in water, mix and rapidly boil. Remove from flame and add mercuric oxide. Reheat for 1 min or until it becomes dark purple. Remove from flame and cool in a basin of cold water. Stain is ready to use. Add 2-4 ml of Glacial acetic acid per 100 ml of solution if desired.
Acid alcohol: Mix one liter 70% alcohol with 10 ml of concentrated hydrochloric acid.
Ammonia water: Mix 2-3 ml of strong ammonia with one liter of tap water.
Alcoholic eosin solution:
Eosin (water soluble) 2 g
Distilled water 160 ml
Alcohol 95% 640 ml
Other reagents: Xylol, absolute alcohol, rectified spirit and methylated spirit are also needed.

**Staining procedure**

1. Put the sections fixed on a glass slide in xylol for 3 min.
2. Then transfer to absolute alcohol for 3 min.
3. Transfer to rectified spirit (80% alcohol) for 2 min.
4. Place in methylated spirit for 2 min.
5. Wash the slide in running water for 1 min and put it in Harris hematoxylin for 3-5 min.
6. Wash in running water for 30 seconds and wash the excess dye in 1% acid alcohol by continuous agitation for 15 seconds.
7. Wash in running water for 30 seconds.
8. Give 2-3 dips in ammonia water solution until tissues attain a blue color.
9. Wash in running water for 2-3 dips.
10. Counter stain with eosin for 2-3 min.
11. Wash in running tap water for 30 seconds.
12. Dehydrate by keeping in increasing concentrations of alcohol (2-3 dips in 70%, 95% and absolute alcohol).
13. Clear it in xylol and mount with Canada balsam.

**Result**

Nuclei Bright blue
Muscle, keratin Bright pink
Collagen and cytoplasm Pale pink (Figure 14.6)
Erythrocytes Orange red

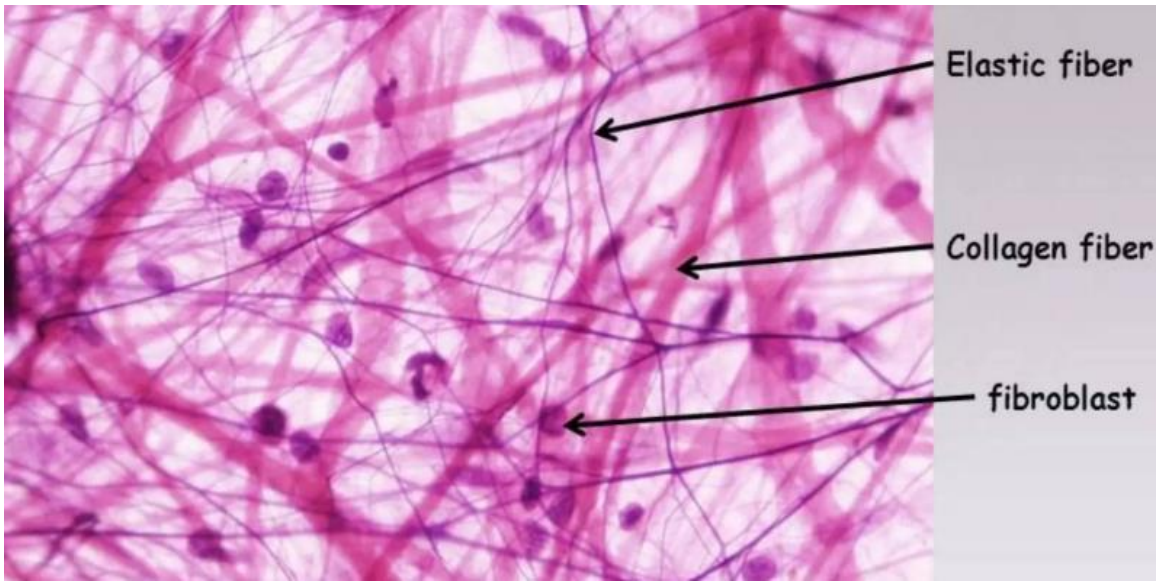


**Figure 14.6: Shows H&E staining of connective tissue**

### 14.4. Notes and Precautions

- Other hematoxylins like Mayer's hematoxylin may also be used. All have different methods of preparation.
- The reagents must be checked daily for deterioration and changed when needed. In the manual method, the xylol and alcohols must be changed daily, hematoxylins once a week, eosin and acid alcohol twice a week, and ammonia water daily. This regimen may be modified by the amount of usage. In the automatic stainer, xylol, alcohols, eosin and acid alcohol, are changed twice a week. Hematoxylins is changed once in two weeks and ammonia water is changed daily.
- The quality of alcohol must be checked before use. This can be done by adding 4-5g of copper sulphate crystals to a Coplin jar containing alcohol. If the color remains unchanged (bluish white) for 10 min, it is acceptable. If the color changes to green the quality of alcohol is unsuitable for processing.

## References


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