PEOPLE'S DEMOCRATIC REPUBLIC OF ALGERIA

Ministry of Higher Education and Scientific Research

University 8 May 1945 Guelma

Faculty of Natural and Life Sciences and Earth and Universe Sciences

Department of Natural and Life Sciences

Course Handout in **:**

Animal biology:

Embryology and Histology

Intended for first-year students in natural and life sciences

Dr. OUMEDDOUR Abdelkader

Academic Year 2025-2026.

Figure list

Table List

List of abbreviations

ABP: Androgen Binding Protein.
ALP: Alkaline Phosphatase.
ANS: Autonomic Nervous System.
ATP: Adenosine Triphosphate.
Ba-P: Basophil Progenitors.
BBB: Blood-Brain Barrier
BMPs : Bone Matrix Proteins.
BMPs : Bone Morphogenic Proteins.
C1-C9: Complement proteins 1-9.
CFU: Colony-Forming Unit.
CFU-Eo: Colony-Forming Unit-Eosinophil
CLP: Common Lymphoid Progenitor.
CMP: Common Myeloid Progenitor.
CNS: Central Nervous System
CS: Chondroitin Sulfate.
DA: Dopamine.
DNA: Deoxyribonucleic Acid.
DS: Dermatan Sulfate
E2: Estradiol.
ECM: Extracellular Matrix.
ENT: Ear, Nose, and Throat.
Eo-P: Eosinophil Progenitors.
EPI: Epinephrine (Adrenaline).
FSH: Follicle-Stimulating Hormone.
GAGs: Glycosaminoglycan Molecules.
GalNAc: N-acetylgalactosamine.
GlcNAc: N-acetylglucosamine.
GMP: CFU-GM: Granulocyte/Macrophage Progenitor Cells.
GM-P: Granulocyte/Monocyte Progenitors.
Hb: Hemoglobin.
hCG: Human Chorionic Gonadotropin.
HS: Heparan Sulfate.
HSCs: Hematopoietic Cells.
IGFs: Insulinlike Growth Factors.
IgG: Immunoglobulin G
IL-1 : Interleukin 1.
IL-6 : Interleukin 6.
KS : Keratan Sulfate.
LH: Luteinizing Hormone.
MC-P: Mast Cell Progenitors
MEP: Megakaryocyte/Erythrocyte Progenitors.
MGG: May-Grünwald Giemsa staining
MGP: Matrix Gla-Protein.
NE: Norepinephrine.
P4: Progesterone.
PDGFs: Platelet-Derived Growth Factors.
PNS: Peripheral Nervous System.
pro-B: B lymphocyte progenitors.
pro-NK: NK cell progenitors.
pro-T: T lymphocyte progenitors.
RBCs: Red Blood Cells.
SHH: Sonic Hedgehog.
SNS: Somatic Nervous System.
TGF-β: Transforming Growth Factor β.
TnC: Troponin C.
TNF-α: Tumor Necrosis Factor α.
TnI: Troponin I.
TnT: Troponin T.
ZP: Zona Pellucida.
ZP1: Zona Pellucida Glycoprotein 1.
ZP2 : Zona Pellucida Glycoprotein 2.
ZP3: Zona Pellucida Glycoprotein 3.
α-SMA: α-Smooth Muscle Actin.

Content

Introduction

The development of living beings begins with the phenomenon of **fertilization**, which forms a fertilized egg, or **zygote**, and ends with birth in viviparous species or hatching in oviparous species. The zygote quickly begins to divide through simple mitoses to form a multicellular organism, the **morula**, which develops a cavity, the blastocoel, and then becomes a **blastula**, marking the end of cleavage. Morphogenetic movements, which occur during gastrulation, allow the rearrangement of the blastomeres of the blastula into three fundamental layers: an outer layer or **ectoderm**, a middle layer present only in triploblasts or **mesoderm**, and an inner layer or **endoderm**. From these three embryonic layers begins **organogenesis** and the formation of the presumptive areas of the embryo, which will be the origin of the four fundamental tissues that form the animal's organs.

The cells derived from the three embryonic layers are grouped to form tissues that vary in their structure, function, and origin. Multicellular animals have four main types of tissues: **epithelial tissues**, **connective tissues**, **muscle tissues**, and **nervous tissues**. Recall that tissues are groups of similar cells, with a more or less abundant extracellular matrix, fulfilling interdependent functions. These tissues combine to form organs, such as the skin, kidneys, or nervous system, which perform specific and specialized functions within the body. Organs are organized into organ systems to fulfill physiological functions: examples include the circulatory system, which includes the heart and blood vessels, and the digestive system, composed of several organs, including the stomach, intestines, liver, and pancreas. These major systems come together to create a whole organism.

Part one: Embryology

A fundamental property of living organisms is their ability to reproduce and thus ensure the continuity of life. Sexual reproduction usually involves the union of two haploid gametes (sperm and eggs) stemming from two different parental individuals (male and female), leading to a diploid offspring in which each parent obtains an equal genetic representation.

The sex of these individuals is genetically determined by the nature of the sex chromosomes: X and "Y" chromosomes in mammals and "Z" and "W" in birds.

- One of the two parents is heterogametic and produces two categories of gametes that differ by the nature of the sex chromosome: male (XY) in mammals and female (ZW) in birds.
- The other parent is homogametic and forms only one category of gametes: female (XX) in mammals and male (ZZ) in birds.

Embryology: is the discipline concerned with the study of embryogenesis, the development of the embryo from a fertilized egg cell.

Embryonic development occurs in several stages in all metazoans:

- Fertilization
- Segmentation
- Gastrulation
- Neurulation
- Organogenesis.

Chapter 01: Gametogenesis

Gametogenesis is the process of formation and differentiation of haploid gametes (sperms and ova) from the diploid primary germ cells, gametogonia (**spermatogonia** and **oogonia**) present in primary sex organs called gonads (testes in male and ovaries in female respectively).

— The differentiation of reproductive cells or gametes is gametogenesis.
— The male gametes are spermatozoa, their formation, spermatogenesis, and takes place in a specialized organ, the male gonad: **testis**.
— The female gametes are the ova = oocytes (or eggs). Their formation is oogenesis takes place in specialized organs, the female gonad: **ovaries** (Figure1.1) [1].

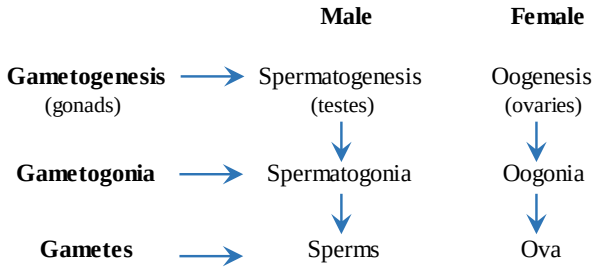

Figure 1.1. Gametogenesis: oogenesis in females and spermatogenesis in males [2].

Meiosis is the cell division that takes place in the germ cells to generate male and female gametes, sperm and egg cells, respectively. Meiosis requires two cell divisions, **meiosis I** and **meiosis II**, to reduce the number of chromosomes to the haploid number of 23. As in mitosis, male and female germ cells (**spermatocytes** and **primary oocytes**) at the beginning of meiosis I replicate their DNA so that each of the 46 chromosomes is duplicated into sister chromatids. In contrast to mitosis, however, **homologous chromosomes** then align themselves in pairs, a process called **synapsis**. Shortly thereafter, meiosis II separates sister chromatids. Each gamete then contains 23 chromosomes [1].

1 Spermatogenesis

Spermatogenesis is the formation of haploid, microscopic and functional male gametes (**spermatozoa**) from the diploid reproductive cells (**spermatogonia**) present in the testes of male organism.

1.1 Steps of spermatogenesis

Spermatogenesis is divided into two parts: Formation of spermatid and spermiogenesis

- **Formation of Spermatid**: It is divided into three phases:

1.1.1 Multiplicative phase (or Mitotic phase)

It involves the rapid mitotic division of diploid primary or primordial germ cells, called **gonocytes**, present in germinal epithelium of the seminiferous tubules of the testes [2, 3].

— This forms large number of diploid and rounded sperm mother cells called **spermatogonia** (*Greec. sperma = seed; gone = offspring*).
— Some spermatogonia act as stem cells (called **Type A spermatogonia**) and go on dividing and adding new cells by repeated mitotic divisions, so forming spermatogenic lineage, but some spermatogonia move inward and enter **growth phase** (called **Type B spermatogonia**) (Figure 1.2) [2, 3].

1.1.2 Growth phase

Characterized by **spermatocytogenesis** in which a diploid **spermatogonium** (*pl. : spermatogonia*) increases in size (about twice) by the accumulation of nutritive materials (derived from germinal cells and not synthesized) in the cytoplasm and replication of DNA, and forms diploid **primary spermatocyte** (2n ch.). During this, the primary spermatocyte prepares itself to enter **meiosis** (Figure 1.2) [2, 3].

1.1.3 Maturation or meiotic phase

Characterized by **meiosis**. The diploid **primary spermatocyte** undergoes meiosis I (reductional or heterotypical division) and forms **two haploid cells** called **secondary spermatocytes**, each containing 23 chromosomes. It is immediately followed by meiosis II (equational or homotypical division) in each secondary spermatocyte to form two haploid **spermatids**, each of which has 23 chromosomes (Figure 1.2). So each diploid spermatogonium produces 4 haploid spermatids [2, 3].

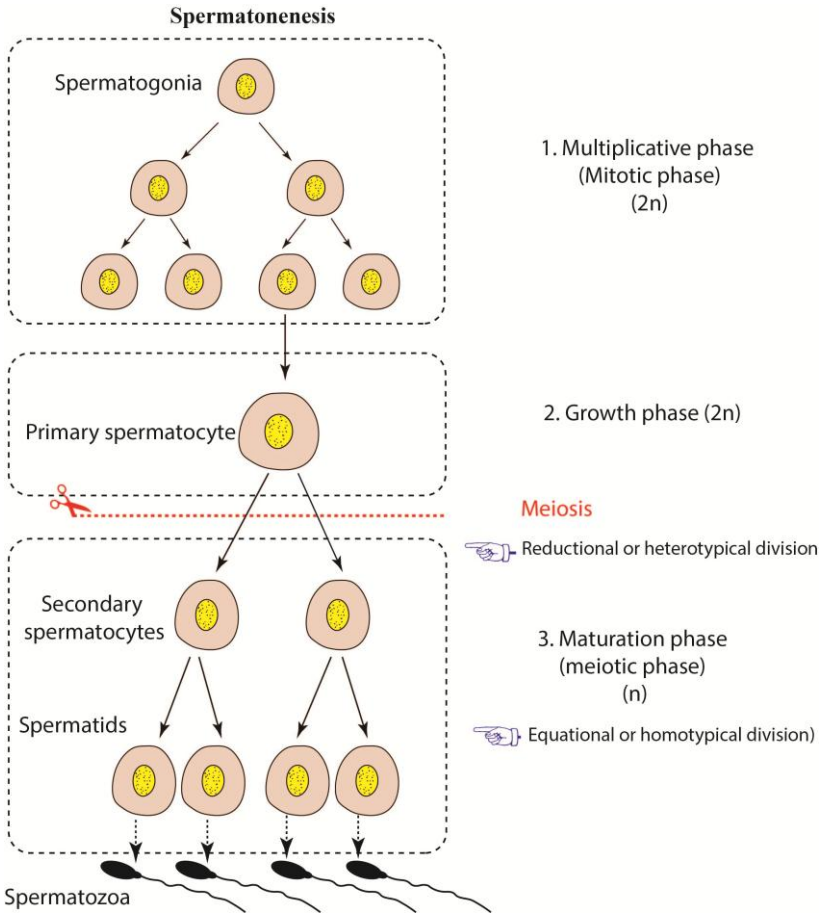

Figure 1.2. The process of spermatogenesis [2].

1.2 Spermiogenesis

The spermiogenesis or spermioteliosis is transformation of a non-motile, rounded and haploid spermatid into a functional and motile **spermatozoa** (*sng*. spermatozoon). The main aim is to increase the sperm motility by reducing weight and development of locomotory structure (Table 1.1) [2, 4].

Table 1.1. Changes in spermatid organelles.

Structure of spermatid	Changes in the sperm
Nucleus	Shrinks and elongated
Golgi complex	Changes to acrosome
Distal centriole	Forms axial filament of sperm tail
Mitochondria	Form mitochondrial spiral of sheath called nebenkem
Cytoplasm	Generally lost except a thin sheath called manchette

Spermiogenesis is comprised of four phases (Figure 1.3):

— **Golgi phase**: involves the packaging of hydrolytic enzymes by the Golgi apparatus into vesicles that fuse with each other to form the acrosomal granule–containing acrosomal vesicle.

— **Cap phase**: The acrosomal vesicle not only enlarges during the cap phase, but also attaches to and partially envelops the nuclear membrane and becomes known as the acrosome.

— **Tail phase**: The tail phase is characterized by the elongation of microtubules on one of the centrioles of the spermatid to become the tail. The microtubules form an **axoneme**. The axoneme contains microtubules that are arranged in a 9 + 2 configuration.

— **Maturation phase**: when the spermatids release their excess cytoplasm, freeing individual spermatozoa, from the syncytium. Sertoli cells phagocytose the cellular remnants of spermatids; this process is known as **spermiation [2]**.

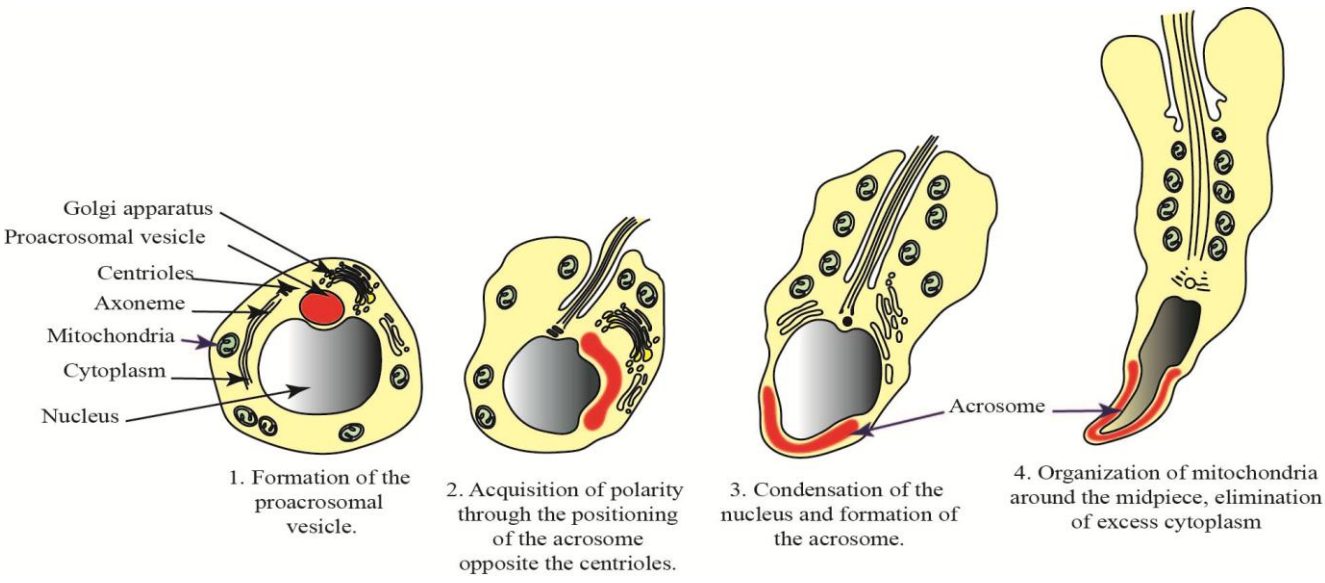

Figure 1.3. Important stages in transformation of the spermatid into the spermatozoon [2].

1.3 Structure of sperm

A spermatozoon consists of a head, a midpiece, and a tail.

— The **head** contains the condensed nucleus and is capped by an apical vesicle filled with hydrolytic enzymes (e.g., acrosin, hyaluronidase, and neuraminidase). This vesicle, the **acrosome**, plays an essential role in fertilization.
— The **midpiece** contains large, helical mitochondria and generates energy for swimming.
— The long **tail** contains microtubules that form part of the propulsion system of the spermatozoon (Figure 1.4) [2].

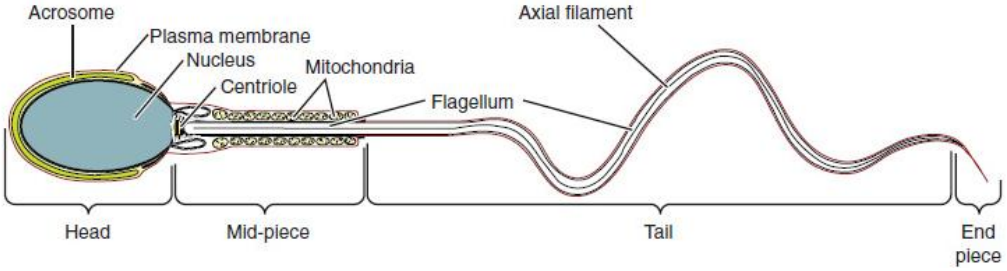

Figure 1.4. Structure of spermatozoon [2].

1.4 Capacitation

Capacitation is a collective term for changes a sperm undergoes when it comes into contact with the female reproductive tract. These changes include reorganisation of membrane proteins, metabolism of membrane phospholipids and reduction in membrane cholesterol levels [4].

1.5 Structure of seminiferous tubules

Each testis possesses approximately 500 sperm-producing seminiferous tubules embedded in a loose vascular connective tissue. The thick seminiferous epithelium (germinal epithelium) is composed of two different epithelial types: **Sertoli** (supporting) **cells** and **spermatogenic cells** that are in the process of differentiation to form spermatozoa (Figure 1.5).

Most of the cells composing the seminiferous epithelium are spermatogenic cells that accomplish the process of spermatogenesis, these include: **spermatogonia** (dark type A spermatogonia, pale type A spermatogonia, type B spermatogonia), **primary spermatocytes (2n)**, **secondary spermatocytes(1n**), **spermatids**; and spermiogenesis, the transformation of spermatids into mature **spermatozoa** (sperm) [2].

The occluding junctions formed between adjacent Sertoli cells subdivide the lumen of the seminiferous tubule into:

— **A basal compartment**, basal to the tight junctions, which is exposed to the underlying vascular connective tissue.
— **An adluminal compartment**, which is isolated from the vascular connective tissue, establishing a blood-testis barrier and protecting the developing gametes from being exposed to the immune system, which would otherwise mount an immune response against the developing gametes.

The functions of Sertoli cells are to:

— Support, protect, and nourish developing spermatogenic cells.
— Phagocytose cell remnants (residual bodies) discarded during the process of spermiogenesis.

— Facilitate the release of mature spermatids into the lumen of the seminiferous tubules via actin-mediated contraction (spermiation)
— Sertoli cells Secrete androgen binding protein (ABP), inhibin, fructose-rich fluid, testicular transferrin, antimüllerian hormone [2].

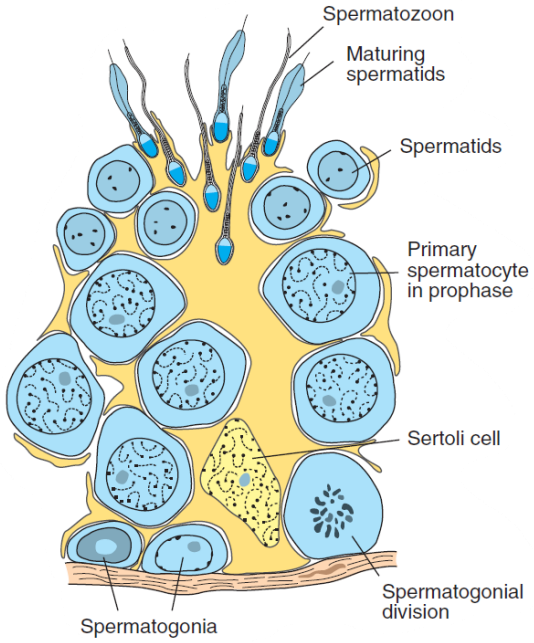

Figure 1.5. Structure of seminiferous tubules [5].

2 Oogenesis

Oogenesis involves the formation of haploid female gametes (ova), from the diploid egg mother cells (oogonia) of ovary of female organism [1, 6].

2.1 Phases of oogenesis

2.1.1 Multiplicative phase (or mitotic phase)

In this certain primary germ cells of germinal epithelium of ovary undergo rapid mitotic divisions to form groups of diploid egg mother cells, **oogonia (Figure 1.6).**

2.1.2 Growth phase

Oogenesis is of very long duration (6-14 days in hen, 3 years in frog and 12-13 years in human female). During growth phase, one oogonium of egg nest is transformed into diploid **primary oocyte**.

2.1.3 Maturation phase (meiosis)

In this, the diploid and fully grown primary oocyte undergoes meiosis-I (reductional division) to form two unequal haploid cells:

— The smaller cell is called first **polar body** (Polocyte) and has very small amount of cytoplasm.
— The larger cell is called **secondary oocyte** and has bulk of nutrient-rich cytoplasm. *Both of these are haploids and each has 23 chromosomes* **(Figure 1.6).**

Secondary oocyte undergoes meiosis-II (equational division) to form two unequal haploid cells. The smaller cell is called second polar body and has very little of cytoplasm. The larger cell is called **ootid** (immature ovum) that differentiates into an ovum.

Meanwhile, first polar body may divide into two. The primary function of formation of polar bodies is to bring haploidy but to retain the whole of the cytoplasm in one ovum to provide food during the development of zygote to form an embryo [1, 6].

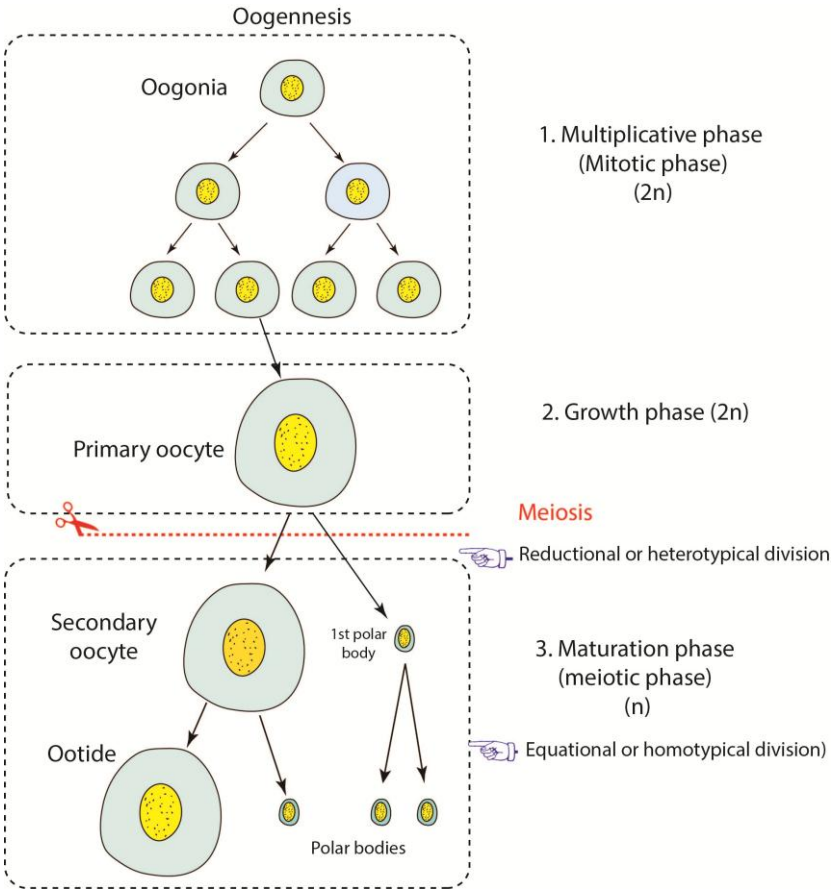

Figure 1.6. Oogenesis in females [2].

2.2 Folliculogenesis and oogenesis: timeline and stages

- **In the embryo**, primordial germ cells multiply during fetal development to form **oogonia**
  - Oogonia are naked, but after meiosis begins, cells from the ovary partially surround the **primary oocytes** to form **primordial follicles** (Figure 1.7).
  - At birth, the ovary contains around 400 000 primordial follicles which contain primary oocytes that remain arrested in the prophase stage of meiotic division I, until sexual maturity.
- **By birth,** the **primary oocytes** are covered by a complete layer of follicular cells, and the complex of primary oocyte and the follicular (granulosa) cells is called a **primary follicle** [1, 6]**.**

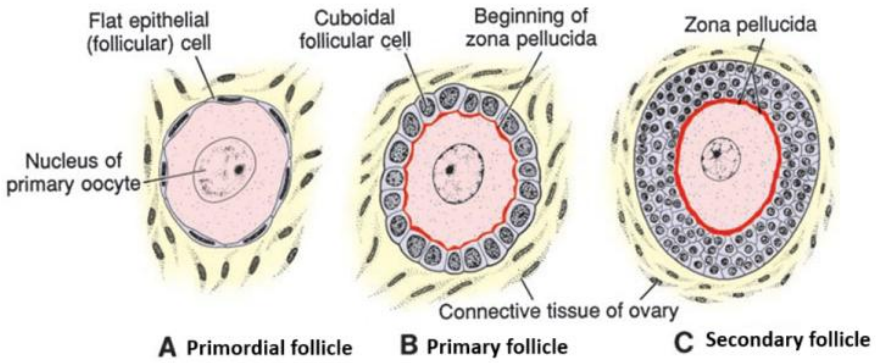

Figure 1.7. Structure of primordial follicle, primary follicle and secondary follicle [5].

— While the primary follicle takes shape, a prominent, translucent, noncellular membrane called the **zona pellucida** forms between the primary oocyte and its enveloping follicular cells.

— **Between birth and puberty:** several of the primary follicles degenerate over time, leaving only a few thousand follicles intact by the time puberty hits (Figure 1.8) [6].

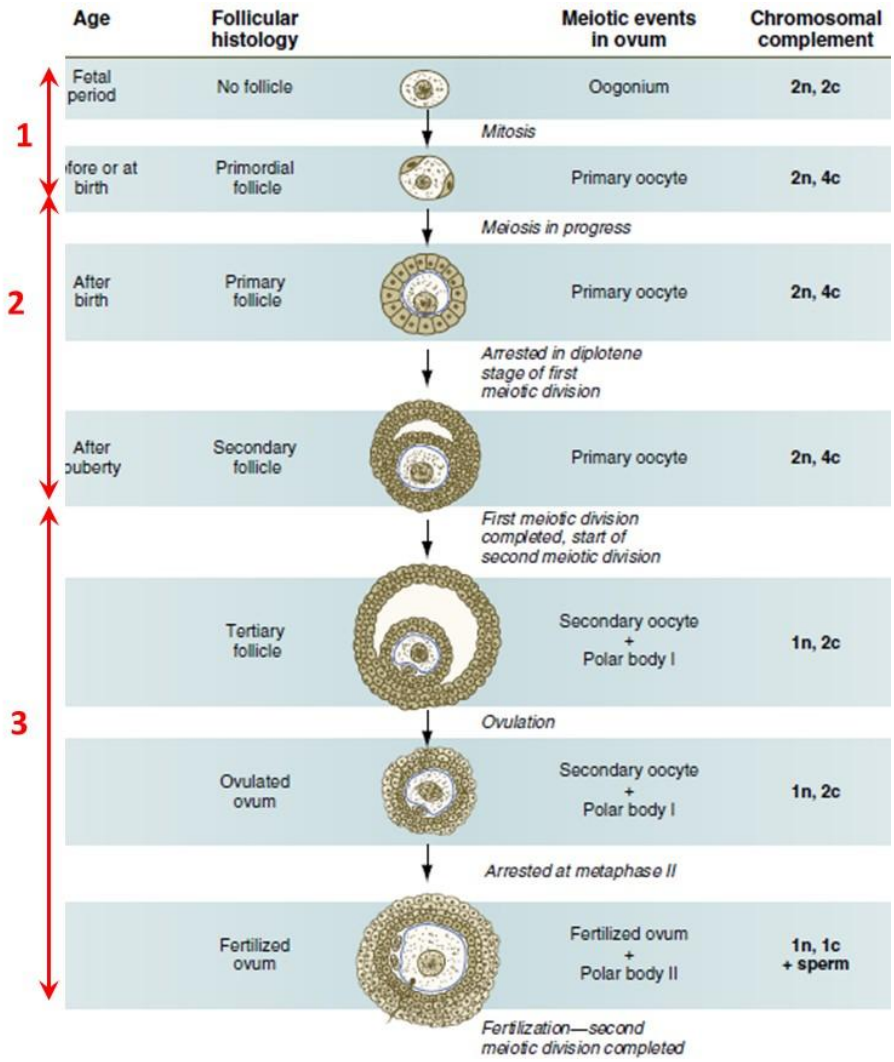

Figure 1.8. Folliculogenesis and meiotic events in ovum [6].

- **After puberty:**

— Many of the **primary follicles** enlarge, because of the increase in size of the oocyte with more than one layer of surrounding granulosa cells. When this happens, the primary follicle has matured into a **secondary follicle,** which completed the first meiotic division. A basement membrane called the **membrana granulosa** surrounds the epithelial **granulosa cells** of the secondary follicle.

— In every month cycle, the second division then completed, and a tertiary follicle (or Graafian follicle, or antral follicle) is formed. This contains a secondary oocyte and polar body I. This second division is not completed, it arrested in metaphase II, and resumed and completed during fertilization [6].

— Tertiary follicles is characterized by a fluid-filled space called the antrum, and two layers of theca: theca interna (highly vascularized and glandular), and theca externa (connective tissuelike).

— The follicle ruptures, and the egg is released. If and when the egg is penetrated by a spermatozoon, this activates the egg, and meiosis II is completed (approximately 3 hours later).

— In a broad sense, Graafian follicles can be divided into two major groups: healthy and **atretic** (Figure 1.9) [6].

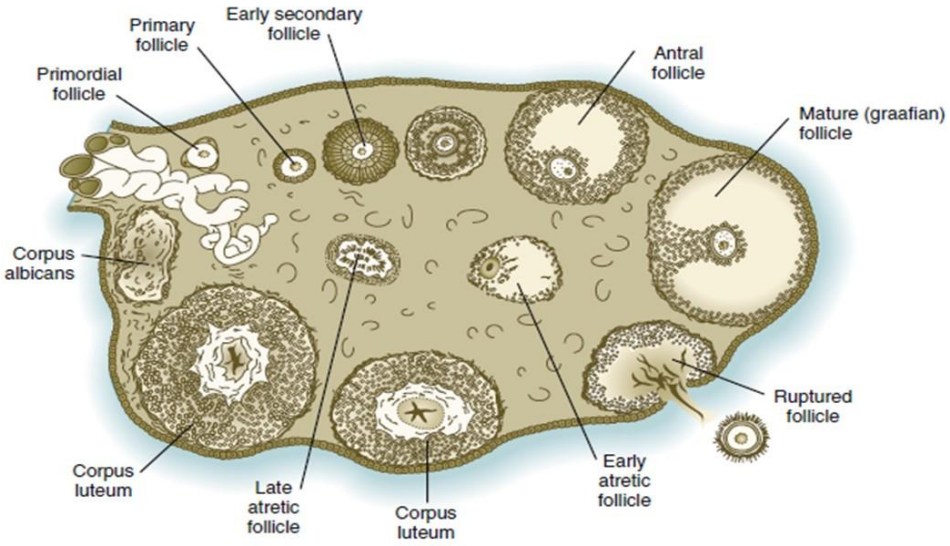

Figure 1.9. The sequence of maturation of follicles within the ovary [6].

2.3 Structure of the mammalian egg

A mature egg (ovum, oocyte) is the biggest cell in the body at 0.1 mm. It contains a large nucleus and within it the DNA material of the egg. The egg is surrounded by the **corona radiata** and the **zona pellucida**.

Graafian follicles display an **antrum** containing follicular fluid, or liquor folliculi, and surrounded by the **granulosa** cells and the **theca externa** (contains the endocrine cells that produce the steroid hormones), **theca interna** (connective tissue layer) and basal lamina (Figure 1.10).

The cytoplasm contains yolk granules that nourish the embryo early in development until it is nourished by its mother. Egg quality is influenced by the nuclear and mitochondrial genome but also by the microenvironment provided by the ovary [2].

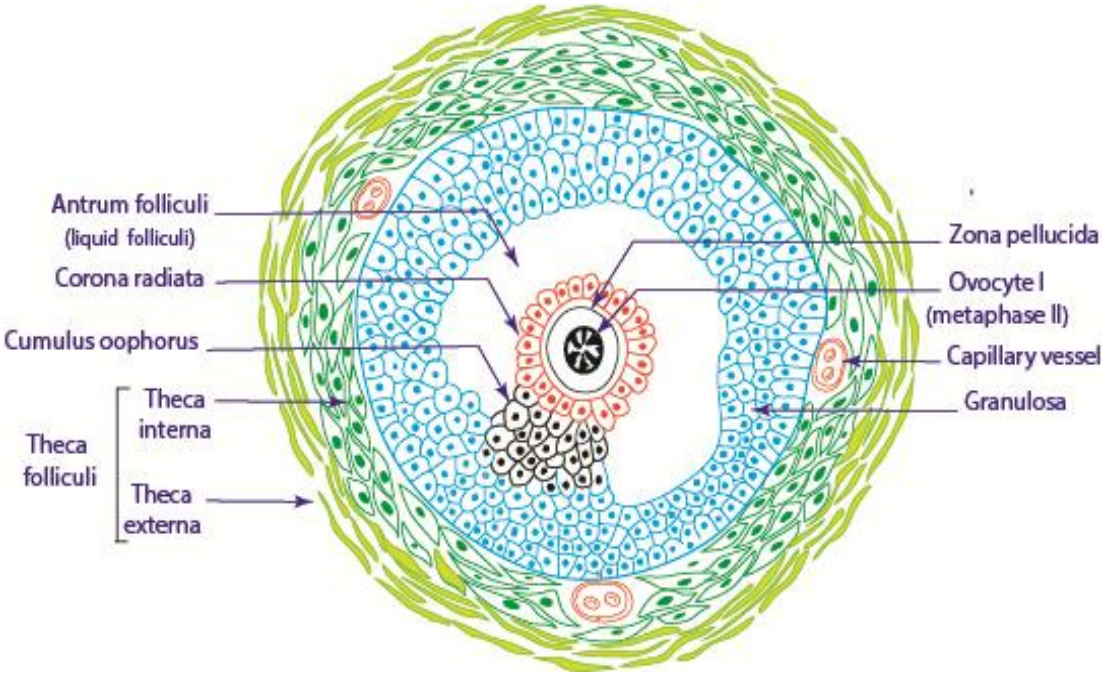

Figure 1.10. Structure of tertiary follicle (Graafian follicle) [2].

2.4 Difference between spermatogenesis and oogenesis

Differences between spermatogenesis and oogenesis are summarized in the table 1.2.

Table 1.2. Difference between spermatogenesis and oogenesis [2]

	Spermatogenesis	**Oogenesis**
	Process	
Location	Occurs entirely in testes	Occurs mostly in ovaries
Meiotic division	Equal division of cells	Unequal division of cytoplasm
Germ line epithelium	Is involved in gamete production	Is not involved in gamete production
Gametes		
Number produced	Four	One (+ 2 or 3 polar bodies)
Size of gametes	Sperm smaller than spermatocytes	Ova larger than oocytes
Timing		
Duration	Uninterrupted process	In arrested stages
Onset	Begins at puberty	Begins in fetus (prenatal)
Release	Continuous	Monthly from puberty (menstrual cycle)
End	Lifelong (but reduce with age)	Terminates with menopause

Chapter 2: Fertilization

Fertilization is the process during which a male gamete (sperm), and a female gamete (oocyte), unite together to form a single diploid cell (**Zygote** or **fertilized egg**).

It is a complex process that begins with a contact between sperm and ovum, and ends up with intermingling of the maternal and paternal pronuclei. Fertilization is a cell-cell recognition process that occurs between two distinct cells: a small asymmetric and motile sperm cell and a large and non-motile egg.

- Fertilization in mammals, reptiles and birds occurs in the oviduct of the female.
- Fertilization in fish and amphibians occurs in the salt and /or fresh water respectively [4].

1 Capacitation

Sperm capacitation is the set of natural physical changes that a sperm undergoes in order to be able to fertilize the ovum. These changes include reorganisation of membrane proteins, metabolism of membrane phospholipids and reduction in membrane cholesterol levels. Capacitation also involves changes in sperm motility, known as hyperactivation, which are thought to aid sperm progression up the oviduct, to enable sperm to move away from the oviductal epithelium and to provide the motive thrust needed for penetration of the zona pellucida

- In the cervix of female, seminal plasma secreted by the accessory glands of the male is removed.
- Uterine secretions cause the removal of cell surface (proteins).
- In the oviduct, glycosaminoglycans and cholesterol of sperm plasma membrane are removed.

2 Stages of fertilization

Fertilization occurs in 4 steps:

1) Gamete recognition and adhesion
2) Acrosome reaction
3) Fusion of membranes and cortical reaction
4) Fusion of male and female pronuclei

In mammals, at the time of ovulation, the oocyte (arrested in metaphase II) is surrounded by the **zona pellucida** and the **corona radiata**.

The oviduct secretes enzymes which weaken the connections between the cells of the corona radiata, which allows the spermatozoa to easily cross this first cellular barrier to reach zona pellucida [7].

2.1 Gamete recognition and adhesion

The gamete recognition and initial binding processes are mediated by the **zona pellucida** of the egg and the **proteins of the head** of the fertilizing spermatozoon. The zona proteins ZP3 and ZP2 are responsible for the initial (primary) and secondary interactions, respectively (Figure 1.11).

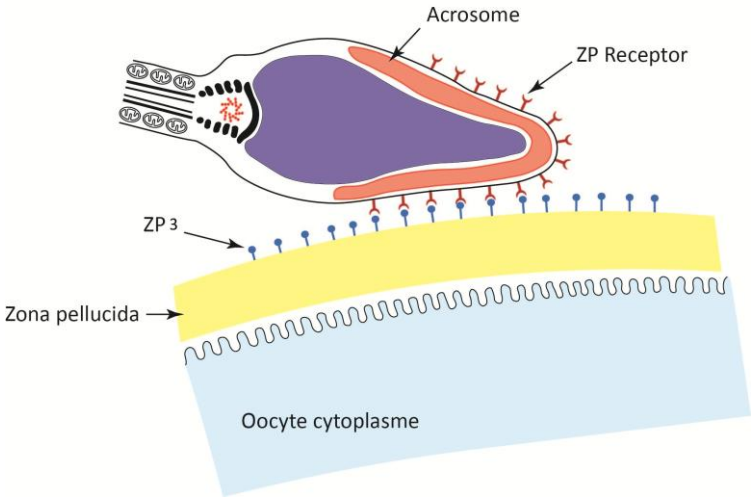

Figure 1.11. Gamete recognition and adhesion [8].

2.2 Acrosome reaction

Adhesion between ZP3 and sperm plasma membrane proteins promotes fusions between the acrosome outer membrane and the sperm head plasma membrane (Figure 1.12). This leads to the formation of membrane pores and the release by exocytosis of the enzymatic contents of the acrosome, among which we find :

— **Acrosin** which destroys ZP1 which reduces the resistance of the zona pellucida;
— **Hyaluronidase** which destroys the hyaluronic acid between associated to zona pellucida,
— **β-N-acetylglucosaminidase** which destroys the bond between ZP2, ZP3 and the sperm, which allows its entry into the perivitelline space;
— **β-neuraminidase** which locally digests ZP3 receptors which prevents any further binding [3, 5].

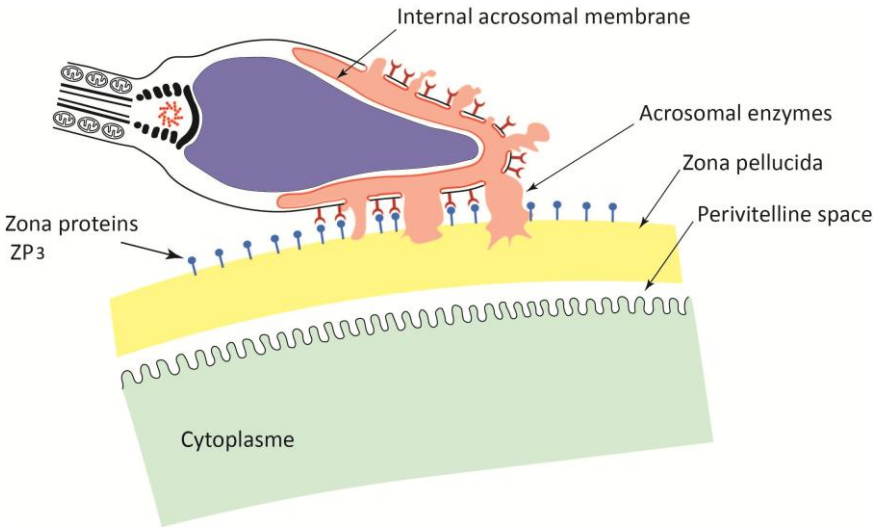

Figure 1.12. Acrosome reaction [8].

2.3 Fusion of plasma membranes and cortical reaction

In mammals, the fusion of plasma membrane is caused by the interaction between the proteins **fertilins** (fertilin β) of the inner membrane of the acrosome and **integrins** of the oocyte membrane, which causes :

— The cortical reaction that involves the fusion of cortical granules and oocyte plasma membrane with exocytosis of cortical granule contents (rich in proteases and proteoglycans) into the ZP (Figure 1.13).
— At this point, the second division of meiosis (blocked in metaphase II) is completed, with the expulsion of the second polar body [2, 3].

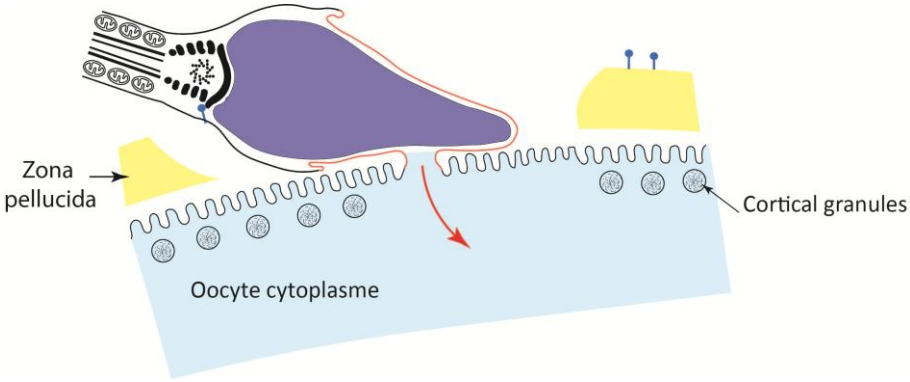

Figure 1.13. Fusion of plasma membranes and cortical reaction [8]

2.4 Fusion of male and female pronuclei

In eggs without yolk (Mammals), the two nuclei migrate towards the center of the egg. In eggs rich in yolk (birds, reptiles, insects) the fusion of the nuclei takes place at the animal pole devoid of yolk.

— The protamines associated to the male nucleus chromatin are replaced by histones.
— The two nuclei fuse to form a single nuclear envelope.
— The paternal and maternal chromosomes individualize again, they pair and organize themselves at the equator of the achromatic spindle at the time of the first cell division [2, 3].

Chapter 3: Cleavage

Cleavage is a period after fertilization, when a one cell embryo starts developing into an early multicellular organism called **morula** and then into a **blastula** (Figure 1.14). It consists of a series of mitotic divisions, which divide the large volume of a fertilized egg into numerous smaller, nucleated cells or **blastomeres.** Embryos of different phyla (sing. *phylum*) divide according to different patterns according to the amount and the distribution of the yolk.

In general, blastomeres formed in the relatively yolk-free **animal pole** are smaller than those on the **vegetal**, yolk-rich pole. During cleavage, small blastomeres (**micromeres**) are formed in the animal pole, whereas big blastomeres (**macromeres**), in the vegetal pole. However, as amount of yolk differs between species, the cleavage pattern observed in amphibians is not universal.

Pattern of embryonic cleavage is determined both by:

1) the position of the mitotic spindle (the cleavage furrow is formed perpendicular to the mitotic spindle and gradually splits the cytoplasm and its contents into two daughter cells)
2) the amount and distribution of yolk.
   - Yolk tends to inhibit cleavage.
   - It slows it down or actually prevents complete cleavage [4].

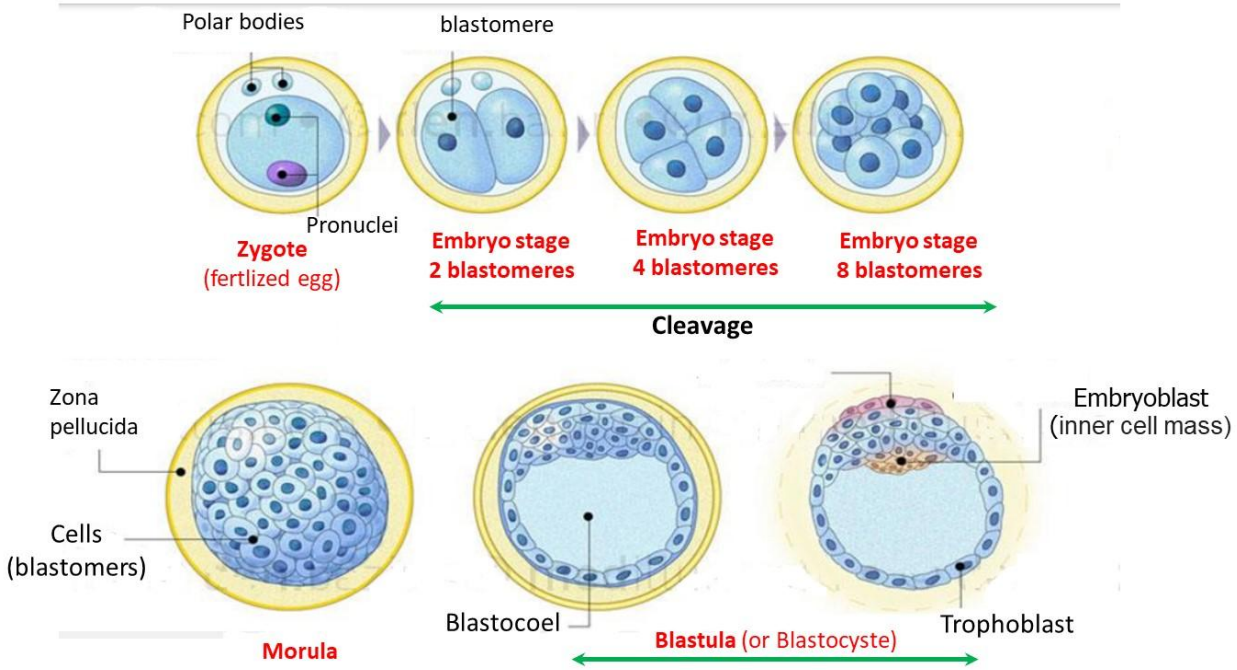

Figure 1.14. Zygote cleavage [9].

According to the distribution of yolk, we can distinguish two poles in the egg:

— The animal pole (or **animal hemisphere),** generally devoid of yolk, and contains the fertilization nucleus

— The vegetal pole (or **vegetal hemisphere)** generally contains the mass of yolk.
The yolk shows a graduation from the animal pole towards the vegetal pole (progressively diminish towards the animal pole). The cytoplasm and nucleus are concentrated in the upper portion or (animal pole) and the yolk material is concentrated in the lower portion (vegetal pole) [2].

1 Types of eggs

1.1 On the basis of amount of yolk present in the eggs

The eggs are classified into four types [10]:

1.1.1 Alecithal egg

They are free of yolk (contains no yolk), e.g. the eggs of eutherian mammals.

1.1.2 Microlecithal eggs (or Oligolecithal)

They contain very small amount or negligible amount of yolk, e.g. eggs of Sea urchin, amphioxus.

1.1.3 Mesolecithal eggs

They contain moderate amount of yolk, e.g., eggs of lungfish, frogs and toads.

1.1.4 Macrolecithal eggs (Megalecithal or Polylecithal)

They contain large amount of yolk, e.g., eggs of insects, sharks, bony fishes, reptiles, birds and egg-laying mammals.

1.2 On the basis of distribution of yolk in cytoplasm

The eggs are classified into three types:

1.2.1 Homolecithal eggs (or Isolecithal)

The very little amount of yolk present is uniformly distributed throughout the ooplasm. e.g. eggs of annelids, molluscs, echinoderms and mammals

1.2.2 Telolecithal eggs

In eggs containing moderate or large quantity of yolk, the distribution of yolk is not uniform. It is concentrated more towards the vegetal pole, e.g. eggs of amphibians, reptiles, birds and egg laying mammal.

1.2.3 Centrolecithal eggs

The very great amount of yolk is localized at the center of the egg, the nucleus lies at the geometric center of the yolk mass, surrounded by a small amount of cytoplasm. A thin cytoplasmic layer covers the surface of the yolk, e.g. eggs of insects [10].

2 Planes of cleavage

During early cleavage, distinct geometrical relationships exist between the blastomeres, i.e., each plane of cell division bears a definite relationship with each other. The planes of division are:

2.1 Meridional plane of cleavage

The cleavage furrow bisects both the poles of the egg passing through the polar axis; the cleavage plane is said to be meridional [11].

2.2 Vertical plane of cleavage

When a furrow passes in any direction from the animal pole towards the vegetal pole. However, it does not pass through the median axis of the egg; it appears on one side of the axis [11].

2.3 Equatorial plane of cleavage

It bisects the egg at right angle to the median axis and half way between the animal and vegetal poles. This type of cleavage plane is exhibited by Sea urchin [11].

2.4 Latitudinal plane of cleavage

It cuts the egg at right angles to the median axis but it passes either above (near the animal pole) or below (near the vegetal pole) the equator of the egg (Figure 1.15) [11].

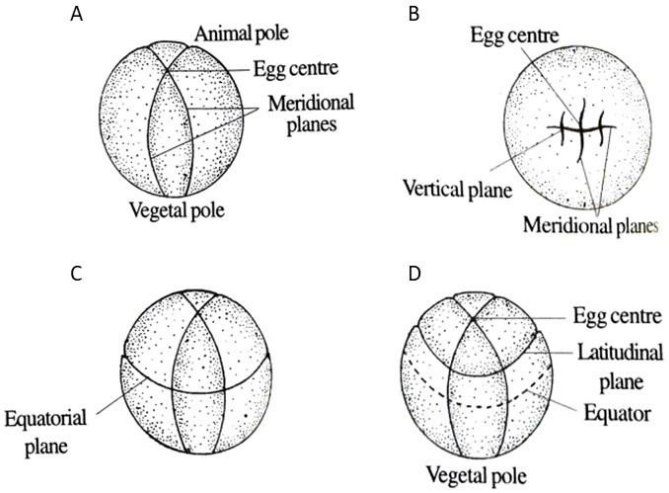

Figure 1.15. Planes of cleavage: (A) meridional, (B) vertical, (C) equatorial, (D) latitudinal [2].

3 Pattern of cleavage

There are mainly two types of cleavage : They are **holoblastic cleavage** when the cleavage furrows divide the entire egg, and **meroblastic cleavage** when segmentation takes place only in a small portion of the egg [11, 12].

3.1 Holoblastic cleavage

The entire egg divides. It is otherwise called total or complete cleavage. In holoblastic cleavage. When the blastomeres are equal in size, the cleavage is said to be **equal**. When the blastomeres are unequal, the cleavage is called **unequal** [11, 12].

Holoblastic cleavage is further divided into four types based upon the symmetry of cleavage. They are:

3.1.1 Radial cleavage

In this cleavage, the blastomeres are arranged radially around the central axis of the egg. The spindles are oriented either parallel or perpendicular to the polar axis (Figure 1.16). The first two cleavage furrows are meridional, and the third cleavage is either:

Equatorial: In microlecithal and isolecithal eggs, cleavage leads to the formation of blastomeres of equal size, and then the cleavage is called **equal holoblastic cleavage**. Ex. Amphioxus and placental mammals [11, 12].

Or

Latitudinal: In mesolecithal and telolocithal eggs, the cleavage is unequal producing two types of blastomeres, namely **micromeres** at animal pole and **macromeres** at vegetal pole. Then the cleavage is called **unequal holoblastic cleavage.** Ex. Amphioxus and frog [11, 12].

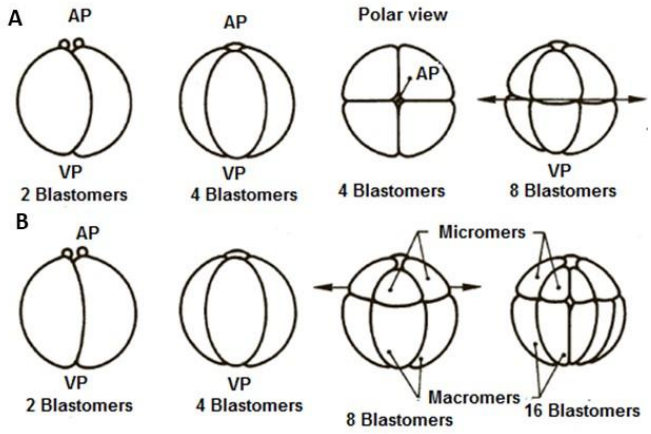

Figure 1.16. (A). Equal radial cleavage, (B). unequal radial cleavage [2].

3.1.2 Bilateral cleavage

Blastomeres are bilaterally arranged around the axis of egg. In bilateral cleavage, one side of the embryo is the mirror image of the other side. Ex. Ascidian and cephalopod molluscs.

In bilateral cleavage, the first cleavage passes through the symmetry, which is in plane the median plane of the resulting embryo. The subsequent cleavages are symmetrical to it [11, 12].

3.1.3 Spiral cleavage

The new blastomeres are slightly displaced giving a spiral appearance to the embryo. Occurs in polyclads (flat worms) nemerteans, annelids, and molluscs except cephalopods [11, 12].

3.1.4 Rotational cleavage

The first cleavage is meridional as in other animals, producing two blastomeres. However, the second cleavage, in one blastomere the plane of cleavage passes through the polar axis, but in the second blastomere, the plane of cleavage is perpendicular to the polar axis (Figure 1.17) [11, 12].

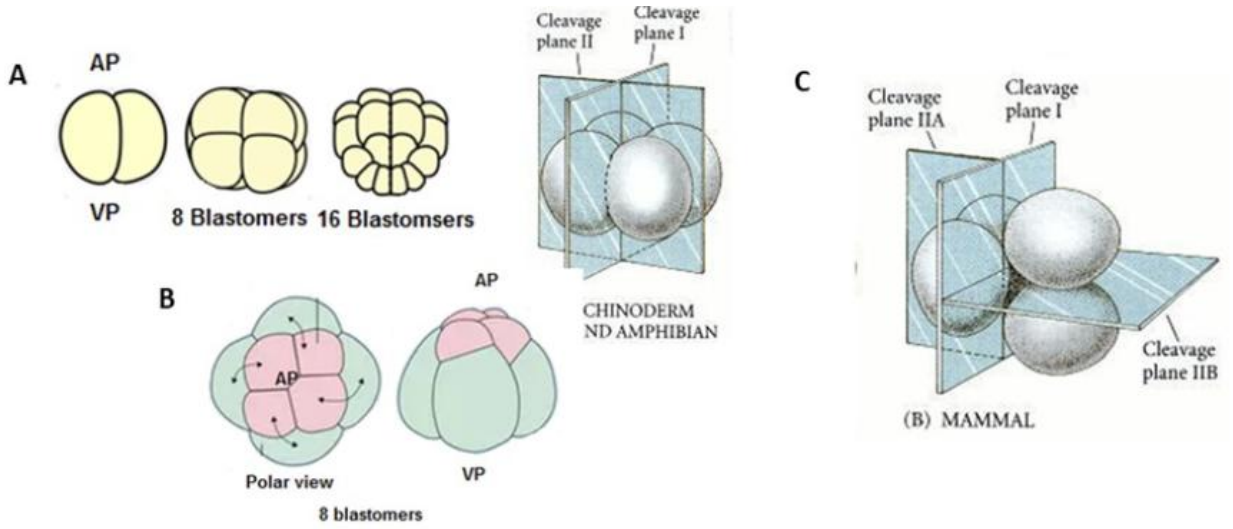

Figure 1.17. (A). Bilateral cleavage, (B). Spiral cleavage, (C). Rotational cleavage [2].

3.2 Meroblastic cleavage

In this type of cleavage, only a portion of the egg divides resulting in the formation of blastoderm. Usually the blastoderm is present in the animal pole and the vegetal pole becomes laden with yolk, which remains in an uncleaved state. It is otherwise called partial or incomplete cleavage. It is characteristic of telolecithal and centrolecithal eggs [11, 12].

The meroblastic cleavage is of two types (Figure 1.18): They are:

3.2.1 Discoidal cleavage

Here the cytoplasm is placed at the animal pole (devoid of yolk) as a disc is called blastodisc and this disc alone divides. It occurs in fishes, reptiles and birds.

3.2.2 Superficial cleavage

The segmentation occurs only in the surface layer of the egg and does not extend into the central yolk. This is characteristic of centrolecithal eggs (insects) [11, 12].

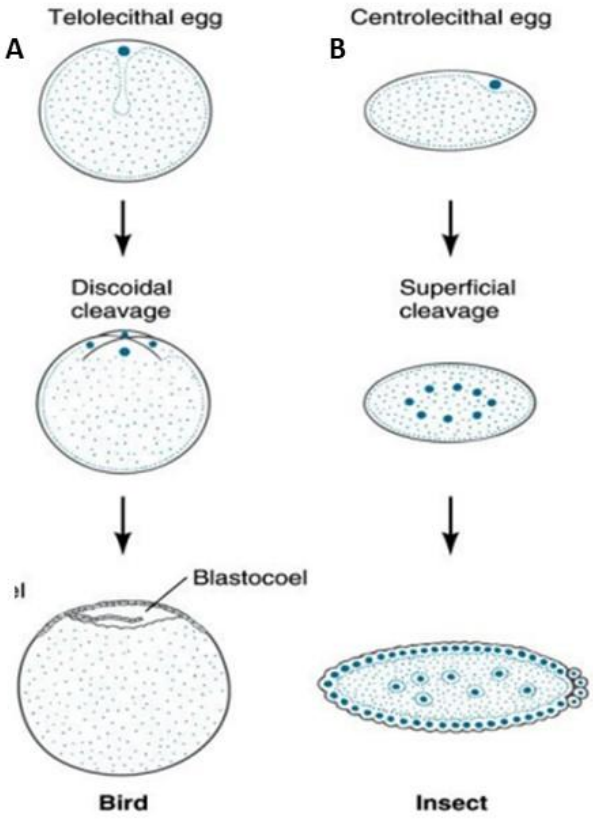

Figure 1.18. Meroblastic cleavage [8].

4 Blastulation

The blastomeres in the early cleavage stage tend to assume a spherical shape. The whole embryo appear to possess a shape of mulberry. This stage is called **morula**.

Further cleavage to morula terminates in **Blastula**. It can be defined as a hollow sphere of blastomeres, surrounding a cavity, the **Blastocoel**. The process of formation of blastula is called **Blastulation**. The cellular layer surrounding the blastocoel is called **Blastoderm** [11, 12].

4.1 Types of blastulae

The types of blastula vary in great deal among different animals depending upon the size of the egg, the amount and distribution pattern of yolk, the rate and number of cleavage division (**Figure** 1.19).

4.1.1 Coeloblastula

It is a hollow blastula containing a large spacious blastocoel. The blastocoele is filled with mucopolysaccharides and the blastoderm is formed of a single layer of cells.

— The blastula resulting from holoblastic equal cleavage, as in the case of echinoderms and amphioxus, is called **equal coeloblastula.**
— Holoblastic unequal cleavage, as in frog, results in **unequal coelobtastula.**

4.1.2 Stereoblastula

In many spirally cleaving eggs of annelids, mollusks, nemerteans and some of the planarians, no blastocoelic cavity appears in the blastula. In them, the smaller micromeres accumulate as a cluster of cells over the larger vegetally placed macromeres.

4.1.3 Periblastula

The superficially cleaving eggs of insects produce a periblastula without a blastocoele cavity as nuclei collect in the peripheral layer.

4.1.4 Discoblastula

It appears at the animal pole as a small multilayered flat disc separated from the yolk by a narrow segmentation or subgerminal cavity. It is found in reptiles, birds and fishes which have large yolky eggs.

4.1.5 Blastocyst

It is the blastula stage of mammals; it consists of a hollow spherical vesicular blastula, containing an inner cell mass (embryoblast) at the animal pole. The outer single layer of cells which encloses the blastocoel is called the **trophoblast.**

In mammals, the blastula forms the blastocyst in the next stage of development. Here the cells in the blastula arrange themselves in two layers:

— The **inner cell mass** (or **embryoblast**), this mass of cells will go on to form the embryo and some embryonic membranes.
— The outer layer called the **trophoblast**, this layer contribute to the placenta and nourish the embryo (**Figure** 1.19) [2, 11, 12].

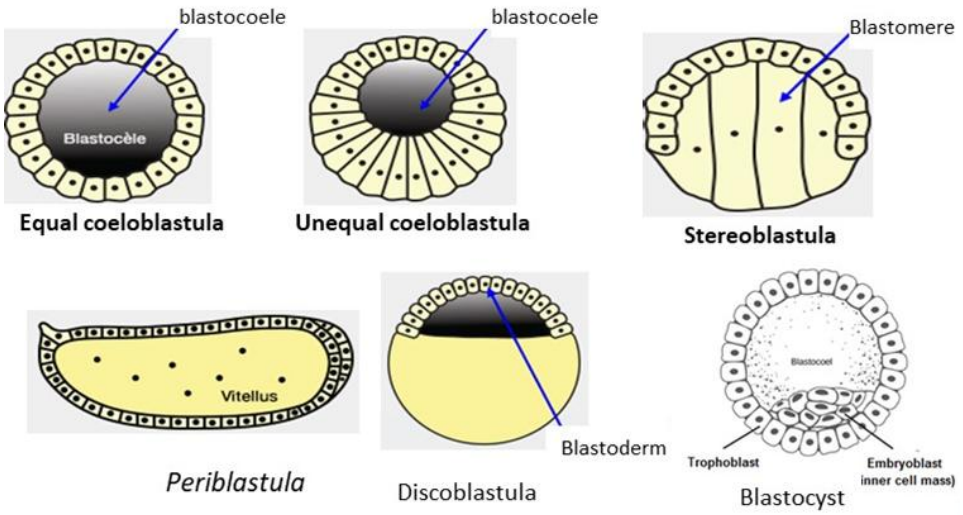

Figure 1.19. Different types of blastulae [2].

Summary of the egg types (yolk amount or distribution) on cleavage and blastulae outcomes is presented in Figure 1.20.

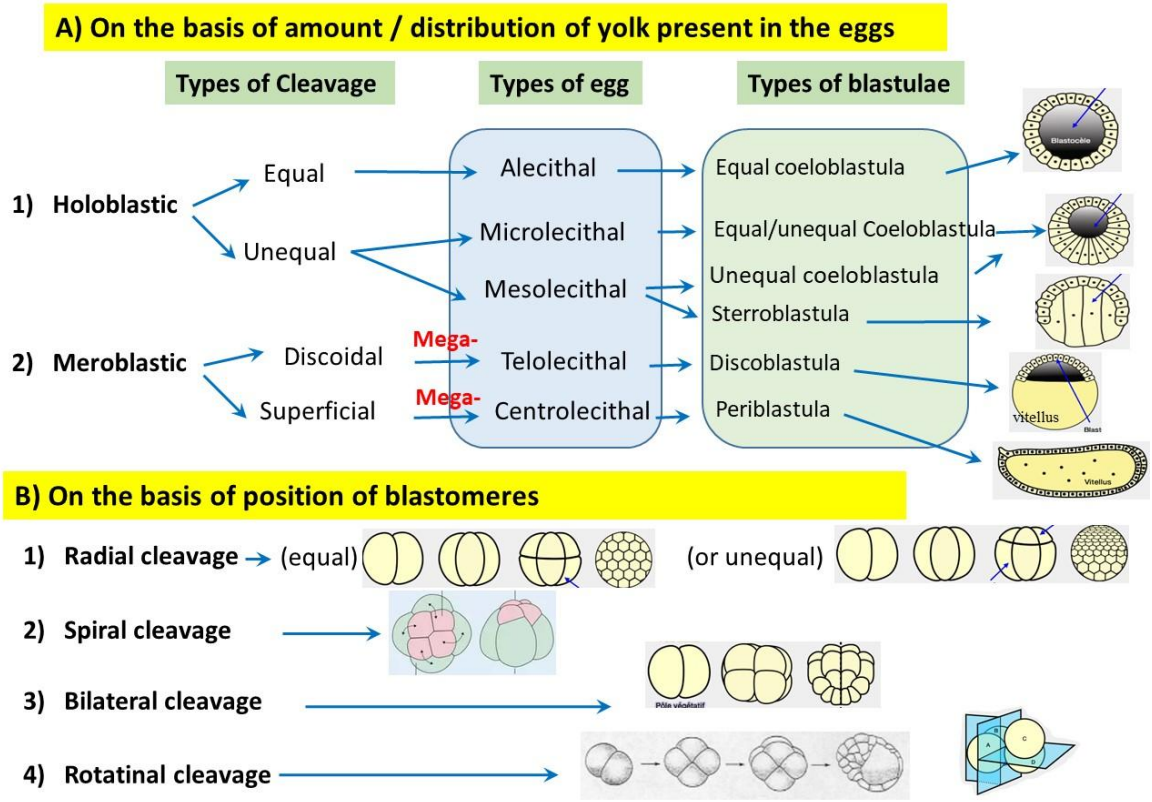

Figure 1.20. Effect of egg types on cleavage and blastulae outcomes [2, 11, 12].

Chapter 4: Gastrulation

After blastula, the next stage in embryonic development is **gastrulation**, in which the cells in the blastula rearrange themselves to form three layers of cells and form the body plan. The embryo during this stage is called a **gastrula**. Gastrulation results in three important outcomes:

— The formation of the embryonic tissues, called **germ layers,** the layers include the **ectoderm** (outer layer), **mesoderm** (middle layer), and **endoderm** (inner layer).
— The formation of the embryonic gut, the **archenteron** (primitive gut).
— The appearance of the major **body axes** : **anteroposterior**, **dorsoventral** and **left-right**.

The specific details of gastrulation are different in among different animal lineages. Although the details of gastrulation differ between various groups of animals, the cellular mechanisms involved in gastrulation are common to all animals. Gastrulation involves changes in cell **motility**, cell **shape**, and **cell adhesion** [13].

1 Pattern of gastrulation

Gastrulation patterns exhibit enormous variation throughout the animal kingdom, they are unified by the five basic types of cell movements that occur during gastrulation:

1.1 Invagination

During invagination, an epithelial sheet bends inward to form an inpocketing. Bending of sheets of cells (epithelial sheet) towards inward of the embryo or cell sheet gets infolded into an embryo. Ex. Sea urchin endoderm (Figure 1.21) [2, 13].

1.2 Ingression

During ingression, cells leave an epithelial sheet by transforming from well-behaved epithelial cells into freely migrating mesenchyme cells. Ex. Sea urchin mesoderm, Drosophila neuroblasts [2, 13].

1.3 Involution

During involution, a tissue sheet rolls inward to form an underlying layer via bulk movement of tissue. Ex. Amphibian mesoderm [2, 13].

1.4 Epiboly

During epiboly, a sheet of cells spreads over other cells by thinning. Ex. Ectoderm formation in amphibians, sea urchin, and tunicates [2, 13].

1.5 Intercalation

During intercalation, two or more rows of cells move between one another, creating an array of cells that is longer (in one or more dimensions) but thinner. The overall change in shape of the tissue results from cell rearrangement. Intercalation can be a powerful means of expanding a tissue sheet [2, 13].

1.6 Convergent-extension

Two or more rows of cells intercalate, but the intercalation is highly directional. Cells converge by intercalating perpendicular to the axis of extension, resulting in the overall extension of the tissue in a preferred direction (Figure 1.21) [2, 13].

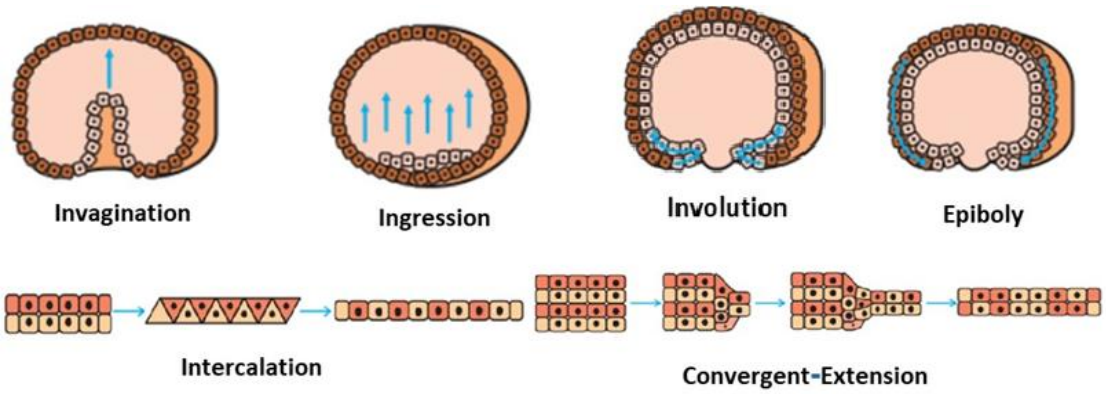

Figure 1.21. Main pattern of gastrulation in animals [8].

2 Beginning of gastrulation in Humans (stage two layers)

The formation of the bilaminar embryonic disc sets the **dorsal/ventral axis** as the **epiblast (primitive ectoderm)** cell layer is positioned dorsal to the **hypoblast**. The anatomical location of the bilaminar disc is found between the amniotic cavity and the primitive yolk sac. The cells of the epiblast stretch to form a semi-sphere known as the amniotic cavity, while the cells of the hypoblast extend to surround the yolk sac. After bilaminar embryonic disc stage, the human blastocyst form a multilayered gastrula with **endoderm**, **mesoderm**, and **ectoderm** (Figure 1.22).

During implantation, the trophoblastic layer surrounding the blastocyst further differentiates into two functionally distinct layers:

→ The outer trophoblast, known as the **syncytiotrophoblast**, releases proteolytic enzymes to assist with endometrial implantation.

→ The inner trophoblast layer, known as the **cytotrophoblast**, is a single sheet of cells surrounding the extraembryonic mesoderm [13].

3 Formation of the third layer: the mesoderm

During the second week gastrulation, the beginning of gastrulation is marked by the appearance of the **primitive streak**, a groove in the caudal end of the epiblast layer. The cranial end of the primitive streak forms a thickening known variously as the **primitive knot**, the **primitive node**, or **Hensen's node**.

The primitive node (or primitive knot) is the organizer for gastrulation in most amniote embryos. In birds, it is known as Hensen's node, and in amphibians, it is known as the Spemann-Mangold organizer. It is induced by the Nieuwkoop center in amphibians, or by the posterior marginal zone in amniotes including birds.

As cells proliferate and migrate toward the midline of the embryo, the thickening elongates to become linear in shape, thus the term primitive steak. It forms immediately posterior to the node and cells from the epiblast migrate here, invaginate, and then form intraembryonic endoderm and mesoderm (**Figure** 1.23) [13].

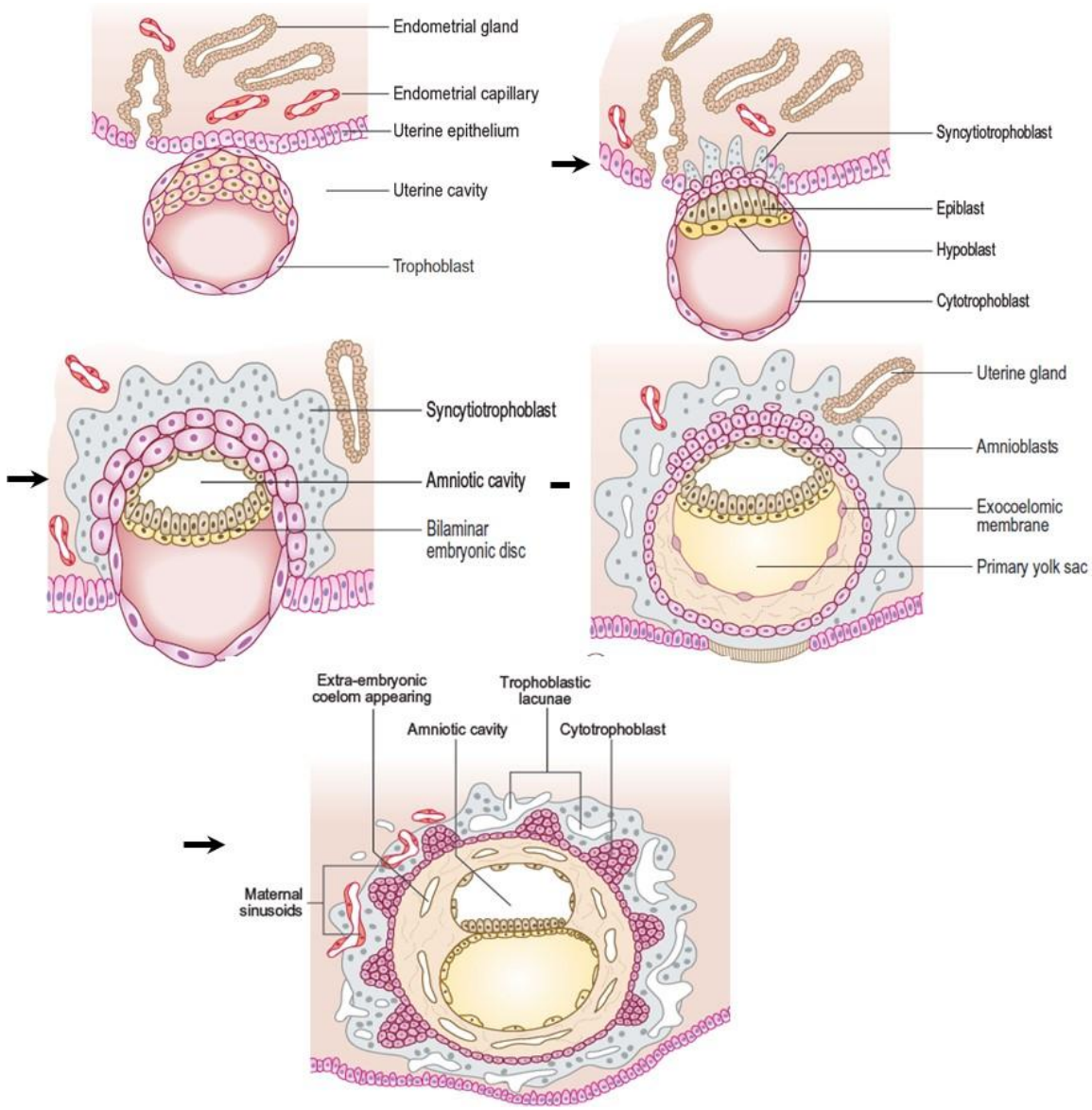

Figure 1.22. Formation of the bilaminar embryonic disc [14].

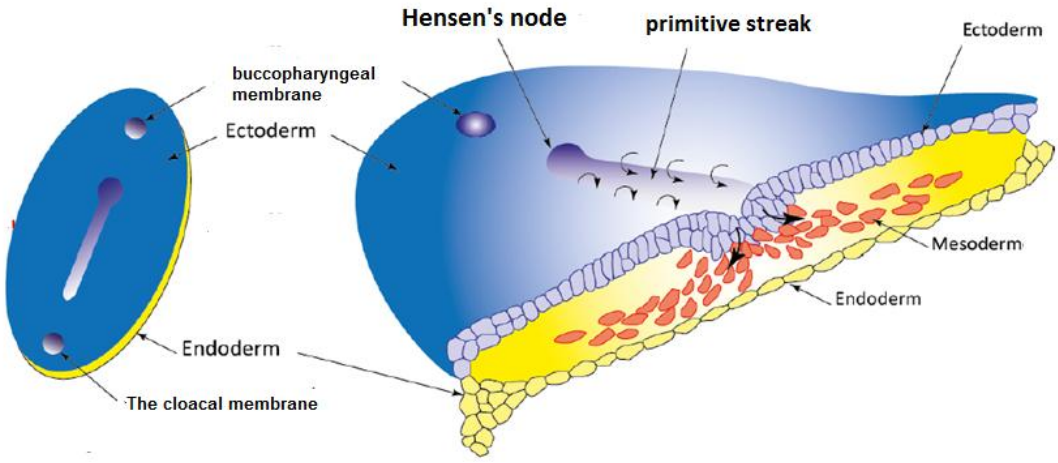

Figure 1.23. Formation of the mesoderm [4].

Chapter 5: Neurulation

Organogenesis is the process by which the three germ tissue layers of the embryo, which are the ectoderm, endoderm, and mesoderm, develop into the internal organs of the organism. Organs form from the germ layers through the differentiation. Organogenesis is the phase of embryonic development that starts at the end of gastrulation and continues until birth.

In vertebrates, one of the primary steps during organogenesis is the formation of the neural system (neurulation). The ectoderm forms epithelial cells and tissues, as well as neuronal tissues [15].

1 The development of ectoderm - neurulation

Immediately after gastrula stage, a vertebrate embryo is known as a **neurula**. This is the stage at which neurulation takes place. The formation of nervous system (**neurulation**) is the beginning of organogenesis. The process of primary neurulation appears to be similar in amphibians, reptiles, birds, and mammals.

Neurulation is a process divided into primary neurulation and secondary neurulation.

→ **Primary neurulation** is a discontinuous process that starts at different points along the rostrum–caudal axis necessary for the **neural tube** closure.

→ **Secondary neurulation** permits the formation of the spinal cord at the lower sacral and caudal level (it pertains to the development of the caudal part of the neural tube at the level of the 31st somite).

The notochord (derived from the mesoderm) plays a very important role in neurulation. The nervous system development begins with neurulation, which includes the following steps:

→ Formation of the **neural plate.**
→ Formation of the neural fold / neural groove.
→ Closure of the neural groove to form the **neural tube** (Figure 1.24) [15].

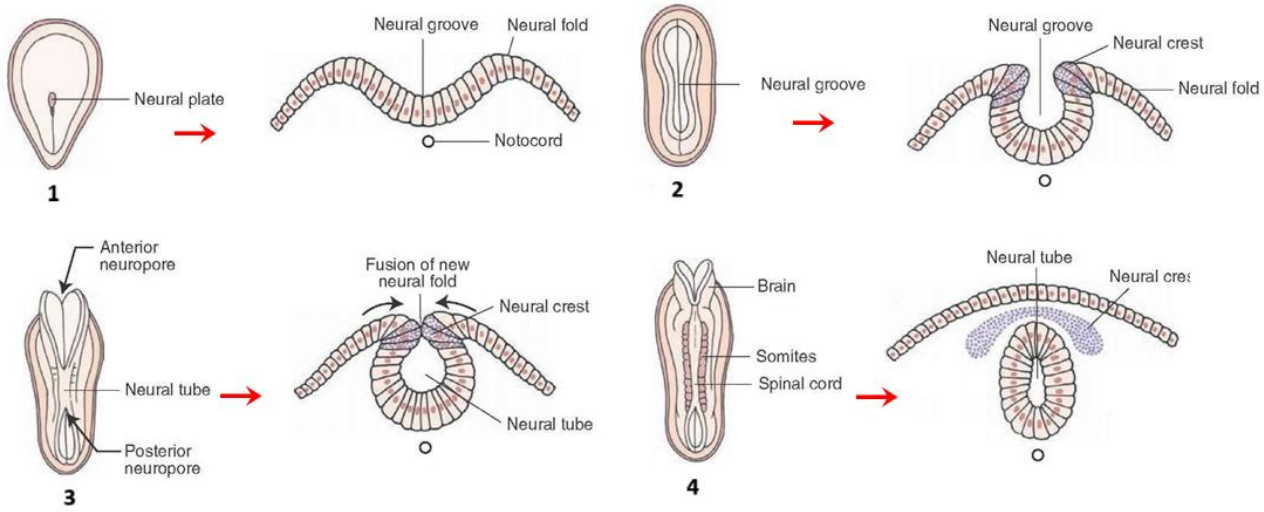

Figure 1.24. Steps of neurulation [16].

Then the neural tube will differentiate into the central nervous system (brain and spinal cord).
As the neural fold is folding into the neural tube, some cells from the ectoderm roll off and become the **neural crest** cells. These cells migrate to different locations of the body and go on to form teeth, craniofacial bones and cartilage, and skin pigmentation [15].

1.1 Early brain structure

1.1.1 Primary vesicles

Rostral neural tube forms three primary brain vesicles (week 4). The most anterior of these embryonic brain vesicles is called the **prosencephalon** which is the embryonic precursor of the forebrain. The middle vesicle is the **mesencephalon** which is the precursor of midbrain structures, and the most posterior is the **rhombencephalon** which will become the hindbrain (Figure 1.25) [15, 17].

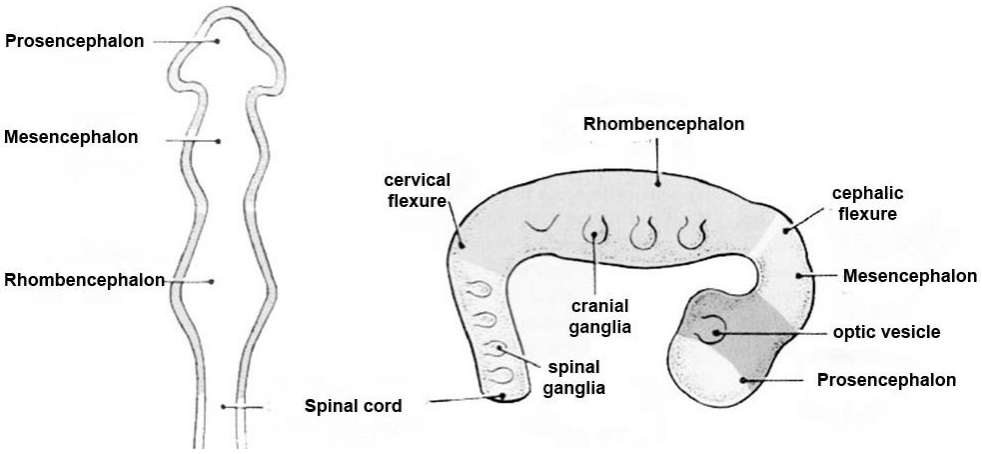

Figure 1.25. The three primary brain vesicles [17].

1.1.2 Secondary vesicles

These three vesicles further subdivide in five secondary brain vesicles (in week 5). The prosencephalon divides into the **telencephalon** and the **diencephalon**, and the rhombencephalon divides into the **metencephalon** and **myelencephalon**. The **mesencephalon** does not further divide. These five subdivisions are aligned along the rostral-caudal axis of the embryo and establish the primary organization of the central nervous system (Figure 1.26) [15, 17].

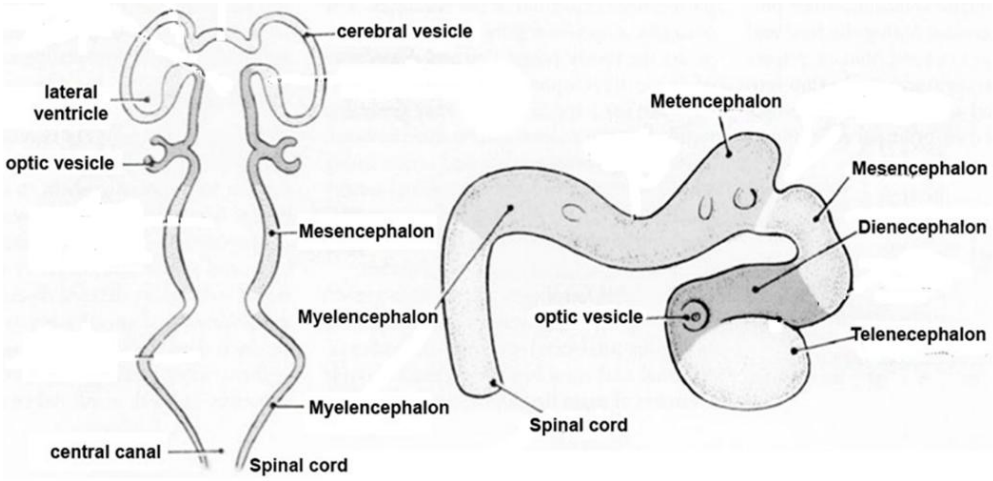

Figure 1.26. The five secondary brain vesicles [17].

2 The development of mesoderm

The mesoderm is one of the three germinal layers that appears in the third week of embryonic development. It is formed through a process called gastrulation. It comprises a wide variety of tissues in the early embryo, including the muscle, the vasculature, blood and heart, the cartilage, and the kidneys [15]. There are four important components , which are:

2.1 Paraxial mesoderm

Called also somites. Mature somites contain two major populations: the **sclerotome** and **dermomyotome**. The sclerotome gives rise to the vertebrae and associated ribs, tendons, and other tissues, such as vascular cells of the dorsal aorta, intervertebral blood vessels, and meninges. The dermomyotome produces two components: the myotome and the dermatome. The **myotome** gives rise to the musculature of the back, rib cage, ventral body wall, and limbs. The **dermatome** gives rise to the dermis of the back, although the term dermomyotome is sometimes used to describe this region because a recent study showed that this central region of the dermomyotome also gave rise to muscles in chick embryos.

Somitogenesis is the process of segmentation of the paraxial mesoderm within the trilaminar embryo body to form pairs of **somites**, or balls of mesoderm. A somite is added either side of the notochord (axial mesoderm) to form a somite pair. The segmentation does not occur in the head region, and begins cranially (head end) and extends caudally (tailward) adding a somite pair at regular time intervals (Figures 1.28). In humans, the first somite pair appears at day 20 and adds caudally at 1 somite pair/90 minutes until on average 44 pairs eventually form [4, 16].

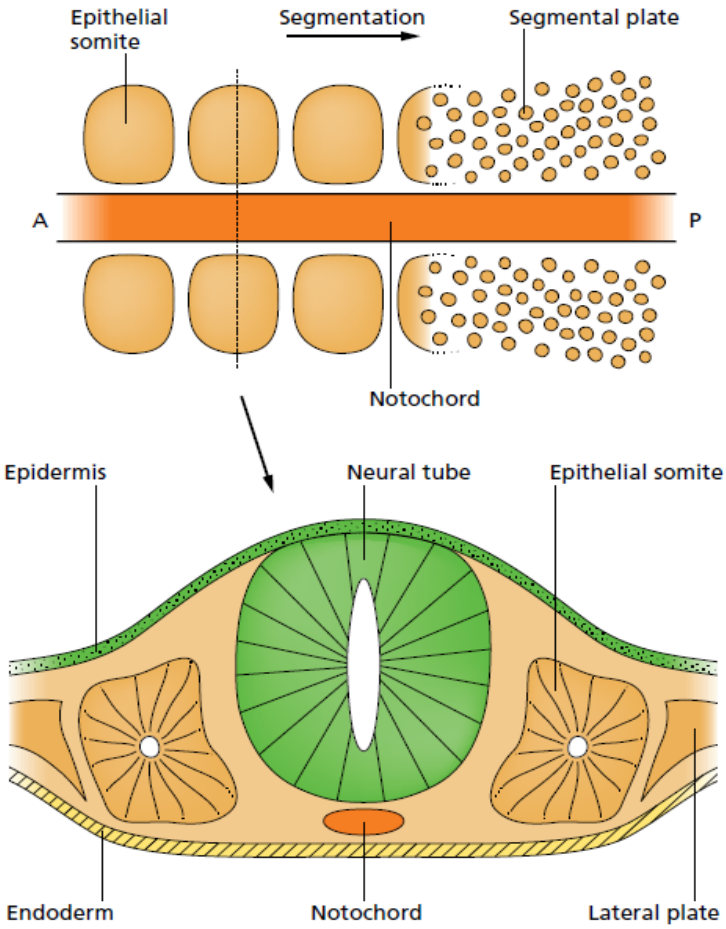

Figure 1.27. The process of the somitogenesis [16]

2.2 Intermediate mesoderm

It forms the urogenital system, including the kidneys (pronephros, mesonephros, and metanephros, which form the kidneys) and gonads (testes or ovaries) (Figure 1.28) [4, 16].

2.3 Lateral plate mesoderm

It is the most lateral, which is subdivided into:

— **Somatic layer** or somatopleure that forms the pelvic skeleton and mesodermal components of the limbs, with the exception of the muscles.
— **Splanchnic layer** or splanchnopleure that gives rise to components of the circulatory system, such as the heart, blood vessels, and blood cells (Figure 1.28) [4, 16].

2.4 Notochord (axial mesoderm)

The notochord secretes signaling molecules like sonic hedgehog (SHH), which direct the differentiation of surrounding tissues. The notochord later degenerates in vertebrates, with remnants forming intervertebral discs [4, 16].

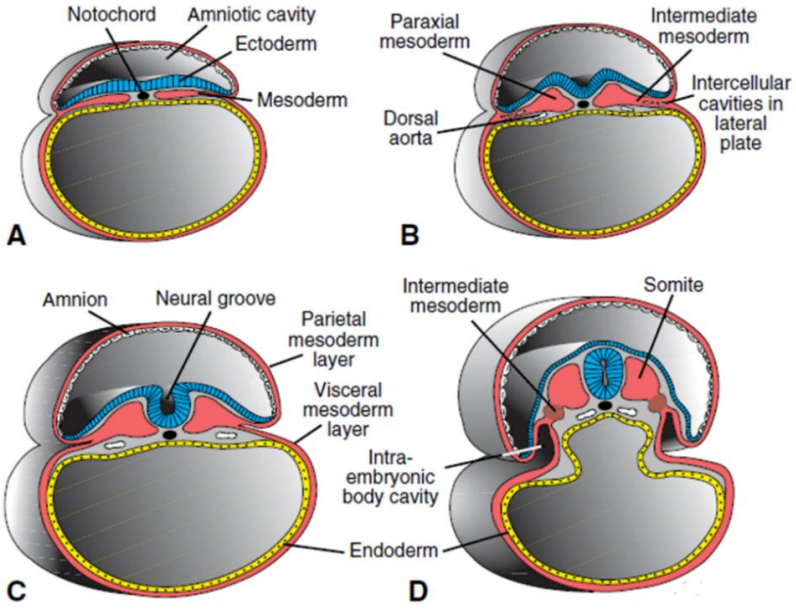

Figure 1.28. The development of mesoderm

3 The development of endoderm

The endoderm germ layer gives rise to a vast array of highly specialized epithelial cell types that line the respiratory and digestive systems; and contributes to associated organs such as thyroid, thymus, lungs, liver, biliary system, and pancreas [4, 16].

Chapter 6: Extraembryonic membranes in birds

These are the membranes that do not form any part of the embryo proper but performs various functions which assist in the development of the embryo. These membranes are formed outside the embryo from the trophoblast only in amniotes (reptiles, birds and mammals) and perform specific functions (Figure 1.29).

Amniotes: These are the vertebrates group whose eggs contain extraembryonic membranes for protecting the embryo. They lay eggs on the land. (e.g., Reptiles, Birds and Mammals).

Anamniotes: These are the vertebrates group whose eggs do not contain extraembryonic membranes during embryonic development. They lay eggs in the water. . (e.g., Fish, Amphibia). There are four types of extraembryonic membranes in birds: **Yolk sac**, **amnion**, **chorion**, **allantois** [2].

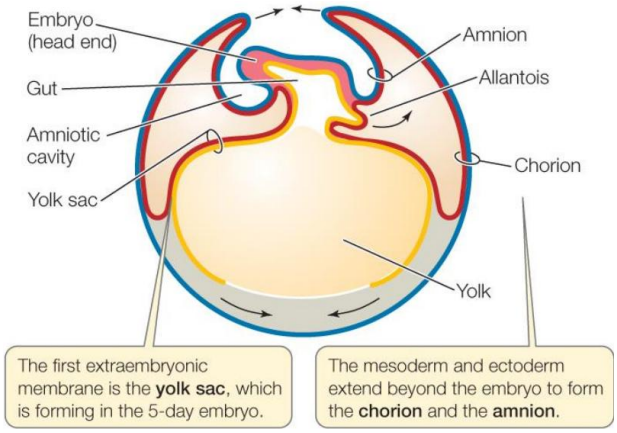

Figure 1.29. Five-day chick embryo [19] .

1 Yolk sac

It is formed of splanchnopleur (inner endoderm and outer mesoderm) and is well developed in reptiles, birds and prototherians having poly lecithal egg. It is formed completely on the 9th day of incubation. In human it is vestigial (Figure 1.30).

Functions of Yolk sac:

— It surrounds the yolk. Its main function is in digestion. It serve as extraembryonic gut.
— It help in digestion of yolk and transfer the digested material to the developing embryo.
— First respiratory organ in the embryo.
— Form yolk sac placenta in the marsupials [2, 4].

2 Amnion

It is innermost fold of somatopleur (inner ectoderm and outer mesoderm) above the embryo. It appears after 30 hours of incubation. Between the amnion and embryo, there is amniotic cavity

filled with amniotic fluid secreted by both embryo and amnion. In this fluid filled cavity embryo floats.

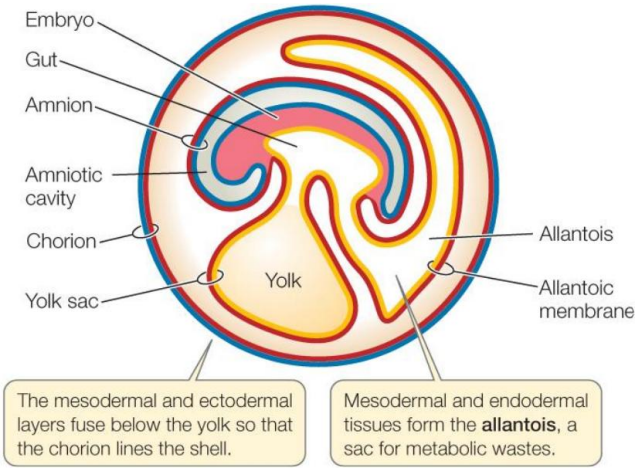

Figure 1.30. Six-day chick embryo [19].

The functions of amnion are:

— Protection of the embryo from the mechanical injury and desiccation.
— Amniotic fluid acts as shock absorber.
— Protect from sudden temperature changes [2, 4].

3 Chorion

Formed of somatopleure (outer ectoderm and inner mesoderm). It forms the outermost boundary. Space between amnion and chorion is called chorionic cavity which further provides protection to the embryo (Figure 1.31) [2, 4].

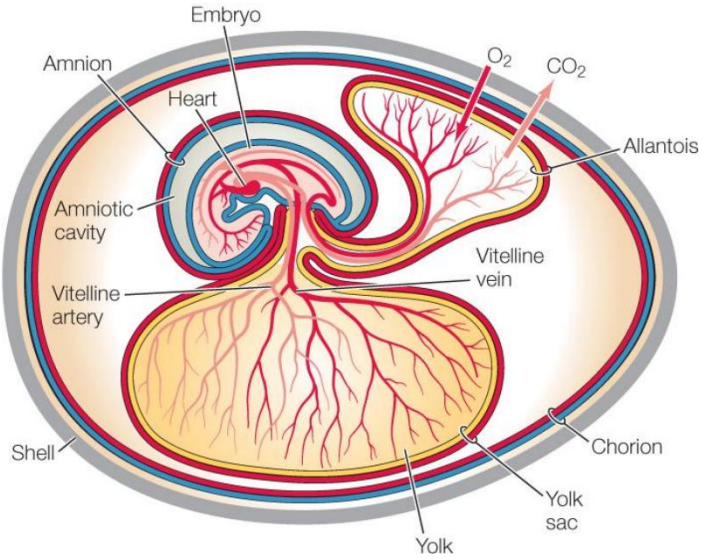

Figure 1.31. 7 day chick embryo showing development of extraembryonic membranes in birds [19].

The functions of chorion are:

— In reptiles, birds and prototherians, chorion along with allantois acts as extra embryonic lung and helps in exchange of gases. However, in primates including human beings, only chorion

forms the placenta (chorionic placenta) while in other eutherian, allantochorion forms allantoic placenta.
— Nutrition and protection [2, 4].

4 Allantois

Formed of splanchnopleure (inner endoderm and outer splanchnic mesoderm). Connected with the hindgut of the embryo. Develops on the third day of incubation from the floor of the hindgut as an outgrowth. It is reduced in human beings.

Functions of Allantois:

— Act as reservoir for the storing the excretory wastes of the embryo. Considered as extra embryonic kidney.
— Also helps in digestion and nutrition from albumen and calcium of the shell.
— It grows in the chorionic cavity. Its outer membrane fuse with the inner membrane of the chorion and forms allantochorion which is highly vascular. Act as extraembryonic lung and provides surface for the gaseous exchange [2, 4].

Chapter 7: Particularities of human embryology.

1 Menstrual cycle

After reaching puberty, also called **menarche** in females, and until the woman enters **menopause** several decades later, monthly cycles in the secretion of hypothalamic, pituitary, and ovarian hormones control a **menstrual cycle**, which results each month in the production of a female gamete and a uterus primed to receive a fertilized embryo. The cycle is divided into two smaller cycles: the **uterine cycle** and **the ovarian cycle** (Table 1.3).

Both the uterine cycle and ovarian cycle are divided into different phases, in other words different stages. Different events occur during each phase. There are three phases in the uterine cycle: menstruation, proliferative phase, and secretory (before period bleeding). There are also three phases in the ovarian cycle: follicular (before ovulation), ovulation (when an egg is released from an ovary), and luteal (after ovulation) [2].

Table 1.3. The menstrual cycle [6].

Cycle	**Pre-ovulation**		**Ovulation**	**Post-ovulation**
Ovarian cycle	Follicular phase			Luteal phase
Uterine cycle	Period	Proliferative phase		Secretory phase

1.1 Uterine cycle

1.1.1 Menstrual phase

This phase begins with the initiation of menses (day 0) and takes 3–5days. The drop of **oestradiol** (E2) and **progesterone** (P4) induces the detachment of the **functionalis layer** (stratum functionalis) and causes endometrial shedding. After this phase, the endometrium is thin, and the **basalis layer** is all that remains [3, 6].

1.1.2 Proliferative phase

This phase starts at the end of menstruation and lasts until the day of ovulation (day 14). The stromal and epithelial cells from the functionalis layer proliferate and regenerate in response to increasing levels of estrogens, secreted from the ovary. The glandular epithelium acquires linear shapes and vascularization. The endometrial thickness increases approximately from 4 to 7mm [3, 6].

1.1.3 Secretory phase

This phase begins at ovulation (day 15) and lasts until menstruation (day 28). After ovulation, the **corpus luteum** starts to secrete high amounts of oestradiol and progesterone. Progesterone, together with estrogenic hormones, causes the uterine mucosa to enter the progestational or secretory stage in preparation for implantation of the embryo. Between days 19 and 21, the endometrial epithelium becomes receptive. However, if there is no implantation, the corpus luteum degenerates and forms a scar tissue called the **corpus albicans**, E2 and P4 levels decrease, the endometrium becomes

ischemic, and glandular secretion stops. The final result is the elimination of the **functionalis layer** (menstrual bleeding or a period) and the start of a new cycle (Figure 1.32) [3, 6].

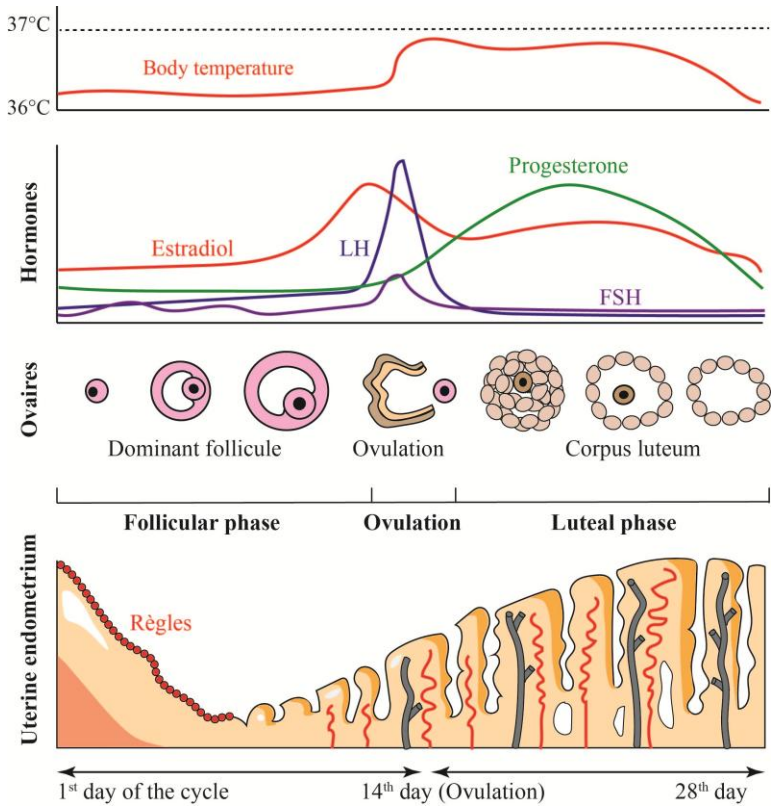

Figure 1.32. Ovarian, endometrial, and hormonal events of the menstrual cycle [6].

1.2 Ovarian cycle

1.2.1 The follicular phase

At the beginning of each ovarian cycle, 15 to 20 primary (preantral) stage follicles are stimulated to grow under the influence of follicle-stimulating hormone (FSH), the hormone is not necessary to promote development of primordial follicles to the primary follicle stage, but without it, these primary follicles die and become atretic. Under normal conditions, only one of these follicles reaches full maturity, and only one oocyte is discharged; the others degenerate and become **atretic follicles**. The dominant follicle becoming evident by day 8–12 reaches a pre-ovulation, mean diameter of between 20–23 mm (**Figure** 1.32) [3, 6].

1.2.2 Ovulation

A peak of **luteinizing hormone** (LH) produced by the pituitary gland 36 hours before ovulation causes the primary oocyte blocked at the end of prophase to complete meiosis I and the follicle to enter the preovulatory stage. Meiosis II is also initiated, but the oocyte is arrested in metaphase approximately 3 hours before ovulation.

High concentration of LH extrude the oocyte, which together with its surrounding granulosa cells from the region of the cumulus oophorus, breaks free (ovulation) and floats out of the ovary [3, 6].

1.2.3 The luteal phase

After ovulation, granulosa cells remaining in the wall of the ruptured follicle, together with cells from the **theca interna**, are vascularized by surrounding vessels. Under the influence of LH, these cells develop a yellowish pigment and change into **luteal cells**, which form the steroidogenic cells of **corpus luteum** and secrete the progesterone hormone.

If fertilization does not occur, the corpus luteum reaches maximum development approximately 9 days after ovulation. Subsequently, the corpus luteum shrinks because of degeneration of luteal cells and forms a mass of fibrotic scar tissue, the corpus albicans. Simultaneously, progesterone production decreases, precipitating **menstrual bleeding** [3, 6].

2 Implantation

Shortly before ovulation, fimbriae of the oviduct begin to sweep over the surface of the ovary, and the tube itself begins to contract rhythmically. Once the oocyte is in the uterine tube, it is propelled by cilia with the rate of transport regulated by the endocrine status during and after ovulation. In humans, the fertilized oocyte reaches the uterine lumen in approximately 3 to 4 days.

Fertilization, the process by which male and female gametes fuse, occurs in the ampullary region of the uterine tube. This is the widest part of the tube and is close to the ovary. Spermatozoa may remain viable in the female reproductive tract for several days. Only 1% of sperm deposited in the vagina enter the cervix, where they may survive for many hours.

Approximately 3 days after fertilization, cells of the compacted embryo divide again to form a 16-cell morula (mulberry). Inner cells of the **morula** constitute the **inner cell mass** or **embryoblast**, and surrounding cells compose the **trophoblast**. The inner cell mass gives rise to tissues of the embryo proper, and the outer cell mass forms the trophoblast, which later contributes to the **placenta** (Figure 1.33) [3, 6].

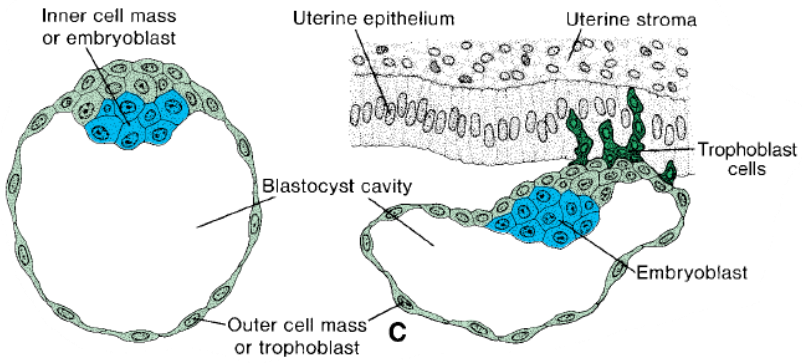

Figure 1.33. Human blastocyst showing inner cell mass and trophoblast cells [5].

In the human, trophoblastic cells over the embryoblast pole begin to penetrate between the epithelial cells of the uterine mucosa about the sixth day. By the end of the first week of development, the human zygote has passed through the morula and blastocyst stages and has begun implantation in the uterine mucosa.

At the eighth day of development, the blastocyst is partially embedded in the endometrial stroma. In the area over the embryoblast, the trophoblast has differentiated into two layers: (1) an inner

layer of mononucleated cells, the **cytotrophoblast**, and (2) an outer multinucleated zone without distinct cell boundaries, the **syncytiotrophoblast**.

Cells of the inner cell mass or embryoblast also differentiate into two layers: (1) a layer of small cuboidal cells adjacent to the blastocyst cavity, known as the **hypoblast layer**, and (2) a layer of high columnar cells adjacent to the amniotic cavity, the **epiblast layer** [5, 6].

3 Evolution of extraembryonic membranes

Together, the layers form a flat disc. At the same time, a small cavity appears within the epiblast. This cavity enlarges to become the **amniotic cavity**. Epiblast cells adjacent to the cytotrophoblast are called **amnioblasts**; together with the rest of the epiblast, they line the amniotic cavity (Figure 1.34) [5, 6].

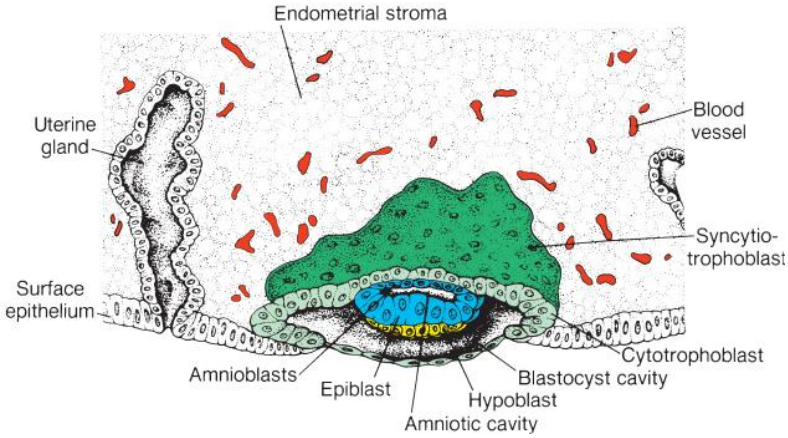

Figure 1.34. A 7.5-day human blastocyst [5].

At the abembryonic pole, flattened cells form a thin membrane, the exocoelomic (Heuser's) membrane that lines the inner surface of the cytotrophoblast. This membrane, together with the hypoblast, forms the lining of the **exocoelomic cavity**, or **primitive yolk sac** [5, 6].

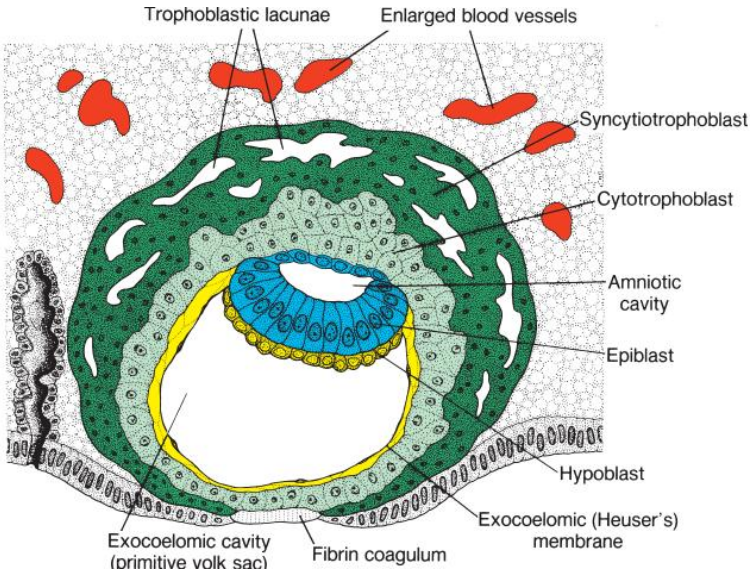

Figure 1.35. Human blastocyst of approximately 12 days [5].

Cells appears between theinner surface of the cytotrophoblast and the outer surface of the exocoelomic cavity. These cells, derived from yolk sac cells, form a fine, loose connective tissue, the extraembryonic mesoderm. Large cavities develop in the extraembryonic mesoderm, and they form a new space known as the **extraembryonic coelom**, or **chorionic cavity** (Figure 1.35) [5, 6].

The extraembryonic mesoderm lining the cytotrophoblast and amnion is called the **extraembryonic somatopleuric mesoderm**; the lining covering the yolk sac is known as the **extraembryonic splanchnopleuric mesoderm.**

In the meantime, the hypoblast produces additional cells that migrate along the inside of the exocoelomic membrane and form a new cavity within the exocoelomic cavity. This new cavity is known as the **secondary yolk** sac or **definitive yolk sac** [5, 6].

4 Placenta

The fetal component of the placenta is derived from the trophoblast and extraembryonic mesoderm (the chorionic plate); the maternal component is derived from the uterine endometrium.

The space between the chorionic and decidual plates is filled with **intervillous lakes** of maternal blood. **Villous trees** (fetal tissue) grow into the maternal blood lakes and are bathed in them. The fetal circulation is at all times separated from the maternal circulation by (1) a syncytial membrane (a chorion derivative) and (2) endothelial cells from fetal capillaries. Hence the human placenta is of the **hemochorial type** [5, 6].

4.1 Function of the placenta

- Exchange of gases (oxygen, carbon dioxide, and carbon monoxide).
- Exchange of nutrients and electrolytes (amino acids, free fatty acids, carbohydrates, and vitamins).
- Transmission of maternal antibodies (IgG).
- Hormone production (progesterone, estriol, human chorionic gonadotropin (hCG) [5, 6].

4.2 Types of placenta

The placentas of different species vary in shape, internal architecture and nature of the interhemal barrier. Anatomically, the placenta is a complex organ and is classified into four types:

4.2.1 Hemochorial (rat, rabbit, human)

The chorionic villi destroy the walls of the maternal capillaries. Blood lacunae are formed in which they bathe directly. The villi only remain in a discoidal area. This is a **discoidal placenta** [20].

4.2.2 Endotheliochorial

Exist in cat and dog. The epithelium of the endometrium and part of the uterine stroma have disappeared. Only four layers remain between the maternal and fetal blood (endothelium of the fetal vessel, extraembryonic mesoblast, cytotrophoblast, endothelium of the uterine vessel). The villi are concentrated in a ring-shaped area: the placenta is called **zonary** [20].

4.2.3 Syndesmochorial (or conjonctivochorial, mésochorial)

Exist in ruminants. Fetal chorionic villi cross the maternal endometrial epithelium. There are only five layers between the maternal and fetal blood. The villi are located at the level of beaches or cotyledons. This is a **cotyledonary** placenta [20].

4.2.4 Epitheliochorial

Exist in pig, horse. The placenta is said to be diffuse. The fetal chorionic villi are pressed against the maternal endometrial epithelium, which is not modified. The fetal blood is separated from the maternal blood by the fetal vessel endothelium, the extraembryonic mesoblast, the cytotrophoblast, the uterine epithelium, the uterine connective tissue, and the uterine vessel endothelium (Figure 1.36) [20].

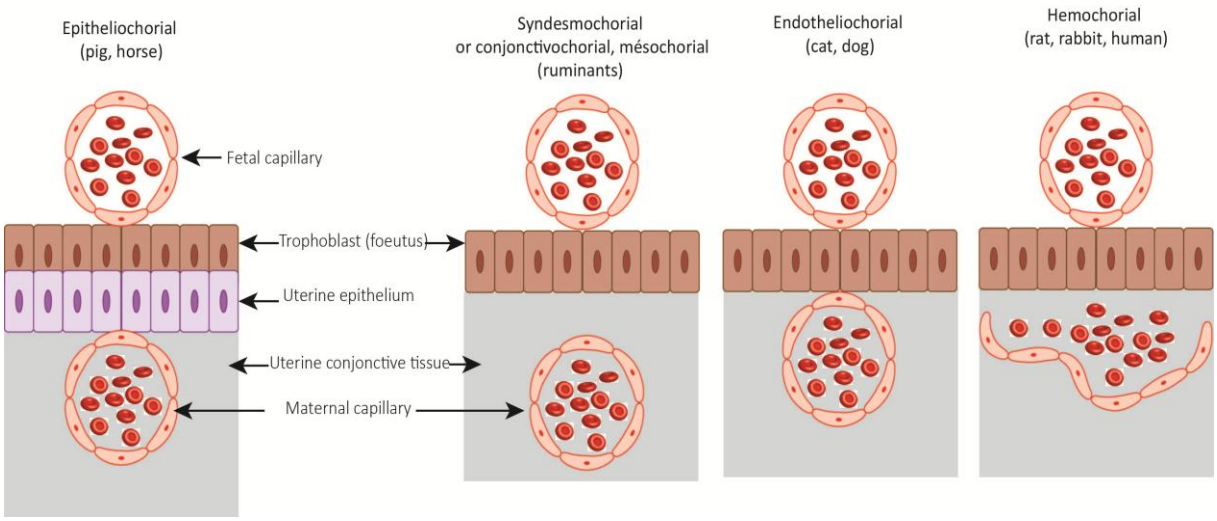

Figure 1.36. Animal placenta types [20].

Part 2: Histology

A group of cells along with intercellular substances that perform a specific function is called tissue.

Tissues are aggregates or groups of cells organized to perform one or more specific functions.

The human body is composed of only four basic types of tissue:

- **Epithelium** (epithelial tissue) covers body surfaces, lines body cavities, and forms glands.
- **Connective tissue** underlies or supports the other three basic tissues, both structurally and functionally.
- **Muscle tissue** is made up of contractile cells and is responsible for movement.
- **Nerve tissue** receives, transmits, and integrates information from outside and inside the body to control the activities of the body

Chapter 1: Epithelial tissue

Epithelial cells form a protective layer on internal and external body surfaces, including the skin and the lining of organs and cavities. The epithelial cells are characterized by:

- Their **morphology**: the Shapes of epithelial cells include squamous (flattened), cuboidal and columnar.
- The existence of **cell-cell interactions** (close apposition of cells).
- Their **cellular polarity** (or apical specializations like microvilli or cilia).Epithelial tissue names.

Each location is associated with a different name of epithelium:

- The epithelium of the skin is called the epidermis and the underlying connective tissue is called the **dermis**,
- The epithelium of the endocardium of the heart and the tunica intima of the vessels is called an **endothelium** .
- The epithelium of serous membrane (or **serosa** = serosal membranes) is called a **mesothelium** : lining the contents and inner walls of body cavities (Figure 2.1).

Epithelia can originate from all three embryonic germ layers

- **Ectoderm**: The epidermis and kératinocytes, oral epithelium, are derived from the **ectoderm**.
- **Endoderm**: the **Endodermis** lining of the gastrointestinal tract and respiratory system.
- **Mesoderm** : the **endothelium** lines the cavity of the vascular system, and the **mesothelium** (serosal membranes) lines the major body cavities, such as the peritoneum (lines the abdomen cavity = peritoneal cavity), pleura (lines the lung cavity = pleural cavity) and **pericardium** (lines the heart cavity = pericardial cavity) (Figure 2.1) [21, 22].

1 Characteristics of epithelial tissues

Epithelial tissues are characterized by:

- the shape of cells: **squamous** (scale-like), **cuboidal** (cube-shaped), or **columnar** (tall and rectangular).
- their distinct **polarity:** their apical surface is free and exposed to a body cavity or the external environment, and a basal surface, which is attached to an underlying basement membrane.
- epithelial cells are bound together by an extensive repertoire of specialized **junctions**, including tight junctions that prevent leakage, desmosomes that provide mechanical strength, and gap junctions that allow for intercellular communication.
- epithelial tissues are **avascular**, meaning they contain no blood vessels of their own and must receive nutrients via diffusion from underlying connective tissues.

— However, they are **innervated**, containing nerve endings that provide sensory reception [21, 22].

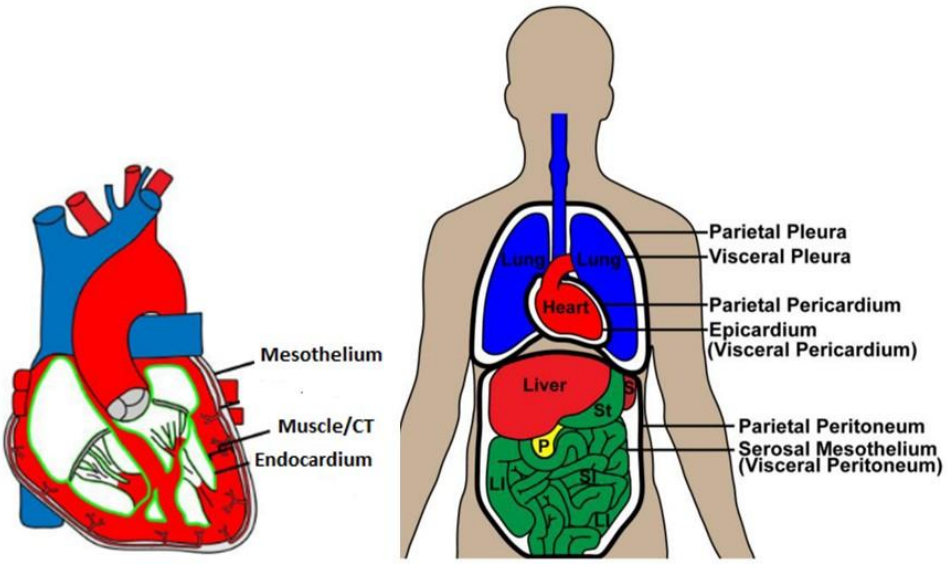

Figure 2.1. Example of some mesothelium and endothelium in human body [23].

2 Cell anchoring junctions

There are four main types of cell-cell junctions that bind the cells together:

2.1 Occluding junctions

The borders of two cells are fused together, often around the whole perimeter of each cell, forming a continuous belt like junction known as a **tight junction** (**Occluding junctions**) or **zonula occludens** (zonula = latin for belt). Proteins in the membrane of adjacent cells called **occludin** interact with each other to produce this tight seal. In the cytoplasm of the cell, occludin interacts with the **actin** cytoskeleton via another proteins called ZO-1 (Figure 2.2) [21, 22].

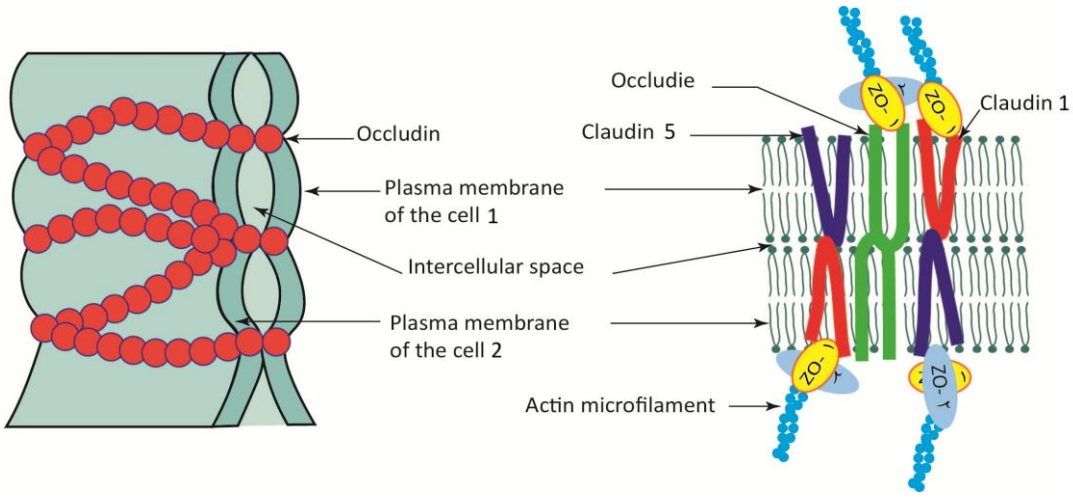

Figure 2.2. Structure of tight junctions [21].

2.2 Adhering junctions

The adherens junction (zonula adherens) lies below the tight junction. In the gap between the two cells, there is a protein called cadherin (E-cadherin) - a cell membrane glycoprotein. The **cadherins** from adjacent cells interact to zipper up the two cells together (Figure 2.3) [21, 22].

Inside the cell, actin filaments (microfilaments) join up the adhesion junctions. These filaments tend to be arranged circumferentially around the cell, as a marginal band.

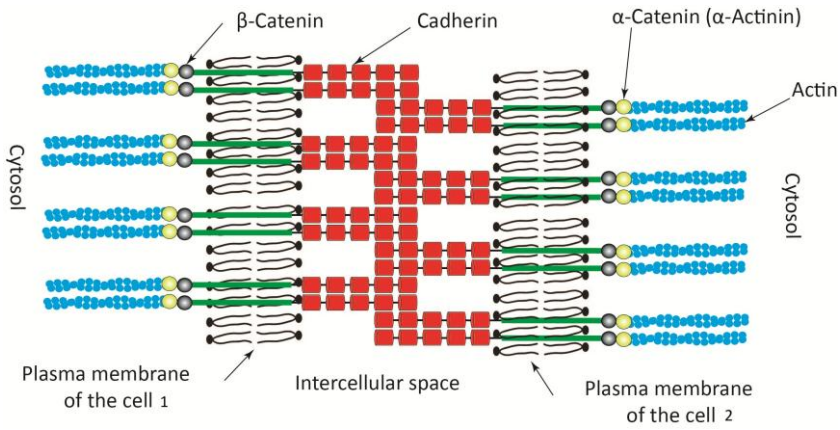

Figure 2.3. Structure of adherens junction [21].

2.3 Desmosomes

A desmosome (macula adherens) is also known as a spot desmosome or macula adherens (macula = latin for spot), because it is circular or spot like in outline, and not belt- or band shaped like adherens junctions. The intermediate filament this case is desmin, not keratin (which is found in epithelial cells). In the cytoplasm of the cell, **cadherins** (**desmogleins** ou **desmocollins**) interacts with **desmin** (in cardiac cells), or **keratin** (found in epithelial cells) via dense cytoplasmic plaque (desmoglobine) (Figure 2.4) [21, 22].

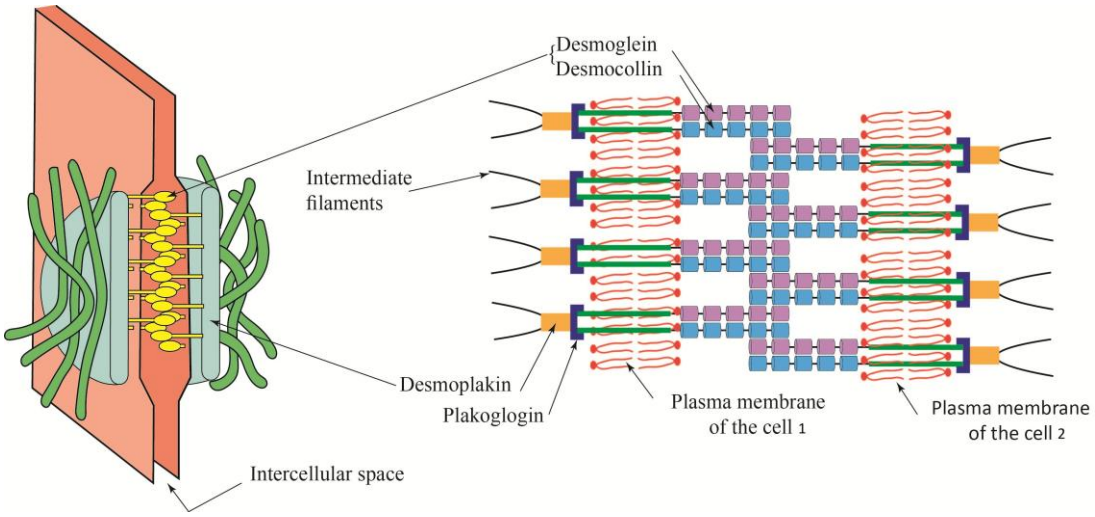

Figure 2.4. Structure of desmosomes [21].

2.4 Gap junctions.

These are communicating junctions. (also known as **nexus**, septate junction): A group of protein molecules called connexins form a structure called a connexon. When connexons from two adjacent cells align, they form a continuous channel between them (Figure 2.5).

This channel is big enough to allow small molecules such as inorganic ions, and other small water soluble molecules (smaller than 1000kDa) to pass between the cells [21, 22].

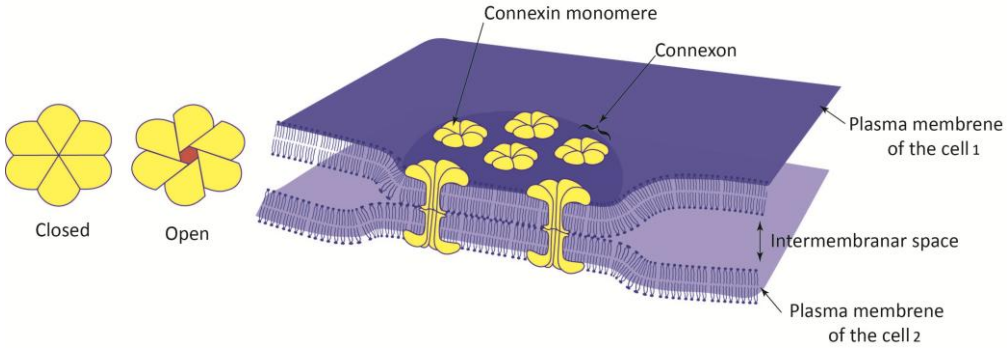

Figure 2.5. Structure of gap junctions [21].

2.5 Hemidesmosomes

They connect the basal surface of epithelial cells via **intermediate filaments** to the underlying basal lamina. The transmembrane proteins of hemidesmosomes are integrin (Figure 1.6) [21, 22].

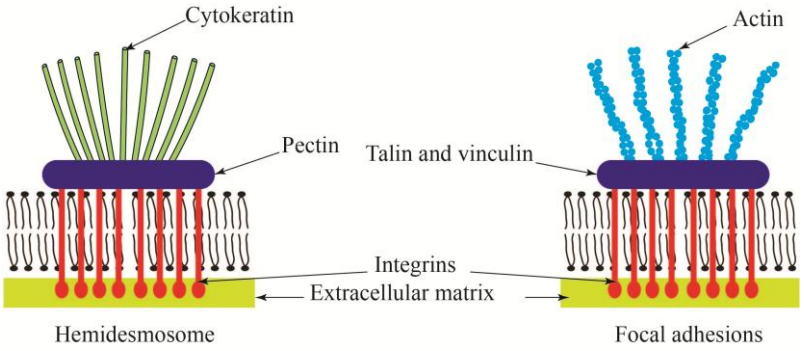

Figure 2.6. Structure of hemidesmosomes [21].

2.6 Focal adhesions

Focal adhesions consist of a cytoplasmic face to which **actin filaments** are bound, a transmembrane connecting region, and an extracellular face that binds to the proteins of the extracellular matrix. The main family of transmembrane proteins involved in focal adhesions are **integrins**. They form a structural link between the actin cytoskeleton and extracellular matrix proteins. They are responsible for attaching long bundles of actin filaments (stress fibers) into the basal lamina (Figure 2.6) [21, 22].

3 Functions of epithelial tissues

- **Protection:** It protects the cells present below against radiation, desiccation, and invasion by pathogens, toxins, and physical trauma (eg, skin).
- **Absorption** occurs via **endocytosis** or **pinocytosis** in various organs (e.g., intestines, the proximal convoluted tubule of the kidney)
- **Secretion** (eg, glands), various molecules (e.g., hormones, mucinogen, proteins) occurs by **exocytosis**.
- **Receptor function:** (eg, gustative and olfactory neuroepithelium)
- **Contractility** (eg, myoepithelial cells).
- **Transportation** : Transport of molecules from one epithelial surface to another occurs by various processes, including :
  - ✓ Diffusion of oxygen and carbon dioxide across the epithelial cells of lung alveoli and capillaries.
  - ✓ Carrier protein-mediated transport of amino acids and glucose across intestinal epithelia.
  - ✓ Vesicle-mediated transport of immunoglobulin A and other molecules [21, 24].

4 Types of epithelium tissue

Epithelial tissue is divided into two types:

- **Covering and lining epithelium**, forms the outer covering of the skin and some internal organs and also forms the inner lining of blood vessels, ducts, body cavities, and the inner lining of the respiratory, digestive, urinary, and reproductive systems.
- **Glandular epithelium** that makes up the secreting portion of glands such as the thyroid gland, adrenal glands, sweat glands, and digestive glands [21, 24].

4.1 Covering epithelial tissue

Epithelial tissue tends to be classified according to four criteria:

- The number of layers of cells:
  - **Simple epithelium** contains only one layer of cells.
  - **Stratified epithelium** contains more than one layer.
  - **Pseudostratified epithelium** appears to have several layers, but in fact, is simple epithelium.
- Shape of cells at free surface*:* It includes **squamous** (flattened), **cuboidal** and **columnar**.
- Surface modifications (if present): They include **cilia** and **microvilli**, and **stereocilia**.
- Function of the epithelium: **Lining epithelia** (Covering epithelia) covers the free surfaces of the body (internal and external). **Glandular epithelium***:* secretory cells or glands [21, 24].

4.1.1 Simple epithelium

Simple epithelium is divided into three main types, and these are named according to the shape of the cells, which differ based on their functions:

- **Simple squamous epithelium**: consists of a single layer of flat cells with a centrally located nucleus that is flattened and oval or spherical. This epithelium most commonly lines the cardiovascular and lymphatic system (heart, blood vessels, lymphatic vessels), where it is

known as endothelium and forms the epithelial layer of serous membranes (peritoneum, pleura, pericardium), where it is called mesothelium (Figure 2.7).

— **Simple cuboidal epithelium**: It is a single layer of cube-shaped cells that are round and have a centrally located nucleus. It covers the surface of the ovary, lines kidney tubules and the secreting portion of some glands like the thyroid gland and ducts of some glands such as the pancreas (Figure 2.7).

— **Simple columnar epithelium**: It is made by a single layer of rectangular cells. Columnar epithelium lines the stomach without any surface structures. However, the free surface of the columnar epithelium lining the small intestine is covered with microvilli. n the trachea, the columnar epithelium is ciliated (Figure 2.7) [21, 24].

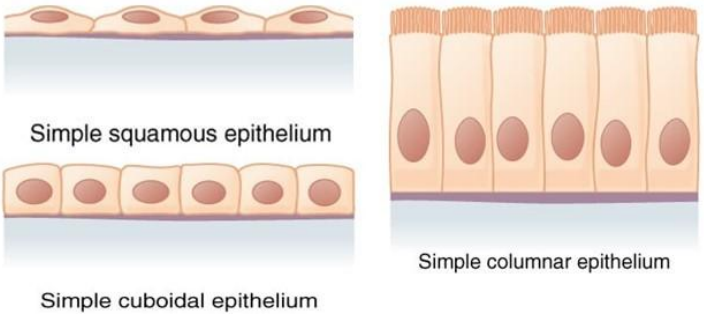

Figure 2.7. Simple epithelia [21].

4.1.2 Pseudostratified epithelium

Pseudostratified epithelium appears as a multilayered tissue because the nuclei of the cells are present at various levels, but in fact, it is a simple epithelium (Figure 2.8). This epithelium lines **epididymis**, larger ducts of many glands, and parts of male **urethra** and airways of most of the **upper respiratory tract** [21, 24].

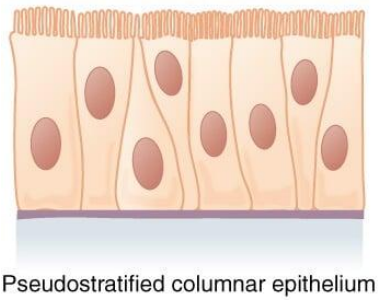

Figure 2.8. Pseudostratified epithelium [21].

4.1.3 Stratified epithelium

A stratified epithelium consists of **several layers** of cells of **various shapes**, and basement membranes are usually absent. As basal cells divide, daughter cells arising from cell divisions are pushed older cells upward toward the apical layer. There are three main types of stratified epithelium: **stratified squamous**, **stratified cuboidal** and **stratified columnar epithelium** (Figure 2.9 et 2.10) [21, 24].

— **Stratified squamous epithelium**: The stratified squamous epithelium has two or more layers of cells. The cells in the apical layer and several layers deep to it are squamous while the cells in deeper layers vary from cuboidal to columnar.

— **Keratinized stratified squamous epithelium:** It develops a tough layer of keratin in the apical segment of cells and several layers deep to it. Keratinized stratified squamous epithelium forms a superficial layer of skin (epidermis) [21, 24].
— **Non-keratinized stratified squamous epithelium**: It lines wet surfaces (lining of mouth, esophagus, part of the epiglottis, part of the pharynx, and vagina) and covers the tongue.
— **Stratified cuboidal epithelium**: It has multiple layers of cells in which the apical layer is made up of cuboidal cells while the deeper layer can be either cuboidal or columnar. It is seen in the excretory ducts of salivary and sweat glands.
— **Stratified columnar epithelium**: It has multiple layers of cells in which the apical layer is made up of columnar cells while the deeper layer can be either cuboidal or columnar. This type of epithelium is present in the conjunctiva of the eyes, parts of the urethra [21, 24].

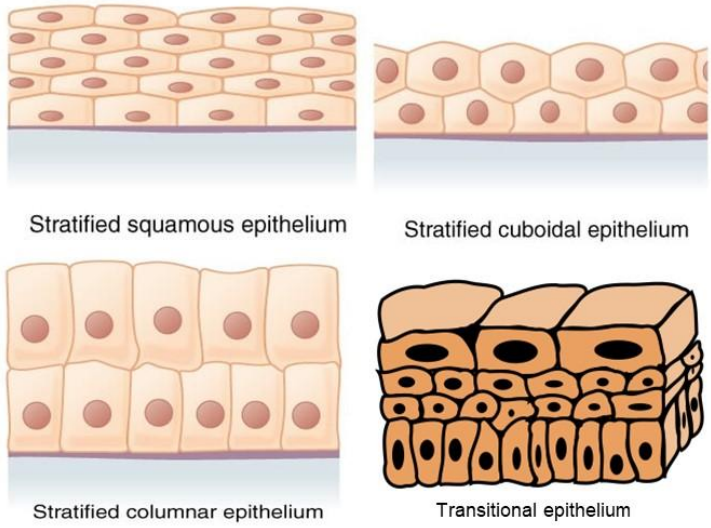

Figure 2.9. Structure of stratified epithelia and transitional epithelium [21].

4.1.4 Transitional epithelium

Transitional epithelium (urothelium) has a variable appearance (transitional):

— In a relaxed or unstretched state, looks like stratified cuboidal epithelium, except apical layer cells tend to be broad and rounded.
— In stretched state, cells become flatter, giving the appearance of stratified squamous epithelium.
— This epithelium can be found in the **ducts of the prostate** gland as well as lining the **urinary bladder** [21, 24].

4.1.5 Epithelial cell renewal

Most epithelial cells have a finite life span less than that of the whole organism

Epithelial cells belong to the category of **continuously renewing cell populations**. The replacement cells are produced by mitotic division of **adult stem cells** residing in different sites (**niches**) in various epithelia.

The renewal of lining epithelium of small intestine is insured by the mitotic activity of **adult stem cells** located in **niches** at the lower portion of the intestinal glands. They differentiate into four principal cell types. **Enterocytes** (columnar absorptive cells), **goblet** cells (mucus-secreting), and **enteroendocrine** cells (regulatory and hormone-secreting), and **Paneth** (antimicrobial peptides secretory cells);

In the stratified squamous epithelium of skin, is renewed by the mitosis of **stratum basale** (**germinativum**), located in the basal layer of the epidermis. As these cells differentiate, they are pushed toward the surface by new cells in the basal layer [21, 25].

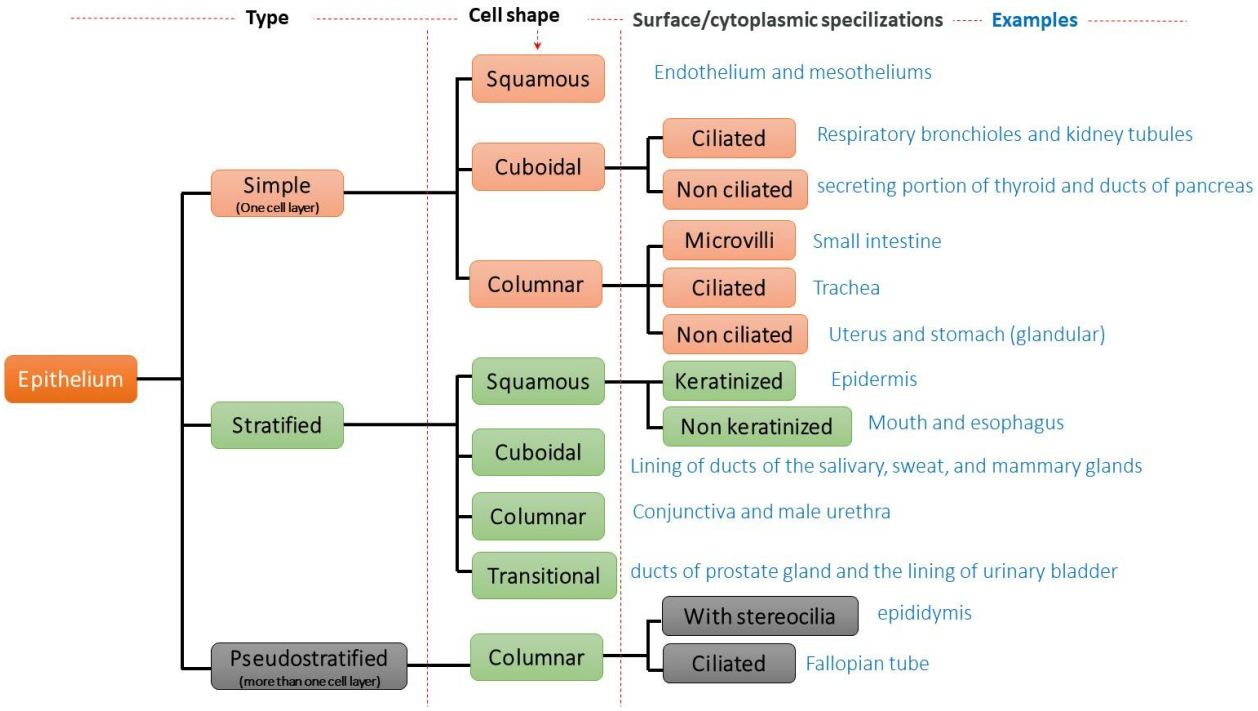

Figure 2.10. Overview of the covering epithelia [21].

4.2 Glandular epithelium

Glandular epithelium can be classified according to the environment in which these glands diversify their secretion product, glands can be classified according into:

— **Exocrine glands:** Release their secretion product (such as sweat, tears, saliva, milk, and digestive juices) through a duct or opening to a body free surface (Gut, skin, respiratory tract…). . (e.g., sweat glands, lacrimal glands, salivary glands, mammary glands, and digestive glands in the stomach, pancreas, and intestines).

— **Endocrine glands:** Release their secretion product (**hormone)** directly into the **blood** and travel to tissues and organs all over the body. . (e.g., the pineal gland, pituitary gland, pancreas, ovaries, testicles, thyroid gland, parathyroid gland, hypothalamus, liver) [21].

4.2.1 Exocrine glands

a) Mode of excretion: the mechanism whereby the gland cells release their secretory product (Figure 2.11)

— **Merocrine:** the cells produce secretions and they reject them externally by exocytosis, the cell integrity being respected (as in the parotid gland).
— **Apocrine:** the secretory product is gradually accumulated at the apical pole, the superficial portion of the cell is detached in the form of large vacuole, the cell integrity being respected (as, in the lactating mammary gland).
— **Holocrine:** Secretory products are stored in the cells of the gland. Entire cells are shed by the gland and become part of the secretion. The lost cells are replaced by other cells deeper in the gland. (as in the sebaceous gland) [21, 26].

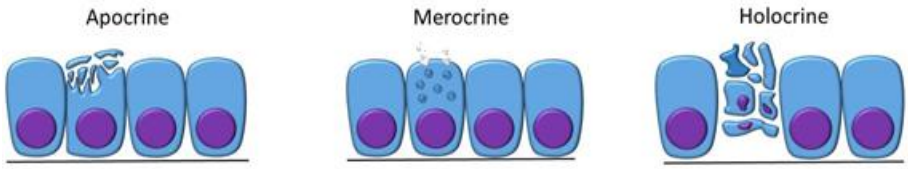

Figure 2.11. Mode of excretion of exocrine glands [21].

b) Histological organization of compound glands

Exocrine glands are usually enclosed in a fibrous **capsule**. The capsule often gives off extensions called **septa** or **trabeculae**, that divide the interior of the gland into compartments called **lobes**, which are visible to the naked eye. Finer connective tissue septa may further subdivide each lobe into microscopic **lobules** (Figure 2.12). Blood vessels, nerves, and the gland's own ducts generally travel through these septa [24, 26].

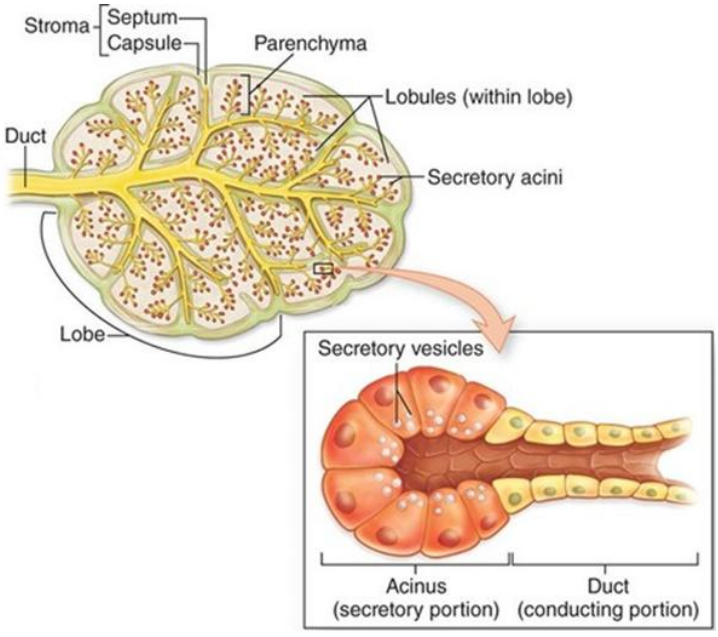

Figure 2.12. Histological organization of compound glands [21].

c) Exocrine glands classification

➢ Exocrine glands may be classified by the number of cells that compose the gland:
— **Unicellular:** a single cell is the entire gland (e.g., **Goblet cell**).
— **Multicellular:** the gland is composed of more than just a single cell (e.g., **submandibular gland**).

- Based on the shape of secretory units, they are classified in:

— **Tubular glands**: the secretory portion is shaped like a tube,

— **Alveolar or acinar glands**: if it is shaped like a flask or grape (Alveolar and acinar glands are both spherical-shaped exocrine glands. They differ in the size of the lumen of the secretory duct. An acinar gland duct has a small lumen while the alveolar gland duct has a large lumen).

- Based on the duct structure, they are classified in:

— **Simple glands**:
  - **Acinous glands** (or alveolar)**:** simple acinar (or simple alveolar).
  - **Tubular glands:** simple tubular, simple branched tubular, simple coiled tubular.

— **Compound glands** :
  - **Acinous glands:** compound acinar.
  - **Tubular glands**: compound tubular.

— **Mixed**: tubolo-acinar glands (or tubule alveolar glands).

- Based on the type of secretion the gland produces: (or the nature of the secretory product)

— **Serous:** watery (e.g., **parotid gland**).

— **Mucous:** viscous (e.g., **Goblet cell**, minor salivary glands of the palate, surface cells of the stomach).

— **Mixed:** serous and mucous (e.g., **sublingual gland**) [24, 26].

1) Unicellular exocrine glands

Unicellular exocrine glands are individual cells that release their products from epithelial cells specialized for secretion directly onto the surface of open body cavities and thus, are considered exocrine. (e.g., **goblet cells** in the intestinal and respiratory epithelium) [24, 26].

2) Multicellular exocrine glands

In general, glands have a **secretory unit** that empty into **excretory duct** that release the secretion into the body surface or free space (Figure 2.13). They have **secretory cells** that are grouped together and organized to act as secretory organs. Structurally, the multicellular exocrine glands are further divided into types on the basis of the shape of the secretory unit and whether the ducts are branched or unbranched [24, 26].

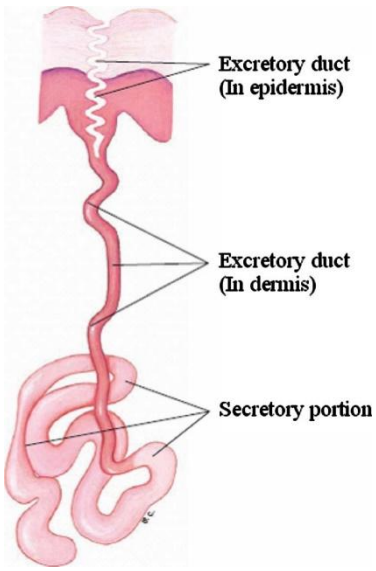

Figure 2.13. Structure of multicellular exocrine glands [21].

a) Simple glands: If the duct of the gland doesn't have branches (or no duct at all).

— **Simple acinar glands**: have a single acinus secretory unit. (e.g., rare in mammals, Peri- and paraurethral glands).

— **Branched acinar glands**: have a branched acinus secretory unit. (e.g., rare in mammals, the mucous glands of the stomach cardia).
— **Tubular glands:** consists of a single straight duct with an elongated or tubular shape to the gland. (e.g., sweat glands (in the soles of the feet), gastric glands (fundic glands), and intestinal glands (called crypts of Lieberkühn).
— **Coiled tubular gland**: the gland is coiled without losing its tubular form. (e.g., sweat glands: eccrine sweat glands).
— **Branched tubular glands:** formed by several secretory units that release their products into the same excretory duct. (e.g., mucous gastric glands (pylorus) , the glands of the endometrium and Brunner's glands of the duodenum) (Figure 2.14) [24, 26].

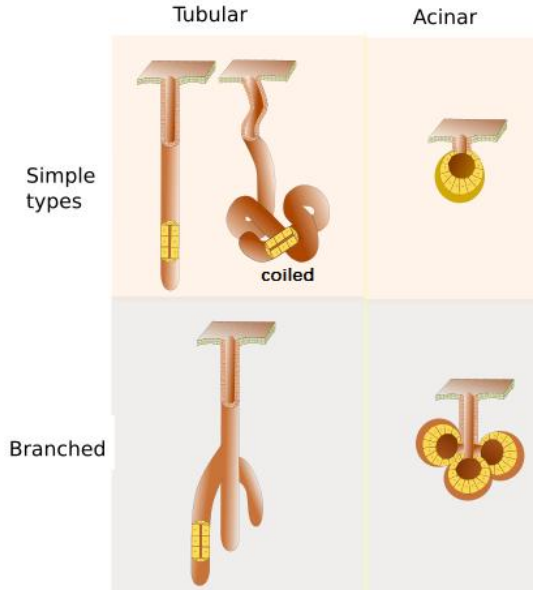

Figure 2.14. Structure of simple glands [21].

b) Compound glands: The excretory duct is branched gives rise to branches in interlobular ducts that contain many secretory units.

— **Compound acinar glands:** alveolar shaped secretory units are formed by pyramid-shaped serous-secretingcells. (e.g., **parotid salivary glands** produce saliva, a watery mixture of enzymes and mucus. The enzymes and the mucus are produced by two distinct cell types, called serous cells and mucous cells; exocrine pancreas).
— **Compound tubular glands**: they are located deep in the submucosa of the duodenum. (e.g., Brunner glands of the duodenal).
— **Compound mixed glands (tubulo-acinar)**: (e.g., mammary gland, lacrimal gland and submandibular salivary gland are tubulo-acinar compound glands) (Figure 2.15) [24, 26].

c) Myoepithelial cells

In the mammary gland, the myoepithelial cells are spindle-shaped cells localized above basement membrane and luminal cells. Found also in sweat glands, lacrimal glands, and salivary glands. Their contraction helps in the excretion of secreted product (Figure 2.16) [24, 26].

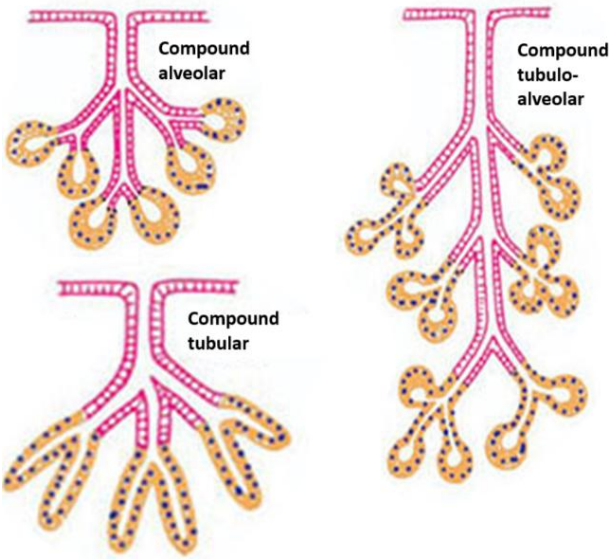

Figure 2.15. Structure of compound glands [24, 26].

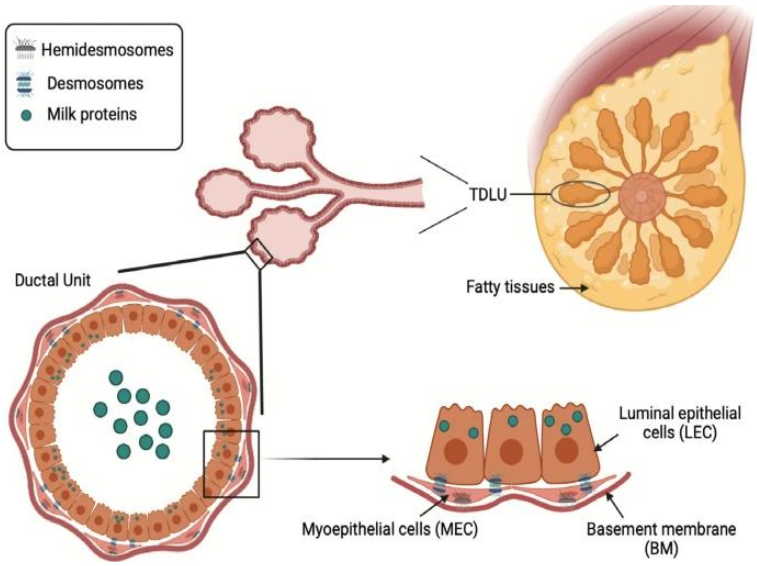

Figure 2.16. Structure of myoepithelial cells [21].

4.2.2 Endocrine glands

Endocrine glands are **ductless** and secrete **hormones** into the blood. Endocrine secretions have far-reaching effects because they are distributed throughout the body by the bloodstream.

Examples of endocrine glands include **pituitary** gland at the base of the brain, the **pineal** gland in the brain, **thyroid** and **parathyroid** glands near larynx (voice box), **adrenal glands** superior to kidneys, **pancreas** near the stomach, **ovaries** in the pelvic cavity, **testes** in the scrotum, **thymus** in the thoracic cavity [24, 26].

4.2.3 Amphicrine glands

Glands that are both exocrine for some products and endocrine for others.

a) Amphicrine homotypic gland: formed by a single type of both endocrine and exocrine cells.

Example the major parenchymal cells in the liver is hepatocytes, they have:

— **Exocrine function**: secretion of bile components in digestive tract;
— **Endocrine function**: release hormones (e.g., Angiotensinogen, Insulin-like growth factor 1, 25-hydroxyvitamin D) in blood.

b) Amphicrine heterotypic gland: two types of cells are found in the parenchyma, some are exocrine others endocrines.

— The pancreas consists in an **exocrine acini** that secretes digestive enzymes into the duodenum.
— The endocrine part is composed of the **Langerhans islets** secrete insulin hormone into the blood stream [24, 26].

Chapter 2: Connective tissue

In general, connective tissue consists of diverse group of **cells** and an **extracellular matrix** (ECM). ECM includes structural (**fibers**) and specialized proteins that constitute the **ground substance**. Connective tissue forms a vast and continuous compartment throughout the body, bounded by the basal laminae of the various epithelia and by the basal or external laminae of muscle cells and nerve-supporting cells. Connective tissue performs a variety of important functions including:

- Innervated tissue (except cartilage).
- Irrigated tissue (except cartilage)
- Have no contact with free surfaces such as body and cavities (except joint cavity).
- Have no contact with the environment [25].

1 Embryonic origin and classification

Mesoderm is the middle of the three primary germ layers of an embryo. It gives rise to **connective tissue**, including embryonic connective tissue (**mesenchyme**), **connective tissue proper** (loose and dense connective tissue), and **specialized connective tissues** (cartilage, bone, adipose tissue, blood and hemopoietic tissue).

2 Composition of connective tissue

Connective tissue consists of cells, and protein fibers, and a ground substance, which together form the extracellular matrix [25, 27].

2.1 Cells

2.1.1 Resident cell population

Fibroblasts are the most common cells in connective tissue. Their nuclei are ovoid or spindle shaped and can be large or small in size depending on their stage of cellular activity. Old cells are fibrocyte, and contractile Cells are myofibroblast. They are responsible for the synthesis of all components of the extracellular matrix (fibers and ground substance) of connective tissue**.**

Mesenchymal cells are undifferentiated pluripotenant embryonic cells that persist to the adult age. They are stellate in shape and located near blood vessels as adventitial cells.

Macrophages, also called tissue histiocytes, are highly phagocytic cells derived from blood monocytes. They are irregular in shape and may be free or fixed type. Their main function is phagocytosis (Figure 2.17).

Macrophages may be named differently in certain organs: they are called **Kupffer cells** in the liver, **osteoclasts** in bone, and **microglial cells** in the central nervous system.

Mast cells develop in bone marrow and differentiate in connective tissue. They are distributed chiefly around small blood vessels. They are oval to round in shape, with a centrally placed nucleus. They have metachromatic granules in cytoplasm (granules have histamine or heparin). Their main

function is the secretion of mediators of inflammation. e.g., histamine, heparin, serine proteases (tryptase and chymase) [25, 27].

2.1.2 Transient cell population

Also named **wandering cell population**, they are leukocytes (white blood cells) that circulate in the bloodstream and migrate into connective tissue in response to immune stimuli. These include neutrophils, eosinophils, basophils, lymphocytes, plasma cells, and monocytes (Figure 2.17).

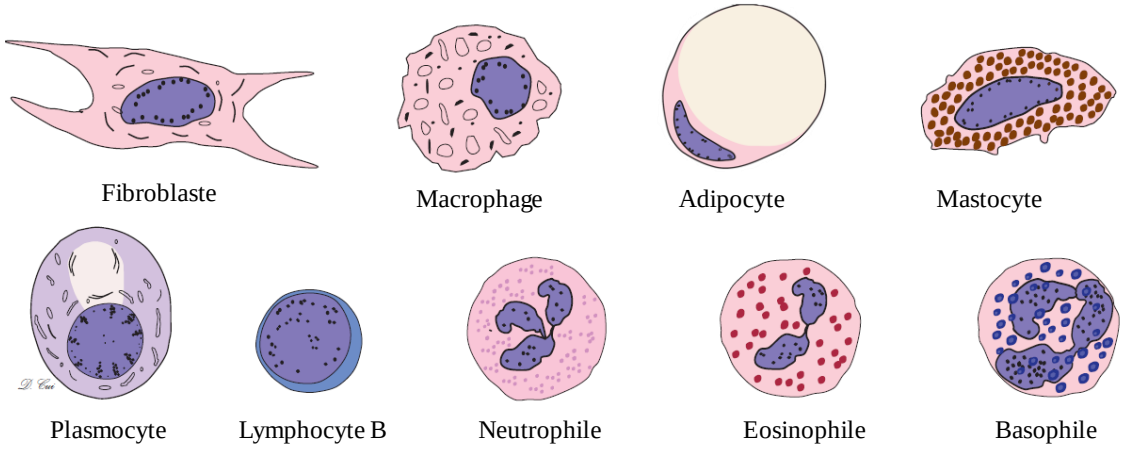

Figure 2.17. The cells of conjunctive tissue [21]

2.2 Extracellular matrix

The ECM is a complex and intricate structural network that surrounds and supports cells within the connective tissue. It contains a variety of fibers such as collagen and elastic fibers that are formed from different types of structural proteins. In addition, the ECM contains a variety of proteoglycans (e.g., aggrecan, syndecan); multiadhesive glycoproteins (such as fibronectin and laminin); and glycosaminoglycans (e.g., dermatan sulfate, keratan sulfate, hyaluronan). The last three groups of molecules constitute the ground substance [25, 27].

2.2.1 Fibers

Collagen fibers and fibrils: They are the most abundant type of connective tissue fiber, and appear as bundles of fine, threadlike subunits of **collagen fibrils**. The collagen molecule that forms collagen fibrils measures is about 300 nm long and 1.5 nm thick and has a head and a tail. A single collagen molecule consists of three polypeptides known as **α chains**. The α chains intertwine, forming a right-handed triple helix. Every third amino acid in the chain is a **glycine** molecule, except at the ends of the α chains. A **hydroxyproline** or **hydroxylysine** frequently precedes each glycine in the chain, and a **proline** frequently follows each glycine in the chain (Figure 2.18).
Fibrillar collagens include types I, II, III, V, and XI collagen molecules. Type I collagen found in loose and dense connective tissue is heterotrimeric while Type II collagen is homotrimeric and present in hyaline and elastic cartilage [25, 27].

Reticular fibers: Reticular fibers provide a supporting framework for the cellular constituents of various tissues and organs. They are composed of type III collagen and are named for their arrangement in a meshlike pattern or network. In loose connective tissue, networks of reticular fibers are found at the boundary of connective tissue and epithelium, as well as surrounding

adipocytes, small blood vessels, nerves, and muscle cells. They are also found in embryonic tissues [25, 27].

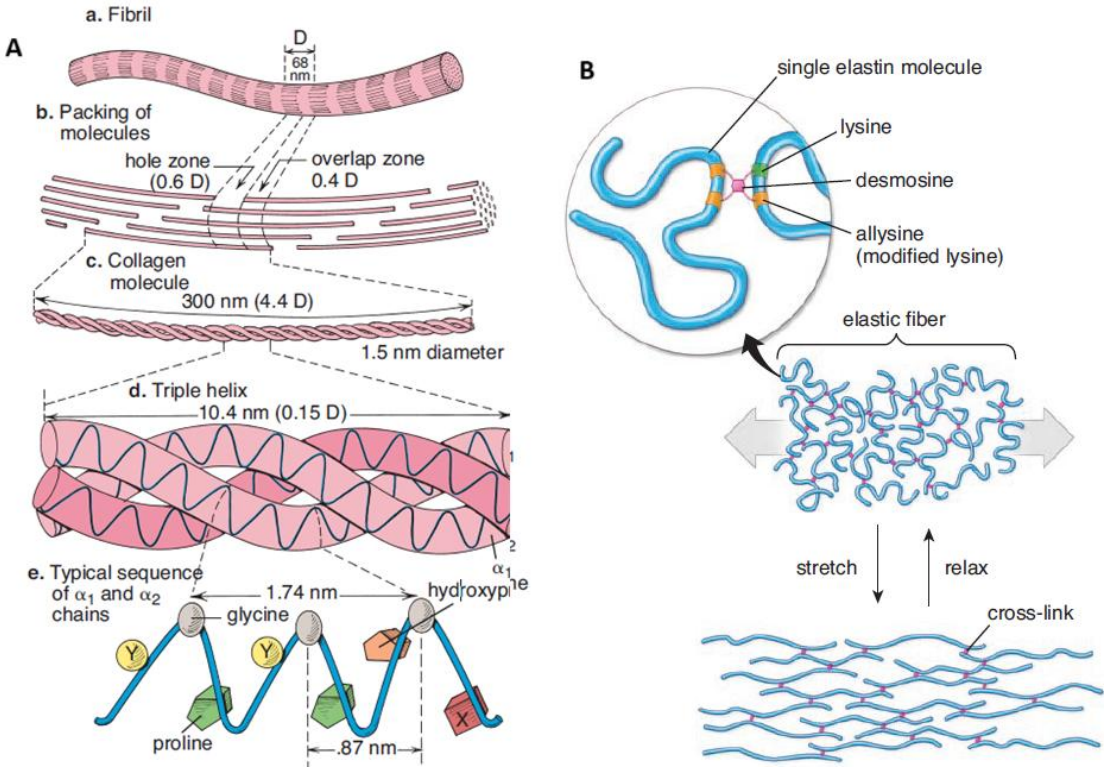

Figure 2.18. Structure of collagen fiber (A) and elastic fibers (B) [21].

Elastic fibers: Elastic fibers are composed of two components, one made up primarily of **elastin** protein and the second being a complex of proteins, rich in **fibrillins**, that form **microfibrils**. The microfibrils serve to orient the elastin in the formation of the elastic fibers. Elastic fibers are very branching and have many connections to each other. This creates the net-like structure (Figure 2.18). They can extend by 100 to 150%. Elastic fibers are produced in the endoplasmic reticulum of the fibroblasts and the smooth muscle cells [25, 27].

2.2.2 Ground substance

Ground substance is a viscous, clear substance with a slippery feel and high water content. It consists predominately of three groups of molecules: **Proteoglycans**, very large macromolecules composed of a core protein; **glycosaminoglycan molecules** (GAGs), which are covalently bound to the proteoglycans; and **multiadhesive glycoproteins**.

Glycosaminoglycan molecules: GAGs are linear polymers of repeating disaccharides with a high negative charge imparted by sulfate and/or carboxyl groups in their structure. These molecules represent long-chain unbranched polysaccharides composed of repeating disaccharide units. The disaccharide units contain either of two modified sugars—**N-acetylgalactosamine** (GalNAc) or **N-acetylglucosamine** (GlcNAc)—and a **uronic acid** such as glucuronate or iduronate. All GAGs (except hyaluronan) are synthesized by connective tissue cells as a covalent, posttranslational modification of proteins called **proteoglycans** (Figure 2.19).

There are four classes of glycosaminoglycans: (1) hyaluronan, (2) chondroitin sulfate (CS)/dermatan sulfate (DS), (3) heparan sulfate (HS)/heparin, and (4) keratan sulfate (KS).

The GAG hyaluronan (or hyaluronic acid) is an exceedingly long, rigid molecule composed of a carbohydrate chain of thousands of sugars rather than the several hundred or fewer sugars found in other GAGs. Hyaluronan polymers are very large (100 to 10,000 kilodaltons) and can displace a large volume of water. They are synthesized by enzymes on the cell surface; therefore, they are not posttranslationally modified like all other GAGs [21, 25, 27].

Proteoglycans indirectly bind to hyaluronan, forming giant macromolecules called **proteoglycan aggregates.**

2.2.3 Multiadhesive glycoproteins

They are multidomain and multifunctional molecules that play an important role in stabilizing the ECM and linking it to the cell surface. Among the best characterized multiadhesive glycoproteins are the following:

Fibronectin (250 to 280 kilodaltons) is the most abundant glycoprotein in connective tissue. Fibronectins are dimer molecules formed from two similar peptides linked by disulfide bonds at a carboxy terminus to form 50-nm long arms. They have multiple functions, one of them is binding integrins on the cell membrane to extracellular molecules such as collagen and glycosaminoglycans (Figure 2.20).

Fibronectin occurs in two principal forms, the **soluble plasma fibronectin** circulating in the blood and the **cellular fibronectin**, that polymerizes into insoluble fibers in the matrix of tissues [21, 25, 27].

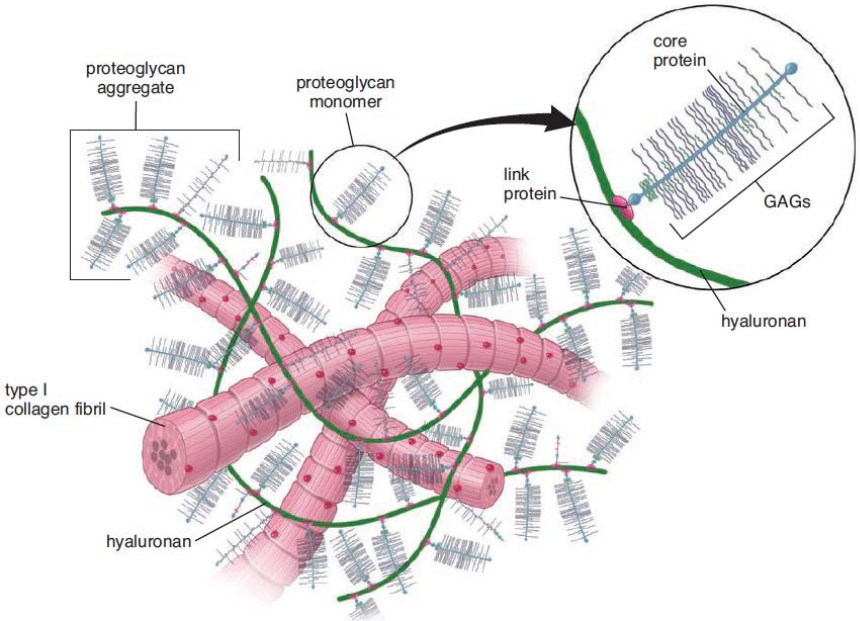

Figure 2.19. Proteoglycan structure [21].

Laminin is a heterotrimer assembled from one heavy α chain and two light β and γ chains, secreted and incorporated into cell-associated extracellular matrices (Figure 2.20). The laminin molecule is a major component of the basement membrane and plays important roles in cell differentiation,

adhesion, and migration. It possesses binding sites for collagen type IV molecules, heparan sulfate, heparin, entactin, laminin, and the laminin receptor on the cell surface [21].

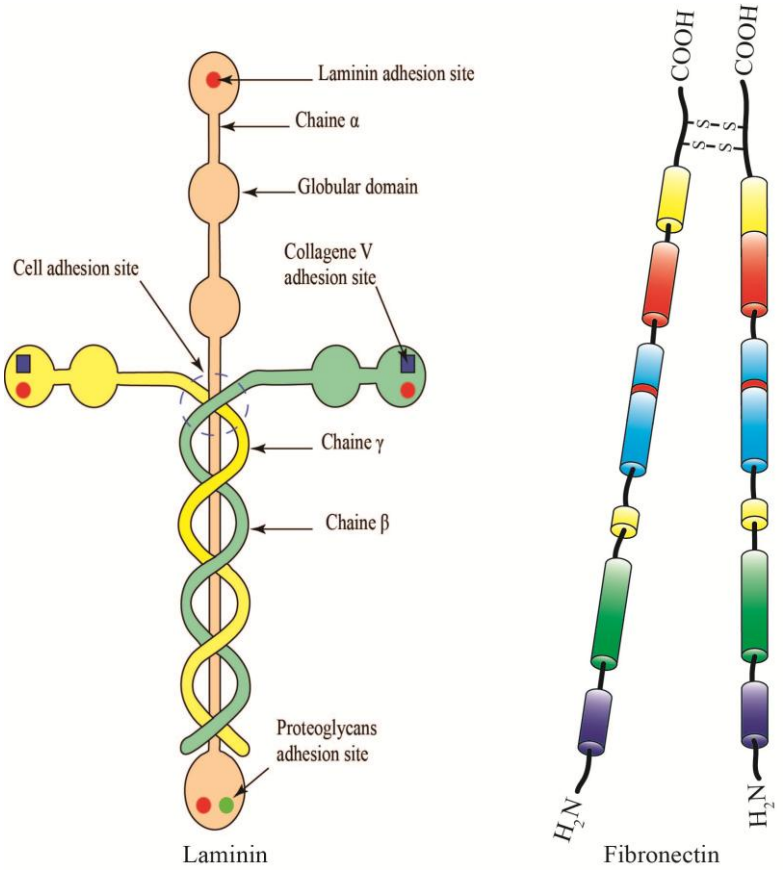

Figure 2.20. Common multiadhesive glycoproteins [21].

3 Classification of connective tissue

Classification of connective tissue is based on the composition and organization of its cellular and extracellular components and on its functions. The classification of connective tissues, include the following subtypes:

3.1 Embryonic connective tissue

Embryonic connective tissue is present in the **umbilical cord** and the embryo. Embryonic connective tissue includes **mucoid connective tissue** (or Mucous connective tissue) and **mesenchyme** [21, 25, 27].

3.1.1 Mucoid connective tissue

It is located within the umbilical cord as **Wharton's jelly** and is a gelatinous tissue primarily composed of a ground substance with few collagen or reticular fibers. Wharton's jelly is largely composed of mesenchymal stem cells and extracellular matrix components, including collagen, chondroitin sulfate, hyaluronic acid, and sulfated proteoglycans [21, 25, 27].

3.1.2 Mesenchyme

Mesenchyme is a loosely organized embryonic connective tissue with undifferentiated cells that produce most of the tissues of the body. Mesenchyme arises from the mesodermal layer of the

trilaminar embryonic disc of the early embryo. As a result, most connective tissues arise from **mesoderm**. For instance, tendons arise from the somatic mesoderm [21, 25, 27].

3.2 Connective tissue proper

Connective tissue proper includes both loose (or areolar) connective tissue and dense connective tissue.

3.2.1 Loose connective tissue

It is the most widely distributed of all connective tissues and the predominant type of connective tissue that joins the cells in the other main tissues (muscle, nerve, and epithelia). It is a cellular connective tissue that consists of a relatively large amount of **amorphous ground substance** of hyaluronan and proteoglycans supported by a **few collagen fibrils** and **elastic fibrils** (Figure 2.21). In addition to fibroblasts, the cell population is heterogeneous, including both indigenous and emigrant connective tissue cells. The loose connective tissue underlying the epithelium in the gastrointestinal tract, and in the optically transparent vitreous body of the eye [21, 25, 27].

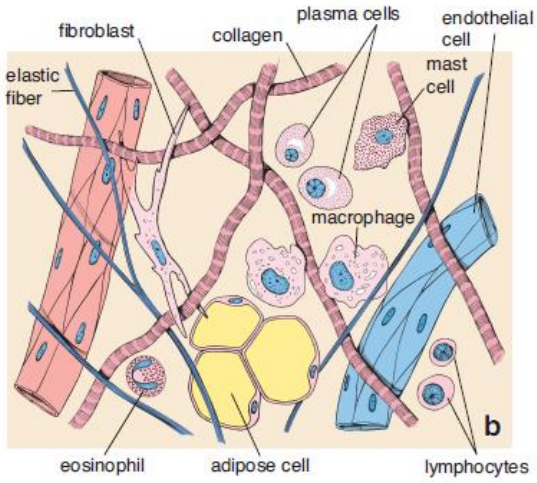

Figure 2.21. Structure of loose connective tissue [21].

3.2.2 Dense connective tissue

Can be divided into:

Dense irregular connective tissue: Consists of few connective tissue cells (fibroblasts), little ground substance and contains mostly collagen fibers (type I collagen fibers, interlaced with a few elastic and reticular fibers). These fibers are arranged in bundles without a definite orientation (oriented in various directions).

Dense irregular connective tissue provides significant strength (high pro -portion of collagen fibers). e.g. The dermis of the skin (deep layer) and capsules of many organs, submucosa of intestine.

Dense regular connective tissue: Consists of fewer cells and ECM, and more fibers, with a predominance of type I collagen fibers. Here, the fibers are arranged into a definite linear pattern. Fibroblasts are arranged linearly in the same orientation. e.g. tendons, ligaments, the inner layer of the skin, and the sclera (the white outer layer of the eye) are all types of dense connective tissue.

Tendons are cord-like structures that attach muscle to bone. They consist of parallel bundles of collagen fibers. Situated between these bundles are rows of fibroblasts called **tendinocytes**. The substance of the tendon is surrounded by a thin connective tissue capsule, the epitendineum.

The tendon is subdivided into fascicles by **endotendineum**, a connective tissue extension of the **epitendineum**.

Ligaments, like tendons, consist of fibers and fibroblasts arranged in parallel. The fibers of ligaments, however, are less regularly arranged than those of tendons. Ligaments join bone to bone, in some locations, such as in the spinal column (requires some elasticity) contain many more elastic fibers and fewer collagen fibers. These ligaments are called elastic ligaments.
Aponeuroses resemble broad, flattened tendons. Instead of fibers lying in parallel arrays, the fibers of aponeuroses are arranged in multiple layers. The bundles of collagen fibers in one layer tend to be arranged at a 90° angle to those in the neighboring layers. The fibers within each of the layers are arranged in regular arrays; thus, **aponeurosis** is a dense regular connective tissue. This orthogonal array is also found in the **cornea** of the eye and is responsible for its transparency [21, 25, 27].

3.3 Specialized connective tissue

This mainly include cartilage, bone, hemopoietic tissue, blood, adipose tissue, and lymphatic tissue (see the following chapters for details) [21, 25, 27].

Chapter 3: Cartilage tissue

Cartilage is an avascular tissue that consists of **chondrocytes** and an extensive extracellular matrix. More than 95% of cartilage volume consists of extracellular matrix, which is a functional element of this tissue. The chondrocytes are sparse but essential participants in producing and maintaining the matrix. The high ratio of glycosaminoglycans (GAGs) to **type II collagen** fibers in the cartilage matrix facilitates the diffusion of substances between blood vessels in the surrounding connective tissue and the chondrocytes embedded within the matrix, thereby supporting tissue viability [26].

1 Cartilage types

Three types of cartilage that differ in appearance and mechanical properties are distinguished on the basis of characteristics of their matrix:

— **Hyaline cartilage** is characterized by matrix containing type II collagen fibers, GAGs, proteoglycans, and multiadhesive glycoproteins.
— **Elastic cartilage** is characterized by elastic fibers and elastic lamellae in addition to the matrix material of hyaline cartilage.
— **Fibrocartilage** is characterized by abundant type I collagen fibers, GAGs, proteoglycans, and multiadhesive glycoproteins [26]..

1.1 Hyaline cartilage

In adults, it is found in the rings of the trachea, the cartilaginous skeleton of the larynx, and articular cartilages. As a connective tissue, it presents the usual constituents to consider: extracellular matrix (ground substance and fibers) and cells.

Hyaline cartilage matrix is composed of:

1.1.1 Ground Substance

The ground substance contains **proteoglycans** whose constituent **glycosaminoglycans** are essentially molecules of **chondroitin sulfate** and **keratan sulfate**, which give it a basophilia that decreases with age (as the ground substance then becomes enriched with a protein substance related to keratin). Proteoglycans readily associate into large molecular aggregates (aggrecans) formed around a backbone of **hyaluronic acid** onto which up to 100 proteoglycan molecules attach [26, 27].

1.1.2 Adhesion glycoproteins

They ensure interactions between chondrocytes and matrix molecules. They have clinical value as markers of cartilage turnover and degeneration.

— **Anchorin CII** (cartilage annexin V), a small molecule that functions as a collagen receptor on chondrocytes,
— **Tenascin** and fibronectin, which also help anchor chondrocytes to the matrix [26, 27].

1.1.3 Fibers

Type II collagen constitutes the majority of the fibrils; type IX collagen facilitates the interaction of fibrils with proteoglycan molecules in the matrix; type XI collagen regulates fibril size; and type

X collagen organizes collagen fibrils into a three-dimensional hexagonal network, essential for their proper mechanical function. Furthermore, type VI collagen is also present in the matrix, mainly at the periphery of chondrocytes, where it contributes to their attachment to the matrix structure (Figure 2.22).

The fibers are fine around the chondroplasts that they surround (pericellular baskets), thicker in the interterritorial regions where their arrangement is determined by the mechanical stresses to which the specific cartilaginous tissue is subjected [26, 27].

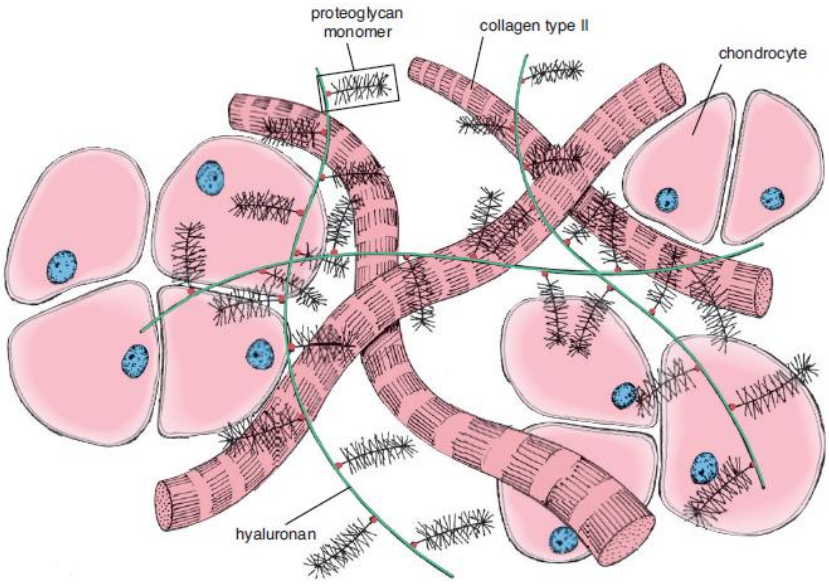

Figure 2.22. Molecular structure of hyaline cartilage matrix [21].

1.1.4 Cells

The cells or chondrocytes are most often grouped in pairs or more. They are located inside cavities: the chondroplasts, which seem to be surrounded by a capsule, which is, in fact, formed by a condensation of the ground substance reinforced inwardly by a layer formed of collagens X and XI.

In adults, chondrocytes are large cells with clear cytoplasm, poor in organelles, containing some inclusions of lipids and glycogen. However, when engaged in a growth process, chondrocytes ensure the synthesis of the constituents of the ground substance and are then richer in organelles [26, 27].

1.1.5 The perichondrium

The perichondrium is a dense layer of fibrous connective tissue that surrounds the surfaces of most hyaline cartilage (except at articular surfaces, like in joints) and elastic cartilage. It is essential for the growth, maintenance, and repair of the cartilage it envelops. The perichondrium is organized into two distinct layers (figure 2.23):

Outer fibrous layer (*Stratum fibrosum*): This layer is composed primarily of dense, irregular connective tissue. It is rich in type I collagen fibers and fibroblasts (the cells that produce this collagenous matrix). It provides structural strength and resistance to deformation, protecting the underlying delicate cartilage, acts as an attachment point for ligaments and tendons, integrating the

cartilage into the surrounding tissues, and barrier: Serves as a barrier against infection or inflammation.

Inner cellular layer (*Stratum chondrogenicum / chondrogenicum*): This layer is thinner and more delicate than the fibrous layer. It is characterized by the presence of specialized cells, including: **chondrogenic Cells** that are undifferentiated mesenchymal stem cells (progenitor cells) that are capable of differentiating into chondroblasts. **Chondroblasts** that are the active, cartilage-forming cells. They are derived from the chondrogenic cells and are responsible for producing the new cartilage matrix (collagen and proteoglycans). This layer is specifically responsible for the appositional **growth** and **repair** of cartilage [26, 27].

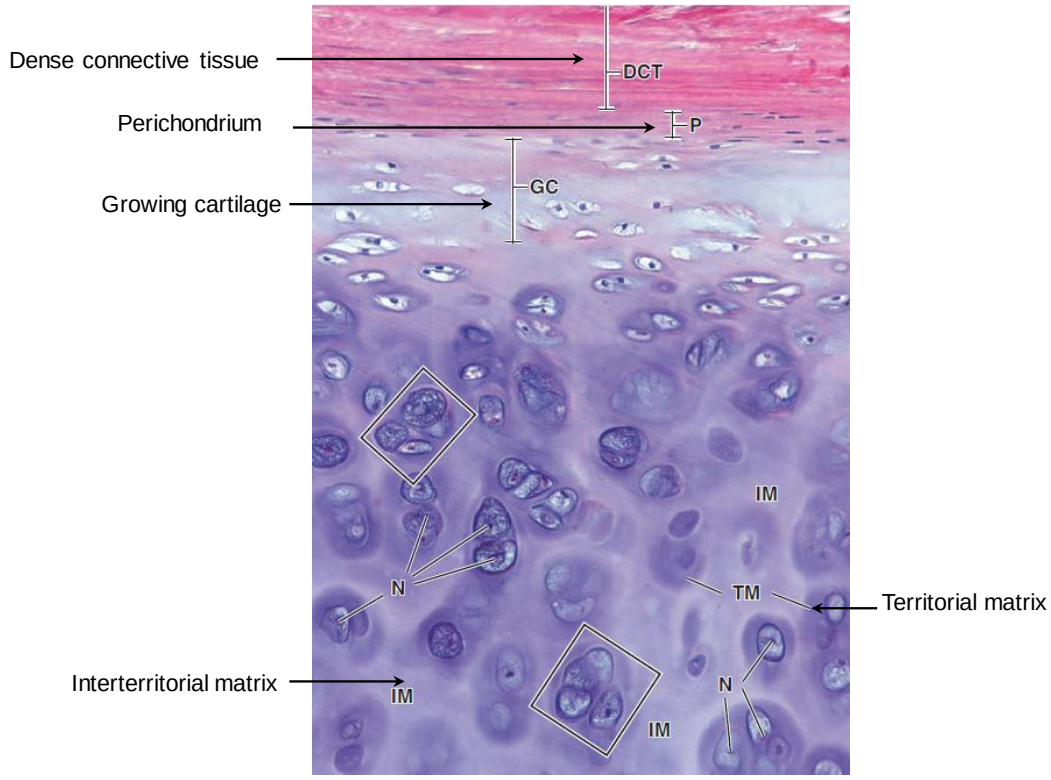

Figure 2.23. Structure of hyaline cartilage [21].

1.1.6 Chondrocytes and isogenous groups

In hyaline cartilage, chondrocytes are distributed either singularly or in clusters called **isogenous groups**. These are clusters of 2, 4, or 8 chondrocytes that are found close together within a single lacuna (space). They are the progeny of a single chondrocyte that has undergone mitotic division [26, 27].

Cartilage matrix can be subdivided by its composition around chondrocytes as (Figure 2.23 and 2.24).

a) Capsular matrix (Pericellular matrix)

This matrix is uniquely rich in type VI collagen, which forms a fine microfibrillar network that anchors the chondrocyte to the matrix. It also contains a high concentration of sulfated proteoglycans, hyaluronan, and multiadhesive glycoproteins (e.g., fibronectin, decorin, and

laminin). It is crucial for transmitting mechanical signals from the matrix to the cell and for cell-matrix adhesion (Figure 2.24).

b) Territorial matrix

This matrix surrounds the capsular matrix and encapsulates either a single chondrocyte or an entire isogenous group and contains a randomly arranged network of type II collagen fibrils with smaller quantities of type IX collagen. It also has a lower concentration of sulfated proteoglycans and stains less intensely than the capsular matrix.

c) Interterritorial matrix

This is the bulk of the cartilage matrix, located between the territorial matrices of the various chondrocytes and isogenous groups. It contains the same molecules as the territorial matrix (primarily **type II collagen fibrils** and the massive aggrecan aggregates), but they are organized differently. The collagen fibrils are thicker and are organized into a wider meshwork, and the proteoglycan concentration is slightly lower [21, 26, 27].

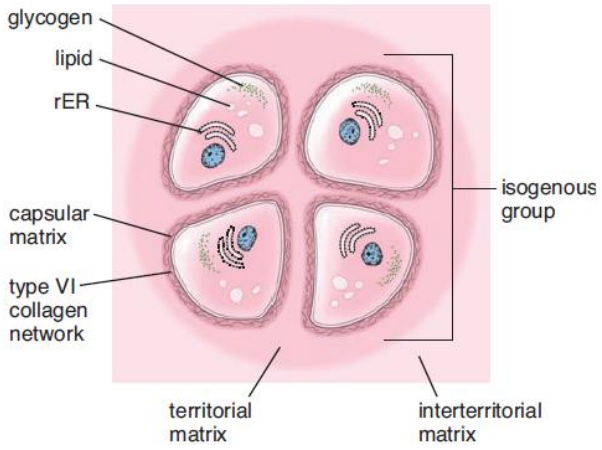

Figure 2.24. Structure of cartilage matrix [21].

1.2 Elastic cartilage

In addition to containing the normal components of hyaline cartilage matrix, elastic cartilage matrix also contains a dense network of branching and anastomosing **elastic fibers** and interconnecting sheets of elastic material. These fibers and lamellae are best demonstrated in paraffin sections with special stains such as **Verhoeff's stain** and **orcein**. The elastic material gives the cartilage elastic properties in addition to the resilience and pliability that are characteristic of hyaline cartilage. Elastic cartilage is found in external ear (auricle), external auditory canal, eustachian tubes, epiglottis, larynx (specifically the corniculate and cuneiform cartilages).

The primary function of elastic cartilage is to provide strong, flexible, and elastic support to soft tissues. It provides shape and resilience, allowing it to bend and deform but always return to its original shape without damage [26, 27].

1.3 Fibrous cartilage

Fibrous cartilage is particularly rich in **type I collagen fibers**, most often arranged in well-organized bundles that delineate areas of ground substance where chondrocytes concentrate. Fibrous cartilage is found in areas of **ligament** and **tendon insertions** and especially in **intervertebral discs**, **menisci**, and the deep part of **articular cartilage** [26, 27].

2 Cartilage growth

All cartilage arises from **mesenchyme**. At sites where cartilage is to form, the mesenchymal cells lose their processes, round up, proliferate, and crowd together in a dense aggregate of chondrogenic cells (a center of chondrification). The cells in the interior of the mass are chondroblasts, which lay down fibers and ground substance. As the amount of intercellular material increases, the cells become isolated in individual compartments (lacunae) and take on the characteristics of chondrocytes.

New chondroblasts are recruited from the more peripherally placed immature cells. The perichondrium is derived by condensation of the mesenchyme that surrounds the developing cartilage. Further growth [26, 27].

Cartilage grows in two ways: appositional growth, interstitial growth. Appositional growth is ensured by the deep layer of the perichondrium, whose cells differentiate into chondroblasts that produce the constituents of the cartilaginous matrix in which they subsequently become trapped.

Perichondral or appositional growth: where new cartilage is added to the surface from the perichondrium, fibroblasts of the perichondrium differentiate into chondrocytes.
Interstitial growth (rare in adults): where chondrocytes within the cartilage divide and secrete new matrix, causing internal expansion. Articular cartilage, devoid of perichondrium, does not regenerate [26, 27].

3 Cartilage repair

Because of its avascularity, mammalian cartilage has a limited capacity to restore itself after injury. Damaged regions of cartilage become **necrotic**, and these areas are then filled in by connective tissue from the perichondrium. Some of this connective tissue may slowly differentiate into cartilage, but most remains as dense irregular connective tissue that may later calcify or even ossify [26, 27].

4 Cartilage nutrition

Cartilaginous tissue lacks blood and lymphatic vessels.

- Most cartilages are nourished by diffusion through the ECM from the capillaries of the perichondrium.
- Articular cartilages are nourished from the synovial fluid [26, 27].

5 Functions of cartilage

- Temporary cartilages of the developing skeleton
- Cartilaginous models: templates for the long bones of the fetal skeleton;
- Growth Plate cartilages (Epiphyseal plates): growth of bones in length.
- Permanent cartilages of adult Joints
- Reduction of friction in joints; mobility and shock absorption (intervertebral discs).
- Cartilages of the ENT (ear, nose, and throat) Sphere and airways.
- Maintenance of the openness of the lumens of the trachea and bronches; maintenance of the shape of structures (nose and ear) [26, 27].

Chapter 4: Bone tissue

Bone is a highly **vascularized** connective tissue characterized by a **mineralized extracellular matrix**. Bone is a specialized form of connective tissue that, like other connective tissues, consists of **cells** and **extracellular matrix**. The feature that distinguishes bone from other connective tissues is the mineralization of its matrix, which produces an extremely hard tissue capable of providing support and protection [21].

1 Functions of Bone and the Skeletal System

- **Mechanical function:** one of the most resistant tissues in the body, capable of providing support and protection.
- **Metabolic function:** bone also serves as a storage site for calcium and phosphate. Both calcium and phosphate can be mobilized from the bone matrix and taken up by the blood as needed to maintain appropriate levels throughout the body.
- **Hematopoietic function**: the bone marrow space, contain the hematopoietic marrow, that forms the three lineages of blood cells (the erythroid, myeloid and the monocytic/lymphoid lineages) [26].

2 Composition of bone tissue

2.1 The inorganic/mineral component

It comprises of calcium and phosphate in the form of needle-like or thin plates of hydroxyapatite crystals [$Ca_{10}(PO_4)_6(OH)_2$]. These are conjugated to a small proportion of magnesium carbonate, sodium and potassium ions.

2.2 The organic matrix

It is composed of collagen and non-collagenous organic materials.

2.2.1 Collagenous proteins

The major structural component of bone matrix is **type I collagen** and, to a lesser extent, **type V collagen**. Trace amounts of other types such as type III, XI, and XIII collagens have also been found in the matrix. All collagen molecules constitute about 90% of the total weight of the bone matrix proteins [21, 25, 27].

2.2.2 Noncollagenous proteins

The matrix also contains other matrix (noncollagenous) proteins that constitute the ground substance of bone. As a minor component of bone, constituting only 10% of the total weight of bone matrix proteins, they are essential to bone development, growth, remodeling, and repair.

Proteoglycan macromolecules: they contain a core protein with various numbers of covalently attached side chains of **glycosaminoglycans** (hyaluronan, chondroitin sulfate, and keratan sulfate). They contribute to the compressive strength of bone. They are also responsible for binding growth factors and may inhibit mineralization.

Multiadhesive glycoproteins are responsible for attachment of bone cells and collagen fibers to the mineralized ground substance. Some of the more important glycoproteins are **osteonectin**

(which serves as a glue between the collagen and hydroxyapatite crystals) and **sialoproteins** such as **osteopontin** (which mediates attachment of cells to bone matrix) and sialoprotein I and II (which mediate cell attachment and initiate calcium phosphate formation during the mineralization process).

Bone-specific, vitamin K–dependent proteins, which include **osteocalcin** (which captures calcium from the circulation and attracts and stimulates osteoclasts in bone remodeling), protein S (which assists in the removal of cells undergoing apoptosis), and matrix Gla-protein (MGP) (which participates in the development of vascular calcifications).

Growth factors and cytokines, which are small regulatory proteins including insulinlike growth factors (IGFs), tumor necrosis factor α (TNF-α), transforming growth factor β (TGF-β), platelet-derived growth factors (PDGFs), bone morphogenic proteins (BMPs), and interleukins (IL-1, IL-6) [21, 25, 27]..

2.3 Bone cells

Five designated cell types are associated with bone tissue: osteoprogenitor cells, osteoblasts, osteocytes, bone-lining cells, and osteoclasts (Figure 2.25 and 2.26). With the exception of the osteoclast, each of these cells may be regarded as a differentiated form of the same basic cell type [21, 25, 27].

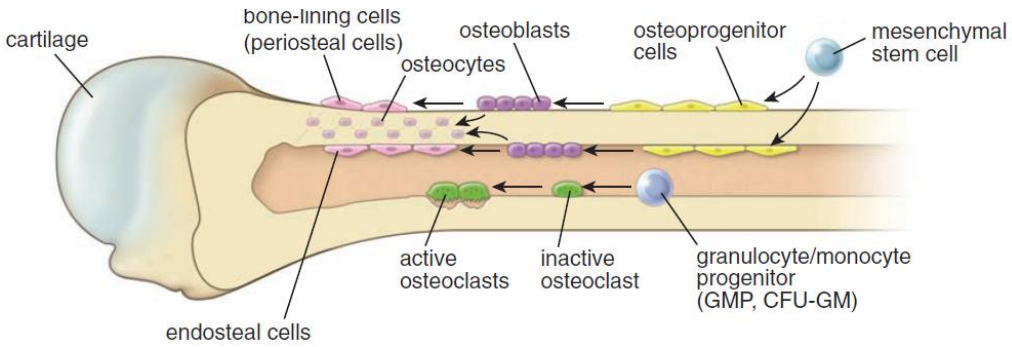

Figure 2.25. Schematic drawing of cells associated with bone [21].

2.3.1 Osteoprogenitor Cells

Osteoprogenitor cells are found on the external and internal surfaces of bones and may also reside in the microvasculature supplying bone. Morphologically, they comprise the **periosteal cells** that form the innermost layer of the periosteum and the **endosteal cells** that line the marrow cavities, the osteonal (Haversian) canals, and the perforating (Volkmann's) canals (Figure 2.26) [21, 25, 27].

Osteogenesis, the process of new bone formation, requires a population of renewable osteoprogenitor cells (osteoblast precursor cells). Osteoprogenitor cells are derived from mesenchymal stem cells in the bone marrow that have the potential to differentiate into many different cell types including fibroblasts, osteoblasts, adipocytes, chondrocytes, and muscle cells [21, 25, 27].

2.3.2 Osteoblasts

They are recognized in the light microscope by their cuboidal or polygonal shape and their aggregation into a single layer of cells lying in apposition to the forming bone.

The osteoblast is the differentiated bone-forming cell that secretes bone matrix. The osteoblast secretes both type I collagen and bone matrix proteins (BMPs), which constitute the initial unmineralized bone, or **osteoid**. The osteoblast is also responsible for the calcification of bone matrix (Figure 2.26) [21, 25, 27].

2.3.3 Osteocytes

The osteocyte is the mature bone cell enclosed by bone matrix that it previously secreted as an osteoblast. Each osteocyte occupies a space, or **lacuna**, that conforms to the shape of the cell. Osteocytes extend cytoplasmic processes through the canaliculi in the matrix to contact processes of neighboring osteocytes and bone-lining cells by means of gap junctions (Figure 2.26) [21, 25, 27].

Osteocytes are the cells responsible for maintaining the bone matrix. One of the roles of osteocytes is **mechanotransduction**, the process by which the osteocyte responds to mechanical forces applied to the bone. Osteocytes can synthesize new matrix, as well as participate in matrix degradation. Such activities help to maintain calcium homeostasis [21, 25, 27].

2.3.4 Bone-lining cells

Bone-lining cells are derived from osteoblasts and cover bone that is not remodeling. Bone-lining cells on external bone surfaces are called **periosteal cells**, and those lining internal bone surfaces are often called **endosteal cells** [21, 25, 27]**.**

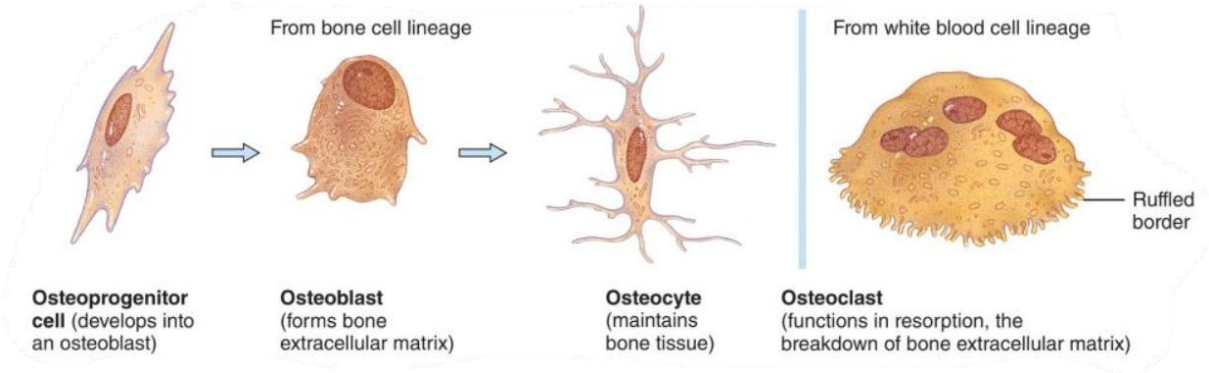

Figure 2.26. Bone tissue cells [30].

2.3.5 Osteoclasts

Osteoclasts are large, multinucleated cells found at sites where bone is being removed. Osteoclasts are derived from the fusion of mononuclear hemopoietic progenitor cells (granulocyte/macrophage progenitor cells (GMP, CFU-GM)) under the influence of multiple cytokines. The osteoclast is responsible for **bone resorption**.

The cell is conspicuous not only because of its large size but also because of its marked acidophilia. It also exhibits a strong histochemical reaction for acid phosphatase because of the numerous lysosomes that it contains.

Osteoclasts resorb bone tissue by releasing protons and lysosomal **hydrolases** into the constricted microenvironment of the extracellular space. Among these enzymes, include **cathepsin K** (a cysteine protease) and matrix **metalloproteinases**, which degrade collagen and other proteins of the bone matrix.

When actively resorbing bone, osteoclasts exhibit three specialized regions (Figure 2.27):

- The **ruffled border** is the part of the cell in direct contact with bone. It contains numerous deep plasma membrane infoldings forming microvillous-type structures responsible for increasing surface area for the exocytosis of hydrolytic enzymes and secretion of protons by ATP–dependent proton pumps, as well as endocytosis of degradation products and bone debris.
- The **clear zone** (sealing zone) is a ringlike perimeter of cytoplasm adjacent to the ruffled border that demarcates the bone area being resorbed. Essentially, the clear zone is a compartment at the site of the ruffled border where resorption and degradation of the matrix occurs. It contains abundant actin filaments but essentially lacks other organelles.
- The **basolateral region** functions in the exocytosis of digested material. Transport vesicles containing degraded bone material endocytosed at the ruffled border fuse here with the cell membrane to release their contents [21, 25, 27].

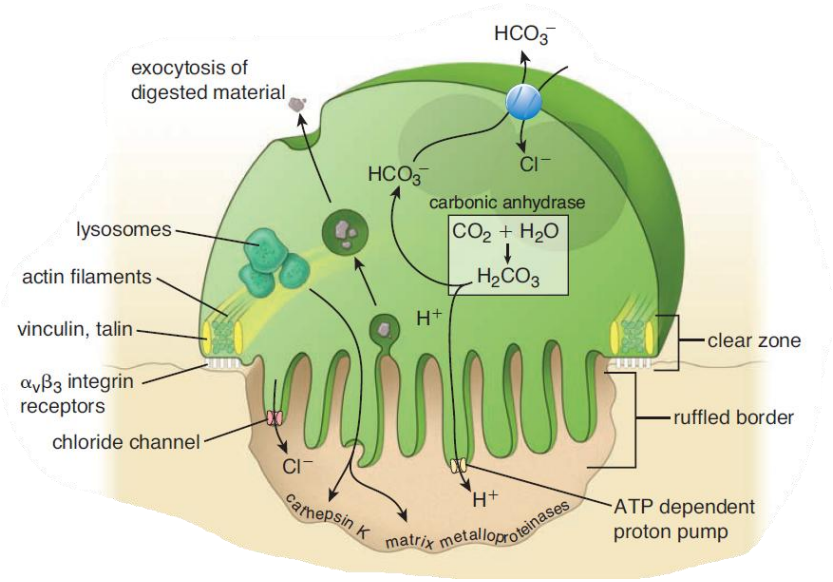

Figure 2.27. Schematic drawing of an osteoclast and its three regions [21].

2.4 Bone structure

Bone tissue is primarily classified into two main types: **compact bone (cortical bone)**, the dense outer layer that provides strength and protection, and **spongy bone (cancellous or trabecular bone)**, the porous, lighter inner tissue that contains bone marrow and forms a network of trabeculae.

Compact bone is characterized by a highly organized, dense structure composed of repeating functional units called **osteons** (or Haversian systems). Each **osteon** is a cylindrical structure made of concentric layers of calcified matrix called lamellae. At the core of every osteon runs a **Haversian canal** (central canal), which contains blood vessels and nerve fibers, providing nourishment and innervation to the living bone cells. These **Haversian canals** are interconnected horizontally by **Volkmann canals** (perforating canals), creating a vast, integrated network for vascular supply and communication throughout the hard tissue.

In contrast, **spongy bone** does not contain **osteons**. Its lattice-like structure of trabeculae is designed to withstand stress and house the bone marrow (Figure 2.28) [30].

2.5 Bone formation

The development of a bone is traditionally classified as **endochondral** in which a cartilage model serves as the precursor of the bone, or **intramembranous ossification**.

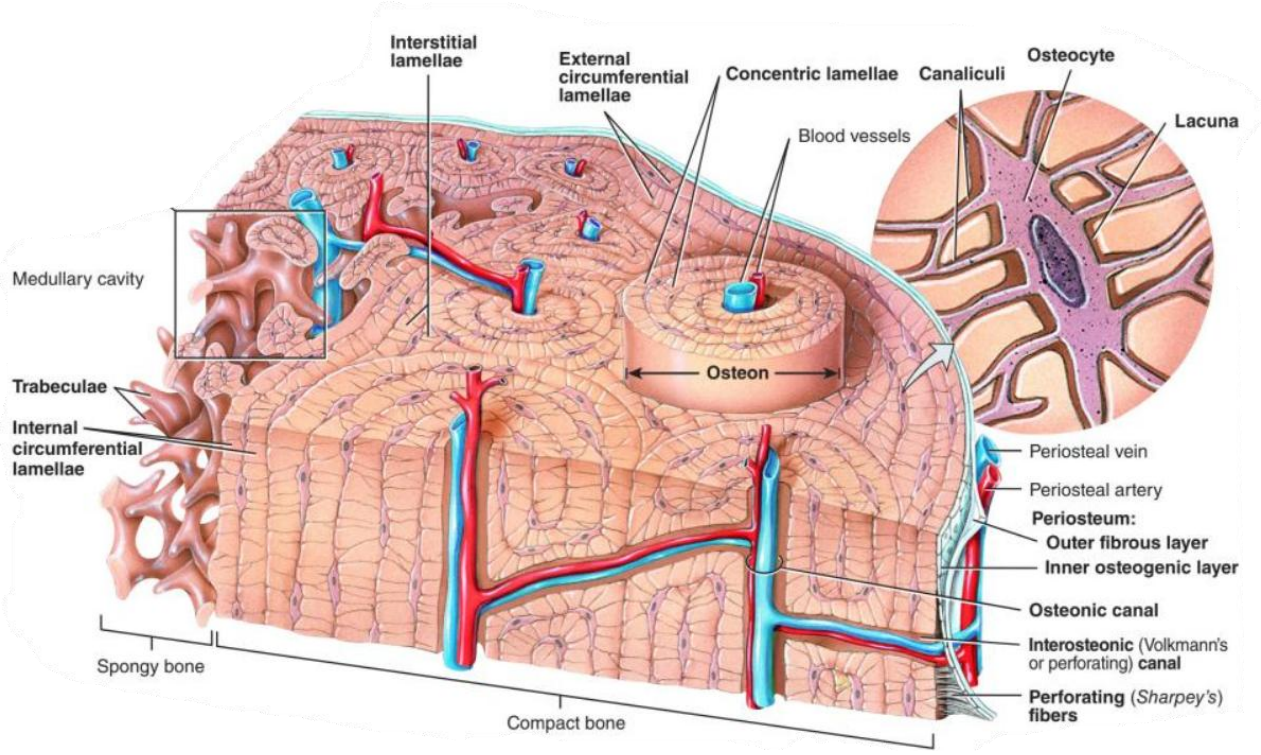

Figure 2.28. Structure of bone tissue [30].

2.5.1 Intramembranous ossification

In intramembranous ossification, bone is formed by differentiation of mesenchymal cells into osteoprogenitor cells that give rise to osteoblasts.

During the eighth week of gestation, some of the mesenchymal cells within the mesenchyme migrate and aggregate in specific areas, the sites where bone is destined to form. This condensation of cells initiates the process of intramembranous ossification [21, 25, 27].

2.5.2 Endochondral ossification

Endochondral ossification is the process by which the long bones of the body form, and is most commonly the way in which damaged adult bone heals (i.e., fracture healing). In endochondral ossification, cartilage serves as a precursor or template for future bone. Endochondral ossification also begins with the proliferation and aggregation of mesenchymal cells at the site of the future bone. The **mesenchymal** cells initially express type II collagen and differentiate into chondroblasts that, in turn, secrete the extracellular matrix that constitutes cartilage, but cellular hypertrophy and environmental constraints trigger the production of alkaline phosphatase, which allows for the mineralization of the cartilage. Blood vessels penetrate the calcifying cartilage, providing the cells necessary for ossification. Infiltrating osteoblasts use the mineralized cartilage as scaffolding, laying down layers of osteoid that will calcify and produce trabecular bone [21, 25, 27].

2.6 Mineralization of extracellular matrix

Mineralization occurs in the extracellular matrix of bone, cartilage and in the dentin, cementum, and enamel of teeth. In places where the mineralization of bone and cartilage is initiated, the local concentration of Ca^{2+} and PO_4 ions in the matrix must exceed the normal threshold level. Several events are responsible for this mineralization:

- The high Ca^{2++} concentration stimulates the osteoblasts to secrete alkaline phosphatase (ALP), which increases the local concentration of PO_4 ions. The high PO_4 concentration stimulates further increases in Ca^{2+} concentration where mineralization will be initiated.
- At this stage of high extracellular Ca^{2+} and PO_4 concentration, the osteoblasts release small matrix vesicles into the bony matrix by exocytosis. The matrix vesicles contain ALP and pyrophosphatase that cleave $PO_4^{\sim}$ ions from other molecules of the matrix.
- The matrix vesicles that accumulate Ca^{2+} and cleave PO_4 ions cause the local isoelectric point to increase, which results in crystallization of CaPO4 in the surrounding matrix vesicles.
- The $CaPO_4$ crystals initiate matrix mineralization by the formation and deposition of hydroxyapatite crystals $[Ca_{10}(PO_4)_6(OH)_2]$ in the matrix surrounding the osteoblasts.

Once the initial crystals of hydroxyapatite have precipitated, they grow rapidly by accretion until they join neighboring crystals produced around other matrix vesicles [21, 25, 27].

Chapter 5: Blood tissue

Blood is a specialized connective tissue composed of formed elements (erythrocytes, leukocytes, and platelets) and a fluid component called plasma. Blood circulates within a closed system of vessels and transports nutrients, waste products, hormones, proteins, ions, oxygen (O_2), carbon dioxide (CO_2), and formed elements. It also regulates body temperature and helps maintain osmotic and acid-base balance. Blood cells have a short lifespan and are continuously replaced. Blood is composed of plasma and formed elements [21, 24].

1 Plasma

Plasma consists of 90% water; 9% organic compounds (proteins, amino acids, and hormones); and 1% inorganic salts, dissolved gases, and nutrients [24].

1.1 Plasma proteins

Albumin, a small protein, maintains osmotic pressure within the vascular system and helps transport certain metabolites.

- γ-globulins are antibodies (immunoglobulins).
- ß-globulins and α-globulins transport metal ions (e.g., iron and copper) and lipids in the form of lipoproteins.
- Coagulation proteins, including fibrinogen, a soluble protein that is converted to fibrin during blood clotting.
- Complement proteins (C1-C9) which are part of the innate immune system, and they are involved in non-specific immune defense and initiate the inflammatory process [21, 24].

1.2 Serum

Serum is the yellowish fluid that remains after blood clotting. It is similar to plasma but lacks fibrinogen and clotting factors.

2 Formed elements of blood

2.1 Erythrocytes (Red blood Cells)

Red blood cells are round, **anucleate**, biconcave cells that appear as biconcave discs, with a diameter of 7.5 µm and a thickness of 2 µm at their periphery. The average lifespan of a red blood cell is 120 days. They originate from the bone marrow (the myeloid lineage). Aged red blood cells are fragile and express oligosaccharides on their membrane surface that are recognized by splenic, hepatic, and bone marrow macrophages, which destroy them.

The carbohydrate determinants for blood groups A, B, and O are located on the external surface of the erythrocyte plasma membrane. Mature erythrocytes lack organelles but are filled with hemoglobin (Hb). Hemoglobin is a protein composed of four polypeptide chains, each covalently bound to a heme group. Erythrocytes also contain soluble enzymes responsible for glycolysis, the hexose monophosphate pathway, and the production of adenosine triphosphate (ATP) [21, 24].

Reticulocytes are the young forms of red blood cells (0.2-2% of RBCs). Reticulocytes are not visible with May-Grünwald Giemsa (MGG) staining; they require a special stain for visualization: brilliant cresyl blue. Nuclear debris is observed (Figure 2.29).

The red blood cell count is 4.6 to 6.2 × 10^{12}/L in men, 4.1 to 5.4 × 10^{12}/L in women, and 4.1 to 5.4 × 10^{12}/L in children [21, 24].

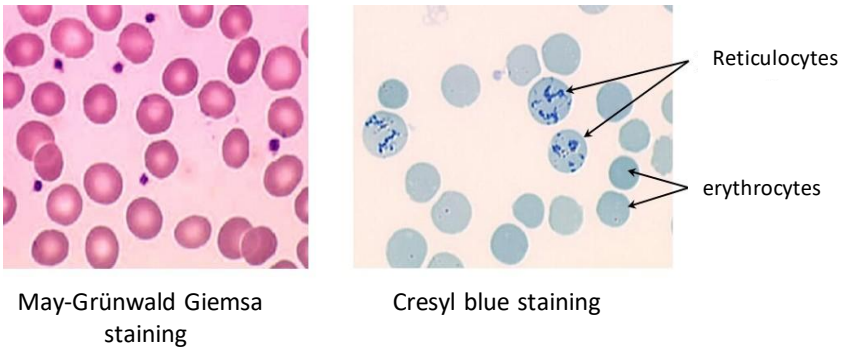

Figure 2.29. Difference between red blood cells and reticulocytes [31].

2.2 Leukocytes or white blood cells

Among leukocytes, we distinguish leukocytes with a generally homogeneous appearing cytoplasm (hyaline leukocytes) which belong to two populations: lymphocytes and monocytes; and leukocytes whose cytoplasm contains large granules, the granulocytes [21, 24].

2.2.1 Lymphocytes

Lymphocytes are present in the blood, lymph, and all lymphoid organs. In the blood, lymphocytes represent 20 to 40% of leukocytes. They are about 7 µm in diameter with a nucleus occupying almost the entire cell (Figure 2.30).

Two main types of lymphocytes coexist: T lymphocytes and B lymphocytes. They appear the same under light microscopy (Figure 2). To distinguish the different lymphocyte populations, characteristic CD membrane proteins are detected.

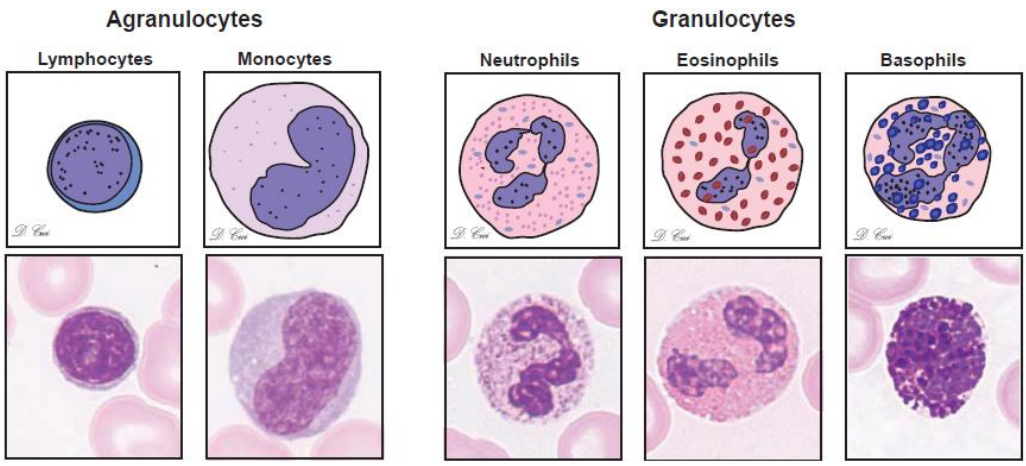

Figure 2.30. Peripheral blood cell types [32]

T and B lymphocytes get their names from the organ where they mature:
— the thymus for T lymphocytes.
the human equivalent of the avian Bursa of Fabricius for B lymphocytes, which is the bone marrow [21, 32].
Two aspects of blood lymphocytes: The small lymphocyte with a flattened or indented nucleus and scant cytoplasm, and the large lymphocyte with a quadrangular nucleus and more abundant cytoplasm.
Some B lymphocytes can transform into plasma cells. This occurs in lymphoid tissue (nodes) and there are no circulating plasma cells [21, 32].

2.2.2 Monocytes

Monocytes are large cells (15 to 30 µm) with a characteristic horseshoe-shaped nucleus (Figure 2). Their appearance is very polymorphic, particularly their indented but non-lobulated nucleus, often appearing shredded, with convoluted shapes. The plasma membrane has irregular contours. The cytoplasm contains numerous lysosomes. These cells are very mobile thanks to pseudopodia. Monocytes represent 3 to 10% of white blood cells. Their half-life in the blood is about ten hours; they then migrate into tissues, differentiating into two distinct cell types: macrophages and myeloid dendritic cells. Monocytes have a primarily phagocytic function and, more accessorily, an antigen-presenting cell function (Figure 2.30) [21, 32].

2.2.3 Macrophages

Macrophages arise from the differentiation of monocytes as they pass into the tissues. They are large cells (20 to 70 µm) with very irregular contours marked by numerous pseudopodia. Their number is estimated at two billion per kg of tissue on average. During their differentiation, macrophages acquire an abundant protein biosynthesis machinery with a well-developed rough endoplasmic reticulum and Golgi apparatus. Depending on their location in the body, macrophages are named differently:

Macrophages (tissues), histiocytes (loose connective tissues), alveolar macrophages (lungs), mesangial cells (kidneys), astrocytes (brain), Kupffer cells (liver), osteoclasts (bone) [21, 32].

2.2.4 Mast Cells

Mast cells are distributed throughout the body, particularly in mucosal tissues, where they are often found close to blood vessels and peripheral nerves. This strategic location gives them a high chance of encountering potential pathogens. There are 3,000 to 25,000 mast cells per mm^3 of tissue [21, 32].

2.2.5 Granulocytes

Cells of the granulocyte lineage have numerous granules, hence their name granulocytes. They have a multilobed or segmented nucleus, which is why they are also called polymorphonuclear leukocytes. The properties of the intracellular granules allow the distinction of three cell types: neutrophils, basophils, and eosinophils. Their names derive from the affinity of their cytoplasmic granules for certain basic dyes like methylene blue and azures or acidic dyes like eosin. May-Grünwald Giemsa staining uses this property to easily distinguish them (Figure 2.30) [21, 32].

a) Neutrophilic Granulocytes

Neutrophilic granulocytes, or neutrophils, are the most abundant white blood cells (50-70%), measuring 10-15 µm with a multi-lobed nucleus. They have a short blood half-life of under 24 hours, with many marginated on vessel walls. Their key functional feature is a arsenal of three distinct granule types: azurophilic (primary) granules that are large, lysosomal, and contain hydrolases and myeloperoxidase; specific (secondary) granules that are smaller and contain lysozyme and lactoferrin; and tertiary (storage) granules. These granules work in concert to destroy phagocytosed pathogens.

The main function of neutrophilic granulocytes is the defense of the organism against pathogens: bacteria and many viruses [21, 32].

b) Eosinophilic Granulocytes

Eosinophilic granulocytes, or eosinophils, are white blood cells (10-15 µm) characterized by a bilobed nucleus and a cytoplasm packed with distinctive granules. Their most notable feature is the large, oval-specific granules containing a crystalline core of cationic proteins, which are highly toxic to parasites. While they circulate briefly in the blood (6-8 hours), constituting 1-3% of leukocytes, they primarily reside in tissues, especially the submucosa, for 8-12 days. Their principal function is to defend the body against helminth parasites [21, 32].

c) Basophilic Granulocytes

Basophilic granulocytes, or basophils, are the rarest (<1%) and smallest (10-12 µm) white blood cells. They are characterized by a large, lobed nucleus and granules that stain metachromatically with basic dyes. These granules are rich in histamine, which is released during allergic reactions. Basophils are functionally closely related to mast cells, sharing the high-affinity IgE receptor. Their primary role is in defense against parasites, but they are notoriously difficult to study due to a short blood half-life and a lack of unique surface markers [21, 32].

2.3 Blood Platelets (or Thrombocytes)

Platelets, or thrombocytes, are small (2-5 µm), anucleate cell fragments present in blood at 150,000-400,000 per mm^3. Their cytoplasm is organized into a peripheral hyalomere, containing a cytoskeleton of microfilaments and microtubules, and a central granulomere, which stores granules. These granules include alpha granules with lytic enzymes and dense granules containing serotonin and calcium. Their primary function is in hemostasis, where they adhere to exposed collagen at injury sites via pseudopodia, initiating clot formation [21, 32].

3 Hematopoiesis

It is a continuous, essential physiological process where new blood cells are produced from hematopoietic stem cells in the bone marrow to replace old cells and meet the body's ongoing needs for red blood cells, white blood cells, and platelets.

The cells that reside inside the bone cavities are called stromal cells. They are represented by different cell types (specialized fibroblasts, endothelial cells, osteoblasts, and perhaps even adipocytes). Maturing hematopoietic cells (HSCs) receive signals through adhesion molecules expressed by stromal cells.

The HSC differentiates into two progenitor cells which lose their self-renewal capacity (Figure 2.31). They give rise to the major blood cell lineages:

The Common Lymphoid Progenitor (CLP) which generates the lymphoid lineage. CLPs differentiate into B lymphocyte progenitors (pro-B), T lymphocyte progenitors (pro-T), and NK cell progenitors (pro-NK);

The Common Myeloid Progenitor (CMP) which generates the myeloid lineage of leukocytes but also the erythrocytic and megakaryocytic lineages. CMPs differentiate into granulocyte/monocyte progenitors (GM-P), mast cell progenitors (MC-P), eosinophil progenitors (Eo-P), basophil progenitors (Ba-P), and megakaryocyte/erythrocyte progenitors (MEP). These progenitors are also called CFU (e.g., CFU-Eo) [21, 32].

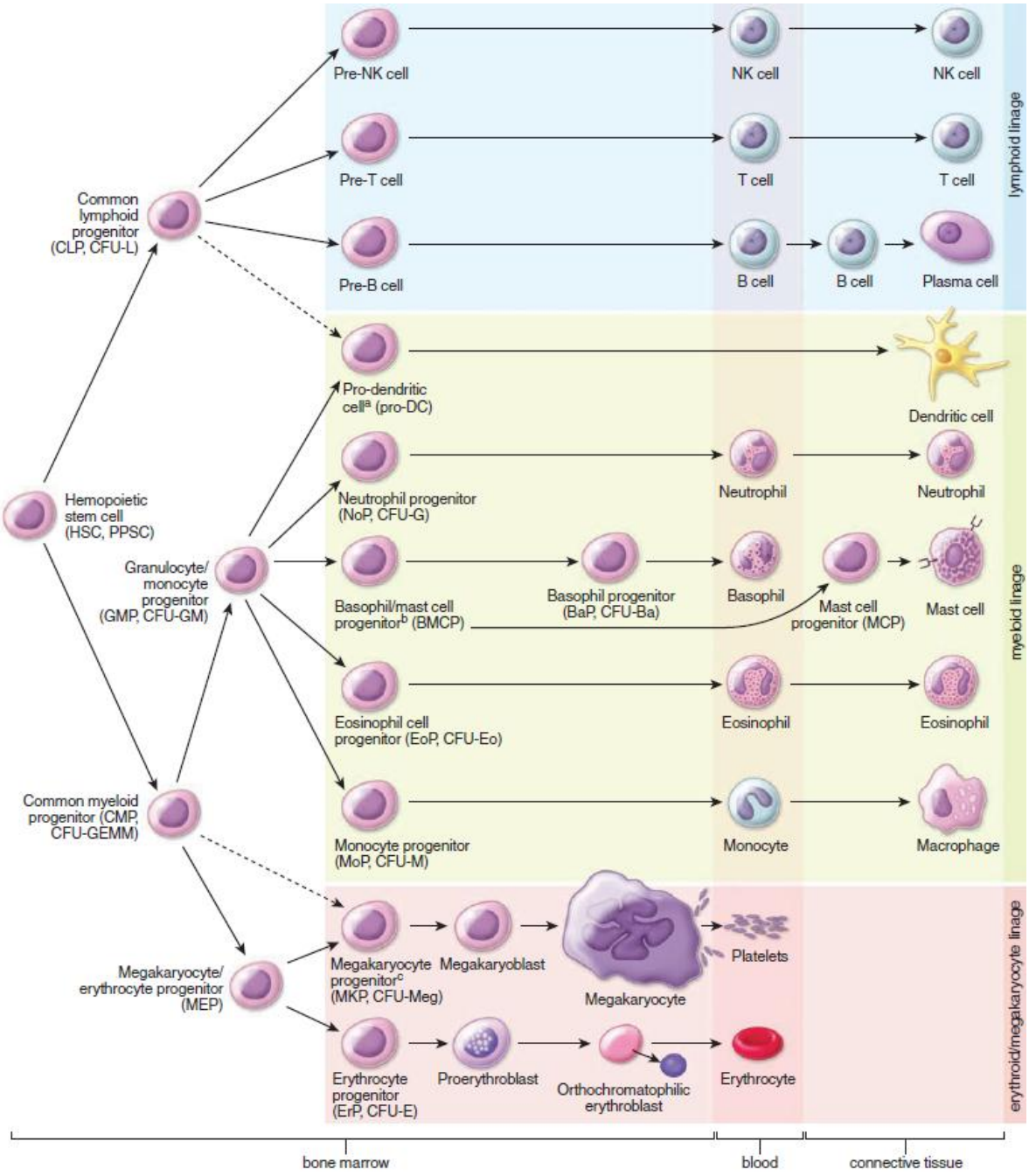

Figure 2.31. Differentiation of pluripotent stem cells during hematopoiesis [21].

4 Bone Marrow

Bone marrow, a gelatinous, vascular connective tissue located in the medullary cavity, is richly supplied with cells responsible for hematopoiesis. Bone marrow is located in the cavities of spongy bone and in the medullary cavity of diaphyses. A distinction is made between red hematopoietic marrow, which is a hematopoietic tissue, and yellow marrow, which is infiltrated with adipose lobules. Gray marrow is marrow where the adipose tissue is in turn replaced by fibrous tissue [21].

Chapter 6: Muscle tissue

Muscle is classified according to the appearance of the contractile cells. Two principal types of muscle are recognized: **striated muscle**, in which the cells exhibit cross-striations at the light microscope level, **smooth muscle**, in which the cells do not exhibit cross-striations.

Striated muscle tissue is further subclassified on the basis of its location: **Skeletal muscle** is attached to bone and is responsible for movement of the axial and appendicular skeleton and for maintenance of body position and posture. **Cardiac muscle** is a type of striated muscle found in the wall of the heart and in the base of the large veins that empty into the heart [21, 32].

1 Skeletal muscle

1.1 Striated muscle structure

Skeletal muscle is composed of long, cylindrical, multinucleated cells ranging in length from 20 to 130 µm, which undergo voluntary contraction to facilitate movement of the body or its parts.

Striated muscle is surrounded by **epimysium**, a dense irregular connective tissue rich in collagen surrounding the entire muscle. The **perimysium**, a dense connective tissue less rich in collagen that derives from the epimysium, surrounds the bundles (fascicles) of muscle fibers. The **endomysium**, composed of reticular fibers and an external lamina (basal lamina), surrounds each individual muscle cell (Figure 2.32) [21, 32].

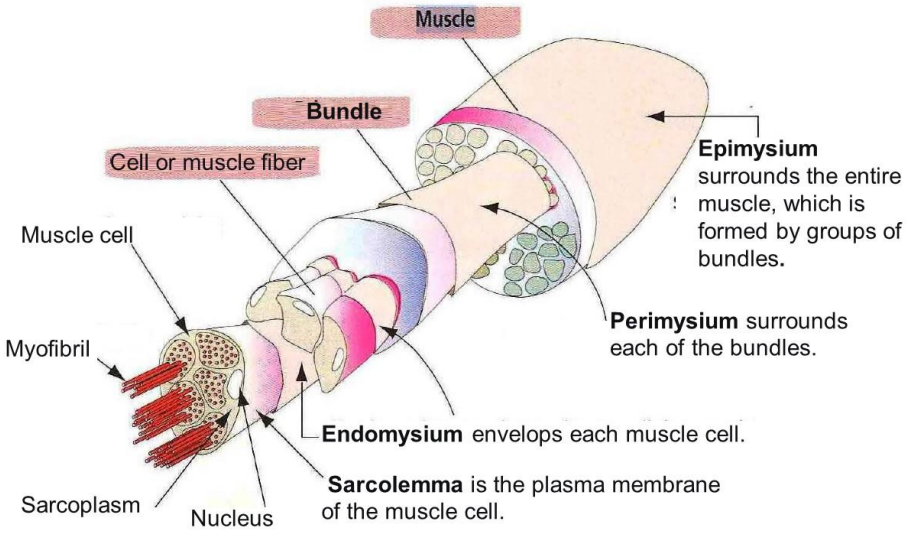

Figure 2.32. Structure of skeletal muscle [24].

Skeletal muscle fibers are **multinucleated** cells; the nuclei have a peripheral position and are located just beneath the cell membrane. The plasma membrane surrounds the cell and is lined by a **basal lamina**: together they form the **sarcolemma** (Figure 2.33).

Small **satellite cells**, which have a single nucleus and act as regenerative cells, are located in shallow depressions on the surface of the muscle cell, sharing the external lamina of the muscle fiber. The muscle cells of skeletal striated muscles are also called **rhabdomyocytes**.

The **T-system** (or transverse tubules) is a network of transverse canaliculi that are tubular invaginations of the plasma membrane surrounding the myofibrils at the **A-band-I-band junctions**. These tubules branch and anastomose but generally remain in a single plane. Each sarcomere has a set of T-tubules at each A-band and I-band interface. Thus, T-tubules extend deep inside the fiber and facilitate the conduction of depolarization waves along the sarcolemma [21, 32].

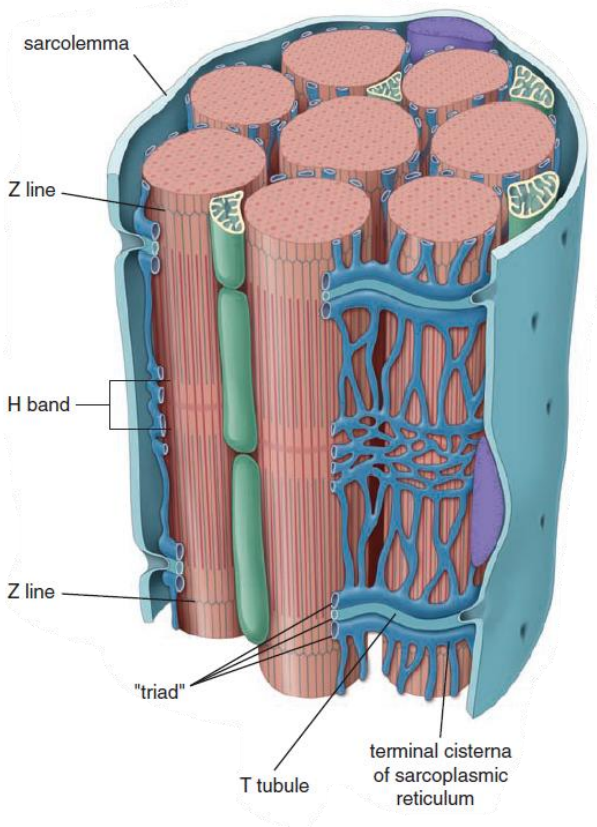

Figure 2.33. Structure of the organization of striated muscle fiber [21].

The sarcoplasmic reticulum (stores Ca^{++}) consists of a network of anastomosing longitudinal canaliculi and saccules, which come into close contact with the A and I bands as well as the **T-tubules** (forming a **triad**). The sarcoplasmic reticulum forms a network around each myofibril and exhibits dilated terminal cisternae at each **A-I junction**. Striated muscle fibers possess numerous elongated **mitochondria**, aligned between the myofibrilles. Striated muscle fibers are rich in **glycogen granules** and **lipofuscin** dispersed throughout the sarcoplasm.

The cytoplasm (**sarcoplasm**) of the muscle cell contains longitudinal networks of cylindrical myofibrils, each 1 to 2 μm in diameter. They extend the entire length of the cell and are precisely aligned with neighboring myofibrils. This parallel arrangement of myofibrils is responsible for the transverse striations of light and dark bands characteristic of skeletal muscles observed in longitudinal section.

Myofibrils are heterogeneous; they are composed of a regular succession of dark bands or discs, **A-discs** (anisotropic under polarized light), and light bands or discs, **I-bands** (isotropic under

polarized light). The center of each A-band is occupied by a pale zone, the **H-band**, which is divided by the **M-line**. Each I-band is bisected by a thin dark line, the **Z-disc** (Z-line) (Figure 2.34). The region of the myofibril between two successive Z-discs, known as the sarcomere, has a length of 2.5 μm and is considered the contractile unit of skeletal muscle fibers.

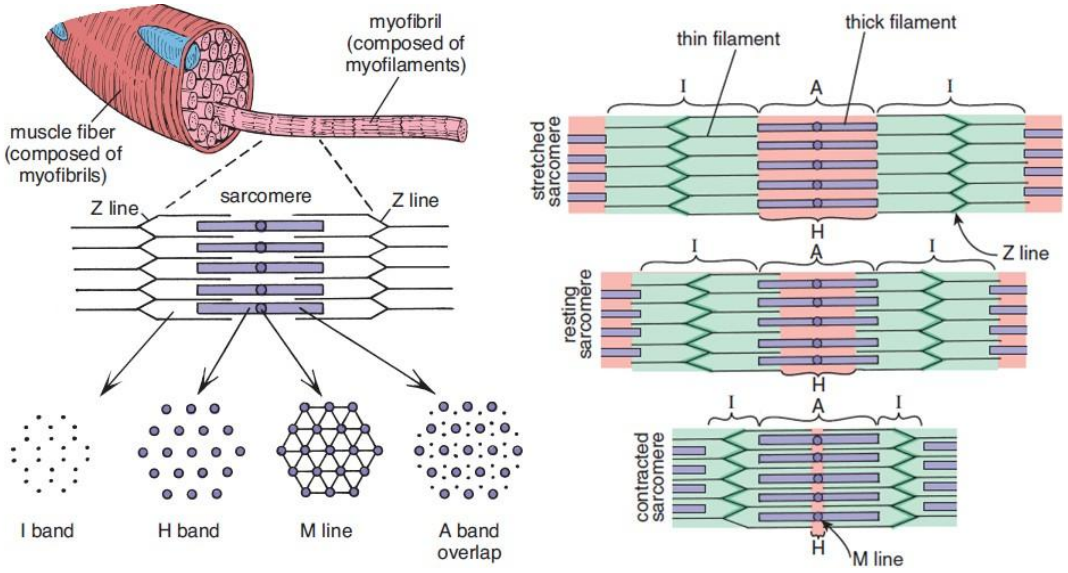

Figure 2.34. Organization of a skeletal muscle and sarcomeres in different functional stages [21].

1.2 The thin myofilament (Actin)

Thin myofilaments result from the helical association of two F-actin molecules (Figure 2.35). A molecule of **tropomyosin** lies in the groove of this helix, while at each point where the two F-actin molecules cross, a molecule of **troponin** is attached. Troponin is composed of three subunits: troponin C (TnC), troponin I (TnI), and troponin T (TnT) [21, 32].

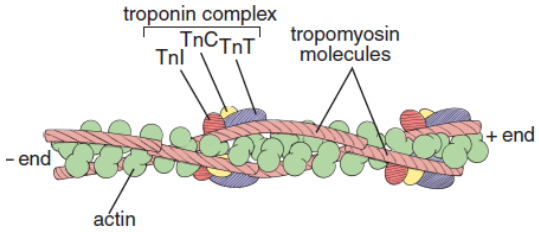

Figure 2.35. Structure of thin myofilament [21].

1.3 The thick myofilament

The thick myofilament is composed of myosin. It consists of two heavy chains and four light chains. It is formed by a straight, rod-like portion (L-meromyosin) and a globular head (H-meromyosin) at one of its ends (Figure 2.36). The straight portion results from the association of the two heavy chains, which are coiled into a helix and provided with binding sites for neighboring myosin molecules. The head is double: it is formed from the two separated ends (N-terminal) of the heavy chains [21, 32].

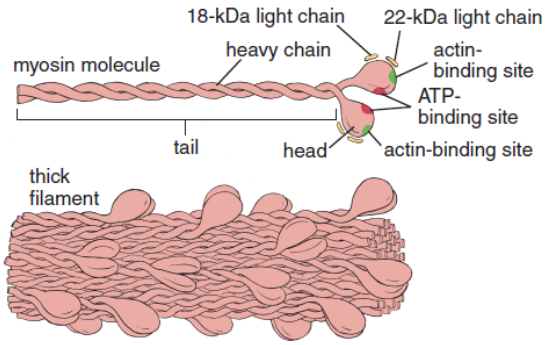

Figure 2.36. Structure of the thick myofilament [21].

1.4 Other proteins associated with myofilaments

Titin is a long, fibrous protein that connects the end of the thick filaments to the Z-discs; it has elastic properties that allow the sarcomere to return to its original length after contraction. **Nebulin**, which lacks elastic properties, is a protein that extends along the thin filaments and helps maintain their structure (Figure 2.37). Finally, the myofibrilles are laterally connected by a network of **desmin** intermediate filaments (see slide 2.31). The Z-disc, a filamentous structure, anchors the thin filaments via **α-actinin** [21, 32].

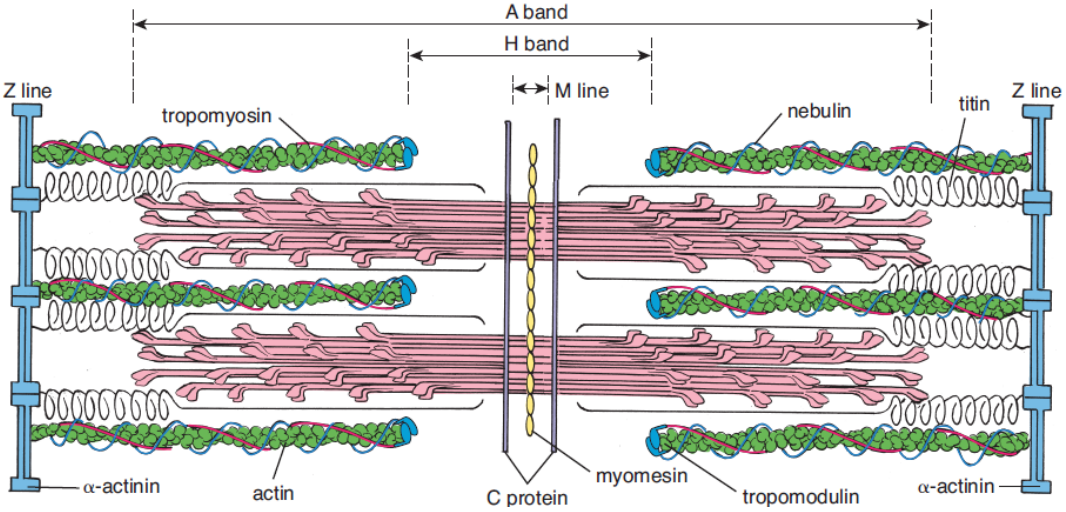

Figure 2.37. Molecular structure of a sarcomere [21].

1.5 Contraction of striated muscle

The contraction of muscle fibers results in shortening, which can reach up to 60% of their initial length. This process is summarized in the following sequence of events (Figure 2.38):

— The depolarization of the plasma membrane propagates along the T-tubule system and is then transmitted to the sarcoplasmic reticulum.
— The depolarization of the sarcoplasmic reticulum membrane triggers the release of Ca^{++} ions into the cytosol through transmembrane Ca^{++} channels (at the A-I junctions).

— At rest, the myosin-binding sites on the actin filaments are partially covered by tropomyosin. Furthermore, troponin I (TnI) is bound to actin and hinders myosin-actin interaction.
— The binding of Ca^{++} to troponin C causes a slight displacement of the tropomyosin molecule, which breaks the TnI-actin bond. This uncovers the myosin-binding sites (active state), leading to actin-myosin contact.
— The actin-myosin contact triggers the activation of myosin's (actin-dependent) ATPase, which catalyzes the hydrolysis of ATP into ADP and Pi. The products of this reaction remain attached to the myosin head, leading to the binding of actin to myosin and a conformational change in the myosin head. The orientation of the myosin head relative to the actin filament is at an angle of approximately 90°.
— The release of the phosphate from the myosin head is associated with the release of energy, causing a stronger binding of myosin to actin and a 45° rotation of the myosin head. This power stroke results in a displacement of about 10 nanometers. The subsequent release of ADP leaves the myosin head still anchored to actin.
— Another ATP molecule binds, reducing the affinity of the myosin head for the actin filament, causing it to detach. A new cycle can then begin.

Relaxation occurs when the concentration of Ca^{++} in the cytosol is sufficiently reduced. Consequently, tropomyosin returns to its resting position, covering the actin-binding sites and restoring the resting state [21, 32].

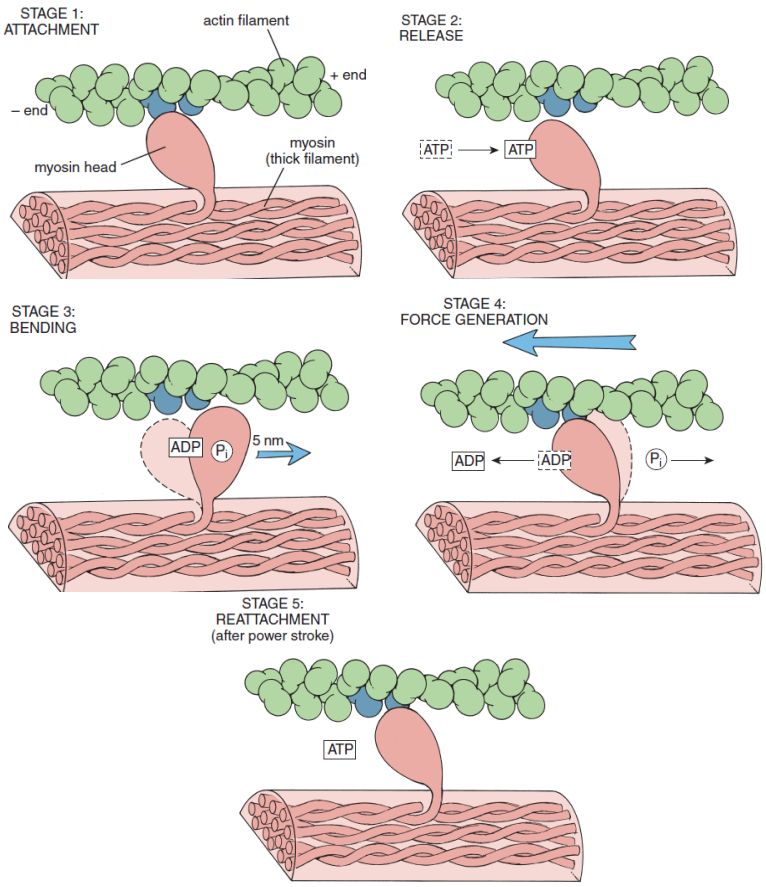

Figure 2.38. Contraction cycle in skeletal muscle cells [21].

1.6 The neuromuscular junction

The neuromuscular junction, or motor end plate, is the specialized synapse that provides the exclusive innervation for each skeletal muscle fiber. It is composed of three distinct regions (Figure 2.39):

— The **presynaptic region**, located at the axon terminal, contains synaptic vesicles filled with neurotransmitter (acetylcholine).
— The **synaptic cleft**, the space between the two membranes, is rich in acetylcholinesterase, the enzyme that degrades the neurotransmitter.
— The **postsynaptic region**, which is the plasma membrane of the muscle cell (rhabdomyocyte), is equipped with specific acetylcholine receptors to receive the chemical signal [21, 32].

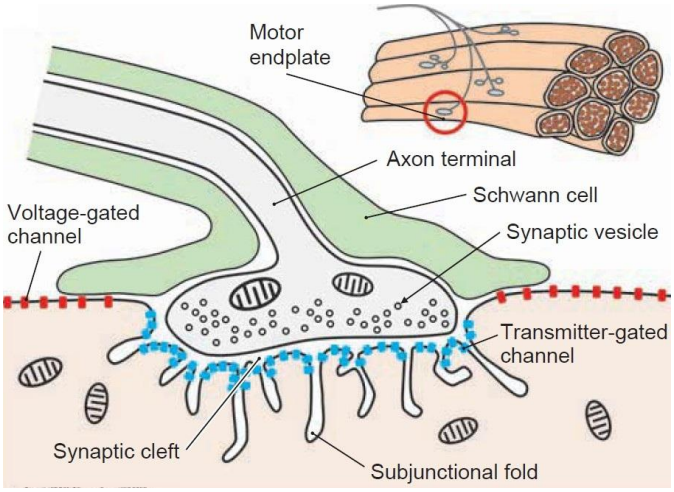

Figure 2.39. Structure of neuromuscular junction [32].

2 Cardiac striated muscle tissue (Myocardium)

2.1 Characteristics of cardiac muscle cells

Cardiac muscle cells, or cardiomyocytes, are distinguished from skeletal muscle cells by their cylindrical shape and smaller size (approximately 80 to 130 µm in length) (Figure 2.40). One of their major characteristics is the presence of bifurcated ends, which allow them to interconnect closely with adjacent cells to form a continuous muscular network via specialized junctions called intercalated discs (Eberth's lines).

— Cardiomyocytes contract in a **rhythmic** and spontaneous manner, an activity modulated by the autonomic nervous system and hormones.
— They typically contain a single central nucleus and are rich in organelles essential for their incessant activity.
— Numerous mitochondria, glycogen granules, and myoglobin are present.
— Their myofibrils are organized around the nucleus, leaving a central area containing organelles.
— Unlike skeletal muscle, cardiac muscle has a very limited capacity for regeneration; damage is generally repaired through the formation of fibrous scar tissue [21, 32].

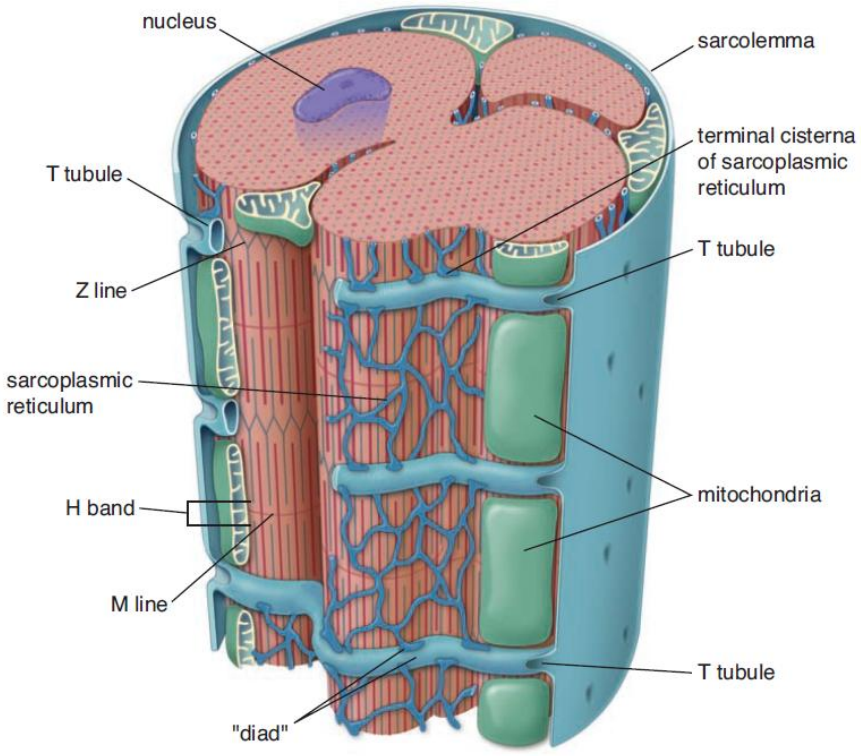

Figure 2.40. Structure of cardiac muscle fiber [21].

2.2 Structural components of cardiac muscle cells

They differ from those of skeletal muscle in the following ways:

T-tubules are larger than those in skeletal muscle and are lined by an external lamina. The sarcolemma invaginates to form T-tubules at the level of the Z-discs (rather than at the A-I junctions as in skeletal striated muscle). These T-tubules can be interconnected by longitudinal tubules that extend along the length of several sarcomeres.

— During relaxation, Ca^{++} slowly leaks into the sarcoplasm, which contributes to the automatic rhythm. Ca^{++} also enters cardiac muscle cells from the extracellular environment via voltage-dependent Ca^{++} channels in the T-tubules and sarcolemma.
— The endoplasmic reticulum, less abundant than in skeletal striated muscle fibers, consists of longitudinal tubules that are interrupted on either side of the Z-discs and interconnected by transverse tubules.
— The mitochondria, generally small in size and very numerous, are located between the myofibrilles.
— The end of the cardiac cell is not straight but forms a stepped pattern. The myofibrilles inside the cell attach to it via their Z-discs, which anchor to the cell membrane (sarcolemma) to form a structure called a dense plaque.
— Nexus-like differentiations, zonula adhaerens, and desmosomes are distributed; the former are found on the lateral (horizontal) parts of the cell end, while the others are located on its transverse part (Figure 2.41) [21, 32].

3 Smooth muscle tissue

Smooth muscle cells are spindle-shaped, non-striated cells of variable size depending on their location (Figure 2.42). Their central nucleus is surrounded by cytoplasmic organelles involved in the synthesis of extracellular matrix components. Enclosed by a basal lamina, these cells organize into layers, bundles, or helices depending on the tissue.

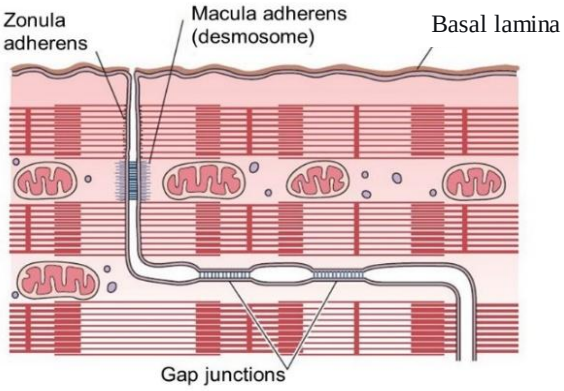

Figure 2.41. Structure of the cardiac cell junctions [21]

There are two types of smooth muscle: **Unitary** (or **single-unit**) **visceral smooth muscle** consists of cells interconnected by gap junctions, enabling coordinated, automatic contractions, as seen in the intestine. **Multi-unit smooth muscle**, in contrast, comprises independent cells, each individually innervated, allowing for more precise and localized control, such as in the iris of the eye or the walls of large arteries (Figure 2.42) [21, 32].

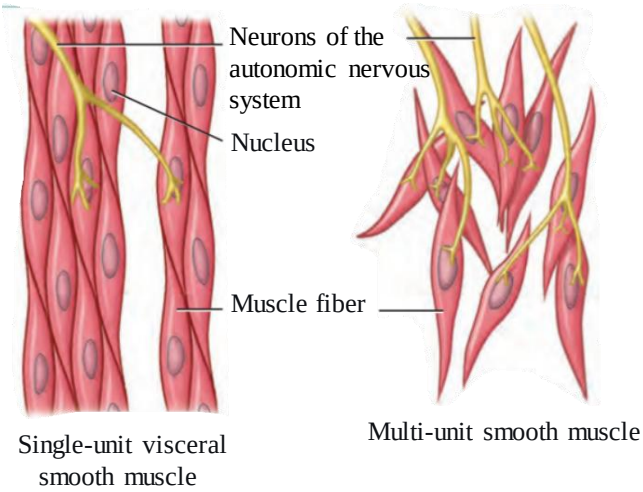

Figure 2.42. Structure of unitary visceral smooth muscle (A) and multi-unit smooth muscle (B) [30].

3.1 Myofilaments in smooth muscle

The actin and myosin filaments are not organized into myofibrils. They attach to peripheral (sarcolemmal) and cytoplasmic (sarcoplasmic) dense bodies and are aligned obliquely relative to the longitudinal axis of the smooth muscle cell [21, 32].

3.2 Thin myofilaments

Thin myofilaments are actin filaments similar to those in striated muscle fibers. They insert into dense bodies composed of **α-actinin**, **vinculin**, and **talin**, which line the deep surface of the sarcolemma [21, 32].

3.3 Thick myofilaments

Thick myofilaments, consisting of myosin filaments, are unstable; it is believed that most of them form only when the fiber is stimulated. Unlike in striated muscle, the myosin heads all point in the same direction [21, 32].

3.4 Cytoskeleton, sarcolemma, and sarcoplasmic reticulum

The smooth muscle cell possesses a specialized internal structure characterized by a cytoskeleton of intermediate filaments (**desmin** or **vimentin**) anchored to dense bodies located in the sarcoplasm or on the cell membrane. The sarcolemma exhibits numerous invaginations (caveolae) and pinocytotic vesicles. Communication and cohesion between cells are ensured by different types of junctions: Gap junctions for the propagation of excitation, as well as desmosomes and tight junctions [21, 32].

3.5 Smooth muscle contraction

Smooth muscle contraction is characterized by its slowness and prolonged duration, resulting from a lower rate of ATP hydrolysis compared to skeletal muscle (Figure 2.43). Its initiation depends on a transient increase in cytosolic Ca^{++}, which activates a specific molecular mechanism:

- Calcium binds to **calmodulin**, forming a complex that activates a key enzyme, myosin light-chain kinase.
- This enzyme phosphorylates the myosin light chains, thereby enabling interaction with actin.
- Simultaneously, calcium lifts the inhibition exerted by two regulatory proteins: it neutralizes the blocking effect of caldesmon (associated with tropomyosin) on the active sites of actin and promotes the inactivation of calponin, another inhibitory protein.
- Once phosphorylated, myosin engages in a continuous cycle of binding to and detaching from actin, fueled by ATP hydrolysis, until it is dephosphorylated by a phosphatase, which induces relaxation.
- The initiating stimuli vary depending on the type of smooth muscle: neural (vascular), mechanical via stretching (visceral), hormonal (oxytocin in the uterus or adrenaline elsewhere), reflecting a fine adaptation to diverse physiological functions [21, 32].

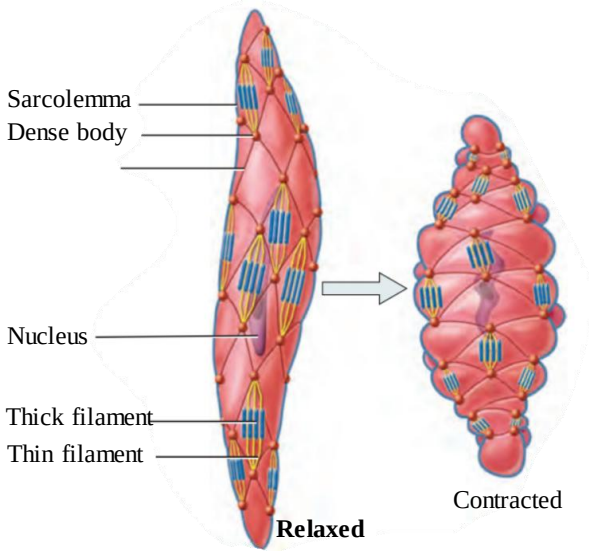

Figure 2.43. Microscopic anatomy of a relaxed and contracted smooth muscle fiber [30].

3.6 Innervation

Smooth muscle innervation is provided by **sympathetic** (noradrenergic) and **parasympathetic** (cholinergic) nerves of the autonomic nervous system.

3.7 Varieties of non-muscle contractile cells

Among non-muscle contractile cells are:

— **Myoepithelial** cells are specialized cells, of both muscle and epithelial type, found in exocrine glands. Their primary function is to contract in response to neural signals, causing the expulsion of secretory products from the glands.

— **Myofibroblasts** are key cells in tissue repair and fibrosis, derived from fibroblasts and characterized by their ability to contract thanks to α-smooth muscle actin (α-SMA) and to produce extracellular matrix [21, 32].

Chapter 7. Nerve tissue

Anatomically, the nervous system is divided into the following:

— The **central nervous system** (CNS) consists of the **brain** and the **spinal cord**, which are located in the cranial cavity and spinal canal, respectively.

— The **peripheral nervous system** (PNS) consists of **cranial**, **spinal**, and **peripheral nerves** that conduct impulses from (efferent or motor nerves) and to (the afferent or sensory nerves of) the CNS, collections of nerve cell bodies endings (both motor and sensory).

Functionally, the nervous system is divided into the following:

— The **somatic nervous system** (SNS) consists of somatic (*Gr. soma, body*) parts of the CNS and PNS. The SNS controls functions that are under conscious voluntary control with the exception of reflex arcs.

— The **autonomic nervous system (ANS)** consists of autonomic parts of the CNS and PNS. The ANS provides efferent involuntary motor innervation to smooth muscle, the conducting system of the heart, and glands. It also provides afferent sensory innervation from the viscera (pain and autonomic reflexes).

1 Neuron classification

Functionally, they can be grouped into three general categories (Figure 2.44):

Sensory neurons convey impulses from receptors to the CNS.

Motor neurons convey impulses from the CNS or ganglia to effector cells.

Interneurons, also called intercalated neurons, form a communicating and integrating network between the sensory and motor neurons [21, 25].

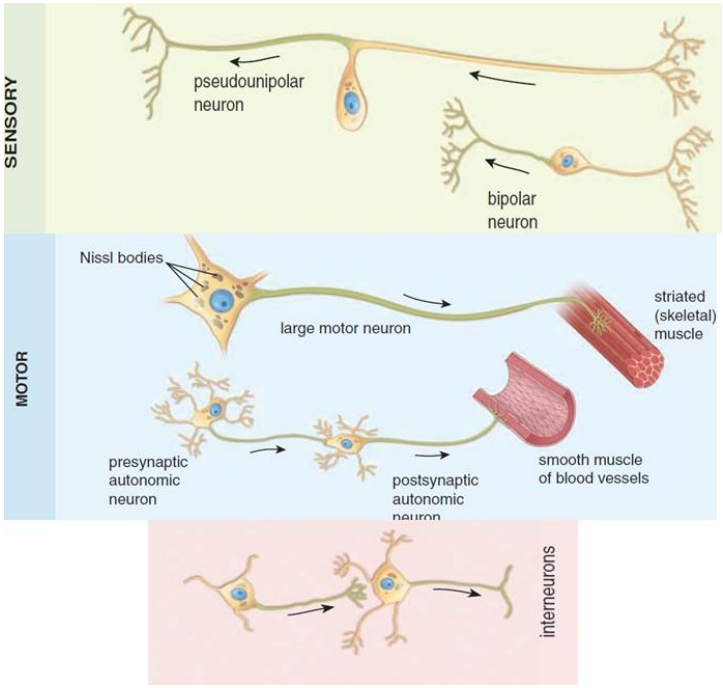

Figure 2.44. Neuron categories.

Anatomically, most neurons can be characterized as the following:

— **Multipolar neurons** have one axon and two or more dendrites (Motor neurons and interneurons constitute most of the multipolar neurons in the nervous system) (Figure 2.45 C-H).

— **Bipolar neurons** have one axon and one dendrite. Bipolar neurons are rare. They are most often associated with the receptors for the special senses (taste, smell, hearing, sight, and equilibrium) (Figure 2.45 A).

— **Pseudounipolar** (unipolar) neurons have one process, the axon that divides close to the cell body into two long axonal branches (Figure 2.45 B) [21, 25].

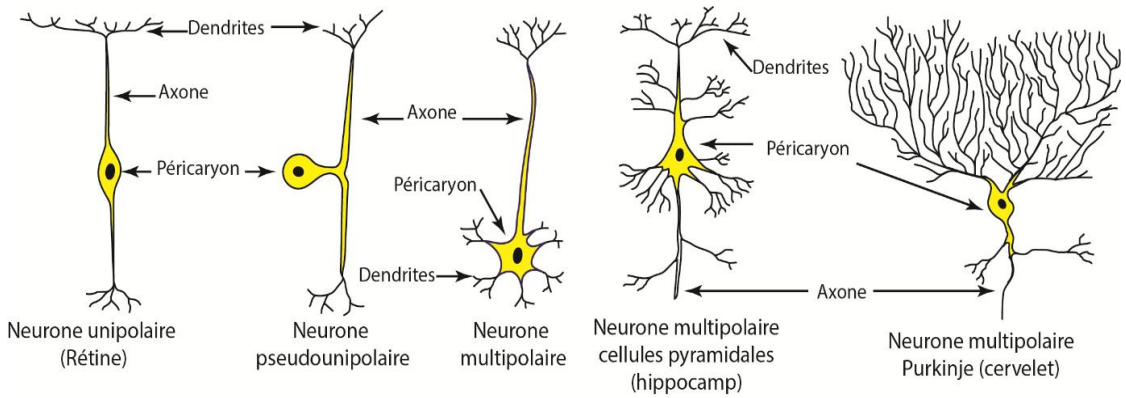

Figure 2.45. Anatomic classification of neurons [24].

2 Composition of nerve tissue

The **neuron** or **nerve cell** is the functional unit of the nervous system. It consists of a cell body, containing the nucleus, and several processes of varying length. Nerve cells are specialized to receive stimuli from other cells and to conduct electrical impulses to other parts of the system via their processes. Several neurons are typically involved in sending impulses from one part of the system to another.

Supporting cells are nonconducting cells that are located close to the neurons. They are referred to as **neuroglial** cells or simply **glia**. The CNS contains four types of glial cells: **oligodendrocytes**, **astrocytes**, **microglia**, and **ependymal cells** [21, 25].

2.1 The neuron

The functional components of a neuron include the **cell body**, **axon**, **dendrites**, and **synaptic junctions**. The cell body (perikaryon) of a neuron contains the nucleus and those organelles that maintain the cell. Most neurons have only one axon, usually the longest process extending from the cell body to a specialized terminal (synapse). A neuron usually has many more dendrites, shorter processes that transmit impulses from the periphery toward the cell body (Figure 2.46) [21, 25].

2.1.1 Cell Body

The cell body (**soma** or **perikaryon**) is the part of a neuron that contains the **nucleus** and other organelles, forming a biosynthetic hub for protein synthesis and general cell maintenance. Its structure includes a large, central nucleus, cytoplasm with organelles like the rough endoplasmic reticulum (**Nissl bodies**), Golgi apparatus, mitochondria, and lysosomes, as well as cytoskeletal elements (**neurofilaments** and **microtubules**). Dendrites and the axon also emerge from the cell body, facilitating information reception and transmission, respectively. The **mitochondria** are found throughout the cell body, dendrites, and axons [21, 25].

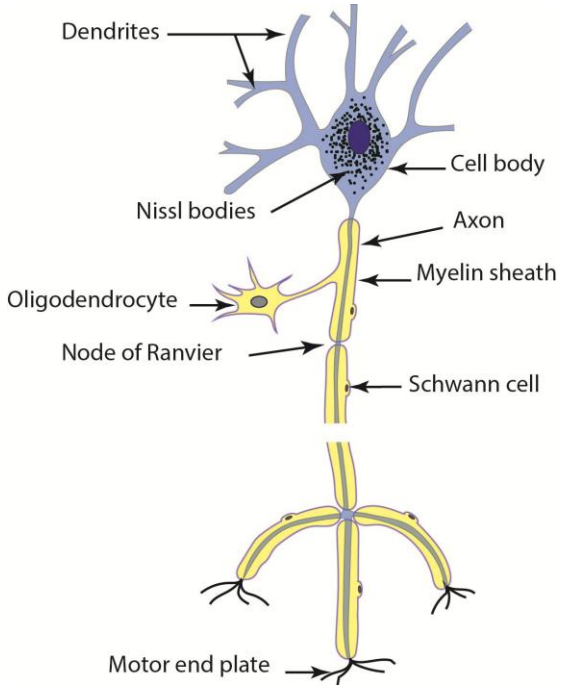

Figure 2.46. Structure of neuron [21].

2.1.2 Dendrites and axons

Dendrites possess the same constituents as the perikaryon from which they extend; they are generally short and most often highly branched. Axons, which are devoid of Nissl bodies, are of variable length and rich in neurofilaments and neurotubules, they constitute the nerve fibers.

Nerve fibers are surrounded by a sheath of neuroglial cells, **Schwann cells** for peripheral fibers, and oligodendroglia for central fibers. This neuroglial sheath may or may not form a **myelin sheath** around the nerve fiber, which then becomes either a **myelinated** fiber or an **unmyelinated fiber** [21, 25].

2.1.3 Synapses

Synapses are specialized junctions between neurons that facilitate the transmission of impulses from one neuron (presynaptic) to another neuron (postsynaptic). Synapses between neurons may be classified morphologically as the following:

— **Axodendritic:** These synapses occur between axons and dendrites. In the CNS, some axodendritic synapses possess dendritic spines, a dynamic projection containing actin fi laments. Their function is associated with long-term memory and learning.
— **Axosomatic:** These synapses occur between axons and the cell body.
— **Axoaxonic:** These synapses occur between axons and axons (Figure 2.47) [21, 25].

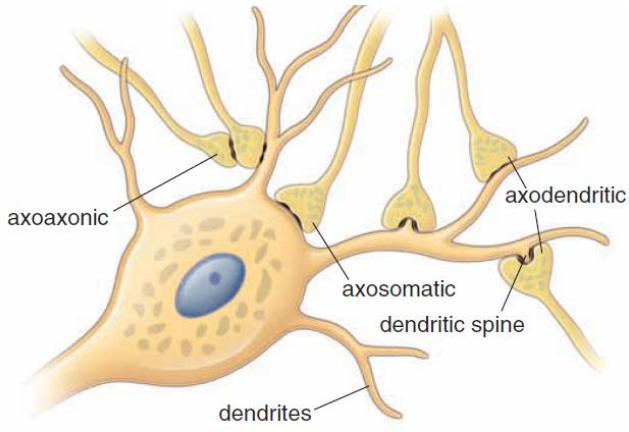

Figure 2.47. Morphologic classification of synapses [21].

Synapses are classified as chemical or electrical:

— **Chemical synapses**: Conduction of impulses is achieved by the release of chemical substances (neuro transmitters) from the presynaptic neuron. **Neurotransmitters**: **Catecholamines** such as **norepinephrine** (NE), **epinephrine** (EPI, adrenaline), and **dopamine** (DA).(Figure 2.48)
— **Electrical synapses**: these synapses contain gap junctions that permit movement of ions between cells and consequently permit the direct spread of electrical current from one cell to another.

A typical chemical synapse contains a **presynaptic element**, **synaptic cleft**, and **postsynaptic membrane** [21, 25].

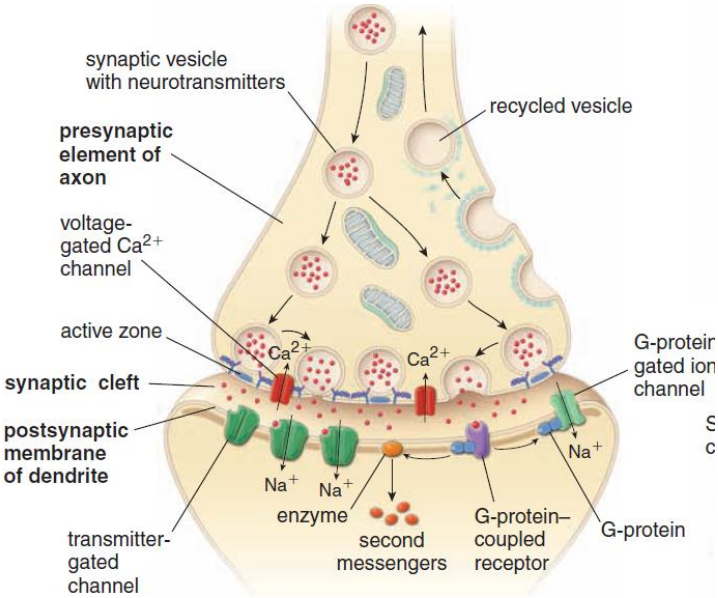

Figure 2.48. Structure of chemical synapse [21].

2.2 Supporting cells, the neuroglia

There are five types of central neuroglia:

2.2.1 Astrocytes

Astrocytes are star-shaped glial cells whose function is to provide necessary nutrients and regulate the chemical environment. They possess cytoplasmic extensions that end in expansions called **astrocytic endfeet** playing a crucial role in regulating the **blood-brain barrier** (BBB), regulate blood flow, and ensure the nutrient supply and energy metabolism of the nervous system.

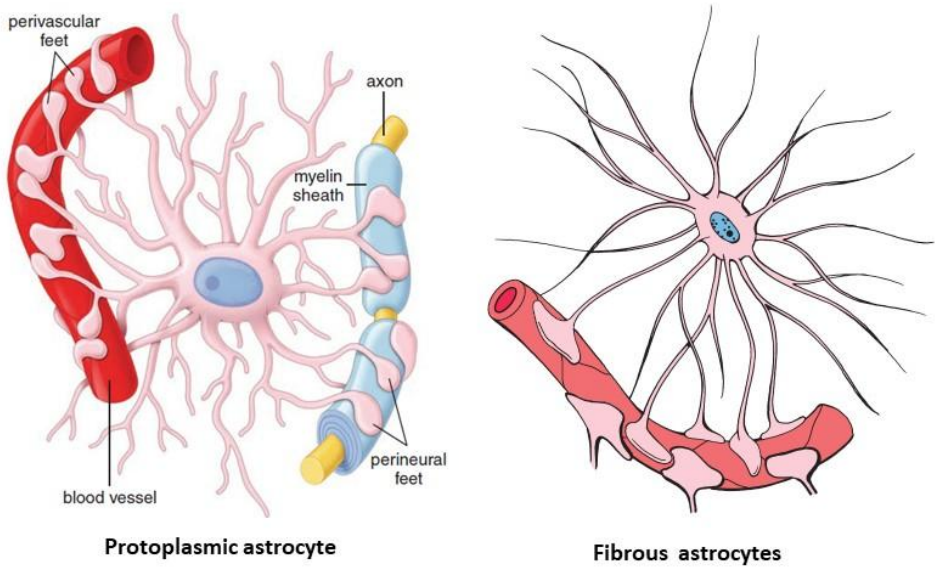

Figure 2.49. Astrocytes types [21].

Astrocytes, present in the CNS, are divided into two categories : (1) **fibrous astrocytes** and (2) protoplasmic astrocytes. Fibrous astrocytes predominate in the white matter and possess long, thin, sparsely branched extensions. **Protoplasmic astrocytes** reside primarily in the gray matter and have short extensions with numerous short branches. The ends of the astrocytic extensions are called astrocytic end-feet. (Figure 2.49) [21, 25].

2.2.2 Oligodendrocytes

Oligodendrocytes are glial cells in the CNS that form the insulating **myelin sheath** around neuronal axons, which facilitates the rapid transmission of electrical signals. Derived from progenitor cells, these cells wrap axons with a lipid-rich structure that appears as white matter in the brain. Each oligodendrocyte can myelinate multiple axons, (Figure 2.50) [21, 25].

2.2.3 Schwann cells

Schwann cells are glial cells of the PNS that form the myelin sheath around axons, which speeds up nerve impulse conduction. They also support non-myelinated axons [21, 25].

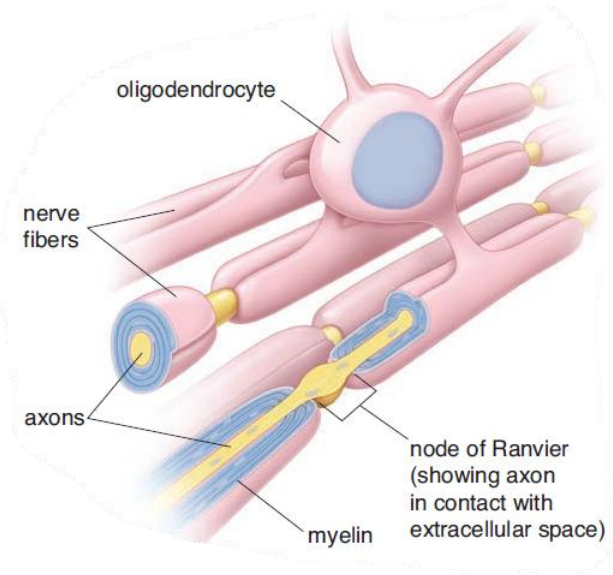

Figure 2.50. Structure of oligodendrocytes [21].

2.2.4 Microglia

They are inconspicuous mobile cells with small, dark, elongated nuclei that possess phagocytotic properties. Microglia possess phagocytotic properties (Figure 2.51) [21, 25].

2.2.5 Ependymal cells

They are columnar cells that line the ventricles of the brain and the central canal of the spinal cord. Ependymal cells form the epithelial-like lining of the ventricles of the brain and spinal canal (Figure 2.51) [21, 25].

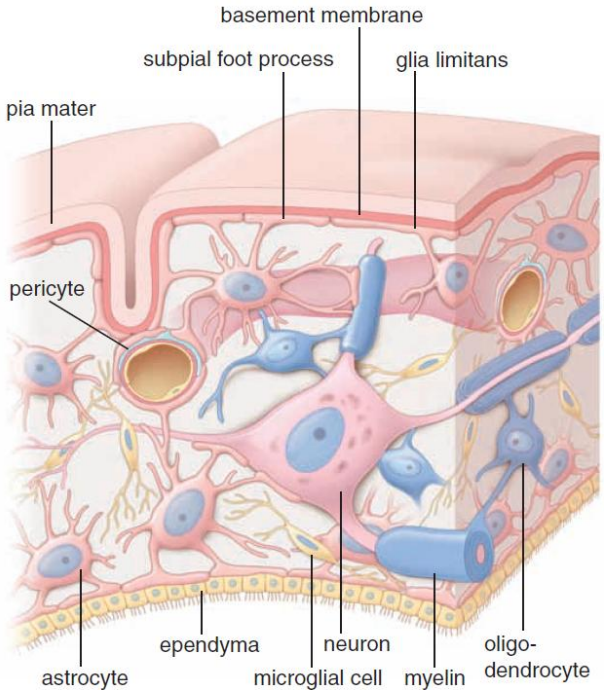

Figure 2.51. The neuroglia of central nervous system [21].

References

1. Larsen, W., et al., *Embryologie humaine*2017: De Boeck supérieur.
2. Moigne, A.L., *Biologie du développement - 7e édition: Cours et questions de révision*2009: Dunod.
3. Tachdjian, G., et al., *Embryologie et histologie humaines*2016: Elsevier Health Sciences France.
4. Gilbert, S.F. and U.S. Gilbert, *Developmental Biology (Loose Leaf)*2010: Oxford University Press, Incorporated.
5. Sadler, T.W., *Langman's Medical Embryology With CD*2010: CBS Publishers & Distributors.
6. Carlson, B.M., *Human Embryology and Developmental Biology*2018: Elsevier.
7. Moigne, A.L. and J. Foucrier, *Biologie du développement - 7e éd.: Cours et questions de révision*2020: Dunod.
8. Müller, W.A., *Developmental Biology*2012: Springer New York.
9. Cochard, L.R. and A.N. Dueñas, *Netter's Atlas of Human Embryology - E-BOOK: Netter's Atlas of Human Embryology - E-BOOK*2023: Elsevier.
10. Fusco, G. and A. Minelli, *The Biology of Reproduction*2019: Cambridge University Press.
11. SUBRAMANIAN, M.A., *Developmental Biology*2019: MJP Publisher.
12. Manglik, R., E.G.P. Experts, and E.G. Community, *Developmental Biology and Cytogenetics*2024: EduGorilla Publication.
13. Chanoine, C., *Bases cellulaires et moléculaires du développement - 3e édition revue et augmentée*2020: Ellipses.
14. Mitchell, B. and R. Sharma, *Embryology E-Book: Embryology E-Book*2012: Churchill Livingstone.
15. Schoenwolf, G.C., et al., *Larsen's Human Embryology*2014: Churchill Livingstone.
16. Slack, J.M.W., *Essential Developmental Biology*2005: Wiley.
17. Jinkins, J.R., *Atlas of Neuroradiologic Embryology, Anatomy, and Variants*2000: Lippincott Williams & Wilkins.
18. Tani, S., et al., *Understanding paraxial mesoderm development and sclerotome specification for skeletal repair*. Experimental & Molecular Medicine, 2020. **52**(8): p. 1166-1177.
19. online, b.n. *Extra-embryonic Membranes in Chick – Definition, Types, Development, Functions*. biology notes online 2025 2025]; Available from: https://biologynotesonline.com/extra-embryonic-membranes-in-chick-definition-types-development-functions/.
20. Benirschke, K. and P.D.P. Kaufmann, *Pathology of the Human Placenta*2013: Springer New York.
21. Ross, M.H. and W. Pawlina, *Histology: A Text and Atlas : with Correlated Cell and Molecular Biology*2011: Wolters Kluwer/Lippincott Williams & Wilkins Health.
22. Junqueira, L.C.U. and J. Carneiro, *Basic Histology: Text & Atlas*2003: Lange Medical Books, McGraw-Hill, Medical Pub. Division.
23. Winters, N.I. and D.M. Bader, *Development of the serosal mesothelium.* Journal of Developmental Biology, 2013. **1**(2): p. 64-81.
24. Kierszenbaum, A.L., P. Validire-Charpy, and P. Validire, *Histologie et biologie cellulaire: Une introduction à l'anatomie pathologique*2006: De Boeck Supérieur.
25. Stevens, A., et al., *Histologie humaine*2006: Elsevier.
26. Poirier, J., et al., *Histologie: Les Tissus*2006: Masson.
27. Pawlina, W., *Histology: A Text and Atlas: With Correlated Cell and Molecular Biology*2023: Wolters Kluwer Health.
28. Niki, T., *Immunohistochemistry of Laminin-5 in Lung Carcinoma.* Handbook of Immunohistochemistry and in Situ Hybridization of Human Carcinomas: Molecular Genetics; Lung and Breast Carcinomas, 2004. **1**: p. 155.
29. Brahme, A., *Comprehensive biomedical physics*2014: Newnes.
30. Tortora, G.J. and B.H. Derrickson, *Principles of Anatomy and Physiology*2018: Wiley.

31. Tkachuk, D.C., J.V. Hirschmann, and M.M. Wintrobe, *Wintrobe's Atlas of Clinical Hematology*2007: Wolters Kluwer Health/Lippincott Williams & Wilkins.
32. Cui, D., *Atlas of Histology: With Functional and Clinical Correlations*2011: Wolters Kluwer Health.