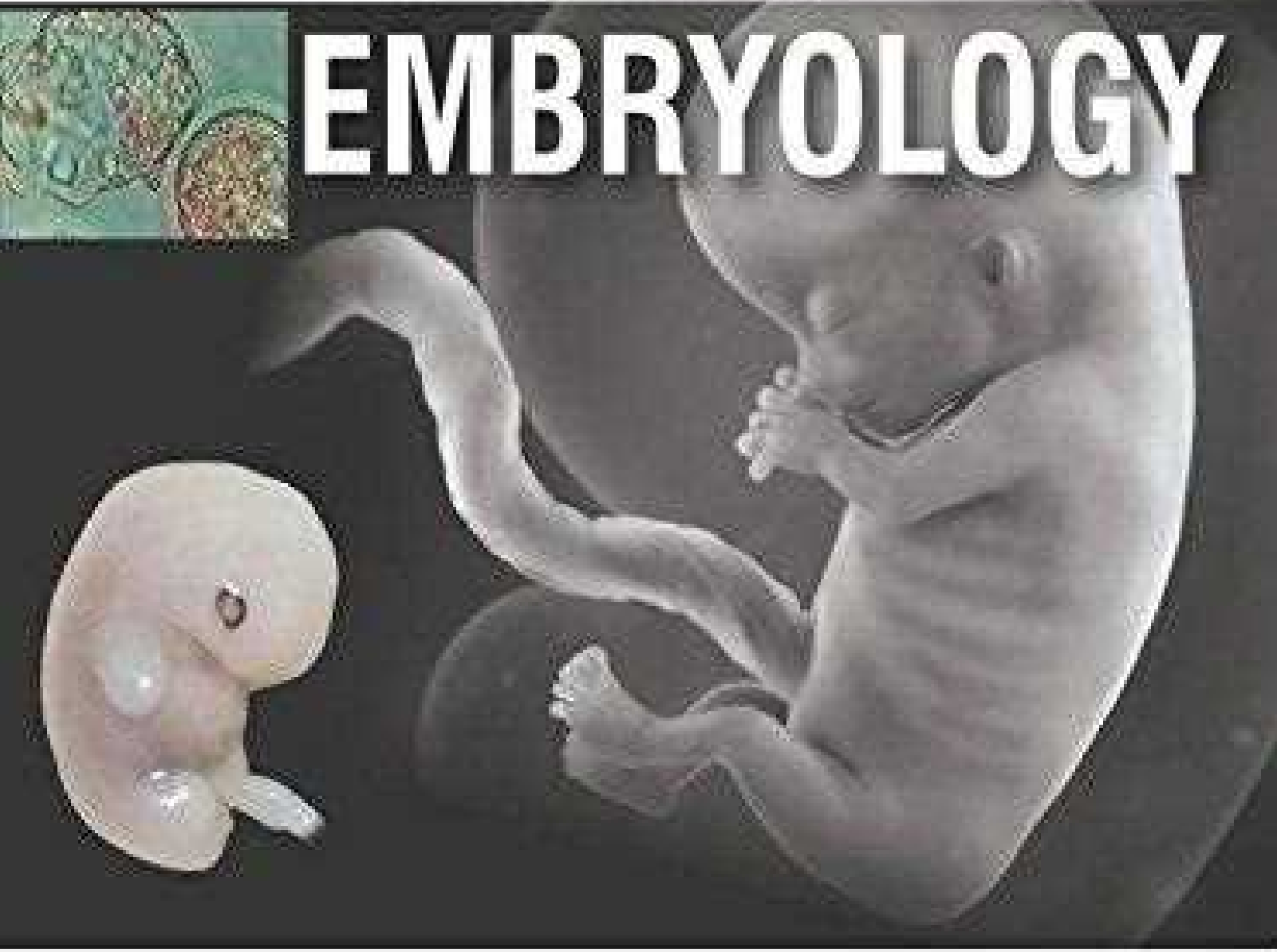
ELEVENTH EDITION

Inderbir Singh's

HUMAN EMBRYOLOGY



Edited by

V Subhadra Devi

Inderbir Singh's

HUMAN EMBRYOLOGY

Late Professor Inderbir Singh
(1930–2014)

Tribute to a Legend

Professor Inderbir Singh, a legendary anatomist, is renowned for being a pillar in the education of generations of medical graduates across the globe. He was one of the greatest teachers of his time. He was a passionate writer who poured his soul into his work. His eagle's eye for details and meticulous way of writing made his books immensely popular amongst students. He managed his lifetime to become enmeshed in millions of hearts. He was conferred the title of Professor Emeritus by Maharshi Dayanand University, Rohtak.

On 12th May, 2014, he was awarded posthumously with **Emeritus Teacher Award** ***by*** **National Board of Examination** ***for making invaluable contribution in teaching of Anatomy.*** This award is given to honour legends who have made tremendous contribution in the field of medical education. He was a visionary for his time, and the legacies he left behind are his various textbooks on *Gross Anatomy*, *Histology*, *Neuroanatomy* and *Embryology*. Although his mortal frame is not present amongst us, his genius will live on forever.

Inderbir Singh's

HUMAN EMBRYOLOGY

ELEVENTH EDITION

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Dedicated to

My husband Dr VH Rao who has been my inspiration and the driving force for all my accomplishments in both personal and professional life.

Preface to the Eleventh Edition

During the publication of my earlier book - "Basic Histology – A Color Atlas and Text" the publishers proposed to me to revise the embryology book written by late Prof Inderbir Singh. Notwithstanding 35 years of experience in teaching embryology and several publications in human developmental anatomy, I was skeptical because it is simply difficult for anyone to match the simplicity of expression and sheer elegance of images so diligently originated by Prof. Singh. With the encouragement provided by the publishers and colleagues, I have taken the proverbial plunge.

When I started my career as a medical teacher way back in 1981, I used to reproduce the diagrams from Prof. Inderbir Singh's embryology on black board. With the evolution of technology, I have initially transcribed the figures on to OHP sheets and recently upgraded several of them into 3D images, some of which are included in the present edition of the book.

Like all its previous editions, this is also a one person effort which clearly offers scope for improvement. Suggestions from academics, students and professionals are welcome for incorporation in the coming editions.

I thank all my students who are my inspiration for revising this book. I am thankful to all staff and students in the Department of Anatomy, SV Medical College and Sri Venkateswara Institute of Medical Sciences, Tirupati, Andhra Pradesh, India, for their continuous support and constructive feedback at different stages while this book is evolving. I make a special mention of Mr. K Thyagaraju, Assistant Professor, for drawing and Photoshop editing several of the figures. Some of the figures in the present edition originated from the research carried out by the postgraduate students in my lab.

I am also thankful to Shri Jitendar P Vij (Group Chairman), Mr Ankit Vij (Group President) of M/s Jaypee Brothers Medical Publishers (P) Ltd, New Delhi, India, for kindly agreeing to publish this book, and the production team especially Ms. Ritu Sharma, Dr Madhu Chaudhary, Dr Pinky Chauhan and Ms. Samina Khan for their dedicated work.

V Subhadra Devi

Preface to the First Edition

This book on human embryology has been written keeping in mind the requirements of undergraduate medical students. The subject of embryology has traditionally been studied from imported textbooks of anatomy or of embryology. Experience has shown that the treatment of the subject in most of these books is way above the head of the average medical student in India. The difficulty has increased from year to year as there has been, and continues to be, progressive deterioration in the standards of the teaching of English in our schools and colleges. The combination of unfamiliar sophistications of language and of an involved technical subject, has very often left the student bewildered.

In this book, care has been taken to ensure that the text provides all the information necessary for an intelligent understanding of the essential features of the development of various organs and tissues of the human body. At the same time, several innovations have been used to make the subject easy to understand.

Firstly, the language has been kept simple. Care has been taken not to compress too many facts into an involved sentence. New words are clearly explained.

Secondly, simultaneous references to the development of more than one structure have been avoided as far as possible. While this has necessitated some repetition, it is hoped that this has removed one of the greatest factors leading to confusion in the study of this subject.

Thirdly, almost every step in development has been shown in a simple, easy to understand, illustration. To avoid confusion, only structures relevant to the discussion are shown. As far as possible, the drawings have been oriented as in adult anatomy to facilitate comprehension.

Fourthly, the chapters have been arranged so that all structures referred to at a particular stage have already been adequately introduced.

In an effort of this kind it is inevitable that some errors of omission, and of commission, are liable to creep in. To obviate as many of these as possible a number of eminent anatomists were requested to read through the text. Their suggestions have greatly added to the accuracy and usefulness of this book. Nevertheless, scope for further improvement remains, and the author would welcome suggestions to this end both from teachers and from students.

Rohtak
January 1976

Inderbir Singh

Contents

Chapter 1

Introduction and Some Preliminary Considerations

HIGHLIGHTS

- *Embryology:* It is the study of the development of an individual before birth (*prenatal period*).
  Embryo (G): (en = within; bruein= to swell or to be full); Logos = study
  Natal = birth; Prenatal = before birth; Postnatal = after birth
- *Embryo:* It is the developing individual during the first 2 months or 8 weeks of intrauterine life.
- *Fetus:* It is the developing individual from the 3rd month or 9th week of intrauterine life to the time of birth.
- Development before birth is called *prenatal development*, and that after birth is called *postnatal development*.
- There are three stages in prenatal development. They are *(1) preimplantation, (2) embryonic and (3) fetal periods.*
- *Gonads:* They are the sex organs that produce sex cells or *gametes*. The *testis* is the male gonad and the *ovary* is the female gonad. Male gametes are called spermatozoa. Female gametes are called *ova*.
- *Gametogenesis:* It is the process of production of gametes in gonads or sex organs. In males it is known as *spermatogenesis* and in females as *oogenesis*.
- *Fertilization:* It is the process of fusion of male and female gametes. It takes place in the uterine tube of female genital tract.
- *Zygote:* It is the single cell that results from fertilization.
- *Development:* It is a process where something grows or changes and becomes more advanced.
- *Growth:* It is a quantitative change that increases the size.
- *Ontogeny:* Complete life cycle of an organism.
- *Phylogeny:* Evolutionary history of a group of organisms.
- *Differentiation:* It is a qualitative change in structure for an assigned function.
- *Organizer:* Any part of the embryo which exerts stimulus on an adjacent part.
- *Cell potency:* It is the potential to differentiate into different cell types.

BASIC QUALITIES OF LIVING ORGANISMS

The three basic qualities of living organism are:

1. *Protection*: Protection from different environmental conditions like heat, cold, rain, famines, etc. by making provision for food, water, clothing and shelter.
2. *Growth*: It includes both physical (increase in height, weight) and mental (intelligence, social behavior) growth by proper nutrition, customs and practices in the society.
3. *Propagation of species*: Propagation of species by reproduction of new individuals to prevent extinction of species.

Nature facilitates for nurturing these three basic qualities.

REPRODUCTION

- Reproduction is a mechanism to produce new generations continuously.
- For reproduction, vertebrates require the presence of two different sexes, i.e. male and female that differ in external and internal sex characters.

- The internal sex organs (gonads) produce gametes that differ in each sex.

Gonads and Gametes

- Gonads are the paired sex glands that are responsible for the production of *gametes* or *sex cells* that carry out the special function of reproduction. The male sex cells (spermatozoa) are produced in the male *gonads* (testes) while the female sex cells (ova) are produced in female gonads (ovaries).
- The formation of spermatozoa in testis is called *spermatogenesis*, while the formation of ova in the ovary is called *oogenesis*. The two are collectively referred to as *gametogenesis*.
- The development of a new individual begins at the movement when one male *gamete* (*sperm* or *spermatozoon*) meets and fuses with one female gamete *(ovum* or *oocyte*). The process of fusion of male and female gametes is called *fertilization.*
- The zygote multiplies and reorganizes to form the miniature new individual called *embryo* that grows and matures as *fetus* in the mother's womb and delivered at the end of term of pregnancy.

DEVELOPMENT OF A HUMAN BEING

Development is a process where someone or something grows or changes and becomes more advanced. Human development is a continuous process that does not stop at birth. It continues after birth for increase in the size of the body, eruption of teeth, etc. Development before birth is called *prenatal development*, and that after birth is called *postnatal development.* Each period is further subdivided into several stages (Fig. 1.1).

Prenatal Development

There are three stages in prenatal development. They are:
1. Preimplantation/pre-embryonic period
2. Embryonic period
3. Fetal period.

Preimplantation/Pre-embryonic Period

It extends from fusion of male and female gametes to form single-celled zygote to formation of primitive germ layers of developing organism. It includes 1st and 2nd weeks of intrauterine development. The following morphogenetic events take place during this period.
1. *Fertilization*: Fusion of male and female gametes resulting in the formation of zygote.
2. *Cleavage*: A series of mitotic divisions of zygote resulting in the formation of morula.
3. Transportation of cleaving zygote, i.e. morula along the fallopian tube toward the uterus.
4. *Blastocyst*: Structural and functional specialization and reorganization of cells (blastomeres) of cleaving zygote that becomes blastocyst.
5. *Implantation*: Process of attachment of blastocyst to the uterine endometrium is called implantation.

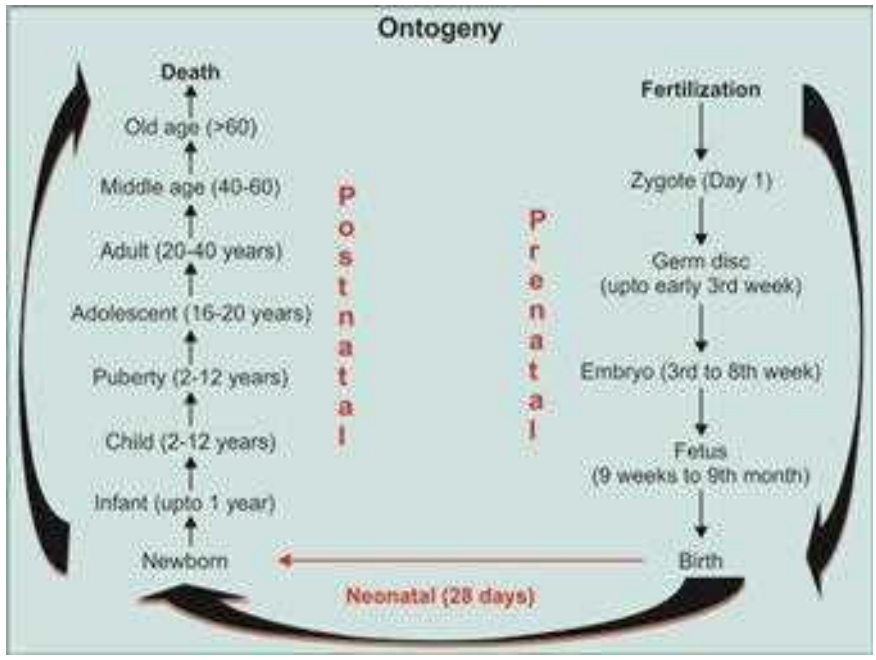


Fig. 1.1: Ontogeny/Life cycle of a human

6. *Specialization of primordial embryonic tissue*: It involves specialization of blastomeres to form embryonic structures *(embryoblast)* and supportive/nutritive structures *(trophoblast)*.
7. *Differentiation of embryoblast*—to form the primitive two layered *(bilaminar)* germ disc having ectoderm and endoderm.
8. Differentiation of trophoblast into *cytotrophoblast* and *syncytiotrophoblast*.

Embryonic Period

It extends from 3rd week of intrauterine life to 8th week of intrauterine life. The following morphogenetic events take place during this period.

1. *Trilaminar germ disc differentiation*: Formation of three layered germ disc with the appearance of mesoderm in between ectoderm and endoderm.
2. *Early organogenesis*: Formation of primordia of various organs like lungs, heart, liver, etc.
3. *Formation of extraembryonic supportive organs and membranes*: Placenta, umbilical cord, amnion, allantois.

Fetal Period

It extends from 9th week to 9th month. This period includes the following:

1. Growth of fetus in all dimensions
2. Specialization of various body structures.

Postnatal Period of Development

It extends from birth of an individual to adulthood. The various stages in postnatal development are as follows:

1. *Neonatal period*: It extends from birth to 28 days after birth. These first 4 weeks are critical in the life of the newborn/neonate as various systems especially respiratory and cardiovascular have to make adjustments with the external/extrauterine environment.
   Neonatology: The branch of medicine that takes care of neonates is called *neonatology*.
   Perinatology: It is the branch of medicine that takes care of the fetus and newborn from 28th week of intrauterine life to 6th day of extrauterine life.
2. *Infancy*: It extends from 1 month to 1 year and the newborn during this period is called *infant*.
3. *Childhood*: It extends from 2nd year to 12th year of age and an individual is called a child. It is the period of rapid growth and development. This age is also called pediatric age.
   Pediatrics and pediatrician: The medical branch that deals with infants and children is called *pediatrics*. The specialist who treats them is known as *pediatrician.*
4. *Puberty*: It extends from 12 years to 16 years. There will be rapid physical growth and development of secondary sex characters and it depends on the interaction of sex hormones and growth hormones.
5. *Adolescence*: It extends from 17 years to 20 years. During this period, there will be rapid physical growth and sexual maturation. The reproductive ability is established.
6. *Adulthood*: It extends from 21 years to 40 years.
7. *Middle age*: It extends from 40 years to 60 years.
8. *Old age*: It extends from more than 60 years to death.

Ontogeny: Complete life cycle of an organism involving both prenatal and postnatal developments is called ontogeny. It is the expression of blue print of life hidden in genes. It includes progressive changes followed by retrogressive changes. It involves various processes like cell division, differentiation and growth.

Phylogeny: Evolutionary/ancestral history of a group of organisms is called phylogeny. It includes developmental changes in various organs (e.g. kidney, heart) and organ systems (e.g. respiratory, skeletal) starting from fishes, amphibians, reptiles, birds and mammals.
Ontogeny repeats phylogeny: Life cycle of an organism repeats its ancestral history. This is observed in the development of certain organs viz. heart, lung and kidney.
In this book, we will study prenatal development only.

EMBRYOLOGY

- It is the science that deals with the processes and regulations in the prenatal growth and development of an organism/individual in the female genital tract. It begins with the fusion of male and female gametes (fertilization) in the fallopian tube up to the birth as a neonate.
- Prenatal development involves repeated division of most of the cells in the body resulting in growth in size, complexity, structural and functional differentiation of body.
- Embryology includes the study of startling integration of various complex molecular, cellular and structural processes that are accountable for the growth and development of a 9-month-old neonate containing 5-7 $\times 10^{12}$ cells from a single-celled zygote. It is also called *developmental anatomy.*

SUBDIVISIONS OF EMBRYOLOGY

General embryology: It is the study of development during pre-embryonic and embryonic periods (first 8 weeks after fertilization). During this period, the single-celled zygote is converted by cell multiplication, migration and

reorganization into a miniature form of an individual with various organs and organ systems of the body.

Systemic embryology: It is detailed study of formation of primordia and their structural and early functional organization into various organs and systems of the body. It is further subdivided into development of cardiovascular system, digestive system, urinary system, genital system, etc.

Comparative embryology: It is the study of embryos in different species of animals.

Experimental embryology: It is for understanding the effects of certain drugs, environmental changes that are induced (exposure to radiation, stress) on the growth and development of embryos and fetuses of lower animals. The knowledge gained from these experiments can be used for avoiding the harmful effects in the human development. It is a vigorous and promising branch of embryology.

Biochemical and molecular aspects in embryology: Chromosomes, gene sequencing, regulation.

Teratology: This is a branch of embryology that deals with abnormal embryonic and fetal development, i.e. congenital abnormalities or birth defects.

IMPORTANCE OF EMBRYOLOGY IN THE MEDICAL PROFESSION

Normal development: This subject tells us how a single cell (the fertilized ovum, i.e. zygote) develops into a newborn, containing numerous tissues and organs.

Normal adult anatomy: This knowledge helps us to understand many complicated facts of adult anatomy like the location and relations of organs to one another. *Examples*—on the location of heart on left side of thoracic cavity, liver on right side of abdominal cavity and its closeness to stomach.

Developmental abnormalities: Embryology helps us understand why some children are born with organs that are abnormal. Appreciation of the factors responsible for abnormal development assists us in preventing, or treating, such abnormalities. *Examples*—exposure to radiation during pregnancy, use of certain medications during pregnancy or a genetic abnormality that exists in family.

Understanding postnatal and adulthood diseases: The mechanisms (molecular and cellular) taking place during the development of embryo play a key role in the development of a wide range of diseases in adult life. *Examples*—that can vary from absence of an ear or presence of an extra finger to hypertension, diabetes, depression, cardiovascular and renal diseases. This is known as *fetal programming* of adult diseases.

Health care strategies for better reproductive outcome: Knowledge of embryology facilitates interpretation of the results of various techniques like fetal ultrasound, amniocentesis, and chorionic villous biopsy. Based on the results, appropriate treatment can be planned. *Example*—performing surgeries for correction of a defect in the diaphragm prenatally; postnatal correction of a cardiac defect; medical line of management of a diabetic or hypertensive mother.

Therapeutic procedures for infertility/fertility-related problems: If the woman is unable to conceive by natural methods, alternate methods like cloning and in vitro fertilization can be planned. For spacing the pregnancies, various birth control methods (medical and surgical) are available. A basic knowledge of embryology is required for understanding the mechanism of action of these methods.

Stem cell therapy: Cells forming tissues in the embryo are called *stem cells*. These are undifferentiated cells that can differentiate into specialized cell types. It is an uncommitted cell and depending on the signal it receives, it can develop into many specialized cells. These cells are capable of treating certain diseases in postnatal life.

BASIC PROCESSES IN EMBRYOLOGY

Growth and *differentiation* are the two basic processes involved in the conversion of a single-celled zygote into a multicellular human newborn.

Growth

It is a quantitative change, i.e. Increase in the bulk. Growth of cells is either by synthesizing new protoplasm in the interphase (G1, S and G2) of cell cycle or reproduction of individual cells of body by mitotic cell divisions. There are four types of growth. They are as follows:

1. *Multiplicative:* This type of growth is the predominant type observed during prenatal period. It is increase in cell number by succession of mitotic divisions without increase in cell size. *Example*—blastomeres. During prenatal and postnatal development, many cells die by apoptosis (programmed cell death) or they lose the power to grow and divide to form definitive contours of the organs. *Examples*—the neurons do not divide during postnatal period. The cells of epidermis, intestinal epithelium and blood cells are continuously produced to replenish the cells lost by wear and tear. The liver cells do not divide normally but, if there is loss of two-thirds of liver (removal) they multiply.
2. *Auxetic*: This type of growth is seen in oocytes and certain neurons. The increase in cell size is due to increase in its cytoplasmic content. This alters the nuclear-cytoplasmic

ratio without alterations in structural genes. If the ratio is altered, it makes the structural genes in nuclear DNA ineffective. This can cause degradation of cytoplasmic proteins. To provide nutrition, there will be cells that surround these larger cells. *Example*—satellite cells around the larger neurons and follicular cells around oocyte.

3. *Accretionary:* Increased accumulation of intercellular substance resulting in overall growth of structure. This causes increase in length. *Example*—increase in length of bone and cartilage.
4. *Appositional:* Addition of new layers on previously formed ones. It takes place at the edges, is seen in rigid structures and is responsible for contours. *Example*—increase in width of bone by addition of lamellae.

Differentiation

It is a qualitative change in structure with an assigned function. Different types of differentiation are as follows:

- *Chemodifferentiation:* It is an invisible differentiation that takes place at molecular level. The substances producing this type of differentiation are called organizers.
- *Histodifferentiation:* It takes place at tissue level.
- *Organodifferentiation/Organogenesis:* This is at organ level and is the basis for organ remodeling.
- *Functional differentiation:* Hemodynamic changes in blood vessels.

Organizer

Any part of the embryo which exerts a morphogenetic stimulus on an adjacent part or parts. There are three types of organizers:

1. *Primary organizer: Example*—blastopore/primitive streak that induces differentiation of notochord and secondary/intraembryonic mesoderm.
2. *Secondary organizer: Example*—notochord acts as a secondary organizer in stimulating the development of brain and spinal cord.
3. *Tertiary organizer: Example*—neural tube is the tertiary organizer that induces segmentation of paraxial mesoderm into somites.

Stem Cells

- These are undifferentiated cells that are capable of giving rise to more number of cells of same type by replication from which some other kinds of cells arise by differentiation (Fig. 1.2).
- There are two types of stem cells: (1) the *embryonic* and (2) *adult/somatic*. Embryonic stem cells are present during embryonic development. Adult stem cells are formed during embryonic development that are tissue-specific and remain so throughout the life of an individual.
- They are the basis for the formation of a tissue and an organ in the body.
- They have the capacity of self-renewal and differentiation.
- Stem cells are classified depending on their potency (cell potency) to differentiate into different cell types.
- Accordingly the cells are named as (Table 1.1):
  - *Totipotent cells:* They can form all the cell types in the embryo in addition to extraembryonic or placental cells. Embryonic cells within the first couple of cell divisions after fertilization are the only cells that are totipotent. *Example*—zygote, early blastomeres.
  - *Pluripotent:* It can give rise to all of the cell types that make up the body. Embryonic stem cells are considered pluripotent. *Example*—inner cell mass.
  - *Multipotent:* They can develop into more than one cell type, but are more limited than pluripotent cells. *Example*—adult stem cells (mesenchymal cells), cord blood stem cells and hematopoietic cells.

Clinical correlation

Process of differentiation

- For understanding the various events that lead to the formation of an embryo or fetus knowledge of developmental processes of growth and differentiation are important. It provides explanation for how an entire individual is produced from a single cell the zygote.
- The cells resulting from the division of zygote are totipotent and are capable of forming an embryo and a new adult. Gradually these cells lose their totipotency and are converted into specialized cells that form various organs like liver, heart, brain, etc. by the process of differentiation. With continuous division, the specialization of embryonic cells gets restricted and is called determination.
- The nucleus of a cell contains copies of genetic material (genes) for the synthesis of proteins. During the process of differentiation either the cell will form new proteins or lose its ability to form proteins. Differentiation of cells regulates the expression of genes.

Stem cell therapy

- ***Regeneration of tissues and organs:*** Example—use of stem cells underneath the skin for skin grafting in burns cases.
- ***Treatment of cardiovascular and neurological diseases:*** Regeneration of blood vessels. Use of embryonic stem cells in treating Alzheimer's and Parkinson's diseases.
- ***Replacement of deficient cells:*** Example—cardiac muscle cells in heart diseases, insulin producing cells in type 1 diabetes.
- ***Treatment of blood disorders:*** Treatment of leukemia, sickle cell anemia.

  - *Oligopotent:* It can develop into cells of one category only. *Example*—vascular stem cells that form endothelium and smooth muscle; lymphoid or myeloid stem cells that form blood cells.
  - *Unipotent:* It can develop into only one type of cell. *Example*—liver cell, muscle cell.

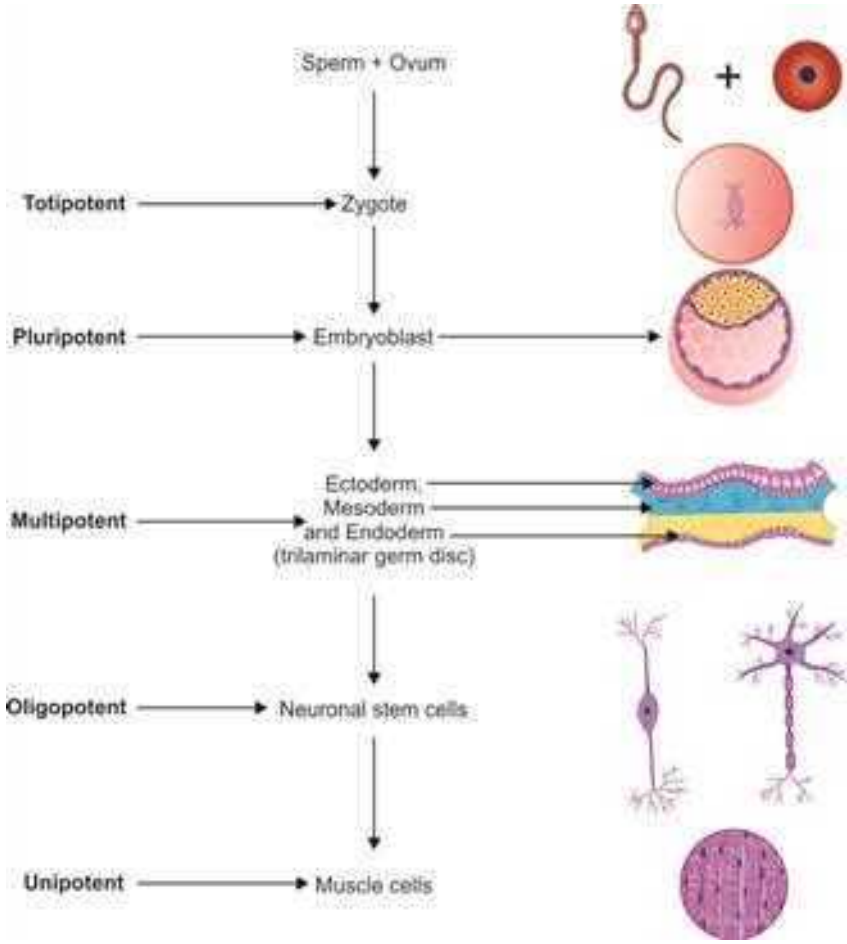


Fig. 1.2: Classification of stem cells

TABLE 1.1: Classification of different types of stem cells

Types of stem cells	*Capacity to differentiate*	*Examples*
Totipotent cells	Can form embryonic and extraembryonic cells	Zygote, early blastomeres
Pluripotent	All cell types of embryonic body but not that of placenta and umbilical cord	Inner cell mass
Multipotent	More than one category of cells (limited types)	Hematopoietic stem cells, cord blood stem cells
Oligopotent	Only one category of cells	Vascular stem cells
Unipotent	Only one type of cells	Liver cells

REVIEW QUESTIONS

1. Name different types of growth with examples.
2. What is differentiation? Name the different types of differentiation.
3. Name different types of Organizers with examples.
4. What are stem cells? Name the different types with examples.

Chapter 2

Genetics and Molecular Biology in Embryology

HIGHLIGHTS

- Genetics is a branch of biology that deals with transmission of *inherited characters* (*traits*) from parent to offspring at the time of fertilization. Some of the *characters/traits* are *dominant* and some are *recessive*.
- Characters of parents are transmitted to offspring through codes borne on strands of DNA. Genes are made of such strands of DNA. They are located on chromosomes. Different forms of each gene are called *alleles*.
- A typical cell contains 46 chromosomes (= *diploid number*). A gamete contains 23 chromosomes (= *haploid number*). The diploid number of chromosomes is restored as a result of fertilization.
- The 46 chromosomes in each cell can be divided into 44 *autosomes* and 2 *sex chromosomes*. The sex-chromosomes are XX in female and XY in male.
- Multiplication of cells takes place by *cell division*. The usual method of cell division, seen in most tissues, is called *mitosis*. Daughter cells resulting from a mitotic division are similar to the parent cell, and have the same number of chromosomes (46).
- A special kind of cell division takes place in the testis and ovary for formation of gametes. It is called meiosis. The gametes resulting from *meiosis* have the haploid number of chromosomes (23). The various gametes formed do not have the same genetic content.
- Embryology includes *development*, *differentiation*, morphogenetic processes and *controlled growth*. These processes are controlled by genes. Most of these genes produce *transcription factors* that control transcription of RNA.
- The parts of a chromosome are two chromatids joined by a centromere. Depending on the position of centromere the chromosomes are classified.
- Karyotyping is the process by which chromosomes can be classified individually.
- Sex-chromatin is the small, dark-staining, condensed mass of inactivated X-chromosome within the nucleus of nondividing cell, i.e. during interphase.
- A *pedigree chart* is prepared to understand the pattern of occurrence (inheritance) of the disease in the families.

GENETIC BASIS OF DEVELOPMENTAL ANATOMY

- Embryology includes development, differentiation, morphogenetic processes (cell migration, transformation, folding, invagination, evagination, apoptosis, etc.) and controlled growth.
- Genetics is a branch of biology that deals with transmission of *inherited characters* (*traits*) from parent to offspring at the time of fertilization. Some of the *characters/traits* are *dominant* and some are *recessive.*
- Inheritance of characters is determined by *factors (genes)* that are passed on from one generation to another. Different forms of each gene are called *alleles.*
- Genetics is the study of genes. Genetics deals with:
  - Inheritance of characters
    - Physical and mental
    - Normal and abnormal

- In individual and family
- In a race or population

– Mode of transmission of characters from generation to generation
– Hereditary factors (genes) and their expression during development (prenatal—embryonic) and life (postnatal).

GENES

- Genes are carriers of blueprints for formation of cells, tissues, organs, and organism. Genes are made up of a nucleic acid called *deoxyribonucleic acid* (DNA) and all information is stored in the molecules of this substance. The genes are strung together to form structures containing long chains of DNA known as *chromosomes*.
- Genes are involved in the synthesis of proteins. Proteins are the most important constituents of our body. They make up the greater part of each cell and of intercellular substances. Enzymes, hormones and antibodies are also proteins.
- The nature and functions of a cell depend on the proteins synthesized by it. It is, therefore, not surprising that one cell differs from another because of the differences in the proteins that constitute it.
- Genes exert their influence on cellular functions by synthesis of proteins. The proteins synthesized differ from cell to cell and within the same cell at different times. This provides the basic mechanism for control of any process, including embryonic development.
- Proteins are the building blocks and are made of smaller units called *amino acids*. Differences in genes cause the building of different amino acids and proteins. These differences make individuals with different traits, e.g. hair color, eye color, skin color, blood groups, etc.
- We now know that genes control the development and functioning of cells, by determining what types of proteins will be synthesized within them. Thus, genes play an important role in the development of tissues and organs of an individual.
- A gene gives only the potential for the development of a trait. How this potential is achieved depends partly on the interaction between the genes and the interaction of the gene with the environment. For example, genetic tendency of overweight is influenced by environmental factors like food, exercise, stress, etc.
- Vast amount of information about individual genes and the various factors that are produced by them to control developmental processes step by step is available in the literature.

To understand genetic processes, we have to first know some facts about DNA structure.

Basic Structure of DNA

- Each of the 100 trillion cells in our body except the red blood cells contains the genetic information (blueprint) of the individual (entire human genome). It is the DNA that contains the entire genetic code for almost every organism and provides template for protein synthesis. *Watson and Crick* 1953 described the structure of DNA. DNA in a chromosome is in the form of very fine fibers. Each fiber consists of two strands that are twisted spirally to form what is called a *double helix* resembling a ladder (Fig. 2.1).
- The two strands are linked to each other at regular intervals. Each strand of the DNA fiber consists of a chain of nucleotides. Each nucleotide consists of a sugar, i.e. deoxyribose, a molecule of phosphate and a base (Fig. 2.2). The phosphate of one nucleotide is linked to the sugar of the next nucleotide.
- The deoxyribose and phosphate molecules are always the same and provide for the structure (side of the ladder). The only difference between individuals is the order and arrangement of the four bases (rungs of the ladder). The base that is attached to the sugar molecule may be *adenine, guanine, cytosine or thymine*.
- The two strands of a DNA fiber are joined together by the linkage of a base on one strand with a base on the opposite strand (Fig. 2.2). This linkage is peculiar in that adenine on one strand is always linked to thymine on the other strand, while cytosine is always linked to guanine. Thus, the two strands are complementary and the arrangement of bases on one strand can be predicted

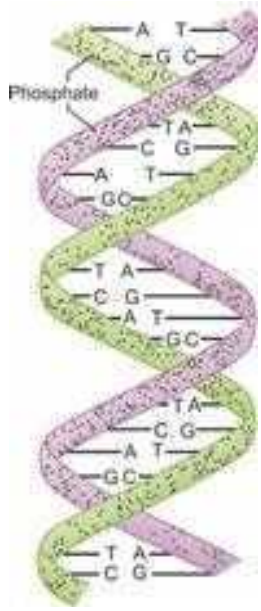


Fig. 2.1: DNA double helix

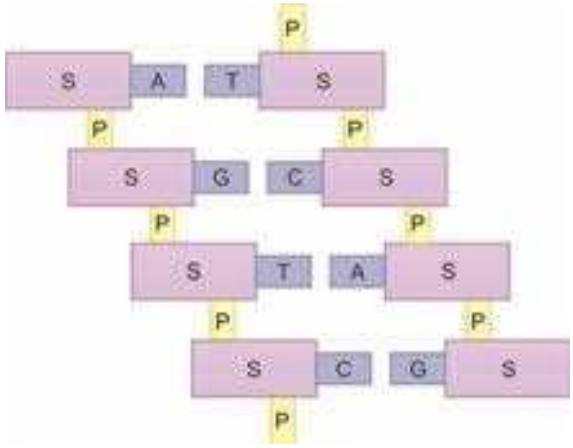


Fig. 2.2: Linkage of two chains of nucleotides to form part of a DNA molecule

from the other. The order in which these four bases are arranged along the length of a strand of DNA determines the nature of the protein that can be synthesized under its influence.

- Every protein is made up of a series of amino acids; the nature of the protein depending upon the amino acids present, and the sequence in which they are arranged. Amino acids may be obtained from food or may be synthesized within the cell. Under the influence of DNA, these amino acids are linked together in a particular sequence to form proteins.

Ribonucleic Acid

In addition to DNA, cells contain another important nucleic acid called *ribonucleic acid* (RNA). The structure of a molecule of RNA corresponds fairly closely to that of one strand of a DNA molecule, with the following important differences.

- RNA contains the sugar ribose instead of deoxyribose.
- Instead of the base thymine, it contains uracil.

Ribonucleic acid is present both in the nucleus and in the cytoplasm of a cell. It is present in three main forms, namely *messenger RNA* (mRNA), *transfer RNA* (tRNA) and *ribosomal RNA*. Messenger RNA acts as an intermediary between the DNA of the chromosome and the amino acids present in the cytoplasm and play a vital role in the synthesis of proteins from amino acids.

Synthesis of Protein

- A protein is made up of amino acids that are linked together in a definite sequence. This sequence is determined by the order in which the bases are arranged in a strand of DNA.
- Each amino acid is represented in the DNA molecule by a sequence of three bases (*triplet code*).
- The four bases in DNA are represented by their first letter, i.e. *adenine (A), cytosine (C), thymine (T) and guanine (G)*. They can be arranged in various combinations so that as many as sixty-four code "words" can be formed from these four bases.
- There are only about 20 amino acids that have to be coded for so that each amino acid has more than one code. The code for a complete polypeptide chain is formed when the codes for its constituent amino acids are arranged in proper sequence. That part of the DNA molecule that bears the code for a complete polypeptide chain constitutes a *structural gene* or *cistron*.
- At this stage, it must be emphasized that a chromosome is very long and thread-like. Only short lengths of the fiber are involved in protein synthesis at a particular time. The main steps in the synthesis of a protein may now be summarized as follows.
  - The two strands of a DNA fiber separate from each other (over the area bearing a particular cistron) so that the ends of the bases that were linked to the opposite strand are now free.
  - A molecule of mRNA is synthesized using one DNA strand as a guide (or *template*), in such a way that one guanine base is formed opposite each cytosine base of the DNA strand, cytosine is formed opposite guanine, adenine is formed opposite thymine, and uracil is formed opposite adenine. In this way, the code for the sequence in which amino acids are to be linked is passed on from DNA of the chromosome to mRNA. This process is called *transcription*. That part of the mRNA strand that bears the code for one amino acid is called a *codon*.
  - This molecule of mRNA now separates from the DNA strand and moves from the nucleus to the cytoplasm (passing through a nuclear pore).
  - In the cytoplasm, the mRNA becomes attached to a ribosome.
  - The cytoplasm also contains another form of RNA called tRNA. In fact, there are about 20 different types of tRNA each corresponding to one amino acid. On one side, tRNA becomes attached to an amino acid. On the other side, it bears a code of three bases (*anticodon*) that are complementary to the bases coding for its amino acid on mRNA. Under the influence of the ribosome, several units of tRNA, along with their amino acids, become arranged alongside the strand of mRNA in the sequence determined by the code on mRNA. This process is called *translation*.

– The amino acids now become linked to each other to form a polypeptide chain. From the above, it will be clear that the amino acids are linked up exactly in the order in which their codes are arranged on mRNA, which in turn, is based on the code on the DNA molecule. Chains of amino acids formed in this way constitute polypeptide chains. Proteins are formed by union of polypeptide chains.

The flow of information from DNA to RNA and finally to protein has been described as the "central dogma of molecular biology".

Control of Development of Embryo

- Certain regions of the embryo have the ability to influence the differentiation of neighboring regions. For example, the influence exerted by the optic vesicle on the overlying surface ectoderm to differentiate into lens vesicle. If the optic vesicle is removed, the lens vesicle fails to form. Conversely, if the optic vesicle is transplanted elsewhere (e.g. under the skin of abdomen) the overlying skin there forms the lens vesicle. This experiment shows that the optic vesicle induces the differentiation of lens vesicle. The influence exerted by an area (optic vesicle) is called *induction* whereas the area exerting induction is called *organizer*. In interactions between tissues, one is *inductor* and the other is *responder*. Capacity to respond to the inductor is called *competence*. The factors that influence the competence to respond are called *competence factors*.
- Many inductive interactions are between epithelium and mesenchyme, i.e. *epithelial mesenchymal interactions*. For example, development of liver and pancreas due to interaction between endoderm of gut and adjacent mesoderm and endoderm of ureteric bud and metanephric blastema of mesodermal origin to form nephron. The interaction between optic vesicle (neuroectodermal derivative) and lens vesicle (surface ectodermal derivative) is an example for *epithelial to epithelial interaction*.
- The blastopore, the primary organizer mentioned in Chapter 1 when removed results in total failure of development of embryo.
- It is now known that the organizers exert their influence by elaborating *chemical substances*, which are probably complex proteins, including enzymes.
- The chemical substances elaborated by the organizer may be *inductors* that stimulate tissue differentiation or *inhibitors* that have a restraining influence on differentiation.
- With the advent of molecular biology, the production of organizers, inductors and inhibitors are controlled by genes.
- A study of controlling mechanisms can be termed as *"Genetic control of development" or "Molecular control of development"*.

Molecular (Genetic) Control of Growth, Differentiation and Development

- Several genes and gene families play important roles in the development of embryo. Most of these genes produce *transcription factors* that control transcription of RNA.
- Transcription factors play an important role in gene expression as they can switch genes on and off by activating or repressing them.
- Many transcription factors control other genes, which regulate fundamental embryological processes of induction, segmentation, migration, differentiation and apoptosis (programmed cell death). These fundamental differentiation factors are mediated by growth and differentiation factors, growth factor receptors and various cytoplasmic proteins.

Components Required for Expression of a Gene

- Several components are required for gene expression. These are:
  1. *Growth factors*—act as *cell signaling molecules* for induction of cellular differentiation.
  2. *Receptors*—present on cell membrane and they recognize and respond to growth factors.
  3. Activation of *signal transducing proteins* that is present within the cell cytoplasm.
  4. Activation of *transcription factor*, which binds to DNA in the nucleus and finally leads to transcription (gene expression).
- Thus two different categories of molecules play an important role in embryonic development. They are *signaling molecules* and *transcription factors*.
- The *signaling molecules* like growth factors are present outside the cell and exert their effects on neighboring cells, or on cells located at a distance. They act by binding to the receptors on the plasma membrane of the cell and ultimately activate the transcription factors.
- The *transcription factors* are gene regulatory proteins, which are present in the nucleus and are responsible for gene expression and are therefore important molecules for control of embryonic development.

The various growth and differentiation factors and their functions are presented in Table 2.1.

TABLE 2.1: Growth and differentiation factors

Growth factor families	*Functions*
Epidermal growth factor (EGF)	Growth and proliferation of cells of ectodermal and mesodermal origin
Transforming growth factors (TGFs) (TGF B1 to TGF B5)	Formation of extracellular matrix, epithelial branching, myoblast proliferation
Bone morphogenetic factors (BMP 1-9)	Bone formation, cell division, cell migration, apoptosis
Müllerian-inhibiting factor (MIF)	Regression of paramesonephric duct
Nodal	Formation of primitive streak, formation of mesoderm
Lefty	Determination of body asymmetry
Sonic Hedgehog (SHH)	Neural tube formation, somite differentiation
WNT proteins	Development of midbrain, some and urogenital differentiation, limb patterning
Fibroblast growth factors (FGFs)	Mesoderm differentiation, angiogenesis, growth of axon, limb development, development of brain, outgrowth of genital tubercle
Insulin-like growth factors (IGFs)	IGF-1 bone growth IGF-2 fetal growth
Nerve growth factors (NGFs)	Growth of sensory and sympathetic neurons

CHROMOSOMES

Haploid and Diploid Chromosomes

- The number of chromosomes in each cell is fixed for a given species and in human beings, it is 46. This is referred to as the *diploid* (or double) number.
- However, in spermatozoa and ova, the number of chromosomes is only half the diploid number, i.e. 23. This is called the *haploid* (or half) number.
- After fertilization, the resulting zygote has 23 chromosomes from the sperm (or father), and 23 from the ovum (or mother). The diploid number is thus restored.

Autosomes and Sex Chromosomes

- The 46 chromosomes in each cell can be divided into 44 *autosomes* and 2 *sex chromosomes*. The sex chromosomes may be of two kinds, X or Y.
- In a male, there are 44 autosomes, one X-chromosome and one Y-chromosome; while in a woman, there are 44 autosomes and two X-chromosomes in each cell (Fig. 2.3).
- When we study the 44 autosomes, we find that they really consist of 22 pairs, the two chromosomes forming a pair being exactly alike (*homologous chromosomes*).
- In a woman, the two X-chromosomes form another such pair; in a man, this pair is represented by one X- and one Y-chromosome.
- One chromosome of each pair is derived from the mother and the other from the father.

To understand the structure of the gametes and to study how they are formed, it is necessary to first review some facts regarding chromosomes and cell division.

Chromosome Structure

In a resting cell, chromosomes are not visible under a light microscope, as their chromatin material is highly dispersed. However, during cell division, the chromatin network in the nucleus becomes condensed into a number of chromosomes. The appearance of a typical chromosome is illustrated in Figure 2.4.

It is made up of two rod-shaped structures or *chromatids* placed more or less parallel to each other. The chromatids are united to each other at a light staining area called

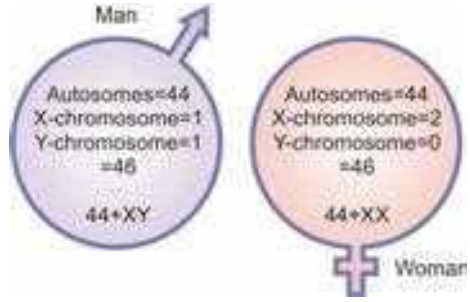


Fig. 2.3: Number of chromosomes in the somatic cell of a man and a woman

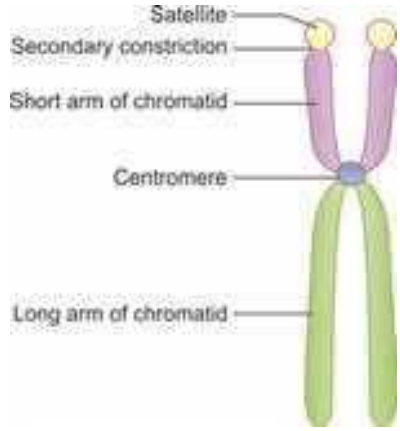


Fig. 2.4: Diagram to show the parts of a typical chromosome

the c*entromere* (or *kinetochore*). Each chromatid has two arms, one on either side of the centromere. Individual chromosomes differ from one another in total length, in the relative length of the two arms and in various other characteristics; these differences enable us to identify each chromosome individually. Classification of chromosomes in this way is called *karyotyping*. Karyotyping makes it possible for us to detect abnormalities in chromosome number or in individual chromosomes.

Significance of Chromosomes

The entire human body develops from the fertilized ovum. It is, therefore, obvious that the fertilized ovum contains all the information necessary for formation of the numerous tissues and organs of the body, and for their orderly assembly and function. Each cell of the body inherits from the fertilized ovum, all the directions that are necessary for it to carry out its functions throughout life. This tremendous volume of information is stored within the chromosomes of each cell.

Each chromosome bears on itself a very large number of structures called *genes*.

Traits (*characters*) of an individual are determined by genes carried on his (or her) chromosomes. As we have seen half of these are inherited from the father and half from mother. We have seen above that chromosomes are made up predominantly of a nucleic acid called *DNA*, and all information is stored in molecules of this substance.

When the need arises, this information is used to direct the activities of the cell by synthesizing appropriate proteins. To understand how this becomes possible, we must consider the structure of DNA in some detail.

Duplication of Chromosomes

One of the most remarkable properties of chromosomes is that they are able to duplicate themselves. Duplication of chromosomes involves the duplication (or replication) of DNA. This takes place as follows (Fig. 2.5):

- The two strands of the DNA molecule to be duplicated unwind and separate from each other so that their bases are "free".
- A new strand is now synthesized opposite each original strand of DNA in such a way that adenine is formed opposite thymine; guanine is formed opposite cytosine, and vice versa.
- This new strand becomes linked to the original strand of DNA to form a new molecule.
- As the same process has taken place in relation to each of the two original strands, we now have two complete molecules of DNA.
- It will be noted that each molecule has one strand that belonged to the original molecule and one strand that is new. It will also be noted that the two molecules formed are identical to the original molecule.

Structure of Fully Formed Chromosomes

- Each chromosome consists of two parallel rod-like elements that are called chromatids (Fig. 2.6).
- The two chromatids are joined to each other at a narrow area that is light staining and is called the *centromere* (or kinetochore). In this region, the chromatin of each chromatid is most highly coiled and, therefore, appears to be thinnest. The chromatids appear to the "constricted" here and this region is called the *primary constriction*.

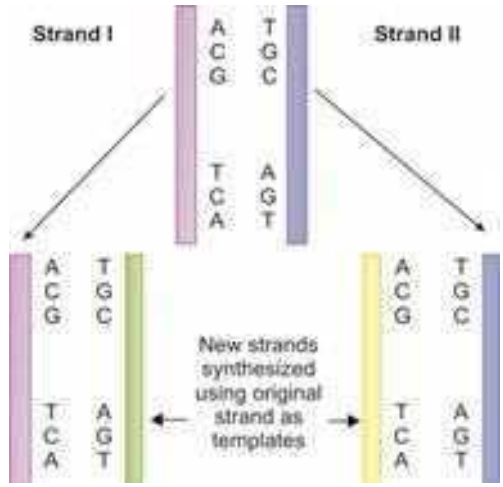


Fig. 2.5: Scheme to show how a DNA molecule is duplicated

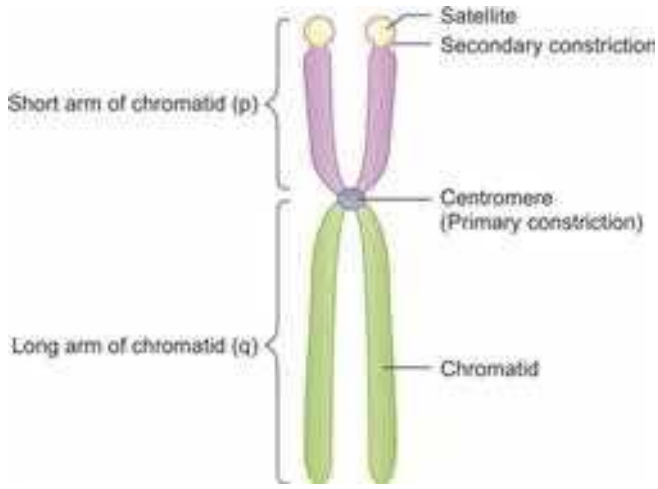


Fig. 2.6: Diagram to show the terms applied to some parts of a typical chromosome

- Typically, the centromere is not midway between the two ends of the chromatids, but somewhat toward one end (Fig. 2.6). As a result, each chromatid can be said to have a long arm (denoted by letter q) and a short arm (denoted by letter p). Based on the position of centromere the chromosomes (Fig. 2.7) are classified as:
  1. *Metacentric*: Centromere is centrally placed and the two arms are of equal length.
  2. *Submetacentric*: Centromere is slightly away from the center and the two arms are only slightly different in length.
  3. *Acrocentric*: Centromere is nearer to one end and the difference in length of arms is marked.
  4. *Telocentric*: Centromere lies at one end.
- Differences in the total length of chromosomes and in the position of the centromere are important factors in distinguishing individual chromosomes from each other.
- Additional help in identification is obtained by the presence in some chromosomes of *secondary constrictions*. Such constrictions lie near one end of the chromatid. The part of the chromatid "distal" to the constriction may appear to be a rounded body almost separate from the rest of the chromatid; such regions are called *satellite bodies*.
- Secondary constrictions are concerned with the formation of nucleoli and are, therefore, called *nucleolar organizing centers*.
- Considerable help in identification of individual chromosomes is also obtained by the use of special staining procedures by which each chromatid can be seen to consist of a number of dark and light staining transverse bands.
- Chromosomes are distinguishable only during mitosis. In the interphase (between successive mitoses), the chromosomes elongate and assume the form of long threads. These threads are called *chromonemata* (singular = chromonema).

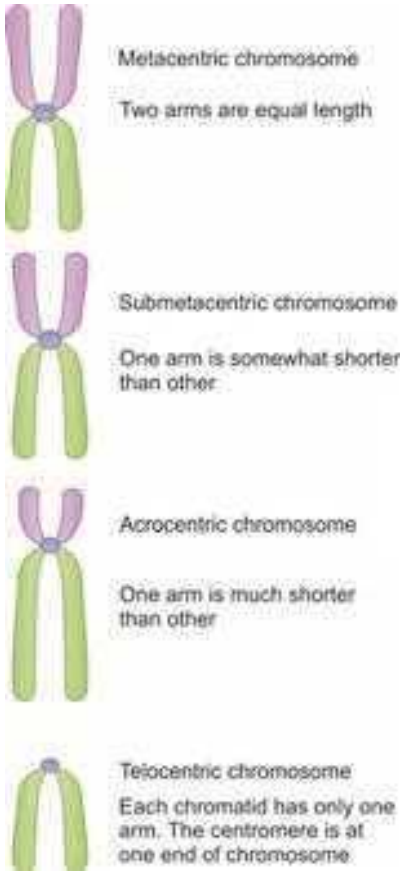


Fig. 2.7: Nomenclature used for different chromosomes based on differences in lengths of the arms of each chromatid

Karyotyping

- It is the procedure by which chromosomes can be mapped individually in an individual by applying the criteria described above.
- For this purpose, a sample of blood from the individual is put into a suitable medium in which lymphocytes can multiply. After a few hours, a drug (colchicine, colcemid) that arrests cell division at a stage when chromosomes are most distinct is added to the medium.
- The dividing cells are then treated with hypotonic saline so that they swell up. This facilitates the proper spreading out of chromosomes.
- A suspension containing the dividing cells is spread out on a slide and suitably stained.
- Cells in which the chromosomes are well spread out (without overlap) are photographed.
- The photographs are cut out and the chromosomes arranged in proper sequence.
- In this way, a map of chromosomes is obtained, and abnormalities in their number or form can be identified.
- In many cases, specific chromosomal abnormalities can be correlated with specific diseases.

According to Denver system of classification, the chromosomes including sex chromosomes are arranged into seven groups based on their length, position of centromere and presence of satellite bodies as shown in Table 2.2. Karyotypes of a normal male and a female are shown in Figures 2.8 and 2.9.

Sex Chromatin

- Small, dark-staining, condensed mass of inactivated X-chromosome within the nucleus of nondividing cell, i.e. during interphase.
- Usually located just inside the nuclear membrane of the interphase nucleus.

TABLE 2.2: Classification of chromosomes

Group	*Pairs of chromosomes*	*Features*
A	1–3	Long, metacentric
B	4, 5	Long, submetacentric
C	6–12 + X-chromosome	Medium, submetacentric
D	13–15	Medium, acrocentric Satellite bodies +
E	16–18	Short, submetacentric
F	19, 20	Short, metacentric
G	21, 22 + Y-chromosome	Short, acrocentric Satellite bodies +

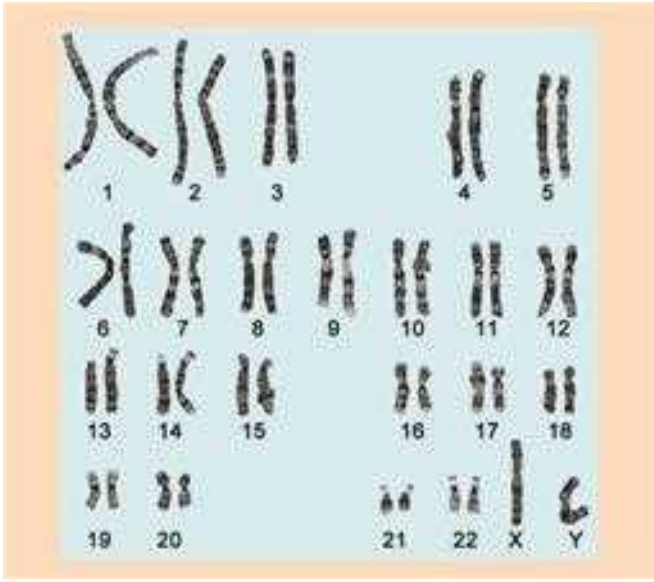


Fig. 2.8: Karyotype—Normal male

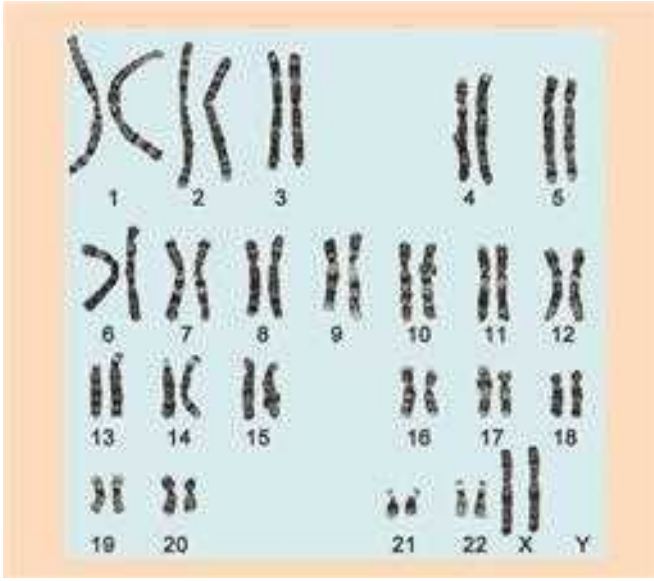


Fig. 2.9: Karyotype—Normal female

- Present in most female mammals in the nuclei of all cells except the germ cells.
- Inactive mammalian X-chromosome is always late-replicating, and in eutherian mammals, it is heterochromatic and hypermethylated.
- The term "sex chromatin" comprises two superficially dissimilar structures:
  1. Barr body, present in epithelial (oral, skin, vaginal, urethral, corneal) and other tissue cells (placenta, dental pulp, skin fibroblasts)
  2. Drumstick/Davidson body in polymorphonuclear leukocytes.

Study of sex chromatin is a relatively simple diagnostic test for certain genetic abnormalities.

Barr Body (Fig. 2.10A)

- Barr bodies are most commonly situated at the periphery of the nucleus.
- Count: Sex differences: males 1–2%; females 20–80%.
- Measurement: Approximately 1 μm.
- Barr bodies have several distinct shapes: Planoconvex/Wedge-shaped/Rectangular.
- Maximum no. of Barr bodies/nucleus = 0 (or) 1.
- Maximum no. of Barr bodies in diploid cells = No. of X-chromosomes – 1, in tetraploids, it is two less than the no. of X-chromosomes, and in octaploids, it is four less than the no. of X-chromosomes. Patients with 4X-chromosomes have three Barr bodies.

Drumstick (Davidson Body) (Fig. 2.10B)

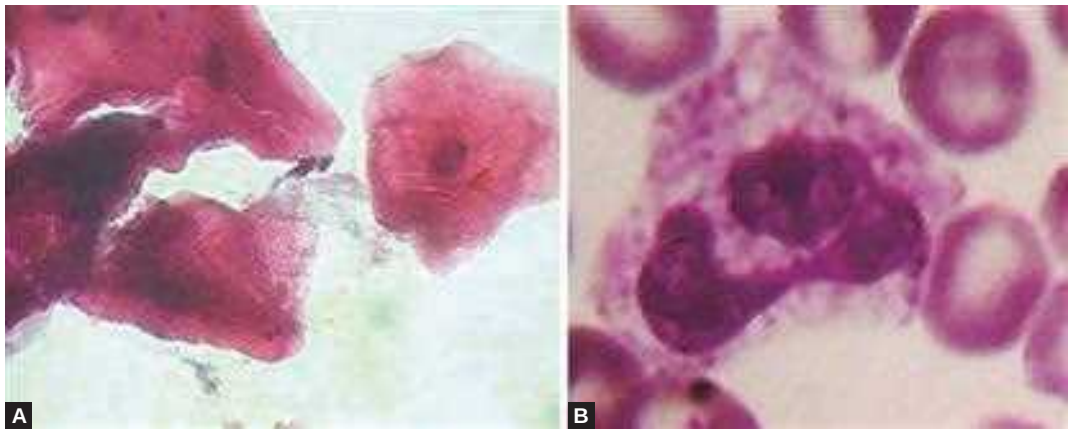
- Appears as a deeply stained body attached to the nucleus of the polymorphonuclear leukocytes.
- Appendage is attached to a lobe of nucleus by a filament of variable length and thickness.
- It consists of head of about 1.5 μm in diameter.
- Seen in all types of polymorphonuclear leukocytes but only that in neutrophils to be considered.
- Incidence: 2–3% or 6/500 cells in normal females.
- It is a highly condensed X chromosome which, in the presence of another X chromosome, may be extruded from the main body of the nucleus of polymorphonuclear leukocytes.

Chromosomal Abnormalities

Chromosomal abnormalities are classified broadly into numerical and structural. In each, those involving autosomes and those involving sex chromosomes are included (Tables 2.3 and 2.4).

Allele

A normal (somatic) cell has two variants (alleles) for a trait/character. A gamete (sperm, egg) contains one allele,



Figs 2.10A and B: Sex chromatin: (A) Barr body in buccal smear; (B) Drumstick (Davidson body) in neutrophil

TABLE 2.3: Structural abnormalities of chromosomes

Abnormality	*Feature*	*Clinical condition*
Deletion	Loss of segment of a chromosome	• Wolf-Hirschhorn syndrome—4p- • Cri-du-chat syndrome—5p-
Microdeletion	Deletion detected by high-resolution banding	Proximal part of long arm of 15q • If paternally inherited—Prader-Willi syndrome • If maternally inherited—Angelman syndrome
Inversion	Detachment of a part of chromosome by 2 breaks	Rarely causes problem
Duplication	Abnormal splitting of chromosomes	
Isochromosome	Duplication of one entire chromosome arm and deletion of other chromosome arm	Duplication of genes
Ring chromosome	Chromosome is deleted at both ends Deleted sticky ends adhere to each other in the form of a ring	Clinical manifestations depend on deletion of specific genes
Translocation	Exchange of segments between nonhomologous chromosomes	• May not always produce abnormal phenotype • But can lead to formation of unbalanced gametes • Carries high risk of abnormal progeny

randomly chosen from the two somatic alleles. For example, if you have one allele for brown eyes (represented as B) and one for blue eyes (represented as b), somatic cells have

TABLE 2.4: Numerical abnormalities of chromosomes

Name of the abnormality	*Numerical anomaly*	*Autosomal/Sex chromosomal*
Down syndrome	Trisomy, 21	Autosomal
Edwards' syndrome	Trisomy, 18	Autosomal
Patau syndrome	Trisomy,13	Autosomal
Turner's syndrome	45, XO	Sex chromosomal
Klinefelter's syndrome	47, XXY	Sex chromosomal

alleles for both (Bb) and each gamete will carry one of it (B or b) chosen randomly.

If the two alleles are different (*heterozygous*, e.g. Bb), the trait associated with only one of these will be visible (dominant) while the other will be hidden (recessive). For example, B is dominant, b is recessive. If two alleles are same (dominant/recessive) it is homozygous (e.g. BB/bb).

Punnett Square

- It is a diagram used to predict the result of a genetic cross (Fig. 2.11).
- Used to determine the probability of an offspring having a particular genotype.
- For the above example, it can be shown as follows:
  - *Genotype:* The state of the two alleles at one or more locus associated with a trait/character.
  - *Phenotype:* The state of the observable trait/character.

Genotype and phenotype for brown and blue eyes have been shown in Table 2.5.

Symbols Used for Chromosomal Nomenclature

Symbols used in karyotype have been shown in Table 2.6.

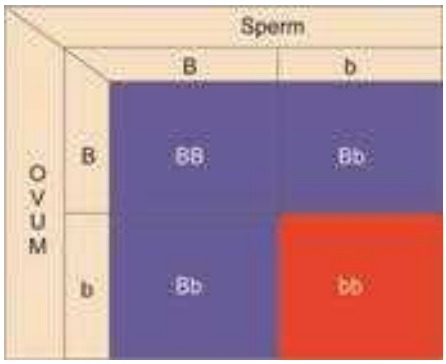


Fig. 2.11: Punnett square diagram

TABLE 2.5: Genotype and phenotype for brown and blue eyes

Genotype	*Phenotype*
BB (homozygous)	Brown eyes
Bb (heterozygous)	Brown eyes
bb (homozygous)	Blue eyes

TABLE 2.6: Symbols used in karyotype

Symbol	*Karyotype*
46,XX	Normal female
46,XY	Normal male
A–G	Chromosome groups
1–22	Autosome numbers
X,Y	Sex chromosomes
46/47	Mosaicism
del	Deletion
dup	Duplication
inv	Inversion
r	Ring chromosome

INHERITANCE OF GENETIC DISORDERS

The pattern of inheritance of genetic disorders facilitates the diagnosis of the disorder, calculation of risk of the disorder in the present and future offspring and for counseling the parents. By obtaining family history, a *pedigree chart* is prepared to understand the pattern of occurrence (inheritance) of the disease in the families. By drawing a Punnett square the percentage risk can be interpreted.

Pedigree Chart

It is a pictorial representation of generations of a family showing the information of family members and their relationship to one another, marriages among cousins (consanguineous) including details of live births, stillbirths and abortions, etc. A pedigree chart shows genetic connections among individuals using standardized symbols. For drawing pedigree charts certain standard symbols are used (Fig. 2.12). Knowledge of probability and Mendelian patterns are required for understanding the basis for a trait. Conclusions are most accurate if they are drawn using large number of pedigrees (generations). A sample pedigree chart is presented in Figure 2.13.

According to the *mode of transmission the genetic disorders* can be classified as follows:

1. Autosomal dominant inheritance
2. Autosomal recessive inheritance
3. X-linked dominant inheritance
4. X-linked recessive inheritance
5. Y-linked inheritance
6. Multifactorial inheritance.

Autosomal Dominant Inheritance (Fig. 2.14)

- The mode of transmission is vertical. An affected person has an affected parent.
- There is 50% of chance of dominant trait being transmitted to offsprings.
- Both males and females are equally affected.

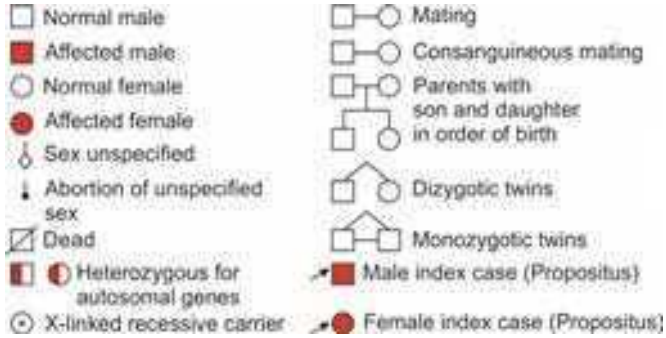


Fig. 2.12: Symbols used in pedigree chart

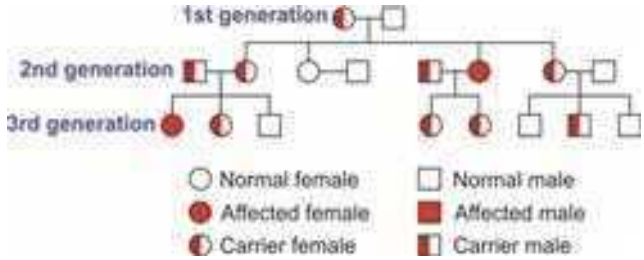


Fig. 2.13: Sample pedigree chart

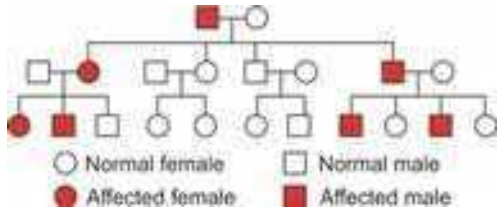


Fig. 2.14: Pedigree chart of autosomal dominant inheritance

- Dominant gene is expressed in heterozygotes.
- Delayed age of onset.
- The trait appears in every generation without skipping.
- An unaffected offspring does not transmit the disease.
- Examples:
  - Achondroplasia
  - Angioneurotic edema
  - Huntington's chorea
  - Multiple neurofibromatosis
  - Osteogenesis imperfecta.

Autosomal Recessive Inheritance (Fig. 2.15)

- Horizontal transmission. The trait appears in sibs and parents are normal.
- History of consanguineous marriage. The parents are blood related. Both the couple are carriers of abnormal gene.
- 25% chance of having an affected child (double dose of abnormal gene) in a carrier couple.
- Early age of onset.
- Both males and females have an equal chance of getting affected.
- Examples:
  - Cystic fibrosis
  - Inborn errors of metabolism—albinism, phenylketonuria
  - Hemoglobinopathies—sickle-cell anemia, thalassemia.

X-linked Dominant Inheritance (Fig. 2.16A)

- Trait is more frequent in females than in males.
- Affected male transmit the trait to all his daughters not to his sons.
- Affected females if homozygote, transmit to all of her children.
- If affected females are heterozygote, transmit the trait to half her children of either sex.
- Example:
  - Vitamin D-resistant rickets
  - Xg blood groups.

X-linked Recessive Inheritance (Fig. 2.16B)

- Females (XX) are the carriers. One X chromosome contains abnormal gene. Allelic gene on other X chromosome is normal.
- Males are the victims. When abnormal gene involves nonhomologous part of single X chromosome of male (XY) disease is expressed. Defective gene has no corresponding allele in Y chromosome to counteract.
- If mother is carrier and father is healthy, 50% of her sons are affected by the disease and 50% of her daughters are carriers.
- Examples:
  - Hemophilia
  - Partial color blindness
  - Glucose-6-phosphate dehydrogenase (G6PD) deficiency
  - Duchenne muscular dystrophy.

Y-linked Inheritance (Fig. 2.15C)

- Y-linked traits are present in all male descendants of affected male.
- The genes that are carried on the Y-chromosome are called *holandric genes*.

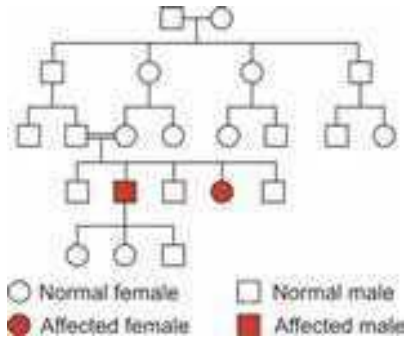
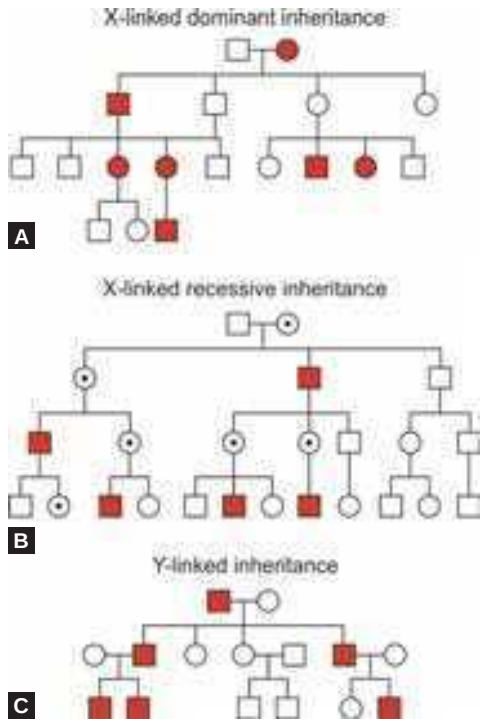


Fig. 2.15: Pedigree chart of autosomal recessive inheritance



Figs 2.16A to C: Pedigree chart of sex-linked inheritance. (A) X-linked dominant inheritance; (B) X-linked recessive inheritance; (C) Y-linked inheritance

- Dominant and recessive pattern will not apply as only one allele is present.
- Example:
  - Hairy pinna.

Multifactorial Inheritance

- It includes genetic and environmental factors like:
  - Drugs—thalidomide, anticancer drugs, antiepileptic drugs and antimalarial drugs.
  - Viral infections—rubella virus, papilloma virus
  - Ionizing radiation—X-rays and radioactive substances like I^{131}.
- Examples:
  - Cleft lip and cleft palate
  - Clubfoot
  - Congenital heart disease
  - Neural tube defects—anencephaly and spina bifida.

CELL DIVISION

- Multiplication of cells takes place by division of pre-existing cells. Such multiplication constitutes an essential feature of embryonic development. Cell multiplication is equally necessary after the birth of the individual for growth and for replacement of dead cells.
- We have seen that chromosomes within the nuclei of cells carry genetic information that controls the development and functioning of various cells and tissues; and, therefore, of the body as a whole. When a cell divides, it is essential that the entire genetic information within it be passed on to both the daughter cells resulting from the division. In other words, the daughter cells must have chromosomes identical in number (and in genetic content) to those in the mother cell. This type of cell division is called mitosis.
- A different kind of cell division called *meiosis* occurs during the formation of the gametes. This consists of two successive divisions called the *first* and *second meiotic divisions*. The cells resulting from these divisions (i.e. gametes) differ from other cells of the body in that
  - the number of chromosomes is reduced to half the normal number.
  - the genetic information in the various gametes produced is not identical.

Mitosis

Many cells of the body have a limited span of functional activity, at the end of which they undergo division into two daughter cells. The daughter cells in turn have their own span of activity, followed by another division. The period during which the cell is actively dividing is the phase of mitosis. The period between two successive divisions is called the *interphase*.

Mitosis is conventionally divided into a number of stages called *prophase, metaphase, anaphase* and *telophase*. The sequence of events of the mitotic cycle is best understood starting with a cell in telophase. At this stage, each chromosome consists of a single chromatid (Fig. 2.17G). With the progress of telophase, the chromatin of the chromosome uncoils and elongates and the chromosome can no longer be identified as such. However, it is believed to retain its identity during the interphase (which follows telophase).

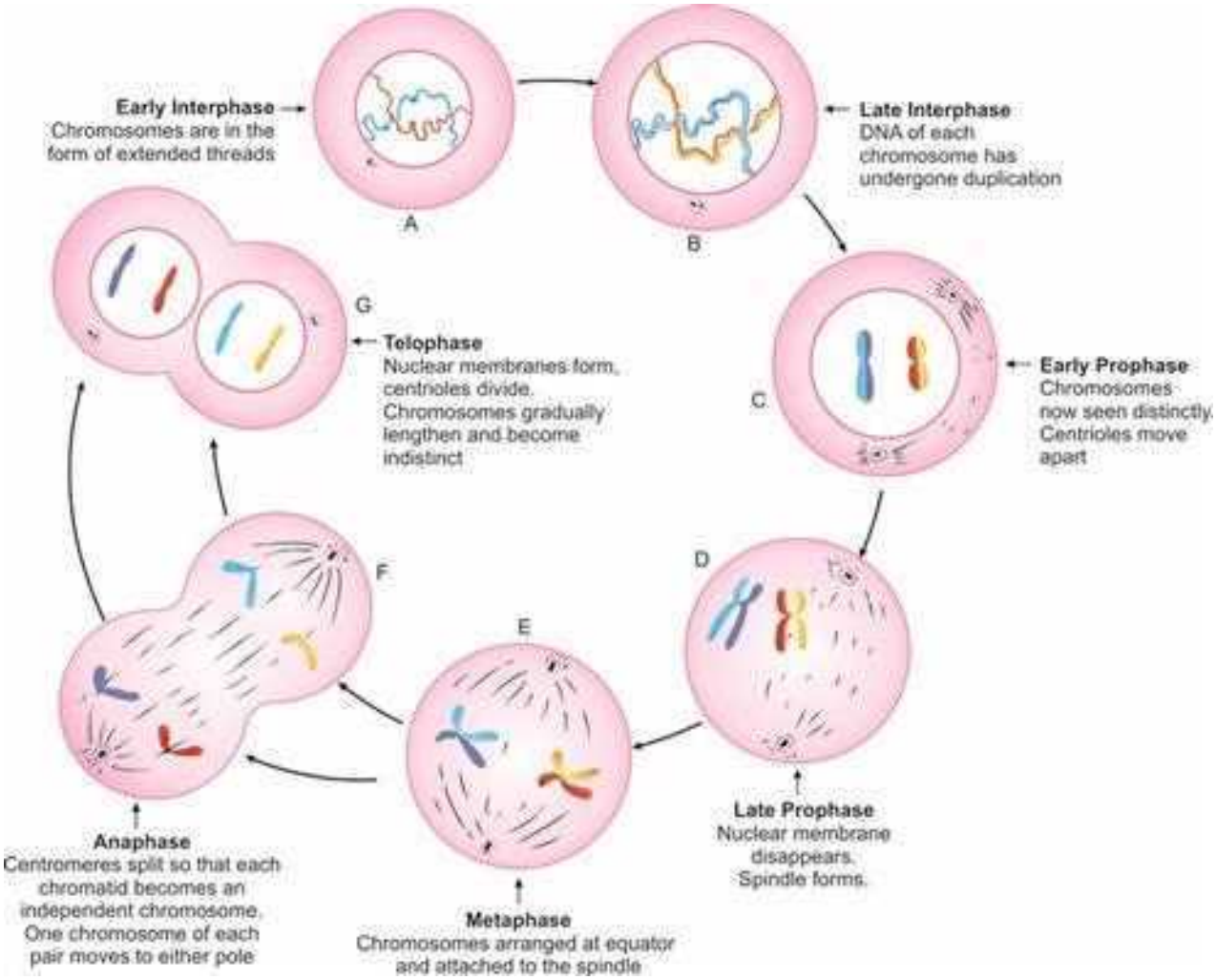
Interphase: This is shown diagrammatically in Figure 2.17A. During a specific period of the interphase, the DNA content of the chromosome is duplicated so that another chromatid identical to the original one is formed; the chromosome is now made up of two chromatids (Fig. 2.17B).

Prophase: When mitosis begins (i.e. during prophase), the chromatin of the chromosome becomes gradually more and more coiled so that the chromosome becomes recognizable as a thread-like structure that gradually acquires a rod-like appearance (Fig. 2.17C). Toward the end of prophase, the two chromatids constituting the chromosome become distinct (Fig. 2.17D) and the chromosome now has the typical structure illustrated in Figure 2.4. While these changes are occurring in chromosomes, a number of other events are also taking place. The two centrioles separate and move to opposite poles of the cell. They produce a number of microtubules that pass from one centriole to the other and form a spindle. Meanwhile the nuclear membrane breaks down and nucleoli disappear (Fig. 2.17D).

Metaphase: With the formation of the spindle, chromosomes move to a position midway between the two centrioles (i.e. at the equator of the cell) where each chromosome becomes attached to microtubules of the spindle by its centromere. This stage is referred to as metaphase (Fig. 2.17E).

Anaphase: The centromere of each chromosome splits longitudinally into two so that the chromatids now become independent chromosomes. At this stage, the cell can be said to contain 46 pairs of chromosomes. One chromosome of each such pair now moves along the spindle to either pole of the cell (Fig. 2.17F).

Telophase: In this phase, the two daughter nuclei are formed by appearance of nuclear membranes. Chromosomes gradually elongate and become indistinct. Nucleoli reappear. The centriole is duplicated at this stage or in early interphase (Fig. 2.17G). The division of the nucleus is accompanied by the division of the cytoplasm. In this process, the organelles are presumably duplicated and each daughter cell comes to have a full complement of them.



Figs 2.17A to G: Scheme to show the main steps of mitosis

Meiosis

The meiosis consists of two successive divisions called the first and second meiotic divisions. During the interphase preceding the first division, duplication of the DNA content of chromosomes takes place as in mitosis. As a result, another chromatid identical to the original one is formed. Thus, each chromosome is now made up of two chromatids.

First Meiotic Division

Prophase: The prophase of the first meiotic division is prolonged and is usually divided into a number of stages as follows:

Leptotene: The chromosomes become visible (as in mitosis). Although each chromosome consists of two chromatids, these cannot be distinguished at this stage (Fig. 2.18A).

Zygotene: The 46 chromosomes in each cell consist of 23 pairs (the X- and Y-chromosomes of a male being taken as a pair). The two chromosomes of each pair come to lie parallel to each other, and are closely apposed. This pairing of chromosomes is also referred to as synapsis or conjugation. The two chromosomes together constitute a bivalent (Fig. 2.18B).

Pachytene: The two chromatids of each chromosome become distinct. The bivalent now has four chromatids in it and is called a *tetrad*. There are two central and two peripheral chromatids, one from each chromosome (Fig. 2.18C). An important event now takes place. The two central chromatids (one belonging to each chromosome of the bivalent) become coiled over each other so that they cross at a number of points. This is called *crossing over*. For sake of simplicity only one such crossing is shown in Figure

2.18D. At the site where the chromatids cross, they become adherent; the points of adherence are called *chiasmata*.

Diplotene: The two chromosomes of a bivalent now try to move apart. As they do so, the chromatids involved in crossing over "break" at the points of crossing and the "loose" pieces become attached to the opposite chromatid. This results in exchange of genetic material between these chromatids. A study of Figure 2.18E will show that each of the four chromatids of the tetrad now has a distinctive genetic content.

Metaphase: As in mitosis the 46 chromosomes become attached to the spindle at the equator, the two chromosomes of a pair being close to each other (Fig. 2.19A).

Anaphase: The anaphase differs from that in mitosis in that there is no splitting of the centromeres. One entire chromosome of each pair moves to each pole of the spindle (Fig. 2.19B). The resulting daughter cells, therefore, have 23 chromosomes, each made up of two chromatids (Fig. 2.19C).

Telophase: The anaphase is followed by the telophase in which two daughter nuclei are formed. The division of the nucleus is followed by division of the cytoplasm.

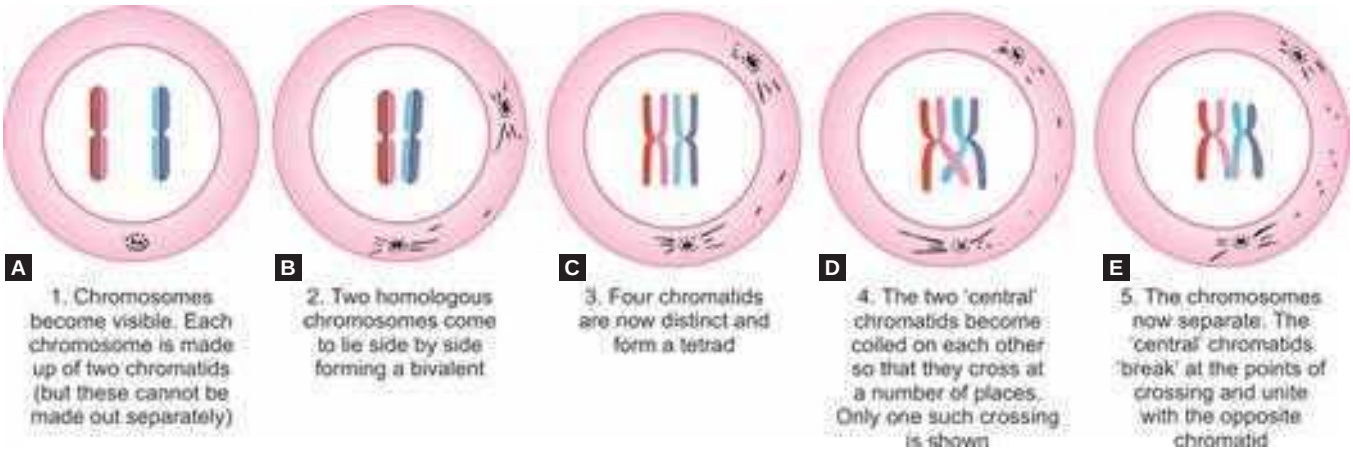
Second Meiotic Division

The first meiotic division is followed by a short *interphase*. This differs from the usual interphase in that *there is no duplication of DNA*. Such duplication is unnecessary as chromosomes of cells resulting from the first division already possess two chromatids each (Fig. 2.19C).

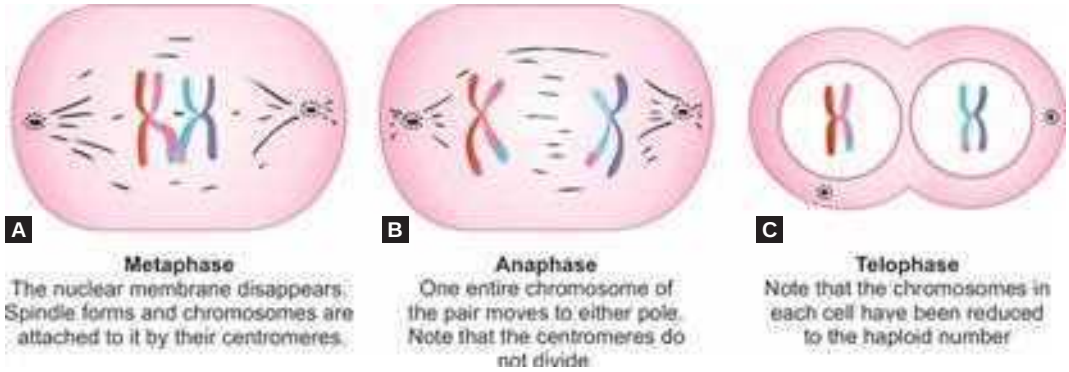
The second meiotic division is similar to mitosis. However, because of the crossing over that has occurred during the first division, the daughter cells are not identical in genetic content (Fig. 2.20).

Significance of Meiosis

- In this kind of cell division, there is reduction of the number of chromosomes from diploid to haploid. At the time of fertilization, the diploid number (46) is restored.



Figs 2.18A to E: Stages in the prophase of the first meiotic division



Figs 2.19A to C: (A) Metaphase; (B) Anaphase; (C) Telophase of the first meiotic division

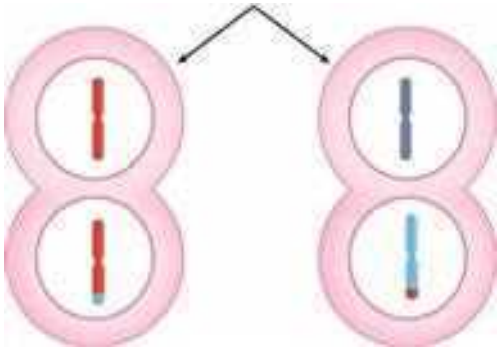


Fig. 2.20: Daughter cells resulting from the second meiotic division. These are not alike because of the crossing-over during first meiotic division

This provides consistency of chromosome number from generation to generation.

- The 46 chromosomes of a cell consist of 23 pairs, one chromosome of each pair being derived from the mother and one from the father. During the first meiotic division, the chromosomes derived from the father and those derived from the mother are distributed between the daughter cells entirely at random.
- This, along with the phenomenon of crossing over, results in thorough shuffling of the genetic material so that the cells produced as a result of various meiotic divisions (i.e. ova or spermatozoa); all have a distinctive genetic content.
- A third step in this process of genetic shuffling takes place at fertilization when there is a combination of randomly selected spermatozoa and ova. It is, therefore, not surprising that no two persons (except identical twins) are alike.

Clinical correlation

Nondisjunction

After splitting of centromere one or more chromosomes fail to migrate properly due to abnormal function of achromatic spindle. This results in one daughter with trisomy and one with monosomy.

- Occurs both in mitosis and meiosis
- Involves both sex chromosomes and autosomes
- Autosomal nondisjunction less viable.
- Mitosis: Nondisjunction in first cleavage division of zygote leads to mosaicism
- Meiosis I: 2 disomic (24) + 2 nullisomic (22) gametes (Fig. 3.25)
- Meiosis II: 2 normal monosomic + 1 abnormal disomic + 1 abnormal nullisomic gametes (Fig. 3.26).

Trisomy

- During gametogenesis:
  - Meiosis I: The 2 chromosomes of a pair go to same pole
  - Meiosis II: A pair of sister chromatids go to same pole
  - Results in a gamete having 24 chromosomes.
- At fertilization:
  - Abnormal gamete + Normal gamete = Trisomy, i.e. 24 chromosomes + 23 chromosomes = 47 chromosomes in zygote.

Monosomy

- During gametogenesis:
  - Meiosis I: The 2 chromosomes of a pair go to one gamete
  - Meiosis II: A pair of sister chromatids go to same gamete
  - Results in a gamete having 22 chromosomes.
- At fertilization:
  - Abnormal gamete + Normal gamete = Monosomy, i.e. 22 chromosomes + 23 chromosomes = 45 chromosomes in zygote.

REVIEW QUESTIONS

1. Write short notes on sex-chromatin.
2. Write short notes on allele.
3. Describe sex linked inheritance.
4. Describe autosomal dominant inheritance.
5. Describe autosomal recessive inheritance.

Chapter 3

Reproductive System, Gametogenesis, Ovarian and Menstrual Cycles

HIGHLIGHTS

- *Reproduction* is the process of formation of a new living organism. For reproduction in higher animals, presence of dimorphic *gametes* and sex organs are required.
- The gametes in males are called *spermatozoa* and are produced in testis. In females, they are called *ova* and are produced in ovary.
- The gametes are derived from *primordial germ cells (PGC)/primitive sex cells*. These cells do not develop in gonads. They are derived from ectoderm or epiblast the first embryonic germ layer.
- The process of formation of gametes is called *gametogenesis*. In males, it is called *spermatogenesis* and in females the *oogenesis*.
- Stages of spermatogenesis are summarized in Flowchart 3.1.
- Spermatozoa are derived from rounded spermatids.
- *Spermiogenesis* is the process of conversion from a typical cell (spermatid) to a specialized cell the spermatozoon.
- A spermatozoon has a head, a neck, a middle piece and a principal piece or tail.
- Stages of *oogenesis* are summarized in Flowchart 3.2.
- An *ovarian follicle* is a rounded structure that contains a developing ovum surrounded by follicular cells. The follicle has a cavity filled with fluid.
- Ovarian follicles have a cellular covering called the theca interna. The cells of the theca interna produce estrogens.
- The follicle gradually increases in size and finally bursts and expels the ovum. This process of shedding of the ovum is called *ovulation*.
- The *corpus luteum* is formed by enlargement and transformation of follicular cells, after-shedding of the ovum. The corpus luteum secretes progesterone, which is essential for maintenance of pregnancy.
- The term *menstrual cycle* is applied to cyclical changes that occur in the endometrium every month. The most obvious feature is a monthly flow of blood (*menstruation*).
- The menstrual cycle is divided into the following phases: *postmenstrual, proliferative, secretory, and menstrual.*
- The menstrual cycle is also divided into the *follicular phase* (in which changes are produced mainly by estrogens) and the *luteal phase* (in which effects of progesterone predominate). Both phases are of roughly equal duration.
- The main changes in the endometrium during menstrual cycle are (a) increase in thickness, (b) growth of uterine glands, (c) changes in epithelial cells lining the glands and (d) increase in thickness and fluid content of the endometrial stroma.
- Just before onset of menstruation, the blood supply to superficial parts of the endometrium is cut off. This part is shed off and there is bleeding.
- The menstrual cycle is influenced by estrogens, progesterone, follicle stimulating hormone (FSH) and luteinizing hormone (LH).

INTRODUCTION

The term "reproduction" means formation of new living organism that closely resembles the parents. The purpose of reproduction is maintenance and propagation of species. It requires the presence of dimorphic gametes. In higher animals it is accomplished by separate male and female sexual organs. The processes involved in reproduction are complicated.

The *gametes* or *germ cells* are produced in the gonads (testis in males and ovary in females). The male gametes are called *spermatozoa* and female gametes the *ova*.

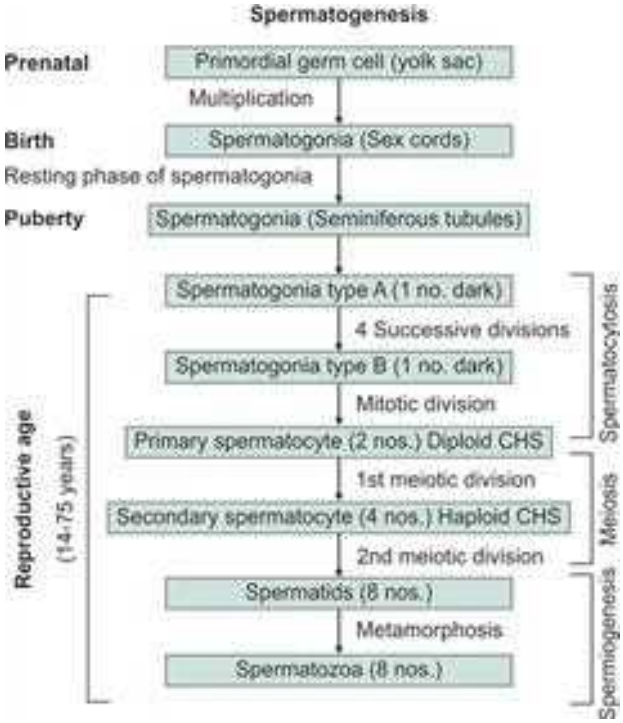
At the time of sexual intercourse the male gametes are introduced into the female reproductive tract where *fertilization* (fusion of male and female gametes) takes place. This initiates the development of a new individual the *embryo* that grows further to be called as *fetus*.

The *growth* and *development* of fertilized egg in the female reproductive system is called *pregnancy*. The woman who bears the fertilized egg that grows for a period of 10 lunar months is called a pregnant woman. At the end of growth period the fetus is delivered and is nourished by mother's milk for a certain period which is known as *lactation*.

MALE REPRODUCTIVE SYSTEM

It consists of external and internal genital organs. External genital organs include penis, scrotum and its contents.

Flowchart 3.1: Stages of spermatogenesis



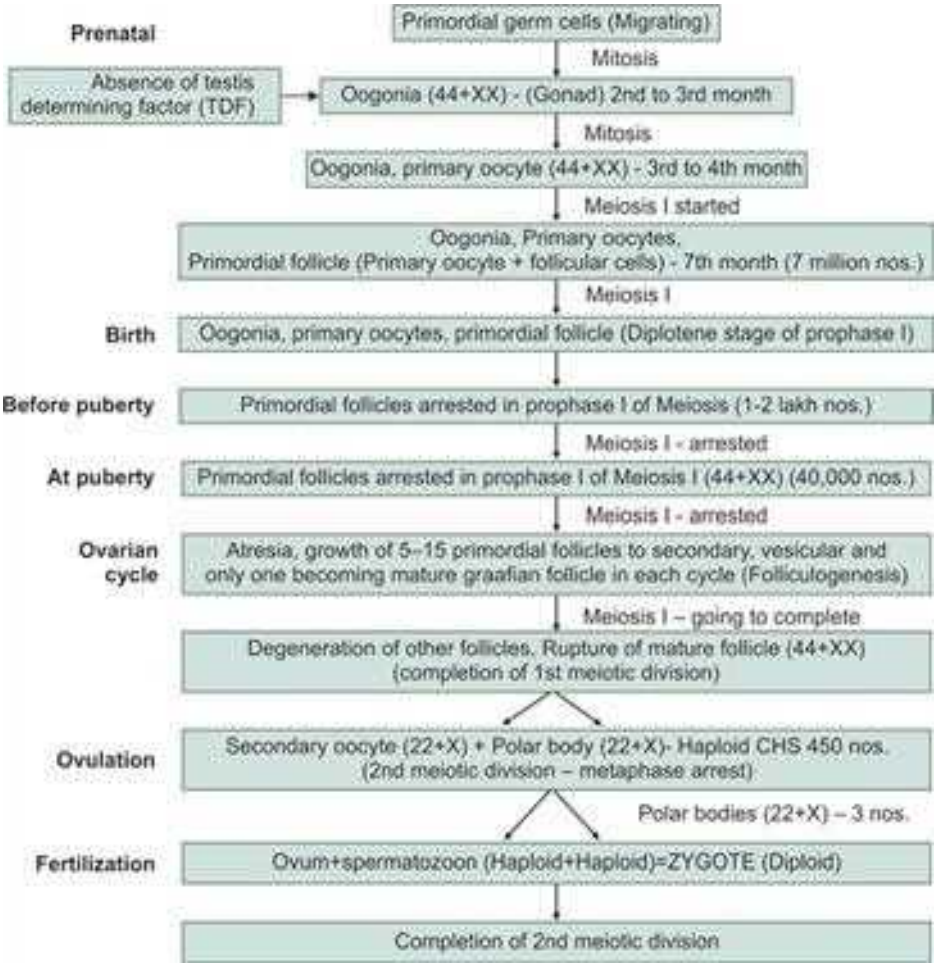
Internal genital organs include the gonads, the duct system and accessory sex organs. Male organs of reproduction (Fig. 3.1) and their functions are:

- *Gonads*: These are a pair of organs known as *testes* (plural)/*testis* (singular). The gonads produce male sex cells and secrete male hormone the testosterone.
- *Duct system*: It includes *epididymis, vas deferens, ejaculatory ducts* and *urethra* that extend from the testes to penis (copulatory organ) and assist in storage, maturation and transport of male sex cells.
- *Accessory sex glands*: These include *seminal vesicle*, *prostate* and *bulbourethral glands*. These secrete fluid that helps in nutrition and transport of sperms. Contraction of smooth muscle that is present in these glands causes a thorough mixture of secretions of accessory glands and spermatozoa which is known as "semen".
- *Penis*: It is a muscular and highly vascular organ that ejaculates or deposits spermatozoa into the vagina of female during sexual intercourse.
- *Scrotum*: The testis is located in the scrotum. It protects the testis and maintains the temperature suitable for spermatogenesis.

Testis

- Testis is oval in shape and is suspended in the scrotal sac by spermatic cord. It is obliquely oriented in scrotal sac. Its upper end is connected to head of epididymis by efferent ductules and is overlapped by it. Lower end is connected to tail of epididymis by areolar tissue. The lateral surface is overlapped by body of epididymis in its posterior part.
- Testis is covered by three covering from outside inwards. They are *tunica vaginalis* (serous layer), *tunica albuginea* (fibrous capsule) and *tunica vasculosa* (vascular membrane). The *tunica albuginea* is thickened on the posterior aspect of the testis to form an incomplete partition called *mediastinum testis*. From the anterior surface of mediastinum number of septa extend into the substance of testis and divide it into 250–300 lobules (Fig. 3.2).
- *Lobule of testis*: Each lobule contains 2–4 tightly coiled *seminiferous tubules* that form the exocrine part of testis and interstitial cells of *Leydig* between them form the endocrine part. The spermatozoa are produced in seminiferous tubules and the process is known as "*spermatogenesis*".
- *Seminiferous tubule*: Seminiferous tubules are structural and functional units of testis. Each seminiferous tubule when uncoiled is about 70–80 cm (2 feet) in length.

Flowchart 3.2: Stages of oogenesis



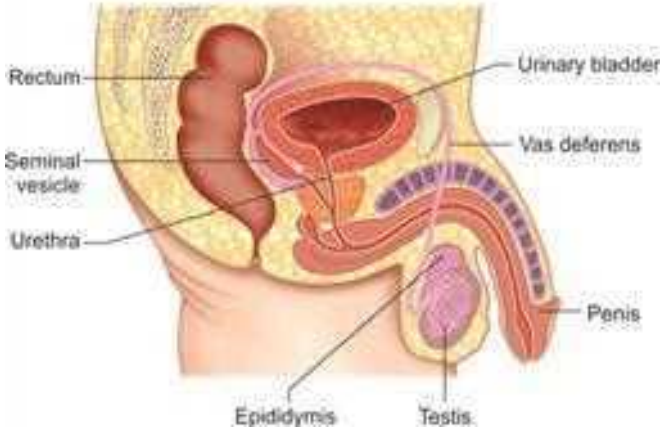


Fig. 3.1: Male reproductive system

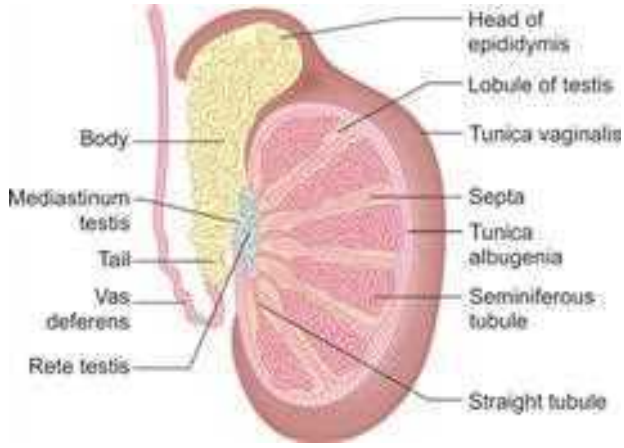


Fig. 3.2: Vertical section of testis, epididymis and vas deferens

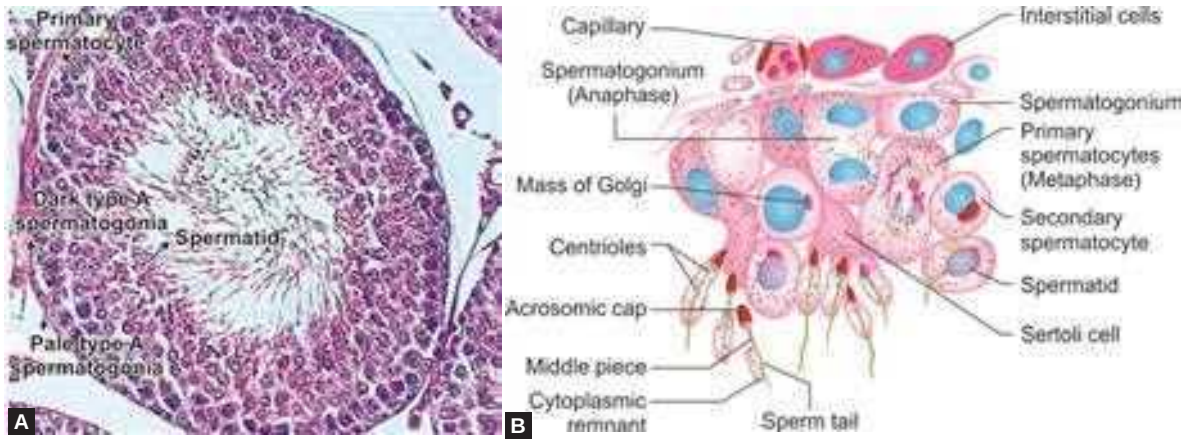
It consists of a coiled part in front and a straight part behind. The straight part extends to mediastinum of testis where it joins with adjacent tubules and forms a plexiform network in mediastinum known as *rete testis*. About 10–20 efferent ductules arise from rete testis and enter the head of epididymis (Fig. 3.2).

- *Seminiferous tubule (Microscopic structure)*: Each seminiferous tubule is covered by basement membrane externally. Internally it is lined by complex stratified epithelium composed of two main types of cells, the *spermatogenic cells* and *supporting cells of Sertoli* (Fig. 3.3A).
  - *Spermatogenic cells are given different names* in different stages of development. Stem cells are known as *spermatogonia* that develop from *primordial germ cells (PGC)*. At puberty the spermatogonia start maturing into *primary spermatocytes*, *secondary spermatocytes*, *spermatids* and *spermatozoa*. The spermatozoa are released into the lumen of seminiferous tubules. Within the basement membrane the spermatogenic cells are arranged in several layers. An outer layer of cuboidal cells with small nuclei enlarge to form *spermatogonia*. Large polyhedral cells with clear nuclei in two or three layers form the *spermatocyte*s, primary and secondary. By meiotic division the *primary spermatocytes* give rise to secondary spermatocyte. *Secondary spermatocytes* undergo another maturation division to form the *spermatids* that metamorphose into spermatozoa. The spermatozoa occupy the luminal aspect of the seminiferous tubule (Fig. 3.3B).
  - *Sertoli cells*: These are polyhedral cells situated between the spermatogenic cells. They extend from the basement membrane to the lumen of the seminiferous tubule. Each is fixed to the basal lamina of seminiferous epithelium. The nucleus is situated toward the basal part of the cell. It forms an elaborate system of thin processes that extend to the lumen. The spermatogenic cells lie in the deep depressions formed by the Sertoli cells. Sertoli cells provide nutrition to the developing spermatozoa, phagocytose the excess cytoplasm of spermatids, control the movement and release of spermatogenic cells and produce the hormone inhibin (Fig. 3.3B).
- *Leydig cells*: These are larger polyhedral cells of 14–21 microns diameter, lying in connective tissue between the seminiferous tubules (Fig. 3.3B). The cells contain fat, phospholipids and Vitamin C and secrete the male hormone testosterone. The function of testosterone is to stimulate development of male genitalia, maintenance of spermatogenesis (formation and maturation of spermatozoa), development and maintenance of secondary sexual characters like growth of beard, male type of hair distribution and male voice.

FEMALE REPRODUCTIVE SYSTEM

It consists of external and internal genital organs. External genital organs are collectively known as *vulva*. Internal genital organs include the *ovaries*, *uterine/fallopian tubes*, *uterus* and *vagina*. Female organs of reproduction (Fig. 3.4) and their functions are:

- *Gonads*: Two in number and are known as *ovaries* (Plural)/*ovary* (singular). The ovaries produce female sex cells (oocytes) and secrete female hormones (estrogen and progesterone), inhibin and relaxin.
- The *uterine tube* or *oviduct* transports the oocyte to uterus and is the seat of fertilization. These are a pair of ducts that convey sperms to reach an ovum, and transport secondary oocytes from ovary to uterine tube for fertilization and fertilized ovum from uterine tube to



Figs 3.3A and B: Cut-section of seminiferous tubule showing cells of spermatogenic lineage and Sertoli cells

uterine cavity. The uterine tube presents two openings and four parts. The two openings are uterine ostium by means of which it communicates with the uterus and the abdominal/pelvic ostium through which it communicates with the peritoneal cavity. The margins of pelvic ostium present finger-like processes called *fimbriae*. From medial to lateral it presents four parts, i.e. intramural, isthmus, ampulla and infundibulum. *Ampulla* is the widest and longest part of uterine tube where *fertilization* takes place. The funnel-shaped lateral end of tube is called "infundibulum".

- The uterus is the seat of *implantation* of fertilized ovum, growth of embryo and fetus during pregnancy and delivery of fetus at labor.
- Vagina is an intromittent organ that receives penis during copulation and is the site of release of sperms. It is the passage for child birth.

Ovaries

- Ovaries are almond-shaped structures. They are intraperitoneal organ and dull gray in color. Before puberty, the surface is smooth. Afterwards due to repeated ovulations the surface becomes irregular. They are located one on either side of uterus below and behind the corresponding uterine tube. They are attached to the posterior layer of broad ligament (Fig. 3.4).
- Each ovary presents two ends, two borders and two surfaces. The tubal end is close to the fimbriae of uterine tube and is attached along with uterine tube to the lateral pelvic wall by suspensory ligament of ovary. The uterine end is connected to the uterus by ligament of ovary.
- Ovaries produce the female gametes the *oocytes* that develop into *ova* after fertilization. They produce the female sex hormones the *progesterone* and *estrogen* that are required for maturation of gametes, receptive uterine endometrium for implantation of fertilized ovum and growth of embryo and fetus.
- *Microscopic structure (Fig. 3.5)*:
  - The surface of ovary is covered by simple cuboidal epithelium known as *germinal epithelium*. Underneath the germinal epithelium is a capsule of dense connective tissue known as *tunica albuginea*. The substance of ovary is divided into outer *cortex* and inner *medulla*.
  - The inner medulla contains loose connective tissue, blood vessels, lymphatics and nerves.
  - The outer cortex contains connective tissue stoma and ovarian follicles. Cortex of ovary is the seat of cyclical changes known as "ovarian cycle" during reproductive age of the female. It contains ovarian follicles in different stages of development.
  - The formation of gametes in female gonads is called *oogenesis* and the development of ovarian follicles is known as *folliculogenesis*.
  - The morphology and morphometry of ovarian follicles varies at different periods of life—prenatal, prepubertal, reproductive and menopausal.

Uterus

- Uterus is a hollow, pear-shaped muscular organ where the embryo/fetus develops.
- It has three parts: (1) the fundus, (2) body and (3) cervix. The dome shaped upper part above the openings of

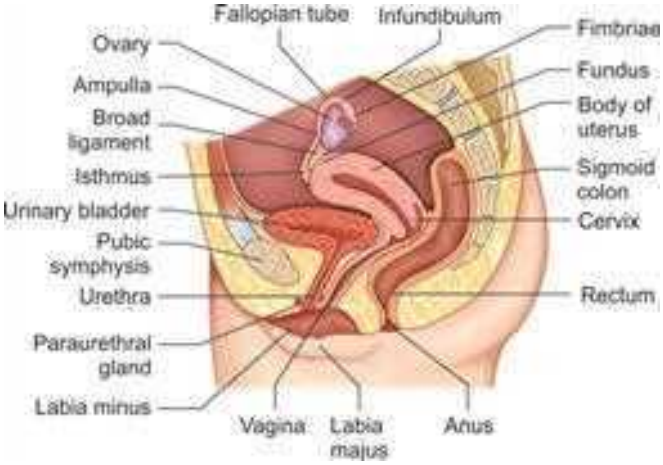


Fig. 3.4: Female reproductive system

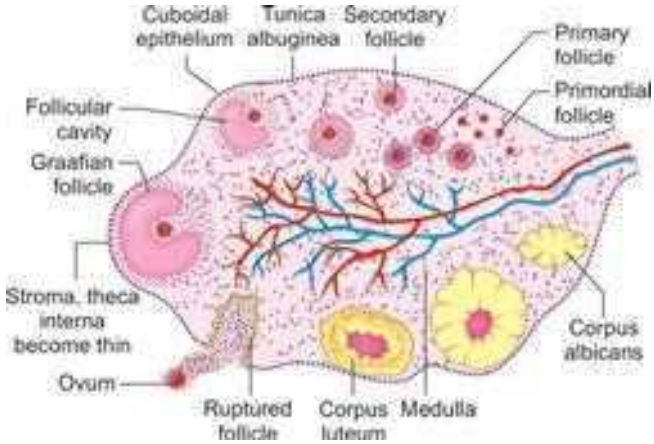


Fig. 3.5: Cut-section of ovary showing various stages of development of ovarian follicles, ovulation and degenerating corpus luteum

uterine tubes is the fundus. Body is the main part that presents a cavity where the fetus grows. Cervix is the lower cylindrical part that projects into the vagina (Fig. 3.4).

- The wall of the uterus is made up of three layers (Fig. 3.6).
  1. The outermost layer or perimetrium is made up of *peritoneum*.
  2. The main thickness of the wall is made up of smooth muscle. This is the *myometrium* and is highly vascular. The muscle fibers run in different directions with each one having a functional role (Table 3.1 and Fig. 3.7). During pregnancy these fibers undergo hypertrophy and hyperplasia.
  3. The innermost layer (corresponding to mucous membrane) is called the *endometrium*. It is this layer which undergoes cyclical changes called "menstrual cycle".

To understand the menstrual cycle it is necessary to know the structure of the uterus.

- The constituents of the endometrium are as follows (Fig. 3.7):
  - The epithelium lining the surface of the endometrium is columnar.
  - The stroma fills the interval between surface epithelium and myometrium. It contains numerous simple tubular glands (uterine glands) that secrete mucus.
  - The arteries that supply the endometrium tend to run vertically toward the surface. Some of these run spirally and supply the whole thickness of the endometrium, while others that remain straight are confined to the basal part.

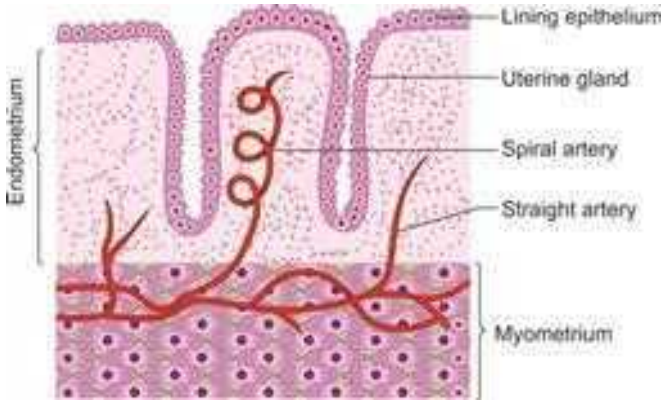


Fig. 3.6: Layers in the wall of uterus and components of uterine endometrium that undergo changes during menstrual cycle

TABLE 3.1: Myometrium—Direction of muscle fiber layers and functions

Direction of muscle fiber layer	*Function*
Outer longitudinal	Expulsion of contents
Middle circular	Retention of products of conception
Inner reticular	Living ligature of uterus

GAMETOGENESIS

The process of formation of gametes is called "gametogenesis". The gametes are derived from *primordial germ cells/ primitive sex cells*.

- *Primordial germ cells (PGC)/Primitive sex cells*: Gametes are derived from PGC during 4th week of development. The PGC appear in the wall of yolk sac (Fig. 3.8) from which they migrate to the developing gonad from the coelomic epithelium and adjacent mesenchyme. The PGC undergo mitotic division during their migration resulting in increase in their number.

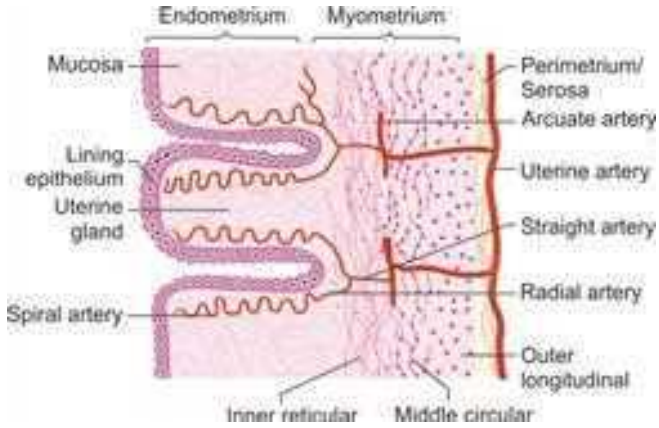


Fig. 3.7: Different strata of endometrium

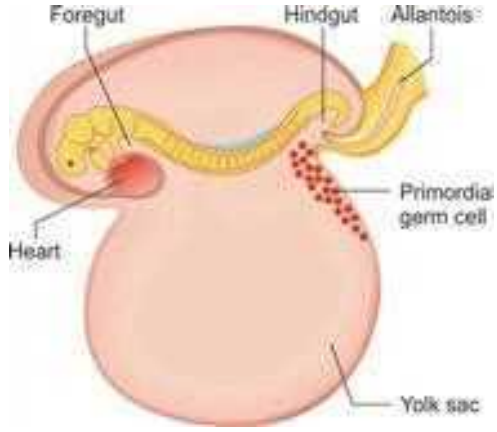


Fig. 3.8: Migrating primordial germ cells at the caudal end of yolk sac

- *Definition*: Gametogenesis can be defined as the process of conversion of primordial or primitive germ cells and their maturation into male and female gametes.
  In males it is called *spermatogenesis* and it takes place in seminiferous tubules of testis. In females it is called oogenesis and it takes place in the cortex of ovary. The differences in gametogenesis between males and females are presented in Table 3.2.
- *Different events in gametogenesis*: The process of gametogenesis includes:
  1. Formation and migration of PGC and their differentiation into male or female sex cells
  2. Mitotic divisions of germ cells
  3. Meiotic reduction in DNA or chromosome content of the germ cells
  4. Differentiation and maturation of germ cells.

  Phase 1 and 2 are similar in both sexes whereas the timing and pattern of phase 3 and 4 differ.
- The PGC are formed in the ectoderm/epiblast of the bilaminar germ disc of human embryo during 2nd week of development. They move to the wall of yolk sac by 4th week (Fig. 3.8). By 5th week they reach the developing gonad.

TABLE 3.2: Differences between spermatogenesis and oogenesis

Spermatogenesis	*Oogenesis*
Continuous process from puberty to death	Cyclical from puberty to menopause
Absent before puberty	Starts before puberty—intrauterine life
Number of gametes released are 200–300 million/ejaculation	400–500 mature ova are released during reproductive life
Meiosis I results in two secondary spermatocytes from one primary spermatocyte	Meiosis I results in one secondary oocyte from one primary oocyte
Meiosis II results in four spermatids from one primary spermatocyte	Meiosis II results in one ovum from one primary oocyte
Relatively short diplotene stage	Prolonged diplotene stage
Four gametes are of equal size	One large gamete (Ovum) and 2/3 polar bodies
Participates in fertilization only after completion of meiosis	Participates in fertilization before completion of meiosis
When the primary spermatocyte divides, its cytoplasm is equally distributed between the two secondary spermatocytes formed.	• When the primary oocyte divides, almost all its cytoplasm goes to the daughter cell, which forms the secondary oocyte. • The other daughter cell (first polar body) receives half the chromosomes of the primary oocyte, but almost no cytoplasm.
Most cytoplasm is shed from spermatozoon	Cytoplasm is conserved in the ovum

- Gonadal differentiation occurs in 6th week.
- The PGC undergo repeated mitotic divisions during their journey. The decision to develop into male sex cells (spermatogonia) and development of testis or female sex cells (oogonia) and ovary development depend on their own sex-chromosome constitution and on the sex determining region of Y (SRY) gene on the Y chromosome.

Spermatogenesis

- *Definition*: It is the process of maturation of male gametes in the wall of seminiferous tubules.
- It involves a series of changes leading to the conversion of spermatogonia into spermatozoa.
- *Time*: In the male, the formation of gametes (spermatozoa) takes place only during the reproductive period, which begins at the age of puberty (12–16 years) and continues even into old age.
- *Duration*: 64–74 days.
- *Stages*: If we look at one of the seminiferous tubules under a microscope, we find that there are many cells of different sizes and shapes (Fig. 3.3B). Most of these represent stages in the formation of spermatozoa, but some (called Sertoli cells) have only a supporting function. The various cell-stages in spermatogenesis are *spermatocytosis*, *meiosis* and *spermiogenesis*. These stages can be described as follows (Figs 3.3, 3.9 to 3.11 and Flowchart 3.1).

Spermatocytosis (Figs 3.3, 3.9 and Flowchart 3.1)

- *It is the process of* conversion of spermatognia to primary spermatocytes. It takes 16 days. It is by repeated mitotic divisions.
- *Formation of stem cells*: The PGCs give rise to spermatogonial stem cells.
- *Cell growth*: From these stem cells a group of cells are formed at regular intervals and are called type A spermatogonia. Production of type A spermatogonia marks the beginning of spermatogenesis.
- *Mitotic divisions*: Type A spermatogonia (44 + X + Y) undergo a limited number of mitotic divisions and form a clone of cells. The last division of cells become Type B spermatogonia. The Type A spermatogonia are dark and Type B are pale in color. Type A spermatogonia are reserve cells. The spermatogonia (Type B) (44 + X + Y) enlarge, or undergo mitosis, to form primary spermatocytes.

Meiotic Divisions (Figs 3.3, 3.9 and Flowchart 3.1)

- It is the process of conversion of primary spermatocytes to secondary spermatocytes and then spermatids. It takes 24 days.

- The primary spermatocytes (44 + X + Y) now divide so that each of them forms two secondary spermatocytes. This is the first meiotic division: it reduces the number of chromosomes to half.
- Each secondary spermatocyte has 22 + X or 22 + Y chromosomes. It divides to form two spermatids. This is the second meiotic division and this time there is no reduction in chromosome number. It is called equation meiosis. There occurs balancing of species specific chromosome number and DNA content by reduction and equation divisions.

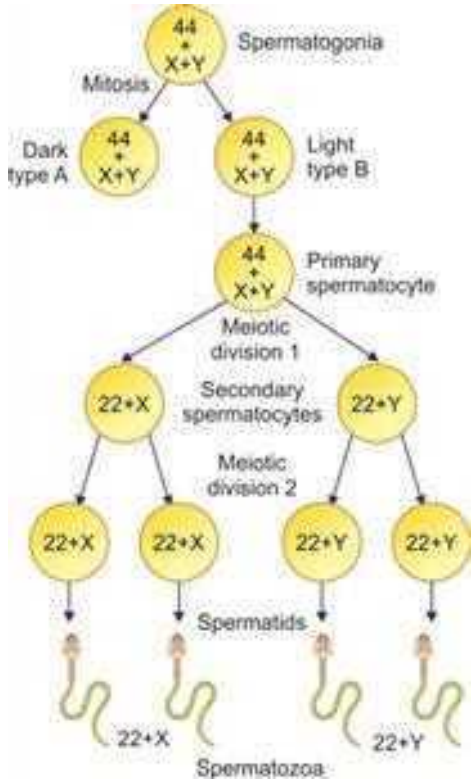


Fig. 3.9: Stages in spermatogenesis. Note the number of chromosomes at each stage

Spermiogenesis (Figs 3.3, 3.9, 3.10 and Flowchart 3.1)

- It is the process of metamorphosis of spermatids to spermatozoa and takes 24 days.
- The process by which a spermatid (22 + X / 22 + Y) gradually changes its shape to become a spermatozoon is called *spermiogenesis (or spermateleosis)*. It is the final stage in the maturation of spermatids into mature, motile spermatozoa.
- The spermatid is a more or less circular cell containing a nucleus, Golgi apparatus, centriole and mitochondria (Fig. 3.10). All these components take part in forming the spermatozoon.
- *Major events in spermiogenesis* (Fig. 3.10)
  - Nuclear morphogenesis and condensation
  - Formation of tail
  - Formation of acrosome
  - Rearrangement of organelles (Mitochondria, centrioles)
  - Shedding of excess cytoplasm.
- *Various processes in spermiogenesis (Figs 3.8 and 3.11)*:
  - Nucleus: Condensation of nucleus and its movement to one pole forms the *head*.
  - Golgi apparatus: The Golgi apparatus is transformed into the *acrosomic cap*. Acrosomal cap covers two-third of nucleus.
  - *Centrosome*: The centriole divides into two parts that are at first close together. The *proximal centriole*

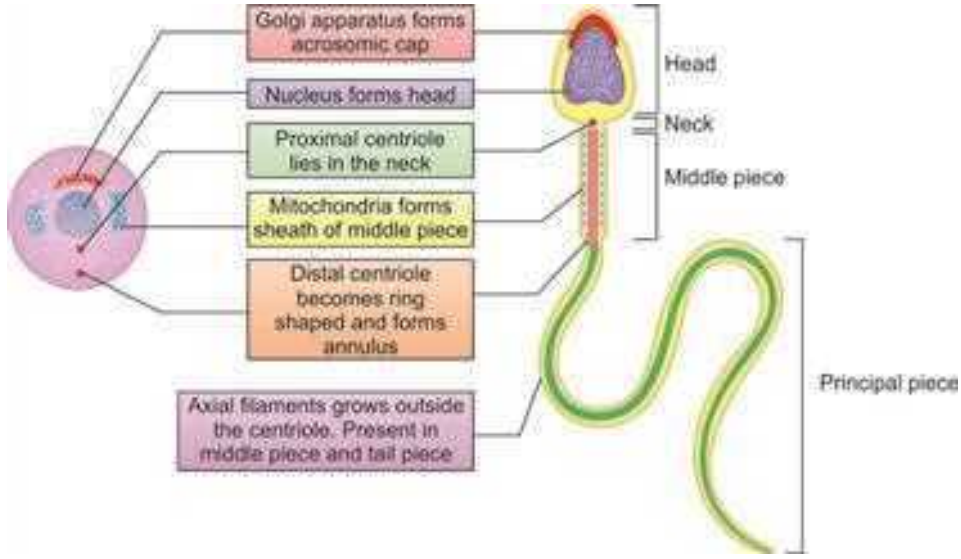
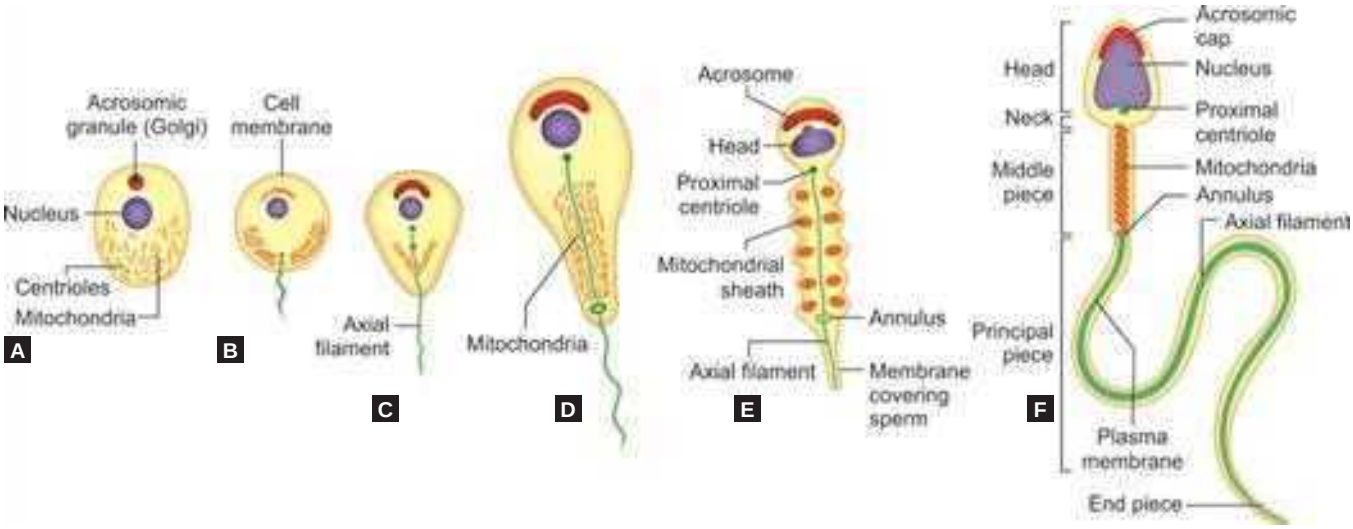


Fig. 3.10: Parts of a spermatozoon and their derivation



Figs 3.11A to F: Stages in spermiogenesis and parts of a spermatozoon

becomes spherical and comes to lie in the neck. The *axial filament* appears to grow out of it. Distal centriole forms the distal end of the middle piece, i.e. annulus. Centrioles are concerned for movement.

- *Mitochondria*: Form a spiral sheath around middle piece. The part of the axial filament between the neck and the annulus becomes surrounded by mitochondria, and together with them forms the middle piece. The remaining part of the axial filament elongates to form the principal piece and tail.
- *Cytoplasm*: Most of it is shed as residual bodies of Renaud and are engulfed by Sertoli cells.
- *Cell membrane:* Persists as a covering for the spermatozoon. Presents specialization for fertilization that includes:
  - Sperm-egg recognition
  - Sperm-egg binding
  - Sperm-egg fusion.

Difference between Spermatogenesis and Spermiogenesis

- Spermatogenesis is the complete process of formation of a spermatozoon from a spermatogonium. It includes the first and second meiotic divisions and spermiogenesis.
- On the other hand, spermiogenesis is the process of transformation of a rounded spermatid into a spermatozoon.

Maturation and Capacitation of Spermatozoa

- When first formed in seminiferous tubules, spermatozoa are immature. They are nonmotile and incapable of fertilizing an ovum.
- A current of fluid in seminiferous tubules carries spermatozoa from the testis to the epididymis. Here they are stored and undergo maturation.
- As spermatozoa pass through the epididymis they undergo a process of maturation. Changes take place in glycoproteins of the plasma membrane covering the sperm head.
- Spermatozoa acquire some motility after maturation, but become fully motile only after ejaculation when they get mixed with secretions of the prostate gland and seminal vesicles.
- Spermatozoa acquire the ability to fertilize an ovum only after they have been in the female genital tract for some time. This final step in their maturation is called capacitation.
- In the process of capacitation, the glycoprotein coat and seminal proteins lying over the surface of the spermatozoon are altered. Spermatozoa usually undergo capacitation in the uterus or uterine tube, under the influence of substances secreted by the female genital tract.
- When a spermatozoon comes in contact with the zona pellucida, changes take place in the membranes over the acrosome and enable release of lysosomal enzymes. This is called the *acrosome reaction*.
- Some enzymes help in digesting the zona pellucida and in penetration of the spermatozoa through it. Changes in the properties of the zona pellucida constitute the *zona reaction*.

Structure of a Mature Spermatozoon

- A spermatozoon is a highly specialized, free swimming, actively motile cell. The spermatozoon has a head, a neck, a middle piece and a principal piece or tail

(Figs 3.10 and 3.11). An axial filament passes through the middle piece and extends into the tail. The spermatozoon measures about 60 µm in length.

- *Head*: The head of the human spermatozoon is piriform in shape and measures 4 µm in length. It is derived from the nucleus, which consists of 23 highly condensed chromosomes. The chromatin in the head of the spermatozoon is extremely condensed. This makes the head highly resistant to various physical stresses. The chemical basis for condensation is the replacement of histones by protamines. The head is covered by a cap-like structure called the *acrosome* (also called the "acrosomic cap" or "galea capitis"). The acrosome contains enzymes that help in penetration of the spermatozoon into the ovum during fertilization (see Chapter 4: Fertilization and Formation of Germ Layers).
- *Neck*: The neck is narrow. It contains a funnel-shaped basal body and a spherical centriole. The basal body is also called the connecting piece because it helps to establish an intimate union between the head and the remainder of the spermatozoon (Fig. 3.10). The basal body is made up of nine segmented rod-like structures. On its proximal side (i.e. toward the head of the spermatozoon), the basal body has a convex articular surface which fits into a depression (implantation fossa) in the head.
- *Middle piece and principal piece*: The axial filament begins just behind the centriole. It passes through the middle piece and most of the tail. At the point where the middle piece joins the tail, the axial filament passes through a ring-like structure called "the annulus". The part of the axial filament, which lies in the middle piece, is surrounded by a spiral sheath made up of mitochondria. The axial filament is really composed of several fibrils arranged as illustrated in Figure 3.12.

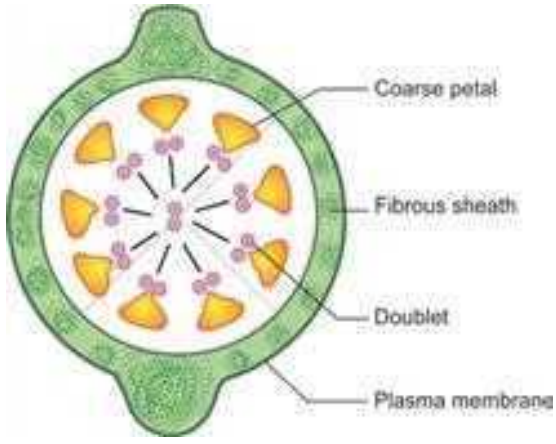


Fig. 3.12: Transverse section across the principal piece (tail) of a spermatozoon to show the arrangement of fibrils

There is a pair of central fibrils, surrounded by nine pairs (doublets) arranged in a circle around the central pair. In addition to the doublets, the axial filament contains nine coarser petal-shaped fibrils, one such fibril lying just outside each doublet. The whole system of fibrils is kept in position by a series of coverings. Immediately outside the fibrils, there is a fibrous sheath. In the region of the middle piece, the fibrous sheath is surrounded by spirally arranged mitochondria.

Finally, the entire spermatozoon is enclosed in a plasma membrane.

Oogenesis

- *Definition*: The process of maturation and differentiation of PGC to oogonia, primary oocytes, secondary oocytes and to mature ova in the female genital tract.
- *Location*: Ovarian cortex.
- *Peculiarities of oogenesis*:
  - Starts before birth (10th week)
  - Stops in the middle (birth to puberty)
  - Restarts at puberty (11–13 years)
  - Continues up to menopause (45–55 years)
- *Processes*: The various processes in oogenesis are:
  - Mitosis
  - Meiosis
  - Growth of follicles
  - Differentiation of follicles
- The cortex contains many large round cells called "oogonia". All the oogonia to be utilized throughout the life of a woman are produced at a very early stage (possibly before birth) and do not multiply thereafter.
- On arrival in the gonad the *primordial germ cells* differentiate into *oogonia*. The oogonia pass through the stages of primary and secondary oocyte and ovum.
- Oogenesis at *different phases of life* (Fig. 3.13 and Flowchart 3.2) can be described as:
  - *Before birth*:
    - *Before 3rd month*: The PGCs undergo mitosis to form oogonia. This occurs in the absence of testicular differentiation factor (TDF).
    - *Before 7th month*: The oogonia continue to divide mitotically. The oogonia are surrounded by a layer of flat epithelial cells. Some of the oogonia enlarge to form *primary oocytes*.
    - *7th month to birth*: Formation of primordial follicles (primary oocyte with its surrounding flat epithelial cells) and multiplication of primary oocytes to produce millions of germ cells occurs. Primary oocyte enters prophase I of meiosis I at that phase the meiosis is arrested by oocyte maturation inhibitor (OMI) factor.

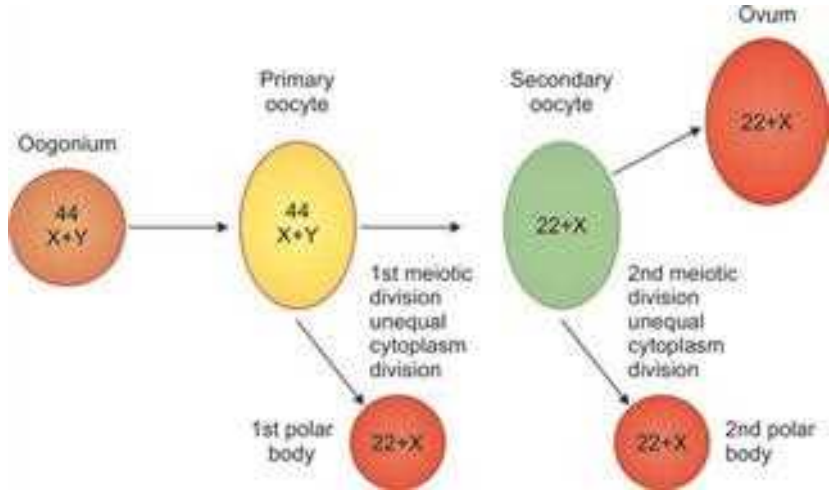


Fig. 3.13: Stages in oogenesis. Compare each stage with the corresponding one in Figure 3.9

- The oogonia are diploid (2n) in chromosome content. Many of these oogonia and primary oocytes degenerate before birth.

– *Birth to puberty*:
  - There will be both maturation and degeneration of primordial follicle resulting in reduction in the number of primary oocytes.
  - At the time of birth all primary oocytes are in the prophase of first meiotic division. At birth approximately two lakh primary oocytes in primordial follicles are present in each ovary.
  - Instead of entering metaphase the primary oocytes enter prolonged *resting or diplotene stage.*

– *After puberty—cyclical preparation for fertilization is known as ovarian cycle.*
  - From the time of birth to puberty there is degeneration of number of primary oocytes. Rest of the primary oocytes remain in prophase and do not complete their first meiotic division until they begin to mature and are ready to ovulate.
  - The first meiotic division of a primary oocyte produces two unequal daughter cells. Each daughter cell has the haploid number of chromosomes (23). The large cell, which receives most of the cytoplasm, is called the *secondary oocyte*, and the smaller cell is known as "the first polar body" (Fig. 3.13). The secondary oocyte immediately enters the second meiotic cell division.
  - Ovulation takes place while the oocyte is in metaphase. The secondary oocyte remains arrested in metaphase till fertilization occurs. The second meiotic division is completed only if fertilization occurs. This division results in two unequal daughter cells. The larger cell is called *ovum*. The smaller daughter cell is called the second polar body. The first polar body may also divide during the second meiotic division (Fig. 3.13) making a total of three polar bodies.
  - If fertilization does not occur, the secondary oocyte fails to complete the second meiotic division and degenerates about 24 hours after ovulation.

Reproductive Period

In an individual, the formation of gametes takes place only during the reproductive period which begins at the age of puberty (10–14 years). In women, it ends between the ages of 45 years and 50 years, but in men it may continue till the age of 60 years or more.

The period of a woman's life in which she can bear children is during the reproductive period. The most obvious feature of this period is a monthly flow of blood from the uterus that is referred to as *menstruation* (or menses). The onset of menstruation (menarche) takes place at about 12 years of age. Menstruation ceases to occur at about 45 years of age, and this is referred to as *menopause*. The monthly menstruation is the external manifestation of a series of cyclic changes taking place in the uterus. These changes constitute the *menstrual cycle*.

Simultaneously, cyclic changes also take place in the ovaries, and these constitute the *ovarian cycle*. The most important event in the ovarian cycle is *ovulation*. During the reproductive life of a female in each month/menstrual cycle several primary and secondary follicles start developing but only one reaches maturity for release of ovum in that cycle.

The process of fusion of sperm and ovum is known as *fertilization*. If fertilization does not take place the

secondary oocyte degenerates. The secondary oocyte at fertilization completes its meiosis II.

In each menstrual cycle, 5–30 primary oocytes in primordial follicles start maturing, but only one of them reaches maturity and is ovulated and the remaining degenerate. During the entire reproductive life of a female, only around 400 ova are discharged (out of 40,000 primary oocytes available).

OVARIAN CYCLE

Definition: Cyclical change in ovaries during 28 day reproductive cycle, terminating in the release of single mature ovum (ovulation) under the influence of anterior pituitary gonadotrophic hormones [follicle stimulating hormone (FSH) and luteinizing hormones (LH)].

Phases: It includes preovulatory, ovulatory and postovulatory phases.

Preovulatory Phase

- In a 28-day reproductive cycle this extends from the 5th to 14th day, i.e. *up to ovulation*. This phase lasts for 8–10 days and may vary from 10–25 days depending on the length of reproductive cycle.
- During this phase under the influence of FSH in earlier stages and both FSH and LH of anterior pituitary in later stages, 10–15 primordial follicles start maturation process known as *folliculogenesis*. But only one follicle matures fully while others undergo degeneration or atresia at different stages of development.

Folliculogenesis

Definition: It is the process of maturation of ovarian follicles. The various types of follicles that are formed during this process of maturation are *primordial, primary, secondary, vesicular, tertiary* and *mature*.

- *Primordial follicles*:
  - The oogonia are surrounded by stromal cells. Some cells of the stroma become flattened and surround a primary oocyte. These flattened cells ultimately form the ovarian follicle and are, therefore, called *follicular cells*.
  - Each primary oocyte covered by a single layer of flattened follicular cells is known as *primordial follicle* (Figs 3.5, 3.14 and Flowchart 3.2). The follicular cells protect the ova and form different types of follicle with progress in their development. Both primary oocyte and granulosa cells are covered by a thin basement membrane.
  - The primordial follicles are the basic reproductive units at puberty. These are seen in the deeper part of cortex of developing ovary. These are formed during intrauterine life by the conversion of oogonia around 10th week of development.
  - The division of primary oocyte is arrested until puberty by oocyte maturation inhibition (OMI) factor produced by the follicular cells that surround the oocyte.
  - At birth approximately two lakh primary oocytes in primordial follicles are present in each ovary. From the time of birth to puberty there is degeneration of number of primary oocytes resulting in reduction in their number to 40,000. Of these 40,000 that were present at puberty around 400–450 mature during the reproductive life of a female and release the ovum. The remaining primary oocytes undergo atresia or degeneration at different phases of maturation.
- *Primary follicles*: The oocyte resumes and completes its first meiotic division, when it starts maturing after puberty, and is preparing for ovulation. From puberty to menopause, i.e. during the reproductive period of a female under the influence of pituitary gonadotrophic hormones—FSH and LH, several primordial follicles (10–50 numbers) start maturing in each month, to be converted to primary follicles.

  Each primary follicle consists of primary oocyte surrounded by a layer of cuboidal or low columnar follicular cells (Figs 3.5 and 3.15). The growing primary

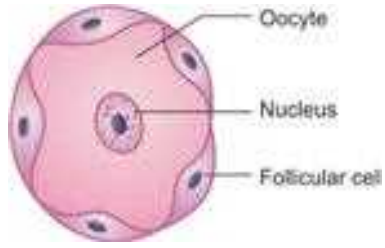


Fig. 3.14: Primordial follicle

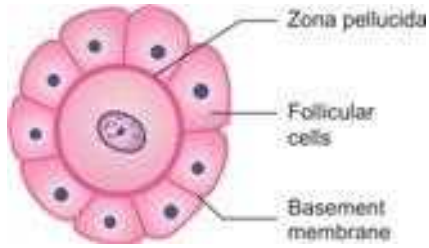


Fig. 3.15: Primary follicle

follicle forms a homogenous layer of glycoprotein in between follicular cells and primary oocyte known as *zona pellucida*.

- *Secondary/Multilaminar follicle*: The follicular cells undergo mitotic division and form several layers of follicular cells surrounding the primary oocyte forming a multi-layered stratum granulosum (Figs 3.5 and 3.16). The primary oocyte increases in size up to 40 microns. Its nucleus is large and vesicular. The follicular cells are now called as "granulosa cells". The primary oocyte receives its nutrition from the granulosa cells up to puberty.
- *Preantral follicle*: Fluid-filled spaces appear between granulosa cells, and such follicle is known as "preantral follicle" (Figs 3.5 and 3.17). The fluid is secreted by granulosa cells.
- *Tertiary/Antral follicle*: With the growth of the follicle, fluid-filled spaces between granulosa cells coalesce and form a bigger cavity known as "follicular antrum" pushing the primary oocyte with surrounding granulosa cells (Figs 3.5 and 3.18). The fluid that is filling the antrum is called liquor folliculi and is secreted by granulosa cells. Because of the proliferation of granulosa cells the size of the follicle increases.
- *Mature/Graafian follicle*: Around 7th day of sexual cycle one of the tertiary follicles increases in size in response to FSH and LH and forms the largest mature follicle that is known as "Graafian follicle" (Figs 3.5 and 3.19). Remaining follicles degenerate and become atretic.
  - A fully mature Graafian follicle is about 3–5 mm in size. It reaches the periphery of the cortex and starts projecting on to the surface of the ovary. The follicular antrum is filled with fluid pushing the

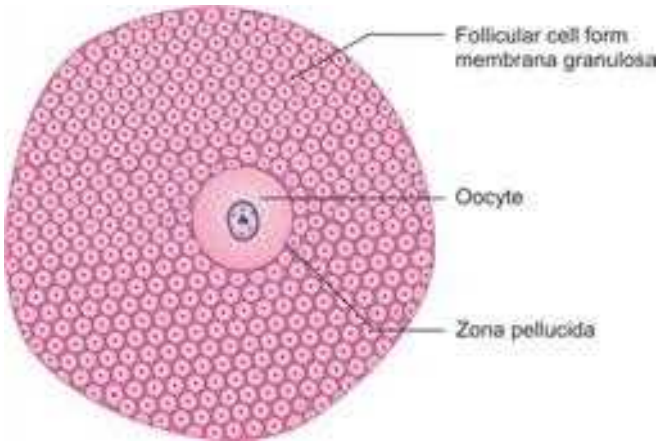


Fig. 3.16: Secondary follicle

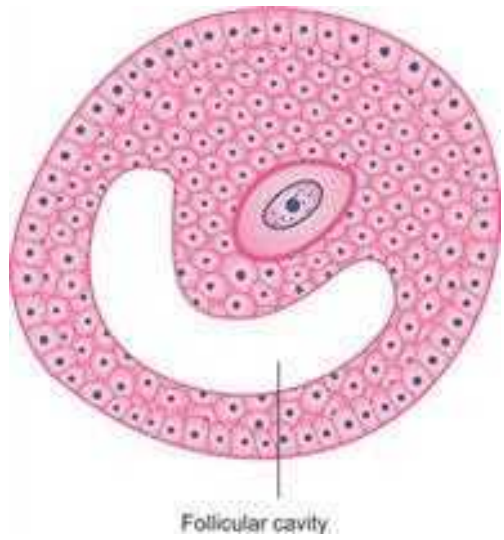


Fig. 3.18: Tertiary/Antral follicle

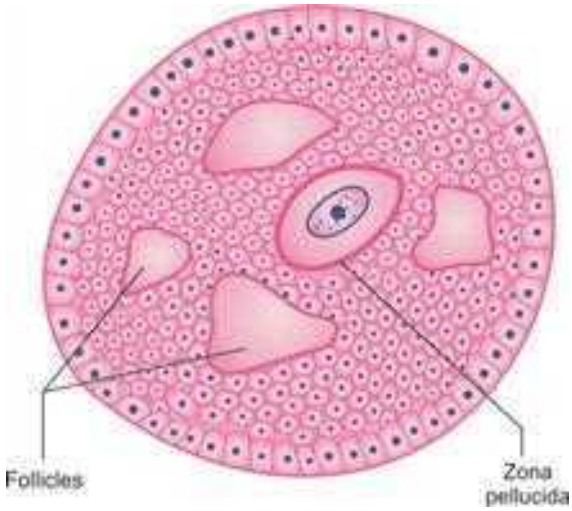


Fig. 3.17: Preantral follicle

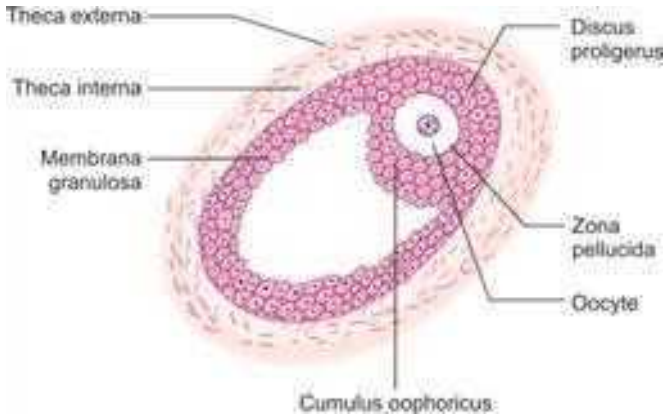


Fig. 3.19: Mature graafian follicle

primary oocyte with a layer of covering cells to one side of the follicle.

- The layer of cells immediately surrounding the oocyte and zona pellucida are called *corona radiata cells*. The projection of granulosa cells covering the primary oocyte projecting into the follicular antrum is called *cumulus oophorous*. The area of attachment of primary oocyte and corona radiata to the wall of follicle is called *discus proligerous*.
- As the follicle expands, the stromal cells surrounding the membrana granulosa become condensed to form a covering called the *theca interna* (theca = cover). Theca interna increases in thickness and becomes more vascular. The cells of the theca interna later secrete a hormone called estrogen; and they are then called the cells of the thecal gland (Fig. 3.19). Outside the theca interna some fibrous tissue becomes condensed to form another covering for the follicle called the *theca externa* (Fig. 3.18). The ovarian follicle is now fully formed.
- The follicle gradually increases in size and finally bursts and expels the ovum. This process of shedding of the ovum is called *ovulation*.
- Just before ovulation the primary oocyte of mature Graafian follicle completes first meiotic division and forms secondary oocyte and first polar body.

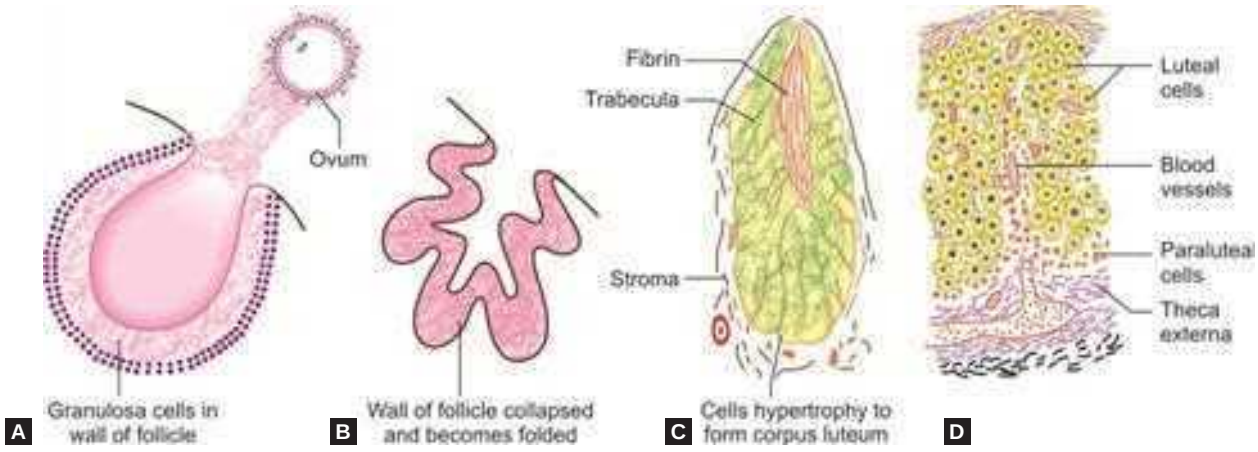
Hormonal control of ovarian cycle and uterine cycle are represented in Figures 3.22 and 3.23.

Ovulatory Phase

The diploid (46 chromosomes) primary oocyte in the mature Graafian follicle completes its meiosis I, forming two cells of unequal size each with haploid number of chromosomes (23). The smaller cell is called first polar body. The larger cell is called secondary oocyte and at this stage the meiosis II begins but it is stopped in metaphase II. At this stage the mature follicle ruptures and releases the secondary oocyte.

Ovulation

- The shedding of the ovum from the ovary is called ovulation. The ovarian follicle is at first very small compared to the thickness of the cortex of the ovary (Figs 3.5 and 3.20).
- As it enlarges the mature Graafian follicle, becomes so big that it not only reaches the surface of the ovary, but also forms a bulging in this situation. Ultimately, the follicle ruptures and the ovum are shed from the ovary (Figs 3.5 and 3.20).
- Just before ovulation the follicle may have a diameter of 15 mm. The stroma and theca on this side of the follicle become very thin. An avascular area (*stigma*) appears over the most convex point of the follicle. At the same time, the cells of the cumulus oophoricus become loosened by accumulation of intercellular fluid between them.
- During the ovulation process there is rupture of mature follicle and release of secondary oocyte in metaphase of 2nd meiotic division.
- At ovulation the secondary oocyte is released from the surface of the ovary into the pelvic cavity together with first polar body and corona radiata cells (Figs 3.5, 3.20 and 3.21). The ovulated oocyte with its surrounding cells swims toward the fimbrial end of fallopian tube.
- In the ampulla of fallopian tube several sperms surround the secondary oocyte with its enclosed carona radiata cells. One sperm penetrates the various barriers surrounding the secondary oocyte. This initiates resuming of meiosis II of secondary oocyte.
- *Stage of meiosis at ovulation*: Completion of meiosis I resulting in secondary oocyte and the first polar body.



Figs 3.20A to D: Stages in the formation of corpus luteum and transformation of follicular cells to luteal cells

- *Time of ovulation*: 14 days ± 1 day before the onset of next menstrual cycle.

Sequence of events occurring during ovulation can be summarized as follows:

- Peak levels of FSH and surge of LH bring about changes in Graafian follicle
- Increase in liquor folliculi within the follicle
- Appearance of perivitelline space
- Withdrawal of processes of follicular cells resulting in homogenous zona pellucida
- Follicular cells showing increased activity
- Mature follicle reaching surface of ovary
- Loosening of cumulus cells with accumulation of intercellular fluid and separation of cells from follicular wall
- Stigma formation—ischemia and bulging of follicular wall
- Marked thinning and separation of supporting collagen fibers and cell layers of stigma with increase in collagenolytic enzymes in liquor and stigma tissue. This results in rupture of follicle.
- Shedding of secondary oocyte with first polar body enclosed in perivitelline space and zona pellucida and surrounded by cells of corona radiate into the peritoneal cavity near the fimbriated end of fallopian tube (Fig. 3.22)
- Entry of released ovum into the fallopian tube is at its fimbriated end.

The following factors may lead to ovulation:

- Ovulation occurs due to high concentration of luteinizing hormones (LH) in blood just before ovulation (Fig. 3.22).
- A high concentration of LH leads to increase activity of the enzyme collagenase, which in turn digests the collagen fibers surrounding the follicle.
- Increase in concentration of *prostaglandins* causes contraction of smooth muscle in the wall of the ovary.
- The increased pressure of fluid in the follicular cavity is also a significant factor for ovulation to occur.
- However, the enzymatic digestion of the follicular wall seems to be the main factor responsible for ovulation.

Clinical correlation

Detection of time of ovulation:

- Basal body temperature recording (Fig. 3.22)—it falls 0.3–0.5°C just before ovulation and increases slightly thereafter. Time of ovulation can be determined by recording the morning temperature during mid-cycle.
- ***Endometrial biopsy:*** to observe changes specific for ovulation under the influence of progesterone
- ***Observation of cervical mucus:*** It is sticky and presents fern pattern.
- ***Hormonal estimation:*** Blood progesterone, estrogen, FSH, LH estimation during mid-cycle—Increased LH and estrogen and decreased FSH at the time of ovulation and increased progesterone after ovulation (Fig. 3.22).
- ***Ultrasonography:*** process of ovulation can be recorded. Corpus luteum detection in ovary.
- Uterine bleeding—Intermenstrual occurs.
- Vaginal smear—Increased cornification of mucosa.
- Mittleschmerz—mid-cycle pain

Conditions affecting ovulation:

- ***Age:*** Anovulatory cycles are common before puberty, initial cycles after puberty, after menopause.
- Pregnancy
- Lactation
- Diseases—nutritional, endocrine and emotional
- Environment—extremes of temperature.

Structure of the Ovum at Ovulation

The ovum that is shed from the ovary is not fully mature. It is a secondary oocyte that is undergoing division to shed off the second polar body (Figs 3.5 and 3.21). At this stage, the ovum has the appearance illustrated in Figure 3.21. Note that it is surrounded by the zona pellucida. Some cells of the corona radiata can be seen sticking to the outside of the zona pellucida. No nucleus is seen, as the nuclear membrane has dissolved for the second meiotic division. A spindle is, however, present. Between the cell membrane (or *vitelline membrane*) and the zona pellucida, a distinct *perivitelline space* is seen. The first polar body lies in this space. Note that the ovum is a very large cell and measures more than 100–120 µm in diameter. In contrast, most other cells of the body measure less than 10 µm (1 µm is 1,000th of a millimeter). Differences between spermatozoon and ovum are presented in Table 3.3.

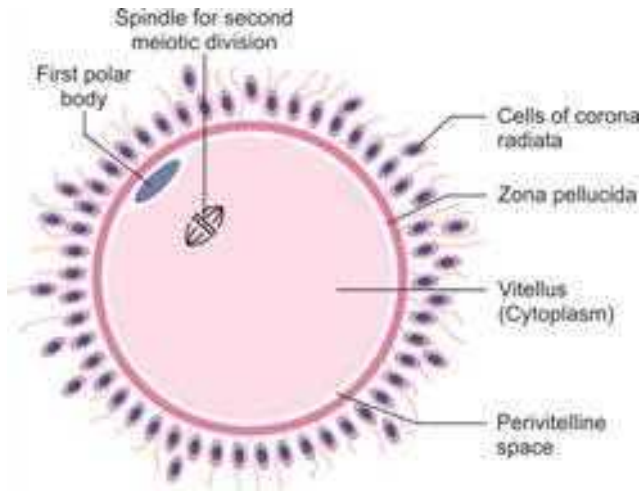


Fig. 3.21: Structure of ovum at the time of ovulation

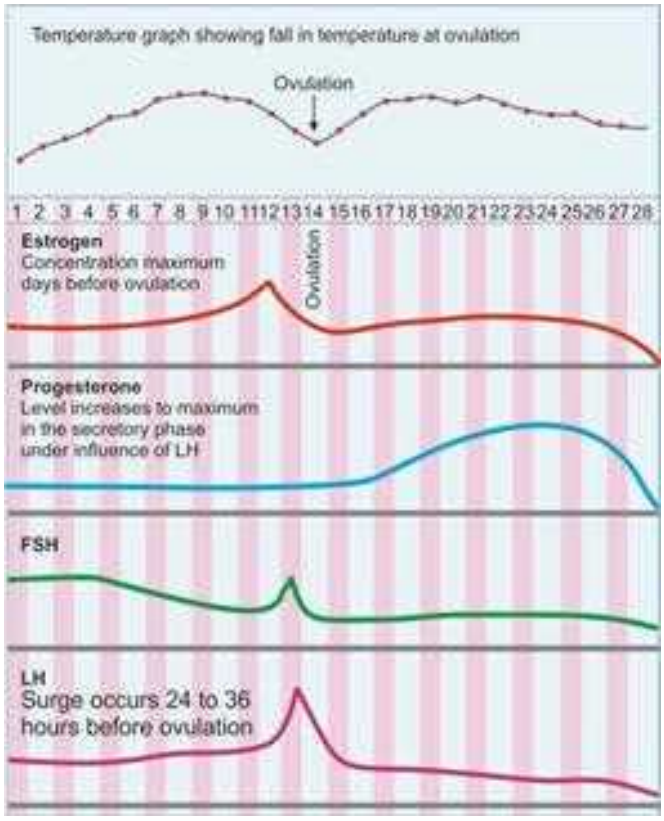


Fig. 3.22: Morning temperature and concentration of hormones FSH, LH, estrogen and progesterone during normal menstrual cycle. Ovulation occurs because of a LH surge just before ovulation

TABLE 3.3: Differences between male and female gametes

Feature	*Spermatozoon*	*Ovum*
Diameter	Small: 3 µ	Very large: 120 µ
Length	60 µ	Small
Shape	Adapted for motility	Adapted to provide ample storage of nutrition for the embryo formed after fertilization
Motility	Highly motile	Immotile
Cytoplasm	Very little	Large amount
Chromosomal types	Spermatozoa are of two chromosomal types (22 + X) and (22 + Y)	All ova have (22 + X) chromosomes

Postovulatory Phase

Fate of the Ovum

The ovum that is shed from the ovary is closely embraced by the fimbriated end of the uterine tube. The ovum is easily carried into the tube partly by the follicular fluid discharged from the follicle and partly by the activity of ciliated cells lining the tube. The ovum slowly travels through the tube toward the uterus, taking 3–4 days to do so. If sexual intercourse takes place at about this time, the spermatozoa deposited in the vagina swim into the uterus and into the ampulla of uterine tube. One of these spermatozoa may fertilize the ovum. If this happens, the fertilized ovum begins to develop into an embryo. The fertilized ovum travels to the uterus and gets implanted in its wall. On the other hand, if the ovum (secondary oocyte) is not fertilized it dies in 12–24 hours. It passes through the uterus into the vagina and is discharged.

Corpus Luteum

- The corpus luteum is an important structure. It mainly secretes the hormone progesterone, but also secretes some estrogen.
- The corpus luteum is formed by enlargement and transformation of follicular cells, after-shedding of the ovum (Figs 3.5 and 3.20A).
- The corpus luteum is derived from the ovarian follicle, after the latter has ruptured to shed the ovum, as follows (Figs 3.5 and 3.20):
  - When the follicle ruptures, its wall collapses and becomes folded (Figs 3.20B and C).
  - At this stage, the follicular cells are small and rounded (Fig. 3.20D). They now rapidly enlarge. As they increase in size, their walls press against those of neighboring cells so that the cells acquire a polyhedral shape (Figs 3.20C and D). Their cytoplasm becomes filled with a yellow pigment called *lutein*. They are now called *luteal cells*. The presence of this yellow pigment gives the structure a yellow color and that is why it is called the corpus luteum (= yellow body). Some cells of the theca interna also enlarge and contribute to the corpus luteum by becoming *paraluteal cells*.
  - The corpus luteum secretes progesterone (Fig. 3.23), which is essential for maintenance of pregnancy.

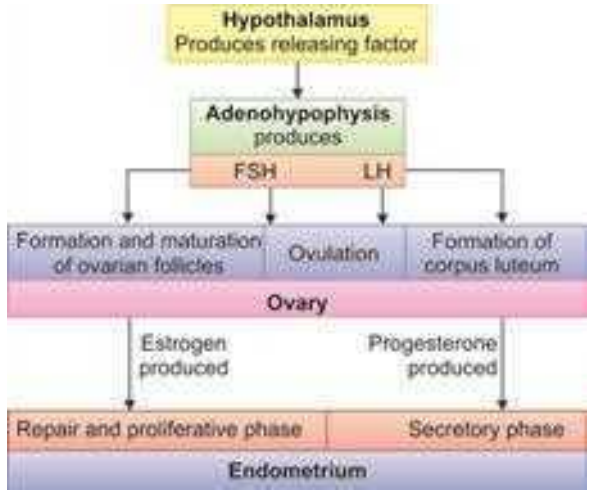


Fig. 3.23: Hormonal control of ovulation and uterine cycles

This secretion has to be poured into the blood like secretions of endocrine glands. All endocrine glands are richly supplied with blood vessels for this purpose. The ovarian follicle itself has no blood vessels, but the surrounding theca interna is full of them. When the corpus luteum is forming, blood vessels from the theca interna invade it and provide it with a rich supply of blood (Fig. 3.20C).

– *The subsequent fate of the corpus luteum depends on whether the ovum is fertilized or not.*
  - If the ovum is *not fertilized*, the corpus luteum persists for about 14 days. During this period it secretes progesterone. It remains relatively small and is called the *corpus luteum of menstruation*. At the end of its functional life, it degenerates and forms a mass of fibrous tissue called the corpus albicans (= white body) (Figs 3.5 and 3.24).
  - If the ovum is *fertilized* and *pregnancy* results, the corpus luteum persists for 3–4 months. This is larger than the corpus luteum of menstruation, and is called the *corpus luteum of pregnancy*.
– The corpus luteum of pregnancy may occupy one-third to half the total volume of the ovary. The progesterone secreted by it is essential for the maintenance of pregnancy in the first few months. After the 4th month, the corpus luteum is no longer needed, as the placenta begins to secrete progesterone. Degeneration of the corpus luteum in the early months of pregnancy is prevented by human chorionic gonadotropin (HCG) secreted by the trophoblast cells of the developing embryo.
– The series of changes that begin with the formation of an ovarian follicle and end with the degeneration of the corpus luteum constitute what is called an *ovarian cycle*. An ovarian cycle has an average duration of 28 days, with ovulation occurring at mid-cycle, i.e. on the 14th day.

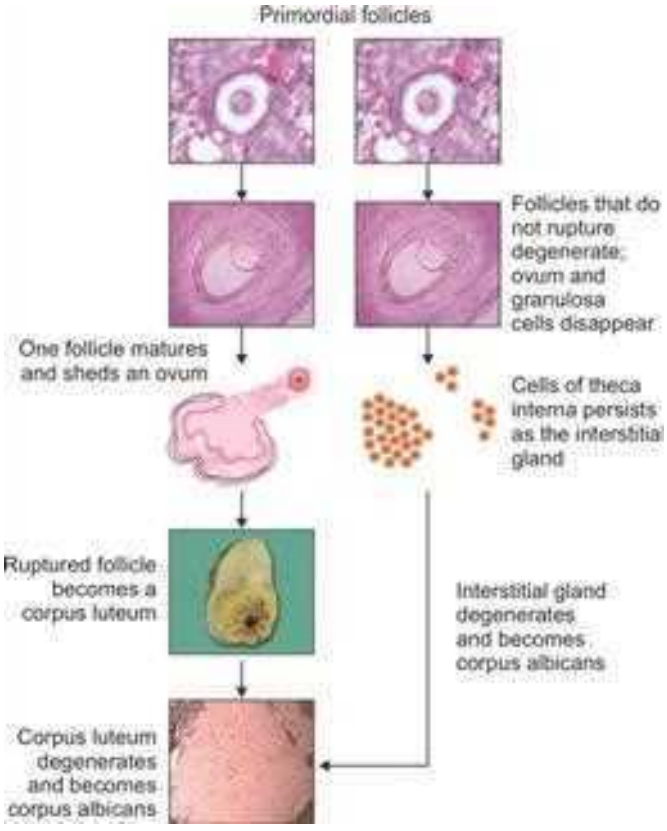


Fig. 3.24: Fate of ovarian follicles

Fate of Ovarian Follicles

We have seen that in each ovarian cycle, one follicle reaches maturity, sheds an ovum, and becomes a corpus luteum. At the same time, several other follicles also begin to develop, but do not reach maturity (Fig. 3.20). It is interesting to note that, contrary to what one might expect, these follicles do not persist into the next ovarian cycle, but undergo degeneration. The ovum and granulosa cells of each follicle disappear. The cells of the theca interna, however, proliferate to form *the interstitial glands*, also called the *corpora atretica* (singular = corpus atreticum). These glands are believed to secrete estrogens. After a period of activity, each gland becomes a mass of scar tissue indistinguishable from the corpus albicans formed from the corpus luteum.

Ovarian Cycle and Hormones

The changes taking place during the ovarian cycle are greatly influenced by certain hormones produced by the hypophysis cerebri (Figs 3.22 and 3.23). The hormones produced by the theca interna and by the corpus luteum in turn influence other parts of the female reproductive system (notably the uterus), resulting in a cycle of changes referred to as the *uterine* or *menstrual cycle*.

Viability of Gametes

An ovum usually degenerates 24 hours after ovulation. However, at the most it may survive for 2 days. Similarly, sperms usually degenerate 48 hours after ejaculation, but may survive up to 4 days in female genital tract.

Clinical correlation

Abnormalities in formation of gametes

Abnormalities of form

Spermatozoa may be too large (giant) or too small (dwarf). The head, body or tail may be duplicated. The ovum may have an unusually large nucleus or two nuclei. Two oocytes may be seen in one follicle.

Chromosomal abnormalities

The gametes may be abnormal in chromosomal content as follows:

- ***Nondisjunction:***
  - During the first meiotic division, the two chromosomes of a pair, instead of separating at anaphase, may both go to the same pole. This is called nondisjunction. The resulting gamete then has 24 chromosomes instead of the normal 23 (Fig. 3.25).
  - During second meiotic division also nondisjunction can take place (Fig. 3.26).
  - At fertilization by this gamete, the zygote will, therefore, have 47 chromosomes; there being three identical chromosomes instead of one of the normal pairs. This is called ***trisomy***. Depending upon the particular chromosomes involved, various trisomy abnormalities are produced.
  - *Down's syndrome:* Trisomy of chromosome 21 results in this condition. In this condition, the child has a broad face, obliquely placed palpebral fissures, epicanthus, a furrowed lower lip, and broad hands with a single transverse crease. Usually, the patients are mentally retarded and have anomalies of the heart.
  - *Extra sex chromosome:* The presence of an extra *X* or *Y* chromosome can give rise to various syndromes associated with abnormal genital development, mental retardation and abnormal growth. Some of these are: *XXX* (abnormal female); *XXY (Klinefelter's syndrome:* abnormal male); *XYY* (abnormal male). In Klinefelter's syndrome, the subject is a male (because of the presence of a Y chromosome). However, the testes are poorly developed leading to sterility and gynecomastia.
  - *Super females:* Patients with *XXX* chromosomes show two masses of sex chromatin in their cells and are sometimes referred to as "super females". However, there is nothing "super" about them. In fact, their bodies show poor sexual development (i.e. they are infantile), and menstruation is scanty. Mental retardation is usual.
  - *Monosomy:* When both chromosomes of a pair go to one gamete (as described above), the other gamete resulting from the division has only 22 chromosomes (instead of the normal 23); and at fertilization, the zygote has only 45 chromosomes. Hence one pair is represented by a single chromosome. This is called *monosomy (Figs 3.25 and 3.26)*.
  - *Turner's syndrome:* The best known example of monosomy is a female with only one X chromosome (Turner's syndrome) (Figs 3.25 and 3.26). In this syndrome, the subject is always female (because of absence of a Y chromosome). There is agenesis of ovaries. Associated deformities include mental retardation, skeletal abnormalities, and folds of skin on the sides of the neck (webbed neck).
  - Such anomalies may affect more than one pair of chromosomes. Alternatively, one pair may be represented by more than three chromosomes. When this happens with the sex chromosomes, individuals with the constitution XXXY, XXXXY, XXYY, or XXXX may be produced.
  - *Triploidy:* Sometimes, a gamete may have the diploid number of chromosomes so that the zygote will have 46 + 23 (i.e. 69) chromosomes. This is called triploidy. Higher multiples of 23 may also be seen. Such fetuses are generally born dead.
- Abnormalities in the process of crossing over can result in a number of chromosomal abnormalities as follows:
  - *Translocation:* Part of a chromosome may get attached to a chromosome of a different pair.
  - *Deletion:* Part of a chromosome may be lost (*deletion*).
  - *Duplication of genes:* The two chromosomes of a pair may break at unequal distances. When each piece joins the opposite chromosome, one chromosome is longer than normal and some of the genes are duplicated. The other chromosome will be shorter than normal, some genes being missing.
  - *Inversion:* A piece separating from a chromosome may get inverted before joining the opposite chromosome. Although the same genes are present, their sequence is disturbed.
- ***Isochromosomes:*** We have seen that during cell division, the centromere splits longitudinally so that each chromatid becomes a separate chromosome. Sometimes, the centromere splits transversely producing two dissimilar chromosomes. One chromosome is made up of the short arms of both chromatids, while the other is made up of the long arms. These are called "isochromosomes".
- ***Mosaicism:*** Chromosomal errors of the type described above may also occur during segmentation of the ovum. This results in a fetus having a mixture of cells with normal and abnormal chromosomes. This is called mosaicism. Such individuals may also show various abnormalities.

Gene abnormalities (gene mutations)

Genes are responsible for normal embryological development. A change in the structure of a gene may occur at the time of gametogenesis. This may give rise to birth defects. The change in the structure or function of a gene is called "gene mutation". At present, many birth defects are known which are caused by gene mutation.

MENSTRUAL CYCLE

- *Definition*: The term "menstrual cycle" is applied to cyclical changes that occur in the endometrium every month (Figs 3.27 and 3.28). It is also called *uterine cycle*.
- *Duration of blood flow*: The most obvious visible feature is a monthly flow of blood (menstruation). The duration of blood flow is 3–6 days.
- *Length of the cycle*: The length of the cycle is usually of 28 days and can vary from 21–40 days. The 1st day of bleeding in the present cycle marks the beginning of

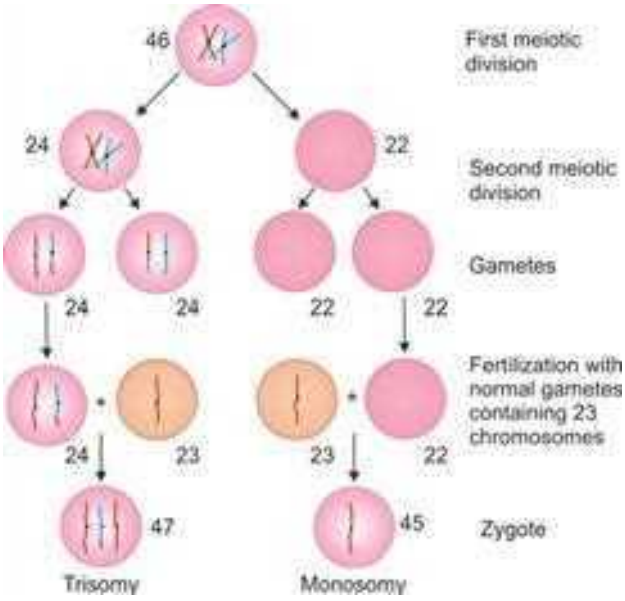


Fig. 3.25: Effects of nondisjunction during first meiotic division

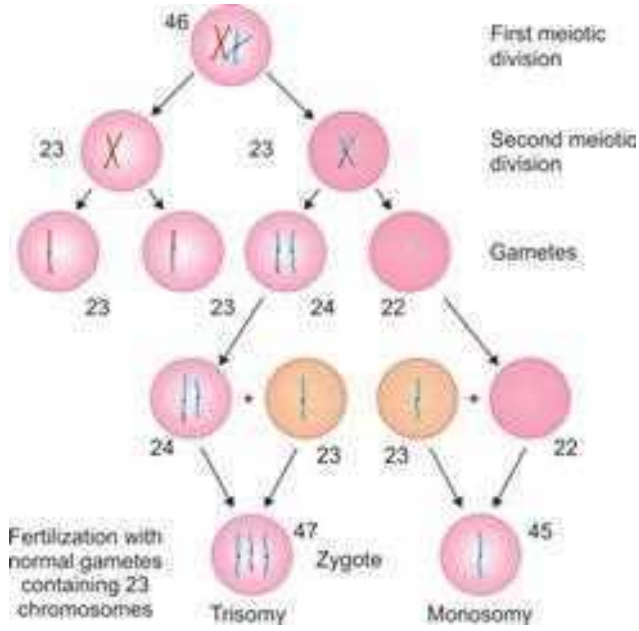


Fig. 3.26: Effects of nondisjunction during second meiotic division

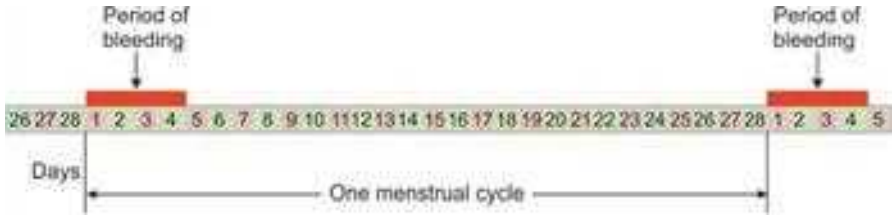


Fig. 3.27: Diagram illustrating the definition of menstrual cycle

the menstrual cycle. Ending of the cycle is the 1st day of bleeding of the next cycle.

- *Purpose*: The purpose of menstrual cycle is to prepare the endometrium for reception of fertilized ovum.

The layers in the wall of uterus and the constituents of endometrium were described in the early part of this chapter and in Figures 3.6 and 3.7.

- *Changes in endometrium*: The main changes in the endometrium are (a) increase in thickness, (b) growth of uterine glands, (c) changes in epithelial cells lining the glands and (d) increase in thickness and fluid content of the endometrial stroma (Fig. 3.29).
- Just before onset of menstruation, the blood supply to superficial parts of the endometrium is cut off (Fig. 3.6). This part is shed off and there is bleeding. The menstrual cycle is influenced by estrogen,progesterone,FSH and LH as represented in Figures 3.22 and 3.23.

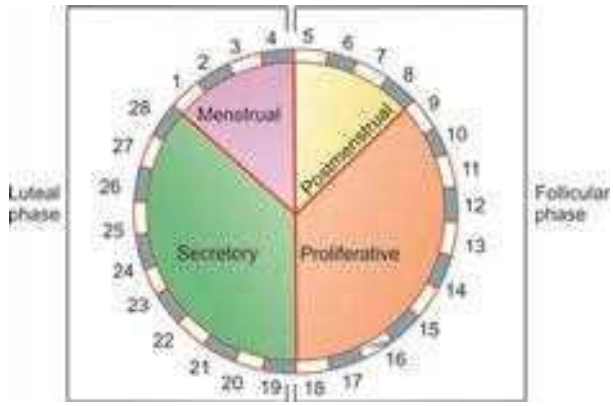


Fig. 3.28: Phases of menstrual cycle

Phases of the Menstrual Cycle

The menstrual cycle is divided into four phases on the basis of changes taking place in the endometrium (Figs 3.28 and 3.29).

i. Postmenstrual
ii. Proliferative
iii. Secretory
iv. Menstrual

- The changes during the postmenstrual phase and during most of the proliferative phase take place under the action of estrogens produced by the developing follicles in the ovary (Figs 3.22 and 3.23). Hence, this period is referred to as the *follicular phase* of the menstrual cycle. The follicular phase constitutes the first half of the menstrual cycle.
- Following ovulation, the corpus luteum is formed and starts secreting progesterone. During the second half of the menstrual cycle, this hormone (along with estrogens) produces striking changes in the endometrium (Figs 3.22 and 3.23). As these changes take place under the influence of the corpus luteum, this half of the menstrual cycle is called the *luteal phase*.
- Just before the onset of the next bleeding, there is lowering of levels of both progesterone and estrogens, and it is believed that this "withdrawal" leads to the onset of menstrual bleeding (Figs 3.22 and 3.23).

The division of the menstrual cycle into the phases mentioned above is, however, arbitrary.

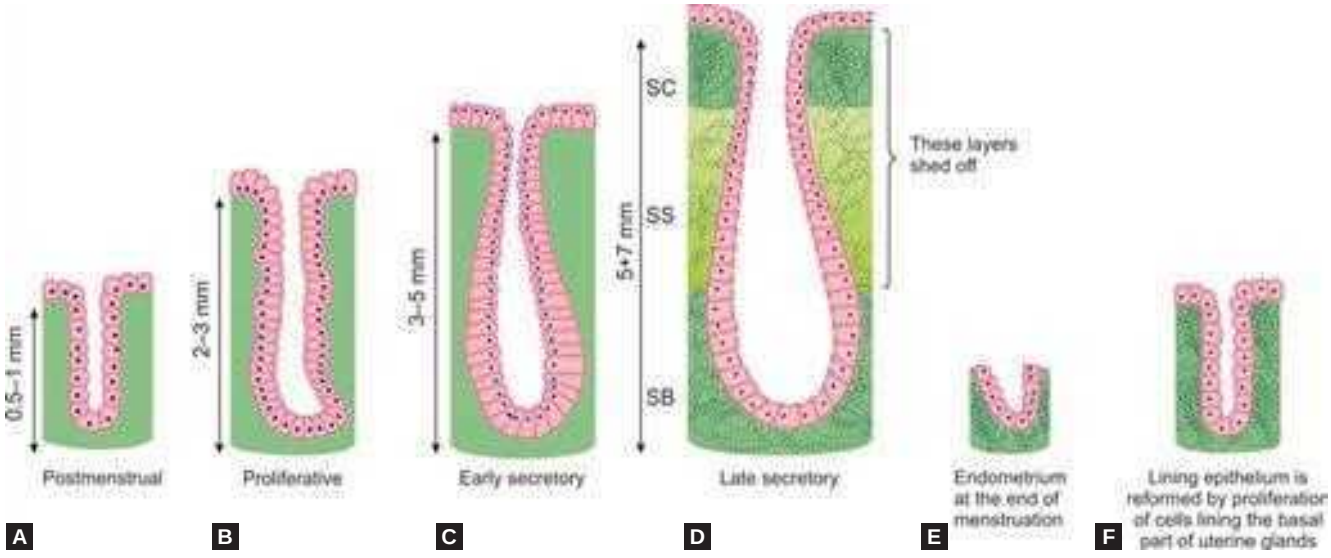
The changes are really continuous and may be summarized as follows:

- *Increase in thickness of endometrium*: The endometrium progressively increases in thickness (Figs 3.29 and 3.30). In the postmenstrual phase it is 0.5–1 mm thick; in the proliferative phase it is 2–3 mm thick; and in the secretory phase its thickness reaches 5–7 mm.
- *Increase in dimensions of uterine glands*: The uterine glands grow in length. At first they are straight (Fig. 3.29A), but gradually become convoluted (Fig. 3.29B). Because of these convolutions, the glands acquire a "saw-toothed" appearance when seen in longitudinal section. The glands also increase in diameter (Fig. 3.29C). The most basal parts of uterine glands, however, remain tubular and do not undergo these changes.
- *Change in epithelial lining of glands*: The epithelium lining the glands is at first cuboidal (Fig. 3.29A). During the proliferative stage it becomes columnar (Fig. 3.29B). Glycogen accumulates in the basal portion of the epithelial cell, pushing the nucleus nearer the lumen (Fig. 3.29C). During the secretory phase the apical part of the cell is shed off as part of the secretion (Fig. 3.29D). The cell again becomes cubical, but the edge toward the lumen becomes irregular (Fig. 3.29D).

Postmenstrual phase: During the postmenstrual phase, the cells of the stroma are uniformly distributed and are compactly arranged (Figs 3.6 and 3.29A).

Proliferative phase: The endometrium increases in thickness, the superficial part of the stroma remains compact, but the part surrounding the bodies of the uterine glands becomes spongy. The deepest part of the stroma also remains compact (Fig. 3.29B). The arteries of the endometrium are small to begin with. They grow in length during the proliferative phase. The stroma can, therefore, be divided into the following three layers from periphery to lumen.

1. Stratum basale (SB): Thin and has a separate blood supply.



Figs 3.29A to F: Changes in uterine epithelium, glands and endometrium during menstrual cycle
Abbreviations: SC, stratum compactum; SS, stratum spongiosum; SB, stratum basale

2. Stratum spongiosum (SS): Thick and edematous.
3. Stratum compactum (SC): Thin and contains compact stroma.

The stratum compactum and spongiosum together are called *functional zone* as these layers are sloughed off during menstruation. The basal layer is retained and is the *regenerative zone* from which regeneration of endometrium occurs

Secretory phase: During the secretory phase, these three layers become better defined. The endometrium becomes soft and edematous, because of the fluid secreted by the uterine glands (Figs 3.29C and D). During the secretory phase, the arteries supplying the superficial two-third of the endometrium become very tortuous, and are called spiral arteries. The arteries to the basal third of the endometrium (which does not participate in the changes associated with the menstrual cycle) remain straight and short. Towards the end of the secretory phase the endometrium is thick, soft, and richly supplied with blood. The secretory activity of the uterine glands not only makes the endometrium soft, but also provides nutrition to the embryo. These changes are, therefore, an obvious preparation for providing a suitable environment for the fertilized ovum, when it reaches the uterus.

Menstrual phase: In the absence of pregnancy, however, these measures are abortive: the superficial parts of the thickened endometrium (stratum compactum and stratum spongiosum) are shed off (Fig. 3.29E), and this is accompanied by menstrual bleeding. Menstrual bleeding causes the endometrium to be shed off bit by bit, and the blood along with shreds of endometrium flows out through the vagina. At the end of menstruation, the endometrium that remains is only 0.5 mm thick. It consists of the stratum basale along with the basal portions of the uterine glands (Fig. 3.29E). The epithelium of these glands rapidly proliferates and reforms the lining epithelium (Fig. 3.29F).

- The endometrial changes associated with the menstrual cycle are confined to the body of the uterus. The cervical mucosa is not affected.

The mechanism for onset of menstrual bleeding is as follows:

- *Constriction of spiral arteries and ischemia of superficial parts of endometrium*: A few hours before the onset of menstrual bleeding the spiral arteries get constricted so that blood supply to superficial parts of the endometrium is cut off. This ischemia leads to degeneration of the endometrium and also damages the walls of the blood vessels themselves.
- *Relaxation of spiral arteries and leakage of blood*: Subsequently when the spiral arteries relax and blood again flows into the endometrium, it leaks out through the damaged blood vessels. This leaking blood is responsible for gradual shedding of endometrium.

Time of Ovulation in Relation to Menstruation

In a 28-day menstrual cycle, ovulation takes place at about the middle of the cycle (Fig. 3.30). The period between ovulation and the *next* menstrual bleeding is constant at about 14 days, but the time of ovulation does not have a constant relationship with the preceding menstruation. This is so because the length of the menstrual cycle may vary from month to month in an individual. Hence, it is difficult to predict the date of the next ovulation from the date of menstruation unless the woman has very regular menstrual periods.

There are many methods of finding out the exact time of ovulation, but the one commonly used is the temperature method. In this technique, the woman's temperature is recorded every morning. When these temperatures are plotted on a graph, we get a curve like that shown in Figure 3.22. The temperature is low during actual menstruation. Subsequently it rises. At about the middle of the cycle, there is a sudden fall in temperature followed by a rise. This rise is believed to indicate that ovulation has occurred.

Clinical correlation

Importance of determining the time of ovulation and "safe period"

- ***Where pregnancy is not desired:*** After ovulation, the ovum is viable (i.e. it can be fertilized) for not more than 2 days. Spermatozoa introduced into the vagina die within 4 days. Therefore, fertilization can occur only if intercourse takes place during a period between 4 days before ovulation to 2 days after ovulation. The remaining days have been regarded as ***safe period*** as far as prevention of pregnancy is concerned. This forms the basis of the so-called ***rhythm-method*** of family planning.
- ***Where pregnancy is desired:*** Knowledge regarding the time of ovulation is also of importance in cases of ***sterility*** (difficulty in having children), where the couple can be advised to have intercourse during the days most favorable for conception.

Correlation between Ovarian and Uterine Cycles

The ovarian and uterine cycles run parallel to each other. Both are of 28 days duration. The uterine cycle is dependent on ovarian cycle.

The menstrual cycle is also divided into the follicular phase (in which changes are produced mainly by estrogens), and the luteal phase (in which effects of progesterone predominate) in correlation with ovarian follicular development (Fig. 3.30). The uterine cycle is dependent on ovarian cycle. The uterine endometrium shows cyclic changes, which are dependent on the hormones secreted by developing ovarian follicles and corpus luteum of the ovary.

HORMONAL CONTROL OF OVARIAN AND UTERINE CYCLES

These cycles are under the control of various hormones, which can be briefly summarized as follows (Fig. 3.22):

- The hypothalamus acts as a major center for the control of reproduction. It secretes the gonadotropin-releasing hormones (GnRH), which in turn controls the secretion of gonadotrophic hormones from the anterior pituitary gland (adenohypophysis).
- There are two gonadotrophic hormones. They are the FSH and the LH.
- In the first half of the menstrual cycle the GnRH acts on the anterior pituitary to release FSH. The FSH acts on the ovary and stimulates the formation and maturation of ovarian follicles (Fig. 3.22).
- The maturing ovarian follicles now start secreting estrogens. The repair and proliferation of endometrium takes place under the influence of estrogens. The endometrial stroma progressively thickens the glands in it, elongate and the spiral arteries begin to grow toward the surface epithelium.
- The level of estrogen rises to a peak about 2 days before ovulation. This leads to sudden increase in the level of LH secreted by the anterior pituitary (LH surge) about 24–36 hours before ovulation (Fig. 3.22). The LH surge leads to ovulation; and the Graafian follicle is transformed to the corpus luteum.
- The LH stimulates the secretion of progesterone by the corpus luteum. Though the secretion of progesterone predominates, some estrogen is also produced. The combined action of estrogen and progesterone stimulates the endometrial glands to secrete glycogen rich mucoid material (Fig. 3.23).
- If fertilization does not occur, the granulosa cells produce the protein inhibin, which acts on the anterior pituitary and inhibits the secretion of gonadotropins. This leads to regression of the corpus luteum.
- Due to the regression of the corpus luteum there is a fall in the blood level of estrogen and progesterone. The withdrawal of these hormones causes the endometrium to regress and triggers the onset of menstruation.
- If fertilization occurs, the corpus luteum does not regress. It continues to secrete progesterone and estrogen. The secretory phase of endometrium continues and menstruation does not occur.

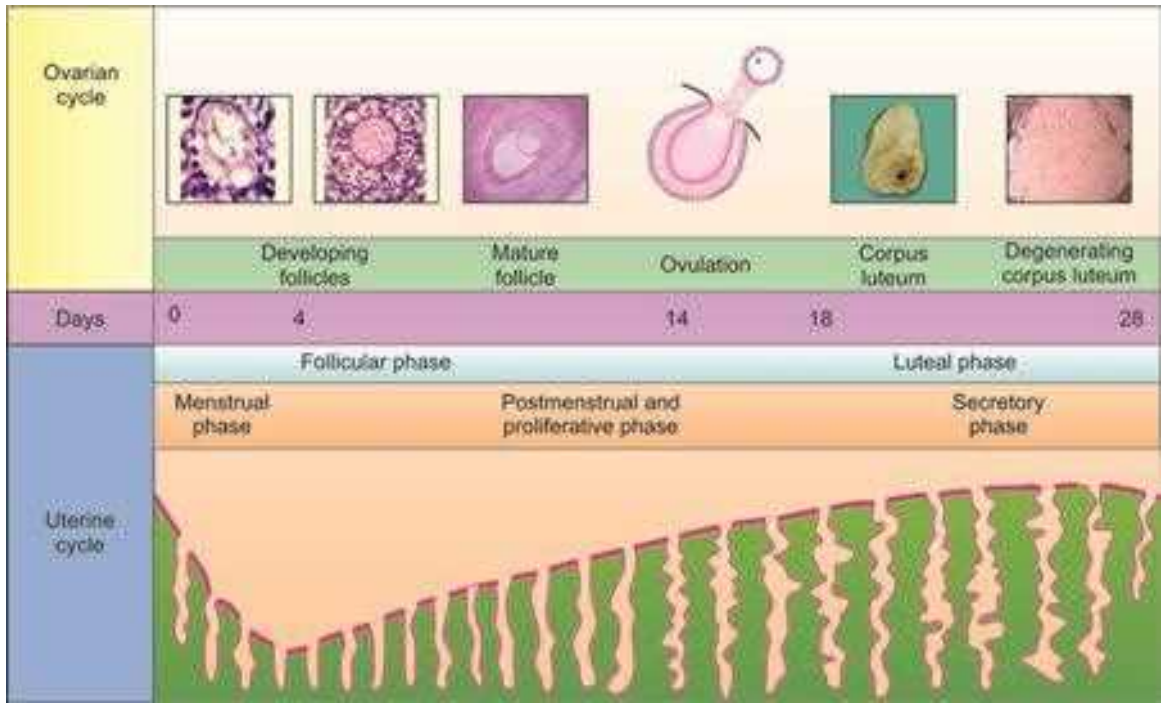


Fig. 3.30: Diagram showing correlation between ovarian and uterine cycles

Molecular and genetic basis of gametogenesis

- During oogenesis the number of layers of granulosal cells increase leading to the formation of theca interna. The oocyte accumulates mRNA molecules that are important for embryogenesis and for formation of zona pellucida.
- Proliferation of granulosa cells is mediated by growth differentiation factor 9, a member of TGF family.

Clinical correlation

Use of hormones in initiation of ovulation

Women of reproductive age with anovulatory cycles require hormonal treatment for treating the inability to conceive in the absence of other reasons for infertility (inability to conceive). The ovulation is induced by human chorionic gonadotrophin (HCG) and clomiphene that suppresses negative feedback of estrogen on adenohypophysis and stimulates release of FSH and LH that induce follicular maturation an ovulation.

Use of hormones for contraception/suppression of ovulation

- Ovulation in a woman (and by corollary, pregnancy) can be prevented by administration of contraceptive pills. The most important ingredients of such pills are progestins (in the form of synthetic compounds). Better results are obtained when a small amount of estrogen is also given.
- In the most common variety of pill (distributed by government agencies in India), the progestin is norethisterone acetate (1 mg); and the estrogen is in the form of estradiol (50 µg). The pills are distributed in packets, each packet containing 28 pills out of which 21 pills contain these hormones, and 7 pills do not (for use in the last 7 days). The use of pills is started 5 days after onset of menstruation. They are taken continuously without any break as long as contraception is desired. Normal menstruation occurs during the 7 days in which pills without hormones are being taken. If the pills are taken regularly there is a regular menstrual cycle of 28 days duration.
- Presence of progesterone in the preovulatory phase prevents occurrence of ovulation. This is because the progesterone in the pill prevents the secretion of FSH and LH by the pituitary. This interferes with the maturation of follicles and ovulation.
- Stoppage of pills reduces levels of these hormones in blood. It is this withdrawal that leads to menstrual bleeding. Such pills have almost 100% success in suppressing maturation of follicles and ovulation.

REVIEW QUESTIONS

1. Explain spermiogenesis.
2. Describe structure of spermatozoon.
3. Enumerate the differences between spermatogenesis and oogenesis.
4. Write short notes on Graafian follicle.
5. Write short notes on corpus luteum.

Chapter 4

Fertilization and Formation of Germ Layers

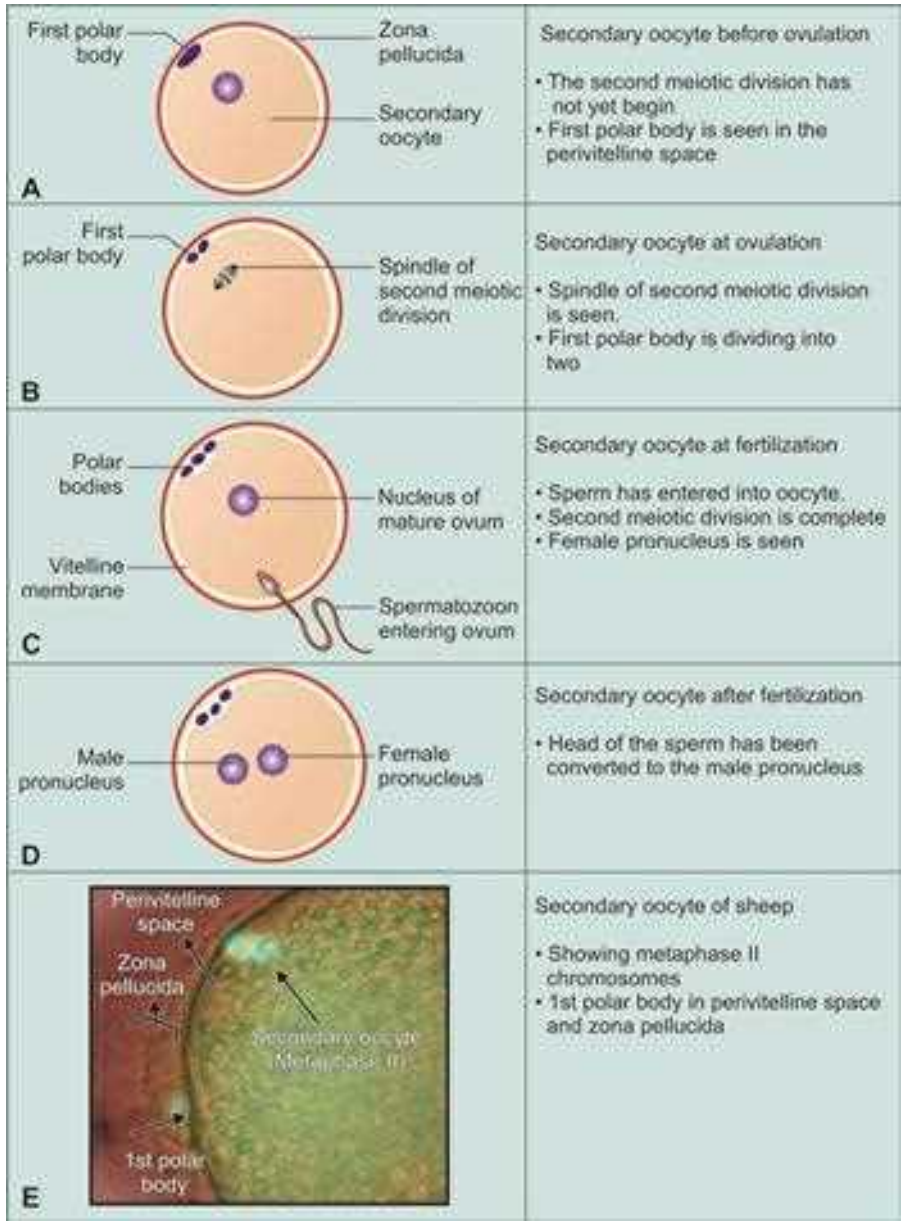
HIGHLIGHTS

- *Fertilization* of the ovum takes place in the ampulla of the uterine. The fertilized ovum is a large cell. It undergoes a series of divisions (*cleavage*).
- When there are 16 cells the ovum is called a *morula*. It has an inner cell mass covered by an outer layer of cells, the trophoblast.
- Fluid partially separates the inner cell mass from trophoblast. The morula now becomes a *blastocyst*.
- The cells of the inner cell mass multiply, and are rearranged to form an *embryonic disc* having two layers. These layers are the *epiblast* and the *hypoblast*. Later, the epiblast differentiates into three germ layers, the *ectoderm* (outer), the *endoderm* (inner) and the *mesoderm* (middle). Cells of the hypoblast become flattened and line the yolk sac.
- A cavity appears on the ectodermal side of the disc. This is the *amniotic cavity*. Another cavity appears on the endodermal side. This is the *yolk sac*.
- At first the walls of the amniotic cavity and yolk sac are in contact with trophoblast. They are soon separated from the latter by *extraembryonic mesoderm*.
- A cavity, the *extraembryonic coelom* appears and splits the extraembryonic mesoderm into a *somatopleuric layer* (in contact with trophoblast) and a *splanchnopleuric layer* (in contact with yolk sac).
- The trophoblast and underlying somatopleuric mesoderm form a membrane called the *chorion*. The cells forming the wall of the amniotic cavity form the *amnion*.
- The amniotic cavity is now attached to trophoblast by mesoderm into which the extraembryonic coelom has not extended. This mesoderm forms the *connecting stalk*.
- If we view the embryonic disc from the ectodermal side near one edge it has a rounded area called the *prochordal plate*. Here ectoderm and endoderm are not separated by mesoderm.
- An elevation, the *primitive streak*, is also seen on the embryonic disc. A line drawn through the prochordal plate and the primitive streak divides the embryonic disc into right and left halves.
- Cells multiplying in the primitive streak move into the interval between ectoderm and endoderm and form the *mesoderm* (third germ layer).
- Caudal to the primitive streak we see a round area called the *cloacal membrane*. It is made up only of ectoderm and endoderm.

INTRODUCTION

In Chapter 3, we have seen that while the ovarian follicle is growing, the oogonium within it undergoes maturation. The oogonium enlarges to form a primary oocyte. The primary oocyte undergoes the first meiotic division to shed off the first polar body and becomes a secondary oocyte (Figs 4.1A and E).

At the time of ovulation, the second meiotic division is in progress and a spindle has formed for separation of the second polar body (Fig. 4.1B). At this stage, the "ovum" enters the infundibulum of the uterine tube and passes into the ampulla (Fig. 4.2).



Figs 4.1A to E: Some stages in the maturation of the ovum: (A) Ovum just before ovulation; (B) Ovum at the time of ovulation; (C) Ovum at the time of fertilization; (D) Ovum just after fertilization; (E) Sheep secondary oocyte showing metaphase II chromosomes and first polar body in perivitelline space (*Image Courtesy:* Dr VH Rao)

FERTILIZATION

- *Definition*: Process of fusion of two highly specialized/highly differentiated/mature, haploid germ cells, an ovum and a spermatozoon resulting in the formation of a most unspecialized/undifferentiated/diploid, mononucleated single cell, the zygote.
- The process of human fertilization is very complicated and comprises of many components and steps. Both male and female gametes have to complete a number of biological processes prior to actual process of fertilization.
- *Fertilization is a signal for completion of second meiotic division***.** Out of a few hundred capacitated sperms, that surround the ovum, only one pierces the zona pellucida and enters the ovum. As soon as one spermatozoon enters the ovum, the second meiotic division is completed, and the second polar body is extruded.

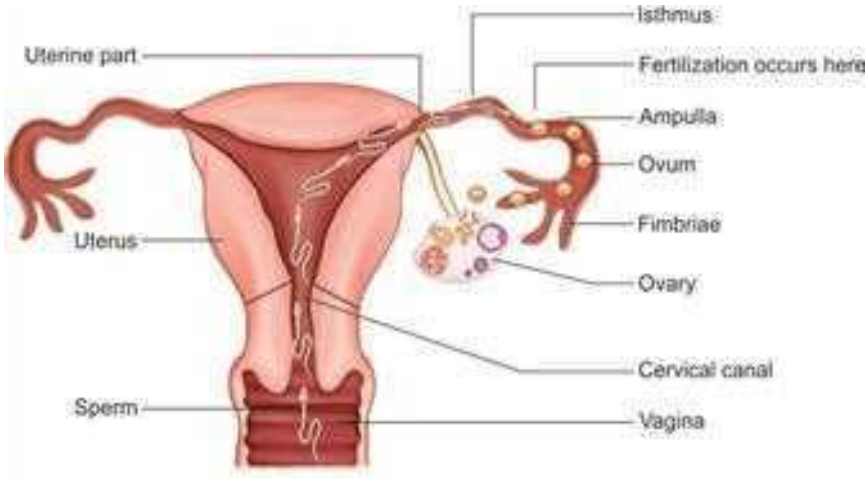
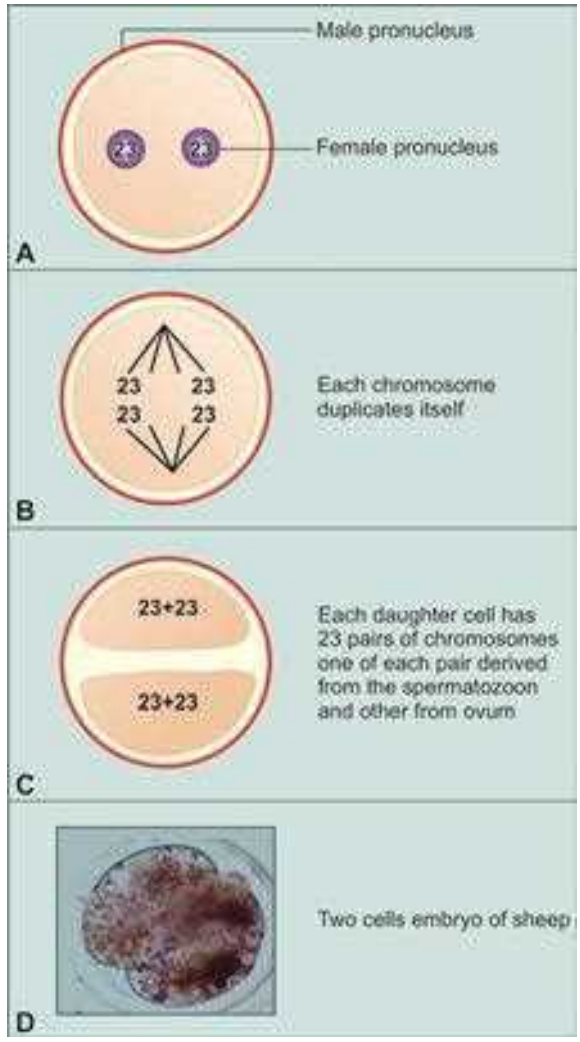


Fig. 4.2: Path taken by the sperm and ovum, for fertilization

Hence, it is a signal for completion of second meiotic division (Fig. 4.1C).

- *Fertilization is fusion of male and female pronuclei* (Fig. 4.1D) as the nucleus of the ovum becomes the female pronucleus. The head of the spermatozoon (which is formed from the nucleus) separates from the middle piece and tail, and transforms itself into the male pronucleus. Soon thereafter, the pronuclei lose their nuclear membranes. The 23 chromosomes of the female pronucleus (Fig. 4.3A) and 23 of the male pronucleus get mixed up and form 23 pairs (46 nos; Fig. 4.3B). Hence, fertilization is the fusion of male and female pronuclei/chromosomes.
- *Fertilization is antithesis of cell division*. Fusion of haploid chromosomes of male (23 nos.) and female (23 nos.) pronuclei results in 46 chromosomes (23 pairs). These undergo changes like those in a typical mitotic division leading to the formation of an embryo having two cells (Figs 4.3C and D). Strictly speaking, there is no one-cell stage of the embryo. The middle piece and the tail soon separate from the head of the sperm and degenerate. Fertilization initiates mitotic division of zygote resulting in the formation of a multicellular organism. Hence, it is antithesis of cell division.
- *Site of fertilization*: Fertilization of the ovum occurs in the ampulla or lateral one-third of uterine tube (Fig. 4.2).
- *Stages*: The various events in fertilization can be described in three stages.
  1. Approximation of gametes
  2. Contact and fusion of gametes
  3. Effects/Results of fertilization.

1. *Approximation of gametes*: It is by transport of male and female gametes in the female genital tract (Fig. 4.4—stage 1).
   a. *Spermatozoon transport*—following events play an important role in successful fertilization.



Figs 4.3A to D: Behavior of chromosomes during fertilization. (A) Male (22 + X/22 + y) and female (22 + X) pronuclei; (B) Duplication of chromosomes and arrangement at equatorial plate; (C) Anaphase and cytokinesis forming two cells; and (D) Sheep—two cells embryo (*Image Courtesy:* Dr VH Rao)

- *Semen*: The semen, also known as *seminal fluid,* is an organic fluid that contains spermatozoa. It includes secretions of seminal vesicle (60%), prostate (25%) and bulbourethral glands (5%) and the spermatozoa (10%). The secretions from seminal vesicle are rich in fructose that provides energy for the spermatozoa. The secretions of prostate contain ions, citric acid, acid phosphatase and fibrinogen (clotting of semen). The secretions of bulbourethral glands act as pre-ejaculatory lubricant. The normal amount of semen produced at each ejaculation is about 2–3 mL. The number of spermatozoa released in each ejaculation is 100 million/mL. The pH of semen is maintained (7.2–7.6) by a base spermine present in it. The semen contains fibrinolysin that liquefies semen in 30 minutes after ejaculation.
- *Maturity and motility of sperms*: During their passage through male genital tract the spermatozoa mature. Movements of tail are important for their motility. Motility is important for penetration of three barriers surrounding the ovum.
- *Transport of sperms*: The prostaglandins present in semen stimulate peristaltic contractions of female genital tract at the time of sexual intercourse. During their transport there will be reduction in the number of spermatozoa due to the constrictions in female genital tract. Movements of their tails through uterus and tubes assisted by muscular contraction are responsible for the movement of spermatozoa. Time taken for transport to uterus is 5–45 minutes.
- *Fate of spermatozoa in female genital tract*: Around 200–500 million sperms are deposited in the female genital tract and about 300–500 spermatozoa only reach the site of fertilization. The life span/viability of spermatozoa after ejaculation is 24–48 hours. They have greater motility; hence, they rapidly lose the fertilizing power. Acidic vaginal pH decreases and alkalinity increases motility of spermatozoa. The spermatozoa are attracted to the ovum by a mechanism known as *chemotaxis*, i.e. release of certain chemicals by the follicular cells.
- *Capacitation*: Capacitation is the final step in maturation of spermatozoon before actual fertilization and it takes place in female genital tract. It is a species-specific interaction between sperm and oocyte. The time required for capacitation is 7 hours. It starts in the uterus and continues into the tubes. The mechanism of capacitation is not known. It involves number of changes including changes in the sperm cell membrane and signal transduction (calcium influx, release of acrosomal enzymes, etc.). Certain major changes in surface glycoproteins (removal of glycoprotein cut and exposing zona binding proteins) is caused by secretions of the female genital tract thus allowing sperm-egg binding. An immunological reaction between the fertilizin on ovum and anti-fertilizin on spermatozoon is also proposed. Follicular fluid is thought to enhance the capacitation.

b. *Ovum transport*: The structure of ovum at ovulation, transport of ovum from ovary to ampulla of uterine tube and the viability of sperm are important for the success of fertilization.
   - *Structure of ovum at ovulation*: At ovulation the ovum contains secondary oocyte with 23 unpaired chromosomes enclosed in vitelline membrane, surrounded by zona pellucida with proteins and corona radiata with matrix rich in hyaluronic acid.
   - *Transport of ovum from ovary to ampulla of uterine tube*: The fimbriae of uterine tube moves over the ovary at ovulation and the ciliary beats of fimbriae sweeps the ovulatory mass into the infundibulum. The ciliary beats of uterine epithelium and muscular contractions of uterine tube are responsible for transcoelomic migration of ovum from the surface of ovary into the ampulla of uterine tube. The ovum reaches ampulla, the site of fertilization in 25 minutes.
   - *Viability of ovum*: The ovum that is released at ovulation is viable for 24–48 hours. In the absence of fertilization it degenerates.

2. *Contact and fusion of gametes*: There are *three barriers* which the sperm has to penetrate before fusing with the ovum (Fig. 4.4—stages 1 and 2). They are:
   1. Corona radiata
   2. Zona pellucida
   3. Vitelline membrane

   Four processes are involved in the penetration of these barriers. They are
   i. Acrosome reaction
   ii. Disintegration of barriers

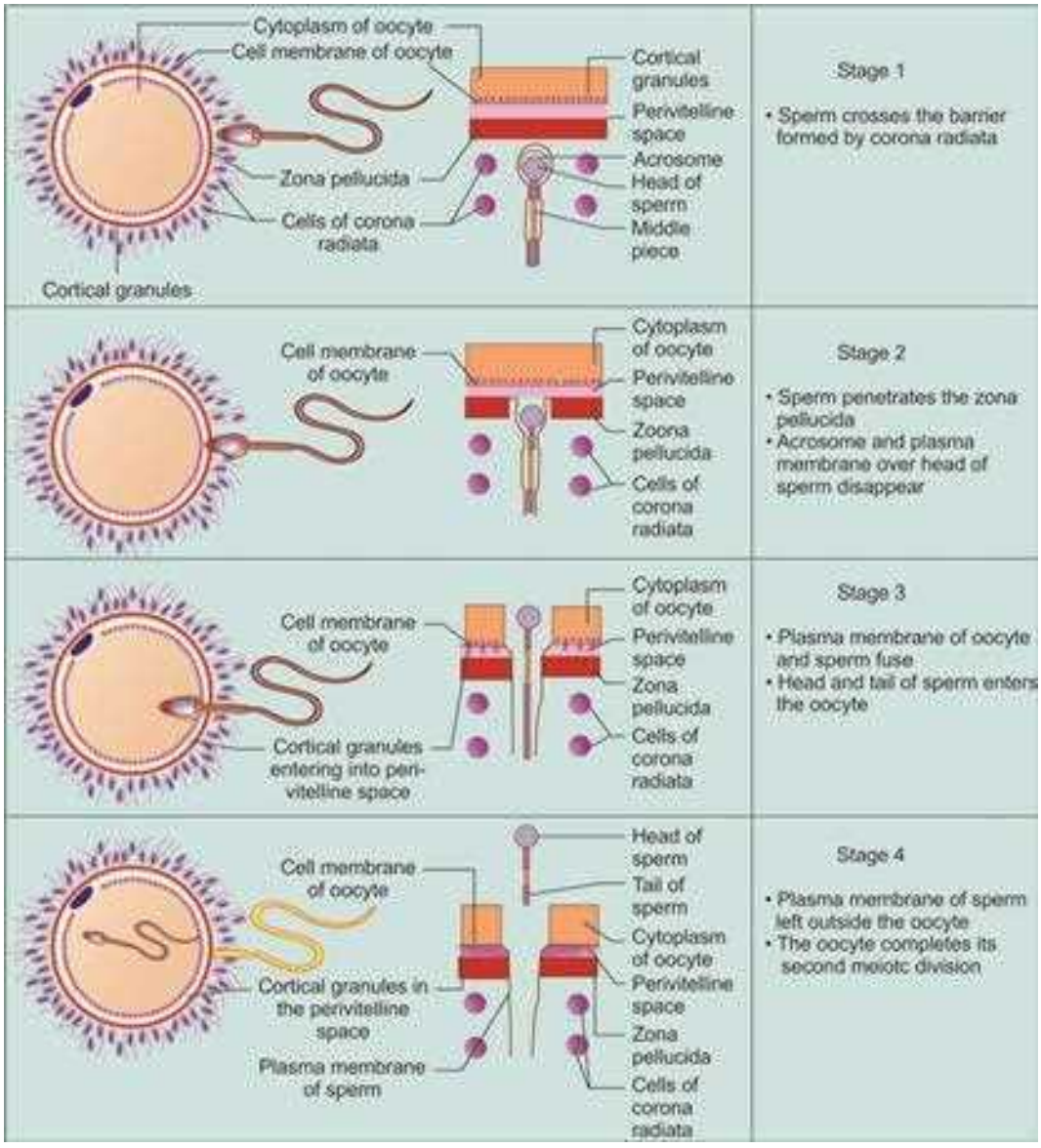
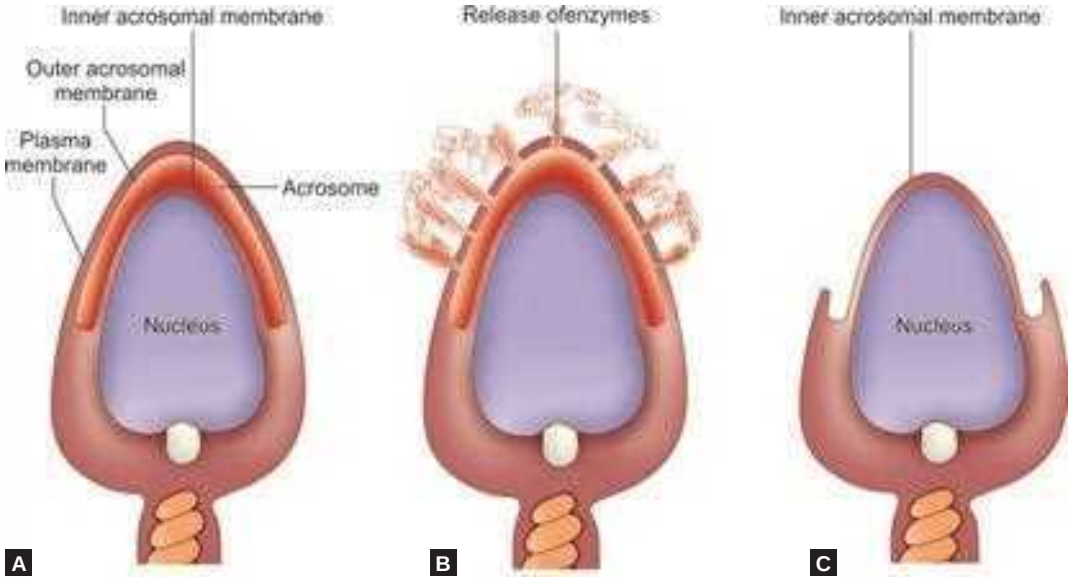


Fig. 4.4: Stages in penetration of a spermatozoon into an ovum

iii. Calcium wave in oocyte cytoplasm
iv. Nuclear fusion

i. *Acrosome reaction*:
   - *Definition*: Process of multiple contacts that capacitated sperm head establishes between plasma membrane and outer membrane of acrosomal cap, and discharging chemical substances that facilitate penetration of barriers around oocyte in succession.
   - *Coverings of sperm head*: The head of the sperm has three coverings. From inside out they are *nuclear envelope, bilaminar acrosomal membrane* containing enzymes for penetration of oocyte and plasma membrane (Fig. 4.5).
   - *Release of acrosomal enzymes*: The acrosome reaction must be completed to facilitate fusion of sperm with the secondary oocyte. It occurs when sperms come into contact with the corona radiata of the oocyte (Figs 4.4—stage 1 and 4.5). Perforations develop in the acrosome. Point fusions of the sperm plasma membrane and the outer acrosomal membrane occur. The acrosome reaction is associated with the release of acrosome enzymes that facilitate penetration of the zona pellucida by the sperm (Figs 4.4—stages 2 and 3 and 4.5B and C). The three acrosome enzymes that are released are *hyaluronidase, the protease enzyme acrosin and*



Figs 4.5A to C: Covering of acrosome and release of acrosomal enzymes

acid phosphatase. The glycoprotein of the zona pellucida is responsible for induction of the acrosomal reaction.

ii. *Disintegration of barriers*: The sperm has to pass through the following three barriers in order. Disintegration of each barrier is by enzyme reaction (Fig. 4.4).
   - *Corona radiata*: Penetration of this first barrier depends on the release of hyaluronidase from the acrosome of sperm, tubal mucosal enzymes and movements of tail of spermatozoon also aids in penetration of corona radiata (Fig. 4.4—stage 1). The hyaluronidase digests the cells of corona radiata.
   - *Zona pellucida*: The glycoproteins on the outer surface of sperm head binds with glycoproteins on the zona pellucida of ovum. This is by binding to Zp3 and Zp2 receptors (Fig. 4.4—stage 2). Acrosin causes digestion of ZP around sperm head. The reaction of zona is to prevent polyspermy. Alterations in the plasma membrane of oocyte and zona pellucida ensure that no other spermatozoon can enter the oocyte. The zona pellucida is altered due to release of lysosomal enzymes by plasma membrane of the oocyte. This process is called *zona reaction*.
   - *Vitelline membrane*: When a spermatozoon comes in contact with the oocyte, plasma membranes of the two cells fuse (Fig. 4.4—stages 3 and 4). This probably occurs at *receptor* sites that are specific for a species. The disintegrin peptide released from sperm head initiates fusion. The vitelline membrane contains integrin peptides. This process takes 30 minutes.

iii. *Calcium wave in oocyte*: The contact of sperm with vitelline membrane of oocyte triggers calcium wave (*depolarization*) in oocyte cytoplasm. This triggers important events at fertilization.
   - Secondary oocyte resumes second meiotic division (Fig. 4.1B).
   - Contact of cortical granules with plasma membrane in the periphery of ooplasm and release of lysosomal enzymes from cortical granules produce vitelline block and prevent polyspermy.
   - Alterations taking place in the plasma membrane of the oocyte, and in the zona pellucida, ensure that no other spermatozoon can enter the oocyte.
   - Metabolic activation of egg**.** Entry of the sperm leads to metabolic changes within the ovum that facilitate its development into an embryo.

iv. *Nuclear fusion*: Both head and tail of the spermatozoon (excluding plasma membrane) enters the cytoplasm of oocyte. Approximation of pronuclei takes place near the middle of cytoplasm of ovum (Fig. 4.1D).
   - Immediately after the entry of sperm head into the cytoplasm of the oocyte the latter completes its second meiotic division, releases ovum with 1N DNA and second polar body. Second polar body extruded into perivitelline space (Fig. 4.1C).
   - Reconstitution of oocyte chromosomes forms female pronucleus (Fig. 4.1D).

- The sperm head makes a rotation of 180° within the oocyte cytoplasm with its nucleus swollen, transforms into a male pronucleus (Fig. 4.1D).
- *Formation of zygote*: Each chromosome in the male and female pronuclei is made up of only one chromatid. Replication of DNA takes place to form a second chromatid in each chromosome (1N–2N DNA) and two centrioles appear. Disappearance of nuclear membranes and splitting of each chromosome into two (as in mitosis) occurs. The ovum is now called *zygote*. Meanwhile a spindle forms between two centrioles and chromosomes from each pronucleus (Haploid chromosomes with 2N DNA) organizes on the spindle equator. One chromosome of each pair moves to each end of the spindle. This leads to formation of two cells, each having 46 chromosomes (Figs 4.3C and D).

3. *Effects/Results of fertilization*: From what has been said above, it will be clear that the results of fertilization are:
   - Completion of second meiotic division of female gamete (secondary oocyte)
   - Restoration of diploid number (46) of chromosomes
   - Determination of chromosomal sex of the future individual to be born
   - Initiation of cleavage (mitotic) division of zygote
   - Determination of polarity and bilateral symmetry of embryo
   - Genetic diversity.

Embryo contains only maternal mitochondria—sperm mitochondria are discarded.

The important points to note at this stage are that:

- The two daughter cells are still surrounded by the zona pellucida (Figs 4.1 and 4.3)
- Each daughter cell is much smaller than the ovum
- With subsequent divisions, the cells become smaller and smaller until they acquire the size of most cells of the body.

Important factors in fertilization are:

- Normally functioning reproductive organs
- Chemotaxis
- Coordination of different processes as mentioned below:
  - Time interval between insemination and ovulation
  - Length of time ovum remains fertilizable
  - Length of time sperm retains fertilizing power
- Number of sperms reaching uterine tube
- Time taken by sperm to reach the ovum
- Other factors of semen which influence fertilization.

SEX DETERMINATION

All ova contain 22 + X chromosomes. However, the spermatozoa are of two types. Half of them have 22 + X chromosomes and the other half of them have 22 + Y chromosomes. These are called as "X-bearing", or "Y-bearing", spermatozoa. An ovum can be fertilized by either type of spermatozoon. If the sperm is X-bearing, the zygote has 44 + X + X chromosomes and the offspring is a girl. If the sperm is Y-bearing the zygote has 44 + X + Y chromosomes and the offspring is a boy. Thus the *sex of a child is "determined" at the time of fertilization depending on the contribution by the spermatozoon*. It will now be clear that *one chromosome of each of the 23 pairs is derived from the mother and the other from the father*.

TEST TUBE BABIES/IN VITRO FERTILIZATION

The so-called test tube babies are produced by the technique of *in vitro fertilization* (in vitro = outside the body, as against in vivo = within the body). This technique is resorted to when normal in vivo fertilization could not be achieved for any one or more reasons mentioned subsequently. It is a form of assisted reproductive technique (ART).

The various steps in this technique are (Fig. 4.6):

- *Pretreatment*: Gonadotropins or clomiphene citrate are administered to the woman to stimulate growth of ovarian follicles.
- *Collection of ova*: Several ova are aspirated from the Graafian follicles by laparoscopy or under ultrasound visualization.
- *Collection of sperms*: Sperms are collected from the husband/donors semen and processed to make them ready for fertilization.
- *Incubation of gametes*: Ova and sperm are incubated together in special media and environment to promote fertilization.
- *Fertilization and early development of the embryo* take place in the culture medium. The process is carefully monitored, up to the 8-cell stage of cleavage.
- *Pretreatment of female with progesterone*: To make the endometrium receptive to the cleaving embryo and its implantation.
- *Reimplantation*: Eight-cell stage embryos (one or more) are introduced into the uterine cavity through cervical canal—embryo transfer. Any remaining embryos are frozen for future use.

The reasons for using the technique can be as follows:

- The number of spermatozoa may be inadequate (Usually about 2–5 mL of semen is ejaculated. Each milliliter contains about 100 million spermatozoa. If the count of spermatozoa is less than 20 million/mL, there may be difficulty in fertilization.
- There may be inadequate motility of spermatozoa.
- There may be obstruction of the uterine tube.
- There may be absence of ovulation.

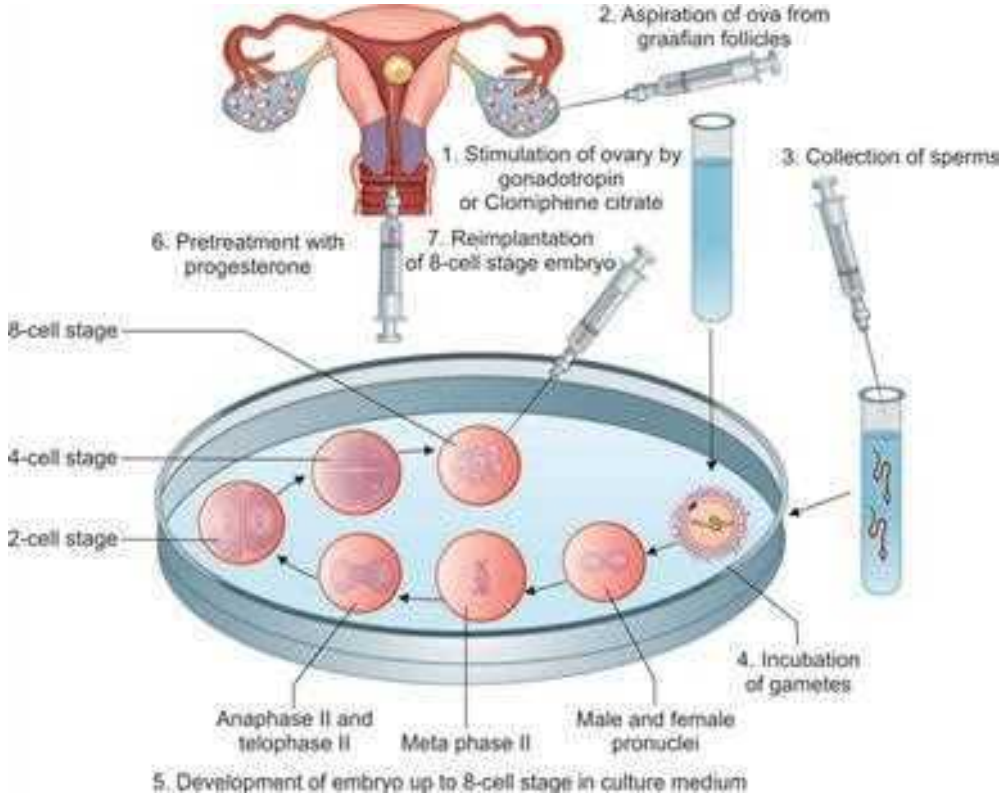


Fig. 4.6: In vitro fertilization (IVF) technique—various steps

CLEAVAGE

The two cells formed as described above (Fig. 4.7A) undergo a series of divisions. One cell divides first so that we have a "3-cell" stage of the embryo (Fig. 4.7B) followed by a "4-cell" stage (Figs 4.7C and 4.8A), a "5-cell" stage, etc. This process of subdivision of the ovum into smaller cells is called *cleavage*.

Definition: It is a process of repeated mitotic divisions of zygote within zona pellucida in rapid succession, giving rise to increasing number of smaller cells called *blastomeres*.

The cleavage divisions start immediately after fertilization and continue as the zygote is passing through the uterine tube toward uterus. The cleaving zygote is covered by zona pellucida.

This journey is facilitated by ciliary beats of uterine epithelium and contraction of uterine tube musculature.

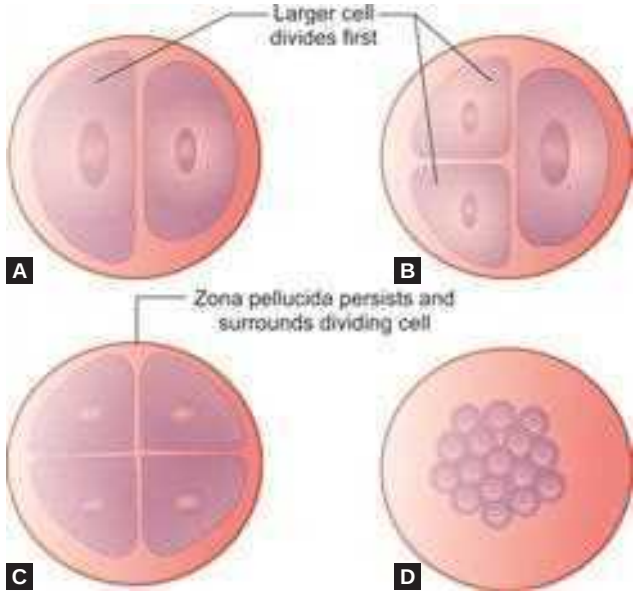
Duration: The cleavage division lasts for 6 days, i.e. up to 7th day after fertilization.

Stages of cleaving egg: During its journey through uterine tube, the cleaving egg passes through the following stages (Figs 4.7 and 4.8). At the first cleavage division the zygote forms one large cell and one small cell. In the next cleavage the larger cell divides first followed by smaller one (Table 4.1).

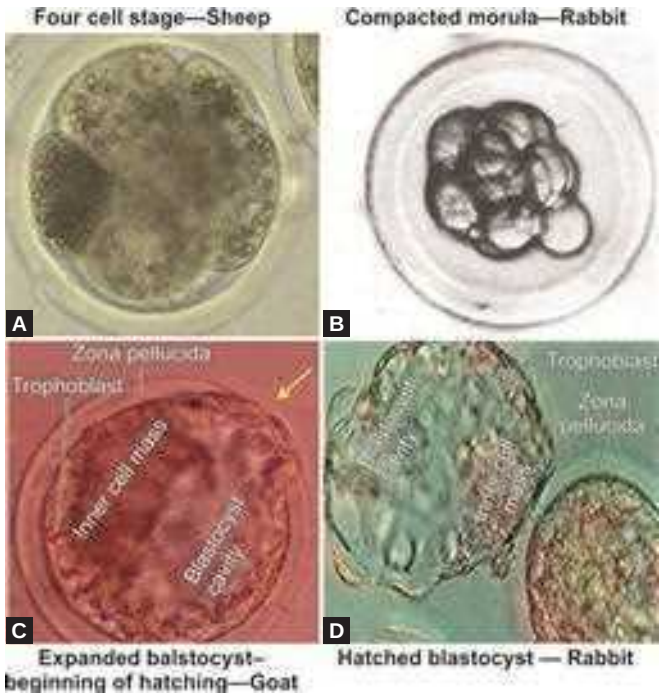
Subdivisions of cleavage: There are three subdivisions in cleavage. They are:

1. Stage of compaction
2. Morula
3. Blastocyst

1. *Stage of compaction*: It starts at third division, i.e. at 8-cell stage. There will be maximal contact between the cells. Outer cells form tight junctions and exhibit polarity. Inner cells form gap junctions. The nutrition to cleaving egg is the meagre store of food in blastomeres and breakdown products of tubal secretion transferred through zona pellucida.
2. *Morula*: As cleavage proceeds the ovum comes to have 16 cells, i.e. fourth division. It now looks like a mulberry and is called the *morula* (Figs 4.7D and 4.8B). It is still surrounded by the zona pellucida. Cells are similar in size and structure. If we cut a section across the morula, we see that it consists of an inner cell mass that is completely surrounded by an outer layer of cells. The cells of the outer layer will later give rise to a structure called the *trophoblast* that forms the coverings of the embryo (Fig. 4.9A). The inner cell mass gives rise to the embryo proper and is, therefore, also called the *embryoblast*. The cells of the trophoblast help to provide nutrition to the embryo.



Figs 4.7A to D: Some stages in segmentation of the fertilized ovum. (A) Two-cells stage, (B) Three-cells stage, (C) Four-cells stage and (D) Morula



Figs 4.8A to D: Some stages in 1st week of embryo. (A) Sheep embryo—four-cells stage; (B) Rabbit embryo—compacted morula; (C) Goat embryo—expanded blastocyst—beginning of hatching; and (D) Rabbit embryo—hatched blastocyst (*Images Courtesy:* Dr VH Rao)

TABLE 4.1: Relationship of cleavage stages and fertilization

Cleavage stage	*Time after fertilization when it can be observed*
One-cell stage	<24 hrs
Two-cells stage	24–36 hrs
3–4 cells stage	36–48 hrs
5–8 cells stage	48–72 hrs
9–16 cells stage	72–96 hrs

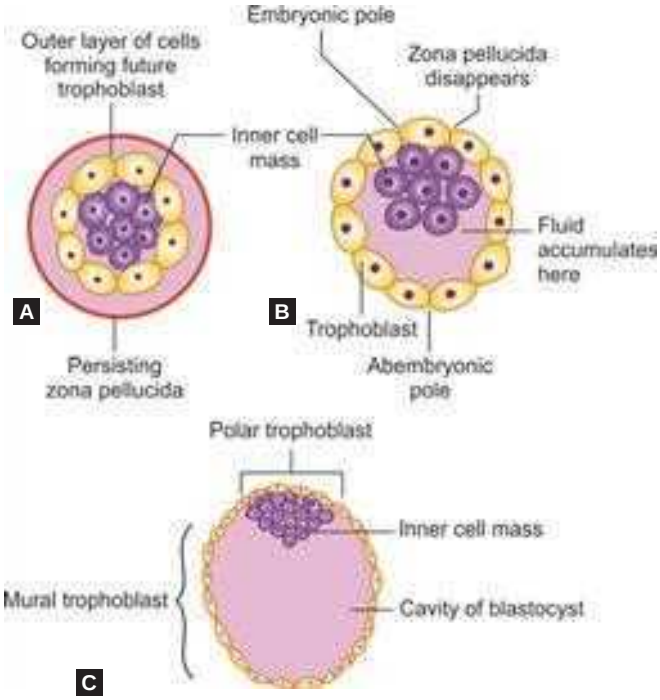
3. *Blastocyst*: Between 4th and 5th day and 32–64 cells stage some fluid passes into the morula from the uterine cavity, and partially separates the cells of the inner cell mass from those of the trophoblast (Figs 4.8C and D and 4.9B). As the quantity of fluid increases, the morula acquires the shape of a cyst. The cells of the trophoblast become flattened and the inner cell mass gets attached to the inner side of the trophoblast on one side only (Figs 4.8C and D and 4.9C). The morula has now become a *blastocyst*. The cavity of the blastocyst is the *blastocoele*. That side of the blastocyst to which the inner cell mass is attached is called the *embryonic or animal pole,* while the opposite side is the *abembryonic pole* (Figs 4.8C and D and 4.9B). The trophoblast divides into the one in contact with embryoblast known as *polar trophoblast* and the rest of it lining the wall of blastocyst is known as *mural trophoblast* (Figs 4.8C and D and 4.9C).

Hatching of blastocyst: Thinning of zonal pellucida starts on 4th day and it disappears on 5th day of fertilization (Fig. 4.8D). Disappearance of zona pellucida initiates attachment of trophoblastic cells to uterine epithelium known as *implantation* on 6th or 7th day after fertilization.

Principal Effects of Cleavage

- There is increase in the number of cells whose size decreases progressively
- Partitioning of cytoplasm of zygote among the blastomeres
- Increased motility of protoplasm facilitating morphogenetic movements and rearrangements in later stages of development
- Approximation in size of cells similar to that of somatic cells characteristic for the species
- Restoration of nuclear cytoplasmic ratio
- There is no increase, rather decrease in protoplasmic volume due to metabolic activity
- Zygote genome is activated on 2nd day after fertilization.

Cleavage is the process whereby a unicellular fertilized ovum (zygote) with exceptionally large ratio of cytoplasm



Figs 4.9A to C: Formation of blastocyst

to nucleus is transformed into a multicellular mass of blastomeres each of which approximates to the ratio found in somatic cells. Thus cleavage facilitates formation of a multicellular organism.

Clinical correlation

Hydatidiform mole: It is a form of abnormal blastocyst that resulted from development of trophoblast/outer cell mass that forms the placenta. There will be little or no embryonic tissue. The moles secrete high levels of HCG and can produce benign (invasive mole) or malignant (***Choriocarcinoma***) tumors. Genetic analysis of moles indicates diploid chromosomes of paternal origin. This results from fertilization of an oocyte without nucleus and duplication of paternal chromosomes to maintain diploid state. Paternal genes regulate the development of trophoblast.

Function of the Zona Pellucida

- The zona pellucida is a specialized extracellular matrix surrounding the developing oocyte. It is formed by secretions from the oocyte and the granulosa cells.
- The trophoblast has the property of being able to stick to the uterine (or other) epithelium and its cells have the capacity to eat up other cells. They can, therefore, invade and burrow into tissues with which they come in contact. As the embryo travels down the uterine tube, and the uppermost part of the uterine cavity, it is prevented from "sticking" to the epithelium by the zona pellucida.
- During its travel in the uterine tube, the embryo receives nutrition, partly from the substances stored within the ovum (e.g. yolk), and partly by diffusion from uterine secretions. By the time a blastocyst is formed, it is necessary for the embryo to acquire additional sources of nutrition. This is achieved when the blastocyst "sticks" to the uterine endometrium, and gets implanted in it.
- However, before implantation, it is necessary for the zona pellucida to disappear. The zona pellucida disappears soon after the morula reaches the uterine lumen. Thus, the function of the zona pellucida is to prevent implantation of the blastocyst at an abnormal site.
- There are four types of zona pellucida glycoproteins ZP1, ZP2, ZP3 and ZP4 which have different roles in different phases of fertilization. ZP2 plays an important role in sperm binding, gamete recognition, penetration and prevention of polyspermy.
- The glycoprotein of the zona pellucida is responsible for induction of the acrosomal reaction. The release of acrosomal enzymes (acrosin) helps the sperm to penetrate the zona. The zona pellucida allows only a sperm of the same species to fertilize the oocyte. Sperms of other species cannot pass through the zona pellucida.
- The zona pellucida is responsible for the zona reaction that prevents any additional spermatozoa from entering the fertilized ovum (zygote).
- The zona pellucida holds the blastomeres of the early embryo together. The developing embryo is genetically different from the mother. This may evoke immunological reactions if embryonic and maternal tissues come in contact. Presence of zona pellucida (which lacks histocompatibility antigens) acts as a barrier that separates maternal tissues from the embryo. After the disappearance of zona pellucida various immunosuppressive cytokines and proteins are produced by the implanting embryo. This blocks the recognition of the embryo as a tissue foreign to the mother.
- To sum up the functions of zona pellucida are: oocyte development, protection of oocyte during its growth and transport in female reproductive tract, spermatozoon binding in fertilization, prevention of polyspermy, development of blastocyst and in preventing ectopic or premature implantation.

FORMATION OF GERM LAYERS

As the blastocyst develops further, it gives rise not only to the tissues and organs of the embryo but also to a number of structures that support the embryo and help it to acquire nutrition.

At a very early stage in development, the embryo proper acquires the form of a three-layered disc. This is called the *embryonic disc* (also called *embryonic area, embryonic shield,* or *germ disc*).

The three layers that constitute this embryonic disc are:

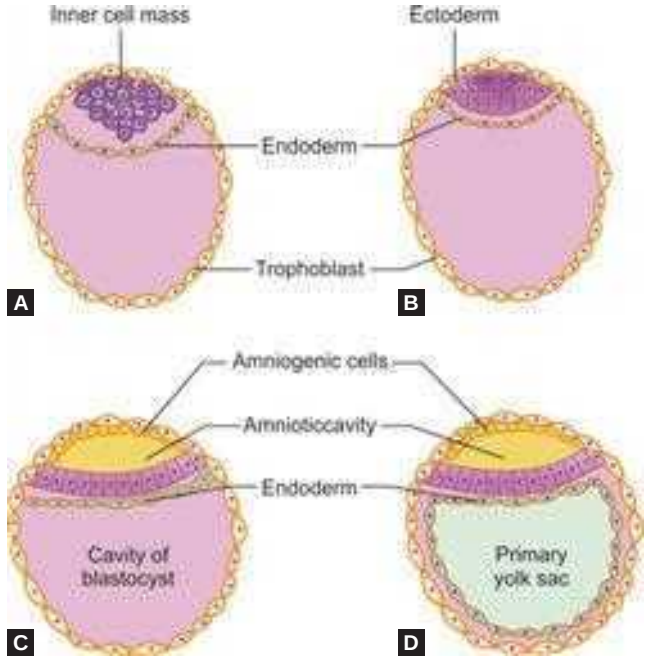
1. Endoderm (endo = inside)
2. Ectoderm (ecto = outside)
3. Mesoderm (meso = in the middle).

These are the *three-germ layers*. All tissues of the body are derived from one or more of these layers. Much of the student's study of embryology concerns itself with learning from which of these germ layers particular tissues and organs develop. In the further development of the blastocyst that we will now consider, it is very important to have a clear concept of the formation of germ layers and of their fate.

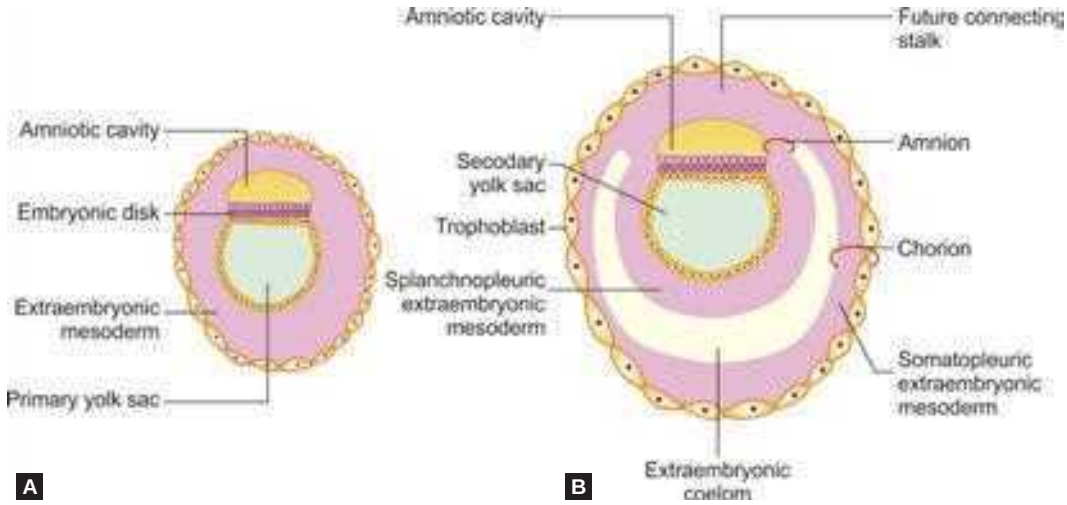
We have seen that the blastocyst is a spherical cyst lined by flattened trophoblastic cells, and that inside it there is a mass of cells, the inner cell mass, attached eccentrically to the trophoblast. Further changes are as follows:

- *Formation of hypoblast:* Some cells of the inner cell mass differentiate (i.e. they become different from others) into flattened cells, that come to line its free surface (lower in Fig. 4.10A). This layer is the *hypoblast/endoderm*.
- *Formation of epiblast*: The remaining cells of the inner cell mass become columnar (Fig. 4.10B). These cells form the *epiblast/ectoderm.* The embryo is now in the form of a disc having two layers (*Bilaminar germ disc*).
- *Formation of amniotic cavity:* A space appears between the epiblast (below) and the trophoblast (above). This is the *amniotic cavity* (Fig. 4.10C), filled by amniotic fluid, or liquor amnii. The roof of this cavity is formed by amniogenic cells derived from the trophoblast, while its floor is formed by the epiblast.
- *Formation of primary yolk sac*: Flattened cells arising from the hypoblast (or, according to some, from trophoblast), spread and line the inside of the blastocystic cavity. (This lining of flattened cells is called "Heuser's membrane"). In this way, a cavity, lined on all sides by cells of endodermal origin, is formed. This cavity is called the *primary yolk sac* (Fig. 4.10D).
- *Formation of extraembryonic mesoderm*: The cells of the trophoblast give origin to a mass of cells called the extraembryonic mesoderm (or primary mesoderm). These cells come to lie between the trophoblast and the flattened endodermal cells lining the yolk sac, thus separating them from each other. These cells also separate the wall of the amniotic cavity from the trophoblast (Fig. 4.11A). This mesoderm is called "extra-embryonic" because it lies outside the embryonic disc. It does not give rise to any tissues of the embryo.
- *Formation of extraembryonic coelom*: Small cavities appear in the extraembryonic mesoderm. Gradually, these join together to form larger spaces and, ultimately, one large cavity is formed. This cavity is called the extraembryonic coelom (Fig. 4.11B) (also called the chorionic cavity). With its formation, the extraembryonic mesoderm is split into two layers. The part lining the inside of the trophoblast, and the outside of the amniotic cavity, is called the parietal or somatopleuric extraembryonic mesoderm (It is also referred to as the chorionic plate). The part lining the outside of the yolk sac is called the visceral or splanchnopleuric extraembryonic mesoderm (Fig. 4.11B).
- *Formation of connecting stalk*: From Figure 4.11B it is clearly seen that the extraembryonic coelom does not extend into that part of the extraembryonic mesoderm which attaches the wall of the amniotic cavity to the trophoblast. The developing embryo, along with the amniotic cavity and the yolk sac, is now suspended in the extraembryonic coelom, and is attached to the wall of the blastocyst (i.e. trophoblast) only by this unsplit part of the extraembryonic mesoderm. This mesoderm forms a structure called the *connecting stalk*.
- *Formation of chorion and amnion*: At this stage, two very important membranes are formed. One is formed by the parietal extraembryonic mesoderm (on the inside) and the overlying trophoblast (on the outside); this is called the *chorion* (Fig. 4.11B).

  The other is the *amnion* which is constituted by amniogenic cells forming the wall of the amniotic cavity



Figs 4.10A to D: Differentiation of endoderm and ectoderm, and the formation of the amniotic cavity and the yolk sac



Figs 4.11A and B: Formation of extraembryonic mesoderm and extraembryonic coelom. Note carefully, the composition of the amnion, and of the chorion

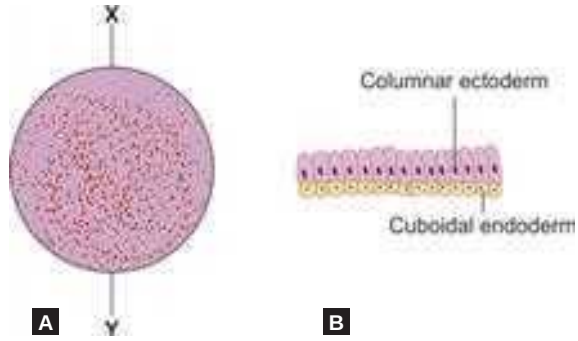
(excluding the ectodermal floor). These cells are derived from the trophoblast.

We have already seen that the amnion is covered by the parietal extraembryonic mesoderm, and that the connecting stalk is attached to it.

The chorion and amnion play an important role in childbirth (parturition) and we will refer to them again.

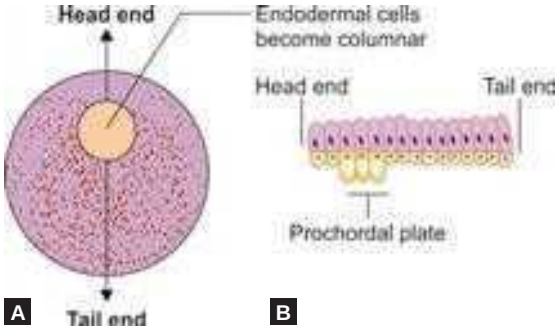
- *Formation of secondary yolk sac*: With the appearance of the extraembryonic mesoderm, and later of the extraembryonic coelom, the yolk sac becomes much smaller than before and is now called the *secondary yolk sac*. This alteration in size is accompanied by a change in the nature of the lining cells. They are no longer flattened but become cubical (Fig. 4.11B).
- *Circular embryonic disc*: At this stage, the embryo proper is a circular disc composed of two layers of cells: (1) the upper layer (toward amniotic cavity) is the epiblast, the cells of which are columnar, while (2) the lower layer (toward yolk sac) is the hypoblast, made up of cubical cells. There is no indication yet of a head or tail end of the embryonic disc (Fig. 4.12).
- *Formation of prochordal plate:* At one circular area near the margin of the disc, the cubical cells of the endoderm become columnar. This area is called the *prochordal plate*.

  The appearance of the prochordal plate determines the central axis of the embryo (i.e. enables us to divide it into right and left halves), and also enables us to distinguish its future head and tail ends (Fig. 4.13).
- *Formation of primitive streak*:
  - A transient structure that forms in the blastula during the early stages of embryonic development.

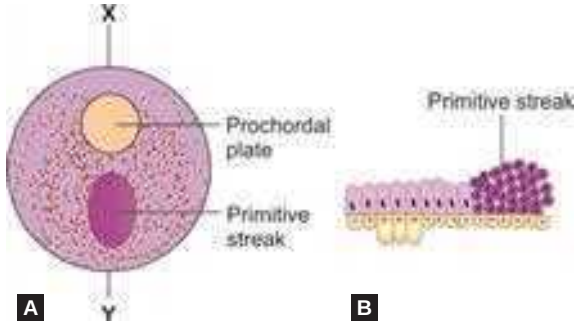


Figs 4.12A and B: Embryonic disc before appearance of a central axis. "B" represents a section along the axis XY shown in "A"

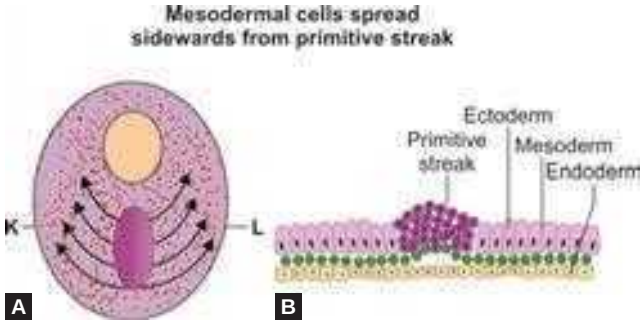
  - Soon after the formation of the prochordal plate (15th day of development) some of the epiblast cells lying along the central axis, near the tail end of the disc and on the dorsal aspect of the embryo, begin to proliferate, and form an elevation that bulges into the amniotic cavity. This elevation is called the primitive streak (Fig. 4.14).
  - The primitive streak is at first a rounded or oval swelling, but with elongation of the embryonic disc it becomes a linear structure lying in the central axis of the disc.
  - With the formation of prochordal plate and primitive streak the shape of embryonic disc changes from circular to oval (Fig. 4.15).
  - Formation of primitive streak marks the beginning of gastrulation.
- *Formation of intraembryonic mesoderm*: The cells that proliferate in the region of the primitive streak



Figs 4.13A and B: Embryonic disc after establishment of a central axis. "B" represents a section along the central axis



Figs 4.14A and B: Appearance of primitive streak. (B) is a section along axis XY shown in (A)



Figs 4.15A and B: Formation of intraembryonic mesoderm. (B) is a section along axis KL in (A)

pass sideways, pushing themselves between the epiblast and hypoblast (Fig. 4.15). These cells form the intraembryonic mesoderm (or secondary mesoderm). Some cells arising from the primitive streak displace the hypoblast and form the layer that is now known as endoderm. Thus, both endoderm and mesoderm are derived from the epiblast. The remaining cells of the epiblast now form the ectoderm.

- *Extensions of intraembryonic mesoderm*: The intraembryonic mesoderm spreads throughout the disc except in the region of the prochordal plate.

  The mesoderm extends cranial to the prochordal plate, and here mesoderm from the two sides becomes continuous across the midline (Fig. 4.16).

  In the region of the prochordal plate, the ectoderm and endoderm remain in contact. In later development, the ectoderm and endoderm mostly persist as a lining epithelium.

  On the other hand, the bulk of the tissues of the body are formed predominantly from mesoderm.

  As there is no mesoderm in the prochordal plate, this region remains relatively thin, and later forms the *buccopharyngeal membrane*.
- The primitive streak gradually elongates, along the central axis of the embryonic disc. The disc also elongates and becomes pear-shaped (Fig. 4.16).
- *Connecting stalk*: When the embryonic disc is first formed, it is suspended (along with amniotic cavity and yolk sac) from the trophoblast by the connecting stalk (Figs 4.11 and 4.17).

  To begin with, the connecting stalk is very broad compared to the size of the embryo.

  As the embryonic disc enlarges in size, and also elongates, the connecting stalk becomes relatively small, and its attachment becomes confined to the region of the tail end of the embryonic disc (Fig. 4.17).

  Some intraembryonic mesoderm arising from the primitive streak passes backward into the connecting stalk (Figs 4.16 and 4.17).

  As it does so, it leaves an area caudal to the primitive streak, where ectoderm and endoderm remain in contact (i.e. mesoderm does not separate them). This region is, therefore, similar to the prochordal plate, and forms the *cloacal membrane* (Fig. 4.16).

Trilaminar germ disc: An embryonic disc made up of three layers. These layers are the ectoderm (outer), endoderm (inner) and mesoderm (middle).

Gastrulation: The process of formation of the primitive streak, endoderm and intraembryonic mesoderm (by the streak) is referred to as gastrulation.

Preorganogenesis period: Development of the embryo from fertilization up to the formation of the bilaminar disc is described as the preorganogenesis period as no organs are as yet recognizable. These events take place in the first 14 days of pregnancy. Anomalies produced by teratogens acting during this period usually result in death of the embryo. These anomalies are, therefore, seldom seen in babies reaching full term.

Embryonic period: Establishment of the primitive streak and formation of intraembryonic mesoderm mark the onset of *gastrulation*. Gastrulation begins in the *3rd week* and most of it will be considered in the next chapter. The 3rd week

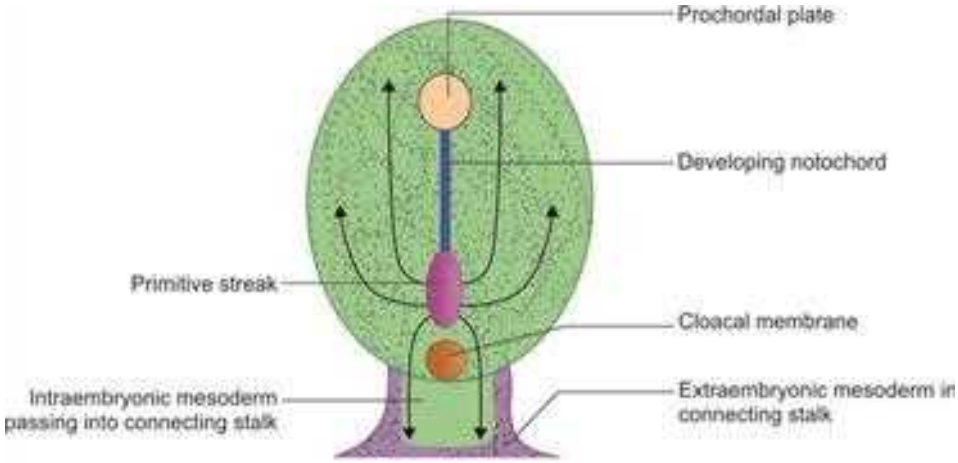


Fig. 4.16: Spread of intraembryonic mesoderm. Note that the mesoderm comes to lie between ectoderm and endoderm in all parts of the embryonic disc except at the (1) prochordal plate (2) cloacal membrane (3) region of the notochord

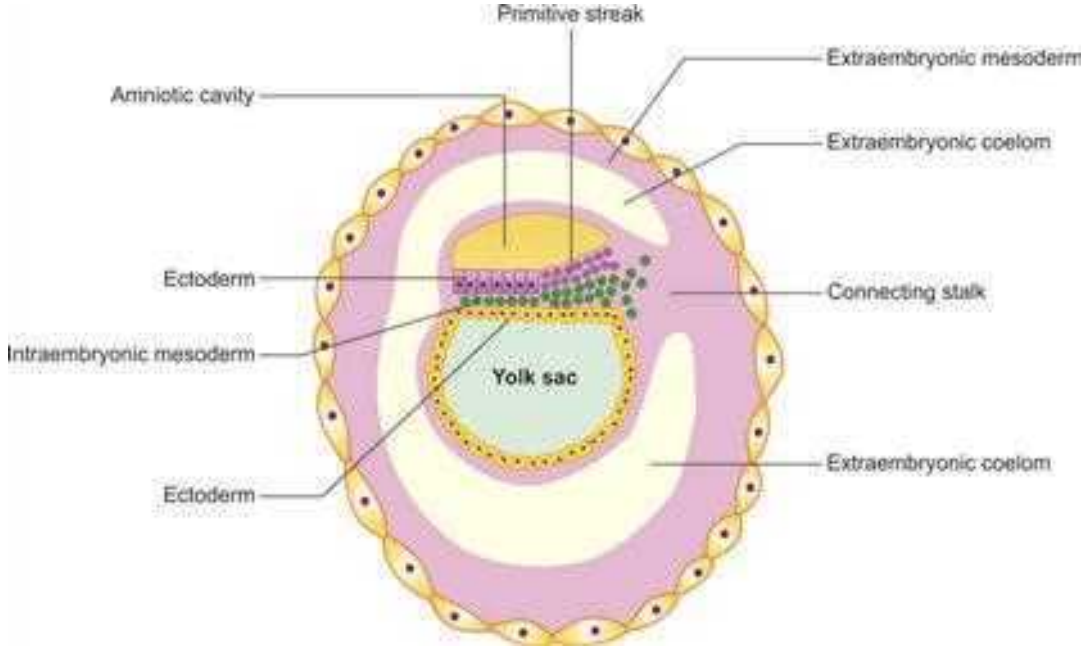


Fig. 4.17: Diagram showing the attachment of the connecting stalk to the caudal end of the embryonic disc. Note the cells of the intraembryonic mesoderm passing into the connecting stalk

marks the beginning of what is termed the embryonic period (3rd–8th week). Most congenital anomalies are produced by teratogens acting during this period.

TIME TABLE OF EVENTS DESCRIBED IN THIS CHAPTER

Time table of events described in this chapter is shown in Table 4.2.

TABLE 4.2: Time table of developmental events

Age in days	*Developmental events*
2	Embryo is at two cells stage
3	Morula is formed
4	Blastocyst is formed
8	Bilaminar disc is formed
14	Prochordal plate and primitive streak is seen
16	Intraembryonic mesoderm is formed/disc is now three layered

Clinical correlation

Use of stem cells in the treatment of diseases

- ***Embryonic stem cells (ESCs):*** The cells of inner cell mass have the potential to differentiate into three different germ layers (ectoderm, endoderm and mesoderm). All the cells, tissues and organs of the body are formed from these three layers. Because of this the cells of the inner cell mass are called *embryonic stem cells*.
- ***Pluripotent cells:*** Embryonic stem cells can be maintained and propagated in an undifferentiated state, in culture, and in laboratories. If these cells are exposed to certain specific growth factors, in culture, the stem cells can form various types of adult cells, e.g. neurons, muscle cells, blood cells, and cartilage cells. These cells are therefore said to be *pluripotent*. It has been observed that when these stem cells are introduced into the living tissues of a person, the local environment helps these stem cells to differentiate into cells similar to those of the tissue in which they are placed.
- ***Embryonic stem cells therapy:*** This technique has tremendous potential for treatment of various diseases. Some of these are Parkinson's disease, Alzheimer disease, diabetes, myocardial infarction, blood diseases, severe burns, osteoporosis, spinal cord injury, to name but a few.
- ***Therapeutic stem cell cloning:*** However, in the ESC therapy, the complication of immune rejection is always present as the genetic constitution of stem cell is different from that of patient. To overcome this problem scientists are working on "therapeutic stem cell cloning". In this procedure the nucleus of patient cell is introduced in the embryonic stem cell. These cells are then allowed to grow in any tissue of the patient. As the tissues arising from the stem cells are now genetically identical to those of the patient rejection is avoided.
- ***Adult stem cells:*** Although the embryonic stem cells are most suitable for therapeutic purposes, stem cells can also be isolated from some adult tissues, e.g. bone marrow, brain and skeletal muscle. However, adult stem cells are difficult to culture in laboratories and have less potential to differentiate in adult tissues.
- ***Ethical issues in ESC therapy:*** As human embryos are needed for stem cell research, some authorities object to it on ethical grounds. The main objections are that it is against nature and it treats the embryo with disrespect.

EMBRYOLOGICAL EXPLANATION FOR CLINICAL CONDITIONS OR ANATOMICAL OBSERVATIONS

Case Scenario 1

A woman of 35 years age with a married life of 8 years and regular menstrual cycles comes with her husband for consultation for infertility. They were not using any contraceptive methods. The doctor advised the following investigations for the couple after a detailed physical examination of both. With your basic knowledge of embryology give explanation for each of these investigations.

- *Seminal analysis of male*
- *Hysterosalpingography of female*
- *Follicular study of female*
- *Blood test for follicle-stimulating hormone (FSH) estimation.*

- It is a case of infertility. To exclude male factor in infertility the husband was advised to undergo seminal analysis for the quantity and pH of semen and for sperm count and morphology.
- To rule out obstruction for the passage of sperms/ovum/fertilized egg in the uterine tube hysterosalpingography was advised.
- Ultrasound-guided follicular study for the number, the size and appearance of antral follicles was advised on 3rd–5th day of menstrual cycle along with blood FSH estimation. During each menstrual cycle around 15 primordial follicles start maturing and pass through the stages of primary, secondary and antral follicles, and one becomes the mature Graafian follicle, which has the potential to be released from the surface of the ovary (ovulation) and enters the uterine tube and remains fertilizable. An antral follicle count of less than 4 indicates poor ovarian reserve and that of more than 10 suggests good ovarian reserve which is favorable for fertilization.
- Follicle-stimulating hormone is advised to correlate the influence of pituitary gonadotropin in the maturity of follicles.

In the above case the seminal analysis is suggestive of low sperm count (less than 20 million/mL) with normal structure and motility of spermatozoa and tubal block on hysterosalpingography and an antral follicular count of 7 and a lower FSH value. What advice is to be given to the couple who wanted to have children?

The couple should be advised about the in vitro fertilization (IVF) technique, as there are no chances for natural method of conception. Because of low antral follicle count, FSH value and the advanced age of the woman (decrease in follicular reserve) the couple should be advised to go for IVF technique.

REVIEW QUESTIONS

1. Define fertilization.
2. Describe the stages involved in fertilization.
3. What is capacitation?
4. What is acrosome reaction?
5. Explain in vitro-fertilization.
6. Describe cleavage.
7. Write short notes on blastocyst.
8. Write short notes on morula.
9. Write short notes on primitive streak.
10. Write short notes on yolk sac.

Chapter 5

Further Development of Embryonic Disc

HIGHLIGHTS

- The cranial end of the primitive streak enlarges to form the *primitive knot*.
- Cells of the primitive knot multiply and pass cranially to form a rod-like structure reaching up to the prochordal plate. This is the *notochordal process*.
- The notochordal process undergoes changes that convert it first into a canal and then into a plate and finally back into a rod-like structure. This is the *notochord*.
- Most of the notochord disappears. Remnants remain as the *nucleus pulposus* of each intervertebral disc.
- A wide strip of ectoderm overlying the notochord becomes thickened and forms the *neural plate* from which the brain and spinal cord develop.
- *Intraembryonic mesoderm* shows three subdivisions. The mesoderm next to the middle line is called the *paraxial mesoderm*. It undergoes segmentation to form *somites*. The mesoderm in the lateral part of the embryonic disc is called the *lateral plate mesoderm*. A cavity called the *intraembryonic coelom* appears in it and splits the mesoderm into a *somatopleuric* layer (in contact with ectoderm) and a *splanchnopleuric* layer (in contact with endoderm). A strip of mesoderm between the lateral plate mesoderm and the paraxial mesoderm is called the *intermediate mesoderm*.
- The intraembryonic coelom later forms the pericardial, pleural and peritoneal cavities.
- The embryonic disc, which is at first flat, undergoes folding at the cranial and caudal ends. These are the *head and tail folds*. Lateral folds also appear. As a result of these folds, the endoderm is converted into a tube, the gut. It is divisible into *foregut, midgut* and *hindgut*.
- After formation of the head fold the gut is closed cranially by the prochordal plate, which is now called the *buccopharyngeal membrane*. Caudally, the gut is closed by the cloacal membrane.
- The *umbilical cord* develops from the connecting stalk. It contains the right and left umbilical arteries, the left umbilical vein, and remnants of the vitellointestinal duct and yolk sac. The ground substance of the umbilical cord is made up of Wharton's jelly derived from mesoderm. The cord is covered by amnion.
- The *allantoic diverticulum* arises from the yolk sac before formation of the gut. After formation of the tail fold, it is seen as a diverticulum of the hindgut.
- The *pericardial cavity* is derived from part of the intraembryonic coelom that lies cranial to the prochordal plate. The developing heart lies ventral to the cavity. After formation of the head fold the pericardial cavity lies ventral to the foregut; and the developing heart is dorsal to the pericardial cavity.
- The *septum transversum* is made of intraembryonic mesoderm that lies cranial to the pericardial cavity. After formation of the head fold, it lies caudal to the pericardium and heart. The liver and the diaphragm develop in relation to the septum transversum.

INTRODUCTION

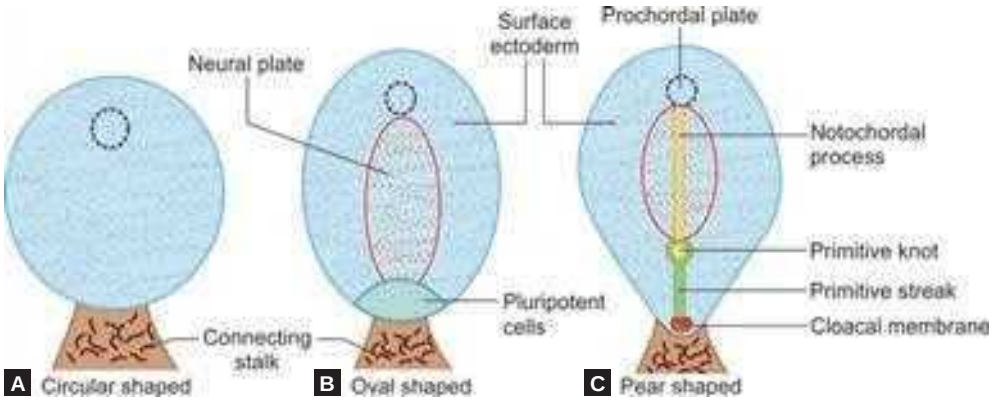
- During the 2nd and 3rd week of development, there is change in the shape of embryonic disc from circular to oval and then pear shape (Figs 5.1A to C). During the 2nd week the *bilaminar embryonic disc* is established with two germ layers (hypoblast and epiblast) and two cavities (amniotic and yolk sac) suspended by connecting stalk (Fig. 5.2).
- The cephalocaudal axis, dorsoventral axis and right left axis are established with the appearance of prochordal plate and migration of connecting stalk. The cells of epiblast at the caudal end of bilaminar embryonic disc are *pluripotent* (Fig. 5.1).
- Pluripotent ectodermal cells form the following:
  - Definitive endoderm
  - Intraembryonic mesoderm
  - Definitive ectoderm
  - Notochord
  - Primordial germ cells (PGC)
- On 15th day of gestation, there is active migration and invagination of these pluripotent ectodermal cells between ectoderm and endoderm forming a narrow median groove and raised lateral margins, the *primitive streak* (Figs 5.1 and 5.2) from which notochord and the third germ layer Intraembryonic mesoderm are formed during the early part of 3rd week resulting in trilaminar germ disc.

FORMATION OF NOTOCHORD

The notochord is a midline structure that develops in the region lying between the cranial end of the primitive streak and the caudal end of the prochordal plate (Figs 4.16 and 5.1).

During its development, the notochord passes through several stages that are as follows:

- The cranial end of the primitive streak becomes thickened. This thickened part of the streak is called the *primitive knot*, *primitive node* or *Henson's node* (Figs 5.1A and 5.3A).



Figs 5.1A to C: Change in the shape of embryonic disc from circular (A) to oval (B) and then pear shape (C)

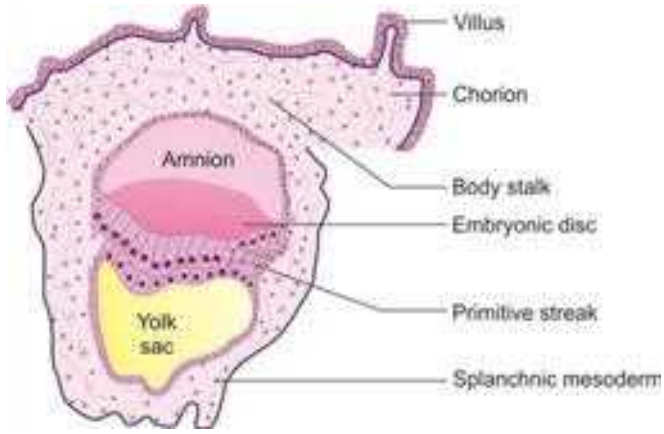
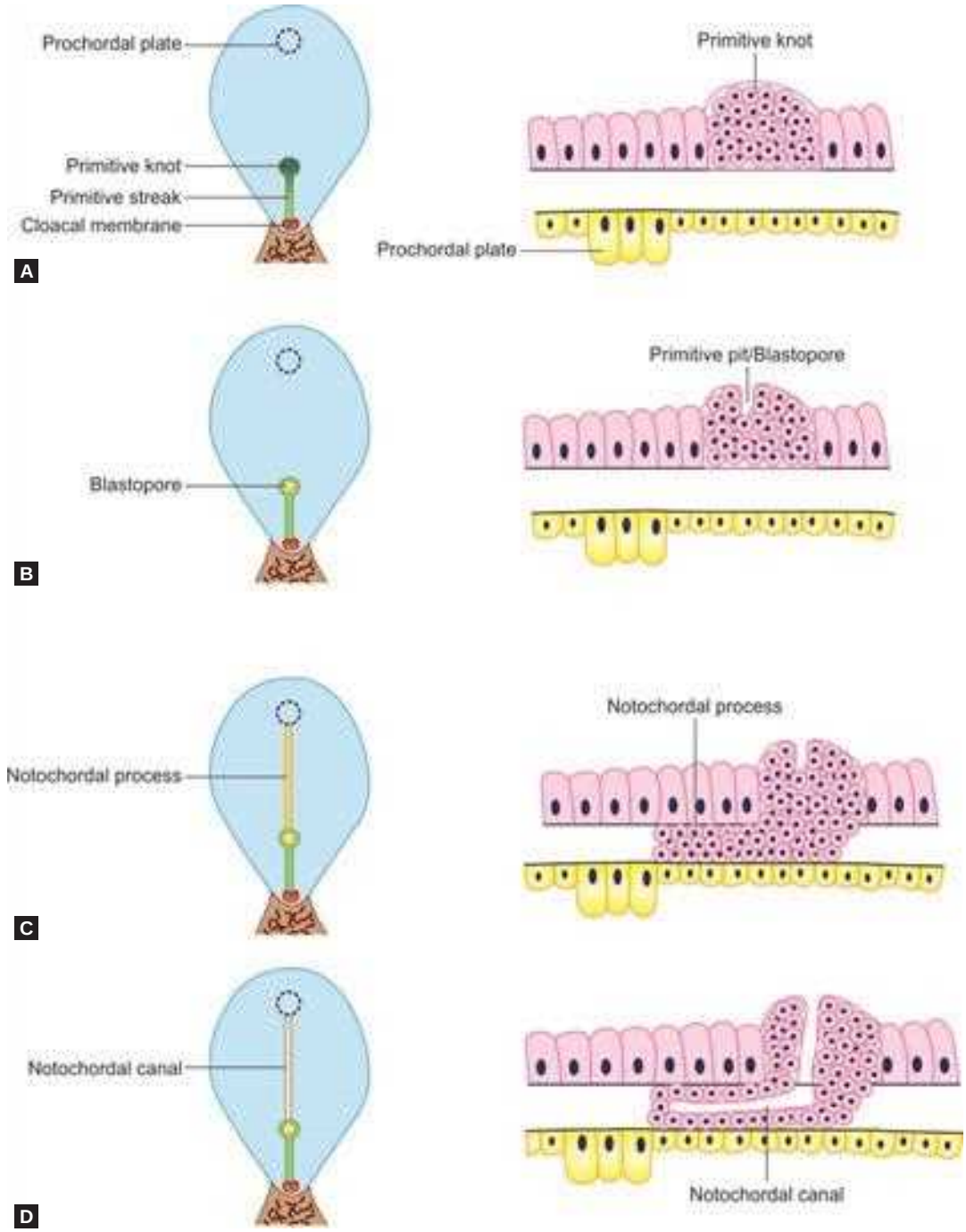


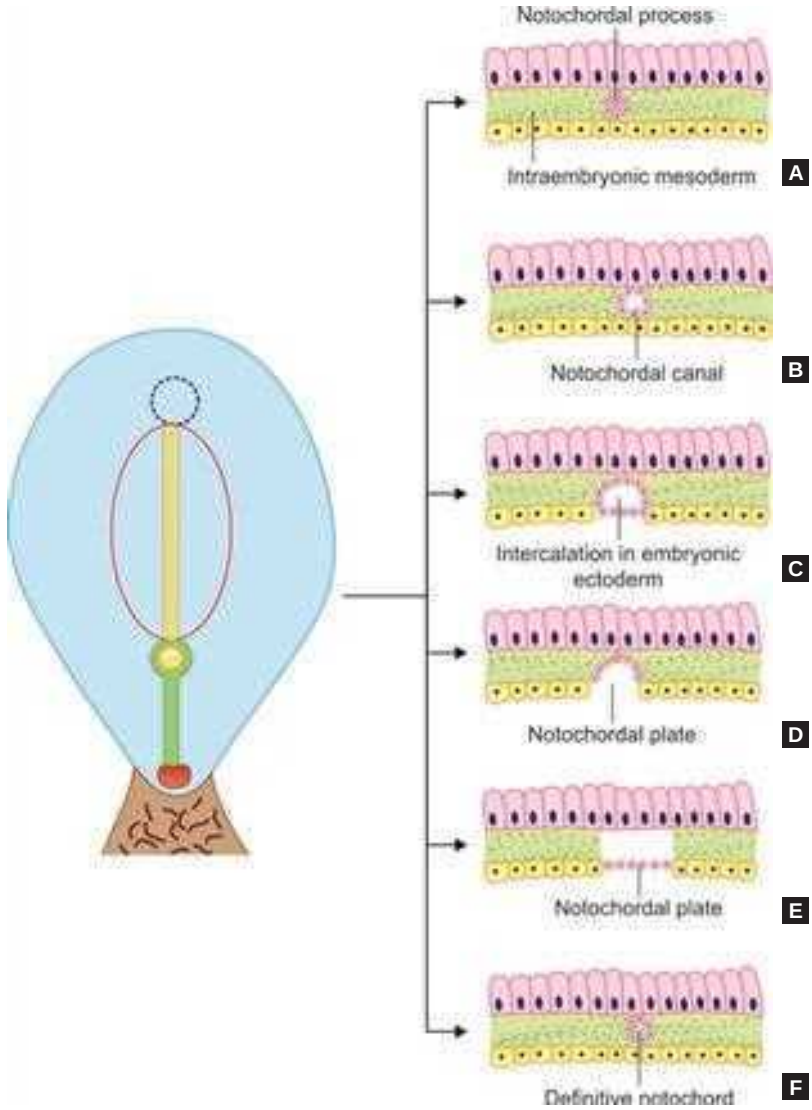
Fig. 5.2: Bilaminar germ disc with amniotic and yolk sac cavities and primitive streak cells



Figs 5.3A to D: Section through embryonic disc showing formation of (A) primitive knot; (B) blastopore; (C) notochordal process and (D) notochordal canal. Note that the notochordal process is deep to ectoderm and that its position is shown diagrammatically

- A depression appears in the center of the primitive knot. This depression is called the *blastopore/primitive pit* (Fig. 5.3B).
- Cells in the primitive knot multiply and pass cranially in the middle line, between the ectoderm and endoderm, reaching up to the caudal margin of the prochordal plate. These cells form a solid cord called the *notochordal process* or *head process* (Figs 5.3C and 5.4A). The cells of this process undergo several stages of rearrangement (Figs 5.1 and 5.2) ending in the formation of a solid rod called the "notochord".

- The cavity of blastopore extends into the notochordal process and converts it into a tube called the *notochordal canal* (Figs 5.3D and 5.4B).
- The cells forming the floor of notochordal canal become intercalated in (i.e. become mixed up with) the cells of the endoderm (Fig. 5.4C). The cells forming the floor of the notochordal canal now separate the canal from the cavity of the yolk sac.
  - The floor of the notochordal canal begins to break down. At first, there are small openings formed in it, but gradually the whole canal comes to communicate with the yolk sac (Fig. 5.4D). The notochordal canal also communicates with the amniotic cavity through the blastopore. Thus, at this stage, the amniotic cavity and the yolk sac are in communication with each other.
  - Gradually the walls of the canal become flattened so that instead of a rounded canal we have a flat plate of cells called the *notochordal plate* (Fig. 5.4E).
  - However, this process of flattening is soon reversed and the notochordal plate again becomes curved to assume the shape of a tube (Fig. 5.4F). Proliferation of cells of this tube converts it into a solid rod of cells. This rod is the *definitive* (i.e. finally formed) *notochord*. It gets completely separated from the endoderm.
  - As the embryo enlarges, the notochord elongates considerably and lies in the midline, in the position to be later occupied by the vertebral column. However, the notochord does not give rise to the vertebral column. Most of it disappears, but parts of it persist in the region of each intervertebral disc



Figs 5.4A to F: Transverse sections through the embryonic disc to illustrate stages in the formation of the notochord

as the *nucleus pulposus* and its cranial continuation the *apical ligament of dens of axis vertebra*.

- The notochord is present in all animals that belong to the phylum Chordata. In some of them, e.g. *Amphioxus*, it persists into adult life and forms the central axis of the body. In others, including man, it appears in the embryo but only small remnants of it remain in the adult.
- *Experiments have shown that formation of the neural tube is induced by the notochord.*

- *Primitive streak is the primary organizer* as it induces formation of notochord and intraembryonic mesoderm. Formation of notochord determines the cranio-caudal axis and right and left sides of embryo.
- *Fate of primitive streak*: It regresses at the end of 3rd week of development and completely disappears by 26th day.

FORMATION OF THE NEURAL TUBE

The details of the formation of the neural tube will be studied later. For the time being, it may be noted that:

- The neural tube gives rise to the brain and the spinal cord
- The neural tube is formed from the ectoderm overlying the notochord and, therefore, extends from the prochordal plate to the primitive knot (Fig. 5.6).
- The neural tube is soon divisible into: (a) a cranial enlarged part that forms the brain, and (b) a caudal tubular part that forms the spinal cord.
- In early embryos, the developing brain forms a large conspicuous mass, on the dorsal aspect. The process of formation of the neural tube is referred to as *neurulation*.

SUBDIVISIONS OF INTRAEMBRYONIC MESODERM

The intraembryonic mesoderm is formed by proliferation of cells in the primitive streak and that it separates the ectoderm and the endoderm, *except* in the following regions that remain bilaminar throughout life (Fig. 5.5):

- *Prochordal plate*: Later becomes *buccopharyngeal membrane* (future oral cavity)—represents the junction of primitive mouth and pharynx.
- *Cloacal membrane*: Later divided into the *anal membrane* (future anal opening) and *urogenital membrane* (future urinary and genital openings). The rupture of these membranes establishes the communication of urinary, genital and digestive systems with the outside.
- In the midline caudal to the prochordal plate, as this place is occupied by the *notochord*.

Cranial to the prochordal plate, the mesoderm of the two sides meets in the midline (Figs 4.16 and 5.5). At the edges of the embryonic disc, the intraembryonic mesoderm is continuous with the extraembryonic mesoderm (Fig. 5.6).

The intraembryonic mesoderm now becomes subdivided into three parts (Figs 5.6 to 5.8):

1. Mesoderm, on either side of the notochord, becomes thick and is called the *paraxial mesoderm*.
2. More laterally, the mesoderm forms a thinner layer called the *lateral plate mesoderm*.
3. Between these two, there is a longitudinal strip called the *intermediate mesoderm*.

Paraxial Mesoderm

At first, the cells of the paraxial mesoderm are homogenously arranged. Later, the mesoderm gets segmented. The

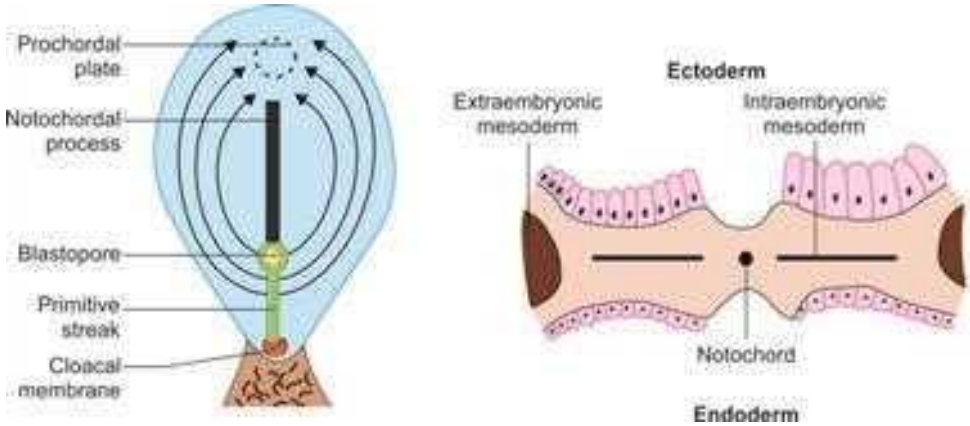


Fig. 5.5: Proliferating intraembryonic mesoderm from primitive node and primitive streak

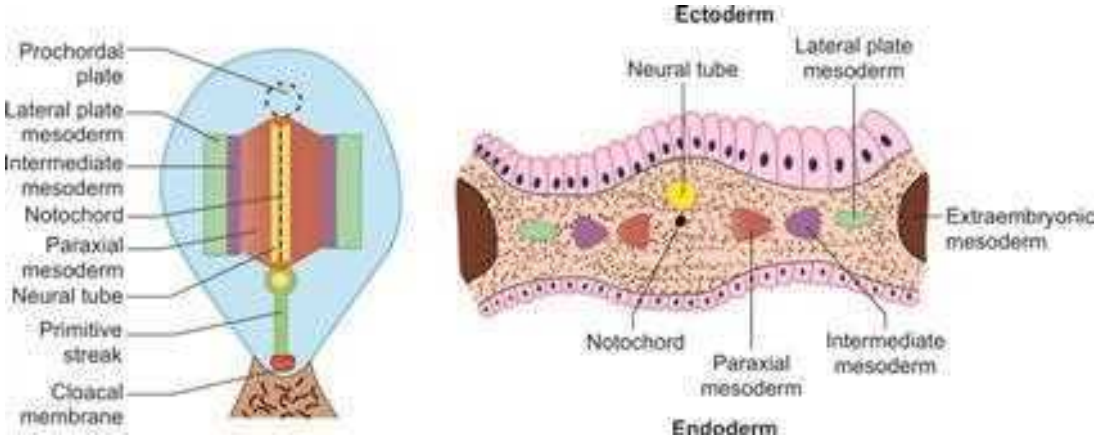


Fig. 5.6: Subdivisions of intraembryonic mesoderm

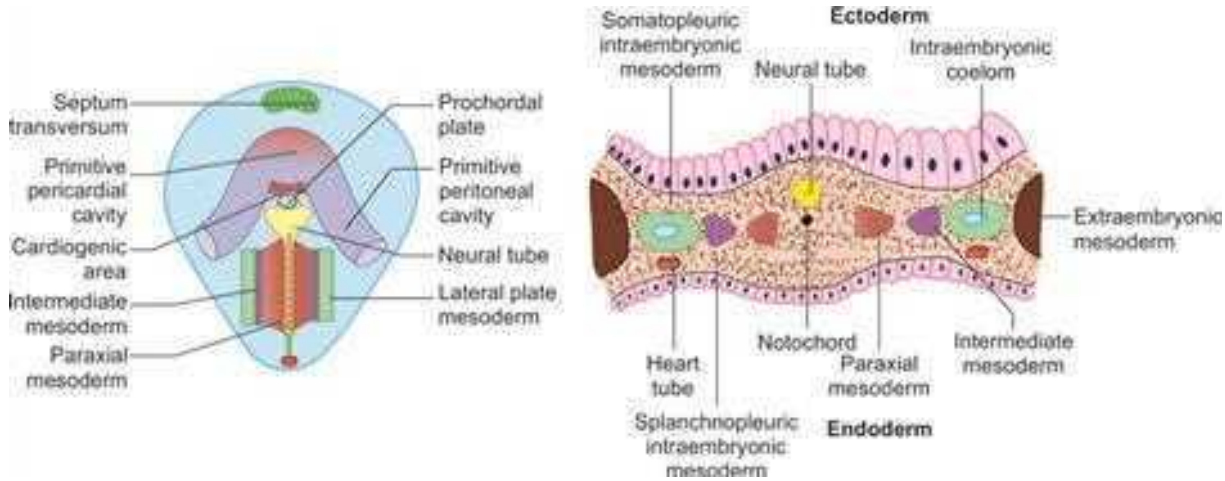


Fig. 5.7: Formation of intraembryonic coelom and its subdivisions

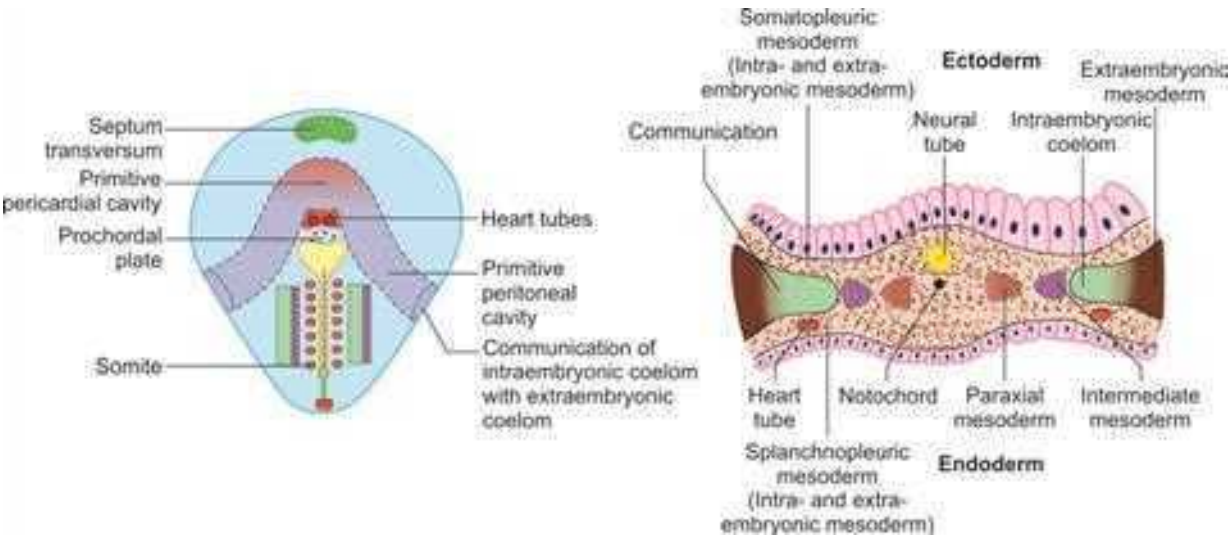


Fig. 5.8: Communication between intraembryonic coelom and extraembryonic coelom. Strucutres in the midline of germ disc before folding

segments are of two categories: (1) somitomeres and (2) somites (Figs 5.7 and 5.8). *Somitomeres* lie in the region of the head. They are rounded structures. There are seven of them. They form the mesoderm and muscles of the head and jaw. *Somites* are cubical and more distinctly segmented. The most cranial somites are formed in the occipital region. New somites are progressively formed caudal to them. Ultimately there are about 44 pairs of somites (4 occipital, 8 cervical, 12 thoracic, 5 lumbar, 5 sacral and 8–10 coccygeal). Occipital somites form muscles of the tongue. Somites form the axial skeleton, skeletal muscle and part of skin. Somitomeres are not confined to the region of somites. In the head region, cranial to somites, somitomeres give origin to some mesenchyme.

Somitomere derived structures are mentioned in Chapter 9. The fate of somites is described in Chapter 10.

LATERAL PLATE MESODERM—FORMATION OF INTRAEMBRYONIC COELOM

- While the paraxial mesoderm is undergoing segmentation, to form the somites, changes are also occurring in the lateral plate mesoderm. Small cavities appear in it. These coalesce (come together) to form one large cavity, called the *intraembryonic coelom*.
- The cavity has the shape of a horseshoe (Fig. 5.7). There are two halves of the cavity (one on either side of the midline) which are joined together cranial to the prochordal plate. At first, this is a closed cavity (Fig. 5.7) but soon it comes to communicate with the extraembryonic coelom (Fig. 5.8).
- With the formation of the intraembryonic coelom, the lateral plate mesoderm splits into:
  - Somatopleuric or parietal layer intraembryonic mesoderm that is in contact with ectoderm.
  - Splanchnopleuric or visceral layer of intraembryonic mesoderm that is in contact with endoderm (Figs 5.7 and 5.8).
- The intraembryonic coelom gives rise to pericardial, pleural, and peritoneal cavities. Their development will be considered later. For the time being, note that the pericardium is formed from that part of the intraembryonic coelom that lies, in the midline, cranial to the prochordal plate.
- The heart is formed in the splanchnopleuric mesoderm forming the floor of this part of the coelom (Figs 5.7 to 5.9). This is, therefore, called the *cardiogenic area* (also called *cardiogenic plate*, *heart-forming plate*).
- Cranial to the cardiogenic area (i.e. at the cranial edge of the embryonic disc) the somatopleuric and splanchnopleuric mesoderms are continuous with each other. The mesoderm here does not get split, as the intraembryonic coelom has not extended into it. This unsplit part of intraembryonic mesoderm forms a structure called the *septum transversum* (Figs 5.7 to 5.9).

INTERMEDIATE MESODERM

The urinary and genital systems are derived from the intermediate mesoderm. This will be discussed in detail in the Chapter 16: Urogenital System.

YOLK SAC

- The formation of the yolk sac has been described in Chapter 4: Fertilization and Formation of Germ Layers (Figs 5.9 and 5.10). We have seen that the *primary yolk sac* is bounded above by cubical endoderm of the embryonic disc and elsewhere by flattened cells lining the inside of the blastocystic cavity.
- With the formation of the extraembryonic mesoderm, and later the extraembryonic coelom, the yolk sac becomes much smaller; it comes to be lined all round by cubical cells; and it is then called the *secondary yolk sac*.

FOLDING OF EMBRYO

The changes that now take place will be best understood by a careful study of Figure 5.10. Note the following:

- There is progressive increase in the size of the embryonic disc due to rapid growth of cells of central part of embryonic disc and rapid growth of somites.
- This causes conversion of flat pear-shaped germ disc into a cylindrical embryo (Fig. 5.10A).
- The head and tail ends of the disc (X and Y), however, remain relatively close together. Hence, the increased length of the disc causes it to bulge upward into the amniotic cavity (Figs 5.10B and C).

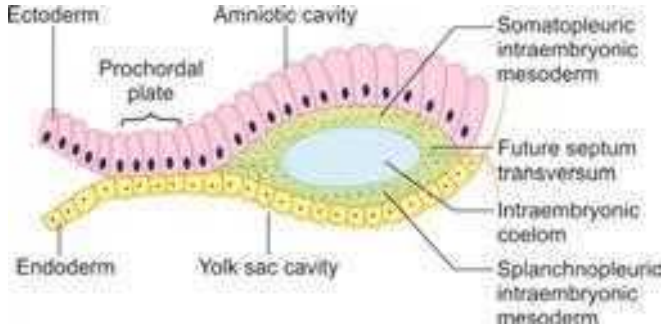
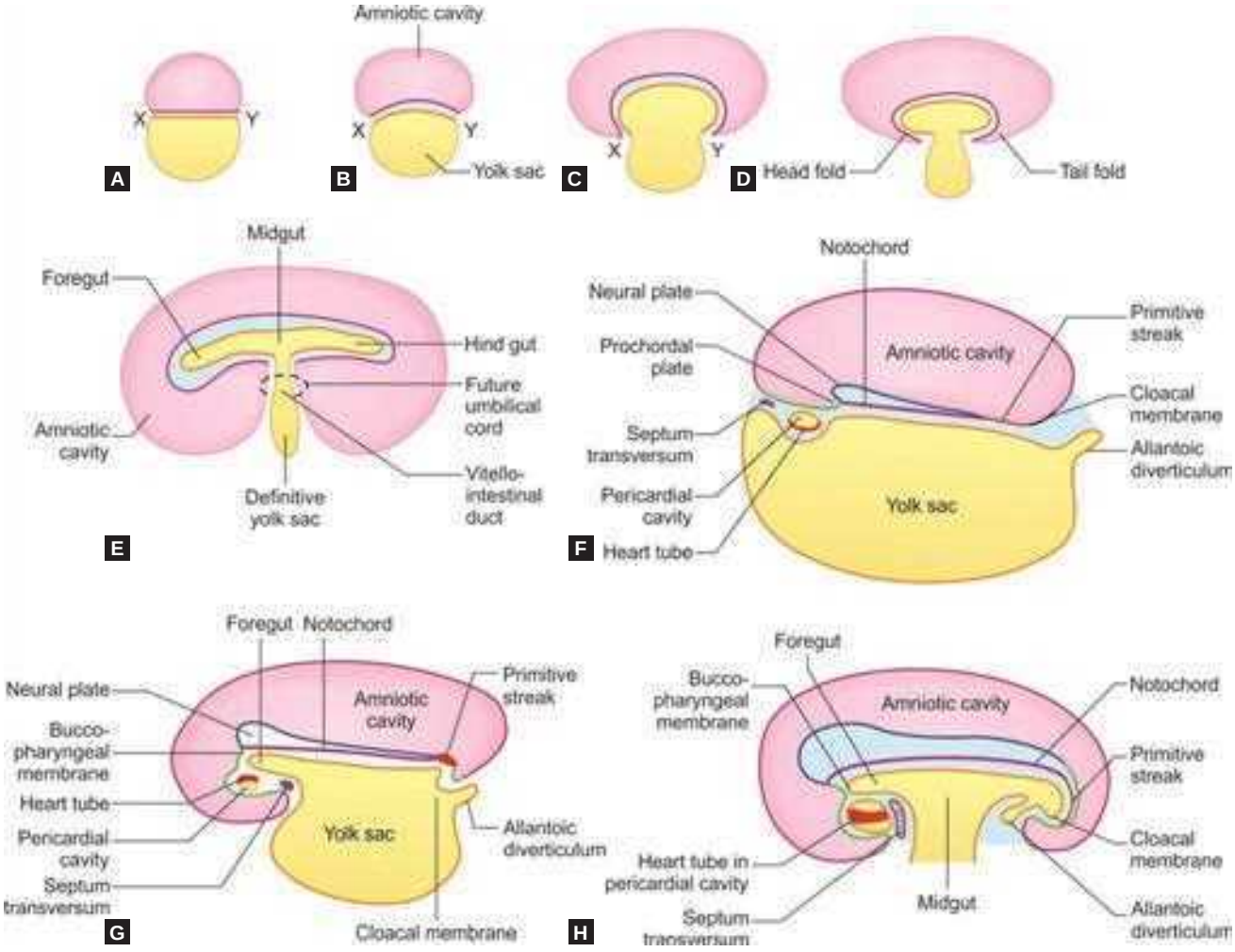


Fig. 5.9: Midline section through cranial end of the embryonic disc to show the relationship of the pericardial cavity to other structures



Figs 5.10A to H: Figures A to E are formation of head and tail folds and establishment of the gut. F to H is embryonic disc-related structures before (F), during (G) and after (H) formation of head and tail folds. Note the changing relationships of septum transversum, pericardium, buccopharyngeal membrane, cloacal membrane and allantois

- With further enlargement, the edges of embryonic disc become folded on itself in the median and in the transverse planes. The folding in the median plane form ventrally directed *head fold* and *tail fold* (Figs 5.10D and E). The folding in the transverse plane forms ventrally directed *lateral folds*.
- Cephalocaudal folding is due to rapid and longitudinal growth of central nervous system (Fig. 5.10F). Lateral foldings are due to rapid growth of somites and convert the embryo into a tubular structure. These are not three separate folds but occur simultaneously and merge into one another. The notochord, neural tube and somites stiffen the dorsal axis of the embryo making it more foldable.
- With the formation of the head and tail folds, parts of the yolk sac become enclosed within the embryo. In this way, a tube lined by endoderm is formed in the embryo. This is the *primitive gut*, from which most of the gastrointestinal tract is derived (Figs 5.10B to E). At first, the gut is in wide communication with the yolk sac. The part of the gut cranial to this communication is called the *foregut*; the part caudal to the communication is called the *hindgut*; while the intervening part is called the *midgut* (Fig. 5.10E). The communication with the yolk sac becomes progressively narrower. As a result of these changes, the yolk sac becomes small and inconspicuous, and is now termed the *definitive yolk sac* (also called the *umbilical vesicle*). The narrow channel connecting it to the gut is called the *vitellointestinal duct* (also called *vitelline duct*; *yolk stalk* or *omphalomesenteric duct*). This duct becomes elongated and eventually disappears.
- As the head and tail folds are forming, similar folds are also formed on each side in transverse or horizontal plane. These are the *lateral folds*. As a result, the embryo

TABLE 5.1: Craniocaudal arrangement of embryonic structures before and after head fold

Before head fold (Cranial to caudal)	*After head fold*			
• Septum transversum • Heart, pericardial cavity • Buccopharyngeal membrane • Neural tube and notochord • Primitive node • Primitive streak • Cloacal membrane • Connecting stalk with allantois	*Ventral to gut (Cranial to caudal)*	*Dorsal to gut (Cranial to caudal)*	*Cranial to gut*	*Caudal to gut*
	• Stomodeum • Pericardial cavity and heart • Septum transversum • Connecting stalk with allantois	• Neural tube and notochord • Primitive node • Primitive streak	Brain vesicle	Proctodeum

comes to be enclosed all around by ectoderm except in the region through which the *vitellointestinal duct (omphalomesenteric duct)* passes. Here, there is a circular aperture which may now be called the *umbilical opening* (Fig. 5.10E).

- The folding facilitates growth and expansion of amniotic cavity that comes to surround the embryo on all sides. In this way, the embryo now floats in the amniotic fluid, which fills the cavity (Fig. 5.10E).
- Convergence of folds on ventral surface forms tubular investment of amnion for connecting stalk. This causes obliteration of extraembryonic coelom. Now, the amnion forms a covering for the umbilical cord.
- The arrangement of embryonic structures from cranial to caudal end of the embryo before and after formation of head and tail folds are presented in Table 5.1 and Figure 5.8 and in Figures 5.10F to H. The events resulting from various folds are presented in Table.5.2.
- The stomodeum is an ectodermal depression at the head end between the bulging head and pericardial bulge. The buccopharyngeal membrane breaks at 4th week and the cloacal membrane at 7th week.

CONNECTING STALK

While discussing the formation of the extraembryonic coelom, we have seen that with the formation of this cavity, the embryo (along with the amniotic cavity and yolk sac) remains attached to the trophoblast only by extraembryonic mesoderm into which the coelom does not extend (Figs 4.17 and 5.11A to D). This extraembryonic mesoderm forms the *connecting stalk*.

We shall see later that the trophoblast and the tissues of the uterus together form an important organ, the *placenta*, which provides the growing embryo with nutrition and with oxygen. It also removes waste products from the embryo. The importance of the connecting stalk is obvious when we see that this is the only connecting link between the embryo and the placenta.

As the embryo grows, the area of attachment of the connecting stalk to it becomes relatively smaller. Gradually this attachment is seen only near the caudal end of the embryonic disc (Figs 5.11D and E). With the formation of the tail fold, the attachment of the connecting stalk moves (with the tail end of the embryonic disc) to the ventral aspect of the embryo. It is now attached in the region of the umbilical opening (Fig. 5.11F).

By now, blood vessels have developed in the embryo, and also in the placenta. These sets of blood vessels are in communication by means of arteries and veins passing through the connecting stalk. At first, there are two arteries and two veins in the connecting stalk, but later the right vein disappears (the left vein is "left").

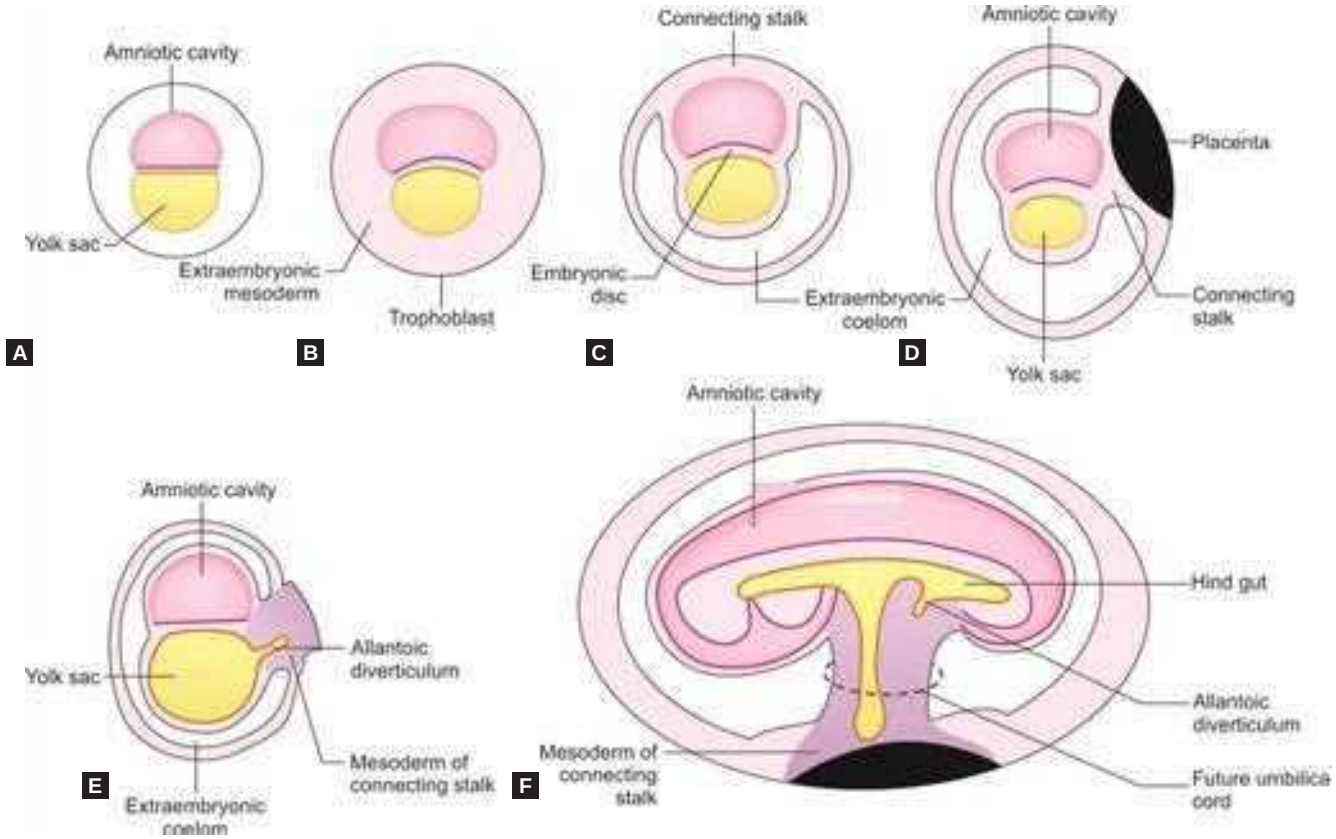
It is clear from Figure 5.12 that, at this stage, the amnion has a circular attachment to the margins of the umbilical opening and forms a wide tube in which the following lie:

- Vitellointestinal duct and remnants of the yolk sac
- Mesoderm (extraembryonic) of the connecting stalk. This mesoderm gets converted into a gelatinous substance called "Wharton's jelly". It protects blood vessels in the umbilical cord.
- Blood vessels that pass from the embryo to placenta
- A small part of the extraembryonic coelom.

This tube of amnion, and the structures within it, constitutes the *umbilical cord* (Fig. 5.13). This cord progressively increases in length to allow free movement of the embryo within the amniotic cavity. At the time of birth of the child (i.e. at full term), the umbilical cord is about half a meter long, and about 2 cm in diameter. It shows marked torsion, which is probably due to fetal movements. An umbilical cord that is either too short or too long can cause problems during delivery of the fetus.

ALLANTOIC DIVERTICULUM

Before the formation of the tail fold, a small endodermal diverticulum called the *allantoic diverticulum* arises from the yolk sac near the caudal end of the embryonic disc (Fig. 5.11E). This diverticulum grows into the mesoderm of the connecting stalk. After the formation of the tail fold, part of this diverticulum is absorbed into the hindgut. It now passes from the ventral side of the hindgut into the connecting stalk (Fig. 5.11F). We will refer to it again while considering the development of the urinary bladder.



Figs 5.11A to F: Stages in the establishment of the umbilical cord, allantoic diverticulum and its relationship to the connecting stalk

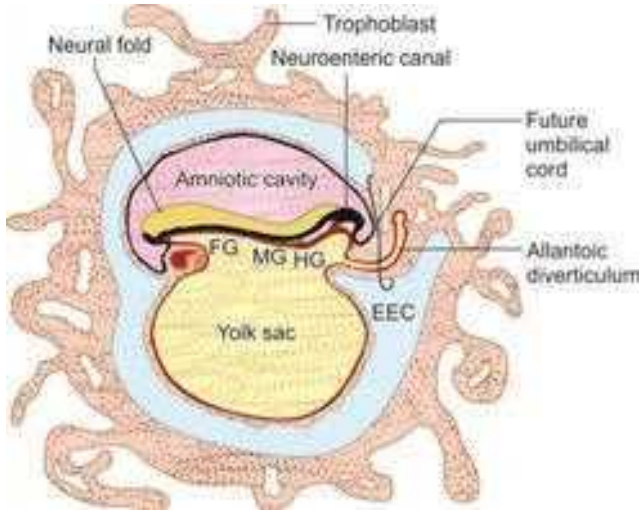


Fig. 5.12: Structures forming the umbilical cord
Abbreviations: FG: Foregut; MG: Midgut; HG: Hindgut; EEC: Extraembryonic coelom

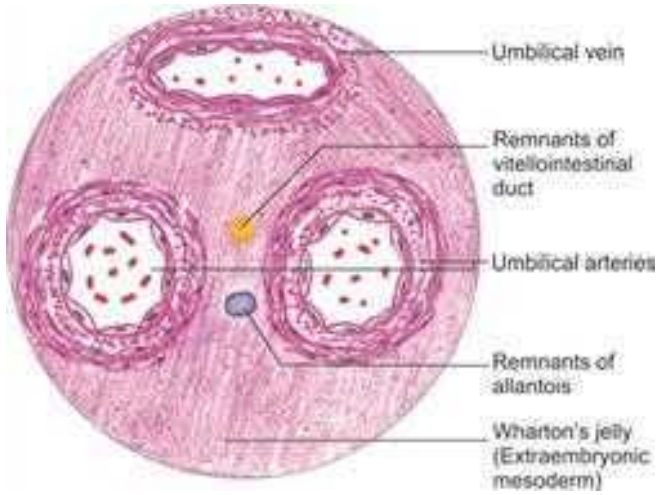


Fig. 5.13: section through umbilical cord

TABLE 5.2: Events resulting from various embryonic folds

Head fold	*Tail fold*	*Lateral fold*
• Formation of *foregut*	• *Formation of hindgut*	• Converts the endoderm into a primitive gut tube
• *Opening of stomodeum* into the amniotic cavity	• *Invagination of ectoderm to form Cloaca*	• The intraembryonic coelom surrounds the gut tube
• *Pericardial cavity* and cardiogenic mesoderm lies *ventral* to the foregut	• *Ventral shifting of connecting stalk*	• The communication between the intra- and extraembryonic coeloms becomes constricted and eventually obliterated
• Transverse mesoderm between the pericardial cavity and the yolk sac—*septum transversum*	• Ventral shifting of allantoic diverticulum	• Converts the endoderm into a primitive gut tube
• *Amniotic cavity* extends ventral to the cranial end of the embryo	• *Amniotic cavity* extension ventral to the caudal end of the embryo	
• *Yolk sac* is constricted from cranial end	• *Constriction of yolk* sac from the caudal end	

EFFECT OF HEAD AND TAIL FOLDS ON POSITIONS OF OTHER STRUCTURES

Just before the formation of the head and tail folds, the structures in the embryonic disc are oriented, as shown in Figure 5.8. A median (midline) section across the disc, at this stage, is shown in Figure 5.10F. From the cranial to the caudal side, the structures seen in the midline are the:

- Septum transversum
- Developing pericardial cavity and the heart
- Prochordal plate
- Neural plate
- Primitive streak
- Cloacal membrane.

Note that the primitive streak is now inconspicuous. After folding, the relative positions of these structures change to that shown in Figures 5.10G and H. The important points to note here are as follows:

- With the formation of the head fold, the developing *pericardial cavity* comes to lie on the ventral side of the embryo, ventral to the foregut. The *heart*, which was developing in the splanchnopleuric mesoderm in the floor of the pericardial cavity (Fig. 5.10F), now lies in the roof of the cavity (Fig. 5.10G). The pericardium enlarges rapidly and forms a conspicuous bulging on the ventral side of the embryo (Fig. 5.10H).
- The *septum transversum*, which was the most cranial structure in the embryonic disc (Fig. 5.8), now lies caudal to the heart (Fig. 5.10G). At a later stage in development, the diaphragm and liver develop in relation to the septum transversum.
- The region of the *prochordal plate* now forms the buccopharyngeal or oral membrane, which closes the foregut cranially. When this membrane breaks down, the foregut communicates with the exterior.
- The most cranial structure of the embryo is now the enlarged cranial part of the neural tube, which later forms the brain (Fig. 5.10G). This enlarges enormously (Fig. 5.10H). There are now two big bulgings on the ventral aspect of the embryo. Cranially, there is the developing brain, and a little below it there is the bulging pericardium (Fig. 5.10H). In between these two, there is a depression called the *stomatodeum* or *stomodeum*, the floor of which is formed by the buccopharyngeal membrane.
- Toward the tail end of the embryo, the primitive streak is now an inconspicuous structure that gradually

Molecular regulation of primitive streak

Migration of primitive streak cells and their specification to form various derivatives are controlled by fibroblast growth factor 8 (FGF-8) produced by primitive streak cells.

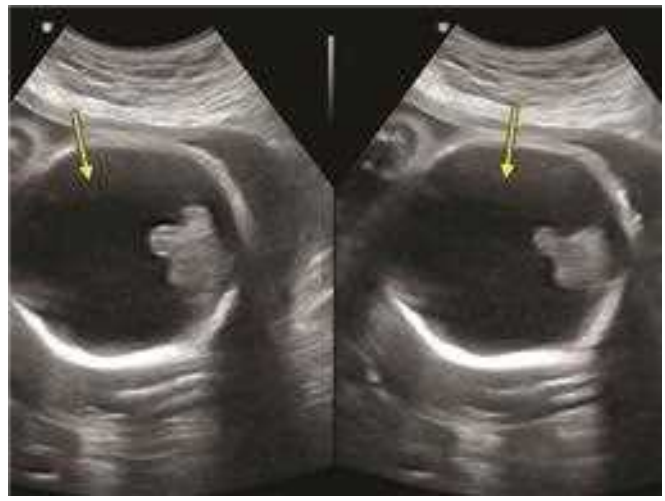


Fig. 5.14: An ultrasound image of fetus with holoprosencephaly
Image Courtesy: Dr Ganesh Kumar and Dr Sasikala

disappears. The distal end of the hindgut is closed by the *cloacal membrane*. At first, this is directed caudally (Fig. 5.10G), but later it comes to face ventrally (Fig. 5.10H).

We have traced the development of the embryo to a stage when the rudiments of the nervous system, the heart and the gut have been formed. We are now in a position to trace the development of individual organ systems in detail. Before we do this, however, we must study the development of the placenta.

Clinical correlation

- ***Teratogenic effects on primitive streak:*** The embryo is highly sensitive to teratogens during 15th to 18th day (3rd week/ gastrulation period) of development as the primitive streak and its derivatives will be affected.
- ***Holoprosencephaly:*** In this condition, the forebrain is small and the two lateral ventricles fuse into a single cavity (Fig. 5.14). The eyes are closely placed (hypertelorism). High doses of alcohol in the mother can cause this condition.
- ***Caudal dysgenesis (Sirenomelia):*** Deficiency of mesoderm in the caudal part of the embryo that normally contributes for the formation of lower limbs, urogenital system and lumbosacral vertebrae will result in abnormalities in these structures. The child is born with fused lower limbs and presents renal, genital and vertebral anomalies including imperforate anus. This condition is more common in mother with diabetes.
- ***Sacrococcygeal teratoma:*** Persistence of pluripotent cells of primitive streak at the caudal end of embryonic disc after 4th week of gestation gives rise to a large tumor called sacrococcygeal teratoma. It can cause obstruction during labor and is usually malignant. It has to be removed within 6 months after birth.
- ***Chordoma:*** Malignant tumor arising from remnants of notochord. It can be seen at cranial or caudal end of notochord.
- ***Serinomelia or caudal dysgenesis:*** Due to deficient development of caudal IEM.

TIME TABLE OF EVENTS DESCRIBED IN THIS CHAPTER

Time table of events described in this chapter is shown in Table 5.3.

TABLE 5.3: Time table of developmental events

Age in days	*Developmental events*
15	Primitive streak appears. Definitive yolk sac is formed
17	Notochordal process appears. Heart tube is seen in cardiogenic area. Allantoic diverticulum is seen.
19	Intraembryonic mesoderm is being formed. Connecting stalk can be distinguished.
21	Neural groove is seen. Head fold begins to form.
23	Closure of the neural tube.

REVIEW QUESTIONS

1. Describe notochord.
2. Write short notes on prochordal plate.
3. Write short notes on allantoic diverticulum.
4. Connecting stalk.

Chapter 6

Placenta, Fetal Membranes and Twinning

HIGHLIGHTS

- The process of attachment of developing embryo to the uterine endometrium is called *implantation*.
- The type of implantation in the human beings is called *interstitial implantation* as the embryo gets buried in the substance of endometrium.
- *Decidua* is the name given to the endometrium after implantation.
- The *placenta* is formed partly from embryonic structures and partly from decidua. Placenta is responsible for transport of nutrients and oxygen to the fetus, and for removal of waste products.
- The essential elements of the placenta are *chorionic villi*. The villi are surrounded by maternal blood. Fetal blood circulates through capillaries in villi. The maternal blood and the fetal blood are separated by a very thin placental membrane (or barrier). All substances passing from mother to fetus (and vice versa) traverse this membrane.
- The fetal tissue that takes part in forming the placenta is chorion. It consists of trophoblast (one layer of cells) resting on extraembryonic mesoderm. Proliferation of cells of the trophoblast leads to formation of two layers: (1) *cytotrophoblast,* which is cellular and (2) *syncytiotrophoblast,* which is a syncytium (cytoplasm with nuclei, but no cell boundaries).
- *Types of villi:* The first-formed villi are called *primary villi*. They consist of a central core of cytotrophoblast covered by syncytiotrophoblast. *Secondary villi* have three layers. From inside out, these are (1) extraembryonic mesoderm, (2) cytotrophoblast and (3) syncytiotrophoblast. In *tertiary villi*, blood capillaries are formed in the extraembryonic mesoderm.
- Villi are surrounded by an *intervillous space* that contains maternal blood. As the placenta enlarges, septa grow into the intervillous space dividing the placenta into lobes. The fully formed placenta is about 6 inches in diameter and about 500 g in weight.
- The placenta is normally attached to the upper part of the body of the uterus. A placenta attached lower down is called *placenta previa*. It can cause problems during childbirth.
- The embryo is surrounded by three large cavities. These are (1) the amniotic cavity, (2) the extraembryonic coelom and (3) the uterine cavity. Enlargement of the amniotic cavity obliterates the extraembryonic coelom, leading to fusion of amnion and chorion. Further enlargement of amniotic cavity obliterates the uterine cavity. Fused amnion and chorion (called membranes) bulge into the cervical canal (during childbirth) and help to dilate it.

FORMATION OF PLACENTA

Introduction

- The placenta is a fetomaternal organ. It connects growing embryo/fetus with the wall of pregnant uterus. It is an organ where there is intimate apposition or fusion of fetal organ to maternal tissue for the purpose of physiological exchange.
- It is a circular or disc-shaped organ of 500 g weight. It has two surfaces and two structural components.
- *Surfaces* (Figs 6.1A and B):
  - *Maternal surface*: It is irregular and is divided into 15–20 small lobules called maternal cotyledons.
  - *Fetal surface*: It is smooth and covered with amnion, and umbilical cord is attached at or near the center of this surface.

- *Structural components*: It has structural components of fetal and maternal origin.
  - Maternal component is contributed by *decidua basalis or decidual plate*.
  - Fetal component is contributed by *chorion frondosum or chorionic plate*.

For proper understanding of the structure and function of placenta, knowledge on *implantation, decidua, trophoblast* and *chorion* are required.

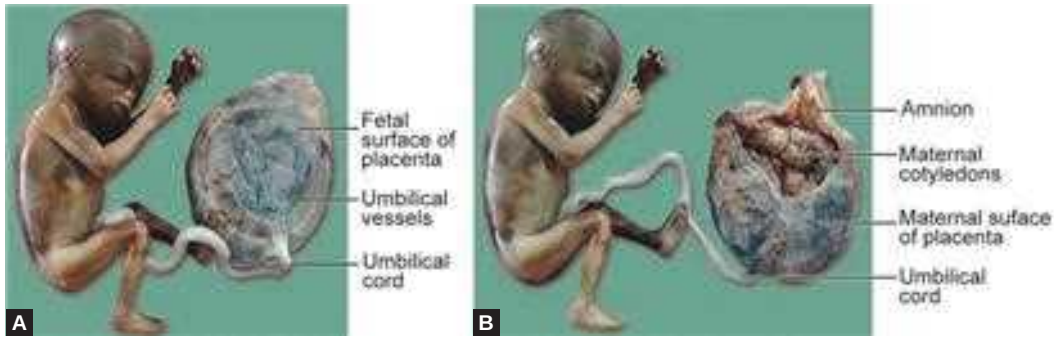
Implantation

Definition: It is the process of attachment of blastocyst to uterine endometrium and subsequent invasion (embedding) of blastocyst (conceptus) into the uterine endometrium in placental animals.

Implantation period: It takes place between 6th and 12th days after fertilization.

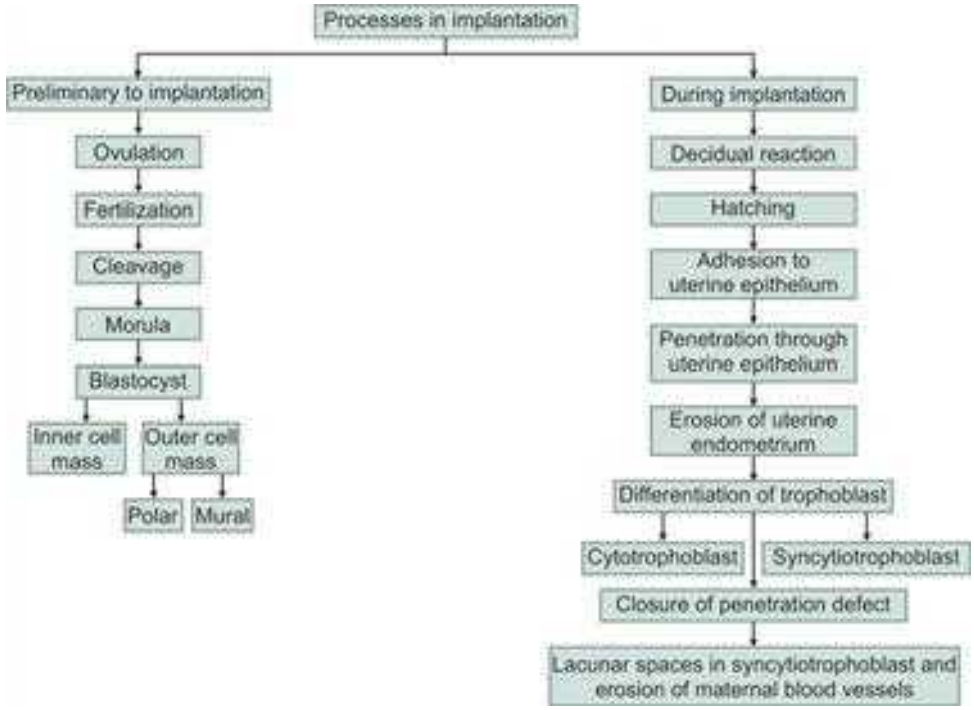
Process of implantation: For understanding the sequence of events, the whole process of implantation can be considered as those occurring preliminary to implantation and those taking place (stages) in implantation. These are simplified in the Flowchart 6.1.

A. Processes preliminary to implantation (Fig. 6.2):
   1. *Release and transport of ovum into the uterine tube*: The ovum with its surrounding zona pellucida and corona radiata cells is shed from the ovary at



Figs 6.1A and B: Fetus, umbilical cord and placenta (fetal and maternal surfaces)

Flowchart 6.1: Processes in implantation



ovulation. Later it travels through the fimbrial end of fallopian tube into the ampulla of uterine tube.

2. *Fertilization of ovum*: The ovum and sperm fuse in the ampulla of uterine tube. The process of fusion of male and female pronuclei is called fertilization. If fertilization occurs, segmentation of the ovum begins.
3. *Cleavage divisions of fertilized ovum and its migration into the fundus of uterus*: The fertilized ovum undergoes series of mitotic divisions and becomes *morula* (16-cell stage) at about 3rd day after fertilization. While cleaving it moves along the uterine tube and reaches the uterus. The morula is still surrounded by the zona pellucida, which prevents it from sticking to the wall of uterine tube/uterus during its journey.
4. *Blastocyst formation*: At about 4th/5th day after fertilization, the cleaving blastomeres reorganize into the central *inner cell mass/embryoblast* (8 cells) and peripheral *outer cell mass/trophoblast* (99 cells) with a central cavity, the blastocyst cavity. The cells of inner cell mass contribute for the formation of embryo proper. The cells of trophoblast have the property of attaching to any tissue with which it comes into contact.
5. *Differentiation of trophoblast cells*: The trophoblast differentiates into *polar trophoblast* (30 cells) and *mural trophoblast* (69 cells). The part of blastocystic trophoblast making contact with endometrium is the polar trophoblast and the remaining is called mural trophoblast (Fig. 4.9C).

B. Processes (stages) at the time of implantation (Figs 6.2 and 6.3):
   - *Decidual reaction/changes in uterine endometrium*:
     - When the morula reaches the uterus, the endometrium is in the secretory phase of menstrual cycle.
     - The change in the endometrial stroma with implantation of blastocyst is called the *decidual reaction*.
   - *Hatching of blastocyst*: The zona pellucida of the cleaving blastocyst that is rolling on the uterine wall gradually becomes thin on 5th day. This thinning is due to the production of trypsin like enzyme that causes dissolution of zona pellucida. By 6th day, the zona pellucida disappears (Fig. 4.8D).
   - *Adhesion of polar trophoblast to columnar uterine epithelium*: The trophoblast has the tendency to stick to the structure with which it comes in contact. Once the zona pellucida disappears, the cells of the trophoblast stick to the uterine endometrium. This is called the beginning of the process of *implantation*

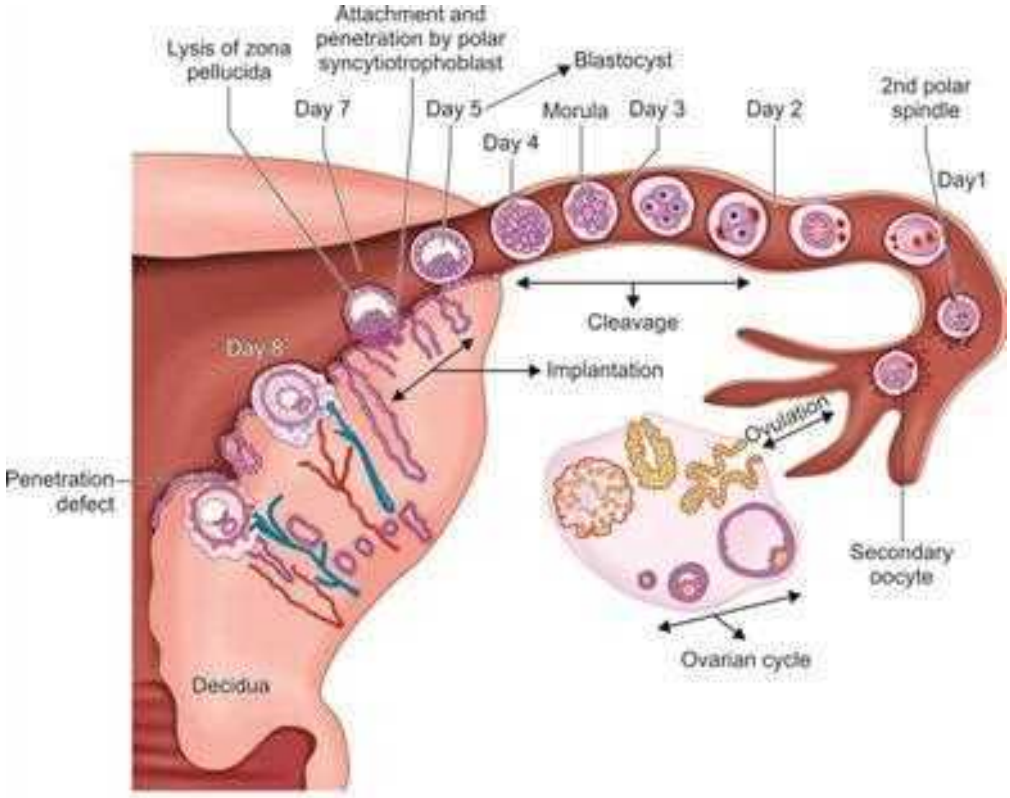
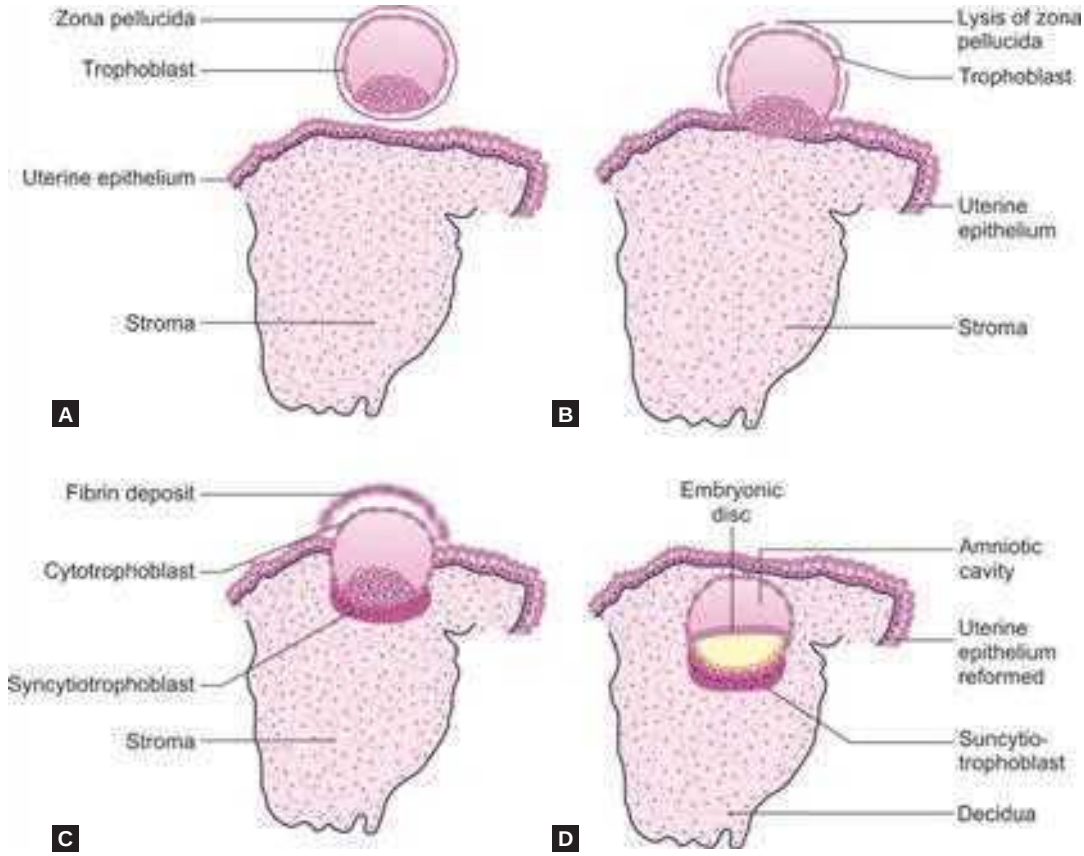


Fig. 6.2: Various processes before and during implantation: ovulation, fertilization, cleavage, blastocyst, trophoblast differentiation, decidual change, hatching of blastocyst, and penetration defect



Figs 6.3A to D: Stages of implantation: (A) Hatching blastocyst; (B) Adhesion of blastocyst to uterine epithelium; (C) Penetration of blastocyst through uterine epithelium and erosion of endometrium; (D) Closure of penetration defect and differentiation of trophoblast and embryoblast

and it takes place on 6th day after fertilization. The disappearance of zona pellucida initiates attachment of polar trophoblastic cells to the columnar uterine epithelium between the mouths of uterine glands.

- *Penetration of blastocyst through uterine epithelium*: The trophoblastic cells have got the penetrating/burrowing nature. The polar trophoblast cells situated over the embryoblast/inner cell mass start penetrating the uterine epithelium to provide passage for the blastocyst. Disarrangement and destruction of epithelial cells occurs due to the penetration of blastocyst.
- *Erosion of the uterine endometrium*: Erosion of stratum compactum and stratum spongiosum by cells of polar trophoblast occurs. This is due to the proteolytic enzymes secreted by both polar trophoblast and uterine epithelium. The blastocyst burrows deeper and deeper into the uterine mucosa till the whole of it comes to lie within the thickness of the endometrium.
- *Differentiation of trophoblast*: By about 8th day of development, the blastocyst has partially embedded into the uterine endometrial stroma. The polar trophoblast over the embryonic pole differentiates into two layers.
  - Cellular/Cytotrophoblast—Langhans layer: The inner layer of cells is cuboidal to low columnar, mononucleated and contains mitotic figures indicating their capacity to divide.
  - Syncytial trophoblast—plasmodial layer: The outer layer of multinucleated cells without mitotic figures. These are formed by the dividing cells of cytotrophoblast that have migrated to the periphery and fused.
- *Closure of penetration defect in uterine epithelium*: Once passage of blastocyst through epithelial surface is completed, closure of penetration defect in surface epithelium takes place around 9th day after fertilization by a coagulum of tissue fluid and debris that forms a fibrin plug.

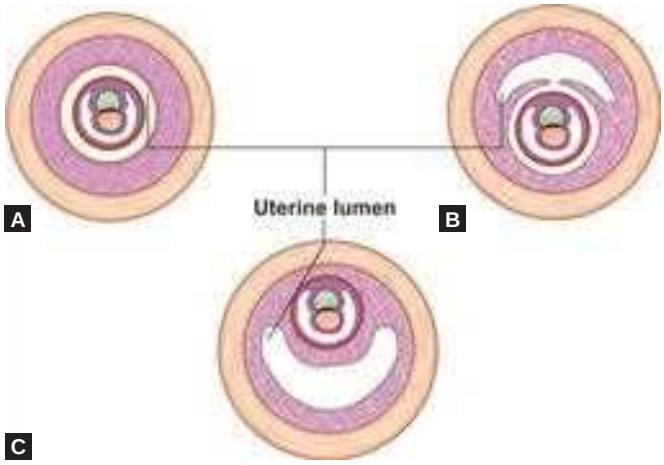
– *Completion of embedding of blastocyst and establishment of nutritive relationship with maternal blood vessels*: By 12th day of fertilization, the blastocyst has completely embedded in the endometrium. Spaces appear in the syncytiotrophoblast that will fuse to form larger lacunae. The syncytiotrophoblast cells erode the maternal capillaries, which become congested and dilated to form sinusoids. This contact between syncytiotrophoblast and maternal sinusoids initiates nutritive relationship between fetus and mother. The lacunae in the syncytial trophoblast become continuous with maternal sinusoids.

Types of Implantation (Figs 6.4A to C):

1. *Central implantation*: Blastocyst is implanted in the uterine cavity, e. g. carnivores—cow.
2. *Eccentric implantation*: Blastocyst is implanted in the uterine crypt, e.g. mouse.
3. *Interstitial implantation*: Blastocyst is implanted in the endometrium of uterine wall. This is the type of implantation in guinea pig and human.

Normal and Abnormal Sites of Implantation

A. Normal site of implantation: The normal site of implantation is the upper part of body of uterus in mid-sagittal plane, in the posterior wall (55%) or in the anterior wall (45%) [Fig. 6.5 (1)].
B. Abnormal sites of implantation [Fig. 6.5 (2-6)]
   – Uterine:
      - *Lower uterine segment*: If the implantation is in the lower uterine segment, it is called *placenta previa* [Fig. 6.5 (2)].



Figs 6.4A to C: Types of implantation: (A) Central; (B) Eccentric; (C) Interstitial

   – Extrauterine:
      - *Tubal implantation*: The most common extrauterine implantation site is in the uterine tube. The various parts in the order of frequency are:
         ◆ Interstitial [Fig. 6.5 (3)]
         ◆ Ampulla [Fig. 6.5 (4)]
         ◆ Isthmus of uterine tube
      - *Abdominal implantation [Fig. 6.5 (5)]*: It is also rare. Implantation can be:
         ◆ Primary: If implantation takes place in relation to the mesentery, it is called primary abdominal implantation and is very rare.
         ◆ Secondary: It is due to reimplantation of tubal or ovarian pregnancy. It usually results from ruptured tubal pregnancy.
      - *Ovarian implantation*: Fertilization and implantation take place in the ovary. It is rare [Fig. 6.5 (6)]. It can cause teratoma.

Implantation—Additional points

- The process of implantation is aided by proteolytic enzymes produced by the trophoblast. The uterine mucosa also aids the process.
- Implantation results due to the mutual interaction between trophoblast cells and endometrium. This interaction is mediated by receptors present on the uterine epithelium and by the secretion of L-selectin and integrins by trophoblastic cells.
- Carbohydrate-binding proteins on trophoblast cells and carbohydrate-binding sites on uterine epithelium facilitate attachment of blastocyst to the uterine wall.
- Interaction between integrin proteins of trophoblast cells and laminin and fibronectin molecules of intercellular stroma of endometrium facilitate invasion of blastocyst and its implantation.
- Principal mechanisms in implantation are:
  – Muscular
  – Adhesive—interaction between polar trophoblast and uterine epithelium—pentasaccharide, lacto-N-fucopentose-1
  – Invasive
  – Immunological.

Decidua

Definition: It is the functional stratum (stratum compactum) of uterine endometrium after the implantation of blastocyst (Fig. 6.2). The word decidua means falling off as this part of endometrium separates and falls off during childbirth.

Change in endometrium: After implantation, the features of the endometrium, which are seen during the secretory phase of the menstrual cycle, are maintained and intensified under the influence of the hormone the human chorionic gonadotropin (hCG) which is secreted by the cells of syncytiotrophoblast. On 17th or 18th day of menstrual cycle, i.e. 5th day after fertilization and at the time of implantation,

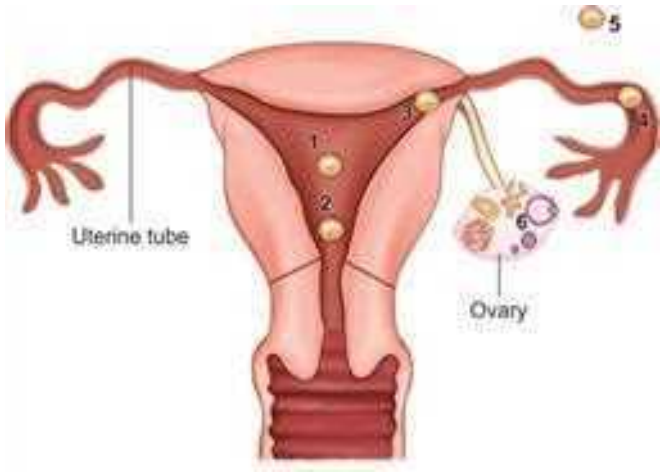


Fig. 6.5: Normal and abnormal sites of implantation: (1) Normal site of implantation in the upper uterine segment; (2) Abnormal sites of implantation in lower uterine segment; (3) Interstitial implantation; (4) Tubal in ampulla of uterine tube; (5) Abdominal implantation; (6) Ovarian implantation

the uterine endometrium is highly modified, edematous and vascular.

Decidual reaction: Due to the higher levels of maternal progesterone and the hCG, the stromal cells enlarge, become vacuolated and filled with glycogen and lipids (decidual transformation). These cells are called *decidual cells*. The intercellular substance increases, and it gives edematous appearance. This change in the endometrial stroma is called the *decidual reaction*. The glycogen and lipids provide nutrition to the early embryo until the placenta takes over this function. The saw-toothed appearance of endometrial glands increases and the blood vessels of endometrium become more tortuous. The decidual reaction is a defensive mechanism to protect the endometrium.

Subdivisions of Decidua (Fig. 6.6 and Flowchart 6.2)

1. *Decidua basalis/Serotina*: The part that contributes for the maternal component of placenta. It is the part that lies deep to the developing blastocyst. The maternal blood vessels (spiral arteries) proliferate in the region of decidua basalis and are filled with blood and dilate to form sinusoids. The decidua basalis consists predominantly of large decidual cells that contain large amounts of lipids and glycogen (that presumably provide a source of nutrition for the embryo). The decidua basalis is also referred to as the *decidual plate*, and is firmly united to the chorion.
2. *Decidua capsularis/Reflexa*: The part of endometrium that surrounds the embryo like a capsule and separates it from the uterine cavity.
3. *Decidua parietalis/Vera*: The part of decidua that lines the rest of uterine cavity.

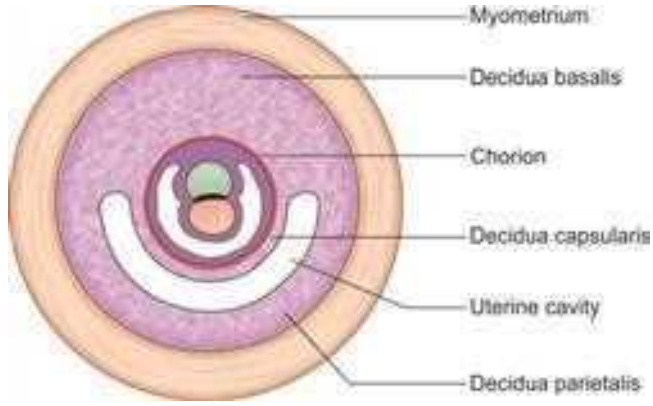


Fig. 6.6: Subdivisions of decidua: basalis, capsularis and parietalis

Fate of decidua: As the conceptus enlarges during development, the decidua capsularis enlarges into the uterine cavity and finally fuses with decidua parietalis during 3rd month of pregnancy thus obliterating the uterine cavity. At the end of pregnancy, the decidua is shed off, along with the placenta and membranes. It is this shedding off which gives the decidua its name (c.f. deciduous trees).

Clinical correlation

Placenta previa

- The normal attachment of placenta is in the upper uterine segment (Fig. 6.7). The attachment of placenta may extend partially or completely into the lower uterine segment. This condition is called *placenta previa*. This is due to the implantation of the blastocyst close to the internal os.
- ***Degrees of placenta previa (Figs 6.8A to D):***
  - *First degree:* Attachment of placenta does not extend to internal os.
  - *Second degree:* Attachment of placenta extends up to internal os but does not cover it.
  - *Third degree:* Placental edge covers the internal os. With the dilatation of internal os at the time of childbirth, the placenta will not occlude the internal os.
  - *Fourth degree:* Placenta completely covers the internal os even when the internal os is completely dilated. This can cause severe bleeding during pregnancy or during parturition.

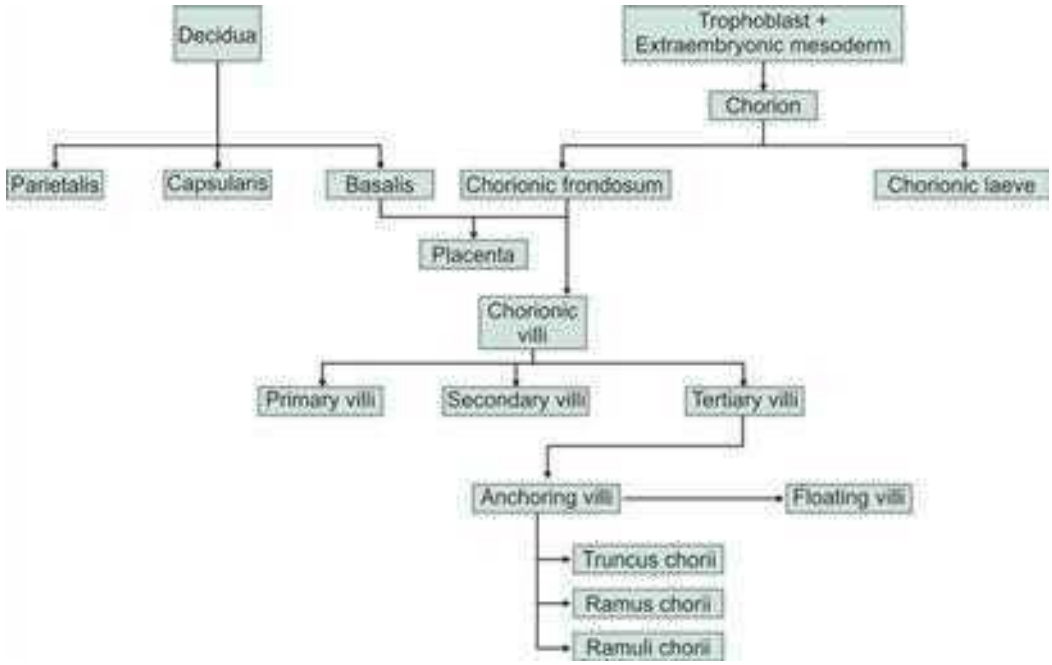
Ectopic pregnancy

- This results from abnormal sites of implantation, i.e. extrauterine pregnancies.
- Ectopic pregnancies do not progress and usually result in death of the embryo. Rarely does this embryo develop to full term.
- The most common ectopic pregnancy is tubal pregnancy with a 95% incidence. Tubal pregnancies are terminated by medical intervention. If it is permitted to progress, it can result in rupture of uterine tube with severe internal bleeding.
- Other types of ectopic pregnancies are abdominal, ovarian.

Trophoblast

It is the first embryonic membrane. The trophoblast cells are the precursor cells of human placenta. At first the cells form

Flowchart 6.2: Components of decidua and chorion



a unilaminar fetal membrane. Later with the formation of blastocyst it becomes bilaminar.

Differentiation of two layers of trophoblast: This is essential for the formation of chorionic villi. The trophoblast is at first made up of a single layer of cells (Figs 6.9 and 6.10A). As these cells multiply, two distinct layers are formed (Fig. 6.10B):

- The cells that are nearest to the decidua (i.e. the most superficial cells) lose their cell boundaries. These cells form a layer of multiple cells without cell outlines and form one continuous sheet of cytoplasm containing many nuclei. Such a tissue is called a syncytium. Hence, this layer of the trophoblast is called the *syncytiotrophoblast* or *plasmodiotrophoblast* (Fig. 6.10B).
- Deep to the syncytium, the cells of the trophoblast retain their cell walls and form the second layer called the *cytotrophoblast* or Langhans' *layer* that rests on extraembryonic mesoderm. This single layer of cuboidal cells with a clear outline is close to the extraembryonic mesoderm.
- All these elements (syncytium, cytotrophoblast and mesoderm) take part in forming chorionic villi.

Chorion and Formation of Chorionic Villi (Flowchart 6.2)

Chorion

Definition: The cellular, outermost extraembryonic membrane composed of trophoblast lined with extraembryonic somatopleuric mesoderm.

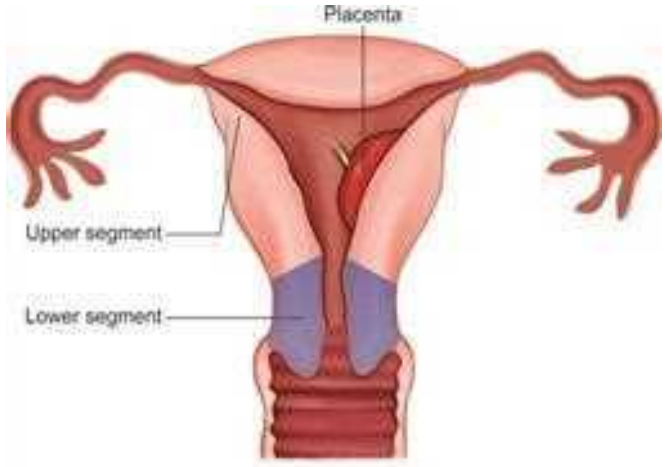
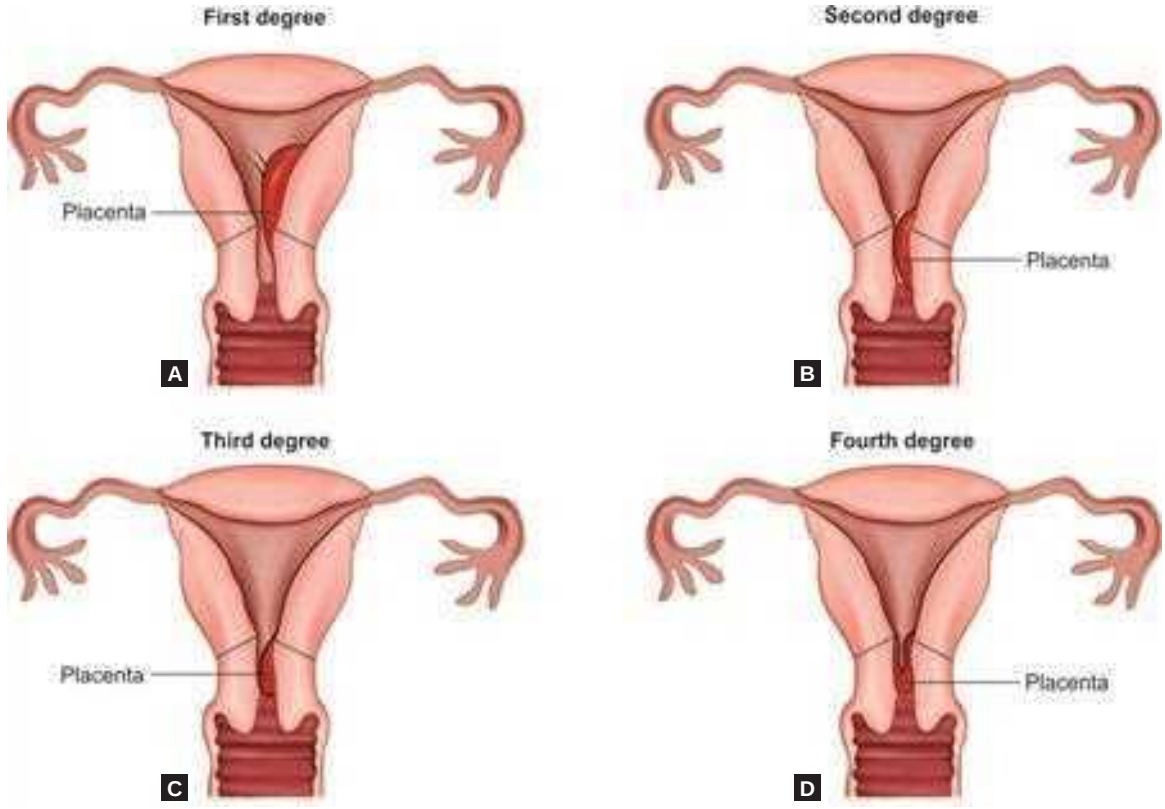


Fig. 6.7: Normal attachment of placenta—upper uterine segment

Formation

- The extraembryonic somatic mesoderm and the two layers of trophoblast (cytotrophoblast and syncytiotrophoblast) contribute for the formation of chorion all around the developing embryo (Fig. 6.9).
- The extraembryonic coelom is now called the chorionic cavity. Embryo and its amniotic and yolk sacs are suspended into it by connecting stalk. The amniotic sac with embryonic epiblast forms its floor and the yolk sac with embryonic hypoblast forms its roof.



Figs 6.8A to D: Different degrees of placenta previa

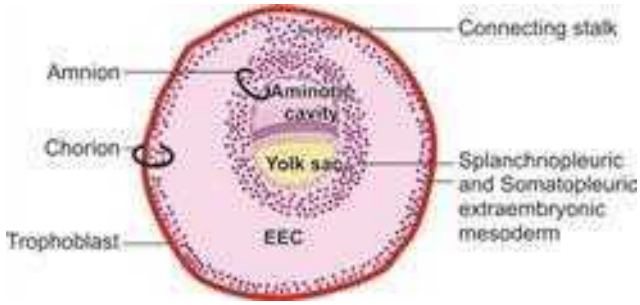


Fig. 6.9: Differentiation of trophoblast and formation of chorion and amnion

- Later blood vessels develop in the extraembryonic mesoderm which forms the chorionic vessels.
- The chorionic villi develop from the chorion and cover the entire chorionic sac until the beginning of 8th week. These become vascularized by allantoic vessels.

Types of Chorion

The chorionic villi are first formed all over the trophoblast and grow into the surrounding decidua (Fig. 6.10).

- *Chorion laeve*: The villi associated with decidua capsularis are transitory. As the gestational sac grows they get compressed and their blood supply is reduced.

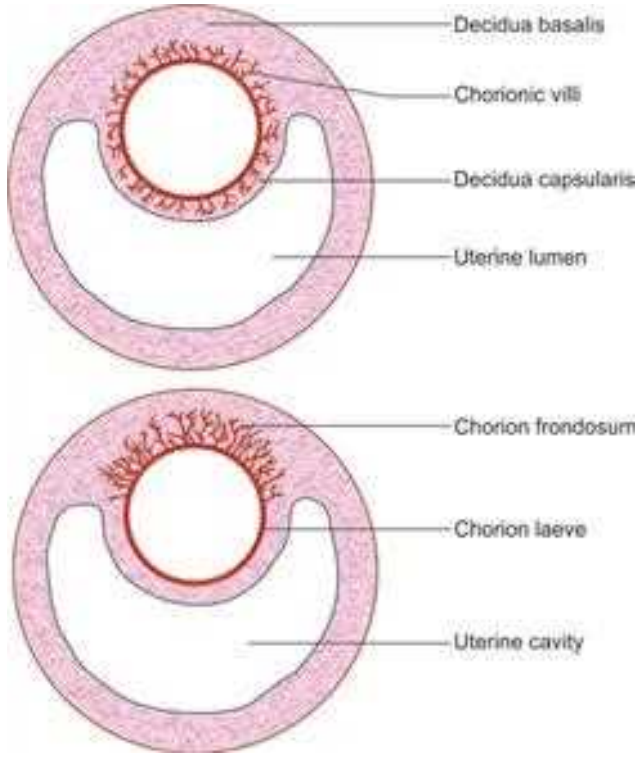


Fig. 6.10: Types of chorion: chorion frondosum in relation to decidua basalis and chorion laeve in relation to decidua capsularis

Because of reduced blood supply these villi soon degenerates producing an avascular bare area of smooth chorion called *chorion laeve*. It regresses in 3rd month of pregnancy.

- *Chorion frondosum*: The chorionic villi associated with decidua basalis retain the vascularity, undergo considerable development and form a bushy area called *chorion frondosum*. This contributes fetal part of placenta as the maternal part is contributed by decidua basalis.

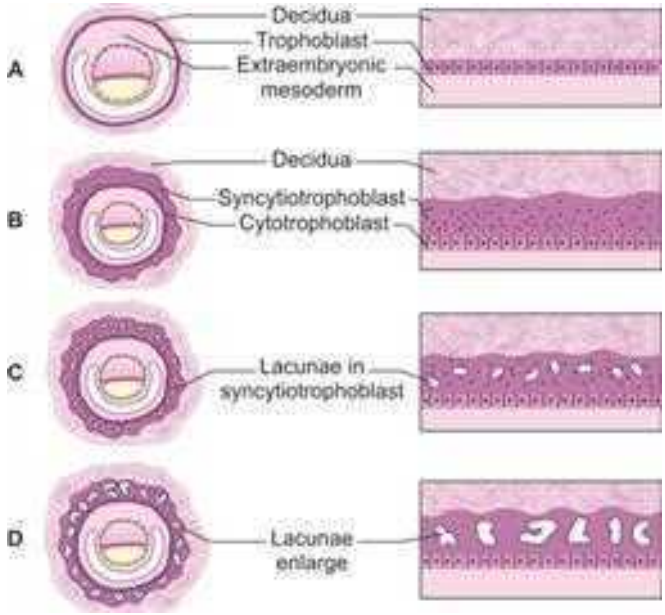
Stages in the Formation of Chorionic Villi

The structural component of chorionic villus differs at different periods of embryonic development. Accordingly there are three types of chorionic villi.

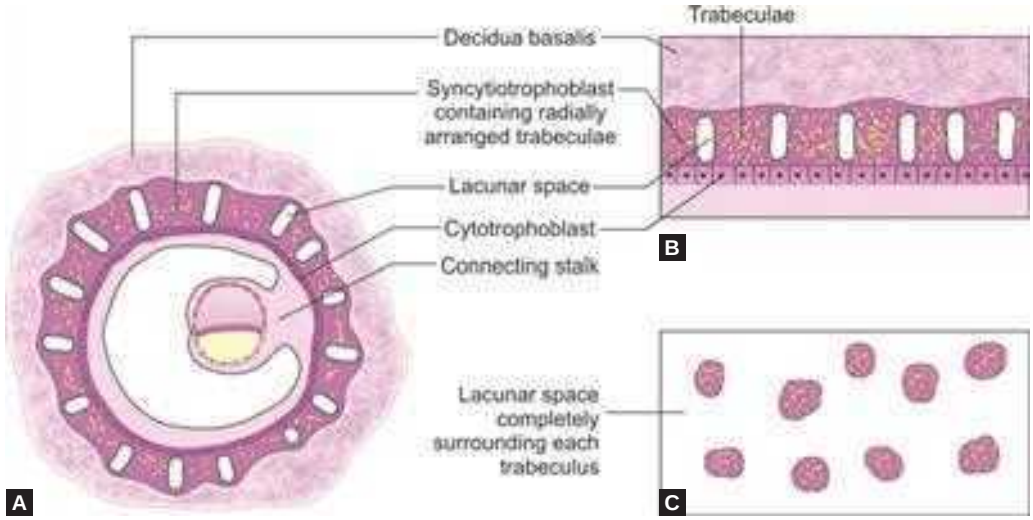
1. *Primary villi*: They consist of a central core of cytotrophoblast covered by a layer of syncytiotrophoblast. Adjoining villi are separated by an intervillous space.
2. *Secondary villi*: These show three layers. Outer syncytiotrophoblast, an intermediate layer of cytotrophoblast, and an inner layer of extraembryonic mesoderm.
3. *Tertiary villi*: These are like secondary villi except that there are fetal blood capillaries in the mesoderm.

Process of villus formation: The various processes in the formation of villi are as follows:

- *Appearance of lacunae and trabeculae in syncytiotrophoblast*: The syncytiotrophoblast grows rapidly and becomes thick. Small cavities called lacunae appear in this layer (Fig. 6.11C). Gradually, the lacunae increase in size. At first they are irregularly arranged (Fig. 6.11D), but gradually, they come to lie radially (Figs 6.12A to C) around the blastocyst. The lacunae are separated from one another by partitions of syncytium, which are called trabeculae. The lacunae gradually communicate with each other, so that eventually one large space is formed. Each trabeculus is now surrounded all around by this lacunar space (Figs 6.12A to C).
- *Erosion of maternal endometrium by syncytiotrophoblast and entry of maternal blood into the syncytial lacunae*: The syncytiotrophoblast in which these changes are occurring grows into the endometrium. As the endometrium is eroded, some of its blood vessels are



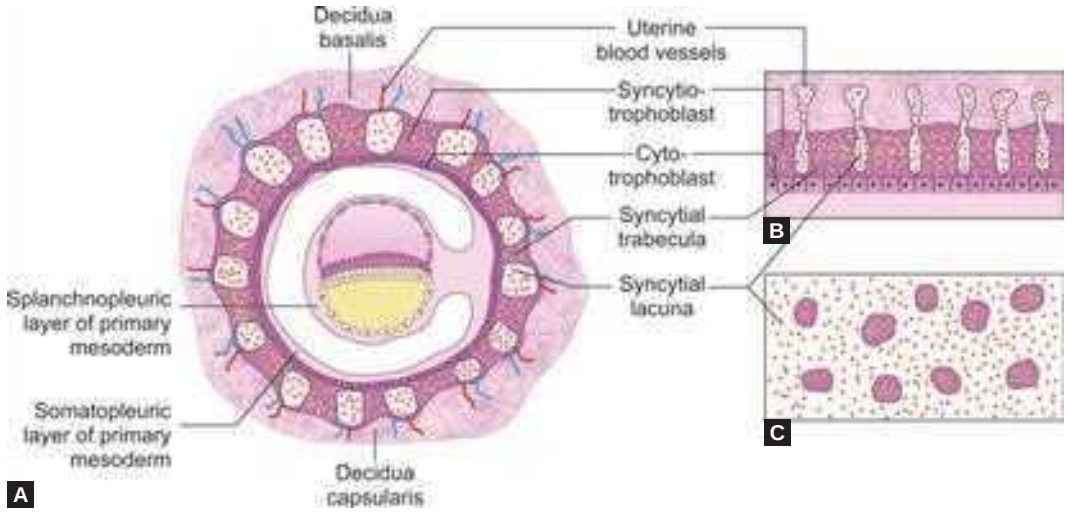
Figs 6.11A to D: Differentiation of trophoblast and early stages in the formation of chorionic villi. (A) Cytotrophoblast in contact with decidua; (B) Formation of syncytiotrophoblast; (C) Lacunae in syncytiotrophoblast; (D) Enlarging lacunae



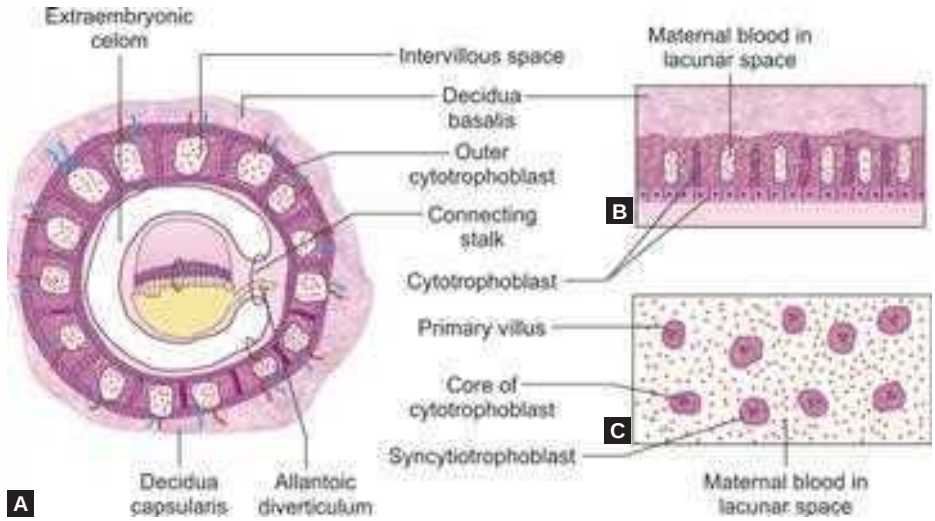
Figs 6.12A to C: (A) Radial arrangement of trabeculae and lacune around the blastocyst; (B) Regularly arranged syncytial trabeculae; (C) Transverse section through trabeculae containing syncytiotrophoblast

opened up, and blood from them fills the lacunar space (Figs 6.13A to C).

- *Formation of primary chorionic villi (Figs 6.14A to C)*: Each trabeculus is, initially, made up entirely of syncytiotrophoblast and in cut section it is surrounded by lacunar space filled with maternal blood. Now the cells of the cytotrophoblast begin to multiply and grow into each trabeculus. The trabeculus thus comes to have a central core of cytotrophoblast covered by an outer layer of syncytium. It is surrounded by maternal blood, filling the lacunar space. The trabeculus is now called a *primary villus* and the lacunar space is now called the *intervillous space*.
- *Formation of secondary chorionic villi (Figs 6.15A to C)*: Extraembryonic mesoderm invades the center of each primary villus. The villus now has a core of mesoderm covered by cytotrophoblast and by syncytium. This structure is called a *secondary villus*.

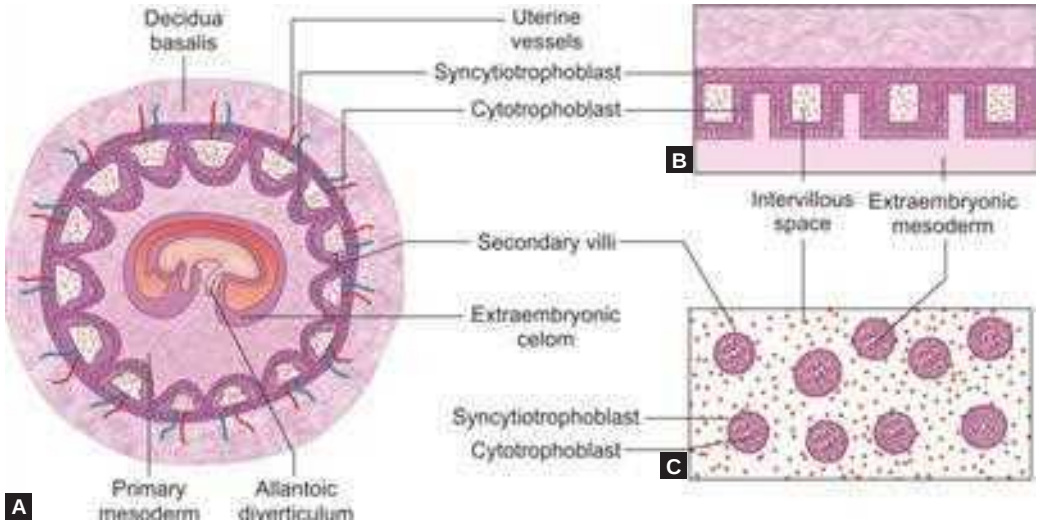


Figs 6.13A to C: (A) Radial arrangement of trabeculae and lacunae around the blastocyst with maternal blood vessels entering lacunar space; (B) Uterine blood vessels in the decidua open into the lacunar space and fill with maternal blood and trabecular filled with syncytiotrophoblast; (C) Transverse section through trabeculae containing syncytiotrophoblast surrounded by lacunar spaces filled with maternal blood

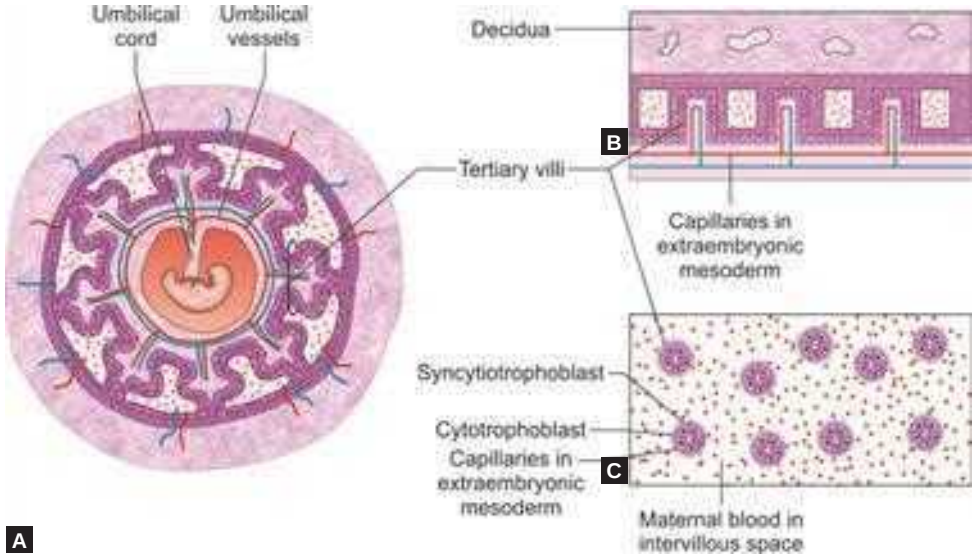


Figs 6.14A to C: (A) Primary chorionic villi and lacunae around the blastocyst with maternal blood vessels entering lacunar space and extensions of cytotrophoblast cells in the center of syncytial trabeculae; (B) Primary villi with central cytotrophoblast cells surrounded by syncytiotrophoblast; (C) Transverse section of primary villi with central cytotrophoblast and peripheral syncytiotrophoblast in contact with maternal blood in intervillous space

- *Formation of tertiary chorionic villi (Figs 6.16A to C)*: Soon thereafter, blood vessels can be seen in the mesoderm forming the core of each villus. With their appearance, the villus is fully formed and is called a *tertiary villus*.
- *Establishment of fetal blood circulation in tertiary villi*: The blood vessels of the villus establish connections with the circulatory system of the embryo. Fetal blood now circulates through the villi, while maternal blood circulates through the intervillous space.
- *Formation of cytotrophoblastic shell*: The cytotrophoblast that grows into the trabeculus (or villus) does not penetrate the entire thickness of syncytium and,



Figs 6.15A to C: (A) Secondary chorionic villi and intervillous space around the blastocyst with maternal in intervillous space and extensions of extraembryonic somatopleuric mesoderm cytotrophoblast cells and syncytiotrophoblast in the trabeculae; (B) Secondary chorionic villi with central extraembryonic mesoderm, intermediate cytotrophoblast cells surrounded by syncytiotrophoblast; (C) Transverse section of secondary villi with central core of extraembryonic mesoderm, intermediate cytotrophoblast and peripheral syncytiotrophoblast in contact with maternal blood in intervillous space



Figs 6.16A to C: (A) Tertiary chorionic villi and intervillous space around the blastocyst with maternal blood in intervillous space and extensions fetal blood capillaries in extraembryonic somatopleuric mesoderm, cytotrophoblast cells and syncytiotrophoblast in the trabeculae; (B) Tertiary chorionic villi with central core of extraembryonic mesoderm with capillaries, intermediate cytotrophoblast cells surrounded by syncytiotrophoblast; (C) Transverse section of tertiary villi with central extraembryonic mesoderm with capillaries, intermediate cytotrophoblast and peripheral syncytiotrophoblast in contact with maternal blood in intervillous space

therefore, does not come in contact with the decidua. At a later stage, however, the cytotrophoblast emerges through the syncytium of each villus. The cells of the cytotrophoblast now spread out to form a layer that completely cuts off the syncytium from the decidua. This layer of cells is called the *cytotrophoblastic shell* (Fig. 6.17). The cells of this shell multiply rapidly and the placenta increases in size.

Subdivisions of Villus (Fig. 6.18)

- The villi that are first formed (as described above) and are attached on the fetal side to the extraembryonic mesoderm and on the maternal side to the cytotrophoblastic shell. They are, therefore, called *anchoring villi*.
- Each anchoring villus consists of a *stem villus* or *truncus chorii*.
- Each stem villus divides into a number of branches called *rami chorii.*

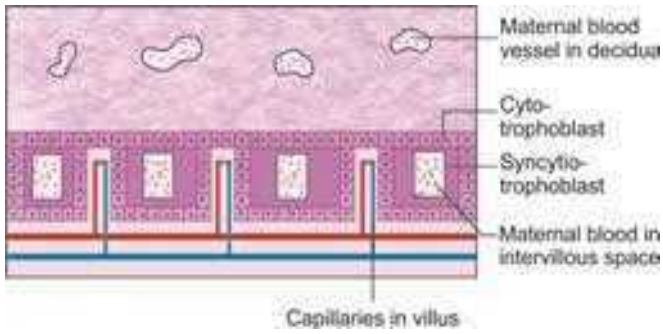


Fig. 6.17: Formation of cytotrophoblast shell

- The rami chorii in turn divide into finer branches called *ramuli chorii*. The ramuli *chorii* are attached to the cytotrophoblastic shell.
- The anchoring villi give off numerous branches that grow into the intervillous space as *free/floating villi*. New villi also sprout from the chorionic side of the intervillous space. Ultimately, almost the whole intervillous space becomes filled with villi. As a result, the surface area available for exchanges between maternal and fetal circulations becomes enormous.
- These, newly formed, villi at first consist only of syncytiotrophoblast. They are subsequently invaded by cytotrophoblast, mesoderm, and blood vessels, and pass through the stages of primary, secondary and tertiary villi as described above.

Further Development of the Placenta

The placenta presents two parts (fetal and maternal), two surfaces (fetal and maternal), two types of cotyledons and a peripheral margin.

Maternal part: This is contributed by decidua basalis or decidual plate of endometrium.

Fetal part: This is contributed by chorion frondosum or chorionic plate. This surface is covered by the fetal membrane amnion and the umbilical cord is attached near the center of this surface.

Maternal surface: The maternal surface is rough and irregular (Fig. 6.19A). It is subdivided into a number of lobes called *maternal cotyledons*. Septa that grow into the intervillous space from the maternal side (Fig. 6.18) divide this surface into 15–20 rough and irregular maternal

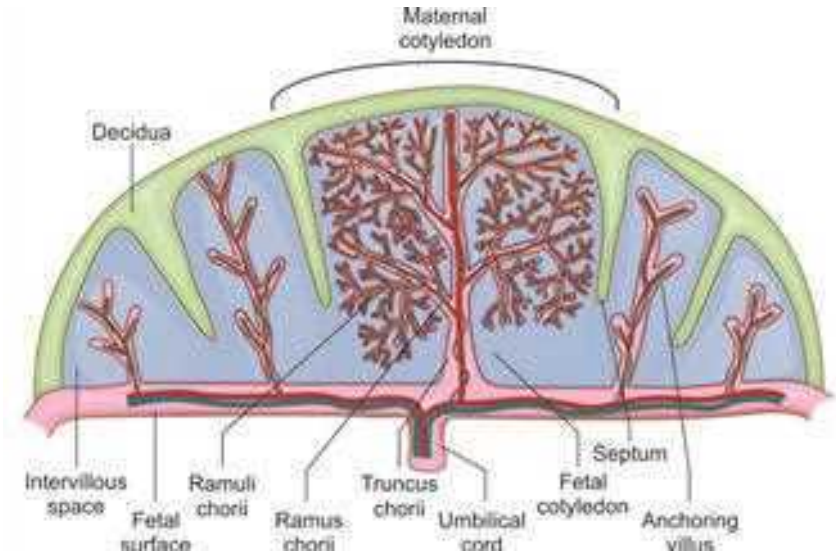
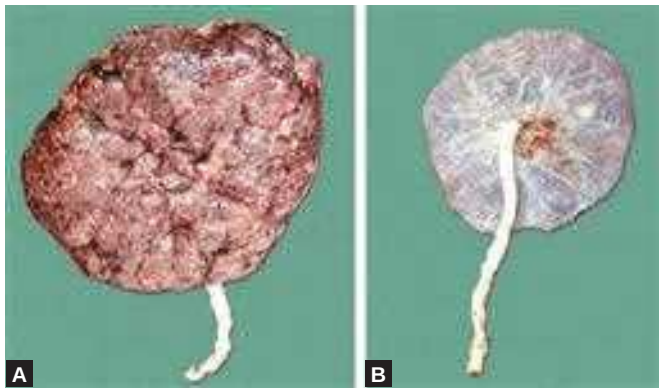


Fig. 6.18: Maternal and fetal cotyledons. Subdivisions of villus—truncus, ramus and ramuli chorii, and anchoring villi

cotyledons. If the placenta is viewed from the maternal side, the bases of the septa are seen as grooves while the cotyledons appear as convex areas bounded by the grooves.

Fetal surface: This surface is smooth and is covered by amnion (Fig. 6.19B). The umbilical cord is attached close to the center of this surface. Umbilical vessels radiate from the umbilical cord beneath the amnion. The fetal part is contributed by chorionic frondosum that is seen as a plate called *chorionic plate*. From the chorionic plate 40–60 extensions (fetal cotyledons) arise and extend toward the decidua basalis. Each *fetal cotyledon* (Fig. 6.18) consists of a stem villus/truncus chorii that show ramifications into number of branches (ramus chorii), each further subdivides (ramuli chorii) like the branches of a tree. Their terminal ramifications look like fingers and are called chorionic villi. The villi that are attached to decidua basalis are called *anchoring villi*. Others float in the maternal blood that flows in between the villi and are called *floating villi* (Fig. 6.18).



Figs 6.19A and B: Placenta: (A) Maternal surface; (B) Fetal surface

Maternal and fetal cotyledons: There are 15–20 maternal cotyledons in placenta. Each maternal cotyledon contains 2–4 anchoring villi and their branches. One anchoring villus and its ramifications (ramus chorii, ramuli chorii and floating villi) constitute a fetal cotyledon.

Peripheral margin of placenta: It presents fetal membrane which is contributed from inside outward by decidua capsularis and parietalis, chorion laeve and amnion. After the birth of the child, the placenta is shed off along with the decidua.

Measurements of placenta at full term:

- Diameter: 15–20 cm
- Thickness: 3 cm
- Weight: 500 g.

Structure of placenta (Fig. 6.20):

- Maternal side—basal plate
  - Stratum spongiosum of decidua basalis containing maternal blood vessels
  - Outer layer of syncytiotrophoblast (Nitabuch's layer)
  - Outer shell of cytotrophoblast
  - Inner layer of syncytiotrophoblast (Rohr's fibrinoid stria).
- Fetal side—chorionic plate
  - Covered by amnion
  - Primary mesoderm with fetal blood vessels
  - Cytotrophoblast
  - Syncytiotrophoblast.

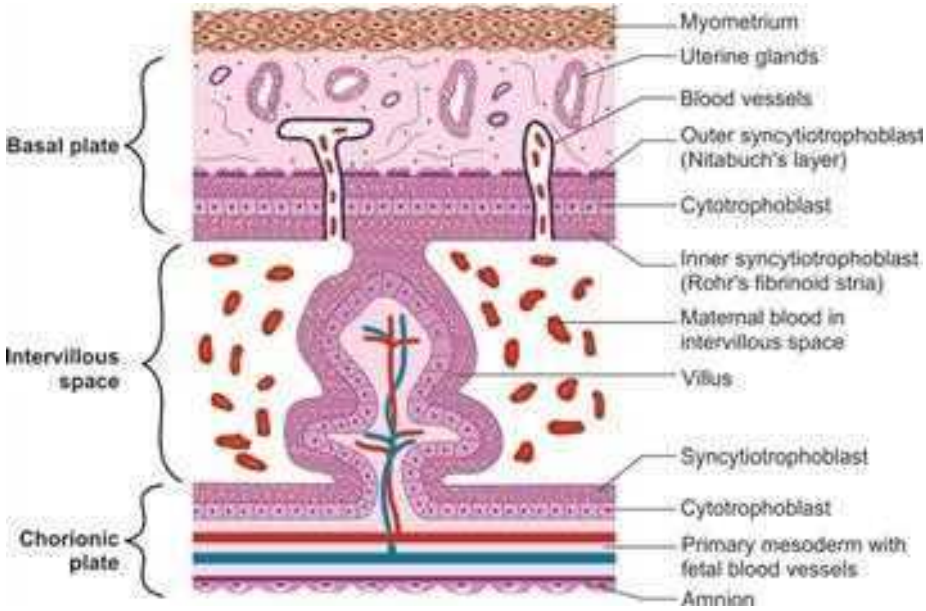


Fig. 6.20: Structural components of placental barrier or membrane

TABLE 6.1: The criteria and descriptive terms used for describing human placenta

Criteria	*Descriptive term*
Extent of maternal and fetal contact	Hemochorial
Source of blood supply to chorion	Chorioallantoic
Presence/absence of decidual reaction	Deciduate
Shape and structure	Discoid, cotyledonous and villus
Nature of blood flow through it	Labyrinthine

- Between basal plate and chorionic plate
  - Intervillous space
    - Volume—140 mL
    - Maternal blood passing through intervillous space—500 mL/minute
    - Volume of fetal blood flowing through fetal villi—400 mL/minute.
  - Stem villi—primary, secondary, tertiary.

Description of human placenta based on certain criteria is presented in Table 6.1.

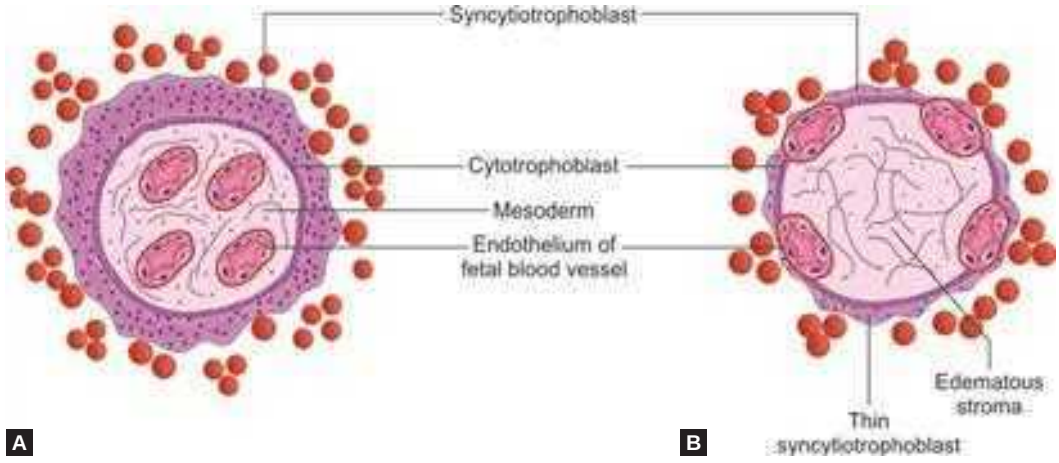
Placental Membrane/Barrier

- In the placenta, maternal blood circulates through the intervillous space and fetal blood circulates through blood vessels in the villi. Though the maternal and fetal bloods are flowing side by side and in opposite directions they do not mix with each other. They are separated by a membrane, made up of the layers of the wall of the villus.
- Tissues intervening between fetal blood in chorionic villi and maternal blood in intervillous space constitute the placental membrane or barrier. All interchanges of oxygen, nutrition and waste products take place through this membrane.
- The constituent structures forming the placental barrier or maternal fetal barrier extending from the maternal erythrocyte to fetal erythrocyte are as follows:
  - In the early part of pregnancy, the barrier presents the following layers (Fig. 6.21A)
    - Endothelium of fetal blood vessels, and its basement membrane
    - Surrounding mesoderm (connective tissue)
    - Cytotrophoblast and its basement membrane
    - Syncytiotrophoblast.
  - In the later part of pregnancy, the efficiency of the membrane is increased due to the reduction in its thickness (Fig. 6.21B) by
    - Disappearance of cytotrophoblast
    - Thinning of syncytiotrophoblast
    - Edematous stroma
    - Peripheral migration of fetal blood vessels
    - It presents endothelium of fetal capillaries resting on basement membrane and syncytiotrophoblast only.
- The total area of this membrane varies from 4 m^2 to 14 m^2. It is interesting to note that this is equal to the total absorptive area of the adult intestinal tract. As in the gut, the effective absorptive area is greatly increased by the presence of numerous microvilli on the surface of the syncytiotrophoblast.
- This membrane, which is at first 0.025 mm thick, is reduced to 0.002 mm. However, toward the end of pregnancy, a fibrinoid deposit appears on the membrane, and this reduces its efficiency.

Functions of Placenta

It has several functions that facilitate growth of the fetus.

- It acts as a temporary organ that allows transport of oxygen, water, electrolytes and nutrients (in the form of carbohydrates, lipids, polypeptides, amino acids



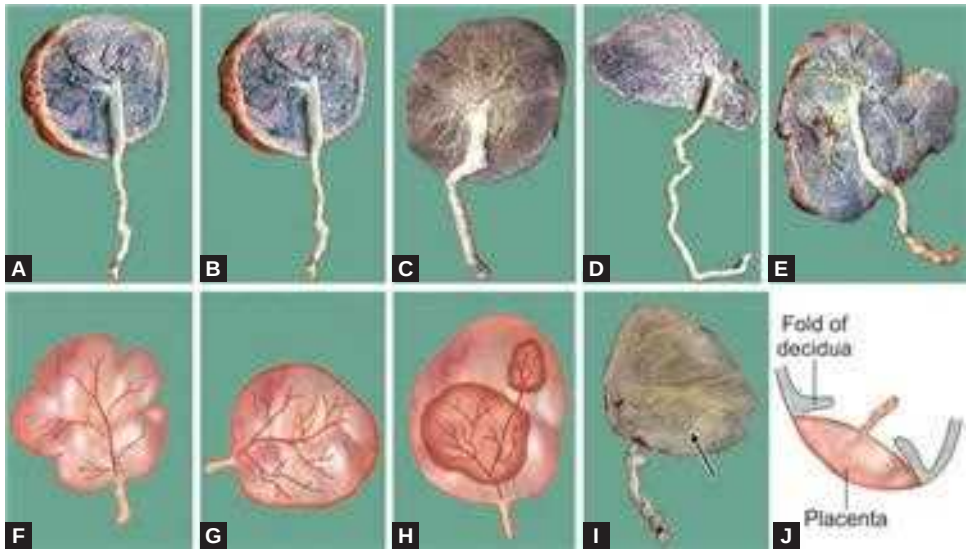
Figs 6.21A and B: Diagram of placental barrier (A) in early part of pregnancy and (B) in later part of pregnancy

and vitamins) from maternal to fetal blood and thus maintains the nutrition of the fetus. A full term fetus takes up about 25 mL of oxygen per minute from maternal blood. Even a short interruption of oxygen supply is fatal for the fetus.

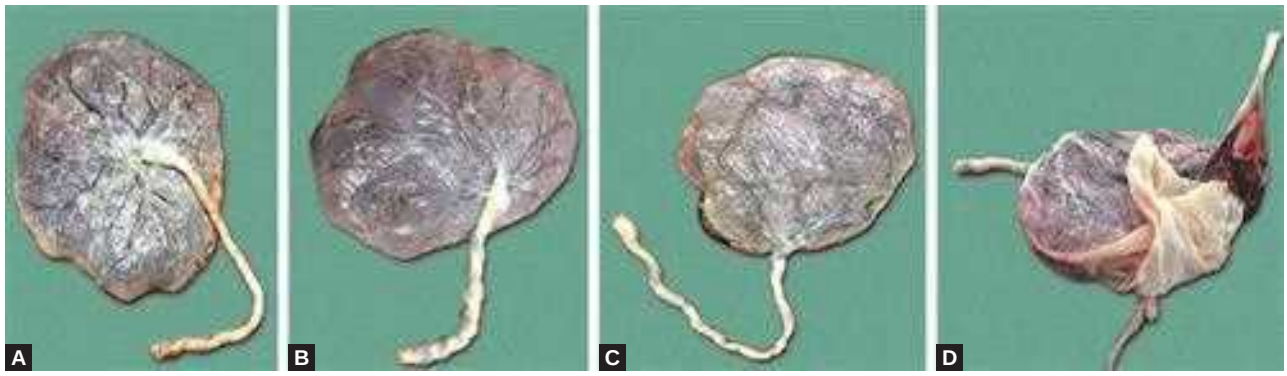
- It eliminates excretion of carbon dioxide, urea and other waste products produced by the fetus into the maternal blood.
- Maternal antibodies [immunoglobulin G (IgG), gamma globulins or immunoglobulins] reaching the fetus through the placenta give the fetus immunity against some infections (e.g. diphtheria and measles).
- The placenta acts as a barrier and prevents many bacteria and other harmful substances from reaching the fetus. However, most viruses (including poliomyelitis, measles and rubella) and some bacteria can pass across it.
- Drugs taken by the mother may also enter the fetal circulation and can produce congenital malformations. As a rule, maternal hormones do not reach the fetus. However, synthetic progestins and synthetic estrogens (e.g. diethylstilboestrol) easily cross the placenta and can have adverse effects on the fetus (including carcinoma in later life).
- While permitting the exchange of several substances between the maternal and fetal blood, it keeps these blood streams separate, thereby preventing antigenic reactions between them.
- The placenta synthesizes several hormones. These are probably produced in the syncytiotrophoblast. Progesterone secreted by the placenta is essential for maintenance of pregnancy after the 4th month (when the corpus luteum degenerates). Estrogens (mainly estriol) produced by the placenta reach maternal blood and promote uterine growth and development of the mammary gland.

Classification of Placenta

1. Based on shape (Figs 6.22A to J):
   - Discoid—round or disc like (Fig. 6.22A)
   - Bidiscoidal—it consists of two discs (Fig. 6.22B)
   - Oval (Fig. 6.22C)
   - Triangular (Fig. 6.22D)
   - Irregular (Fig. 6.22E)
   - Lobed—it divides into lobes (Fig. 6.22F)
   - Diffuse/placenta membranacea (Fig. 6.22G)—chorionic villi persists all-round the blastocyst
   - Placenta succenturiata (Fig. 6.22H)—a small part of the placenta is separated from the rest of it
   - Fenestrated (Fig. 6.22I)—presence of hole or opening in the placenta
   - Circumvallate (Fig. 6.22J)—when peripheral edge of placenta is covered by a circular fold of decidua, it is called circumvallate.
2. According to attachment of umbilical cord (Figs 6.23A to D):
   - Normal—Central insertion (Fig. 6.23A)
   - Paracentral insertion of umbilical cord (Fig. 6.23B)
   - Marginal or battledore placenta (Fig. 6.23C)—Cord is attached to the margin of placenta
   - Velamentous (Fig. 6.23D)—Umbilical cord is attached to the fetal membrane close to the peripheral margin of placenta.



Figs 6.22A to J: Types of placenta based on shape: (A) Discoid; (B) Bidiscoidal; (C) Oval; (D) Triangular; (E) Irregular; (F) Lobed—it divides into lobes; (G) Diffuse or placenta membranacea; (H) Placenta Succenturiata; (I) Fenestrated; (J) Circumvallate



Figs 6.23A to D: Types of placenta based on attachment of umbilical cord: (A) Normal; (B) Paracentral insertion of umbilical cord; (C) Marginal or Battledore placenta; (D) Velamentous

3. According to distribution of umbilical arteries:
   - Disperse type (Fig. 6.22C)—Umbilical arteries show dichotomous branching and show progressive reduction in size
   - Magistral type (Fig. 6.23C)—Arteries present uniform caliber up to the periphery of placenta
   - Furcate (Fig. 6.24)—Blood vessels divide before reaching the placenta.
4. Phylogenetic classification: According to tissues from maternal and fetal parts of placenta contributing for placental barrier (Fig. 6.25) (Table 6.2).

Clinical correlation

- ***Human chorionic gonadotropin*** produced by the placenta is similar in its actions to the luteinizing hormone of the hypophysis cerebri. Gonadotropins are excreted through maternal urine where their presence is used as a test to detect a pregnancy in its early stages.
- Human chorionic somatomammotropin (hCS) has an antiinsulin effect on the mother. This leads to increased plasma levels of glucose and amino acids in the maternal circulation. In this way, it increases availability of these materials for the fetus. It also enhances glucose utilization by the fetus.
- Circulation of blood through the placenta (Fig. 6.26):
  - Blood flow through lacunar spaces in the syncytiotrophoblast begins as early as the 9th day of pregnancy. Thereafter, the maternal blood in the intervillous spaces is constantly in circulation.
  - Blood enters the intervillous space through maternal arteries that open into the space. The pressure of blood drives it right up to the chorionic plate. Blood from the intervillous spaces is drained by veins that also open into the same spaces. In the fully formed placenta, the intervillous spaces contain about 150 mL of blood that is replaced in 15–20 seconds (i.e. three to four times per minute).

FETAL/EXTRAEMBRYONIC MEMBRANES

Definition: Tissues or structures that develop from the zygote but do not form part of embryo proper.

Structures that constitute the fetal membranes are:

- Trophoblast and chorion forming *placenta*
- *Amnion* or ectodermal vesicle covering the embryo or fetus and filled with amniotic fluid
- *Yolk sac*—Primary, secondary, tertiary
- *Allantois* or allantoenteric diverticulum
- Connecting or body stalk and *umbilical cord*
- Functions: These are concerned with protection, nutrition
- Protection—Amniotic membrane and fluid
- Respiration—Placenta
- Excretion
- Nutrition—Amniotic fluid, placenta, umbilical cord
- Clinical importance: They are essential for performing prenatal diagnostic procedures like chorionic villus biopsy and amniocentesis.

Fig. 6.24: Furcate placenta

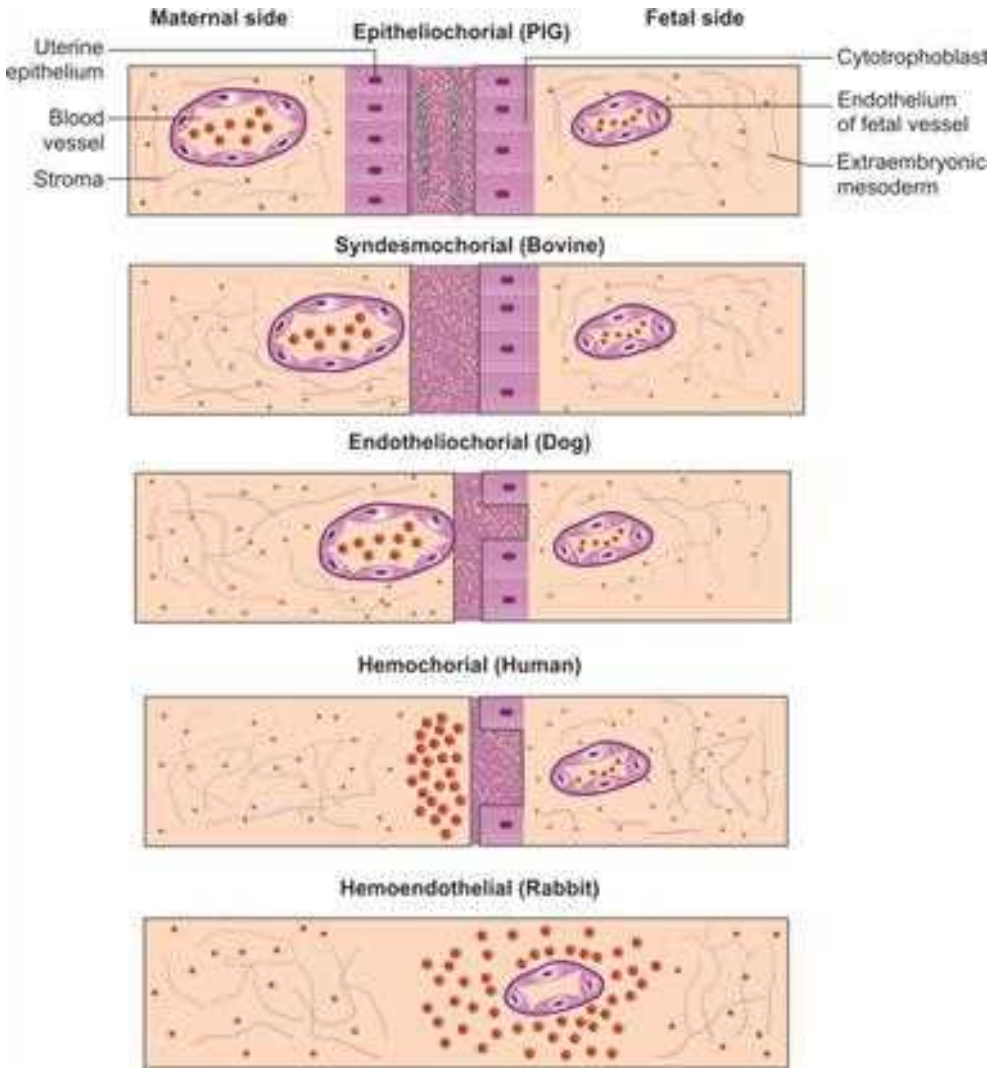


Fig. 6.25: Phylogenetic classification of placenta into epitheliochorial, syndesmochorial, endotheliochorial, hemochorial and hemoendothelial

TABLE 6.2: Types of placenta based on tissues contributing for placental barrier

Type	*Maternal component*	*Fetal component*	*Example*
Epitheliochorial	Endometrial epithelium	Chorion	Pig
Syndesmochorial	Endometrial stroma	Chorion	Bovine
Endotheliochorial	Endothelium of maternal blood vessels	Chorion	Dog
Hemochorial	Maternal blood in intervillous space	Chorion	Human
Hemoendothelial	Maternal blood in intervillous space	Endothelium of fetal blood vessels	Rabbit

Amnion and Amniotic Fluid

- It is the fetal membrane that covers the embryo and forms the amniotic sac that is filled with amniotic fluid (Figs 6.27A and B). It appears in the 2nd week of development.
- *Formation and expansion of amniotic cavity*: The amniotic cavity is lined by extension of cells of ectoderm from the inner cell mass and amniogenic cells from the trophoblast (Figs 6.3 and 6.9). The amniogenic cells line the roof and lateral wall of amniotic sac and its floor is formed by ectodermal cells. The amniotic cavity expands

during the late embryonic period due to the collection of fluid within it. It gradually surround whole embryo, ensheaths the umbilical cord and covers the fetal surface of placenta.

- The amnion consists of two layers: (1) an outer somatopleuric layer of extraembryonic mesoderm and (2) an inner amniogenic cells.
- The amniotic cavity grows at the expense of extraembryonic coelom, which gets obliterated and results in fusion between chorion and amnion.
- *Classification of animals* depending on the presence or absence of amnion.
  - *Amniotes*: Those that contain the amnion are called amniotes, e.g. reptiles, birds, mammals.
  - *Anamniotes*: Those without amnion, e.g. fish, amphibian.
- Functions:
  - Amniotic fluid provides support for the delicate tissues of the growing embryo or fetus.
  - It allows free movement and protects the fetus from external injury. It also avoids adhesion of the fetus to amnion.
  - As pregnancy advances, the quantity of this fluid increases, till at full term it is about 1 L.
  - There is constant exchange of water between the amniotic fluid and maternal blood, the water being completely replaced every 3 hours.
  - Sometime in the 5th month the fetus begins to swallow amniotic fluid. This fluid is absorbed (through the gut) into fetal blood and is transferred through the placenta to maternal blood.
  - When the fetal kidneys start working, the fetus passes urine into the amniotic fluid. This does not cause harm because fetal urine is made up mostly of water (metabolic wastes being removed from blood by the placenta and not through the kidneys).

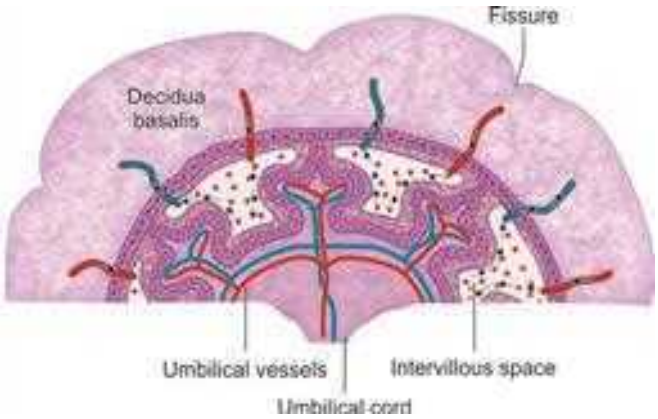


Fig. 6.26: Scheme to show how maternal blood circulates through placenta

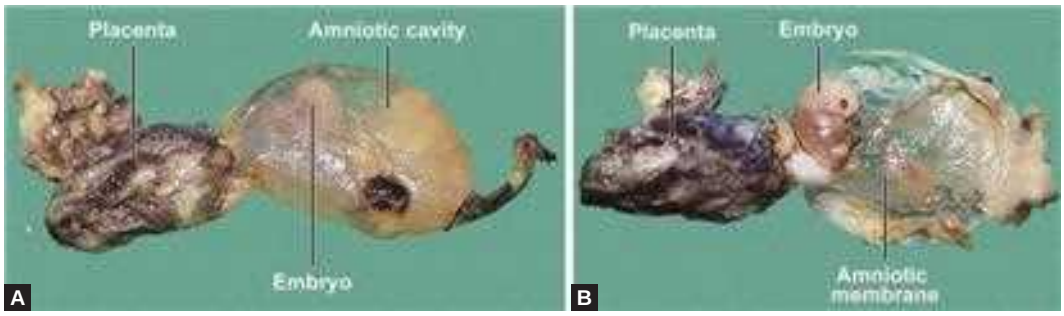
Umbilical Cord

Introduction: It is one of the fetal membranes. Umbilical cord develops from yolk sac and contains its remnants. Umbilical cord is tubular in structure and is covered by amniotic membrane. It contains blood vessels, yolk sac remnants and embryonic connective tissue. One end of it is attached to the anterior abdominal wall of fetus and the other end is fixed to the center of fetal surface of placenta (Figs 6.1A and B). It is 50 cm in length and 2 cm in breadth at full term. If the cord is too long, it can wind round the neck of fetus resulting in strangulation or it can prolapse into the cervical canal. If the cord is too short, it can cause difficulty during delivery of the fetus.

Function: The umbilical vessels transport oxygen and nutrients from the placenta to the developing fetus and eliminate carbon dioxide from fetal circulation into the placenta.

Formation of Umbilical Cord

- During 2nd week of development with the appearance of extraembryonic coelom, the extraembryonic mesoderm is divided into somatopleuric and splanchnopleuric layers around the entire conceptus except at one area.



Figs 6.27A and B: (A) Embryo covered with amniotic membrane and floating in the amniotic fluid; (B) Embryo after opening the amniotic cavity

This unsplit area of extraembryonic mesoderm forms the connecting stalk (Fig. 6.9).

- The connecting stalk contains the primary mesoderm and suspends the developing bilaminar embryonic disc along with amniotic and yolk sac cavities in the blastocyst/chorionic cavity (Fig. 6.9).
- With the establishment of cephalocaudal axis of the embryo, the connecting stalk moves toward the caudal end of the embryo. An evagination from the caudal end of secondary yolk sac known as allantoic diverticulum extends into the primary mesoderm of connecting stalk during 3rd week of development (Figs 6.14 and 6.15).
- During 3rd week of development, extraembryonic blood vessels develop in the chorion and connecting stalk and are known as umbilical vessels (Fig. 6.16).
- During the 4th week of development with the formation of head fold, tail fold and lateral folds of the embryo, the connecting stalk with its constituent allantoic diverticulum moves to the ventral surface of the developing embryo. With the incorporation of yolk sac into the head and tail folds of the embryo, contributing for the foregut and hind gut respectively the midgut between the two is in communication with the extraembryonic part of yolk sac known as definitive yolk sac (Fig. 6.28). This communication between midgut and definitive yolk sac is known as vitellointestinal duct and lies close to the connecting stalk. Because of the formation of embryonic folds, the amniotic membrane forms a tubular investment enclosing the connecting stalk along with allantoic diverticulum, vitellointestinal duct and umbilical vessels forming the umbilical cord.

Components: Components of umbilical cord vary with gestational age of the fetus. They are:

- *Umbilical arteries:* Two umbilical arteries known as right and left umbilical arteries that are derived from ventral division of internal iliac arteries. These transport deoxygenated blood from the fetus to the chorionic villi of placenta.
- *Umbilical veins*: In the early part of gestation, two umbilical veins known as right and left umbilical veins are present. During later part of pregnancy, the right umbilical vein disappears leaving the left umbilical vein that conveys oxygenated blood from the placenta to fetus.
- *Wharton's jelly*: It is primary or intraembryonic mesodermal cells of connecting stalk that have undergone mucoid degeneration to protect the umbilical vessels.
- *Allantoic diverticulum*: It is a ventral projection of hindgut into the connecting stalk. The proximal part of diverticulum gets incorporated into the apex of urinary bladder and its distal part undergoes fibrosis to form urachus.
- *Vitellointestinal duct*: It is the communication between the midgut and extraembryonic part of yolk sac. In late fetal life, it disappears.
- *Communication between intra- and extraembryonic coelom*: The coelomic cavity exists around the vitellointestinal duct at the fetal end of umbilical cord up to 10th week of development. During this period, there is herniation of U-shaped midgut loop into the extraembryonic coelom which is known as *physiological hernia*.

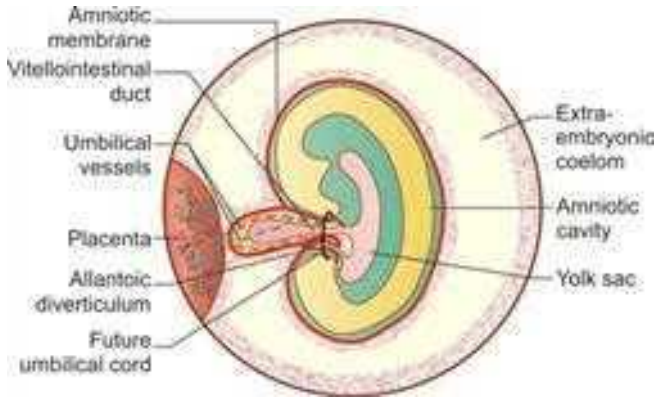


Fig. 6.28: Developing embryo with incorporation of yolk sac into the head and tail folds and its communication with the extraembryonic part of yolk sac

Clinical correlation

Amniotic fluid

- ***Amniotic fluid:*** It is a clear, watery fluid (98%) and contains 2% solids (inorganic salts, urea, proteins, sugars).
  - Source: Fetal/maternal/both
    - Amnion, fetal kidney, fetal lung, placenta
    - Amnio-fetomaternal exchange
  - Amount
    - 10th–20th week: 25–400 mL
    - Increases up to 6th month, then decreases. At 28 weeks, it is 800 mL and at term it is 400 mL
  - Abnormal production
    - Hydramnios—more than 2 L of amniotic fluid will be present. In some cases, hydramnios is associated with atresia of the esophagus, which prevents swallowing of amniotic fluid by the fetus
    - Oligoamnios—scanty amniotic fluid. It is sometimes associated with renal agenesis, as no urine is added to the amniotic fluid.

      Both conditions can cause abnormalities in the fetus. They can also cause difficulties during childbirth.

Clinical importance of amniotic fluid

Amniocentesis: It is a technique to collect amniotic fluid. The fluid is collected either through cervix or anterior abdominal wall. This procedure is usually done during 15–20 weeks of pregnancy. There is risk of fetal injury or preterm delivery in performing this procedure.

The indications for this procedure are:

- Maternal age
- Bad obstetric history
- Cytogenetic analysis: Diagnosis of trisomy's, sex-linked disorders
- Biochemical analysis: Enzyme estimations—gross fetal anomalies—alpha-fetoproteins, surfactant
- Metabolic disorders:
  - Lipid—Tay-Sachs disease
  - Mucopolysacharides—Hurler's syndrome
  - Carbohydrate—Pompe's disease
  - Purine—Lesch-Nyhan syndrome
- Amniotic stem cells: Production of embryonic cells in stem cell therapy for defects of mesenchymal, hematopoietic, neural, epithelial or endothelial cell origin.

Amniotic bands

Tears in the amnion results in amniotic bands. These bands may encircle parts of fetus particularly the limbs. Amputation of limb, ring like constrictions of limb, other abnormalities including craniofacial malformations can occur. This condition results from infections or toxins affecting the fetus or fetal membranes or both.

Clinical correlation

Umbilical cord

- Single umbilical artery: Instead of normal two umbilical arteries a single umbilical artery will be present. Usually the left umbilical artery is absent. Its incidence is 1% and is associated with fetal anomalies.
- Cord blood therapies: Cord blood is the source of stem cells that are used for various disorders.
  - Hematopoietic stem cells
  - Cardiovascular diseases—myocardial infarction
  - Genetic diseases
  - Brain injury
  - Type I diabetes.
- Wharton's jelly: Mesenchymal stem cells—cartilage, bone.
- Umbilical cord cyst (Fig. 6.29)

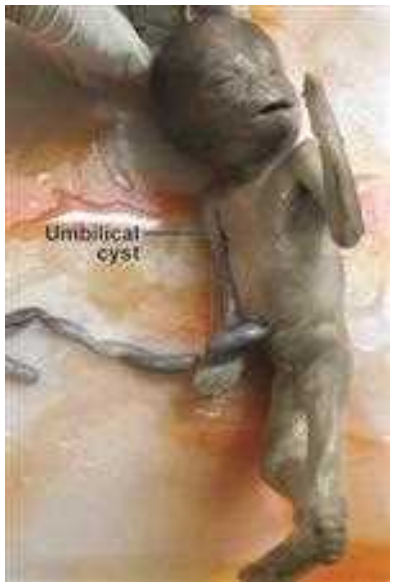


Fig. 6.29: Fetus with umbilical cyst

Mutual Relationship of Amniotic Cavity, Extraembryonic Coelom and Uterine Cavity

We have so far considered the fetal membranes (amnion and chorion), and the placenta, mainly in relation to the fetus. Let us now see their relationships to the uterine cavity. These are important, as they help us to understand some aspects of the process of childbirth. The changing relationships will be best understood by first reviewing Figures 6.2, 6.3, 6.6 and 6.9 and then by studying Figures 6.30 to 6.32.

In Figure 6.30, we see three cavities, namely (1) the uterine cavity, (2) the extraembryonic coelom and (3) the amniotic cavity. The outer wall of the extraembryonic coelom is formed by chorion and the inner wall by amnion. As the amniotic cavity enlarges, the extraembryonic coelom becomes smaller and smaller. It is eventually obliterated

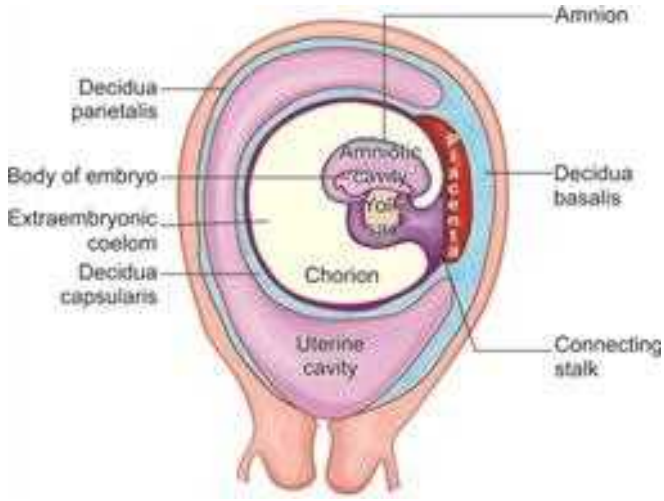


Fig. 6.30: Relationship of amniotic cavity, extraembryonic coelom and uterine cavity

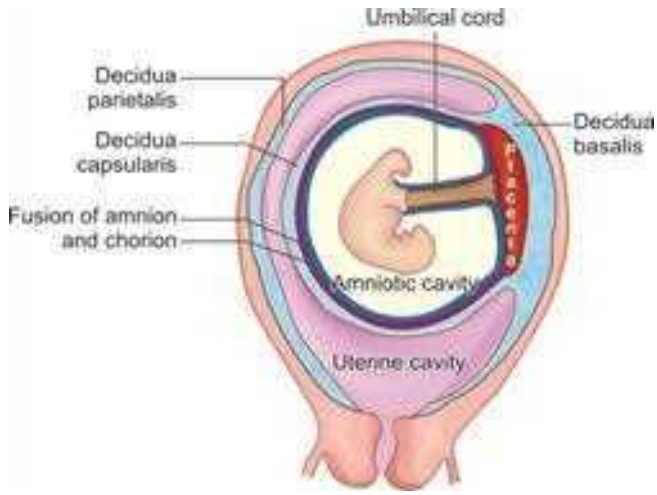


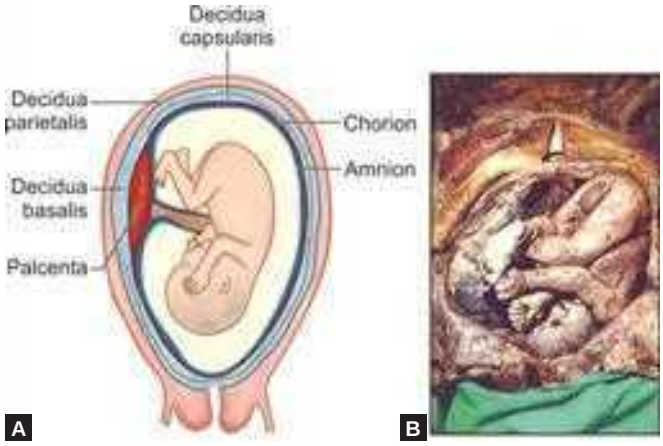
Fig. 6.31: Relationship of amniotic cavity and uterine cavity after obliteration of the extraembryonic coelom

by fusion of amnion and chorion. The fused chorion and amnion form the amniochorionic membrane.

From Figure 6.31, it will be seen that the wall of the amniotic cavity is now formed by (1) amnion, (2) chorion and (3) decidua capsularis, all three being fused to one another. Further expansion of the amniotic cavity occurs at the expense of the uterine cavity. Gradually, the decidua capsularis fuses with the decidua parietalis, and the uterine cavity is also obliterated (Figs 6.32A and B).

Still, further expansion of the amniotic cavity is achieved by enlargement of the uterus. Enlargement of the amniotic cavity is accompanied by an increase in the amount of amniotic fluid. At the time of parturition (childbirth), the fused amnion and chorion (amniochorionic membrane) (along with the greatly thinned out decidua capsularis), constitute what are called the membranes.

As the uterine muscle contracts, increased pressure in the amniotic fluid causes these membranes to bulge into the cervical canal. This bulging helps to dilate this canal. The bulging membranes can be felt through the vagina and are referred to as the bag of waters. Ultimately the membranes rupture. Amniotic fluid flows out into the vagina. After the child is delivered, the placenta and the membranes, along with all parts of the decidua, separate from the wall of the uterus and are expelled from it.



Figs 6.32A and B: (A) Amniotic cavity after obliteration of the extraembryonic coelom and uterine cavity; (B) A full term fetus and placenta in uterine cavity of a cadaver

MULTIPLE BIRTHS AND TWINNING

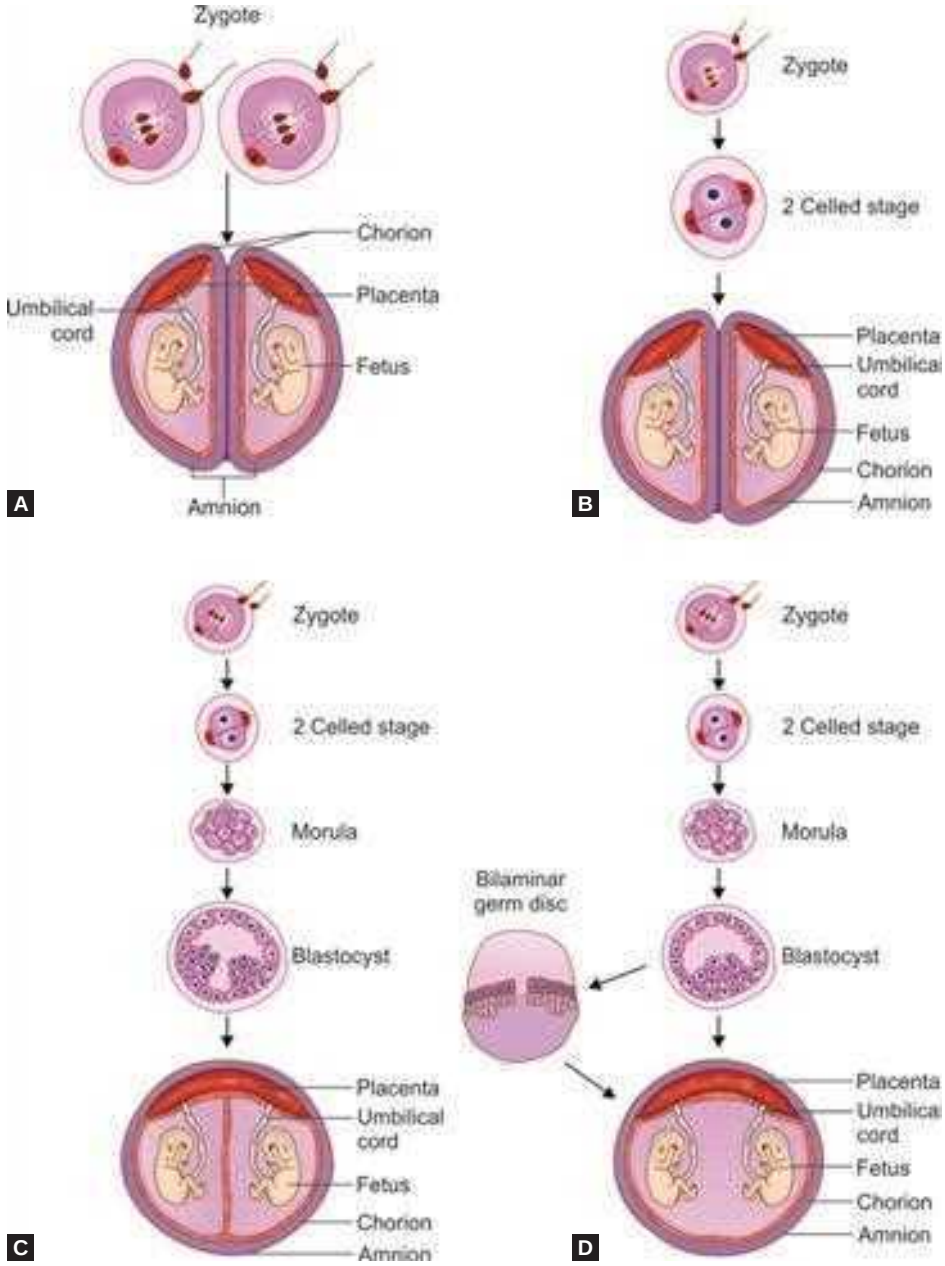
Multiple births: If more than one fetus is carried to term in a single pregnancy. When a mother gives birth to two infants at the same time, they are called twins. Three (triplets), four (quadruplets) or even more infants are sometimes born simultaneously.

Types of twinning: Twins can be produced in two ways (Table 6.3):

- *Dizygotic twins*: Two ova may be shed simultaneously from the ovary. Each of them may be fertilized and may develop in the usual manner. This results in twins that are called *dizygotic* or *fraternal twins*. As each of them develops from a separate ovum, they have independent genetic constitutions. These twins, therefore, need not be of the same sex, nor do they resemble each other any more than children of the same parents that are born separately. Each fetus has its own chorionic and amniotic sacs (*bichorial, biamniotic*). Dizygotic twinning is more common in human beings than monozygotic twinning (Fig. 6.33A).
- *Monozygotic twins*: Twins can also arise from a single fertilized ovum. These are called *monozygotic* or *maternal twins*. The genetic constitution of the two twins is exactly the same. Hence they are of the same sex. They are also exactly alike in appearance. Monozygotic twins are produced in one of the following ways:
  - *Early blastomere separation*: The cells formed in the first few divisions of the zygote are totipotent, i.e. each cell is capable of developing into a complete embryo. The two cells formed by the first division may separate and develop independently. In such a case, the fetuses will have separate chorionic and amniotic sacs (*bichorial, biamniotic*) as in dizygotic twins. The percentage incidence is 25–30% and can result up to 3rd day after fertilization as separation takes place after the first cellular division (Fig. 6.33B).

TABLE 6.3: Differences in features between monozygotic and dizygotic twins

Feature	*Monozygotic twins*	*Dizygotic twins*
No. of ova fertilized	Fertilization of a single ovum	Fertilization of two separate ova
Incidence	More common	Less common
Sex of embryos/fetuses	Similar sex	Same or different sexes
Appearance	Identical in every way including the HLA genes	Unlike/fraternal twins
Genetic constitution	Identical genetic constitution	Genetically dissimilar
No. of amnion, chorion, placenta	Majority diamniotic, monochorionic	Two amnions, chorions and placentae



Figs 6.33A to D: Twinning: (A) Dizygotic twins resulting from fertilization of two different ova—bichorial, biamniotic; (B to D) Monozygotic twins: (B) Bichorial, biamniotic; (C) Monochorial, biamniotic; (D) Monochorial, monoamniotic

- *Duplication of inner cell mass*: The embryo may develop normally up to the stage of the morula. However, when the blastocyst is formed, two inner cell masses form within it and each develops into a complete fetus. In this case, the two fetuses have a common chorionic sac but each lies in an independent amniotic cavity (*monochorial, biamniotic*). The percentage incidence is 70–75%. Separation takes place a little later in the development but before the blastocyst has defined the roles of each cell, i.e. between 4th and 7th day after fertilization (Fig. 6.33C).
- *Duplication of embryonic disc*: The inner cell mass may split into two; or two embryonic axes may be established in one inner cell mass. By this we mean that two separate embryonic discs are formed within it, each with its own prochordal plate and primitive streak. In this case, the two fetuses share a common chorion as well as a common amniotic cavity (*monochorial, monoamniotic*). The percentage

incidence is 1–2%. Separation takes place at the stage when the amniotic bag is already being formed, i.e. between 8th and 12th day after fertilization (Fig. 6.33D).

Multiple births may occur by subdivision of one zygote into more than two parts, by the simultaneous fertilization of more than two ova, or by a combination of both these factors (Figs 6.34 and 6.35).

Clinical correlation

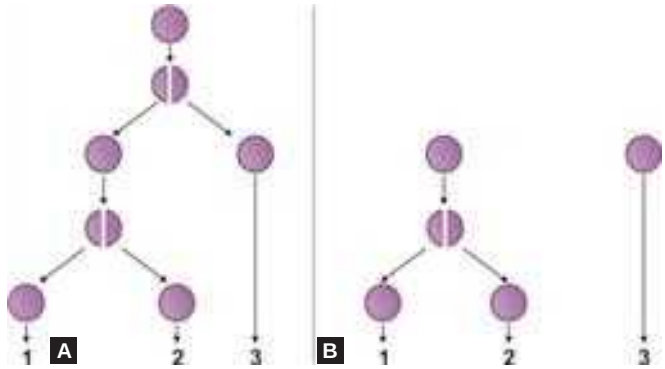
Hazards of monochorionic monoamniotic twinning

Incomplete duplication of disc (Figs 6.36A to D): This results in the formation of conjoined twins or double monsters or Siamese twins. Incomplete separation of monozygotic twins results in the birth of two infants that are joined together in some part of the body. In some cases, it is possible to separate them by operation, but most of them are born dead. Depending on the degree of incomplete separation or fusion, different types of conjoined twins result:

- Craniopagus—twins united at head
- Thoracopagus—twins showing fusion of thorax
- Pygopagus—fusion at sacral region
- Cephalothoracopagus—fusion of thorax and head.

Acephalic, acardiac fetus: Sometimes the two twins do not undergo equal development, possibly as a result of unequal blood supply (Fig. 6.37). The underdeveloped fetus may possess no heart of its own and may depend upon the other fetus for its blood supply. Unequal division of embryonic axis/unequal blood supply are responsible for this type of anomaly.

Parasitic twins: Sometimes the second conceptus may be represented as a mass attached to other fetus, or may be embedded within its body (Fig. 6.38). This results from cessation of development of one embryo/fetus which is called parasitic as it is incompletely developed and is wholly dependent on the complete embryo/fetus for its growth and development.



Figs 6.34A and B: Derivation of triplets (A) from one ovum and (B) from two ova

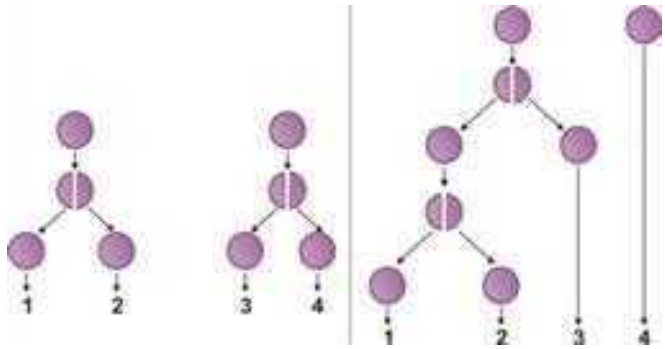
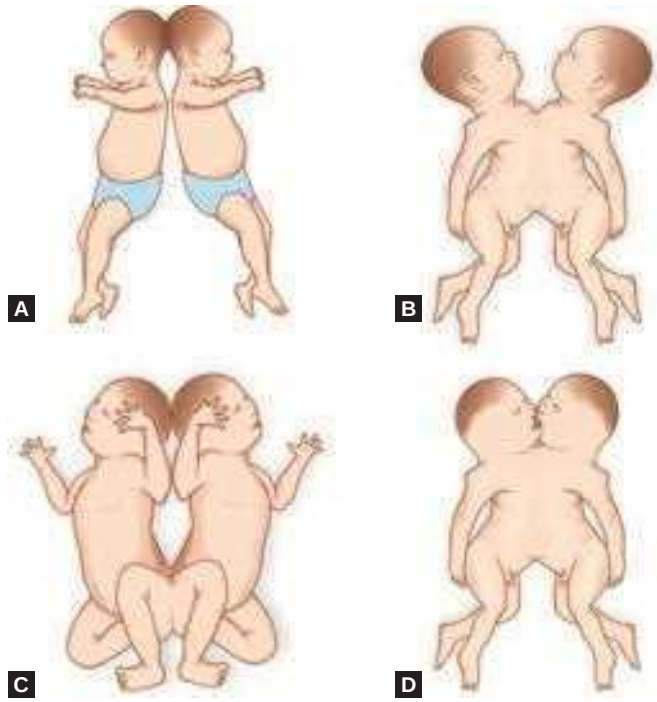


Fig. 6.35: Two ways in which quadruplets may be derived from two ova



Figs 6.36A to D: Conjoined twins. (A) Craniopagus; (B) Thoracopagus; (C) Pygopagus; (D) Cephalothoracopagus—fusion of thorax and head

EMBRYOLOGICAL BASIS FOR CLINICAL CONDITIONS OR ANATOMICAL OBSERVATIONS

Case Scenario 1

A 28-year married woman with a history of regular menstrual cycles comes to the obstetrician with a history of amenorrhea of 2 months and a complaint of severe lower abdominal pain on right side. What is your explanation for her condition? What investigations you will suggest? What treatment you advice? Give the embryological explanation for the condition.

- Amenorrhea is absence of menstruation in a woman of reproductive age. The physiological cause of amenorrhea is pregnancy and lactation. In the present case, the cause for amenorrhea is pregnancy.
- The cause for severe pain in abdomen in the present case is most probably an ectopic pregnancy. Normal

Fig. 6.37: Acephalic acardiac fetus
(*Specimen Courtesy:* Dr K Ashalatha)

Fig. 6.38: Parasitic twins

Figs 6.39A to C: Tubal pregnancy. (A) Separated tube with intact gestational sac; (B) Tubal gestation with sac opened; (C) Developing embryo in fallopian tube

site of implantation of blastocyst is in the upper uterine segment. The most common site of extrauterine implantation is in the uterine tube. It is a case of ectopic pregnancy with implantation of blastocyst in the right uterine tube.

- It is diagnosed by a pelvic examination for location of pain/mass in the abdomen. Ultrasound examination for confirmation of the embryo in right uterine tube.
- The treatment is surgical removal of right uterine tube with the implanted embryo and sending it for histopathological examination for confirmation. If the conceptus is removed intact it presents the embryo in closed gestational sac (Fig. 6.39A) and when it is removed with tube and subjected for examination by opening the tube the amniotic cavity and the attached conceptus can be identified (Fig. 6.39B).
- As the cleaving blastocyst is passing through the uterine tube for implantation in the uterus, it is prevented from adhering to tubal mucosa by the zona pellucida. The zona pellucida of the cleaving blastocyst that is rolling on the uterine wall gradually becomes thin on 5th day. By 6th day the zona pellucida disappears. If fertilized ovum cannot reach the uterus by 5th day of fertilization, the implantation of blastocyst takes place in the extrauterine site and in the present case in the uterine tube (Fig. 6.39C).

REVIEW QUESTIONS

1. What are the abnormal sites of implantation?
2. Explain the decidual reaction.
3. What are the different types of decidua?
4. What is chorion? What are the different types of chorion?
5. Explain the stages in the formation of chorionic villi.
6. Describe placental barrier.
7. Describe umbilical cord.
8. Write short notes on dizygotic twins.
9. Write short notes on monozygotic twins.

Chapter 7

Formation of Tissues of the Body

HIGHLIGHTS

- The four basic tissues of the body are (1) epithelial, (2) connective, (3) muscular and (4) nervous tissues.
- *Epithelia* may originate from all three germ layers, i.e. (1) ectoderm, (2) endoderm and (3) mesoderm.
- Epithelia lining the *external surfaces* of the body, and terminal parts of passages opening to the outside are ectodermal in origin.
- Epithelium lining the *gut*, and of organs that develop as diverticula of the gut, is endodermal in origin.
- Epithelium lining most of the *urogenital tract* is derived from mesoderm. In some parts, it is endodermal in origin.
- *Mesenchyme* is made up of cells that can give rise to cartilage, bone, muscle, blood and connective tissues.
- *Blood cells* are derived from mesenchyme in bone marrow, liver and spleen. Lymphocytes are formed mainly in lymphoid tissues.
- Most *bones* are formed by *endochondral ossification*, in which a cartilaginous model is first formed and is later replaced by bone. Some bones are formed by direct ossification of membrane (*intramembranous ossification*).
- An area where ossification starts is called a *center of ossification*. In the case of long bones, the shaft (or diaphysis) is formed by extension of ossification from the *primary center of ossification*. Secondary centers (of variable number) appear for bone ends. The part of bone ossified from a secondary center is called an *epiphysis*.
- In growing bone, the diaphysis and epiphysis are separated by the *epiphyseal plate* (which is made up of cartilage). Growth in length of a bone takes place mainly at the epiphyseal plate.
- The portion of diaphysis adjoining the epiphyseal plate is called the *metaphysis*.
- *Skeletal muscle* is derived partly from somites and partly from mesenchyme of the region. Most *smooth muscle* is formed from mesenchyme related to viscera and blood vessels. *Cardiac muscle* is formed from mesoderm related to the developing heart.
- *Neurons* and many *neuroglial cells* are formed in the neural tube. The myelin sheaths of peripheral nerves are derived from *Schwann cells,* while in the central nervous system they are derived from *oligodendrocytes.*

INTRODUCTION

Functional differentiation of cells of germ layers and organogenesis takes place during the embryonic period. The contributions by various germ layers are:

- The *ectoderm* differentiates into surface ectoderm, neuroectoderm and neural crest cells (Table 7.1).
- The *endoderm* contributes for the formation of digestive and respiratory systems (Table 7.2).
- The *intraembryonic mesoderm* is divided into three parts, i.e. paraxial, intermediate and lateral plate mesoderm. The musculoskeletal, blood vascular and parts of urinary and genital systems develop from them (Table 7.3).

The four basic tissues of the human body are derivatives of germ layers. They are as follows:

1. *Epithelial tissue:* Epithelium consists of cells arranged in the form of continuous sheets. Epithelia line the external and internal surfaces of the body and of body cavities.
2. *Connective tissue:* Connective tissue proper includes loose connective tissue, dense connective tissue and

TABLE 7.1: Derivatives of ectoderm

Skin and appendages	*Eye*	*Ear*	*Nose*	*Oral cavity and gastrointestinal tract*	*Urogenital system*
Epidermis	Lens of eye	Utricle	Epithelial lining of nasal cavity	• Epithelial lining of anterior two-third of tongue • Hard palate • Sides of the mouth	Epithelial lining of distal penile urethra
Hairs and nails	Corneal epithelium	Semicircular ducts	Paranasal air sinuses	Ameloblasts	Parts of female external genitalia
Sebaceous and sweat glands	Conjunctiva	Epithelial lining of external auditory meatus	Olfactory placode including olfactory nerve	Parotid glands and ducts	
Arrectores pilorum muscle	Lacrimal gland	Outer lining of tympanic membrane		Epithelial lining of lower anal canal	
Mammary glands	Nasolacrimal duct				
	Muscles of iris				

TABLE 7.2: Derivatives of endoderm

Oral cavity and gastrointestinal tract	*Respiratory system*	*Ear*	*Endocrine glands*	*Urogenital system*
Epithelial lining of the posterior third of the tongue, floor of the mouth, palatoglossal and palatopharyngeal folds, soft palate, crypts of palatine tonsil	Epithelial lining and glands of the trachea, bronchi, and lungs	Epithelial lining of the auditory tube and middle ear cavity	Principal and oxyphil cells of the parathyroid glands	Epithelial lining of the urinary bladder
Epithelial lining of entire gastrointestinal tract except part of mouth and anal canal			Epithelial reticular cells and thymic corpuscles	Epithelial lining of the vagina
Hepatocytes and epithelial lining of the biliary tree			Thyroid follicular cells	Epithelial lining of the female urethra and most of the male urethra
Acinar cells, islet cells, and the epithelial lining of the pancreatic ducts				
Sublingual and submandibular glands and ducts				

adipose tissue. Blood, cartilage and bone are special connective tissues.

3. *Muscular tissue:* This is of three types: (1) skeletal, (2) cardiac and (3) smooth.
4. *Nervous tissue:* This tissue consists of neurons (nerve cells), nerve cell processes (axons and dendrites) and cells of neuroglia.

In the present chapter, we shall study the formation of these basic tissues.

EPITHELIA

An epithelium may be derived from ectoderm, endoderm and mesoderm. In general, ectoderm gives rise to epithelia covering the external surfaces of the body; and some surfaces near the exterior. Endoderm gives origin to the epithelium of most of the gut; and of structures arising as diverticula from the gut (e.g. the liver and pancreas). Mesoderm gives origin to the epithelial lining of the greater part of the urogenital tract.

TABLE 7.3: Derivatives of mesoderm

Paraxial mesoderm	*Intermediate mesoderm*	*Lateral plate mesoderm*
• Skeletal muscles of: • Trunk • Limbs • Head and neck	Kidneys Ureters Trigone of urinary bladder	Bones: • Sternum • Limb bones
Extraocular muscles	Gonads: Testes and ovaries	Serous membranes of body cavities: • Pleura • Pericardium • Peritoneum
Intrinsic muscles of tongue	Genital ducts	Layers in the wall of gastrointestinal tract (GIT) • Lamina propria • Muscularis mucosae • Submucosa • Muscularis externa • Adventitia
Bones: • Vertebrae • Ribs • Cranial	Dorsal part of prostatic urethra (Males)	Blood cells • Microglia • Kupffer cells
Dermis of skin		Cardiovascular system Lymphatic systems
Dura mater		Spleen
		Suprarenal cortex
		Laryngeal cartilages

Epithelia Derived from Ectoderm

- *Skin and its appendages:* Epithelium of skin, hair follicles, sweat glands, sebaceous glands, and mammary glands.
- *Special senses:* Epithelium over cornea and conjunctiva, external acoustic meatus and outer surface of tympanic membrane.
- *Digestive system:* Epithelium of some parts of the mouth, lower part of anal canal.
- *Urogenital system:* Terminal part of male urethra, parts of female external genitalia.

Epithelia Derived from Endoderm

- *Digestive system:* Epithelium of the entire gut *except* part of the mouth and anal canal (lined by ectoderm).
- *Respiratory system:* Epithelium of respiratory tract.
- *Urogenital system:* Epithelium over part of urinary bladder, urethra and vagina.
- *Special senses:* Epithelium of auditory tube and middle ear.

Epithelia Derived from Mesoderm

- *Blood vascular system:* Endothelium lining the heart, blood vessels and lymphatics.
- *Coelomic cavities*: Mesothelium lining the pericardial, peritoneal and pleural cavities; and cavities of joints.
- *Urogenital system*: Tubules of kidneys, ureter, trigone of urinary bladder; uterine tubes, uterus, part of vagina; and testis and its duct system.

Glands

Almost all glands, both exocrine and endocrine, develop as downgrowths (diverticulum) from the epithelial surface into the underlying tissue (Figs 7.1A and B). Later the development of these downward extensions differs in exocrine and endocrine glands.

Exocrine Glands

- The gland may be derived from elements formed by branching of one diverticulum (e.g. parotid) or may be formed from several diverticula (e.g. lacrimal gland, prostate). The opening of the duct (or ducts) is usually situated at the site of the original outgrowth.
- The diverticula are generally solid to begin with (Fig. 7.1B) and are canalized later (Fig. 7.1C). The proximal parts of the diverticula form the duct system. The distal parts of the diverticula form the secretory elements (Fig. 7.1D).

Endocrine Glands

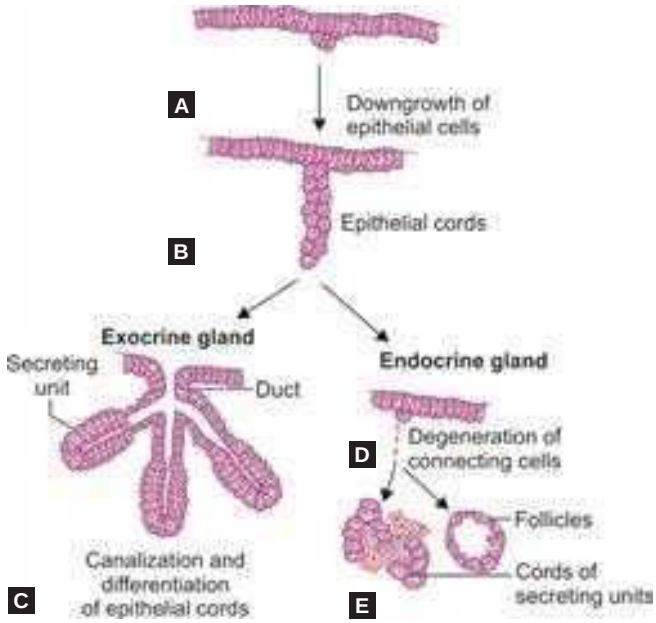
- In the case of endocrine glands (e.g. thyroid, anterior part of hypophysis cerebri), the gland loses all contact with the epithelial surface by degeneration of cells connecting the secretory elements with the surface epithelium from which it takes origin (Fig. 7.1D).
- The cells of secretory portion get organized in the form of cords (pituitary) or follicles (thyroid) and are surrounded by capillaries (Fig. 7.1E).

Depending on the epithelium from which they take origin, glands may be:

- Ectodermal, e.g. sweat glands, mammary glands
- Endodermal, e.g. pancreas, liver, submandibular and sublingual salivary glands
- Mesodermal, e.g. adrenal cortex
- Mixed origin, e.g. prostate.

Mesenchyme

- A small proportion of mesodermal cells give rise to epithelia. The remaining cells that make up the bulk of mesoderm get converted into a loose tissue called *mesenchyme* (Fig. 7.2).



Figs 7.1A to E: Stages in the development of exocrine and endocrine glands

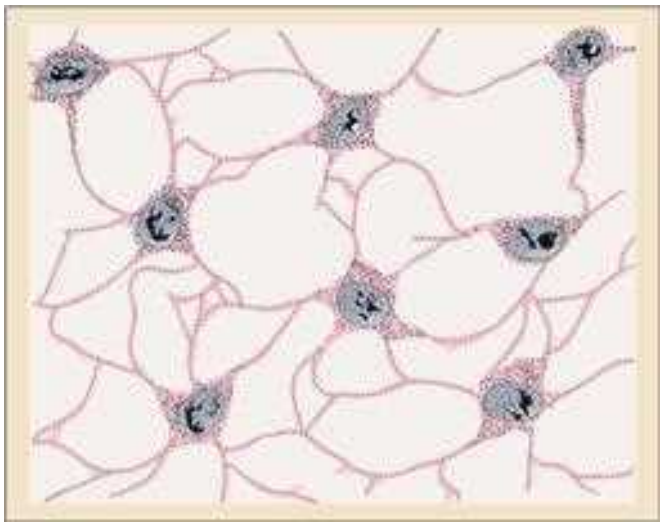


Fig. 7.2: Mesenchymal cells. Note the delicate cytoplasmic processes joining the cells to one another

- Mesenchymal cells have the ability to form many different kinds of cells that in turn give rise to various tissues (Fig. 7.3). They are:
  - *Chondroblasts* form cartilage
  - *Osteoblasts* form bone
  - *Myoblasts* form muscle
  - *Lymphoblasts* and *hemocytoblasts* form various cells of blood
  - *Endothelial cells* form blood vessels and the primitive heart tubes
- However, after all these tissues have been formed, many mesenchymal cells are still left and they give rise to cells of various types of connective tissue.

CONNECTIVE TISSUE

As the name suggests, connective tissue serves as a connecting system binding, supporting and strengthening all other body tissues. Connective tissue consists of three components, i.e. (1) cells, (2) fibers and (3) ground substance. The fibers and ground substance are synthesized by the cells of the connective tissue.

Formation of Loose Connective Tissue

At the site of formation of loose connective tissue, the mesenchymal cells get converted into fibroblasts. Fibroblasts secrete the ground substance and synthesize the collagen, reticular and elastic fibers. Some mesenchymal cells present in the developing connective tissue also get converted into histiocytes, mast cells, plasma cells and fat cells (Fig. 7.3).

Formation of Blood

- Blood is a specialized fluid connective tissue, which acts as a major transport system within the body. The formation of cells of blood begins very early in embryonic life (before somites have appeared) and continues throughout life. Blood formation is especially rapid in the embryo to provide for increase in blood volume with the growth of the embryo.
- In the 3rd week of embryonic life, formation of blood vessels and blood cells is first seen in the wall of the yolk sac, around the allantoic diverticulum and in the connecting stalk (Fig. 7.4A). In these situations, clusters of mesodermal cells aggregate to form *blood islands* (Fig. 7.4B). These mesodermal cells are then converted to precursor cells (*hemangioblasts*) that give rise to blood vessels and blood cells (Fig. 7.4C). Cells, which are present in the center of the blood island, form the precursors of all blood cells (*hematopoietic stem cells*). Cells at the periphery of the island form the precursors of blood vessels (*angioblasts*; Fig. 7.4D).
- Blood cells arising in the blood islands of the yolk sac are temporary. They are soon replaced by permanent stem cells, which arise from the mesoderm surrounding the developing aorta.
- These stem cells first form colonies in the liver. In the late embryonic period, formation of blood starts in the liver, which remains an important site of blood cell formation till the 6th month of intrauterine life.
- Almost near the middle of prenatal life, definitive hematopoietic stem cells from the liver migrate to

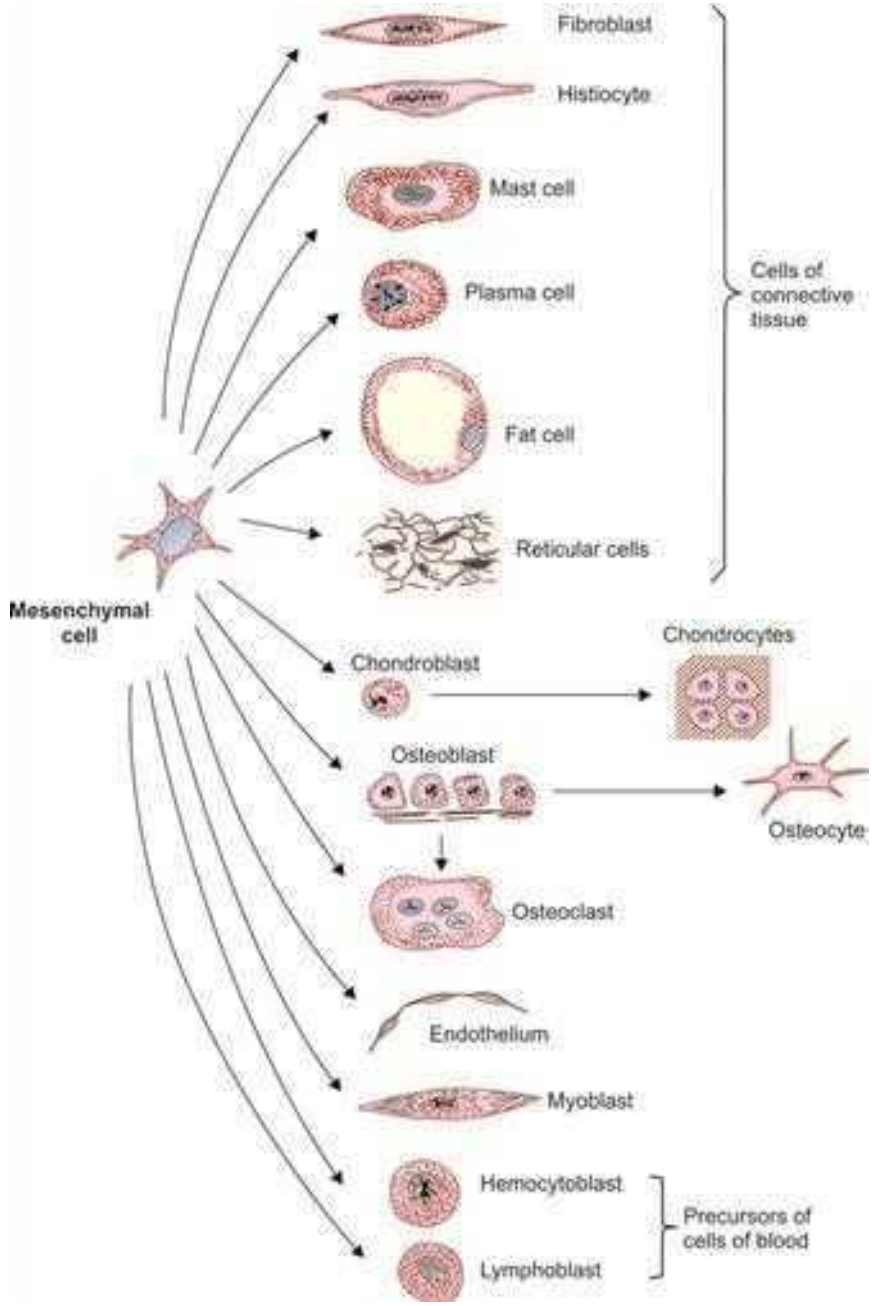
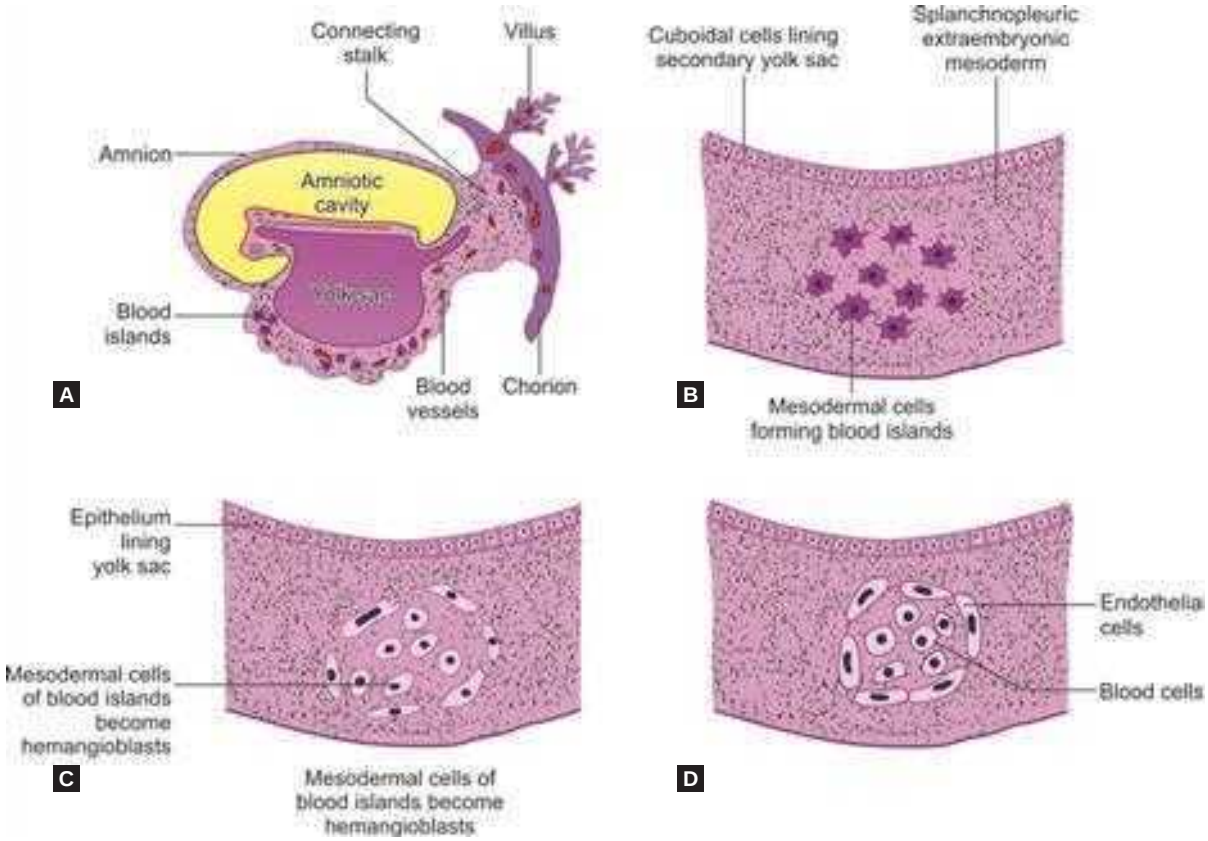


Fig. 7.3: Derivatives of mesenchymal cells

colonize the bone marrow. At the time of birth, blood formation is mainly in the bone marrow. Here totipotent hemal stem cells give rise to pluripotent lymphoid stem cells and pluripotent hemal stem cells (Fig. 7.5). These stem cells form *colony-forming units (CFUs)*.

- Cells of one particular CFU are committed to differentiate only into one line of blood cells, i.e. erythrocytes, megakaryocyte, granulocytes, monocytes, macrophages and lymphocytes (Fig. 7.5). In the case of erythrocytes, stem cells divide so rapidly that they seem to burst. They are therefore called *burst-forming units (BFUs)*. Their daughter cells then form CFUs.
- In the adult, main sites of blood formation are bone marrow, lymph nodes, thymus and spleen.

The precursors of various types of blood cells are generally regarded as being of mesodermal in origin.



Figs 7.4A to D: Formation of blood cells and blood vessels from a blood island. (A) In the embryo during 3rd week; (B) Formation of blood islands; (C) Formation of hemangioblasts; (D) Formation of blood cells and endothelial cells

However, blood forming cells differentiating in relation to the wall of the yolk sac and probably in the liver may be endodermal in origin.

Formation of Cartilage

- Cartilage is formed from mesenchyme. At a site where cartilage is to be formed, mesenchymal cells become closely packed. This is called a *mesenchymal condensation*. The mesenchymal cells then become rounded and get converted into cartilage forming cells or *chondroblasts*.
- Under the influence of chondroblasts, the *intercellular substance* of cartilage is laid down. Some chondroblasts get imprisoned within the substance of this developing cartilage and are called *chondrocytes*. Some fibers also develop in the intercellular substance.
- In *hyaline cartilage*, collagen fibers are present, but are not seen easily. In *fibrocartilage*, collagen fibers are numerous and very obvious. In some situations, the intercellular substance is permeated by elastic fibers, forming *elastic cartilage*.
- Mesenchymal cells surrounding the surface of the developing cartilage form a fibrous membrane, the *perichondrium*.

Bone

To understand the formation of bone, it is necessary for students to know its normal structure. This can be read from the author's Textbook of Human Histology. Some features of bone can be seen in Figures 7.6 and 7.7

- A regular parallel arrangement of collagen sheets called lamellae are stacked one above the other in the bone (Fig. 7.6A).
- The thickness of bone depends on the number of layers stacked (Fig. 7.6B).
- The spaces between the lamellae will be occupied by the osteocytes and their processes (Fig. 7.6C).
- With the mineralization of the sheets of lamellae, the spaces occupied by the osteocytes and their processes become lacunae and canaliculi respectively (Fig. 7.6D).
- Through their processes the osteocytes of adjacent lamellae are connected to one another and also with the

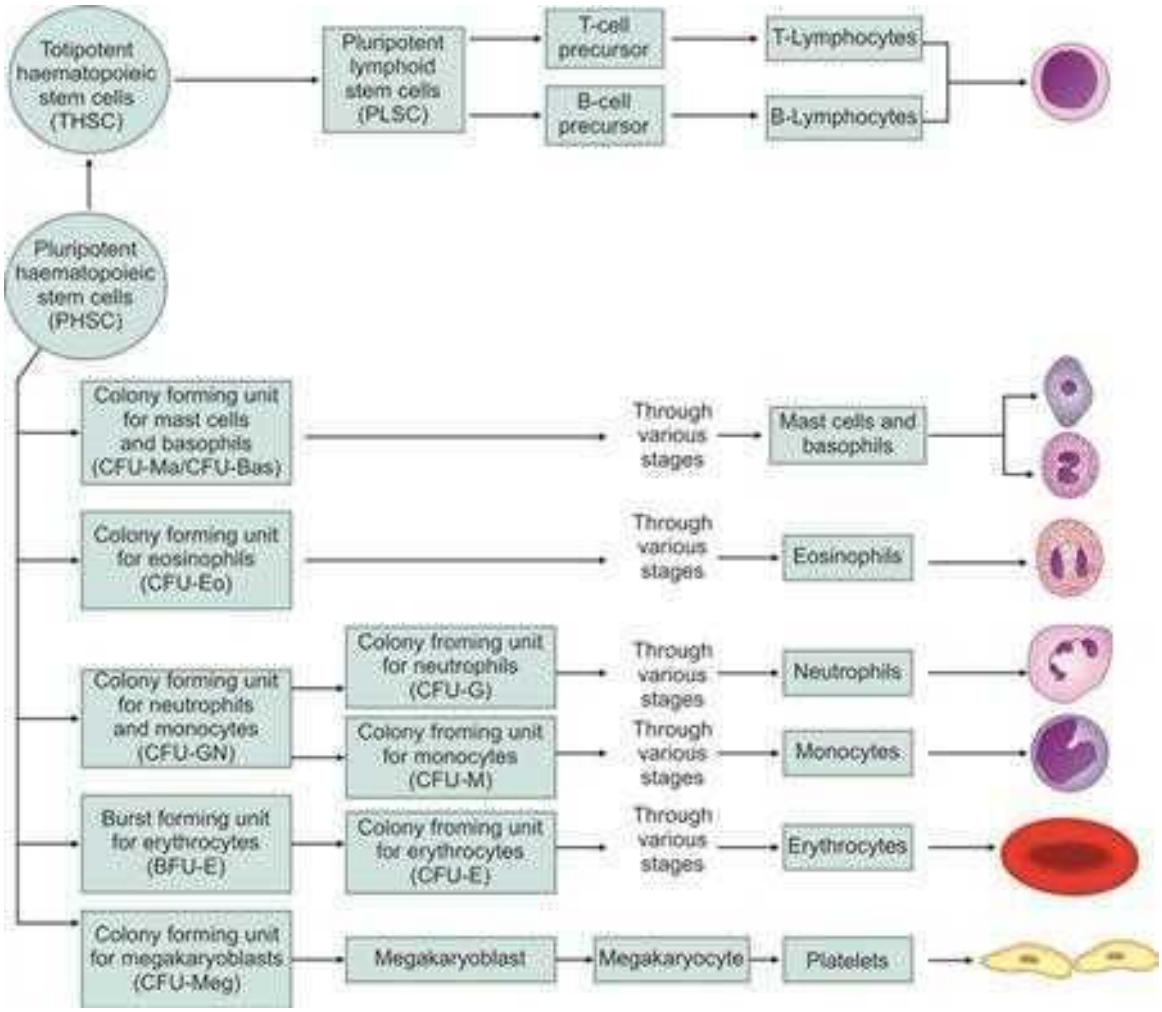


Fig. 7.5: Scheme showing the terms applied to precursors of various blood cells

blood vessels in the bone marrow and periosteal vessels for the supply of nutrition to the developing bone cells.

- Both compact and spongy bone contain lamellar organization. In a spongy bone, trabeculae (lamellae) are seen around marrow spaces (Fig. 7.7A). In the compact bone the great majority of lamellae are arranged concentrically around longitudinal vascular channels within the bone to form cylindrical structure called Haversian system (Fig. 7.7B).

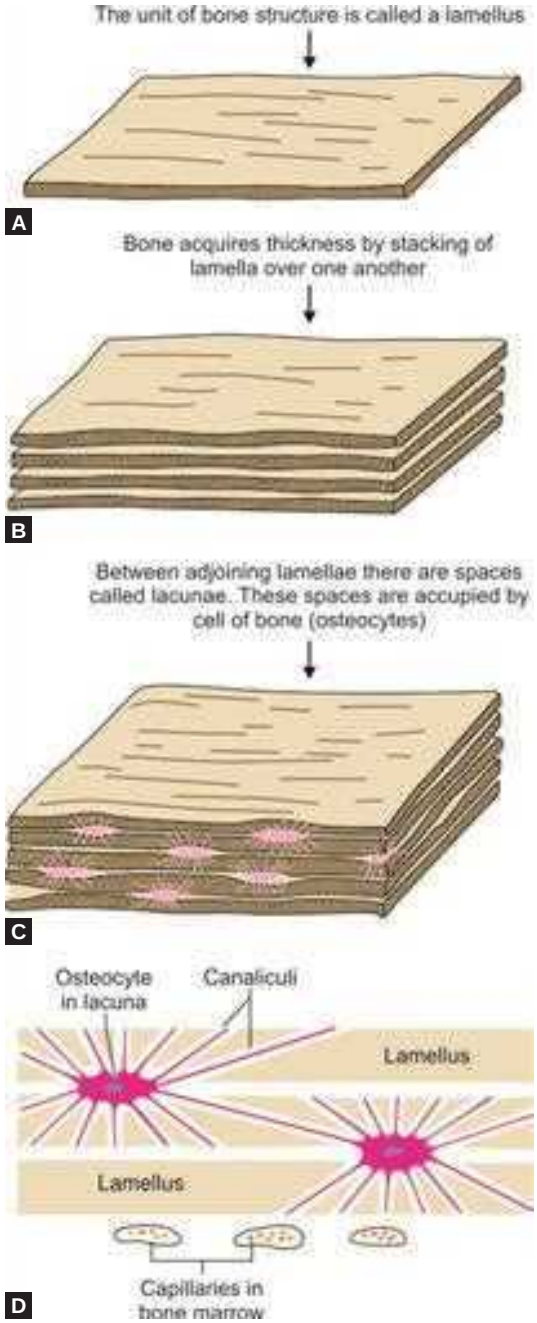
Cells of Bone

Three main types of cells are present in bone.

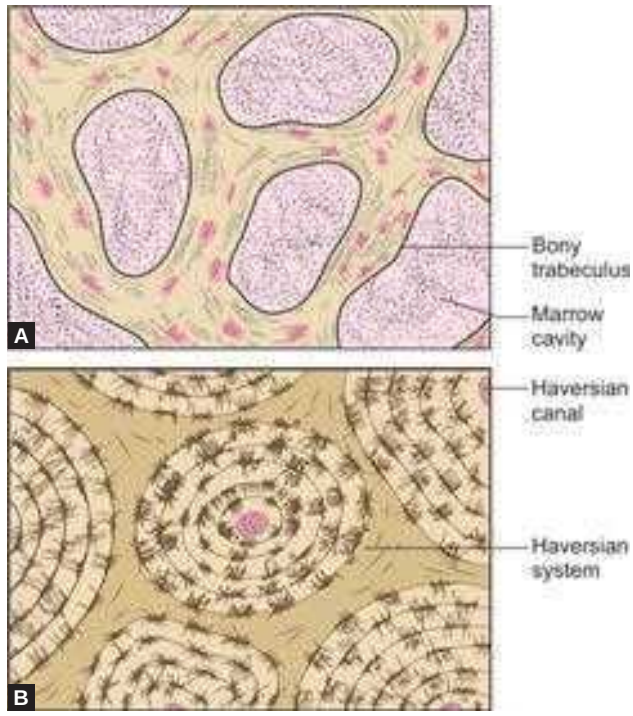
1. *Osteocytes* are cells that are seen in mature bone.
2. *Osteoblasts* are bone forming cells. These cells are, therefore, seen wherever bone is being laid down. They have abundant basophilic cytoplasm and are arranged in regular rows, looking very much like an epithelial lining (Fig. 7.8).
3. *Osteoclasts are*, on the other hand, responsible for bone removal. They are large multinucleated cells and are seen in regions where bone is being absorbed (Fig. 7.8).

Formation of Bone

- All bone is of *mesodermal origin*. The process of bone formation is called *ossification*.
- There are two methods of bone formation. Both involve transformation of a pre-existing mesenchymal model of tissue into bone tissue. They are:
  1. Intramembranous ossification
  2. Endochondral ossification.



Figs 7.6A to D: (A to C) Scheme to show that bone is made up of lamellae; (D) Position of osteocytes among the lamellae



Figs 7.7A and B: (A) Structure of spongy bone; (B) Structure of compact bone

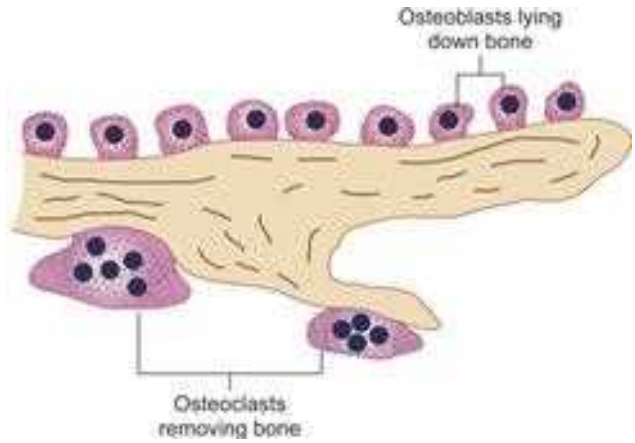
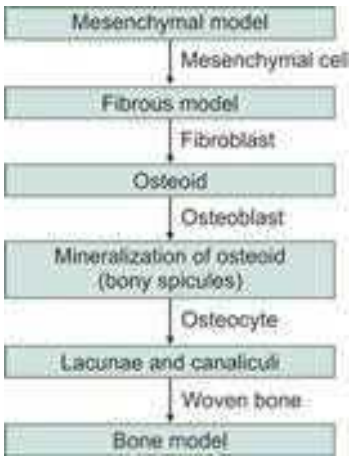


Fig. 7.8: Relationship of osteoblasts and osteoclasts to developing bone

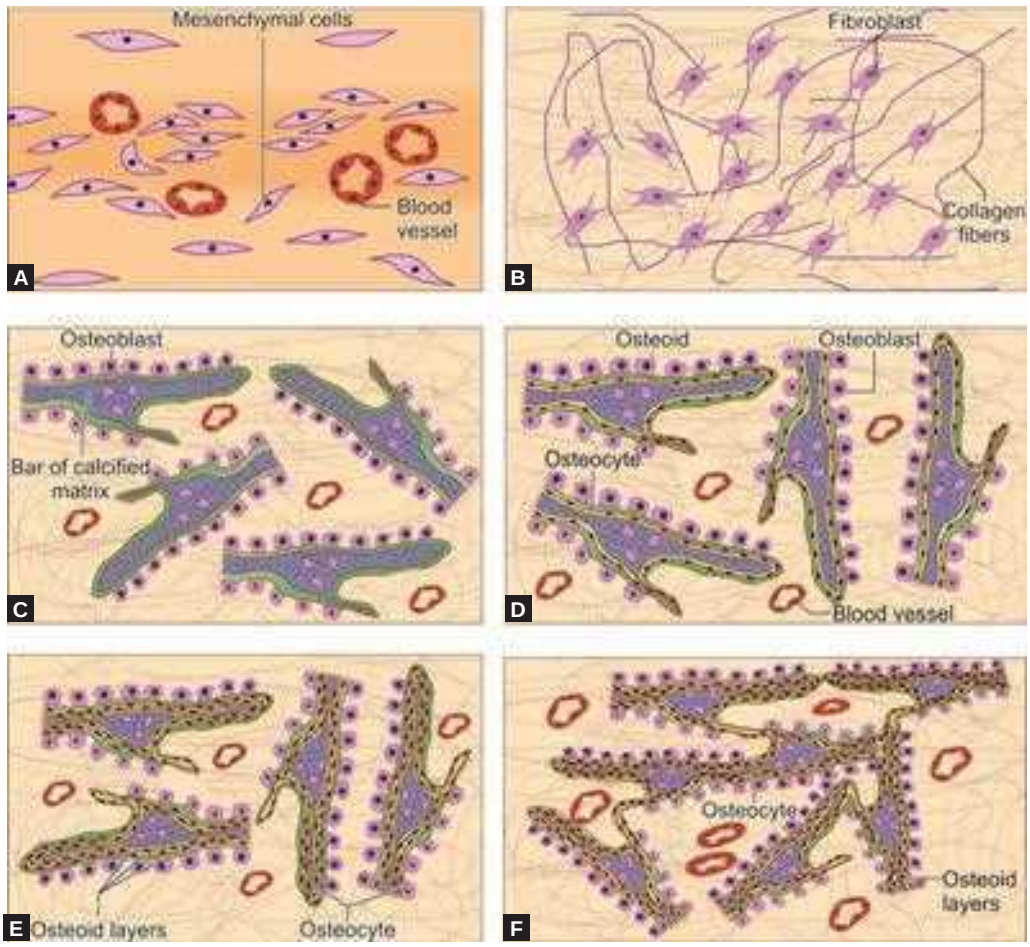
Intramembranous Ossification

- It is direct conversion of mesenchymal tissue into bone. In some situations (e.g. the vault of the skull), formation of bone is not preceded by formation of a cartilaginous model. Instead, bone is laid down directly in a fibrous membrane.
- This is called *intramembranous ossification* and these bones are called *membrane bones*. These include the bones of the vault of the skull, the mandible and the clavicle.
- It is observed in bones of vault of skull, mandible and clavicle.
- The various steps involved in this type of bone formation are (Flowchart 7.1):

Flowchart 7.1: Intramembranous ossification



1. *Mesenchymal condensation:* Star-shaped mesenchymal cells of loose connective tissue aggregate in the area where bone is to be formed. The mesenchymal cells are converted to spindle-shaped cells thus forming a condensed mesenchymal tissue model (Fig. 7.9A).
2. *Conversion into a fibrous membrane:* Spindle-shaped mesenchymal cells differentiate into fibroblasts. Fibroblasts lay down collagen fibers converting the mesenchymal model into a fibrous model (Fig. 7.9B).
3. *Osteoblast and osteoid formation:* Fibroblasts get converted into osteoblasts and start laying down the early bone matrix, i.e. osteoid (uncalcified bone). It contains fibers and ground substance which are products of osteoblasts. This forms the center of ossification (Fig. 7.9C).



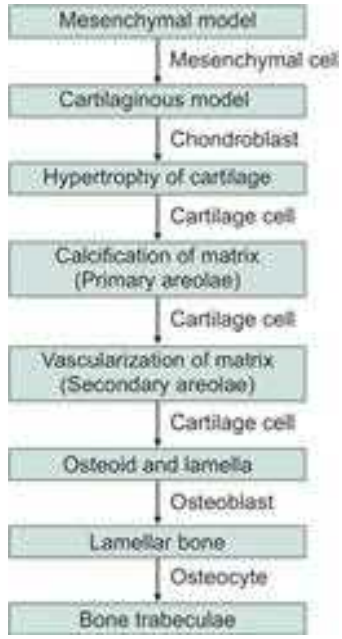
Figs 7.9A to F: Stages in intramembranous ossification

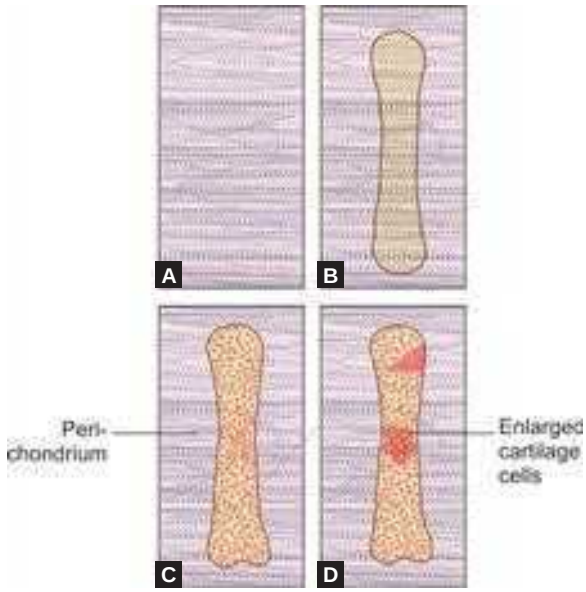
4. *Mineralization of osteoid:* Secretion of alkaline phosphatase by osteoblasts and deposition of hydroxyapatite crystals of calcium converts the osteoid into calcified bone matrix. Mineralized osteoid is seen as a spicule of bone (Fig. 7.9D).
5. *Conversion of osteoblast to osteocyte and formation of woven bone:* The osteoblasts trapped in matrix lacunae (shell around the bone cell) become osteocytes. Processes of osteoblasts traverse through mineralized canals of calcified matrix (tunnels) called *canaliculi*. The collagen fiber bundles run in different directions giving the appearance of a woven bone. The spicules are irregularly arranged with spaces between them (Fig. 7.9E).
6. *Progressive bone formation:* Fusion of adjacent spicules forms the bone model (Fig. 7.9F).
7. *Remodeling into lamellar bone:* Woven bone gets transformed into lamellar bone due to resorption by osteoclasts and bone deposition by osteoblasts. This results in formation of mature compact or spongy bone.

Endochondral Ossification

- Here mesenchymal cells differentiate into cartilage cells that are later replaced by bone. In most parts of the embryo, bone formation is preceded by the formation of a *cartilaginous model* that closely resembles the bone to be formed. This cartilage is subsequently replaced by (not converted into) bone.
- This kind of bone formation is called *endochondral ossification*. Bones formed in this way are, therefore, called *cartilage bones*.
- Majority of bones of the body are formed by endochondral ossification. This type of ossification is seen in all long bones except clavicle, bones of base of skull, vertebrae and ribs.
- The essential steps in the formation of bone by endochondral ossification are (Flowchart 7.2):
  1. *Mesenchymal condensation:* At the site where the bone is to be formed, the mesenchymal cells become closely packed to form a mesenchymal condensation (Figs 7.10A and B).
  2. *Cartilaginous model:* Some mesenchymal cells become chondroblasts and lay down hyaline cartilage (Fig. 7.10C). Mesenchymal cells on the surface of the cartilage form a membrane called the *perichondrium*. This membrane is vascular and contains osteogenic cells.
  3. *Cartilage cell hypertrophy:* The cells of the cartilage are at first small and irregularly arranged. However, in the area where bone formation is to begin, the cells enlarge considerably (Fig. 7.10D).
  4. *Calcification of intercellular matrix:* The intercellular substance between the enlarged cartilage cells becomes calcified, under the influence of an enzyme called *alkaline phosphatase*, which is secreted by the cartilage cells. The nutrition to the cells is thus cut off and they die, leaving behind empty spaces called *primary areolae* (Figs 7.11A and B).

Flowchart 7.2: Endochondral ossification

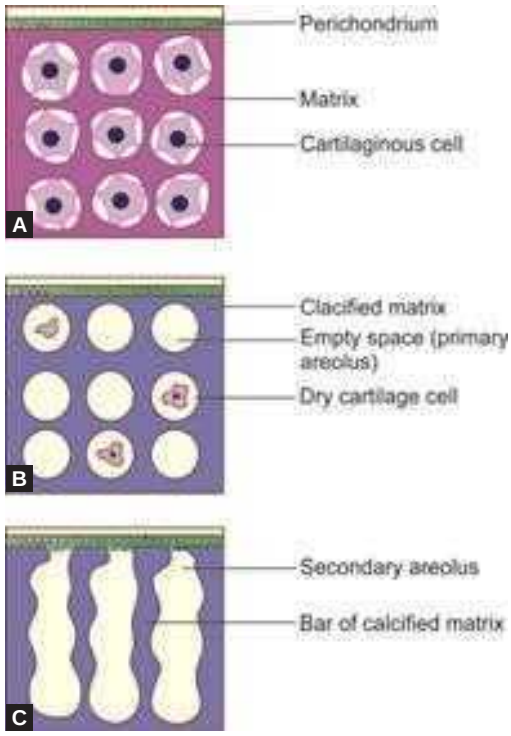




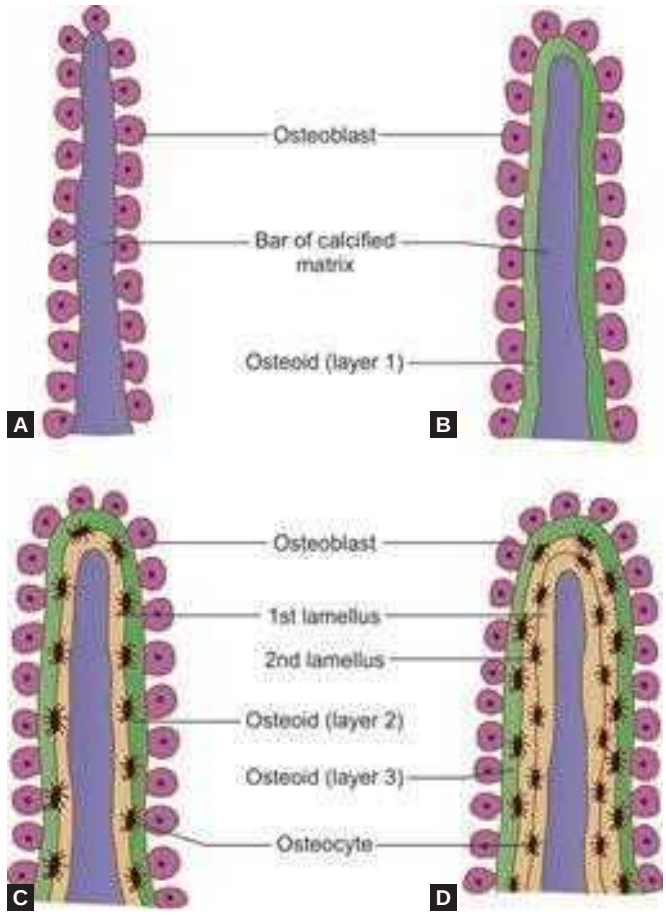
Figs 7.10A to D: Endochondral ossification. (A and B) Mesenchymal condensation; (C) Cartilaginous model with perichondrium; (D) Enlarged cartilage cells (ECC) at the site of bone formation

5. *Vascularization of cartilaginous matrix:* Some blood vessels of the perichondrium (which may be called *periosteum* as soon as bone is formed) now invade the calcified cartilaginous matrix. They are accompanied by osteogenic cells. This mass of vessels and cells is called the *periosteal bud*. It eats away much of the calcified matrix forming the walls of the primary areolae, and thus creates large cavities called *secondary areolae* (Fig. 7.11C).
6. *Osteoid and lamella formation:* The walls of the secondary areolae are formed by thin layers of calcified matrix that have not been dissolved. The osteogenic cells become osteoblasts and arrange themselves along the surfaces of these bars, or plates, of calcified cartilaginous matrix (Fig. 7.12A). These osteoblasts now lay down a layer of ossein fibrils embedded in a gelatinous intercellular matrix (Fig. 7.12B). This material is called *osteoid*. It is calcified and a *lamellus* of bone is formed (Fig. 7.12C).
7. *Formation of trabeculae:* The osteoblasts now lay down another layer of osteoid over the first lamellus. This is also calcified. Thus two lamellae of bone are formed. Some osteoblasts that get caught between the lamellae form *osteocytes*. As more lamellae are laid down, bony trabeculae are formed (Fig. 7.12D).

- The calcified matrix of cartilage only acts as a support for the developing trabeculae and is not itself converted into bone.
- At this stage, the ossifying cartilage shows a central area (1 in Fig. 7.13A) where bone has been formed. As we move away from this area we see:
  - A region where the cartilaginous matrix has been calcified and surrounds dead, and dying, cartilage cells (2 in Fig. 7.13A).
  - A zone of hypertrophied cartilage cells, in an uncalcified matrix (3 in Fig. 7.13A).
  - A normal cartilage (4 in Fig. 7.13A) in which there is considerable mitotic activity.
- If we see the same cartilage a little latter (Fig. 7.13B), we find that ossification has now extended into zone 2, and simultaneously the matrix in zone 3 has become calcified. The deeper cells of zone 4 have meanwhile hypertrophied, while the more superficial ones have multiplied to form zone 5.



Figs 7.11A to C: Endochondral ossification. (A and B) Formation of primary areolae; (C) Formation of secondary areolae



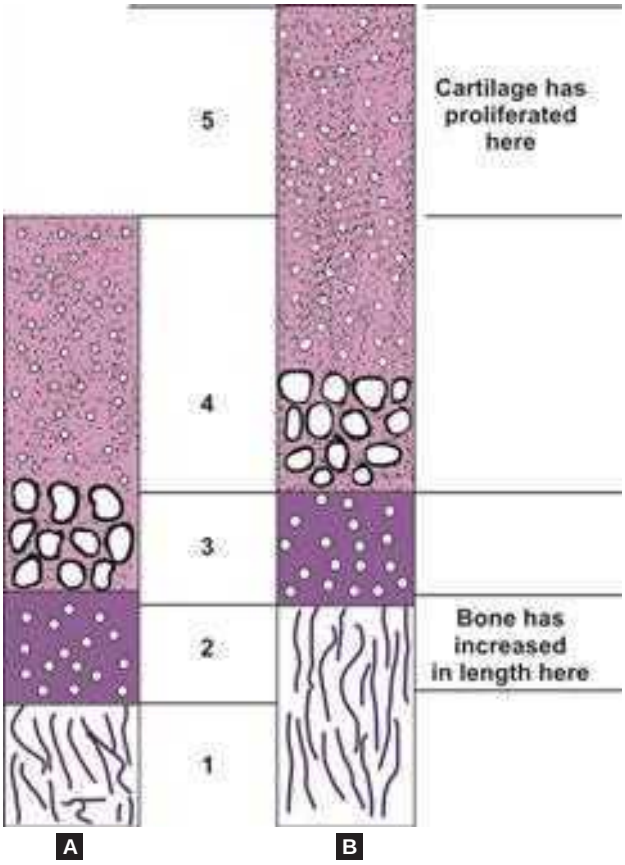
Figs 7.12A to D: Endochondral ossification. Stages in the formation of bony lamellae

In this way, formation of new cartilage keeps pace with the loss due to replacement by bone. The total effect is that the ossifying cartilage progressively increases in size.

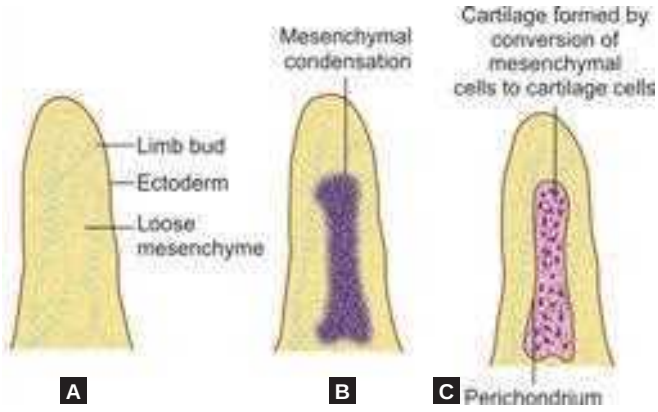
Development of a Typical Long Bone

The various stages in the formation of a long bone are:

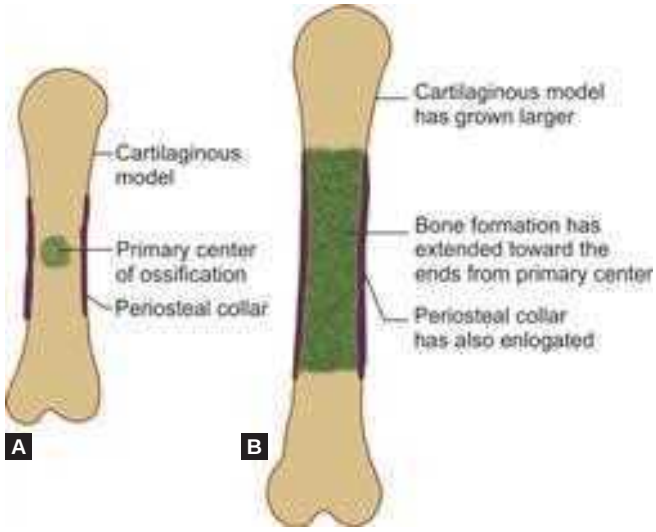
1. A *mesenchymal condensation* is seen in the limb bud in the region where the bone is to be formed (Figs 7.14A and B).
2. This mesenchymal condensation is converted into a *cartilaginous model*. This model closely resembles the bone to be formed. It is covered by perichondrium that has a superficial fibrous layer and a deeper layer that has osteogenic cells (Fig. 7.14C).
3. Endochondral ossification starts in a small area of the shaft as described above. This area is called the *primary center of ossification* (Fig. 7.15A).
4. Gradually, bone formation extends from the primary center toward the ends of the shaft. This is accompanied by enlargement of the cartilaginous model (Fig. 7.15B).
5. Soon after the appearance of the primary center, and onset of endochondral ossification in it, the perichondrium (which may now be called *periosteum*) becomes active. The osteogenic cells in its deeper layer lay down bone *on the surface of* the cartilaginous model by *intramembranous ossification*. This periosteal bone completely surrounds the cartilaginous shaft and is, therefore, called the *periosteal collar* (Fig. 7.16A). It is first formed only around the region of the primary center but rapidly extends toward the ends of the cartilaginous model.
6. The periosteal collar acts as a splint, and gives strength to the cartilaginous model, at the site where it is weakened by the formation of secondary areolae. We shall see that most of the shaft of the bone is derived from this periosteal collar and is, therefore, intramembranous in origin.
7. At about the time of birth, the developing bone consists of a part called the *diaphysis* (or shaft) (that is bony, and



Figs 7.13A and B: Scheme to show the growth in length of a bone



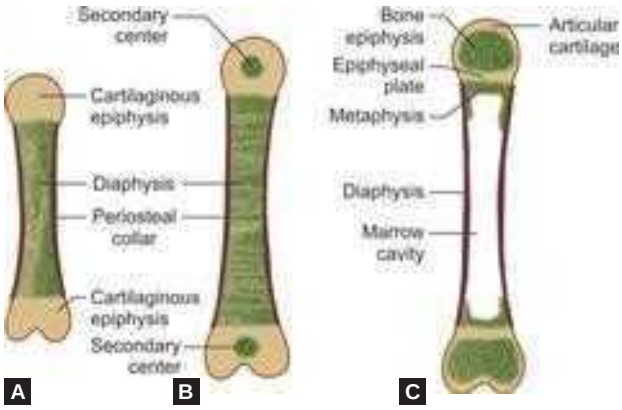
Figs 7.14A to C: Formation of a typical long bone. Establishment of cartilaginous model



Figs 7.15A and B: Formation of a typical long bone. Primary center of ossification and periosteal collar

has been formed by extension of the primary center of ossification); and ends that are cartilaginous (Fig. 7.16A). At varying times after birth, *secondary centers* of endochondral ossification appear in the cartilages forming the ends of the bone (Fig. 7.16B). These centers enlarge until the ends become bony (Fig. 7.16C). More than one secondary center of ossification may appear at either end. The portion of bone formed from one secondary center is called an *epiphysis*.

8. For a considerable time after birth, the bone of the diaphysis and the bone of the epiphysis are separated by a plate of cartilage called the *epiphyseal cartilage*, or *epiphyseal plate*. This is formed by cartilage into which ossification has not extended either from the diaphysis or from the epiphysis. We shall see that this plate plays a vital role in growth of the bone.

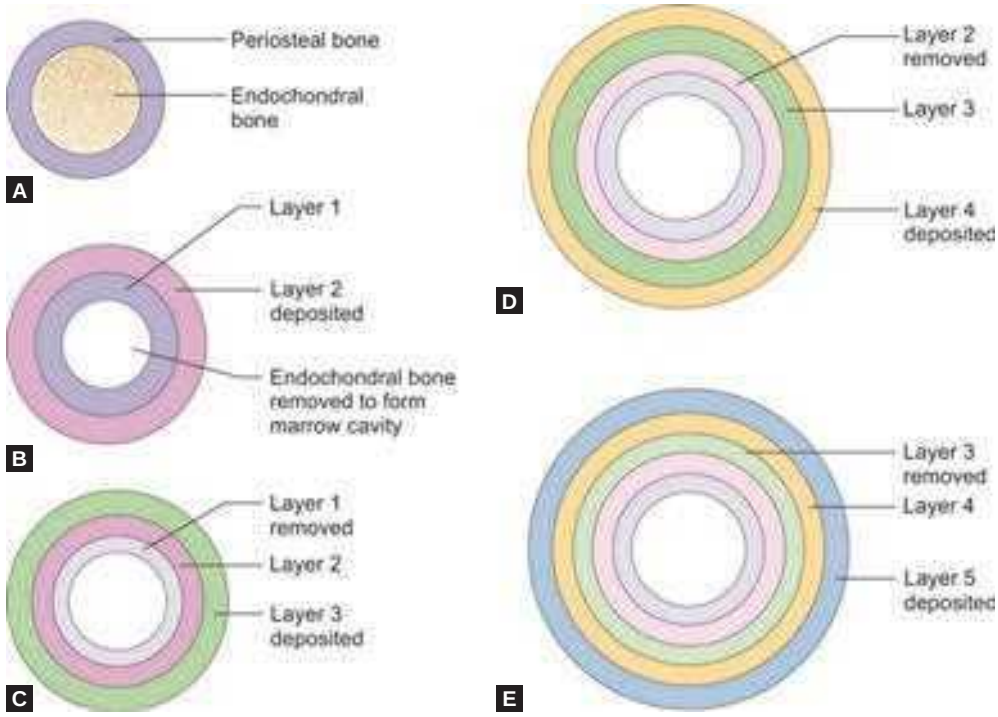


Figs 7.16A to C: Formation of a typical long bone. Secondary centers of ossification

Growth of a Long Bone

A growing bone increases both in length and in thickness.

- We have seen that the periosteum lays down a layer of bone around the shaft of the cartilaginous model. This periosteal collar gradually extends over the whole length of the diaphysis. As more layers of bone are laid down over it, the periosteal bone becomes thicker and thicker.
- However, it is neither necessary nor desirable for it to become too thick. Hence, osteoclasts come to line the internal surface of the shaft and remove bone from this aspect. As bone is laid down outside the shaft, it is removed from the inside. The shaft thus grows in diameter, and at the same time, its wall does not become too thick (Figs 7.17A to E). The osteoclasts also remove the trabeculae lying in the center of the bone that were formed by endochondral ossification. In this way, a *marrow cavity* is formed.
- As the shaft increases in diameter, there is a corresponding increase in the size of the marrow cavity. This cavity also extends toward the ends of the diaphysis but does not reach the epiphyseal plate. Gradually, most of the bone



Figs 7.17A to E: Formation of a typical long bone. Increase in thickness. Shaft is ultimately made up almost entirely of periosteal bone formed by the process of intramembranous ossification

formed from the primary center (i.e. of endochondral origin) is removed, except near the ends, so that the wall of the shaft is made up purely of periosteal bone formed by the process of intramembranous ossification.

- To understand how a bone grows in length, we will now have a closer look at the epiphyseal plate. Depending on the arrangement of its cells, three zones can be recognized (Fig. 7.18).
  1. *Zone of resting cartilage:* Here, the cells are small and irregularly arranged.
  2. *Zone of proliferating cartilage:* Here, the cells are larger and are undergoing repeated mitosis. As they multiply, they come to be arranged in parallel columns, separated by bars of intercellular matrix.
  3. *Zone of calcification:* Here, the cells become still larger and the matrix becomes calcified.
- Next to the zone of calcification, there is a zone where cartilage cells are dead and the calcified matrix is being replaced by bone. Growth in length of the bone takes place by continuous transformation of the epiphyseal cartilage to bone (Figs 7.18 and 7.19) in this zone (i.e. on the diaphyseal surface of epiphyseal cartilage). At the same time, the thickness of the epiphyseal cartilage is maintained by active multiplication of cells in the zone of proliferation.

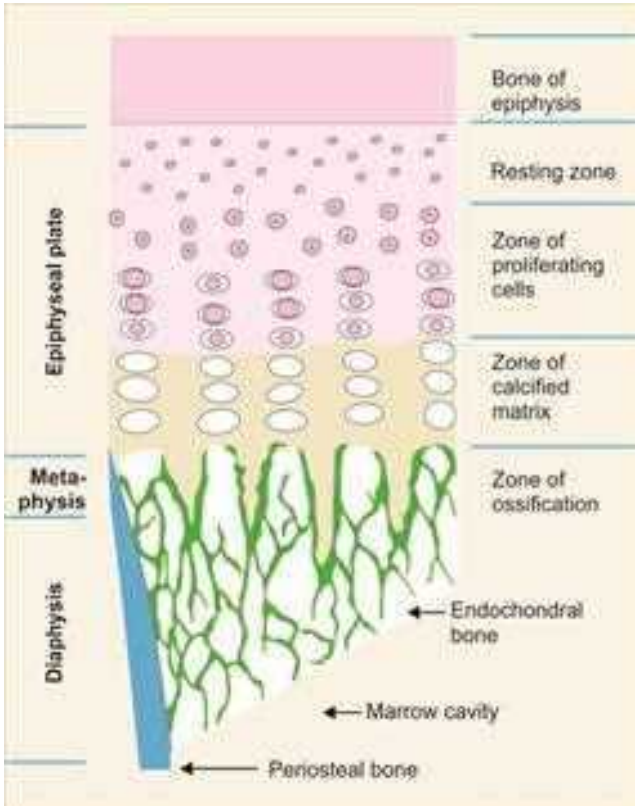


Fig. 7.18: Structure of epiphyseal cartilage

- When the bone has attained its full length, cells in the epiphyseal cartilage stop proliferating. The process of ossification, however, continues to extend into it until the whole of the epiphyseal plate is converted into bone. The bone of the diaphysis and epiphysis then becomes continuous. This is called *fusion of epiphysis*.

Metaphysis

The portion of diaphysis adjoining the epiphyseal plate is called the *metaphysis*. It is a region of active bone formation and, for this reason, it is highly vascular. The metaphysis does not have a marrow cavity. Numerous muscles and ligaments are usually attached to the bone in this region. Even after bone growth has ceased, the calcium-turnover function of bone is most active in the metaphysis, which acts as a storehouse of calcium. This region is frequently the site of infection.

Interstitial and Appositional Growth

Tissues grow by two methods. In some of them, growth takes place by multiplication of cells (or by increase in intercellular material) throughout the substance of the tissue. This is called *interstitial growth*. As a result, the tissue expands equally in all directions and its shape is maintained. Cartilage (and most other tissues) grows in this way. On the other hand, bone grows only by deposition of more bone on its surface, or at its ends. This is called *appositional growth*.

Remodeling

We have seen above that when a tissue grows by interstitial growth it is easy for it to maintain its shape. However, this is not true of bone which can grow only by apposition. This will be clear from Figure 7.20. In this Figure, the brown

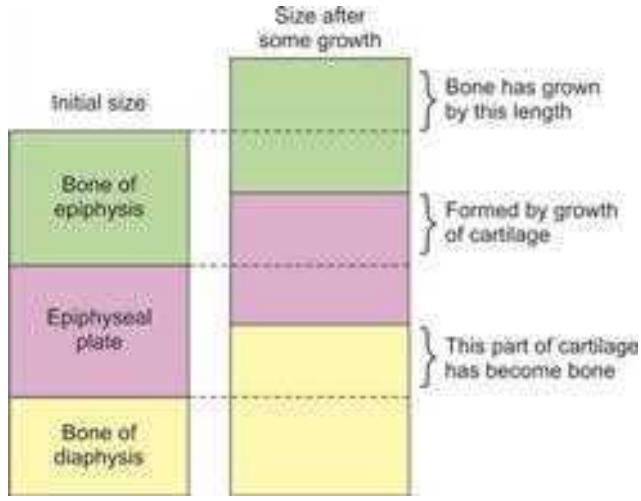


Fig. 7.19: Growth in length of bone at epiphyseal cartilage

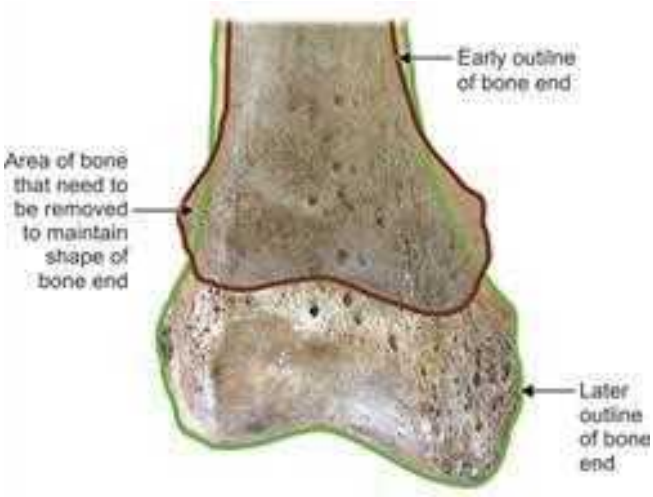


Fig. 7.20: Remodeling of bone ends during growth

line represents the shape of a bone end. The green line represents the same bone end after it has grown for some time. It will be clear that some areas of the original bone have to disappear if proper shape is to be maintained. This process of removal of unwanted bone is called *remodeling*.

The trabeculae of spongy bone and the Haversian systems of compact bone are so arranged that they are best fitted to bear the stresses imposed on them. This arrangement can change with change in stresses acting on the bone. This process is often called *internal remodeling*.

Molecular and genetic basis of bone formation

- Sex-determining region of Y (SRY) related high mobility group box transcription factors Sox9, Sox5, Sox6 regulate differentiation of chondrocytes.
- Vascular endothelial growth factor (VEGF) is needed for invasion of capillaries into the cartilage.
- Runx2 and Osterix genes control osteoblastic differentiation.
- Fibroblast growth factors (FGFs) and fibroblast growth factor receptors (FGFRs) also play an important role in development of skeleton. Together they regulate the events in bone development. FGFR1 is active in osteogenic differentiation and FGFR2 in cell proliferation. Mutations in these receptors are linked to skeletal dysplasia.

Clinical correlation

Anomalies of bone formation

Bone and cartilage formation may sometimes be abnormal as a result of various genetic and environmental factors. The anomalies may be localized to a particular part of the skeleton, or may be generalized. Anomalies of individual parts of the skeleton are considered in Chapter 10.

Some anomalies that affect the skeleton as a whole are as follows:

- ***Dyschondroplasia or enchondromatosis:*** Disorderly and excessive proliferation of cartilage cells in the epiphyseal plate, or the failure of normally formed cartilage to be replaced by bone, leads to the formation of irregular masses of cartilage within the metaphysis.
- ***Multiple exostoses or diaphyseal aclasis:*** Abnormal masses of bone may be formed in the region of the metaphysis and may protrude from the bone. Such a protrusion is called an exostosis. This condition may be a result of interference with the process of remodeling of bone ends.
- ***Osteogenesis imperfecta:*** Defective calcification of bone and may result in multiple fractures.
- ***Fibrous dysplasia:*** Parts of bone may be replaced by fibrous tissue.
- ***Osteosclerosis:*** Bones may show increased density. One disease characterized by increased bone density is known as *osteopetrosis,* or *marble bone disease*.
- ***Achondroplasia:*** In this condition, there is insufficient, or disorderly, formation of bone in the region of the epiphyseal cartilage. This interferes with growth of long bones. The individual does not grow in height and becomes a dwarf (Fig. 7.21). A similar condition in which the limbs are of normal length, but in which the vertebral column remains short, is called *chondro-osteodystrophy*.
- ***Cleidocranial dysostosis:*** Anomalous bone formation may be confined to membrane bones. One such condition in which the clavicle is absent and there are deformities of the skull. On the other hand, anomalies like achondroplasia and exostoses are confined to cartilage bones.
- Generalized underdevelopment (***dwarfism***), or overdevelopment (***gigantism***) of bone may be present. Sometimes all bones of one-half of the body are affected (***asymmetric development***).
- Overdevelopment or underdevelopment may be localized, e.g. to a digit, or to a limb.

MUSCULAR TISSUE

There are three different types of muscles. They are (1) skeletal, (2) cardiac and (3) smooth muscles. The primitive mesenchymal cells differentiate into spindle-shaped cells with longitudinal striations called *myoblasts* or *premuscle*

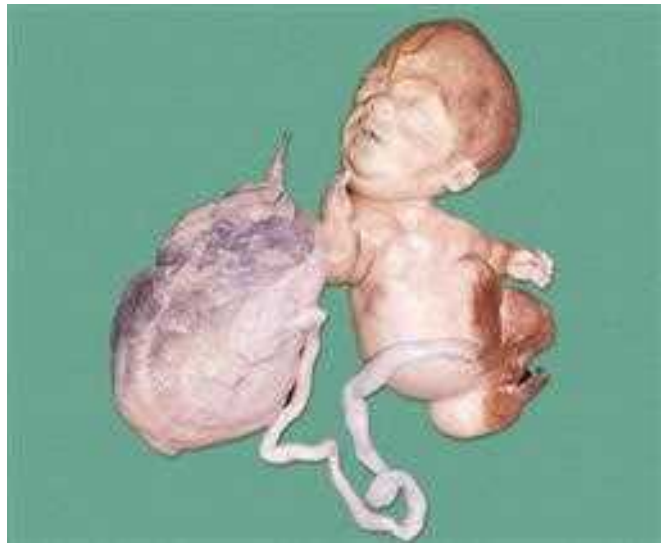


Fig. 7.21: Achondroplasia

cells. These cells differentiate into skeletal/cardiac/smooth muscle cells.

Skeletal Muscle

Skeletal muscle is derived from somites and also from mesenchyme of the region. The description of somites and the muscles derived from them will be discussed in Chapter 10. In this chapter, the myogenesis of skeletal muscle will be considered in brief.

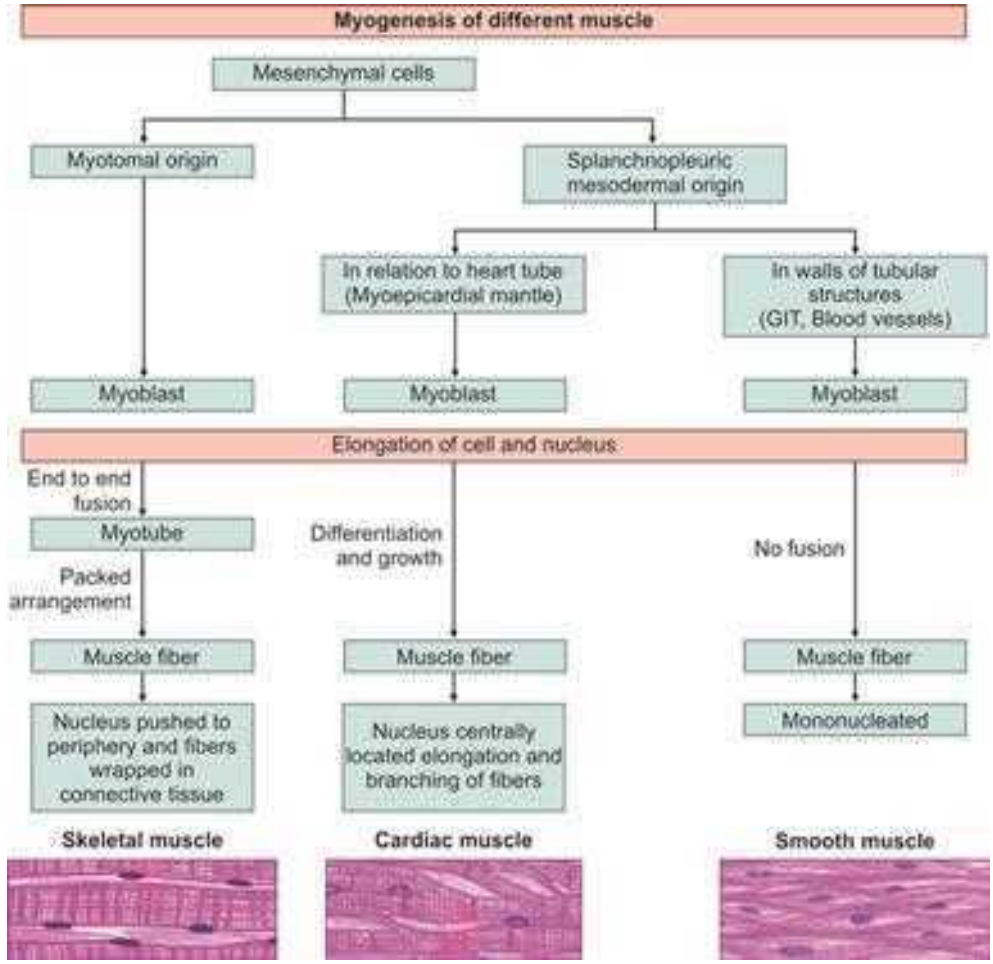
Myogenesis of skeletal muscle (Flowchart 7.3; Fig. 7.22): The mesenchymal cells of myotomal origin elongate and become the myoblasts. During differentiation, the premuscle cells or myoblasts fuse end to end form and form myotubes which are multinucleated, cylindrical syncytial cells (structural syncytium). Myotubes join to form the multinucleated muscle fibers. Protein synthesis in myoblasts forms myofilaments and myofibrils. The nuclei in a muscle fiber are pushed to the periphery and aggregations of muscle fibers are wrapped in connective tissue to form individual muscles.

Smooth Muscle

Almost all smooth muscle is formed from mesenchyme. The origin of smooth muscle at various locations with exceptions is as follows:

- This muscle is derived from splanchnopleuric mesoderm in relation with gastrointestinal tract (GIT) and respiratory system.
- Intermediate mesoderm forms the smooth muscle in relation to urogenital system.
- In situ differentiation of general/splanchnic/somatic mesoderm forms the muscle cells in blood vessels and lymph vessels.
- The neuroectodermal optic cup forms the smooth muscles of iris.

Flowchart 7.3: Myogenesis of different muscles



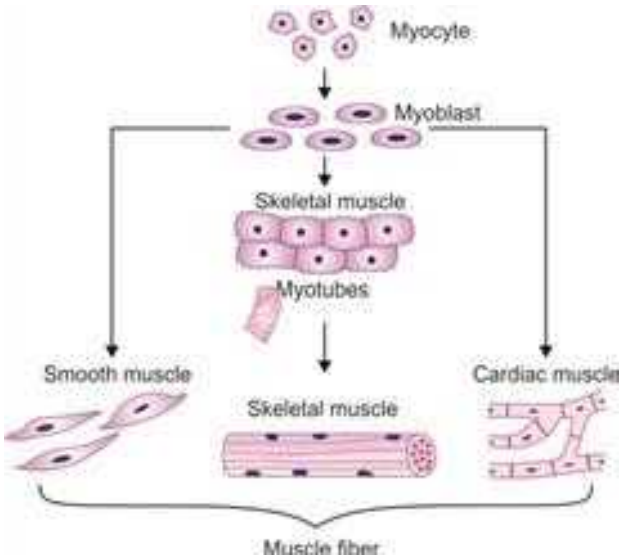


Fig. 7.22: Myogenesis of different muscle tissues

- *Exception:* Arrectores pilorum of skin and myoepithelial cells of sweat glands develop from surface ectoderm.

Myogenesis of smooth muscle (Flowchart 7.3; Fig. 7.22): The mesenchymal cells of splanchnopleuric mesoderm in relation to various viscera (e.g. GIT) elongate and become the spindle-shaped cells with oval nucleus called myoblasts. During differentiation the premuscle cells or myoblasts do not fuse. Protein granules are arranged in rows. The muscle fibers are mononucleated.

Cardiac Muscle

This is derived from splanchnopleuric mesoderm in relation to the developing heart tubes and pericardium**.**

Myogenesis of cardiac muscle (Flowchart 7.3; Fig. 7.22): The mesenchymal cells of splanchnopleuric mesoderm in relation to the developing heart (myoepicardial mantle) elongate and become the myoblasts. Differentiation and growth of each myoblast forms muscle fiber. The muscle fibers show elongation and branching. The cell membranes at the ends and branches of adjacent fibers adhere to form intercalated discs. Thus cardiac muscle is a functional syncytium.

NERVOUS TISSUE

Nervous tissue consists of cells, fibers and blood vessels. Two different categories of cells are found in the nervous tissues, i.e. (1) neurons and (2) neuroglial cells. The neurons are cells that generate and conduct nerve impulses (excitable), while the neuroglial cells are supporting (nonexcitable) structures. Each neuron has many processes. They are a single long process called *axons* and number of smaller processes called *dendrites*. The axons of several neurons collect to form nerves.

The nervous system and special sense organs are derived from specialized surface ectoderm called *neuroectoderm*. The neuroectoderm differentiates to form neural tube, neural crest cell and ectodermal placodes. The neuroectoderm forms the central nervous system and the neural crest cells to peripheral nervous system. The ectodermal placodes contribute for the formation of cranial sensory ganglia, inner ear and hypophysis cerebri. Neurons and neuroglia are derived from the wall of a specialized ectodermal structure called *neural tube*. Blood vessels of the nervous tissue are not derived from the neural tube but enter it from surrounding *mesoderm*.

Formation of Neurons and Neuroglial Cells

Histogenesis of Neural Tube

The developing neural tube presents a lumen or cavity that contains cerebrospinal fluid and a wall. Initially the all of the neural tube is lined by single layer of columnar cells. This layer proliferates to form pseudostratified neuroepithelial (germinal) layer, the cells of which extend between internal and external limiting membranes (Fig. 7.23). The pattern of formation of various cells from neuroepithelium differs before and after closure of neural tube.

- *Before closure of neural tube*:
  - The neuroepithelial layer [Fig. 7.23(A)] forms the neurons, neuroglial cells and ependymal cells.
  - DNA synthesis occurs in the cells nearer to external limiting membrane after which the nucleus migrates toward internal limiting membrane [Fig. 7.23(B)].
  - These cells loose contact with external limiting membrane.
  - The separated cell undergoes mitosis at this stage, and the two daughter cells lie parallel to the lumen and are near the internal limiting membrane [Fig. 7.23(C)].
  - Following this the cells elongate and reach the external limiting membrane to continue DNA synthesis [Fig. 7.23(D)].
- *After closure of neural tube*:
  - The neuroepithelial layer gives different types of cells, the neuroblasts that are not capable of DNA synthesis.
  - The pattern of mitosis changes. Some of the neuroepithelial cells continue mitosis with axis parallel to internal limiting membrane. Others undergo mitosis at right angles to the internal limiting membrane [Fig. 7.23(E)] resulting in

migration of one daughter cell to be detached from the internal limiting membrane that migrates toward the external limiting membrane to become a free neuroblast [Fig. 7.23(E)].
- Because of migration of neuroblast, a mantle layer is formed in the wall of the neural tube.

Formation of Three Layered Neural Tube

The neurons and many neuroglial cells are formed in the neural tube. These proliferate (Fig. 7.24A) to form three layers in the wall of neural tube (Fig. 7.24B).

1. Nearest to the lumen of the tube is the *matrix cell layer* (primitive ependymal or germinal layer). Ependymal (or neuroepithelial) cells give rise both to neuroblasts and to neuroglia. However, these two cell types are not formed simultaneously. The neuroblasts are formed first. Neuroglial cells are formed after the differentiation of neuroblasts is completed. The cells of this layer undergo mitotic division and give rise to neuroblasts first. Subsequently when the production of neuroblasts ceases they form spongioblasts followed by ependymal cells (Fig. 7.24C).
2. Next comes the *mantle layer* in which are seen the migrated neuroblasts that differentiate into nerve cells. After cessation of production of neuroblasts, the cells of germinal layer form spongioblasts (neuroglial cells).
3. The outermost layer, termed the *marginal zone*, contains no nerve cells. It consists of a reticulum formed by protoplasmic processes of developing neuroglial cells (*spongioblasts*). It provides a framework into which the processes (axons) of nerve cells developing in the mantle layer can grow.

Fate of Layers of Neural Tube after Cessation of Production of Cells

After cessation of production of neuroblasts and spongioblasts, the neuroepithelial cells form the ependymal layer lining the lumen of neural tube. The mantle layer forms the gray matter of central nervous system. The marginal layer forms the white matter of the central nervous system.

Stages in the Formation of Nerve Cells

1. *Stage of apolar neuroblast:* One of the germinal cells passes from the germinal layer to the mantle layer and becomes an *apolar neuroblast* (Fig. 7.25).
2. *Stage of bipolar neuroblast:* Two processes develop and convert the apolar neuroblast to a *bipolar neuroblast* (Fig. 7.25).
3. *Stage of unipolar neuroblast:* One of the processes of the neuroblast disappears, and it can now be called a *unipolar neuroblast* (Fig. 7.25).

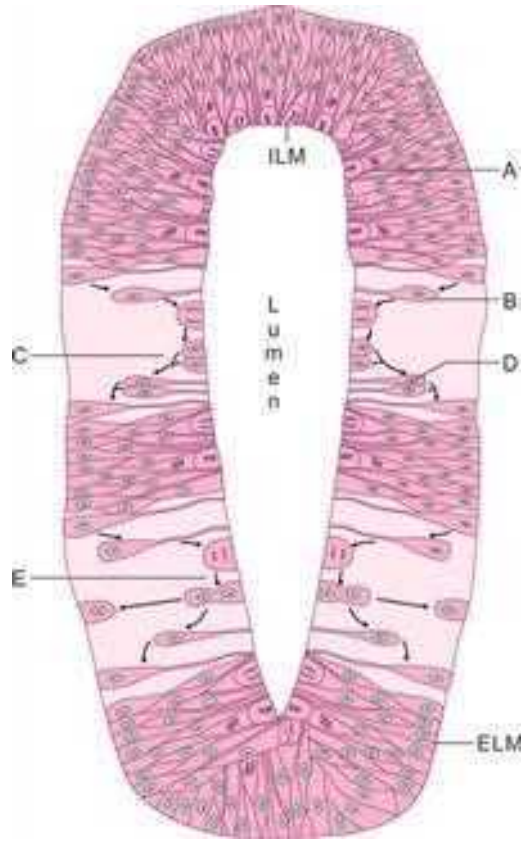
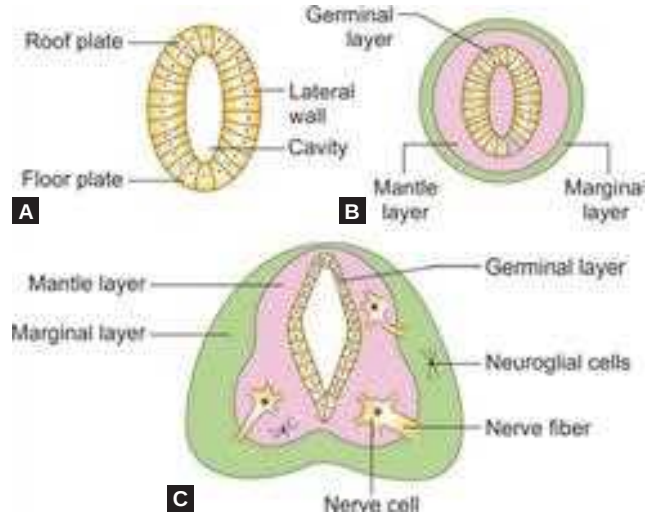


Fig. 7.23: (A to E) Histogenesis of neural tube before and after closure. Pattern of formation of cells (A to D) before and (E) after closure of neural tube. (A) Neuroepithelial cells extend between internal limiting membrane (ILM) and external limiting membrane (ELM); (B) DNA synthesis in cells close to EEM and their migration from ELM; (C) Mitosis parallel to ILM; (D) Elongation to reach ELM; (E) Mitosis at right angles to ILM and migration of daughter cell and formation of neuroblast



Figs 7.24A to C: Layers of neural tube. (A) Cavity and wall of neural tube; (B and C) Differentiation of three layers of wall and formation of nerve cells, processes and neuroglia

4. *Stage of multipolar neuroblast:* The process of the cell (which does not disappear) now elongates, and on the side opposite to it numerous smaller processes form. At this stage, the cell is called a *multipolar neuroblast* (Fig. 7.25).

- *Differentiation of processes of neuroblast:* The main process of the multipolar neuroblast now grows into the marginal layer, and becomes the *axon* of the nerve cell (Fig. 7.24B). The axon can grow to a considerable length. It may either remain within the central nervous system, or may grow out of it as an efferent nerve fiber of a peripheral nerve. At its destination, it establishes connections, either with the cell bodies and dendrites of other neurons or with an effector organ (e.g. muscle). The smaller processes of the neuroblast are the *dendrites*. These ramify and establish connections with other nerve cells.
- At first the cytoplasm of the nerve cell is homogeneous. Later *Nissl's granules* make their appearance. After their formation, neurons lose the ability to divide.
- *Formation of neuroglial cells:* Majority of neuroglial cells are formed from germinal cells of the ependymal layer after it ceases to produce neuroblasts. These cells (*glioblasts*) migrate to the mantle and marginal zones. In the mantle zone, they form *medulloblasts* (also called *spongioblasts*), which differentiate into *astroblasts*, and subsequently into *astrocytes*. There are two types of astrocytes, i.e. (1) protoplasmic and (2) fibrous. In the marginal zone the glioblasts differentiate into *oligodendroblasts* that become *oligodendrocytes*. The oligodendrocytes form myelin sheath around the axons of ascending and descending tracts. There is a third type of neuroglial cell called *microglia*. This type does not develop from the cells of the neural tube, but migrates into it along with blood vessels. These cells are believed to be of mesodermal origin (Table 7.4).

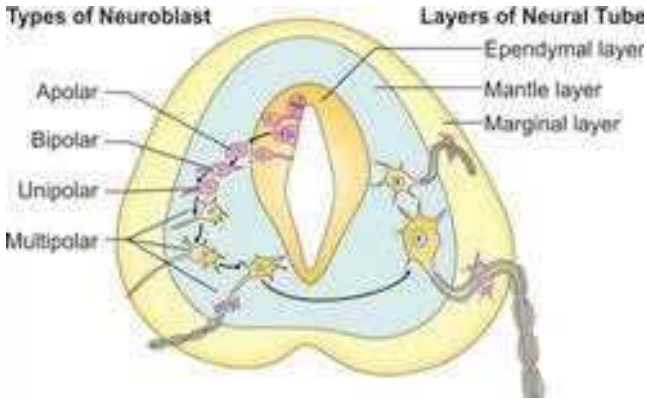


Fig. 7.25: Stages in the formation of neuroblast—apolar, bipolar, unipolar and multipolar

TABLE 7.4: Development of various cells of nervous tissue

Neuroepithelial derivatives (from wall of neural tube)	*Neural crest derivatives*	*Mesodermal/ Mesenchymal derivatives*
A. Neuron bodies inside CNS	*A. Neuron bodies outside CNS*	Microglia
1. Multipolar neurons	1. Dorsal root ganglia	
2. Bipolar neurons	2. Sensory ganglia of cranial nerves 5th, 7th to 10th	
3. Preganglionic sympathetic and parasympathetic neurons	3. Autonomic ganglia—postganglionic sympathetic and parasympathetic neurons	
B. Neuroglia of CNS	*B. Neuroglia of PNS*	
1. Protoplasmic and fibrous astrocytes	1. Satellite cells	
2. Oligodendrocytes	2. Schwann cells	
3. Ependymal cells		

Formation of Myelin Sheath

- Nerve fibers, which remain within the brain and spinal cord, receive support from, and are ensheathed by, neuroglial cells. The nerve fibers, which leave the central nervous system to become constituents of peripheral nerves, acquire a special sheath called the *neurolemma*. This sheath is derived from cells of neural crest origin called *Schwann cells*. At a later stage of development, a large number of nerve fibers, both inside and outside the central nervous system, develop another sheath between the neurolemma and the axon. This is called the *myelin sheath*. The myelin sheaths of peripheral nerves are derived from the same Schwann cells that form the neurolemma. In the central nervous system, there are no Schwann cells and the myelin sheath is formed by neuroglial cells called *oligodendrocytes*.
- The relationship of an axon to a Schwann cell in myelinated and unmyelinated fibers is illustrated in Figure 7.26. Note the following points:

Myelinated Nerve Fiber

- A single axon comes in contact with a single Schwann cell [Fig. 7.26(A)].

- The axon invaginates cytoplasm of a Schwann cell and thus comes to be completely surrounded by it [Fig. 7.26(B)].
- Along the line of invagination, the cell membrane of the Schwann cell becomes drawn into form a double-layered mesentery-like membrane called the *mesaxon* [Fig. 7.26(C)].
- The mesaxon elongates and becomes spirally wound around the axon. Some fatty substances are deposited between adjacent layers of the mesaxon and, together with it, form the *myelin sheath* [Fig. 7.26(D)].

Unmyelinated Nerve Fiber

- A number of axons come in contact with a single Schwann cell [Fig. 7.26(E)].
- They invaginate into the cytoplasm of a single Schwann cell [Fig. 7.26(F)].
- There is no elongation of the mesaxon as several axons may invaginate the same Schwann cell [Fig. 7.26(G)].
- Nerve fibers in different parts of the brain and spinal cord become myelinated at different stages of development. The process begins during the 4th month of intrauterine life, but is not completed until the child is 2–3 years old. Nerve fibers become fully functional only after they have acquired their myelin sheaths.
- The blood vessels of the brain, and their surrounding connective tissue, are not derived from the neural tube. These are mesodermal in origin and invade the developing brain and spinal cord from the surrounding mesoderm.
- The development of the *pia mater* and the *arachnoid mater* (*leptomeninges*) is not definitely understood.

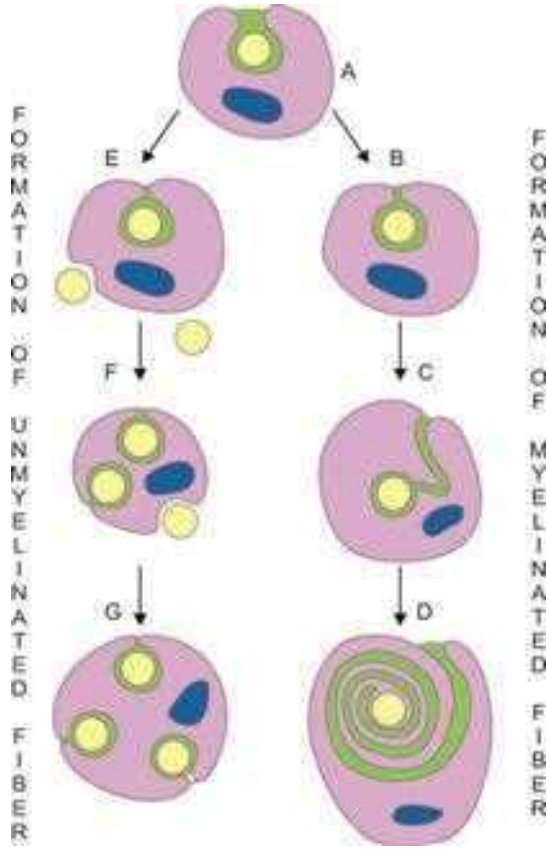


Fig. 7.26: Process of formation of (A to D) myelinated and (E to G) unmyelinated axons

According to some workers, these are derived from the neural crest. The dura mater develops from the mesoderm surrounding the neural tube.

REVIEW QUESTIONS

1. Name five derivatives of ectoderm.
2. Name the derivatives of mesenchymal cells.
3. Name the stages in the formation of nerve cells.
4. Describe the stages in intramembranous ossification.
5. Describe the stages in endochondral ossification.

Chapter 8

Integumentary System (Skin and Its Appendages, Mammary Gland)

HIGHLIGHTS

- Skin is the largest organ in the body.
- The two layers of skin are (1) epidermis and (2) dermis.
- The epidermis is derived from surface ectoderm.
- The dermis is formed by mesenchyme derived from dermatomes of somites.
- Nails develop from ectoderm at the tip of each digit. Later this ectoderm migrates to the dorsal aspect.
- Hairs are derived from surface ectoderm which is modified to form hair follicles.
- Sebaceous glands (ectoderm) arise as diverticula from hair follicles.
- Sweat glands develop as downgrowths from the epidermis that are later canalized.
- Mammary glands arise from surface ectoderm. They are formed along a milk line extending from axilla to the inguinal region.

SKIN

The skin is derived from three diverse components (Fig. 8.1), i.e. (1) surface ectoderm, (2) underlying mesoderm and (3) neural crest cells.

Epidermis

- The epidermis is derived from the surface ectoderm. This is, at first, single layered (Fig. 8.2A).
- During 2nd month, it presents two layers, i.e. (1) a superficial layer of flat cells called *periderm/epitrichium* and (2) a deep layer of cuboidal cells called *basal/germinative* layer (Fig. 8.2B).
- Later the cells of basal layer proliferate to form a third *intermediate* layer (Fig. 8.2C).
- The basal layer is known as *stratum germinativum* as these cells proliferate to form the various layers of epidermis.
- During 3rd to 5th month because of proliferation of cells, the epidermis becomes typical stratified squamous epithelium consisting of five layers of strata (Fig. 8.2D). They are *stratum germinativum, stratum spinosum, stratum granulosum, stratum lucidum and stratum corneum*.
- Up to the end of 5th month, there will be continuous keratinization, desquamation and replacement of peridermal cells by those arising from basal layer.
- *Vernix caseosa*: Many of the superficial layers of epidermis are shed off. These get mixed up with secretions of sebaceous glands and hairs to form a whitish sticky substance (*vernix caseosa*) which covers the skin of the newborn infant (Fig. 8.2D). The vernix caseosa has a protective function as it prevents the skin from maceration by amniotic fluid.
- After 5th month, the periderm disappears as the cells are cast off and appear in amniotic fluid. In the place of periderm, the stratum corneum forms.
- Proliferation of stratum germinativum extends into the developing dermis as *epidermal ridges*. They appear by 11th week and become permanent by 18th week (Figs 8.2A to D).
- *Finger and palm prints* are grooves on the surface of palms of the hand and soles of the feet including digits that are formed by epidermal ridges. Soon, thereafter,

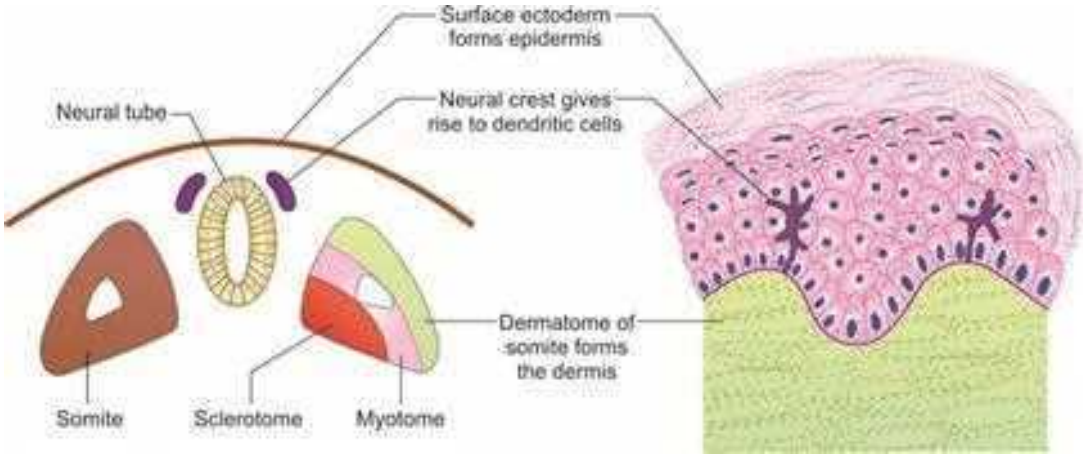
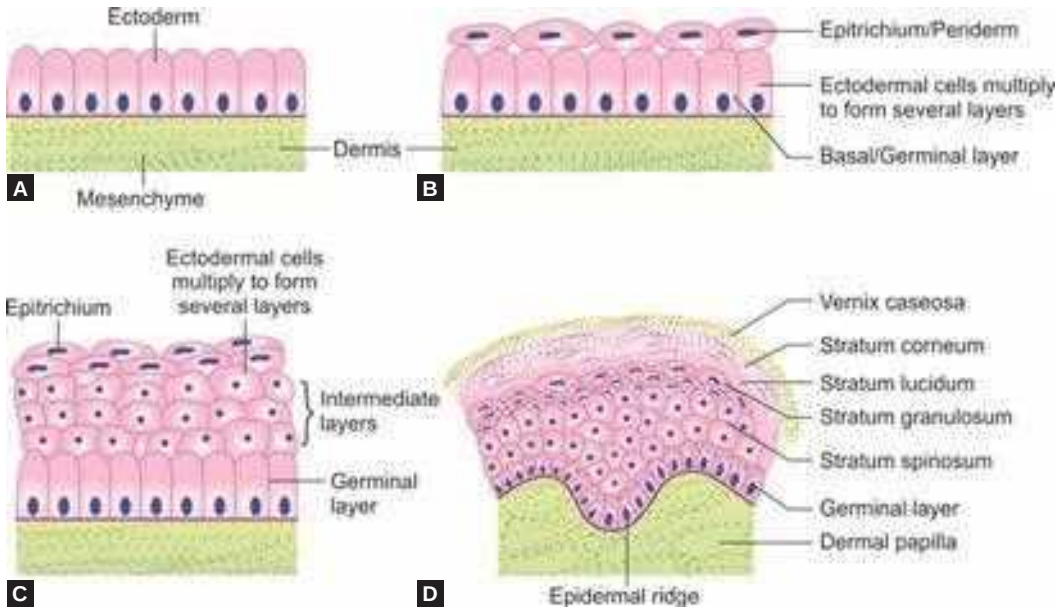


Fig. 8.1: Derivation of components of the skin



Figs 8.2A to D: Stages in the development of the epidermis

characteristic patterns (whorls, composite, loop and arch) are formed on the tips of fingers and toes. The patterns are genetically determined, and are different for each person. Similarly the epidermal ridges form patterns on palm and sole also (Figs 8.3A and B).

- At birth, all the layers of adult epidermis are present.

Melanoblasts

- The melanoblasts (or dendritic cells) of the epidermis are derived from the neural crest (Fig. 8.1).
- These invade the epidermis during 3rd month. Later they invade dermoepidermal junction and become melanocytes. These cells synthesize melanin pigments responsible for skin and hair color.
- Cell bodies of melanocytes are confined to basal layers of the epidermis. Their processes extend between the epidermal cells. Melanocytes begin producing melanin before birth and distribute it to the epidermal cells.
- Cells of Merkel and Langerhans cells appear in the epidermis between 8 weeks and 12 weeks of intrauterine life.

Dermis

- The dermis is formed by condensation and differentiation of mesenchyme underlying the surface ectoderm (Figs 8.2A to D). The three sources of origin of this mesenchyme in different parts of the body are:

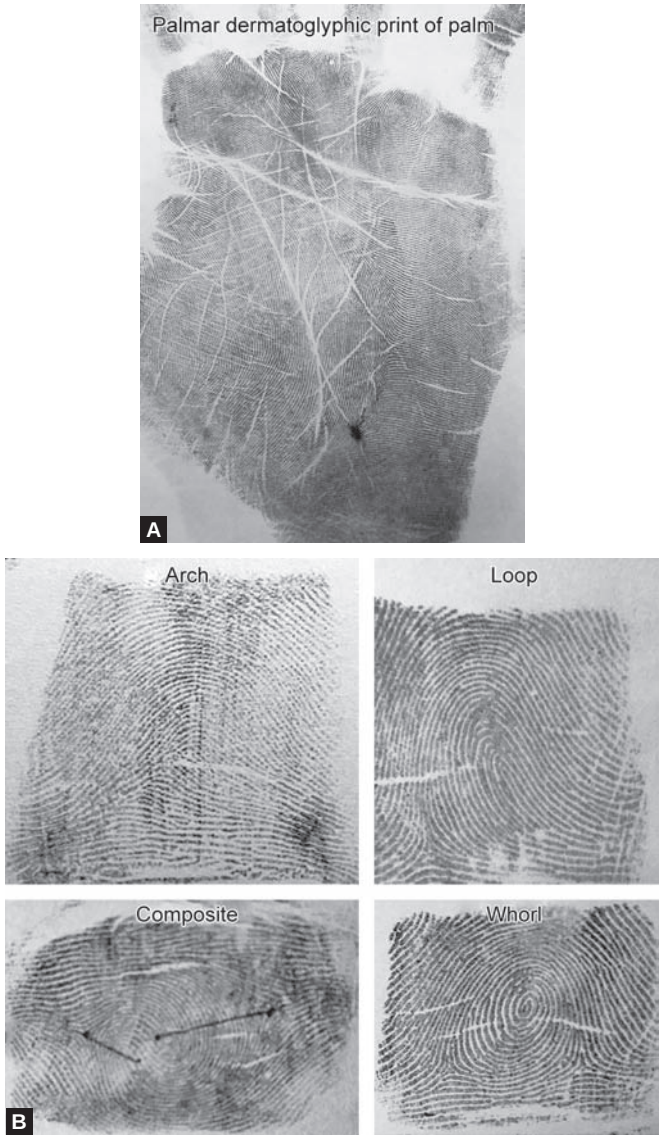
  - The *dermatomes* give rise only to the dermis on the dorsal aspect of the head and trunk.
  - The dermis of the limbs and that on the lateral and ventral aspects of the trunk arises from *lateral plate mesoderm*.
  - The dermis over most of the head and over the anterior aspect of the neck is derived from the *neural crest*.
- By 11th week mesenchymal cells begin to produce collagen and elastic fibers.
- The line of junction between dermis and epidermis is at first straight (Figs 8.2A and B) subsequently, during 3rd and 4th months the epidermis shows regularly spaced thickenings that project into the dermis (Fig. 8.2D). The portions of dermis intervening between these projections form the *dermal papillae*. Still later, surface elevations (*epidermal ridges*) are formed by further thickening of the epidermis in the same situation.
- The dermis differentiates into two layers: (1) a superficial papillary layer and (2) a deep reticular layer.

APPENDAGES OF SKIN

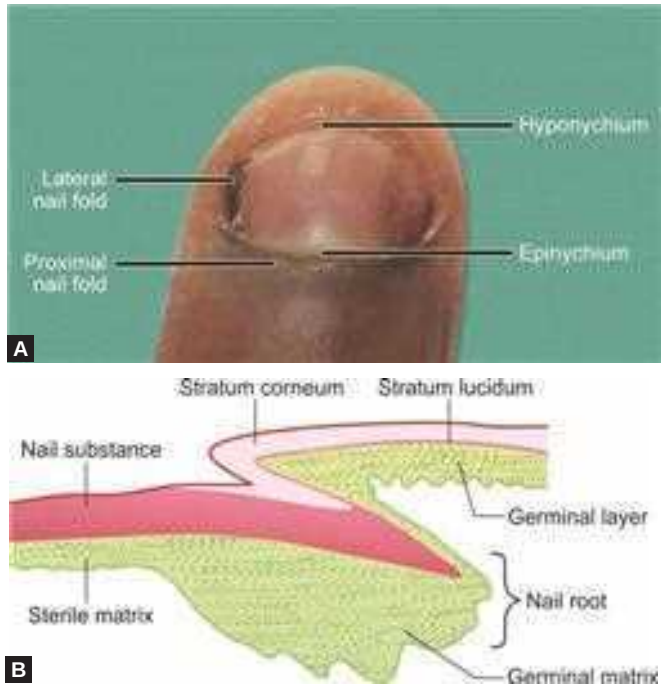
The *appendages of skin* are associated structures that are derived from the epidermis and dermis that usually are located adjacent to the skin and serve a specific function, e.g. prevention of heat loss, sensation. These include nails, hairs and glands.

Nails

- Nails develop from the surface ectoderm.
- The ectoderm at the tip of each digit becomes thickened at 10th week to form a *primary nail field*.
- Subsequently, this thickening migrates from the tip of the digit onto its dorsal aspect and is surrounded by U-shaped nail folds of epidermis (Fig. 8.4A).
- The cells in the most proximal part of the nail field proliferate to form the root of the nail. Here the cells of



Figs 8.3A and B: Dermatoglyphics: Epidermal ridges forming (A) patterns on palm and (B) patterns on fingers—arch, loop, composite and whorl



Figs 8.4A and B: Derivation of nail. (A) Parts of nail; (B) Developing nail root and substance

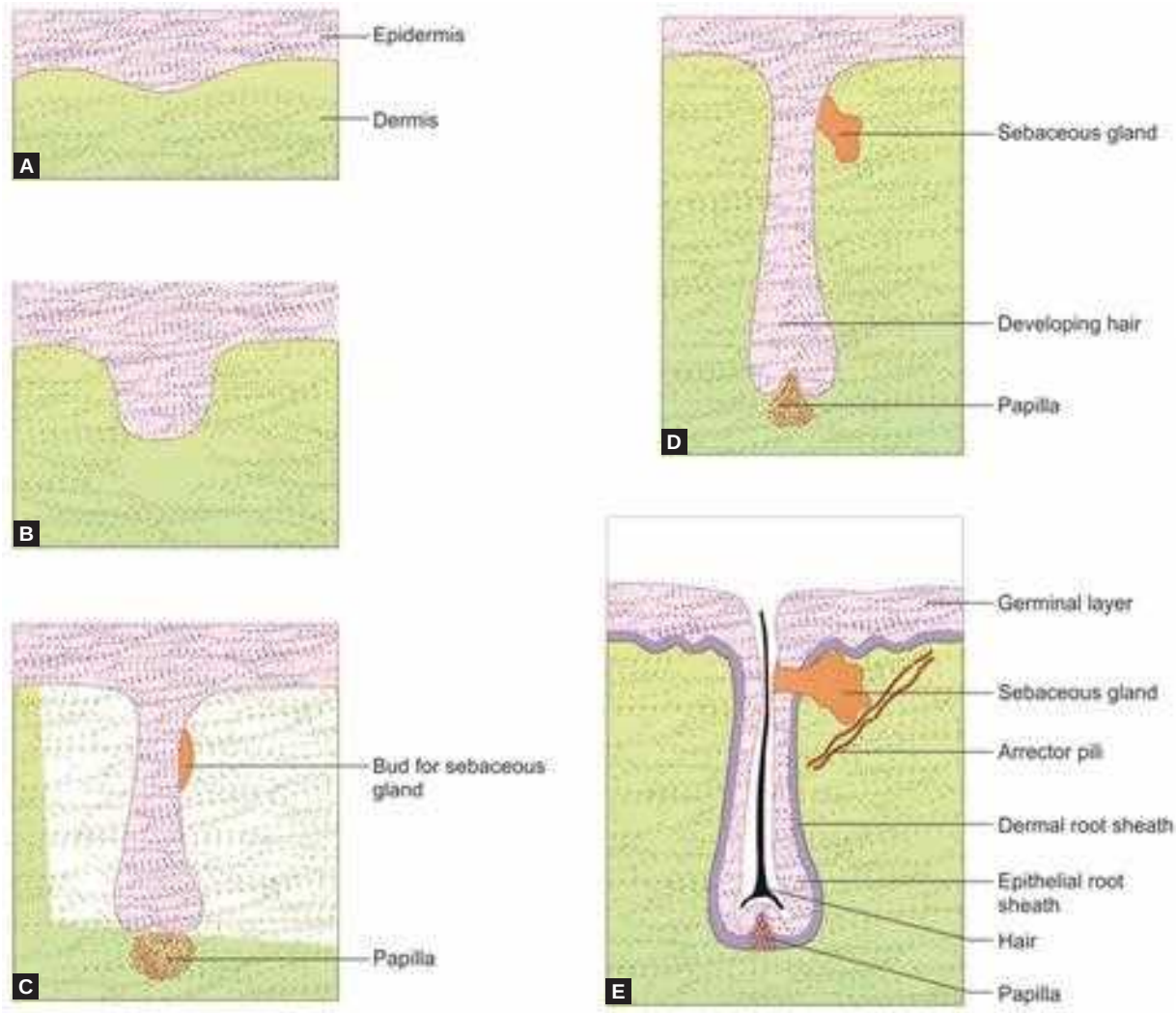
the germinal layer multiply to form a thick layer of cells called the *germinal matrix* (Figs 8.4A and B).

- As the cells in this matrix multiply, they are transformed into the nail substance/*nail plate* which corresponds to stratum lucidum of the skin (Fig. 8.4B).
- At first the stratum corneum covers the surface of the nail but later it disappears except over the proximal part of nail plate.
- The part of epidermis overlapping proximal part of nail plate is called *eponychium.* The epidermis below free margin of nail is called *hyponychium* (Fig. 8.4A).

Migration of primary nail fields from the tips of the digits to their dorsal aspect explains why the skin of the dorsal aspect of the terminal part of the digits is supplied by nerves of the ventral aspect.

Hair

- Hair is also derived from surface ectoderm. At the site where a *hair follicle* is to form, the germinal layer of the epidermis proliferates to form a cylindrical mass that grows down into the dermis (Figs 8.5A and B).
- The lower end of this downgrowth becomes expanded and is invaginated by a condensation of mesoderm, which forms the *papilla* (Figs 8.5C and D).
- The hair itself is formed by proliferation of germinal cells overlying the papilla. As the hair grows to the surface, the cells forming the wall of the downgrowth surround it and form the *epithelial root sheath* (Fig. 8.5E).
- An additional *dermal root sheath* is formed from the surrounding mesenchymal cells (Fig. 8.5E).



Figs 8.5A to E: Development of a hair follicle and sebaceous glands

- A thin band of smooth muscle *(arrector pili)* is formed by mesodermal cells. It gets attached to the dermal root sheath. A typical hair follicle is thus formed (Fig. 8.5E).

Glands of Skin

The glands of skin and their functions are as follows:

- *Sebaceous glands*: These are located near the hair follicle. They function as waterproof for the surface for protection.
- *Eccrine sweat glands*: These are present in deep dermis. They are involved in maintenance of body temperature.
- *Apocrine sweat glands*: These are located near hair follicles in armpit, groin, around nipple. Their secretions are odor producing and sexual attractant (emotional).
- *Ceruminous glands*: These are present in external auditory canal and produce earwax.
- *Mammary glands*: These glands secrete milk after parturition.

Sebaceous Glands

- A sebaceous gland is formed as a bud arising from ectodermal cells forming the wall of a hair follicle (*epithelial root sheath*; Fig. 8.5C).
- The bud grows into the adjacent dermis and divides into number of branches, the primordia of alveoli and ducts (Figs 8.5D and E).
- The central cells of alveoli degenerate and produce oily sebum that is released on to the surface of skin.
- In the glans penis and labia minora, the sebaceous glands develop independent of hair follicle from epidermis.
- The arrector pili muscle is a small bundle of smooth muscle fibers that differentiate from the mesenchyme adjacent to hair follicle and is attached to the dermal sheath of hair follicle and the papillary layer of dermis (Fig. 8.5E).

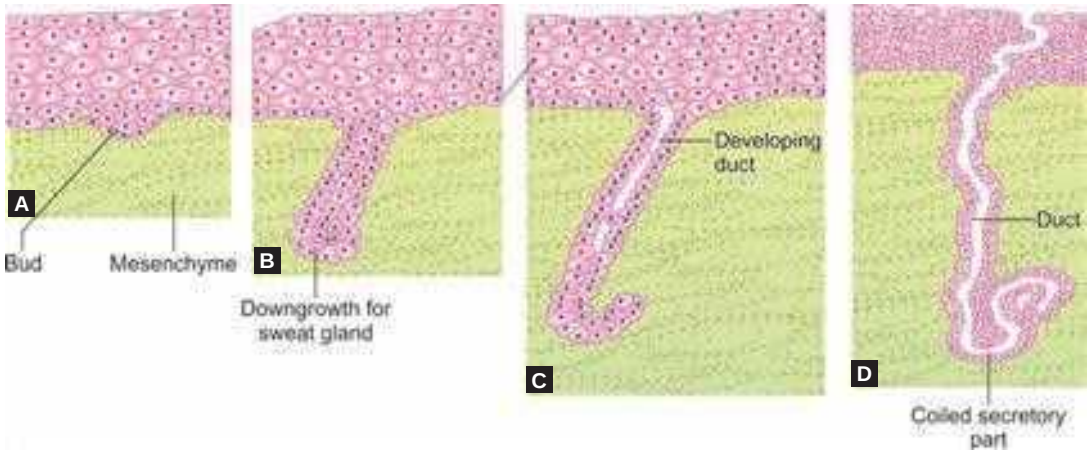
Some stages in the formation of a sebaceous gland are shown in Figures 8.5C to E.

Sweat Glands

- There are two types of sweat glands: (1) the *eccrine* and (2) *apocrine*.
- *Eccrine* sweat gland develops as a downgrowth from the epidermis (Fig. 8.6A) into the underlying dermis around 20 weeks. The downgrowth is at first solid, but later canalized (Figs 8.6B and C). The lower end of the downgrowth becomes coiled (Fig. 8.6D) and forms the secretory part of the gland. The upper end is straight and forms the duct of the sweat gland that opens on to the surface of the epidermis by sweat pore. The eccrine sweat glands start functioning from the time of birth, their mechanism of secretion is merocrine and they take part in temperature control.
- *Apocrine* sweat glands begin to develop during puberty. They are seen in axilla, in the areolae of nipples, pubic and perineal areas. They develop from the hair follicles in the form of buds and open into the hair follicles. They are named apocrine as a part of the secretory cell is shed as secretion.

Molecular and genetic basis of skin development

- In the presence of Wnt signaling and absence of response to fibroblast growth factor (FGF) signaling, the ectodermal cells express bone morphogenetic proteins (BMPs), and become committed to develop into epidermis.
- The cells that are not responding to Wnt signaling may receive BMP and FGF inhibitory signals from underlying mesenchyme thus facilitating development of skin appendages.
- Pax3 genes are active in neural crest cells migration and their differentiation into melanoblasts, melanocytes and to start their function of producing melanin pigment.



Figs 8.6A to D: Development of a sweat gland

Clinical correlation

Anomalies of skin and its appendages

- ***Aplasia:*** The skin may fail to develop in certain regions.
- ***Dysplasia:*** The skin may be abnormal in structure. Numerous varieties of dysplasia are described. There may be congenital growths of the skin. Dysplasia may be part of maldevelopment of various ectodermal derivatives including hair, teeth, sweat glands and sebaceous glands.

Pigment disorders

- ***Albinism:*** Absence of pigment in skin, hair and eyes occurs because melanocytes are unable to synthesize melanin. In this autosomal recessive genetic condition, skin is depigmented all over the body. This should be distinguished from vitiligo.
- ***Piebaldism:*** A rare autosomal dominant disorder with patchy areas of absence of hair pigment (patches of white hair on forehead—white forelock) due to disordered development of melanocytes. Mutations in CD117 gene result in this condition.
- ***Vitiligo:*** It is an autoimmune disorder that presents as patchy loss of pigment that includes skin, hair and oral mucosa (Fig. 8.7) which is not congenital. In vitiligo, the absence of pigment is patchy. In the affected areas, there is degeneration of already existing melanocytes. It is an autoimmune disease.
- ***Waardenburg syndrome:*** It is due to defects in the migration and proliferation of neural crest cells from which the melanocytes are derived. It presents white patches of skin and hair, iris of different colors and deafness (loss of pigment cells in stria vascularis of cochlea). This condition can result from mutations in Pax3 gene.

Keratinization defect

- ***Ichthyosis:*** Hyperkeratinization of skin is a hereditary disorder of autosomal recessive or X-linked inheritance.
- ***Harlequin fetus:*** A severe form of excessive keratinization that can result in an ugly appearance of fetus.

Hair distribution abnormalities

- ***Congenital alopecia:*** Hair may be absent over the scalp. The eyebrows and eyelashes may also be absent.
- ***Atrichia:*** Absence of hair in any part of the body. It is usually associated with other ectodermal defects, e.g. in teeth and nails.
- ***Hypertrichosis:*** Overgrowth of hair either localized (in lumbar region covering spina bifida occulta) or generalized.

Anonychia

- Nails may be absent. Occasionally they may show over development.

Dermatoglyphics

- The epidermal ridges produce typical patterns on the fingertips, palms of the hand and soles of the feet. These are permanent identification marks of an individual. These patterns are called dermatoglyphics and have genetic basis. Study of these patterns is used in medico-legal investigation, for establishing identity of an individual and in medical genetics (Fig. 8.3). It is used as a diagnostic tool in individuals with chromosomal abnormalities (Down's syndrome) and in certain medical conditions (cancers, hypertension, mental illness, diabetes).

Mammary Glands

- Mammary glands are modified sweat glands and are ectodermal in development.
- In some animals (e.g. bitches), a series of mammary glands are present on either side of the midline, on the ventral surface of the trunk. These are situated along a line that extends from the axilla to the inguinal region (*mammary line or ridge*).
- In the human embryo, these lines appear around 7th week of development extending from root of upper limb to root of lower limb (Figs 8.8A and B). The ectoderm becomes thickened along this line to form mammary ridges or lines (Figs 8.8A and B). Most of this line soon disappears except in thoracic region.
- Each mammary gland develops from a part of this line that overlies the pectoral region. In the region where the mammary gland is to form, a thickened mass of

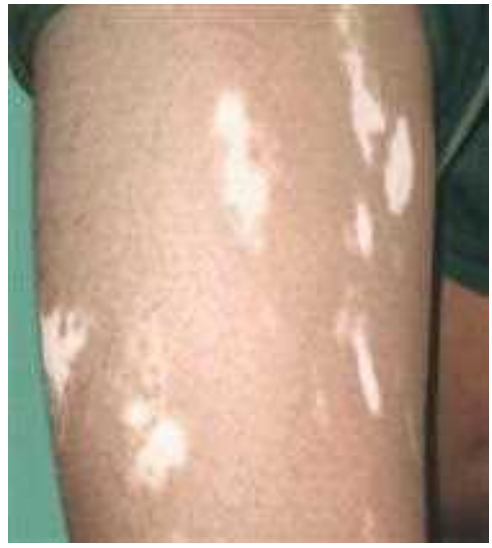
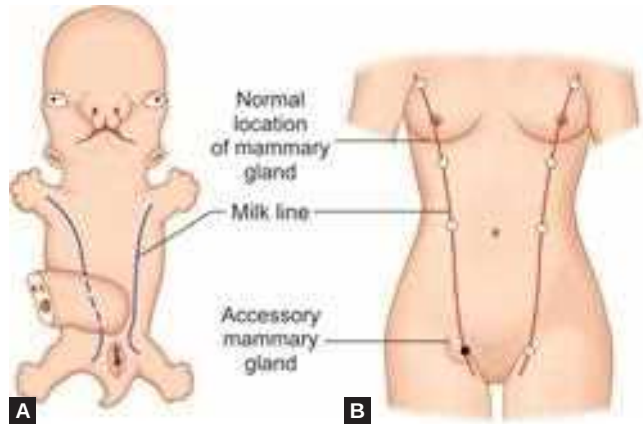
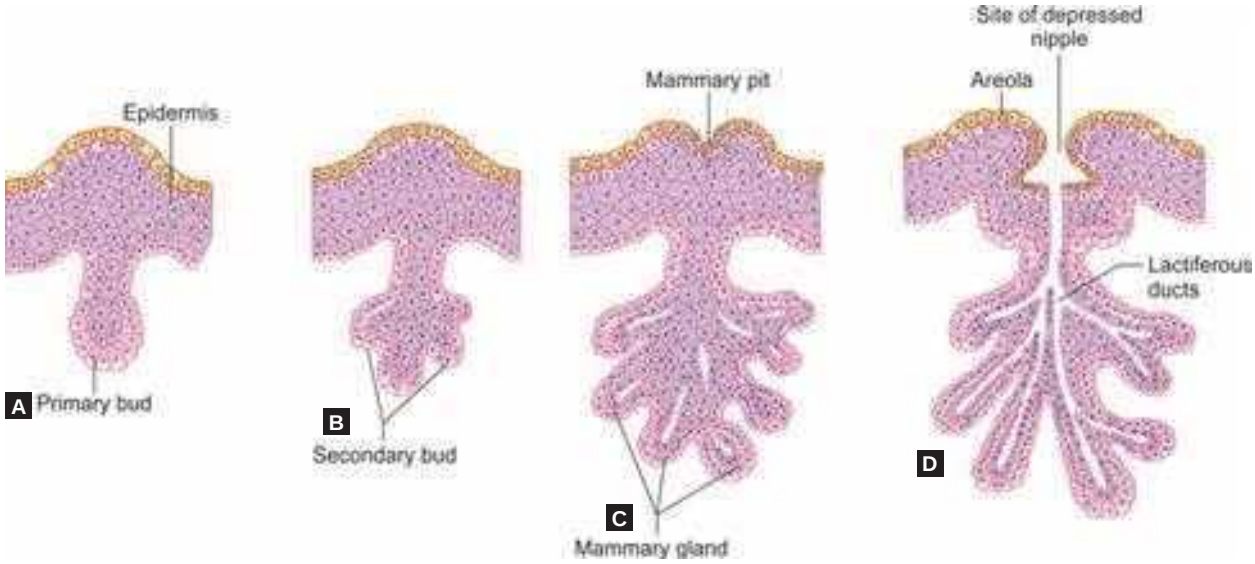


Fig. 8.7: Vitiligo—absence of pigmentation (*Image Courtesy:* Dr Suguna)



Figs 8.8A and B: Mammary ridge/line. (A) The mammary line passing from the axilla to the inguinal region in fetus; (B) Adult mammary gland in pectoral region and accessory mammary gland in inguinal region



Figs 8.9A to D: Stages in the development of the mammary gland

epidermal cells is seen projecting into the dermis as primary bud (Figs 8.8 and 8.9A). From this thickened mass, 16–20 solid outgrowths arise, and grow into the underlying dermis as secondary bud (Figs 8.9B and C).

- The thickened mass of epidermis (and the outgrowths) gets canalized (Figs 8.9C and D).
- The secretory elements (*alveoli*) of the gland are formed by proliferation of terminal parts of the outgrowths. The proximal end of each outgrowth forms one *lactiferous duct*. The ducts at first open into a pit formed by cavitation of the original epithelial thickening. However, the growth of underlying mesoderm progressively pushes the wall of this pit outward, until it becomes elevated above the surface and forms the *nipple* by the time of birth.
- The mammary gland remains rudimentary in the male. In females, the ducts and secretory elements undergo extensive development during puberty and pregnancy.

Clinical correlation

Developmental anomalies of mammary gland

Amastia: The gland may be absent on one or both sides.

Athelia: The nipple may be absent.

Polythelia and polymastia: Supernumerary nipples may be present anywhere along the milk line. They may remain rudimentary (polythelia) or may form accessory mammary glands (polymastia). Accessory breasts may be found away from the milk line. They have been observed in the neck, cheeks, femoral triangle and vulva (Figs 8.8A and B).

Inverted or crater nipple: The nipple may fail to form resulting in lactiferous ducts opening into a pit. This causes difficulty in suckling.

Size variations: The gland may be abnormally small (*micromastia*) or abnormally large (*macromastia*).

Gynecomastia: The male breast may enlarge as in the normal female and may even secrete milk.

TIME TABLE OF SOME EVENTS DESCRIBED IN THIS CHAPTER

Time table of some events described in this chapter is shown in Table 8.1.

TABLE 8.1: Time table of developmental events

Age	*Developmental events*
7th week	Mammary line is established
8th week	Melanoblasts start appearing
11th week	Epidermal ridges appear; collagen and elastic fibers appear
1st to 3rd month	Cells of neural crest migrate to skin
Before 2nd month	Surface ectoderm is single layered
3rd month	Melanoblasts invade epidermis
2nd to 4th month	Surface ectoderm becomes multiple layered
3rd to 4th month	Dermal papillae are formed, nails appear
5th month	Development of eccrine sweat glands, rudiments of lactiferous ducts

EMBRYOLOGICAL EXPLANATION FOR CLINICAL CONDITIONS OR ANATOMICAL OBSERVATIONS IN SKIN

Case Scenario 1

An adult male attended dermatology outpatient department (OPD) with number of white patches on the front of leg (Fig. 8.7) that were increasing in number and size over the past 6 months. There is no history of trauma such as cuts, burns or ulceration. There is no history of fever or jaundice or history of use of any medicines. What is the name given to

this condition? What is the explanation for the occurrence of white patches? What are the complications of it?

- The white patches are due to the absence of melanin pigment. This condition is called vitiligo.
- Vitiligo is one of the pigmentary disorders of integumentary system. It is due to destruction of pigment forming cells the melanocytes which are derived from neural crest cells. There is history of progression in the number and area of skin patches. This condition has to be differentiated from leukoderma where white patches are present but there will be history of trauma. By asking the history the possibility of drug induced or other causes will be excluded. In the absence of a history of trauma and other tests to rule out diabetes and hyperthyroidism, it is diagnosed as vitiligo.
- Exact cause of this condition is not known. It is thought to be an autoimmune disorder. It can be hereditary.
- Lack of melanin causes sunburns and increased risk of carcinoma of skin on prolonged exposure to sun. Some people will have lack of pigmentation of the eyes and hearing loss.

For understanding different types of pigment disorders refer to pigment disorders mentioned under clinical correlation in this chapter.

REVIEW QUESTIONS

1. Name the developmental derivatives of skin.
2. Write notes on development of sweat glands.
3. Write short notes on development of nail.
4. Write short notes on development of mammary gland.

Chapter 9

Pharyngeal Arches

HIGHLIGHTS

- *Pharyngeal arches* are rod-like thickenings of mesoderm present in the wall of the foregut.
- At first there are six arches. The fifth arch disappears and only five remain.
- The ventral ends of the arches of the right and left sides meet in the middle line in the floor of the pharynx.
- In the interval between any two arches, the endoderm (lining the pharynx) is pushed outward to form a series of pouches. These are called *endodermal, or pharyngeal, pouches*.
- Opposite each pouch the surface ectoderm dips inward as an *ectodermal cleft*.
- Each pharyngeal arch contains a *skeletal element* (cartilage that may later form bone), *striated muscle* supplied by the nerve of the arch, and an *arterial arch*.
- The cartilage of the first arch (*Meckel's cartilage*) gives origin to the *incus* and *malleus* (of middle ear).
- The cartilage of the second arch forms the *stapes*, the *styloid process* and *part of the hyoid bone*.
- The cartilage of the third arch forms the *greater part of the hyoid bone*.
- The cartilages of the fourth and sixth arches give rise to the *cartilages of the larynx*.
- The nerves of the pharyngeal arches are as follows: First arch = mandibular; second arch = facial; third arch = glossopharyngeal; fourth arch = superior laryngeal; fifth arch = recurrent laryngeal. The muscles supplied by these nerves are derived from the mesoderm of the arch concerned.
- The *external acoustic meatus* develops from the first ectodermal cleft.
- The first endodermal pouch (and part of second) gives off a diverticulum called the *tubotympanic recess*. The *middle ear* and the *auditory tube* develop from the tubotympanic recess.
- The *palatine tonsil* arises from the second pouch.
- The *inferior parathyroid* gland and the *thymus* are derived from the third pouch.
- The *superior parathyroid gland* is derived from the fourth pouch.
- The *thyroid gland* develops mainly from the thyroglossal duct. This duct is formed as a median diverticulum arising from the floor of the pharynx (at the foramen cecum).

INTRODUCTION

- The formation of the foregut has been considered in Chapter 5: Further Development of Embryonic Disc. With the establishment of the head fold, part of yolk sac gets incorporated into it and forms the foregut. The foregut is bounded ventrally by the pericardium, and dorsally by the developing brain (Chapter 5: Fig. 5.10H). Cranially, it is at first separated from the stomatodeum by the buccopharyngeal membrane. When this membrane breaks down, the foregut opens to the exterior through the stomatodeum.
- At this stage, the head is represented by the bulging caused by the developing brain (Chapter 5: Fig. 5.10H), while the pericardium may be considered as occupying the region of the future thorax. The two are separated by the stomatodeum which is the future mouth. It is, thus, apparent that a neck is not yet present.

PHARYNGEAL/BRANCHIAL ARCHES

- The neck is formed by the elongation of the region between the stomatodeum and the pericardium. This is achieved, partly, by a "descent" of the developing heart. However, this elongation is due mainly to the appearance of a series of mesodermal thickenings in the wall of the cranial-most part of the foregut, i.e. future pharynx. These mesodermal thickenings are called the *pharyngeal, or branchial, arches* (Fig. 9.1).
- The cranial most part of foregut, i.e. pharyngeal part is funnel shaped to begin with. It is compressed dorso-ventrally and presents a *ventral wall* or *floor*, *dorsal wall* or *roof* and two *lateral walls*. A coronal section through the foregut (the part destined to form the pharynx), before the appearance of the pharyngeal arches, is shown in Figure 9. 2A. At this stage, the endodermal wall of the foregut is separated from the surface ectoderm by a layer of mesoderm.
- Soon, thereafter, the mesoderm comes to be arranged in the form of six cylindrical bars that run dorsoventrally in the side wall of the foregut in craniocaudal sequence. Each of these "bars" grows ventrally in the floor of the developing pharynx and fuses with the corresponding "bar" of the opposite side to form a *pharyngeal or branchial arch*. Each bar when viewed from the front is horse-shoe shaped. The arches are numbered craniocaudally as I–VI. In the interval between any two adjoining arches, the endoderm extends outward in the form of a pouch (*endodermal or pharyngeal pouch*) to meet the ectoderm which dips into this interval as an *ectodermal cleft* (Fig. 9.2B).
- The pharyngeal arches are six-curved mesodermal thickenings with each arch having an ectodermal covering and an endodermal lining containing a mesodermal core. These provide support to the ventral and lateral walls of primitive pharynx. By the time that the anterior neuropore closes, the first and second pharyngeal arches are present.
- The mesoderm of the arches is derived from paraxial mesoderm and lateral plate mesoderm. It is invaded by neural crest cells that contribute for skeletal elements and connective tissue of head and neck region.

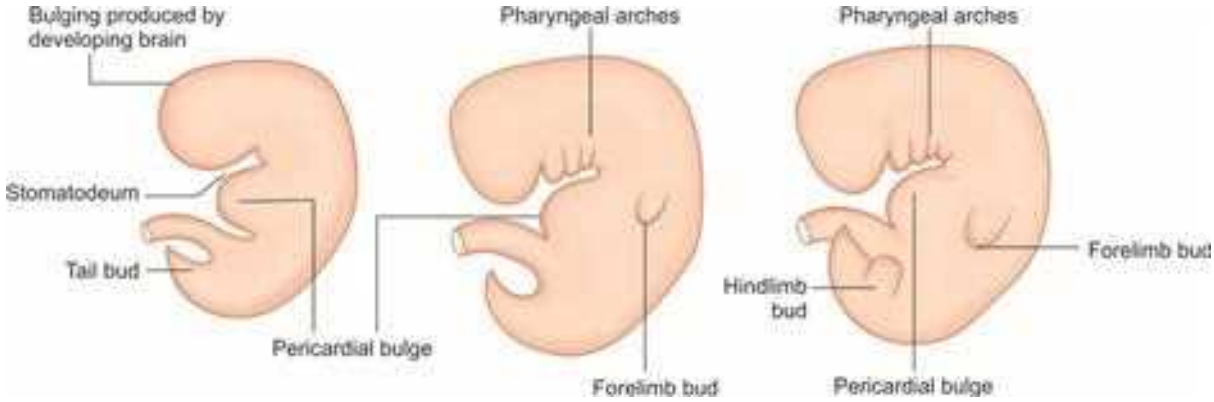
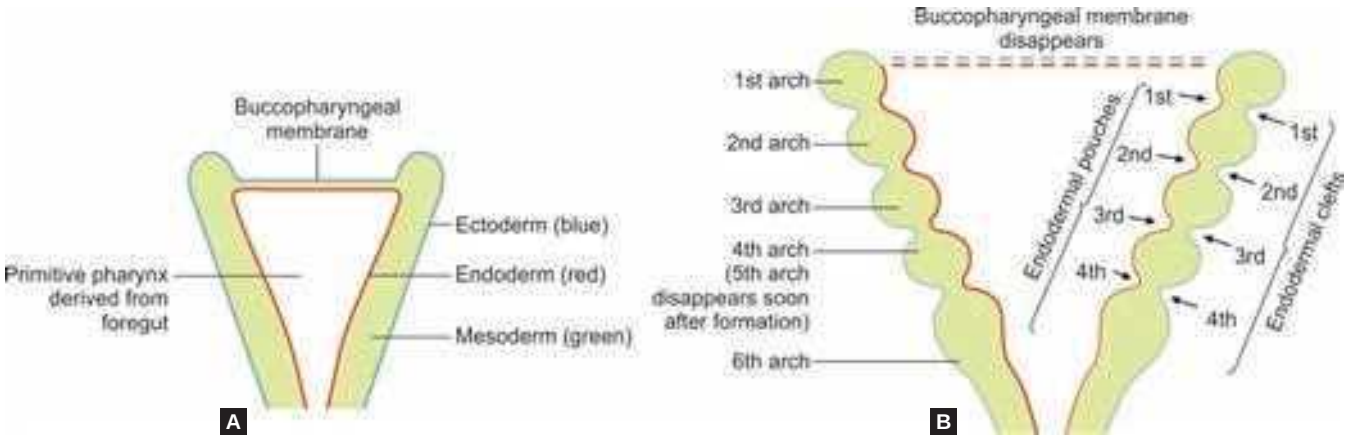


Fig. 9.1: Lateral views of embryos showing the formation of pharyngeal arches between stomatodeum and the pericardial bulge



Figs 9.2A and B: Coronal sections through cranial part of foregut. (A) Before; (B) After formation of pharyngeal arches

- The first arch is also called the *mandibular arch*; and the second, the *hyoid arch*. The third, fourth and sixth arches do not have special names. The fifth arch disappears soon after its formation, so that only five arches remain.

The following structures are formed in the mesoderm of each arch (Fig. 9.3):

- *A skeletal element*: This is cartilaginous to begin with. It may remain cartilaginous, may develop into bone, ligament or may disappear.
- *Striated muscle*: This muscle is supplied by the nerve of the arch (see below). In later development, this musculature may, or may not, retain its attachment to the skeletal elements derived from the arch. It may subdivide to form a number of distinct muscles, which may migrate away from the pharyngeal region. When they do so, however, they carry their nerve with them and their embryological origin can thus be determined from their nerve supply.
- *An arterial arch*: Ventral to the foregut, an artery called the *ventral aorta* develops. Dorsal to the foregut, another artery called the *dorsal aorta*, is formed. A series of arterial arches (*aortic arches*) connect the ventral and dorsal aortae. One such arterial arch lies in each pharyngeal arch. In a subsequent development, the arrangement of these arteries is greatly modified. *The fate of the arterial arches is considered in Chapter 15: Cardiovascular System.*
- *Nerve of the arch*: Each pharyngeal arch is supplied by a nerve derived from hind brain. In addition to supplying the skeletal muscle of the arch, it supplies sensory branches to the overlying ectoderm, and endoderm (Fig. 9.3). In some lower animals, each arch is supplied by two nerves (Fig. 9.4). The nerve of the arch itself runs along the cranial border of the arch. This is called the *posttrematic nerve* of the arch (*trema = trench*). Each arch also receives a branch from the nerve of the succeeding arch. This runs along the caudal border of the arch, and is called the *pretrematic nerve* of the arch. In the human embryo, however, a double innervation is to be seen only in the first pharyngeal arch.

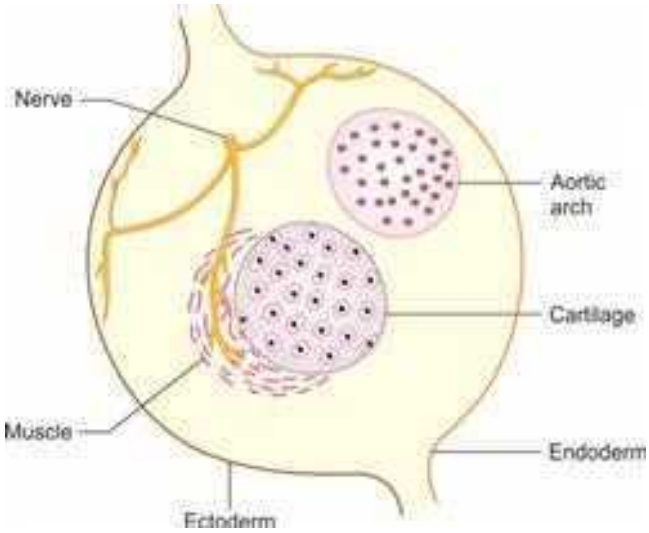


Fig. 9.3: Structures to be seen in a pharyngeal arch

DERIVATIVES OF SKELETAL ELEMENTS

- The cartilage of the first arch is called *Meckel's cartilage* (Fig. 9.5). The *incus* and *malleus* (of the middle ear) and *spine of sphenoid* are derived from its dorsal end. The ventral part of the cartilage is surrounded by the mesenchyme that forms the mandible by membranous ossification. The Meckel's

Fig. 9.4: Arrangement of nerves supplying the pharyngeal arches in some lower animals

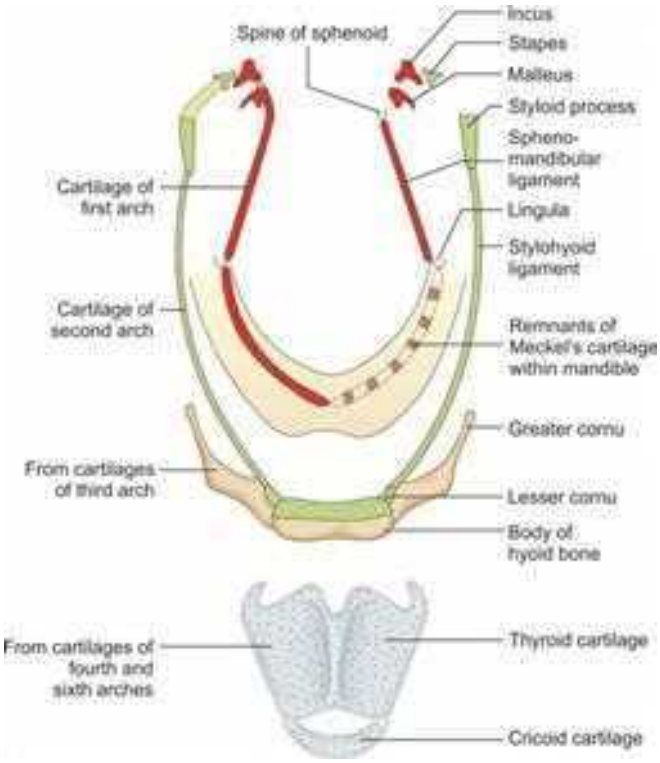


Fig. 9.5: Fate of the cartilages of the pharyngeal arches. The left half of the figure shows an earlier stage of development. The derivatives of the first arch are shown in red, second arch in green, third arch in orange, and fourth, fifth and sixth arches in blue

cartilage is thus trapped in the developing mandible, and is absorbed. The part of the cartilage extending from the region of the middle ear to the mandible disappears, but its sheath (perichondrium) forms the *anterior ligament of the malleus* and the *sphenomandibular ligament*.

The mesenchyme of ventral part of first arch forms the *mandible* and that of dorsal part (maxillary part) forms *bones of the face* including the *maxilla*, *zygomatic bone*, *palatine bone* and *part of temporal bone by* membranous ossification.

- The cartilage of the second arch (Reichert's) forms the following:
  - Dorsal end of the cartilage forms
    - Stapes
    - Styloid process.
  - Ventral part forms
    - Smaller (lesser) cornu of hyoid bone
    - Superior part of body of hyoid bone.
  - Part between ventral and dorsal parts disappears but its perichondrium forms
    - Stylohyoid ligament (from sheath).

  (*Note that all skeletal structures of second arch listed start with "S"*).
- The following structures are formed from the ventral part of cartilage of the third arch and the dorsal part disappears:
  - Greater cornu of hyoid bone
  - Lower part of the body of hyoid bone.
- The cartilages of the larynx are derived from the fourth and sixth arches that get fused with a possible contribution from the fifth arch, but their exact derivation is controversial.
- The skeletal elements of the pharyngeal arches are summarized in Table 9.1.

TABLE 9.1: Skeletal derivatives of pharyngeal arches

I Arch—Mandibular Meckel's cartilage	*II Arch—Hyoid Reichert's cartilage*	*III Arch*	*IV + VI Arches*
Malleus	Stapes	Greater cornu of hyoid	Thyroid
Incus	Styloid process	Lower half of body of hyoid	Cricoid
Mandible	Stylohyoid ligament		Corniculate
Maxilla	Lesser cornu of hyoid		Cuneiform
Zygomatic	Upper half of body of hyoid		Arytenoid
Palatine			Thyrohyoid ligament
Temporal (part)			Epiglottis—from mesenchyme of hypobranchial eminence
Anterior ligament of malleus			
Sphenomandibular ligament			

NERVES AND MUSCLES OF THE ARCHES

- All the muscles derived from a pharyngeal arch are supplied by the nerve of the arch and can, therefore, be identified by their nerve supply. The nerves of the arches and the muscles supplied by them are given in Table 9.2.
- We have already seen that these nerves not only supply muscles, but also innervate the parts of skin and mucous membrane derived from the arches. Some of the nerves (e.g. glossopharyngeal) have only a small motor component and are predominantly sensory.
- As stated above, the first arch has a *double nerve supply*. The mandibular nerve is the *posttrematic nerve* of the first arch, while the *chorda tympani* (*branch of facial nerve*) are the *pretrematic nerve*. This double innervation is reflected in the nerve supply of the anterior two-thirds of the tongue that are derived from the ventral part of the first arch.
- The other arches have only posttrematic nerves. There is a view that *tympanic branch of glossopharyngeal nerve* and *auricular branch of vagus* are pretrematic nerves for second and third arches.

Some recent investigations suggest that mesenchyme giving rise to muscles of the pharyngeal arches is derived from paraxial mesoderm cranial to the occipital somites (i.e. from the region of the preoccipital somites); and that its organization is influenced by neural crest cells. Although paraxial mesoderm here does not form typical somites, it shows partial segmentation into seven masses of mesenchyme called *somitomeres*. The structures derived from the seven somitomeres and from five occipital somites that follow them, have been described as given in Table 9.3.

If we accept this view of the origin of branchial musculature, there would be no significant reason to distinguish between it and muscle derived from somites.

FATE OF ECTODERMAL CLEFTS

- After the formation of the pharyngeal arches, the region of the neck is marked on the outside by a series of grooves, or *ectodermal clefts*.

TABLE 9.2: Nerves, arteries and muscles of pharyngeal arches

Arch	*Nerve of arch*	*Artery of arch*	*Muscles of arch*
First	Maxillary (Sensory) and mandibular (Motor and sensory) branches of trigeminal nerve	Maxillary artery	• Masseter • Temporalis • Medial pterygoid • Lateral pterygoids • Mylohyoid • Anterior belly of digastric • Tensor tympani, and • Tensor palate *All are migratory except T. tympani*
Second	Facial nerve	• Hyoid artery • Stapedial artery	• Stapedius • Stylohyoid • Posterior belly of digastric • Epicranius • Orbicularis oculi • Orbicularis oris • Zygomaticus • Buccinator • Nasal muscles • Platysma • Levator labii superioris, levator labii inferioris • Levator anguli oris • Auricular muscles *All are migratory except stapedius, stylohyoid*
Third	Glossopharyngeal nerve	• Common carotid artery • Internal carotid artery	Stylopharyngeus
Fourth	Superior laryngeal branch of vagus nerve	• Arch of aorta (left side) • Subclavian artery (right side)	• Larynx–Cricothyroid • Muscles of pharynx • Intrinsic muscles of soft palate except tensor palati
Sixth	Recurrent laryngeal branch of vagus nerve	• Ductus arteriosus (left side) • Pulmonary artery (right side)	All intrinsic muscles of larynx *except* cricothyroid

- The dorsal part of the first cleft only persists (between the first and second arches) and develops into the epithelial lining of the *external acoustic meatus* and cuticular layer of tympanic membrane (Fig. 9.6).
- The *pinna* (or auricle) is formed from a series of swellings or hillocks that arise on the first and second arches, where they adjoin the first cleft (for the development of pinna refer to Chapter 20: Development of the Ear). The ventral part of this cleft is obliterated.
- Ectodermal derivatives of first arch include skin over upper and lower jaws and tragus of ear, lips, alveolabial sulci and dental lamina (that forms enamel of tooth).
- Epibranchial placodes are specialized, thickened regions of surface ectoderm in the region of head and neck. The cells of these placodes invaginate to contribute, to the formation of sensory ganglia of cranial nerves 5th, 7th, 9th, 10th in relation with 1st arch and 1st, 2nd and 4th clefts.
- *Cervical sinus:*
  - Overgrowth of second arch mesoderm occurs. The second arch mesoderm overgrows the succeeding arches and comes to overhang them (Fig. 9.7). The space between the overhanging second arch and the third, fourth and sixth arches is called the *cervical sinus*.

TABLE 9.3: Muscles derived from somitomeres and somites

Somitomere/Somites	*Muscles derived*
Somitomeres 1 and 2	Muscles supplied by oculomotor nerve—Superior, inferior and medial recti, inferior oblique and levator palpebrae superioris
Somitomere 3	Superior oblique muscle supplied by trochlear nerve
Somitomere 4	Muscles of first pharyngeal arch supplied by mandibular nerve
Somitomere 5	Lateral rectus muscle supplied by abducent nerve
Somitomere 6	Muscles of the second pharyngeal arch supplied by the facial nerve
Somitomere 7	Stylopharyngeus (from 3rd arch) supplied by glossopharyngeal nerve
Occipital somites 1 and 2	Laryngeal muscles (from 4th to 6th arches) supplied by the vagus nerve
Occipital somites 3 to 5	Muscles of tongue supplied by hypoglossal nerve

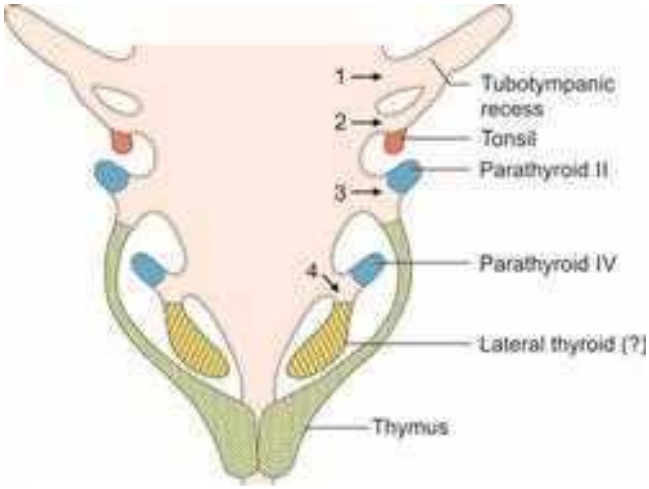


Fig. 9.6: Derivation of pharyngeal pouches including superior and inferior parathyroid glands. Note that the relative position of parathyroid III and IV is reversed during development

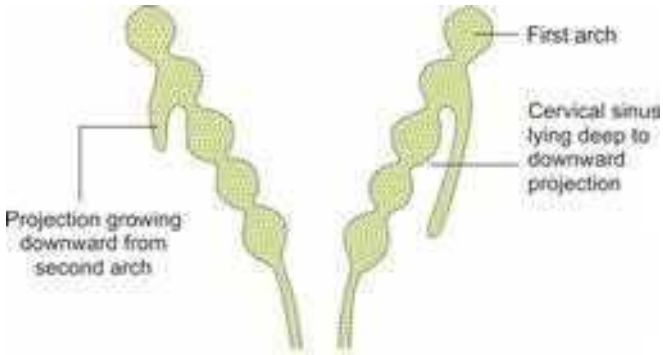


Fig. 9.7: Cervical sinus. The left half of the figure shows an earlier stage than the right half

- Subsequently, the lower overhanging border of the second arch fuses with tissues caudal to the arches (*epicardial ridge*). The side of the neck (which was thus far marked by the ectodermal clefts) now becomes smooth.
- The cavity of the cervical sinus (which is lined by ectoderm) normally gets obliterated. Part of it may persist and give rise to swellings that lie in the neck, along the anterior border of the sternocleidomastoid. These are called *branchial cysts*, and are most commonly located just below the angle of the mandible. If such a cyst opens onto the surface, it becomes a *branchial sinus*. Rarely, a cervical sinus may open into the lumen of the pharynx in the region of the tonsil.

FATE OF ENDODERMAL POUCHES

- There are five endodermal pouches. The derivatives of the pouches are divided into ventral and lateral. The ventral derivatives develop in the floor of pharynx and contribute for the development of tongue. Lateral derivatives except that of 1st are divided into those derived from *ventral* wing and those derived from *dorsal wing*.
- The endodermal pouches take part in the formation of several important organs (Figs 9.6 and 9.8). These are listed below and in Table.9.4.

First Pouch

- Its ventral part is obliterated by formation of the *tongue*.
- Its dorsal part receives a contribution from the dorsal part of the second pouch, and these two together form a diverticulum that grows toward the region of the developing ear. This diverticulum is called the *tubotympanic recess*. The proximal part of this recess gives rise to the *auditory (pharyngotympanic/Eustachian) tube*, and the distal part expands to form the *middle ear (tympanic) cavity*, including the *tympanic (mastoid) antrum*. The auditory tube is the communication between the nasopharynx and middle ear (Fig. 9.6).

Second Pouch

- The epithelium of the ventral part of this pouch contributes to the formation of the *tonsil*.
- The dorsal part takes part in the formation of the *tubotympanic recess*.

Third Pouch

The communication of this pouch with the pharynx gradually narrows and is ultimately cut off. This gives rise to the following structures that lie outside the pharynx.

- Parathyroid III or *inferior parathyroid glands*—from dorsal wing
- *Thymus*—from ventral wing.

TABLE 9.4: Derivatives of endodermal pouches

Pouch	*Derivatives*
1st pharyngeal pouch	• Tympani cavity • Auditory tube • Inner surface of the eardrum
2nd pharyngeal pouch	• Palatine tonsil • Tonsillar fossa
3rd pharyngeal pouch	• Thymus • Inferior parathyroid glands
4th pharyngeal pouch	• Superior parathyroid glands • Thyroid gland?
5th pharyngeal pouch	Ultimobranchial body

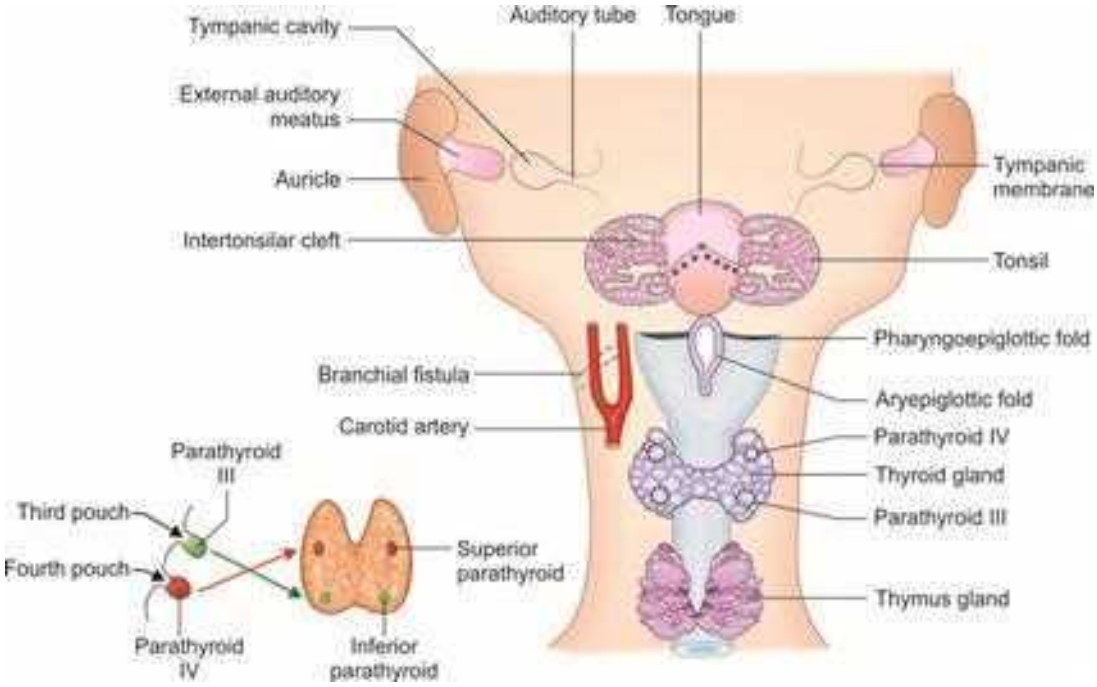


Fig. 9.8: Scheme to show the fate of the pharyngeal pouches

Fourth Pouch

The communication of this pouch with pharynx disappears. The derivatives of this pouch are:

- Dorsal wing forms—Parathyroid IV or superior parathyroid gland
- Ventral wing—may contribute to the *thyroid gland*.

Fifth or Ultimobranchial Pouch

A fifth pouch is seen for a brief period during development. In some species, it gives rise to the *ultimobranchial body*. Its fate in man is controversial.

Caudal-pharyngeal Complex

The fourth pouch joins with fifth pouch and forms caudal pharyngeal complex. The neural crest cells migrate into this complex.

- The superior parathyroid glands arise from this complex
- *Thymic element*: Incorporates into developing thymus.
- *Lateral thyroid*: Fuses with median thyroid element from hypoglossal duct. It arrests further migration of thyroid.
- *Ultimobranchial body*: It is constant in vertebrates. Incorporates into the substance of thyroid rudiment and gives origin to the parafollicular or C cells of the thyroid gland.

DEVELOPMENT OF PALATINE TONSIL

During early part of 3rd month the lining epithelium of ventral part of second pouch proliferates and forms solid cords known as *tonsillar buds* that grow into the surrounding mesoderm. Their central cells degenerate and form the hollow *tonsillar crypts*. The epithelial lines crypts and surrounding mesoderm forms the palatine tonsil. During 3rd to 5th month lymphatic tissue (in situ or adjacent mesenchyme or circulating blood) infiltrates the mesoderm. Capsule of the tonsil is formed by condensed mesoderm. Inward bulging of tonsil into pharynx occurs. Remnant of the pouch is represented by *intratonsillar crypt* (Fig. 9.6).

DEVELOPMENT OF THE THYMUS

The thymus develops from the endoderm of the third pharyngeal pouch (which also gives rise to the inferior parathyroid glands).

- Early in development, this pouch is cut off, both from the pharyngeal wall and from the surface ectoderm. After separation from the inferior parathyroid rudiment, each thymic rudiment has a thinner cranial part and a broader caudal part. The thinner portion forms the cervical part of the thymus. The broader parts, of the two sides, enter the thorax and become united to each

other by connective tissue in front of aortic sac. Descent of heart and aortic sac causes caudal migration of thymic rudiment along with parathyroid III.
- The endodermal cells of the thymus are invaded by vascular mesoderm which contains numerous lymphoblasts. This invading mesenchyme partially breaks up the thymic tissue into isolated masses, and thus gives the organ its lobulated appearance.
- The thymocytes are derived from bone marrow and the cytoreticulum and Hassall's corpuscles are derived from endoderm of 3rd pouch.
- Fragmentation of the cervical part of the thymus may give rise to accessory thymic tissue. Such tissue, present in relation to the superior parathyroid glands, is believed to arise from the fourth pouch.
- The thymus is relatively large at birth. It continues to increase in weight till puberty. Thereafter, it gradually undergoes atrophy.

DEVELOPMENT OF PARATHYROID GLANDS

- Parathyroid glands are derived as follows:
  - Inferior parathyroid glands develop from endoderm of the third pharyngeal pouch (parathyroid III).
  - Superior parathyroid glands develop from endoderm of the fourth pharyngeal pouch (parathyroid IV).
- As the third pouch also gives origin to the thymus, this organ is closely related to parathyroid III. When the thymus descends toward the thorax, parathyroid III is carried caudally along with it for some distance.
- Meanwhile, parathyroid IV is prevented from descending caudally, because of the close relationship of the fourth pouch to the developing thyroid gland. As a result, parathyroid III becomes caudal to parathyroid IV.
- Hence, the parathyroid glands derived from the fourth pouch become the superior parathyroid glands and those derived from the third pouch become the inferior parathyroid glands (Fig. 9.6).
- In keeping with their developmental history, the superior parathyroid glands are relatively constant in position, but the inferior parathyroid glands may descend into the lower part of the neck or even into the anterior mediastinum. Alternatively, they may remain at their site of origin and are then seen near the bifurcation of the common carotid artery.

DEVELOPMENT OF THYROID GLAND

- The *thyroid gland develops mainly from the thyroglossal duct*.
- *Parafollicular cells* are derived from the caudal pharyngeal complex (derived from the fourth and fifth pharyngeal pouches).
- After the formation of the pharyngeal arches, the floor of the pharynx has the appearance shown in Figure 9.9. The medial ends of the two mandibular arches are separated by a midline swelling called the *tuberculum impar*. Immediately behind the tuberculum, the epithelium of the floor of the pharynx shows a thickening in the middle line (Fig. 9.10A). This region is soon depressed below the surface to form a diverticulum called the *thyroglossal duct* (Fig. 9.10B).
- The site of origin of the diverticulum is now seen as a depression called the *foramen cecum*. The diverticulum grows down in the midline into the neck. Its tip soon bifurcates (Fig. 9.10C). Proliferation of the cells of this bifid end gives rise to the two lobes of the thyroid gland.
- The developing thyroid comes into intimate relationship with the caudal pharyngeal complex and fuses with it (Fig. 9.10D). Cells arising from this complex are believed to give origin to the parafollicular cells of the thyroid which may represent the ultimobranchial body of lower animals.

Pharyngeal Apparatus/Branchial Apparatus

It includes:
- Pharyngeal arches (mesoderm)
- Pharyngeal clefts (ectoderm)
- Pharyngeal pouches (endoderm)

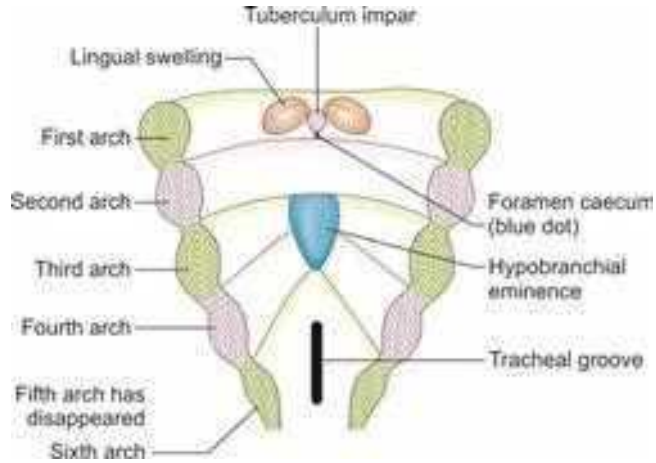
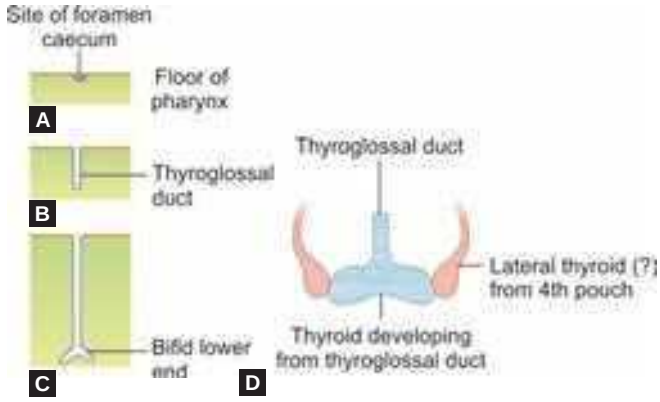


Fig. 9.9: Floor of the pharynx showing the foramen cecum from where the thyroglossal duct arises



Figs 9.10A to D: Stages in the development of the thyroid gland

- Pharyngeal membranes—they are the site of contact of pouches and clefts. Initially, there are four membranes. As most clefts are filled with extension of 2nd arch mesoderm over the others, only first membrane develops. This lies close to external auditory meatus and develops into the *tympanic membrane.*

The pharyngeal apparatus contributes for the formation of scalp, face, neck, definitive mouth, pharynx and larynx.

Clinical correlation

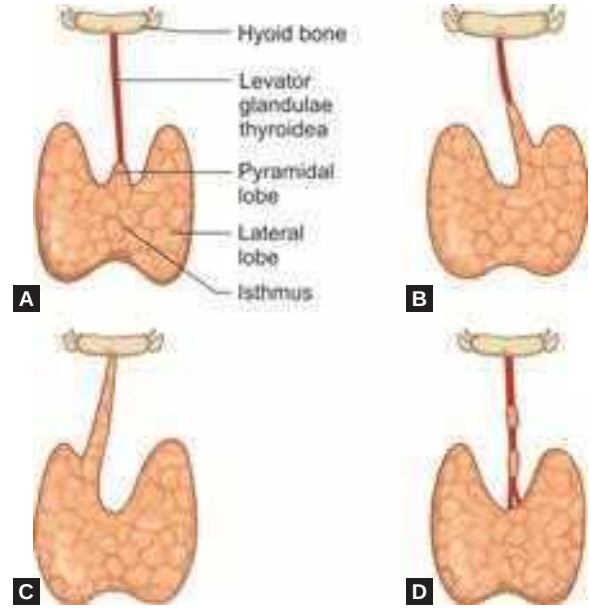
Anomalies of the thyroid gland

- ***Anomalies of shape*:**
  - The pyramidal lobe is present so often that it is regarded as a normal structure. It may arise from the isthmus (Fig. 9.11A) or from one of the lobes (Figs 9.11B and C). It may have no connection with the rest of the thyroid and may be divided into two or more parts (Fig. 9.11D). In extent, it may vary from a short stump (Fig. 9.11A) to a process reaching the hyoid bone (Fig. 9.11C).
  - The isthmus may be absent (Fig. 9.12A).
  - One of the lobes of the gland may be very small (Fig. 9.12B), or absent (Fig. 9.12C).
- ***Anomalies of position (Fig. 9.13)*:**
  - *Lingual thyroid*: The thyroid may lie under the mucosa of the dorsum of the tongue and may form a swelling that may cause difficulty in swallowing.
  - *Intralingual thyroid*: The thyroid may be embedded in the muscular substance of the tongue.
  - *Suprahyoid thyroid*: The gland may lie in the midline of the neck, above the hyoid bone.
  - *Infrahyoid thyroid*: The gland may lie below the hyoid bone, but above its normal position.
  - *Intrathoracic thyroid*: The entire gland, or part of it, may lie in the thorax. Note that when thyroid tissue is present in the anomalous positions described above, an additional thyroid may or may not be present at the normal site.
- ***Ectopic thyroid tissue*:** Small masses of thyroid tissue may be present at abnormal sites.
  - Thyroid tissue has been observed in the larynx, trachea, esophagus, pons, pleura, pericardium and ovaries.
  - Masses of ectopic thyroid tissue have been described in relation to the deep cervical lymph nodes (*lateral aberrant thyroids*) but these are now believed to represent metastases in the lymph nodes from a carcinoma of the thyroid gland.
- ***Remnants of the thyroglossal duct:*** These remnants may persist and lead to the formation of:
  - *Thyroglossal cysts* that may occur anywhere along the course of the duct. They may acquire secondary openings on the surface of the neck to form fistulae.
  - *Thyroglossal fistula* opening at the foramen cecum.
  - *Carcinoma of the thyroglossal duct.*

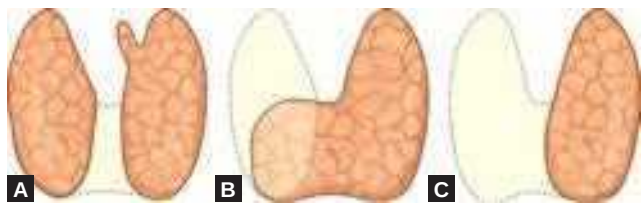
In the surgical removal of thyroglossal cysts and fistulae, it is important to remove all remnants of the thyroglossal duct. In this connection, it has to be remembered that the duct is intimately related to the hyoid bone (Fig. 9.13).

Molecular regulation and genetic basis of pharyngeal arch development

The formation of pharyngeal arches is controlled by pharyngeal arch endoderm. Lateral migration of pharyngeal endodermal cells forms the pharyngeal pouches. This migration is controlled by fibroblast growth factor (FGF-8), bone mineral protein (BMP7), paired box gene (PAX-1), and sonic hedgehog (SHH) gene.



Figs 9.11A to D: Variations in the pyramidal process of the thyroid gland



Figs 9.12A to C: Anomalies of the thyroid gland. The parts of the gland shown in dotted outline are missing

Clinical correlation

***Congenital anomalies because of persistence of cervical sinus*:**

- ***Branchial cyst*:** Persistence of cervical sinus as a cyst along the anterior border of sternomatoid muscle. If the cyst ruptures it results in branchial sinus.
- ***Branchial sinus*:** It can be external or internal.
  - *External*: If the cyst opens outside, usually anterior to sternomastoid.
  - *Internal*: Cyst opens into pharynx, usually in the tonsillar region.
- ***Branchial/Cervical fistula*:** The cyst opens both externally and internally. Connects pharynx with outside.

First-arch syndrome: These are congenital defects caused by a failure of migration of neural crest cells into the first pharyngeal arch. They usually produce facial anomalies.

- ***Treacher Collins syndrome (Mandibulo-facial dysostosis)*:**
  - It is a rare autosomal dominant disorder with 1:50,000 incidences.
  - Clinically, it presents mandibular and malar (zygomatic) hypoplasia, down slanting palpebral fissure and ear defects (external/middle ear, conductive deafness).
  - Genetic defect: Mutation of TCOF1 (Treacle) gene located on chromosome 5
- ***Pierre Robin syndrome*:**
  - *Genetic cause*—anomalies in chromosomes 2, 11 or 17. Genetic dysregulation of *SOX9* gene that controls development of face. The incidence of this condition is 1:8,500.
  - *Clinical presentation*: Unusually small mandible (micrognathia), posterior displacement or retraction of the tongue (glossoptosis) and upper airway obstruction. Cleft palate (incomplete closure of the roof of the mouth) is present in majority of cases. An ultrasound image showing fetus with micrognathia, cleft palate and cleft lip (Fig. 9.14). It can be syndromic or nonsyndromic.

TIME TABLE OF SOME EVENTS IN THE DEVELOPMENT OF PHARYNGEAL ARCHES

Time table of some events described in this chapter is shown in Table 9.5.

TABLE 9.5: Time table of developmental events

Age	*Developmental events*
4th week (22nd day)	Appearance of 1st and 2nd arches
5th week (29th day)	Four arches are seen. Thyroid, parathyroid and thymus start forming
7th week	Thyroid gland reaches its definitive position

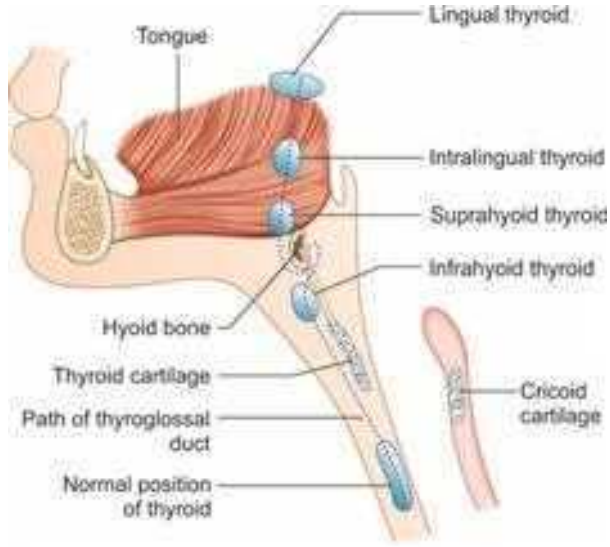


Fig. 9.13: Path of thyroglossal duct. Note that thyroid tissue may come to lie anywhere along the course of this duct

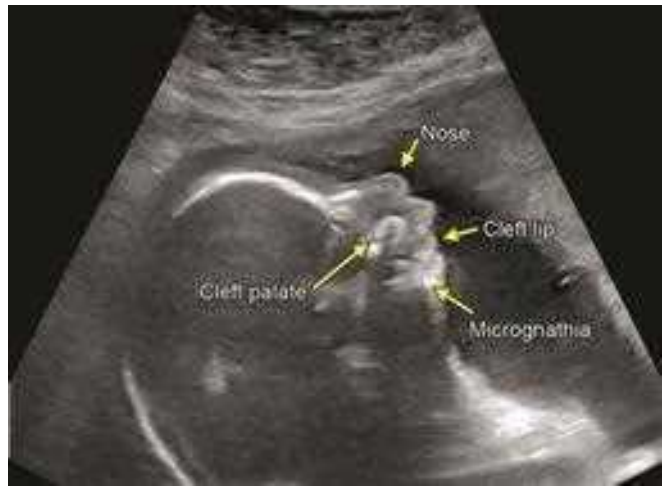


Fig. 9.14: Ultrasound image of a fetus with micrognathia, cleft lip and cleft palate. *Image courtesy:* Dr Ganesh Kumar and Dr Sasikala

EMBRYOLOGICAL EXPLANATION FOR CLINICAL CONDITIONS OR ANATOMICAL OBSERVATIONS

Case Scenario 1

A child of 5 years was brought to the pediatric surgeon with a complaint of recurrent discharge from an opening in the right lateral neck. On examination of the patient, a small external

opening at the middle of anterior border of sternomastoid muscle was observed with a spontaneous thick discharge. Based on the MRI of neck the pediatric surgeon diagnosed it as a cervical fistula with its direction as shown in Figure 9.6. What is the embryological basis for this condition? Give explanation for the position of the fistula.

- It is a case of branchial or cervical fistula. It is a congenital, abnormal tract connecting the skin of the neck with an internal structure. It results from failure of closure of 2nd to 4th branchial clefts.
- Rapid growth of mesenchyme of second arch forms the cervical sinus between the overhanging second arch and subsequent arches. Subsequently, with the obliteration of cervical sinus the subsequent arches are buried and the ectodermal clefts between the arches are obliterated giving a smooth contour to the neck.
- If the cervical sinus persists, it results in the formation of branchial cyst, anywhere along the anterior border of sternomastoid muscle. Rupture of branchial cyst forms the branchial fistula that can open outside. Rarely the fistula opens inside only. It may communicate both inside and outside forming a communication of external opening with the interior of pharynx.
- In the present case, it is a fistula in relation to the 2nd branchial cleft between 3rd and 4th branchial arches. The 2nd branchial arch contributes for the formation of hyoid bone and its endodermal pouch forms the tonsil and the cleft forms the tonsillar fossa. The fistula is travelling between internal and external carotid arteries. Internal opening of fistula is at level of tonsillar fossa. External opening is found along the anterior border of sternocleidomastoid muscle.

REVIEW QUESTIONS

1. What are the derivatives of first pharyngeal arch?
2. Name the nerves of pharyngeal arches.
3. Name the skeletal elements of pharyngeal arches.
4. Describe cervical sinus.
5. Write short notes on derivatives of pharyngeal pouches.
6. Write short notes on development of thymus.
7. Write short notes on development of parathyroid glands.
8. Write short notes on development of thyroid gland.
9. Write short notes on development of palatine tonsil.

Chapter **10**

Skeletal System and Muscular System

HIGHLIGHTS

- Subdivisions of intraembryonic mesoderm are *paraxial mesoderm*, *intermediate mesoderm* and *lateral plate mesoderm*.
- The paraxial mesoderm extends as a longitudinal column on either side of the notochord and the developing neural tube. With the formation of otic vesicle (neuroectodermal thickenings that form the membranous labyrinth of internal ear), the paraxial mesoderm is divided into preotic and postotic parts. The preotic part is unsegmented and is called *head mesoderm* (*somitomeres*). The postotic part undergoes segmentation into 40–45 pairs of segments called *somites*.
- *Somites* undergo division into three parts: (1) the *dermatome* which forms the dermis of the skin; (2) *myotome* which forms skeletal muscle; and (3) *sclerotome* which helps to form the vertebral column and ribs.
- The skull is divided into neurocranium and viscerocranium. Viscerocranium forms the facial skeleton. The neurocranium forms the bones around the brain.
- The neural crest cells enter the head mesoderm and both together contribute for facial skeleton (viscero/splanchnocranium) and membranous neurocranium. The chondrocranium or base of skull rostral to the level of pituitary gland is formed by neural crest cells. The part posterior to it is formed by occipital sclerotomes.
- The *vertebral column* is derived from the sclerotomes of somites. Each sclerotome divides into three parts: (1) cranial; (2) middle; and (3) caudal.
- A *vertebra* is formed by fusion of the caudal part of one sclerotome and the cranial part of the next sclerotome. It is, therefore, intersegmental in position.
- The middle part of the sclerotome forms an *intervertebral disc*, which is therefore segmental in position.
- The *sternum* is formed by fusion of right and left sternal bars.
- The *skull* develops from mesenchyme around the developing brain. Some skull bones are formed in membrane (e.g. parietal); some partly in membrane and partly in cartilage (e.g. sphenoid); and a few entirely in cartilage (e.g. ethmoid).
- The *mandible* is formed in membrane from the mesenchyme of the mandibular process.
- Limbs are first seen as outgrowths (limb buds) from the side wall of the embryo. Each bud grows and gets subdivided to form parts of the limb.
- *Limb bones* develop from mesenchyme of the limb buds. Joints are formed in intervals between bone ends.
- All muscles of the body develop from mesoderm *except* muscles of iris, arrectores pilorum of skin and myoepithelial cells lining ducts of sweat glands.
- *Skeletal muscle* is derived partly from somites and partly from mesenchyme of the region.
- Most *smooth muscle* is formed from mesenchyme related to viscera and blood vessels.
- *Cardiac muscle* is formed from mesoderm related to developing heart.

PART 1: SKELETAL SYSTEM

INTRODUCTION

The intraembryonic mesoderm divides into paraxial, intermediate and lateral plate mesoderm. The paraxial mesoderm extends as a longitudinal column on either side of the notochord and the developing neural tube. The developing *otic capsules* (neuroectodermal thickenings that form the membranous labyrinth of internal ear) divide the paraxial mesoderm into *preotic* and *postotic* parts. The preotic part of paraxial mesoderm is the unsegmented head mesoderm (*somitomeres*). The postotic part of paraxial mesoderm shows 40–45 pairs of segments called *somites* that appear in craniocaudal sequence. Somites undergo division into three parts. These are:

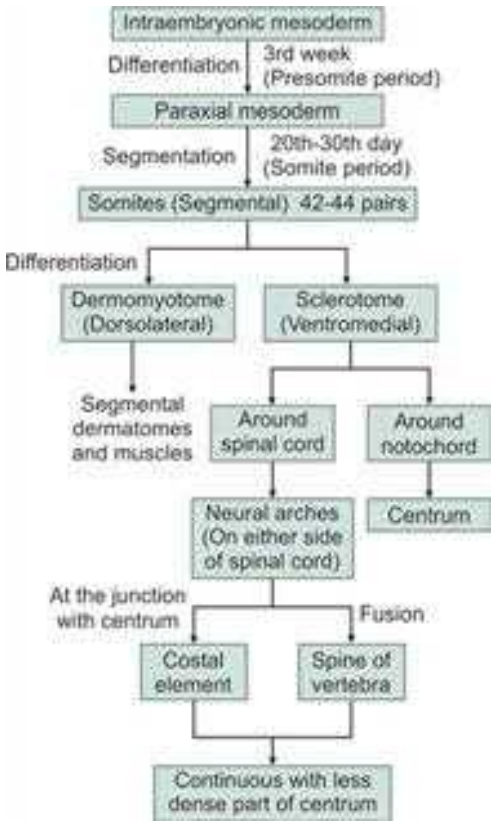
1. *Dermatome* which forms the segmental dermis of the skin.
2. *Myotome* which forms the skeletal muscle.
3. *Sclerotome* which helps to form the vertebral column.

Some books refer them as dermomyotome and sclerotome as shown in Flowchart 10.1.

Skeletal system includes cartilage and bone. The process of formation of cartilage and bone has been considered in Chapter 7. Skeleton is classified into axial skeleton and appendicular skeleton. All bones are of mesodermal origin. Bones can be classified as cartilaginous bones or membranous bones or membrano-cartilaginous bones on the basis of their mode of ossification.

- Most bones of the axial skeleton are derived from sclerotomes of *somites* (paraxial mesoderm) and *head mesoderm*.
- Bones of the shoulder and hip girdle, and of the limbs, arise from *somatopleuric layer of lateral plate mesoderm*.
- Some bones of the face and skull are derivatives of the *mesoderm of pharyngeal arches* that are invaded by neural crest.

Flowchart 10.1: Development of vertebra



SOMITES

The paraxial mesoderm becomes segmented to form 40–45 pairs of somites that lie on either side of the developing neural tube (Figs 5.6 to 5.8) and notochord.

The somites appear between the 20th and 30th day of development. Hence, the 4th week of development is known as *somite period* of development.

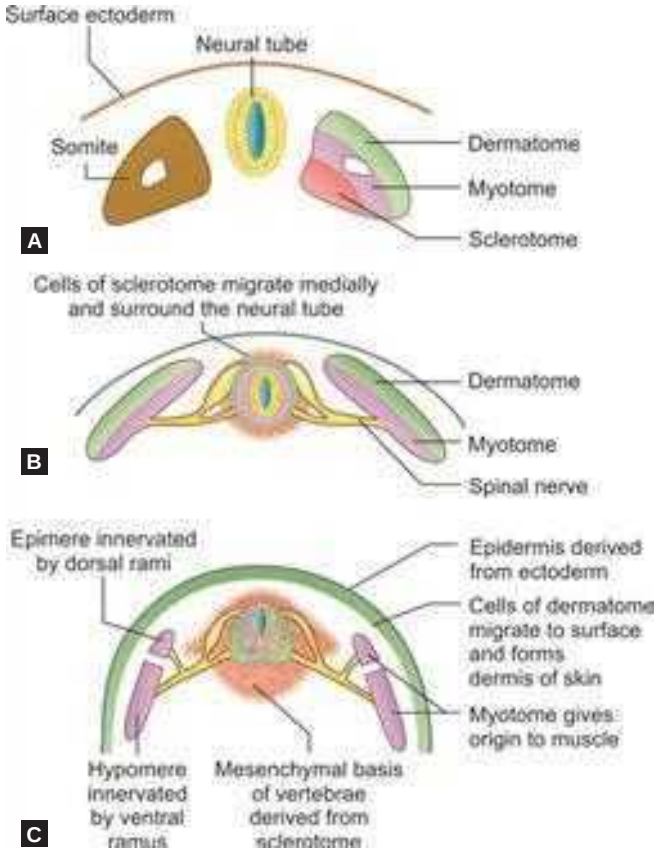
A cross section through a somite shows that it is a triangular structure and has a cavity (Figs 10.1A to C). The somite is divisible into three parts.

1. The ventromedial part is called the *sclerotome*. The cells of the sclerotome migrate medially. They surround the neural tube and give rise to the vertebral column and ribs (Figs 10.1A to C).
2. The lateral part is called the *dermatome*. The cells of this part also migrate, and come to line the deep surface of the ectoderm covering the entire body. These cells give rise to the dermis of the skin and to subcutaneous tissue (Figs 10.1A to C).
3. The intermediate part is the *myotome*. It gives rise to striated muscle as described in the following section (Figs 10.1A to C).

Recently, it has been held that the dermatome only forms dermis on the back of the head and trunk, and that dermis elsewhere is derived from lateral plate mesoderm.

In the cervical, thoracic, lumbar and sacral regions, one spinal nerve innervates each myotome. The number of somites formed in these regions, therefore, corresponds to the number of spinal nerves. In the coccygeal region, the somites exceed the number of spinal nerves but many of them subsequently degenerate. The first cervical somite is caudal to tip of notochord (which becomes the apical ligament of dens of axis vertebra). The first cervical is *not* the most cranial somite to be formed. Cranial to it, there are:

- *Occipital somites* (4–5) which give rise to muscles of the tongue and are supplied by the hypoglossal nerve.
- *Preoccipital* (or *preotic*) *somites* (somitomeres), supplied by the third, 4th and 6th cranial nerves.



Figs 10.1A to C: (A) Somites lying on either side of the neural tube. Note subdivisions of somite. (B) The cells of the sclerotome have migrated medially and now surround the neural tube. The myotome is innervated by nerves growing out of the neural tube. (C) The cells of the dermatome have migrated to form the dermis of the skin

- Total number of somites and their classification craniocaudally is shown in Table 10.1.

DEVELOPMENT OF AXIAL SKELETON

The axial skeleton consists of vertebral column, ribs, sternum and skull.

Vertebral Column

- The vertebral column is formed from the sclerotomes of the somites. The cells of each sclerotome get converted into loose mesenchyme. This mesenchyme migrates medially and surrounds the notochord (Flowchart 10.1 and Fig. 10.2).
- The mesenchyme then extends backward on either side of the neural tube and surrounds it (Flowchart 10.1 and Fig. 10.3). Extensions of this mesenchyme also take place laterally in the position to be subsequently occupied by the transverse processes, and ventrally in the body wall, in the position to be occupied by the ribs.
- For some time, the mesenchyme derived from each somite can be seen as a distinct segment. The mesenchymal cells of each segment are at first uniformly distributed (Fig. 10.4A).
- However, the cells soon become condensed in a region that runs transversely across the middle of the segment. This condensed region is called the *perichordal disc*.

TABLE 10.1: Distribution of somites and their skeletal and muscular derivatives

Somites	*Number of pairs*	*Skeletal elements*	*Musculature*
Preoccipital	3	Base of skull	Extraocular muscles of eyeball
Occipital	4–5		Tongue musculature except palatoglossus
Cervical	8	Vertebra	Striated muscles of trunk, diaphragm, limbs
Thoracic	12	Vertebra and ribs	
Lumbar	5	Vertebra	
Sacral	5		
Coccygeal	8–10		

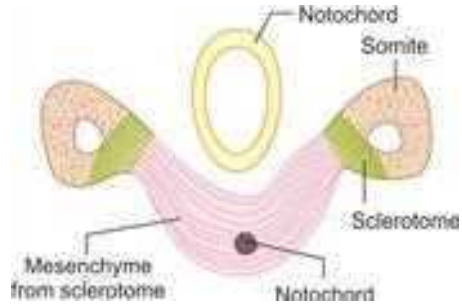


Fig. 10.2: Formation of mesenchymal basis of the body of a vertebra from a sclerotome

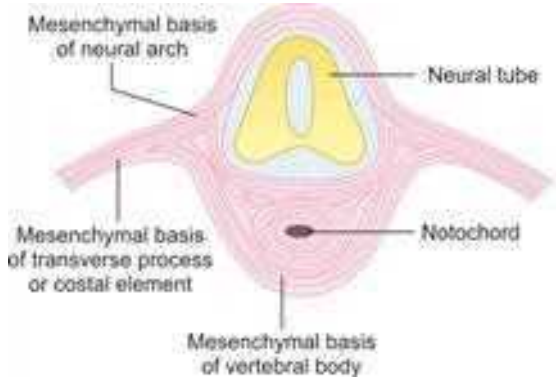


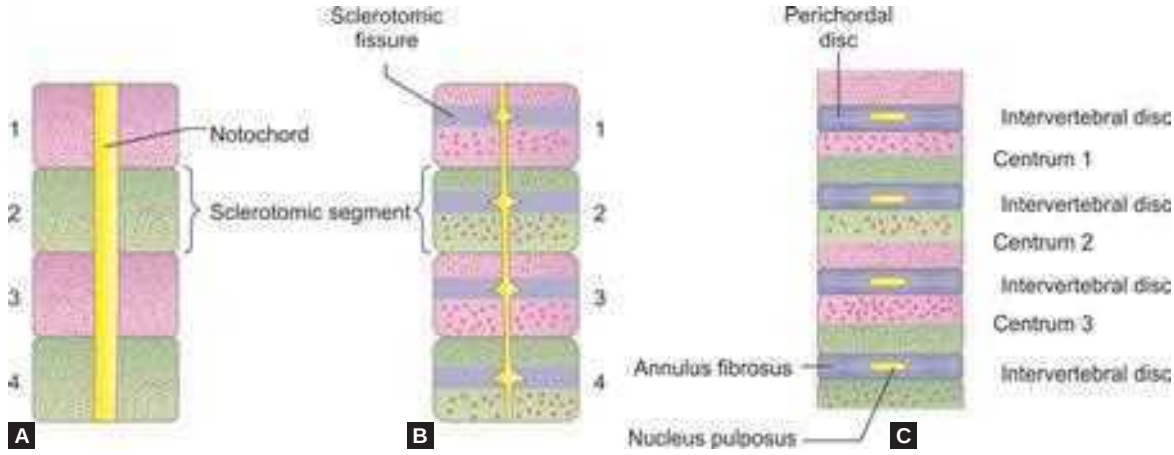
Fig. 10.3: Formation of mesenchymal basis of the neural arch and of the costal element

Above and below it there are less condensed and more condensed parts (Fig. 10.4B).

- The mesenchymal basis of the *body* (or *centrum*) of each vertebra is formed by fusion of the more condensed part of one sclerotomic segment with the less condensed part of adjoining segment (Fig. 10.4C).
- The perichordal disc becomes the *intervertebral disc*.
- The neural arches and their processes are continuous with the less dense part of sclerotomic segment. The neural arch, the transverse processes and the costal elements are formed in the same way as the body.
- The interspinous and intertransverse ligaments are formed in the same manner as the intervertebral disc.
- The notochord disappears in the region of the vertebral bodies. In the region of the intervertebral discs, the notochord becomes expanded and forms the nucleus pulposus (Fig. 10.5).

From the above account, we may note that (Fig. 10.5, Flowchart 10.2):

- The vertebra is an intersegmental structure made up from portions of two somites.
- The intervertebral disc is formed at the center of the somite.
- The transverse processes and ribs are also intersegmental. They separate the muscles derived from two adjoining myotomes.



Figs 10.4A to C: (A) Mesenchyme derived from somites is seen in the form of segments. (B) Each segment has a central condensed part, and cranial less dense and caudal more dense parts. (C) Centrum of a vertebra is formed by fusion of more dense caudal part of one sclerotomic segment with the cranial less dense part of adjacent sclerotomic segment. Hence, it is an intersegmental structure. Each intervertebral disc is derived from the condensed part of one somite. Hence, it is segmental in position

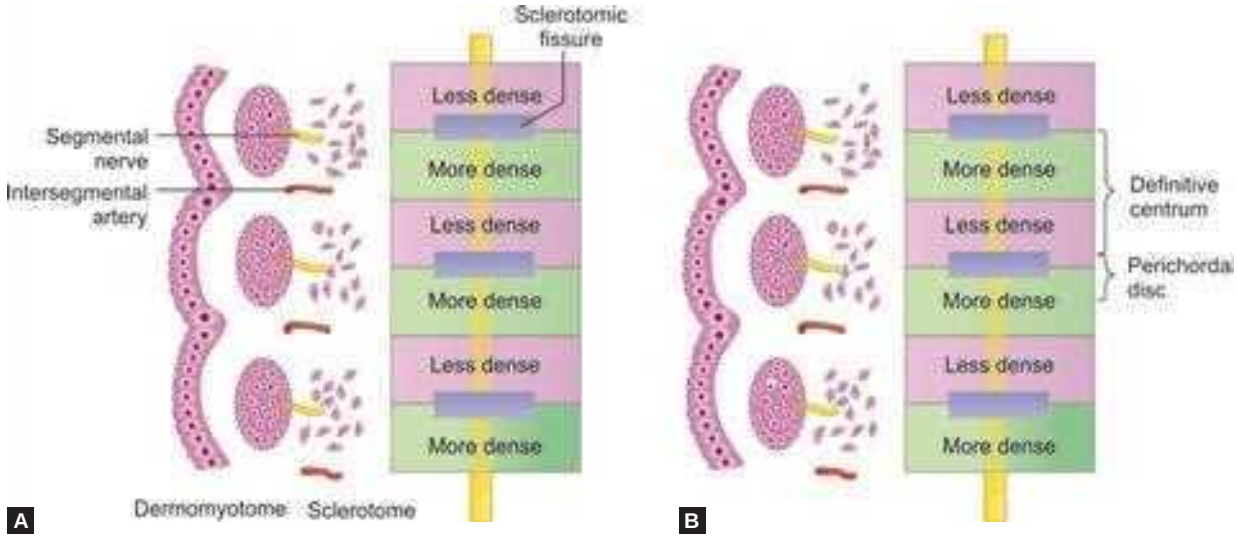
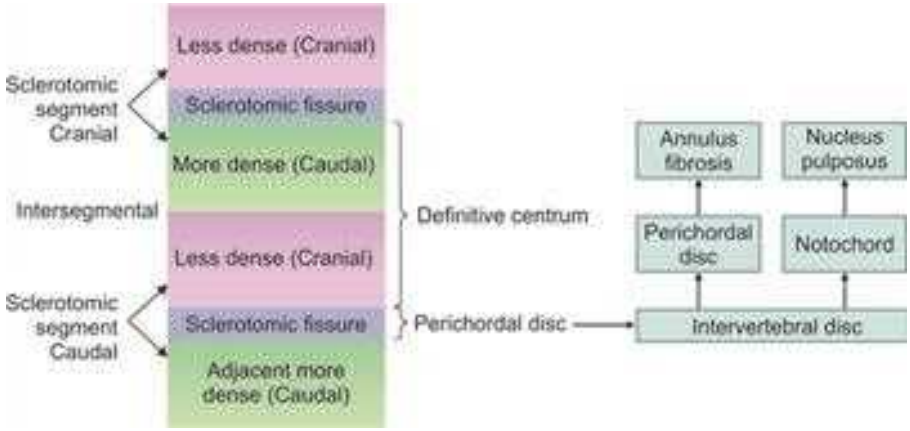
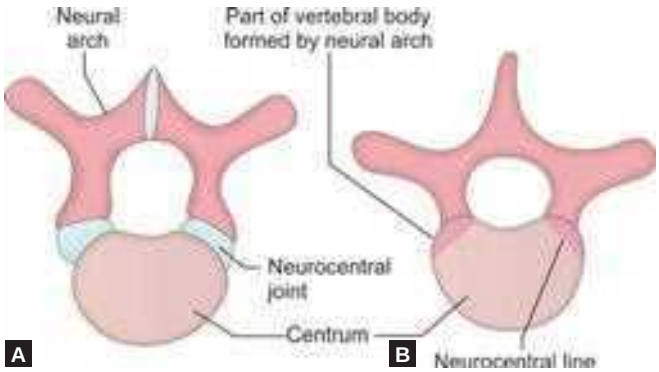


Fig. 10.5: Showing the segmental and intersegmental components of the parts of a vertebra and the nerves and arteries

Flowchart 10.2: Development of vertebra



  - Spinal nerves are segmental structures. They, therefore, emerge from between the two adjacent vertebrae and lie between two adjacent ribs.
  - The blood vessels supplying structures derived from the myotome (e.g. intercostal vessels) are intersegmental like the vertebrae. Therefore, the intercostal and lumbar arteries lie opposite the vertebral bodies.
- The mesenchymal basis of the vertebra is converted into cartilage by the appearance of several centers of chondrification. Three primary centers of ossification appear in the cartilaginous model for each vertebra; one for each neural arch and one for the greater part of the body (centrum).
- At birth, the centrum and the two halves of the neural arch are joined by cartilage (Fig. 10.6A). These are termed neurocentral joints. Note that the posterolateral parts of the vertebral body are formed from the neural arch (Fig. 10.6B). After the centrum and neural arch have fused, the junction between the two is indicated by the neurocentral line.
- In the cervical, thoracic, lumbar and sacral regions, the contributions to the various parts of the vertebrae by the centrum, neural arches and costal elements are shown in Figure 10.7 and Table 10.2.



Figs 10.6A and B: (A) A vertebra at birth consisting of three separate pieces of bone: a centrum and two neural arches. (B) Diagram to show the neurocentral line which is the line along which body and neural arch have fused

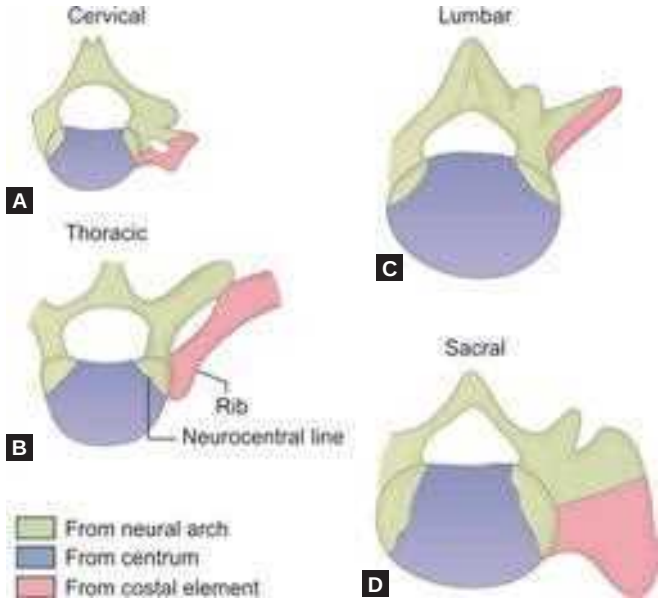


Fig. 10.7: Relative contribution to vertebrae by the centrum, the neural arch and the costal element in different regions. Note that a small part of the body of the vertebra is derived from the neural arch

TABLE 10.2: Adult vertebral and rib derivatives from various embryonic components

Region	Centrum	Neural arch	Costal element	Transverse element
Cervical	B O D Y	Pedicles Laminae Spine Articular processes	Anterior root Anterior tubercle Costotransverse bar Posterior tubercle	Posterior root
Thoracic			Rib	Transverse process
Lumbar			Transverse process	Mammillary process Accessory mammillary process
Sacral			Anterior 2/3rd of lateral mass	Posterior 1/3rd of lateral mass

Clinical correlation

Congenital anomalies of vertebral column

- One or more vertebrae may be absent, the caudal vertebrae being more commonly affected. Absence of the coccyx alone, or of the sacrum and coccyx, may be seen.
- Additional vertebrae may be present. The sacrum may show six segments.
- Part of a vertebra may be missing. Various anomalies result, depending on the part that is absent (Fig. 17.34).
  - The two halves of the neural arch may fail to fuse in the midline. This condition is called *spina bifida* (Fig. 10.8). The gap between the neural arches may not be obvious (*spina bifida occulta*), or may be large enough for meninges and neural elements to bulge out of it (see meningocele and *meningomyelocele*). Spina bifida in a fetus can be recognized by ultrasound examination. Examination of amniotic fluid shows increased levels of alpha-fetoproteins (AFPs) in a case with spina bifida.
  - The vertebral body may ossify from two primary centers which soon fuse. One of these parts may fail to develop, resulting in only half of the body being present. This is called *hemivertebra*. It is usually associated with absence of the corresponding rib.
  - The two halves of the vertebral body may be formed normally but may fail to fuse. The vertebral body then consists of two hemivertebrae. Sometimes the gap between the two halves is large enough for meninges and nerves to bulge forward between them (*anterior spina bifida*).
- Two or more vertebrae that are normally separate may be fused to each other. Such fusion may occur in the cervical region (***Klippel-Feil syndrome***). The atlas vertebra may be fused to the occipital bone (***occipitalization of atlas***) (Fig. 10.9). The fifth lumbar vertebra may be partially or completely fused to the sacrum (***sacralization of 5th lumbar vertebra***).
- Parts of the vertebral column that are normally fused to each other may be separate. The first sacral vertebra may be separate from the rest of the sacrum (***lumbarization of the 1st sacral vertebra***). The odontoid process may be separate from the rest of the axis vertebra.
- The articular facets may be abnormal in orientation, or may be deficient. When there is deficiency of both the inferior articular processes of the fifth lumbar vertebra, the body of the vertebra may slip forward over the sacrum. This is called ***spondylolisthesis***.
- The vertebral canal may be divided into two lateral halves by a projecting shelf of bone, which splits the spinal cord longitudinally into two halves (***diastematomyelia***).
- Ossification of the vertebral bodies may be defective thus reducing the total length of the spine. This can lead to the formation of dwarfs who have a short trunk but have limbs of normal length (***chondro-osteodystrophy***).
- A peculiar tumor arising from cells of the primitive knot may be seen attached to the lower end of the spine. Various tissues may be seen in it. Such a growth is called a ***sacrococcygeal teratoma.***
- Anomalies of the vertebrae are of practical importance in that:
  - They may cause deformities of the spine. The spine may be bent on itself (*congenital scoliosis*). Deformities of cervical vertebrae may lead to tilting of the head to one side and its rotation to the opposite side (*congenital torticollis*). This deformity may be secondary to a contracture of the sternocleidomastoid muscle.
  - The spinal nerves, or even the spinal cord, may be implicated. They may be subjected to abnormal pressure leading to paralysis.
  - They are frequently the cause of backache.

Ribs

- The ribs are derived from ventral extensions of the sclerotomic mesenchyme that forms the vertebral arches. These extensions are present not only in the thoracic region but also in the cervical, lumbar and sacral regions.
- They lie ventral to the mesenchymal basis of the transverse processes with which they are continuous. In the thoracic region, the entire extension (called the

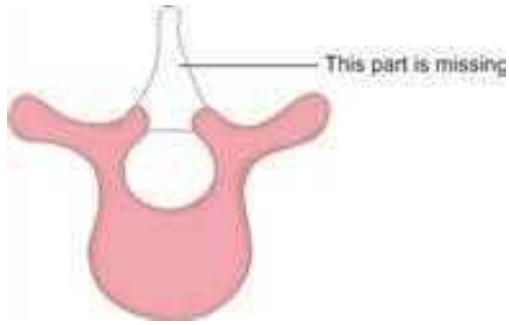


Fig. 10.8: Spina bifida produced by nonfusion of the two halves of the neural arch

primitive costal arch) undergoes chondrification, and subsequent ossification, to form the ribs.

- However, some mesenchyme between it and the developing transverse process does not undergo chondrification: it becomes loose and forms the *costotransverse joint*.
- In the cervical, lumbar and sacral regions, chondrification and ossification of the costal arch are confined to the region in immediate relationship to the transverse process. The bone formed from the arch is fused to the transverse process and is referred to as the *costal element* of the process. The contributions made by the costal element to the cervical, lumbar and sacral vertebrae are shown in Figures 10.7A to D and Table 10.2.

Sternum

The sternum is formed by fusion of two *sternal bars*, or plates, that develop on either side of the midline. Mesenchymal condensations forming at these sites become cartilaginous in the 7th week of intrauterine life. Laterally, the sternal bars are continuous with ribs. The fusion of the two sternal bars first occurs at their cranial end (manubrium) and proceeds caudally (Figs 10.10B and C).

The manubrium and the body of the sternum are ossified, separately. The xiphoid process ossifies only late in life.

Clinical correlation

Anomalies of the sternum and ribs

- Some ribs that are normally present may be missing. Unilateral absence of a rib is often associated with hemivertebra.
- Accessory ribs may be present. Such a rib may be attached to the seventh cervical vertebra (***cervical rib***), or to the first lumbar vertebra (***lumbar rib***).
- When the fusion of the two sternal bars is faulty, the body of the sternum shows a partial or even a complete midline cleft. Minor degrees of nonfusion may result in a bifid xiphoid process or in midline foramina. Transverse clefts may also occur.
- In the condition called ***funnel chest***, the lower part of the sternum and the attached ribs are drawn inward into the thorax. The primary defect is that the central tendon of the diaphragm is abnormally short.
- The upper part of the sternum (and related costal cartilages) may project forward (***pigeon breast***).

Skull

- The bones of skull (cranium) develop around the developing brain.
- Cranial to the first cervical somite there are four *occipital somites*. The mesenchyme arising from the sclerotomes of these somites helps to form part of the base of the skull in the region of the occipital bone.

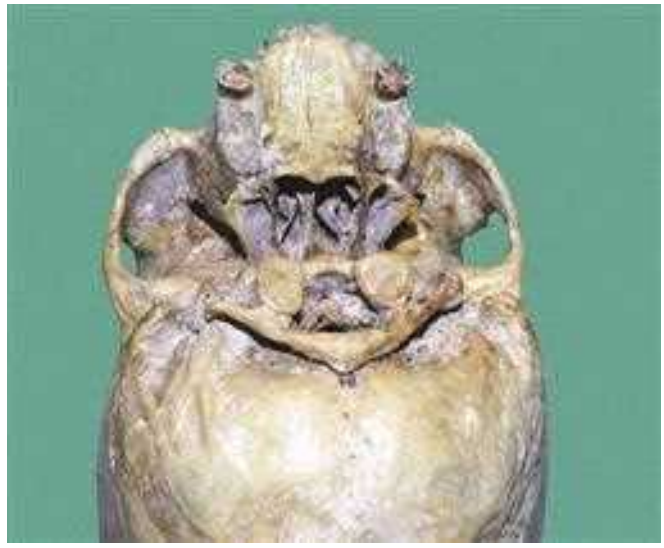
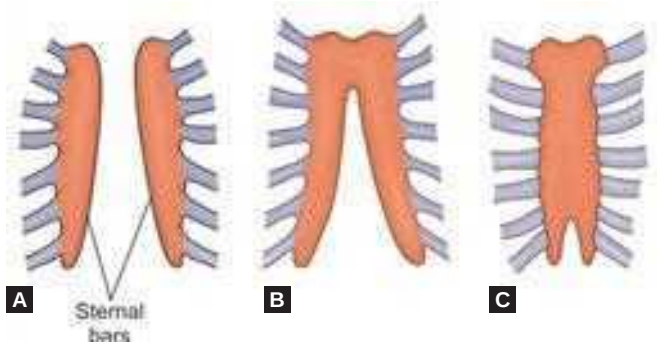


Fig. 10.9: Occipitalization of atlas



Figs 10.10A to C: Development of the sternum. (A) Sternal bars formed on each side of the middle line. (B) The sternal bars begin to fuse with each other at the cranial end. (C) Fusion progresses caudally

- The developing internal ear (*otic vesicle*), and the region of the developing nose, are surrounded by mesenchymal condensations called the *otic*, and *nasal, capsules* respectively. These capsules also take part in forming the mesenchymal basis of the skull.
- The first branchial arch is closely related to the developing skull. It soon shows two subdivisions, called the (1) *mandibular* and (2) *maxillary processes*. Some bones of the skull are formed in the mesoderm of these processes.
- The skull is divided into two parts: (1) the *neurocranium* and *(2) viscerocranium*. The neurocranium forms the bones that encloses the brain and protects it. The viscerocranium forms facial skeleton.
- The neurocranium is divided into *chondrocranium* that forms the bones of base of skull and *membranous neurocranium* that forms the bones of vault of skull.

- The neural crest cells enter the head mesoderm and both together contribute for facial skeleton (viscero/splanchnocranium) and membranous neurocranium.
- The chondrocranium or base of skull up to pituitary gland is formed by occipital sclerotomes and the part rostral to it is formed by neural crest cells.

Chondrocranium (Base of Skull)

- Base of the developing cranium is formed by fusion of several cartilages. Three cartilaginous centers appear in cranial base during 2nd month. They are:
  1. *Parachordal cartilages*: Appear around cephalic part of notochord in the otico-occipital region.
  2. *Polar cartilages*: Appear around hypophysis cerebri in the region of sphenoid.
  3. *Orbitosphenoids, alisphenoids and trabeculae cranii*: Appear between otic and nasal capsules that form the internal ear and nasal cavity respectively.
- The fusion of the cartilages forms the various part of base of skull (chondrocranium) as shown in Figure 10.11.
- Some bones of the skull are formed in membrane, some in cartilage, and some partly in membrane and partly in cartilage, as listed below.

Membranous Neurocranium (Vault of Skull)

- Intramembranous ossification occurs in the mesenchyme at the sides and top of the brain forming calvaria (cranial vault).
- This mesenchyme also receives contribution from neural crest cells.

Viscerocranium (Facial Skeleton)

- The viscerocranium is divided into a *cartilaginous part* and a *membranous part.*
- The cartilaginous viscerocranium is derived from the cartilaginous skeleton of the first two pairs of pharyngeal arches. The membranous viscerocranium forms the following by intramembranous ossification of the maxillary prominence of the first pharyngeal arch. It also receives contribution from neural crest cells.

Bones that are Completely Formed in Membrane

- The *frontal* and *parietal bones* are formed in relation to mesenchyme covering the developing brain.
- The *maxilla* (excluding the premaxilla), *zygomatic* and *palatine bones*, and part of the *temporal* bones, are formed by intramembranous ossification of the mesenchyme of the maxillary process. The *nasal, lacrimal* and *vomer bones* are ossified in the membrane covering the nasal capsule.

Bones that are Completely Formed in Cartilage

- The *ethmoid bone* and the *inferior nasal concha* are derived from the cartilage of the nasal capsule.
- The septal and alar cartilages of the nose represent parts of the capsule that do not undergo ossification.

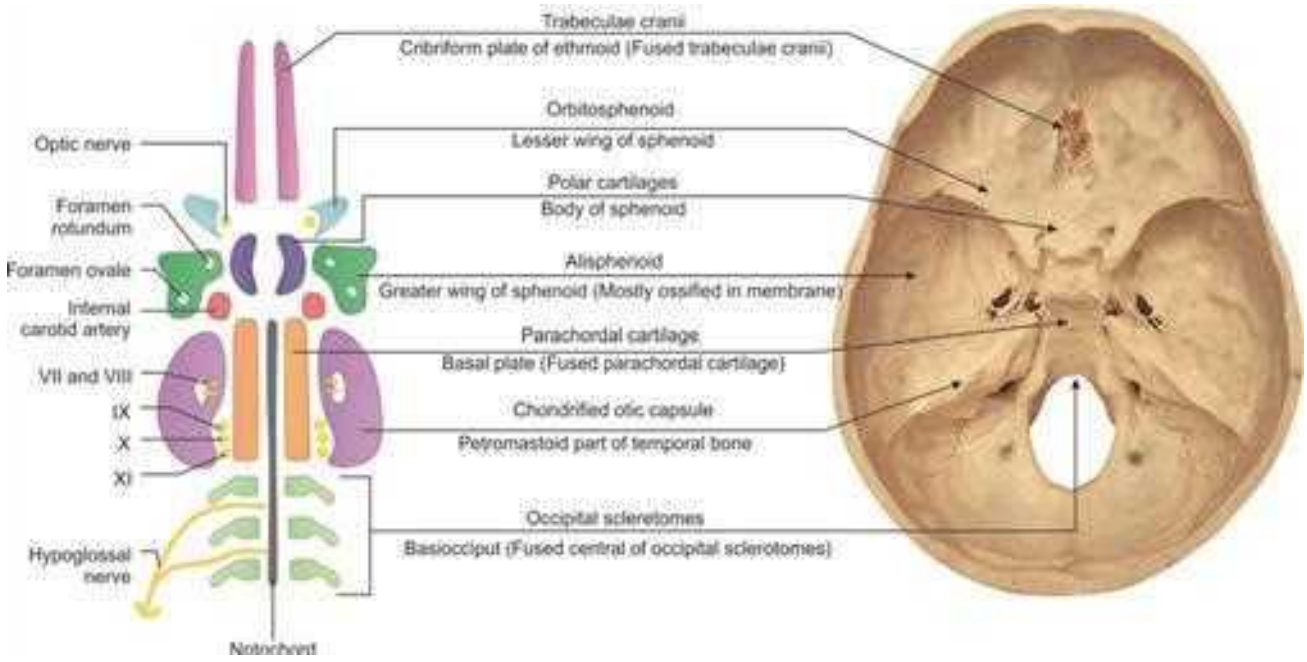


Fig. 10.11: Developmental components of chondrocranium and their derivatives

Bones that are Partly Formed in Cartilage and Partly in Membrane

- *Occipital*: The interparietal part (lying above the superior nuchal lines) is formed in membrane; the rest of the bone is formed by endochondral ossification.
- *Sphenoid*: The lateral part of the greater wing and the pterygoid laminae are formed in membrane; the rest is cartilage bone.
- *Temporal*: The squamous and tympanic parts are formed in membrane. The petrous and mastoid parts are formed by ossification of the cartilage of the otic capsule. The styloid process is derived from the cartilage of the second branchial arch.
- *Mandible*: Most of the bone is formed in membrane in the mesenchyme of the mandibular process. The ventral part of Meckel's cartilage gets embedded in the bone. The condylar and coronoid processes are ossified from secondary cartilages that appear in these situations.

The development of the hyoid bone has been described in Chapter 9.

Clinical correlation

Anomalies of the skull

- The greater part of the vault of the skull is missing in cases of ***anencephaly*** (Fig. 17.33).
- The skull may show various types of deformity. In one syndrome, deformities of the skull are associated with absence of the clavicle (***cleidocranial dysostosis***). Premature union of the sagittal suture gives rise to a boat-shaped skull (***scaphocephaly***). Early union of the coronal suture results in a pointed skull (***acrocephaly***). Asymmetrical union of sutures results in a twisted skull (***plagiocephaly***). When the brain fails to grow the skull remains small (***microcephaly***).
- The bones of the vault of the skull may be widely separated by expansion of the cranial cavity in congenital ***hydrocephalus*** (Fig. 17.35).
- In a rare congenital condition called ***Hand-Schüller-Christian disease***, large defects are seen in the skull bones. The primary defect is in the reticuloendothelial system; the changes in the bones are secondary.
- The occipital bone may be fused to the atlas vertebra (***occipitalization of atlas***) (Fig. 10.9).
- Several genetic disorders of craniofacial development have been described. One syndrome caused by under development of the first branchial arch is ***mandibulofacial dysostosis***.

FORMATION OF LIMBS

- The bones of the limbs, including the bones of the shoulder and pelvic girdles, are formed from mesenchyme of the limb buds. With the exception of the clavicle (which is a membrane bone), they are all formed by endochondral ossification.
- The *limb buds* are paddle-shaped outgrowths that arise from the side wall of the embryo at the beginning of the 2nd month of intrauterine life (Fig. 10.12). Each bud is a mass of mesenchyme covered by ectoderm.
- The mesenchyme of limb buds is derived from (the parietal layer of) the lateral plate mesoderm. This mesenchyme gives rise to bones, connective tissue and some blood vessels. The muscles of the limbs are derived from myotomes of somites which migrate into the limbs.
- The *forelimb buds* appear a little earlier than the *hindlimb buds*. As each forelimb bud grows, it becomes subdivided by constrictions into arm, forearm and hand. The hand itself soon shows outlines of the digits. The interdigital areas show cell death because of which the digits separate from each other (Fig. 10.13). Similar changes occur in the hindlimb.
- While the limb buds are growing, the mesenchymal cells in the buds form cartilaginous models, which subsequently ossify to form the bones of the limb.
- The limb buds are at first directed forward and laterally from the body of the embryo (Fig. 10.14). Each bud has a *preaxial* (or cranial) border and a *postaxial border* (Fig. 10.15). The thumb and great toe are formed on the preaxial border.
- The radius is the preaxial bone of the forearm. In a later development, the forelimb is adducted to the side of the

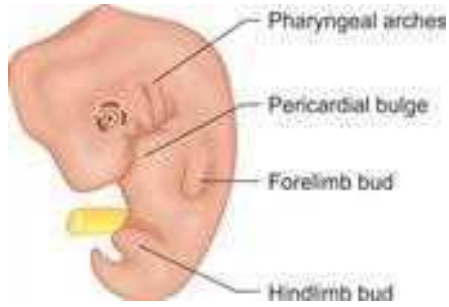


Fig. 10.12: Embryo showing limb buds

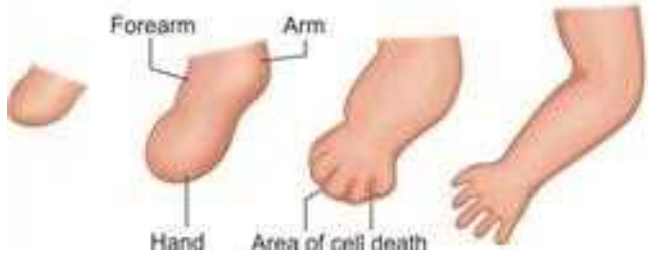


Fig. 10.13: Stages in differentiation of the forelimb bud

body (Fig. 10.15). The original ventral surface forms the anterior surface of the arm, forearm and hand.

- In the case of the lower limb, the tibia is the preaxial bone of the leg. Adduction of this limb is accompanied by medial rotation with the result that the great toe and tibia come to lie on the medial side. The original ventral surface of the limb is represented by the inguinal region, the medial side of the lower part of the thigh, the popliteal surface of the knee, the back of the leg and the sole of the foot.
- The forelimb bud is derived from the part of the body wall belonging to segments C4, C5, C6, C7, C8, T1 and T2. It is, therefore, innervated by the corresponding spinal nerves. The hindlimb bud is formed opposite the segments L2, L3, L4, L5, S1 and S2.

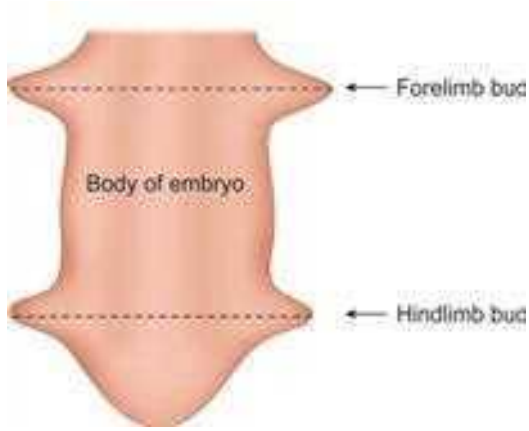


Fig. 10.14: Scheme to show that the longitudinal axis of the limb buds is transverse to the long axis of the embryonic body

Molecular regulation of limb bud development

Three centers in the limb bud determine the three limb axes. They are:

1. ***Apical ectodermal ridge (AER)***: It determines proximal and distal segments. It is essential for limb bud development. At the tip of each limb bud, the ectoderm is thickened to form the AER. This ridge has an inducing effect on underlying mesenchyme causing it to remain undifferentiated and to proliferate. Areas away from the apical ridge undergo differentiation into cartilage, muscle, etc. Sometimes two AERs are formed on a limb bud. This results in formation of a ***supernumerary limb***. Removal of it leads to failure of growth and differentiation of limb called ***phocomelia***.
2. ***Zone of polarizing activity (ZPA)***: It determines cranial to caudal axis (preaxial and postaxial margins).
3. ***Dorsal and ventral ectoderm***: It determines dorsal and ventral axes.

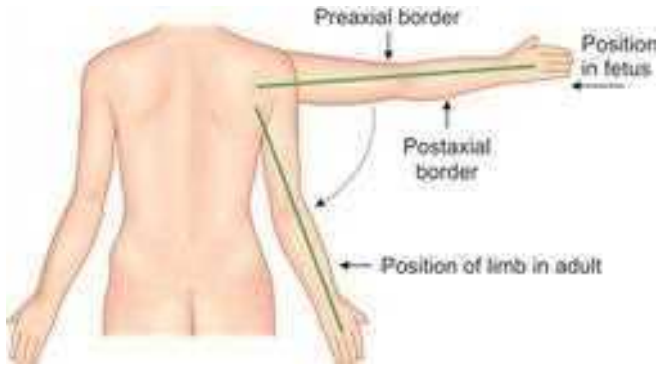


Fig. 10.15: Scheme showing that with "adduction" of the embryonic limb, the preaxial border becomes the lateral border

JOINTS

The tissues of joints are derived from mesenchyme intervening between developing bone ends. This mesenchyme may differentiate into fibrous tissue, forming a *fibrous joint* (*syndesmosis*), or into cartilage forming a cartilaginous joint. In the case of some *cartilaginous joints* (*synchondrosis* or *primary cartilaginous joints*), the cartilage connecting the bones is later ossified, with the result that the two bones become continuous. This is seen, typically, at the joints between the diaphyses and epiphyses of long bones.

At the site where a synovial joint is to be formed, the mesenchyme is usually seen in three layers. The two outer layers are continuous with the perichondrium covering the cartilaginous ends of the articulating bones. The middle layer becomes loose and a cavity is formed in it. The cavity comes to be lined by a mesothelium that forms the synovial membrane (Fig. 10.16). The capsule and other ligaments are derived from the surrounding mesenchyme.

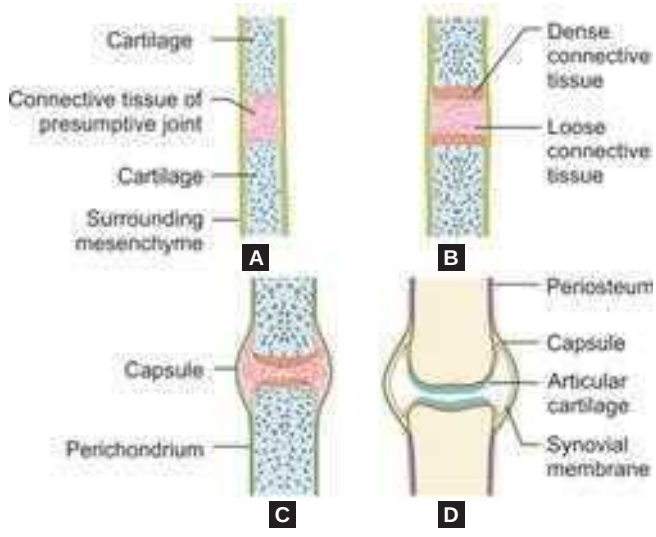


Fig. 10.16: Development of a synovial joint

Clinical correlation

Anomalies of limbs

- One or more limbs of the body may be partially, or completely, absent (***phocomelia, amelia***). These conditions may be produced by ingestion of harmful drugs.
- Part of a limb may be deformed***. Deformities*** are most frequently seen in the region of the ankle and foot, and are of various types. In the most common variety of deformity, the foot shows marked plantar flexion (equinus: like the horse), and inversion (varus). Hence, this condition is called ***talipes equinovarus***, or ***clubfoot*** (Fig. 10.17).
- ***Congenital strictures***, ***congenital amputations*** or ***congenital contractures*** may be present.
- There may be abnormal fusion (bony or fibrous) between different bones of the limb. Adjoining digits may be fused (***syndactyly***). The phalanges of a digit may be fused to one another (***synphalangia***).
- A digit may be abnormally large (***macrodactyly***), or abnormally short (***brachydactyly***). In arachnodactyly, the fingers are long and thin (***spider fingers***).
- Supernumerary digits may be present (***polydactyly***) (Fig. 10.18). A digit (most commonly the thumb) may have an extra phalanx.
- The palm or sole may show a deep longitudinal cleft (***lobster claw***).
- The limbs may remain short in ***achondroplasia*** (Fig. 7.21).
- Sometimes the bone ends forming a joint are imperfectly formed (***congenital dysplasia***). This can lead to ***congenital dislocation***. The hip joint is most commonly affected.

PART 2: MUSCULAR SYSTEM

INTRODUCTION

Majority of the skeletal muscles develop from somites. The cardiac and smooth muscles develop from splanchnic mesoderm. The myogenesis of skeletal, cardiac and smooth muscle are described in Chapter 7. In this chapter, the development of skeletal musculature of body will be considered.

SKELETAL MUSCLE

- Each myotome establishes contact with one segmental nerve. Hence, theoretically, the embryological derivation of a muscle should be indicated by its nerve supply. On this basis, it would be presumed that all the musculature of the body walls and limbs is derived from the myotomes and has subsequently migrated to these regions.
- Such migration of myotomes can be seen in embryos of some lower animals, but not in the human embryo. In man, the myotomes appear to give origin only to the musculature of the trunk, in whole or in part. The occipital myotomes are believed to give rise to the musculature of the tongue, while the extrinsic muscles of the eyeball are regarded as derivatives of the preoccipital myotomes.
- Soon after its formation, each myotome, in the neck and trunk, separates into a small dorsal part (*epimere*) which gives rise to the muscles supplied by the dorsal primary ramus of the spinal nerve, and a larger ventral part (*hypomere*), which gives origin to the muscles supplied by the ventral ramus (Figs 10.1A to C) of spinal nerve. The epimeres give origin to the muscles of the back (epaxial/extensors of the vertebral column), while the hypomeres give origin to the hypoxial/flexor muscles of the body wall and limbs. The intermuscular septum separating these two groups is represented by thoracolumbar fascia.
- Some cells from the ventrolateral region of the dermomyotomes migrate into the parietal layer of lateral

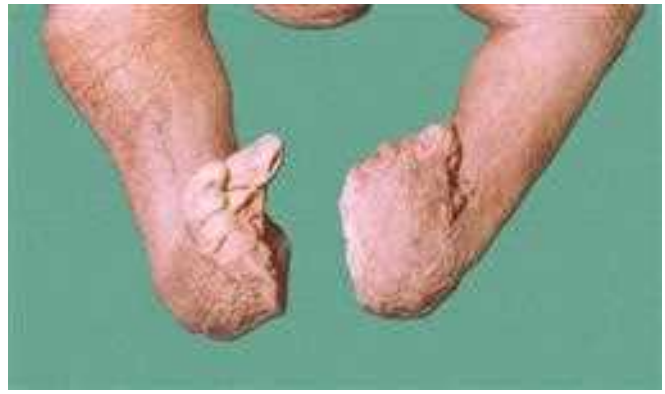


Fig. 10.17: Clubfoot

Fig. 10.18: Polydactyly

plate mesoderm where they form muscles of limbs, and anterolateral muscles of the neck and abdomen.

DEVELOPMENT OF MUSCULAR SYSTEM

The skeletal musculature of the body is derived from mesoderm as described below with exceptions:

- Somatic mesoderm—limb, trunk
- Branchial mesoderm—head and neck
- Splanchnic mesoderm—cardiac, smooth.
- *Exceptions*—ectodermal
  - Musculature of iris
  - Arrectores pilorum of skin
  - Myoepithelial cells of ducts of sweat glands.

Developmentally the skeletal musculature of the body can be divided into *branchial arch derived* and *somite derived* muscles.

Branchial Arch Derived Musculature

Muscles of head and neck and face are derived from pharyngeal arches. They are discussed in Chapter 9.

Somite Derived Skeletal Musculature

The somite derived musculature distribution is divided as follows (Table 10.3, Figs 10.19 to 10.21):

Extraocular Muscles of Eyeball

- These are derived from the three *preoptic myotomes* that are arranged around the developing eyeball (Fig. 10.19).
- They receive innervation from the 3rd (supplies inferior oblique, levator palpebrae superioris and medial, superior and inferior recti), 4th (supplies superior oblique) and 6th (supplies lateral rectus) cranial nerves.

Muscles of Tongue

- All muscles of tongue both intrinsic and extrinsic except palatoglossus are derived from the four *occipital myotomes* (Fig. 10.19) and are supplied by 12th cranial nerve (which is formed by fusion of pre cervical nerves) and the palatoglossus is supplied by vagus nerve.
- The occipital myotomes migrate into the developing tongue (contributed by 1st to 3rd pharyngeal arches) in the floor of mouth.

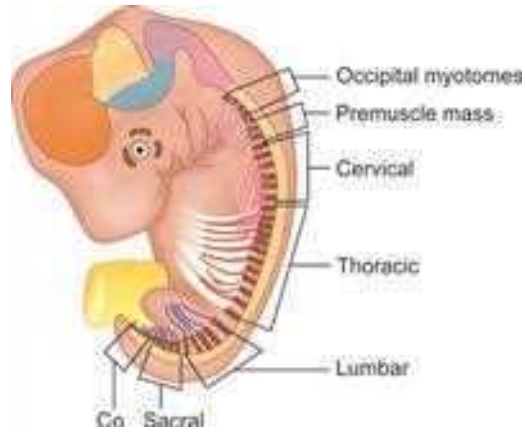
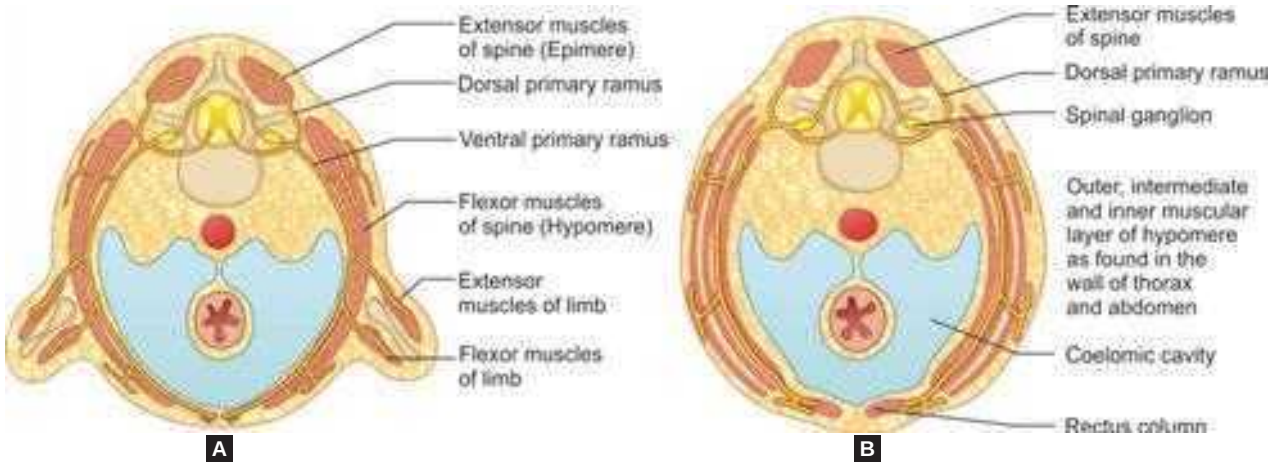


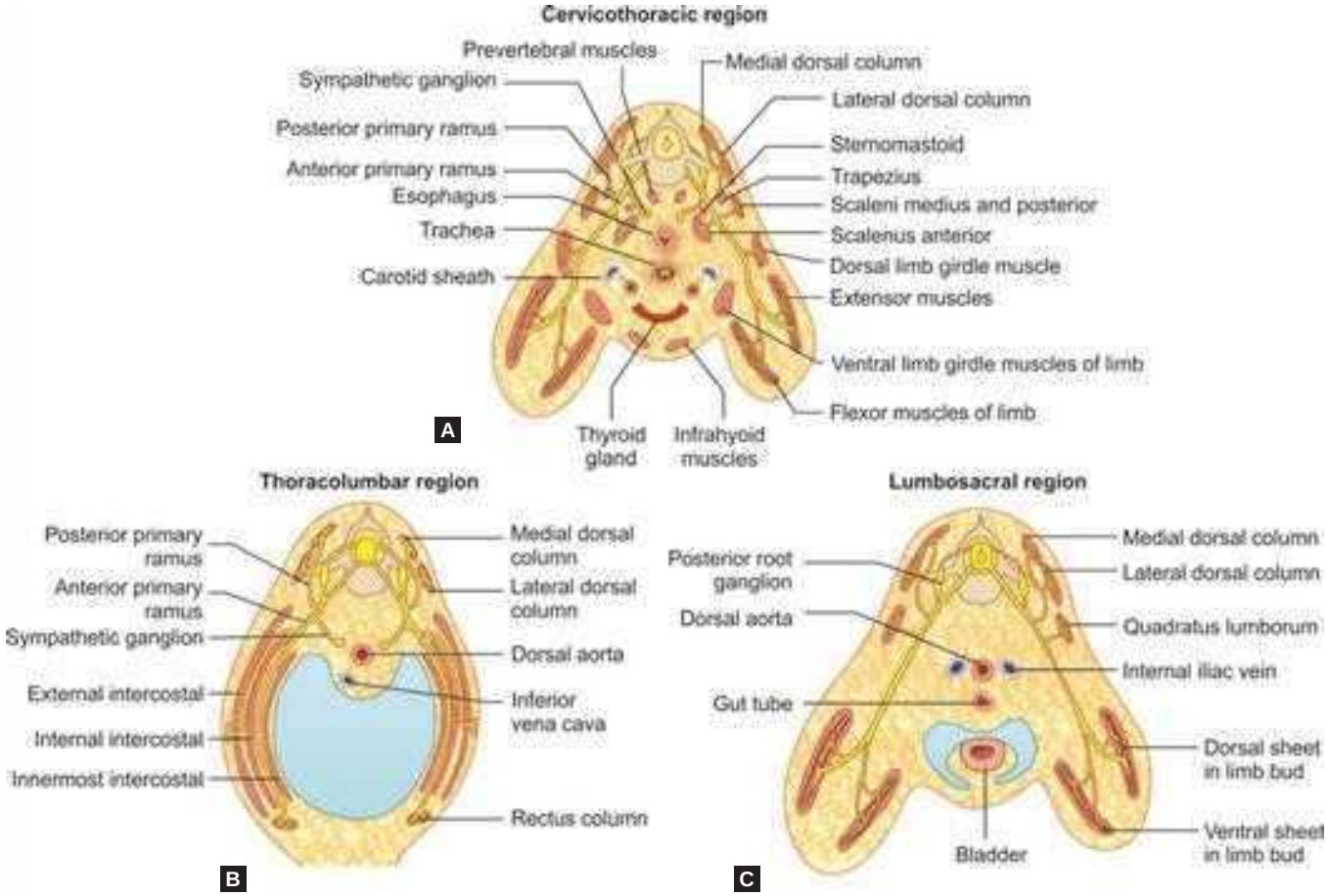
Fig. 10.19: Myotomic segment derived muscles of the body

TABLE 10.3: Somite derived skeletal musculature

Muscle group	*Myotomic segments*	*Cranial/Spinal nerve supply*
Extraocular muscles of eyeball	Preoccipital—3 nos.	3rd, 4th, 6th cranial nerves
Tongue muscles	Occipital—4 nos.	12th cranial nerve
Intrinsic muscles of back—extensors of vertebral column	Dorsal/epaxial divisions of C1–S3/4	C1–S3 or S4
• Anterolateral thoracic and abdominal wall • Intercostals, obliques, transversus • Recuts abdominis, pyramidalis	Ventrolateral/hypaxial divisions of T1–L1	T1–L1
• Upper limb and shoulder girdle • Lower limb and pelvic girdle	Splits into dorsal and ventral masses by in situ developing somatopleuric mesodermal derived bones	• C4–T2—brachial plexus • L2–S3—lumbar and lumbosacral plexuses
Diaphragm	Ventrolateral divisions of C3–C5	Phrenic nerve (C3–C5)
Other muscles • Posterior abdominal wall muscles (quadratus lumborum) • Pelvic diaphragm (levator ani and coccygeus) • External anal sphincter and striated muscles of superficial deep perineal pouches	• Ventrolateral divisions of L1–L5 • Ventrolateral divisions of S2–Co1 • Cloacal sphincter—skin muscle	• L1–L5 • S2–Co1 • S2–Co1



Figs 10.20A and B: Distribution of thoracoabdominal musculature



Figs 10.21A to C: Distribution of limb and trunk musculature

Body Wall (Trunk)

- They are derived from segmental myotomes of somite origin. They are divided into epaxial and hypaxial groups (Fig. 10.20A).
- Epaxial or dorsal groups are supplied by posterior spinal rami. They form the erector spinae group of muscles that act as extensors of vertebral column.
- Hypaxial or ventral groups are supplied by ventral ramus. They extend in ventrolateral direction along the

somatopleuric layer of mesoderm of coelomic cavities and form the flexors of vertebral column that divides into the three layers of muscles of thorax (external, internal and innermost intercostals) and that of anterior abdominal wall (external oblique, internal oblique and transversus abdominis) (Fig. 10.20B).
- The hypaxial musculature in the prevertebral region of neck forms longus and scalene muscles in the midline and rectus (sternalis and abdominis) and infrahyoid muscle on either side of midline.

Limb Muscles

- Myotome derived mesodermal cell migration into the developing limb buds occurs during 5th week.
- For upper limb they are derived from C4 to T2 segments and for lower limb they are derived from L1 to S3 segments (Figs 10.21A to C).
- The muscles are at first organized along the preaxial and postaxial borders of in situ developing somatopleuric mesodermal derived bones. The muscles and the borders of the limbs differentiate into preaxial and postaxial groups. The preaxial muscles are supplied by upper segments of nerves and the postaxial muscles are supplied by lower segmental nerves.
- Regrouping of muscles and spinal nerves results in formation of anterior and posterior groups of muscles of limbs and nerve plexuses in relation to the limbs respectively. The anterior groups are flexors and adductors of the limb and are supplied by anterior divisions of nerve plexuses (e.g. medial and ulnar nerves supply flexors). The posterior groups are extensors and abductors of the limbs and are supplied by posterior divisions of nerve plexuses (e.g. radial nerve supplies the extensors).

Diaphragm

- The musculature develops from C3 to C5 myotomes. It is supplied by phrenic nerves that invade septum transversum.
- The transversus layer of thorax is peeled off due to the down growth of lung buds and comes into contact with the septum transversum to form thoracoabdominal diaphragm.

Other Muscles

- The 2nd sacral to 1st coccygeal myotomes contribute for the formation of pelvic diaphragm, external anal sphincter and striated muscles of genital organs.

TIME TABLE OF SOME EVENTS

Time table of some events has been shown in Table 10.4.

TABLE 10.4: Time table of some developmental events

Age	*Developmental events*
4th week (26th day)	Forelimb bud appears
4th week (28th day)	Hindlimb bud appears
5th week	Limbs become paddle-shaped
6th week (36th day)	Formation of future digits can be seen Cartilaginous models of bone start forming
7th week	Rotation of limbs occurs
8th week (50th day)	The elbow and knee are established The fingers and toes are free Primary centers of ossification are seen in many bones
12th week	Primary centers of ossification are seen in all the long bones

Note: The extremities are most susceptible to teratogens during the 4th–7th weeks; and slightly less susceptible in the 8th week.

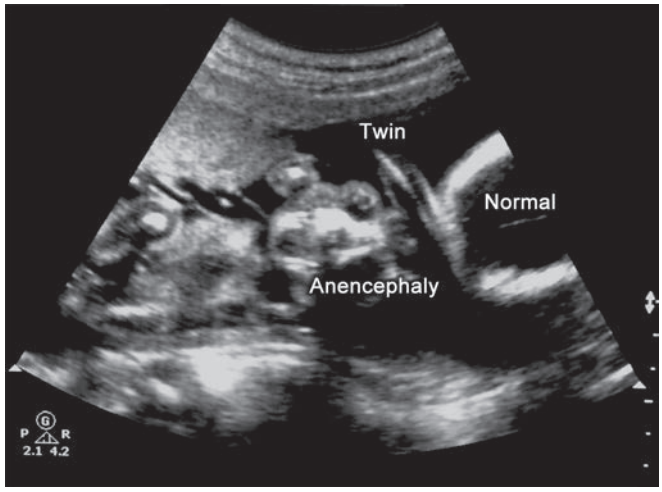


Fig. 10.22: Ultrasound image of twin pregnancy with one normal and one anencephalic fetus. *Image Courtesy:* Dr Ganesh Kumar and Dr Sasikala

Fig. 10.23: Anencephalic fetus

CLINICAL CASE WITH PRENATAL ULTRASOUND AND ABORTED FETAL IMAGES: EMBRYOLOGICAL AND CLINICAL EXPLANATION

A primi of 30 years age came for obstetric checkup at 22nd week and was informed about twin pregnancy on obstetrical examination. She was advised transabdominal fetal ultrasound. The ultrasound picture (Fig. 10.22) presented one fetus with normal head and the other presented absence of skull cap. It was diagnosed as a twin pregnancy with one normal and one anencephalic fetus. The woman was advised to continue the pregnancy and was advised to come for regular checkup. At 36 weeks, polyhydramnios was identified and by emergency cesarean section twin babies were delivered. One was normal and was admitted in neonatal care unit. Another was anencephalic and did not survive (Fig. 10.23). Give the embryological explanation and explanation for continuation of pregnancy and extra care taken in this case.

- In the ultrasound, normal skull image was observed for one fetus and its absence for the other, i.e. anencephalic fetus.
- The ultrasound image of anencephalic fetus presented bulging eyes.
- Failure of closure of cephalic part of neural tube and nonformation of vault of skull resulted in this condition. As the brainstem is intact, the fetus was live born and subsequently died.
- The neural tissue is disorganized due to exposure to amniotic fluid that caused necrosis of nervous tissue.
- Because of one normal fetus, the pregnancy was continued by taking antenatal care to save the normal fetus and a planned preterm cesarean was done.
- A 100% diagnosis of anencephaly can be made by prenatal ultrasound. If it is a singleton, anencephalic pregnancy termination of pregnancy will be advised as the anencephalic fetus has no chance of survival.
- Since it is a multiple gestation with dichorionic and diamniotic pregnancy with one normal fetus the pregnancy was continued.

REVIEW QUESTIONS

1. Describe somites.
2. Explain development of vertebra.
3. Write short notes on development of sternum.
4. Write short notes on developmental components of chondrocranium and their derivatives.
5. Write short notes on development of synovial joint.

Chapter 11

Face, Nose and Palate

HIGHLIGHTS

- The *stomatodeum* (future mouth) is a depression bounded cranially by a bulging produced by the brain, and caudally by a bulging produced by the pericardial cavity.
- Three prominences appear around the stomatodeum. These are the *frontonasal process* (above), and the *right and left mandibular arches* (first pharyngeal arches)
- The mandibular arch divides into a *maxillary process* and a *mandibular process*.
- The right and left mandibular processes meet in the midline and fuse. They form the *lower lip* and *lower jaw*.
- The *upper lip* is formed by fusion of the frontonasal process with the right and left maxillary processes. Failure to fuse completely leads to various forms of *harelip*.
- The *cheeks* are formed by fusion of (the posterior parts of) the maxillary and mandibular processes.
- The *nose* is derived from the frontonasal process.
- The *nasal cavity* is formed from an ectodermal thickening, the *nasal placode*, appears over the frontonasal process. The placode gets depressed below the surface to form the *nasal pit*. The nasal pits enlarge to form the nasal cavity.
- *Paranasal sinuses* appear as outgrowths from the nasal cavity.
- The *palate* is formed by fusion of three components. These are the right and left *palatal processes* (arising from the maxillary process); and the *primitive palate* (derived from the frontonasal process). Deficiency in fusion leads to various forms *of cleft palate*.

INTRODUCTION

- During the 4th week of development, after the formation of the head fold, two prominent bulgings appear on the ventral aspect of the developing embryo, separated by the stomatodeum (Fig. 11.1). They are:
  - Developing brain cranially
  - Pericardium caudally
- The floor of the stomatodeum is formed by the buccopharyngeal membrane, which separates it from the foregut. On each side, the stomatodeum is bounded by first arch.
- Soon, mesoderm covering the developing forebrain proliferates and forms a downward projection that overlaps the upper part of the stomatodeum. This downward projection is called the *frontonasal process* (Fig. 11.2).
- The pharyngeal arches are laid down in the lateral and ventral walls of the most cranial part of the foregut (Chapter 9, Fig. 9.1B). These are also, therefore, in very close relationship to the stomatodeum.

DEVELOPMENT OF THE FACE

- It will now be readily appreciated that the *face* is derived from the structures that lie around the stomatodeum
  - *Unpaired*: Frontonasal process from above.
  - *Paired*: First pharyngeal (or mandibular) arch of each side (Fig. 11.3A).

- Each mandibular arch forms the lateral wall of the stomatodeum (Fig. 11.3A). This arch gives off a bud from its dorsal end. This bud is called the *maxillary process* (Fig. 11.3B). It grows ventromedially cranial to the main part of the arch which is now called the *mandibular process*.
- The five primordia for face development are an unpaired frontonasal process and paired maxillary and mandibular processes.

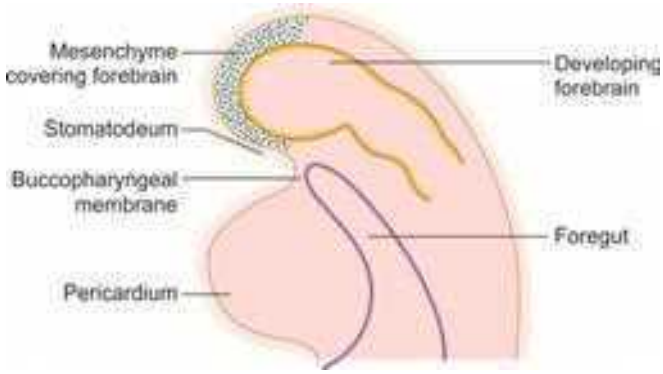


Fig. 11.1: Head end of an embryo just before formation of the frontonasal process

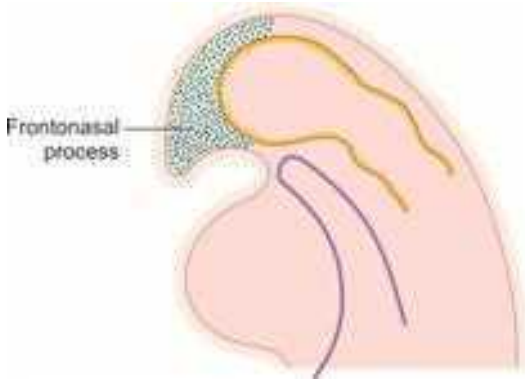


Fig. 11.2: Formation of frontonasal process

- The ectoderm overlying the frontonasal process soon shows bilateral localized thickenings that are situated a little above the stomatodeum (Fig. 11.4A) on either side of midline. These are called the *nasal placodes*. The formation of these placodes is induced by the underlying forebrain. The placodes soon sink below the surface to form *nasal pits* (Fig. 11.4B). The pits are continuous with the stomatodeum below. The edges of each pit are raised above the surface: the medial raised edge is called the *medial nasal process* and the lateral edge is called the *lateral nasal process*. Lateral and cranial to the nasal placodes pair of thickenings appear and are called *lens placodes*.

DEVELOPMENT OF VARIOUS PARTS OF FACE

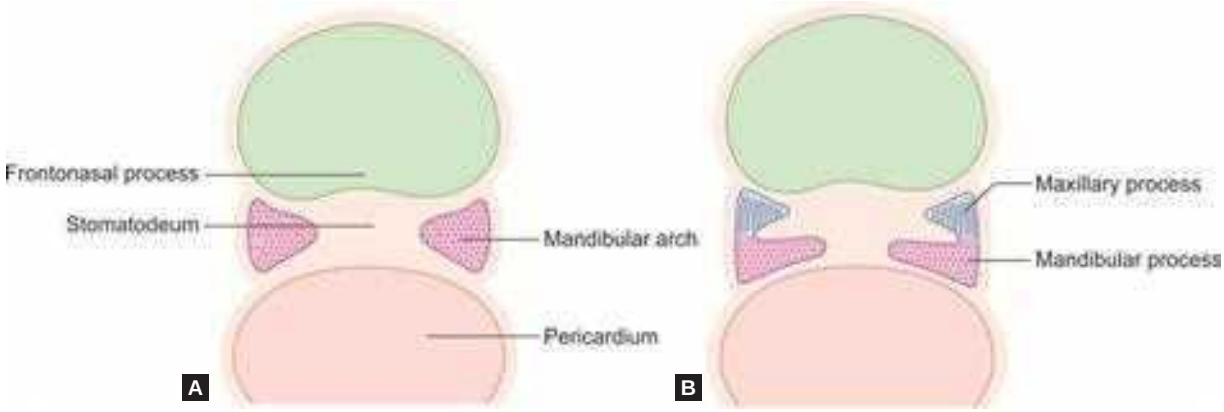
We are now in a position to study the formation of various parts of the face. The various primordia of face, their relation to stomatodeum and the parts derived are shown in Table 11.1.

Lower Lip

The *mandibular processes* of the two sides grow toward each other (Fig. 11.3B) and fuse in the midline (Fig. 11.4A). They now form the lower margin of the stomatodeum. If it is remembered that the mouth develops from the stomatodeum, it will be readily understood that the fused mandibular processes give rise to the *lower lip*, and to the *lower jaw* (Fig. 11.7).

Upper Lip

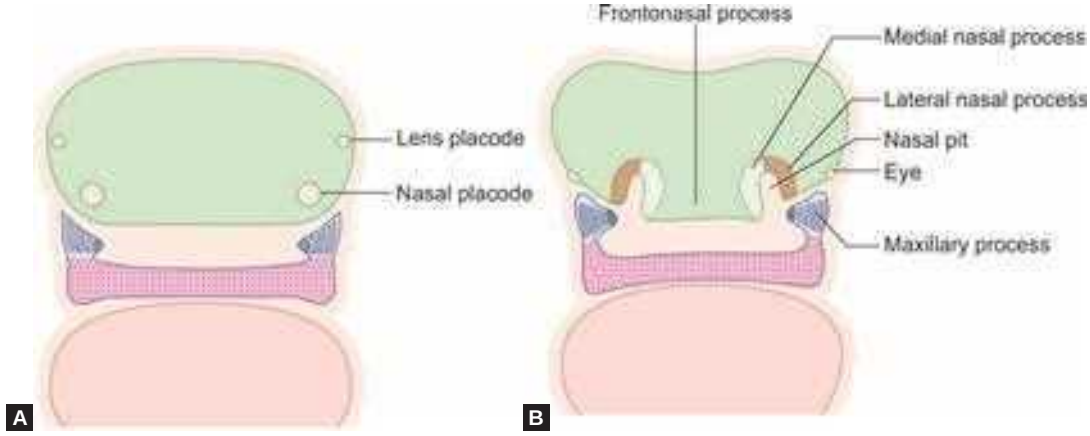
- Each *maxillary process* now grows medially below the developing eye and fuses, first with the *lateral nasal process* (Fig. 11.5), and then with the *medial nasal process* (Fig. 11.6). The medial and lateral nasal processes also fuse with each other. In this way, the



Figs 11.3A and B: Development of face: Formation of mandibular and maxillary processes

nasal pits (now called *external nares*) are cut off from the stomatodeum.

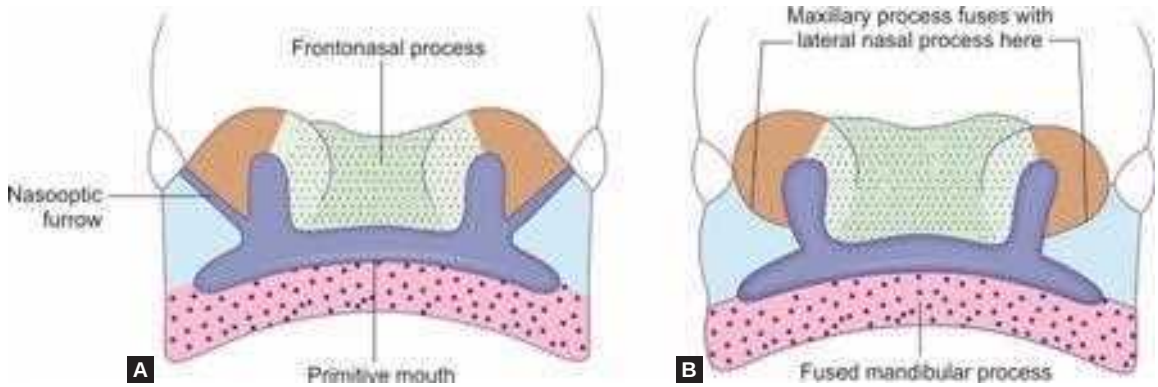
- The maxillary processes undergo considerable growth (Fig. 11.6). At the same time, the frontonasal process becomes much narrower from side to side, with the result that the two external nares come closer together.
- The stomatodeum is now bounded above by the upper lip that is derived as follows (Figs 11.7 and 11.8):



Figs 11.4A and B: Development of face (continued). (A) The right and left mandibular processes fuse and form the lower boundary of the future mouth. The nasal placodes appear over the frontonasal process. The lens placode appears; (B) The nasal placode is converted into the nasal pit. Elevations of the pit form the medial and lateral nasal processes

TABLE 11.1: Face-primordia, their relation to stomatodeum and parts of face contributed by them

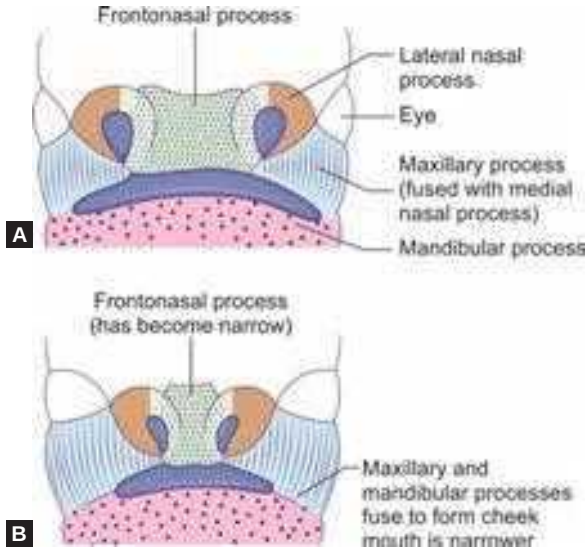
Processes	*Developmental Primordia*	*Relation to stomatodeum*	*Parts of face formed*	*Trigeminal nerve division innervating*
Frontonasal (Unpaired)	Mesenchyme ventral to developing forebrain	Middle part of upper border	• Forehead • External nose • Nasal cavity • Nasal septum • Philtrum of upper lip	Ophthalmic Except philtrum which is innervated by maxillary
Maxillary (Paired)	Mesoderm of dorsal part of 1st arch	Lateral part of upper border	• Lateral parts of upper lip • Upper parts cheeks	Maxillary
Mandibular (Paired)	Mesoderm of ventral part of 1st arch	Lower border	• Chin • Lower lip • Lower parts of cheeks	Mandibular



Figs 11.5A and B: Development of the face (continued). (A) The right and left nasal pits come close to each other. The lateral nasal process is separated from the maxillary process by the nasooptic furrow; (B) The maxillary process fuses with the lateral nasal process obliterating the nasooptic furrow

- The mesodermal basis of the lateral part of the lip is formed from the maxillary process. The overlying skin is derived from ectoderm covering this process.
- The mesodermal basis of the median part of the lip (called *philtrum*) is formed from the frontonasal process. The ectoderm of the maxillary process, however, overgrows this mesoderm to meet that of the opposite maxillary process in the midline (Fig. 11.8). As a result, the skin of the entire upper lip is innervated by the maxillary nerves.

- The *muscles of the face* (including those of the lips) are derived from mesoderm of the *second branchial arch* and are, therefore, supplied by the facial nerve.



Figs 11.6A and B: Development of the face (continued). (A) The maxillary process extends below the nasal pit and fuses with the medial nasal process. In this way, the nasal pit is separated from the stomatodeum; (B) The maxillary and mandibular processes partly fuse to form the cheek. With growth of the maxillary processes the nasal pits come closer to each other

Cheeks

- After formation of the upper and lower lips, the stomatodeum (which can now be called the mouth) is very broad. In its lateral part, it is bounded above by the maxillary process and below by the mandibular process. These processes undergo progressive fusion with each other to form the cheeks (compare Figs 11.6A and B; also see Figs 11.9 and 11.10).
- The maxillary process fuses with the lateral nasal process. This fusion not only occurs in the region of the lip but also extends from the stomatodeum to the medial angle of the developing eye (Figs 11.6 and 11.9B). For some time, this line of fusion is marked by a groove called the *nasooptic furrow* or *nasolacrimal sulcus* (Fig. 11.5A). A strip of ectoderm becomes buried along this furrow and gives rise to the *nasolacrimal duct* (Chapter 19: Development of Eye).

Eye

- The development of the eye itself will be dealt with later (Chapter 19), but a brief reference to it is necessary to form a complete idea of the development of the face.
- The region of the eye is first seen as an ectodermal thickening, the *lens placode*, which appears on the ventrolateral side of the developing forebrain, lateral and cranial to the nasal placode (Fig. 11.4A).
- The lens placode sinks below the surface and is eventually cut off from the surface ectoderm. The developing eyeball produces a bulging in this situation (Fig. 11.5).
- The bulgings of the eyes are at first directed laterally (Figs 11.5 and 11.6), and lie in the angles between the maxillary processes and the lateral nasal processes.
- With the narrowing of the frontonasal process, they come to face forward (Figs 11.6 and 11.7).
- The eyelids are derived from folds of ectoderm that are formed above and below the eyes, and by mesoderm enclosed within the folds.

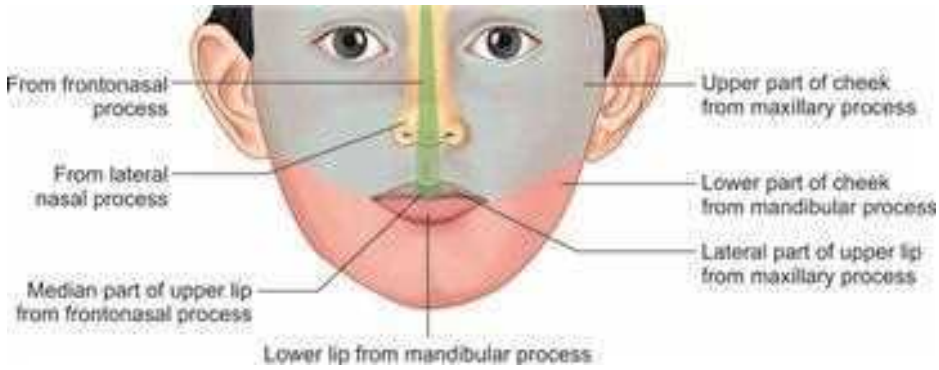


Fig. 11.7: Derivation of parts of the face

Nose

- The nose receives contributions from the *frontonasal process* and from the *medial and lateral nasal processes* of the right and left sides.

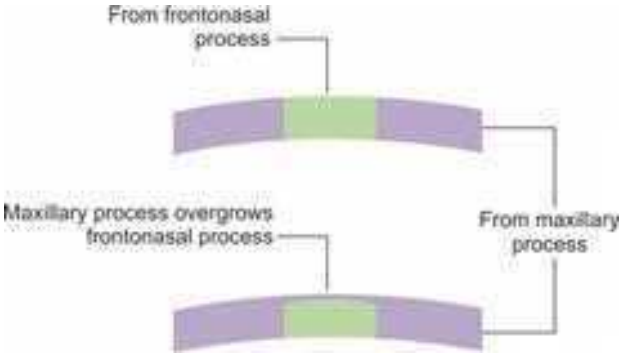
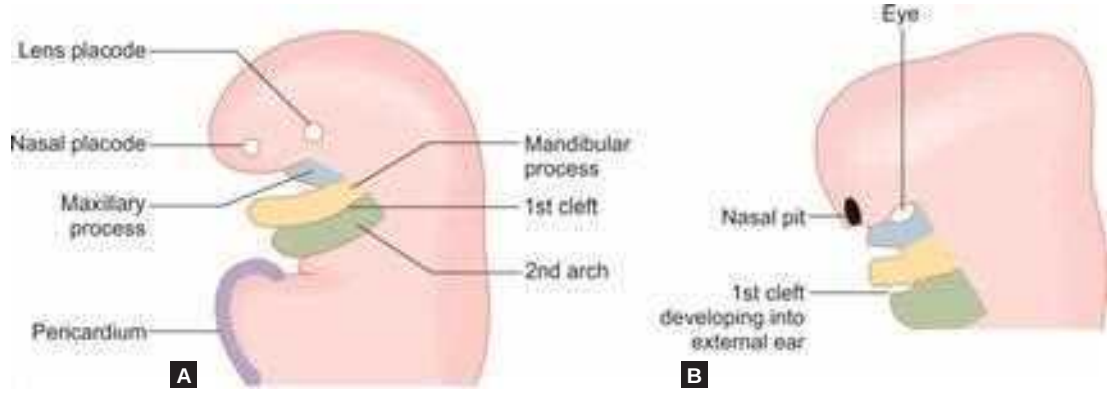


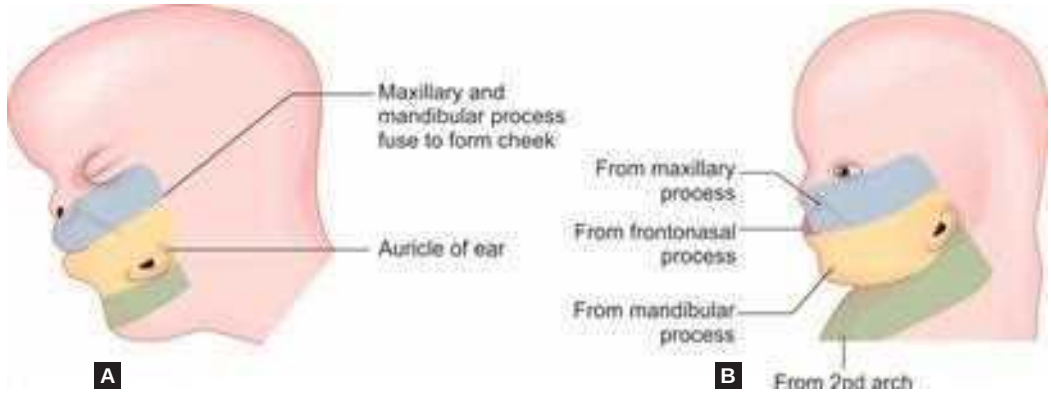
Fig. 11.8: Formation of upper lip: Scheme to show how the maxillary process "overgrows" the frontonasal process

- *External nares* are formed when the nasal pits are cut off from the stomatodeum by the fusion of the maxillary process with the medial nasal process.
- External nares gradually approach each other. This is a result of the fact that the frontonasal process becomes progressively narrower and its deeper part ultimately forms the *nasal septum.*
- Mesoderm becomes heaped up in the median plane to form the prominence of the nose. Simultaneously, a groove appears between the region of the nose and the bulging forebrain (which may now be called the forehead) (Fig. 11.10).
- As the nose becomes prominent, the external nares come to open downward instead of forward (Fig. 11.10).
- The external form of the nose is thus established with the fusion of five processes as follows:
  - Frontonasal process forms the *bridge of the nose.*
  - Fused medial nasal processes form the *dorsum and tip of nose.*
  - Lateral nasal processes form the *alae of the nose.*

The development of the nasal cavity is considered later.



Figs 11.9A and B: Early stages in the development of the face as seen from the lateral aspect



Figs 11.10A and B: Later stage in the development of the face as seen from the lateral aspect

Clinical correlation

Developmental anomalies of the face

It has been seen that the formation of various parts of the face involves fusion of diverse components. This fusion is occasionally incomplete and gives rise to various anomalies.

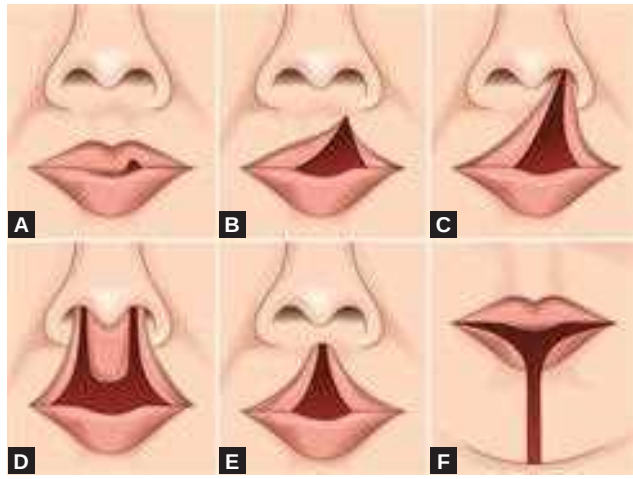
- ***Harelip*:** The upper lip of the hare normally has a cleft. Hence, the term harelip is used for defects of the lips.
  - *Unilateral harelip*: Failure of fusion of maxillary process with medial nasal process on one side (Figs 11.11A to C).
  - *Bilateral harelip*: Failure of fusion of both maxillary processes with the medial nasal process (Fig. 11.11D).
  - *Midline cleft of upper lip*: Defective development of the lowermost part of the frontonasal process may give rise to a midline defect of the upper lip (Fig. 11.11E).
- ***Cleft of lower lip*:** When the two mandibular processes do not fuse with each other the lower lip shows a defect in the midline. The defect usually extends into the jaw (Fig. 11.11F).
- ***Oblique facial cleft*:** Nonfusion of the maxillary and lateral nasal process gives rise to a cleft running from the medial angle of the eye to the mouth (Fig.11.12A). The nasolacrimal duct is not formed.
- Inadequate fusion of the mandibular and maxillary processes with each other may lead to an abnormally wide mouth (***macrostomia***) (Fig. 11.12B). Lack of fusion may be unilateral: this leads to formation of a lateral facial cleft. Too much fusion may result in a small mouth (***microstomia***) (Fig. 11.12C).
- The nose may be bifid. This may be associated with median cleft lip. Both these occur due to bifurcation of the frontonasal process. Occasionally one half of it may be absent. Very rarely the nose forms a cylindrical projection, or ***proboscis*** (Fig. 11.13, 19.11D) jutting out from just below the forehead. This anomaly may sometimes affect only one half of the nose and is usually associated with fusion of the two eyes (***cyclops***).
- The entire first arch may remain underdeveloped on one or both sides, affecting the lower eyelid (coloboma type defect), the maxilla, the mandible, and the external ear. The prominence of the cheek is absent and the ear may be displaced ventrally and caudally. There may be presence of cleft palate and of faulty dentition. This condition is called ***mandibulofacial dysostosis***, ***Treacher Collins syndrome*** or ***first arch syndrome***. This is a genetic condition inherited as autosomal dominant.
- One half of the face may be under developed or overdeveloped.
- The mandible may be small compared to the rest of the face resulting in a receding chin (***retrognathia***). In extreme cases, it may fail to develop (***agnathia***).
- Congenital tumors may be present in relation to the face. These may represent attempts at duplication of some parts.
- The eyes may be widely separated (***hypertelorism***). The nasal bridge is broad. This condition results from the presence of excessive tissue in the frontonasal process.
- The lips may show congenital pits or fistulae. The lip may be double.

External Ear

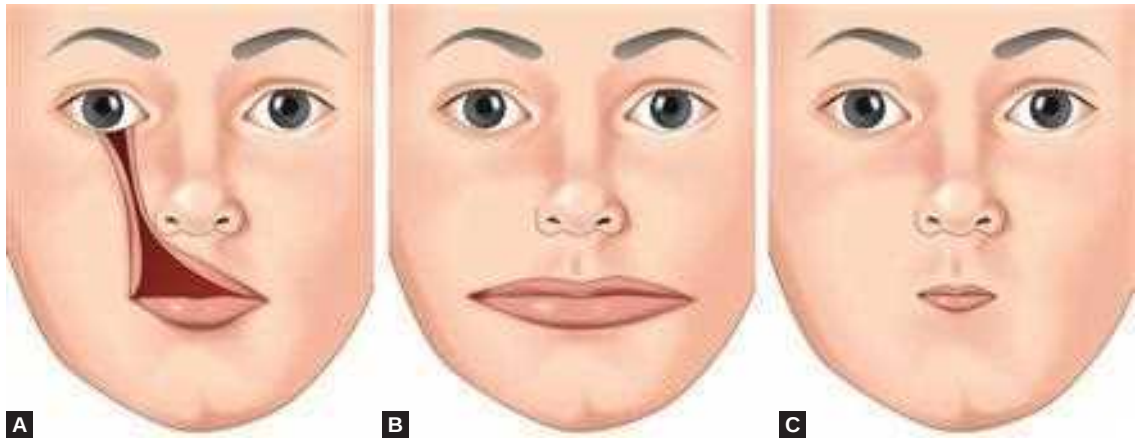
- The external ear is formed around the dorsal part of the first ectodermal cleft (Fig. 11.9B).
- A series of mesodermal thickenings (often called tubercles or hillocks) appear on the mandibular and hyoid arches where they adjoin this cleft.
- The pinna (or auricle) is formed by fusion of these thickenings (Chapter 20: Development of the Ear, Fig. 20.10).
- From a study of Figures 11.9 and 11.10, it will be seen that when first formed, the pinna lies caudal to the developing jaw. It is pushed upward and backward to its definitive position due to the great enlargement of the mandibular process.
- If the mandibular process fails to enlarge, the ears remain low down and it can result in mandibulofacial dysostosis.

Development of Nasal Cavities

- The nasal cavities are formed by extension of the *nasal pits*. We have seen that these pits are at first in open communication with the stomatodeum (Fig. 11.14A). The frontonasal process is between nasal pits.
- Soon the medial and lateral nasal processes fuse, and form a partition between the pit and the stomatodeum. This is called the *primitive palate* (Fig. 11.14B), and is derived from the frontonasal process.
- The nasal pits now deepen to form the *nasal sacs* which expand both dorsally and caudally (Fig. 11.14C). The dorsal part of this sac is, at first, separated from the stomatodeum by a thin membrane called the *bucconasal membrane* (or *nasal fin*). This soon breaks down (Figs 11.14D and 11.15B). The nasal sac now has a ventral orifice that opens on the face (*anterior or external nares*), and a dorsal orifice that opens into the stomatodeum (*primitive posterior nasal aperture*).
- The two nasal sacs are at first widely separated from one another by the frontonasal process (Figs 11.15A and B).



Figs 11.11A to F: Varieties of harelip. For explanation see text



Figs 11.12A to C: (A) Oblique facial cleft; (B) Macrostomia; (C) Microstomia

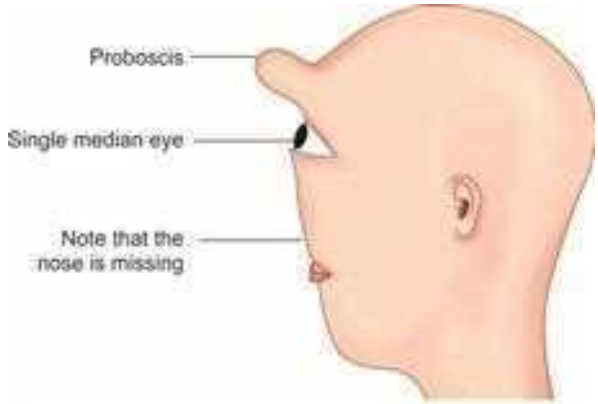
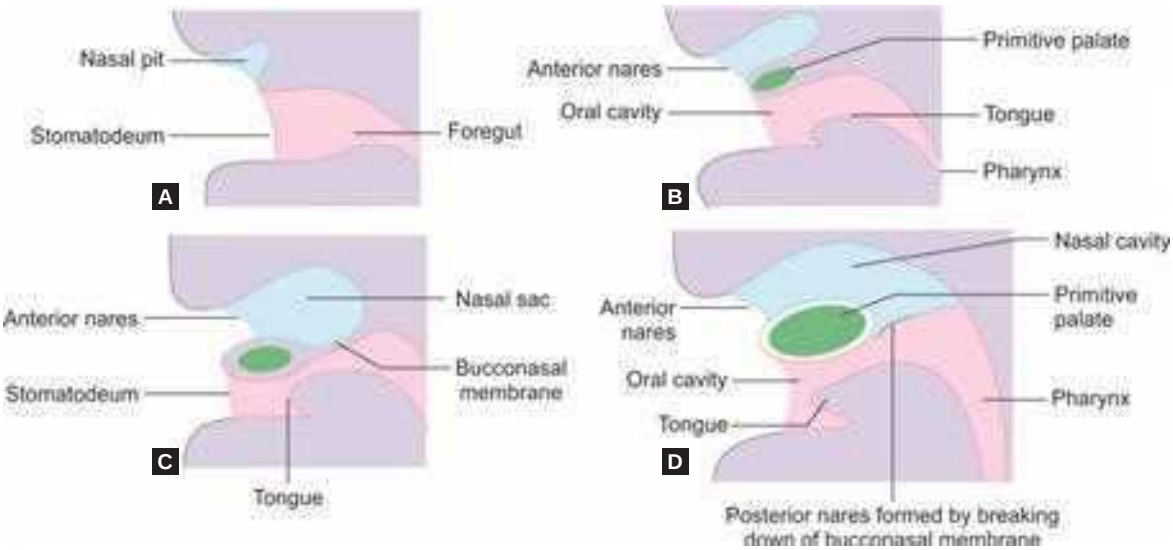
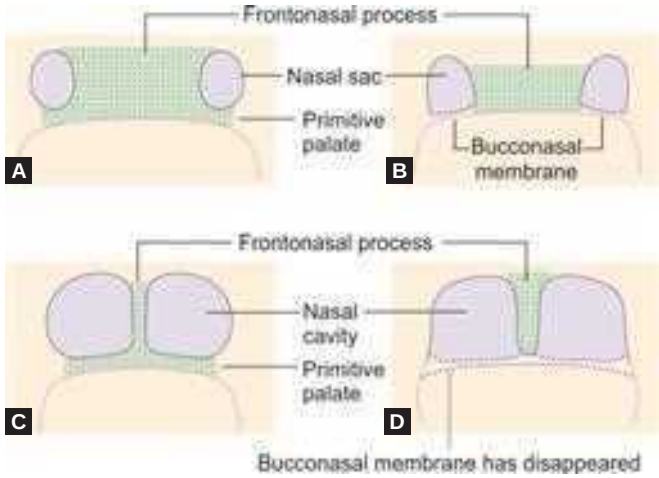


Fig. 11.13: Abnormal face showing single median eye (cyclops). A rod-like projection is seen above the eye (proboscis). Also see Figure 19.11



Figs 11.14A to D: Parasagittal sections through developing nasal cavity. (A) Nasal pit formed; (B) Nasal pit deepens. It is separated from the stomatodeum by the primitive palate; (C) The nasal pit enlarges to form the nasal sac. Posterior to the primitive palate the sac is separated from the oral cavity by the bucconasal membrane; (D) Bucconasal membrane breaks down



Figs 11.15A to D: Formation of the nasal septum. A and C are coronal sections through the anterior part of the nasal sac. B and D are sections through the posterior part. (A) Right and left nasal sacs are widely separated by the frontonasal process. Anterior part of nasal sac is separated from the stomatodeum by the primitive palate; (B) Posterior part of nasal sac is separated from the stomatodeum by the bucconasal membrane; (C) Nasal sacs enlarge and come close together. The frontonasal process is narrow and forms the nasal septum. The lower edge of the septum reaches the primitive palate; (D) Bucconasal membrane breaks down. As a result the posterior part of the nasal sac opens into the stomatodeum

Later, the frontonasal process becomes progressively narrower. This narrowing of the frontonasal process, and the enlargement of the nasal cavities themselves, brings them closer together. The intervening tissue becomes much thinned to from the *nasal septum* (Figs 11.15C and D). The ventral part of the nasal septum is attached below to the primitive palate (Fig. 11.15C). More posteriorly, the septum is at first attached to the bucconasal membrane (Fig. 11.15D), but on disappearance of this membrane it has a free lower edge. The nasal cavities are separated from the mouth by the development of the palate, as described below.

- The *lateral wall* of the nose is derived, on each side, from the lateral nasal process. The *nasal conchae* appear as elevations on the lateral wall of each nasal cavity. The original olfactory placodes form the *olfactory epithelium* that lies in the roof, and adjoining parts of the walls, of the nasal cavity.
- The development of various components of nose can be summarized as follows:
  - Frontonasal process—forms dorsum and tip of nose
  - Nasal pit—original site of it forms the anterior nares (nostrils)
  - Nasal sacs—the elongation of nasal pits form the nasal cavity
  - Bucconasal membrane—rupture of this membrane forms the posterior nares (Choanae).

Clinical correlation

Anomalies of the nasal cavity

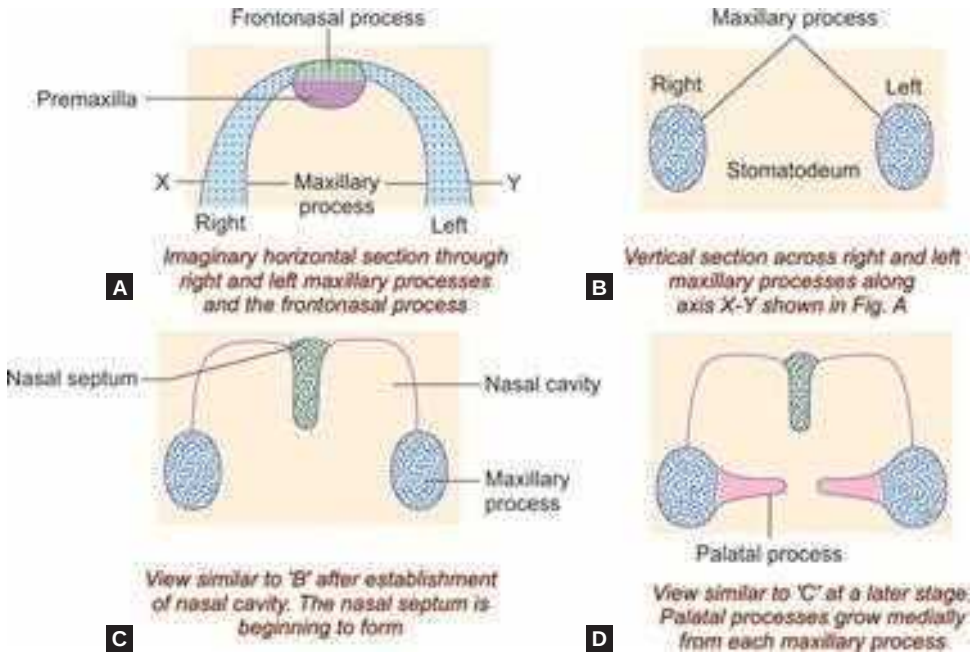
- There may be atresia of the cavity at the external nares, at the posterior nasal aperture, or in the cavity proper. This may be unilateral or bilateral. Very rarely, there may be total absence of the nasal passages.
- Congenital defects in the cribriform plate of the ethmoid bone may lead to a communication between the cranial cavity and the nose.
- The nasal septum may not be in the middle line, i.e. it may be deflected to one side. The septum may be absent.
- The nasal cavity may communicate with the mouth.

Paranasal Sinuses

- The paranasal sinuses appear as *diverticula from the nasal cavity*. The diverticula gradually invade the bones after which they are named, i.e. the sphenoid, maxilla, frontal, ethmoid and then expand.
- They are named accordingly into sphenoidal, maxillary, ethmoidal and frontal air sinuses.
- The paranasal sinuses are ectodermal in origin.
- The maxillary and sphenoidal sinuses begin to develop before birth. The other sinuses develop after birth.
- Enlargement of paranasal sinuses is associated with overall enlargement of the facial skeleton, including the jaws. This provides space in the jaws for growth and eruption of teeth.
- Growth of the facial skeleton is responsible for the gradual change in looks of a baby.

DEVELOPMENT OF PALATE

- To understand the development of the palate, let us have another look at the *maxillary process*. From Figures 11.6 and 11.10, it will be seen that these processes not only form the upper lip but also extend backward on either side of the stomatodeum. They can, therefore, be diagrammatically illustrated as in Figure 11.16A.
- If we cut a coronal section through the region (along the line XY in Fig. 11.16A) the maxillary processes will be seen as in Figure 11.16B. Finally, if we now correlate Figure 11.16B with Figure 11.15D the relationship of the maxillary processes to the developing nasal cavity and mouth is easily understood (Fig. 11.16C).
- From each maxillary process, a palate like shelf grows medially (Fig. 11.16D). This is called the *palatal process* of maxilla.
- We now have three components from which the palate will be formed. These are (Figs 11.17 and 11.18):



Figs 11.16A to D: Development of the palate

  - *Primary/Primitive palate*: Develops from frontonasal process
  - *Secondary palate/Palatal processes*: Develop from maxillary process.
- *Primary palate*: Fusion of the two medial nasal processes of frontonasal process at a deeper level forms a wedge-shaped mass of mesenchyme opposite upper jaw carrying four incisor teeth. The part of the palate derived from the frontonasal process forms the form *premaxilla* or *primary palate* which carries the incisor teeth. This ossifies and represents only small part lying anterior to incisive fossa.
- *Secondary palate*: Tongue develops in the floor of oral cavity. The palatine processes of maxilla are hook like projections on either side of tongue. Later they assume horizontal position above the tongue and fuse with each other forming the secondary palate. At a later stage, the mesoderm in the palate undergoes intramembranous ossification to form the *hard palate*. However, ossification does not extend into the most posterior portion, which remains as the *soft palate*. The secondary palate forms most of the hard palate and whole of soft palate. Soft palate is invaded by muscles migrating from first arch (Tensor palate) and fourth arch (Levator palati, palatoglossus, palatopharyngeus and musculus uvulae).
- The *definitive/permanent palate* is formed by the fusion of these three parts as follows:

1. *Fusion of palatal processes of maxilla with primitive palate*: Each palatal process fuses with the posterior margin of the primitive palate (Fig. 11.18) in a Y-shaped manner. Each limb of Y extends between lateral incisor and canine teeth. The junction of these two components in the midline is represented by incisive fossa.
2. *Fusion of both palatal processes of maxilla*: The two palatal processes fuse with each other in the midline (Fig. 11.19A). Their fusion begins anteriorly and proceeds backward.

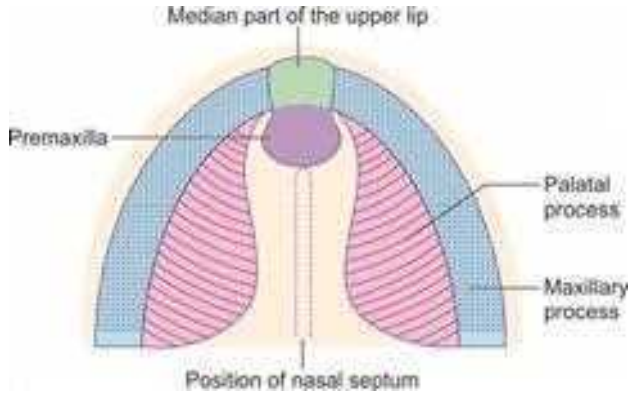


Fig. 11.17: Constituents of the developing palate as seen in a schematic horizontal section through the maxillary processes

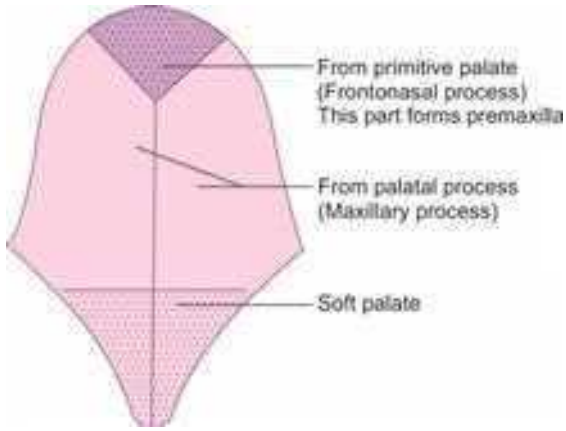


Fig. 11.18: Embryological subdivisions of the palate and the lines of fusion of these subdivisions

3. *Fusion of palatal processes with nasal septum*: The medial edges of the palatal processes fuse with the free lower edge of the nasal septum (Fig. 11.19B), thus separating the two nasal cavities from each other, and from the mouth.
   - Anterior three-fourths of permanent palate is ossified in membrane and forms the hard palate. Posterior one-fourth is the unossified part that forms the soft palate.

Clinical correlation

Cleft palate

Defective fusion of the various components of the palate gives rise to clefts in the palate. These vary considerably in degree as illustrated in Figure 11.20.

***Complete cleft palate*:**

- ***Bilateral complete cleft:*** Failure of fusion of both palatine processes of maxilla with premaxilla. A Y-shaped cleft will be present between primary and secondary palate and between the two halves of secondary palate. It presents bilateral cleft of upper lip also (Fig. 11.20A).
- ***Unilateral complete cleft:*** Nonfusion of one side palatine process of maxilla with premaxilla. It presents unilateral cleft of upper lip (Fig. 11.20B).

***Incomplete cleft palate*:**

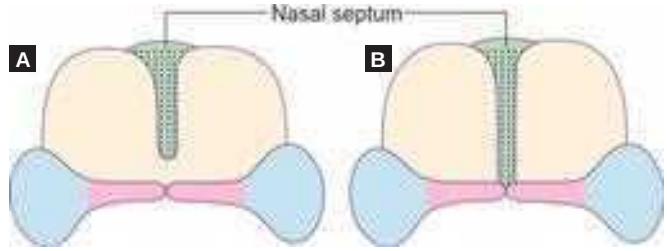
- ***Cleft of hard and soft palate:*** Cleft limited to hard palate (Fig. 11.20C).
- ***Cleft of soft palate:*** Cleft limited to hard palate (Fig. 11.20D).
- ***Bifid uvula:*** Cleft limited to uvula (Fig. 11.20E).

TIME TABLE OF SOME EVENTS IN THE DEVELOPMENT OF FACE, NOSE AND PALATE

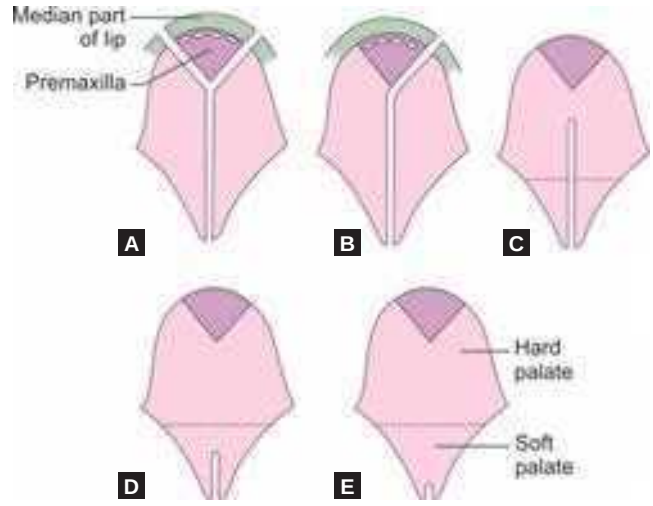
Time table of some events described in this chapter is shown in Table 11.2.

TABLE 11.2: Time table of developmental events

Age	*Developmental events*
4th week (28th day)	• Frontonasal, maxillary and mandibular processes can be identified • Lens and nasal placodes are present
5th week (31–35 days)	Nasal pits are established
6th week	• Tubercles for the development of pinna begin to be formed • On each side, palatal process arises from the maxillary process
7th week	• Eyelids are established • maxillary process fuses with the medial nasal process
8th week	• Eyes shift from a lateral to a frontal position • Bucconasal membrane ruptures
10th week	Palatal processes and nasal septum fuse with each other



Figs 11.19A and B: Separation of nasal cavities from each other, and from the mouth. Compare with Figure 11.16D



Figs 11.20A to E: Varieties of cleft palate. (A) Complete cleft with bilateral harelip; (B) Unilateral cleft palate and cleft of upper lip. The left maxillary process has fused with the premaxilla, but not with the right maxillary process. The cleft is accompanied by unilateral harelip; (C) Midline cleft of hard palate and soft palate; (D) Cleft of soft palate; (E) Bifid uvula

EMBRYOLOGICAL EXPLANATION FOR CLINICAL CONDITIONS OR ANATOMICAL OBSERVATIONS

Case Scenario 1

A mother brings her apparently normal newborn of 20 days old with a complaint of milk coming through nasal passages of the baby during feeding. What could be the cause for nasal regurgitation of fluids? How this condition can be diagnosed. Describe the embryological basis and what are the probable causes for this condition and what advice to be given in this case.

- The cause for nasal regurgitation of fluids is cleft palate.
- It is diagnosed by a physical examination of mouth, nose and palate. In the present case, it is a case of cleft palate. There are different types of cleft palate. It can be associated with the cleft lip. In the present case as the baby is apparently normal, the cleft is ruled out.
- Prenatal ultrasound at 16–20 weeks facilitates diagnosis of this condition.
- The four components that are involved in the development of palate are the paired palatal processes of maxillae, nasal septum and premaxilla. Timing of fusion and extent of fusion of these components plays an important role in the development of normal palate.
- Because of cleft in the palate the newborn is having feeding problems. This can lead to loss of weight. It can lead to repeated ear infections and speech difficulties.
- It is caused by a combination of genetic, viral, toxins and environmental factors. Proper nutrition and prenatal vitamins in antenatal period reduce the incidence of cleft palate. Use of anti-epileptic drugs by the mother especially valproic acid is known to cause this defect. It can be part of a syndrome, e.g. Pierre Robin, DiGeorge, Edwards, and Patau.
- This condition is not life-threatening. This case required surgery to close the cleft within 1st year of life to avoid speech difficulties and hearing problems.

REVIEW QUESTIONS

1. Explain development of face.
2. Explain development of palate.
3. Write short notes on cleft palate.
4. Describe developmental anomalies of face.

Chapter 12

Alimentary System—I: Mouth, Pharynx and Related Structures

HIGHLIGHTS

- The *oral cavity* is derived partly from the stomatodeum (ectoderm) and partly from the foregut (endoderm). These two are separated by the buccopharyngeal membrane which later disappears.
- *Teeth* are formed in relation to the dental lamina. An enlargement of the lamina is formed for each tooth. It is called the *enamel organ*.
- Ameloblasts (derived from ectoderm) form the *enamel*.
- *Odontoblasts* (derived from mesoderm) form *dentine*.
- The *pulp* is formed by mesenchyme that invaginates into the enamel organ.
- Three swellings appear in the floor of the pharynx, in relation to the first pharyngeal arch. These are the right and left *lingual swellings*, and a median swelling the *tuberculum impar*. Another median swelling is formed in relation to the third and fourth arches. This is the *hypobranchial eminence*.
- The *anterior two-thirds of the tongue* is formed from the lingual swellings and the tuberculum impar.
- The *posterior one-third* of the tongue is formed by the cranial part of the hypobranchial eminence.
- *Salivary glands* develop as outgrowths of buccal epithelium.
- The *pharynx* is derived from the foregut.

MOUTH

The mouth is *bidermal* in development. It is derived partly from the *stomatodeum (ectodermal)* and partly from the cranial part of the *foregut (endodermal)*. Hence its epithelial lining is partly ectodermal and partly endodermal and the demarcation between the two is buccopharyngeal membrane. After disappearance of the buccopharyngeal membrane (4th week), both become continuous with each other and the line of junction between the ectoderm and endoderm is difficult to define.

Primitive Oral Cavity

The *stomatodeum* is divided into two parts by developing primitive and definitive palate.

1. *Nasal part*—forms mucus lining of nasal cavity, nasal septum and palate.
2. *Oral part*—forms mucus lining of cheek, lips, gums and enamel of teeth.

Nasal part was described in Chapter 11: Face, Nose and Palate.

The derivatives of oral part can be subdivided into those from ectoderm and those from endoderm.

- Ectodermal developmental derivatives are the major constituents. They are:
  - Epithelium lining inside of lips, cheeks and palate
  - Teeth and gums.
- Endodermal derivatives are the minor constituents that contribute mainly for the floor of oral cavity. They form epithelium of:

- Tongue, floor and soft palate
- Palatoglossal and palatopharyngeal folds.

Definitive Oral Cavity

- The epithelium lining the inside of the lips and cheeks, and the palate, is most probably ectodermal.
- The teeth and gums are also of ectodermal origin.
- The epithelium of the tongue is, however, derived from endoderm (Fig. 12.1).
- Floor of oral cavity is derived from foregut, hence endodermal.
- Alveolabial sulcus is ectodermal.
- Alveolingual sulcus is endodermal.
- *Floor of mouth*: In the region of the floor of the mouth, the mandibular processes take part in the formation of three structures. These are:
  1. Lower lip and lower part of cheeks
  2. Lower jaw
  3. Tongue.
  - At first these regions are not demarcated from each other (Fig. 12.2A). Soon the tongue forms a recognizable swelling, which is separated laterally from the rest of the mandibular process by the *linguogingival sulcus* (Fig. 12.2B) which is endodermal.

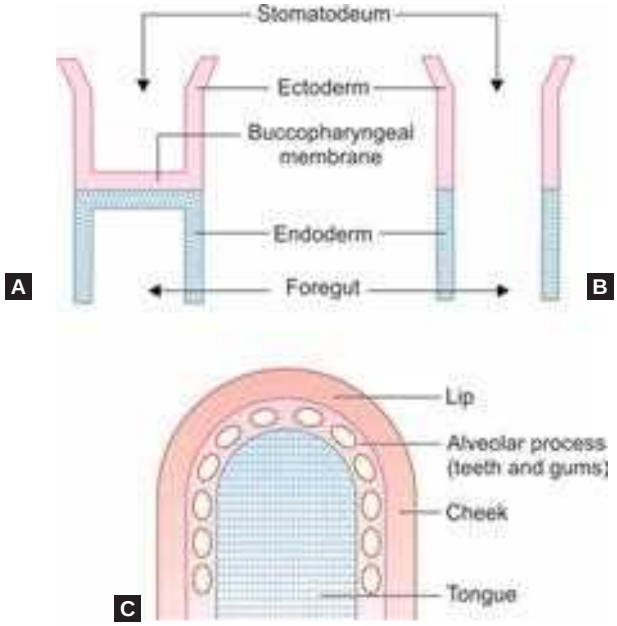


Fig. 12.1: Derivation of the ectodermal part, and endodermal part of the floor of the mouth. (A) Stomatodeum separated from foregut by buccopharyngeal membrane. (B) Buccopharyngeal membrane disappears. (C) Lips, cheeks and gums lined by ectoderm, tongue by endoderm

  - Soon, thereafter, another more laterally placed sulcus makes its appearance. This is called the *labiogingival sulcus* (Fig. 12.2C) which is ectodermal. This sulcus deepens rapidly and the tissues of the mandibular arch lateral to it form the *lower lip* (or cheek). With the deepening of these two sulci, the area lying between them becomes a raised *alveolar process* (Fig. 12.2D). The alveolar process is between the labiogingival and linguogingival sulci. The alveolar process forms the *jaw*, and the *teeth* develop in relation to it. The tongue, the alveolar process (or jaw) and the lips (or cheeks) are thus separated from one another (Fig. 12.3).
- *Roof of mouth*: The roof of the mouth is formed by the palate. The development of the palate has already been considered. The alveolar process of the upper jaw is separated from the upper lip and cheek by appearance of a *labiogingival furrow*, just as in the lower jaw. The medial margin of the alveolus becomes defined when the palate becomes highly arched (Fig. 12.4).

Some anomalies in the region of the mouth are described in Chapter 11.

TEETH

- The teeth are formed in relation to the alveolar process. The epithelium overlying the convex border of this process becomes thickened and projects into the underlying mesoderm. This epithelial thickening is called the *dental lamina* (Figs 12.2C and D). The dental lamina is, in fact, apparent even before the alveolar process itself is defined (Fig. 12.2C).
- As the alveolar process is semicircular in outline (Fig. 12.3), the dental lamina is similarly curved (Fig. 12.5A).
- The dental lamina now shows a series of local thickenings, each of which is destined to form one milk tooth. These thickenings are called *enamel organs*. There are 10 such enamel organs (five on each side) in each alveolar process (Fig. 12.5B).
- The stages in the formation of an enamel organ and the development of a tooth are as follows:
  - *Stage of dental lamina*: Ectoderm over convex upper border of alveolar process thickens and projects into underlying mesoderm as dental lamina which is U shaped and corresponds to alveolar process. As already stated each enamel organ is formed by localized proliferation of the cells of the dental lamina (Figs 12.6A and B).
  - *Bud stage*: During this stage, ten thickening of dental lamina appears five on each side. These are called tooth buds/enamel organs.
  - *Cap stage:* As the enamel organ grows downward into the mesenchyme (of the alveolar process)

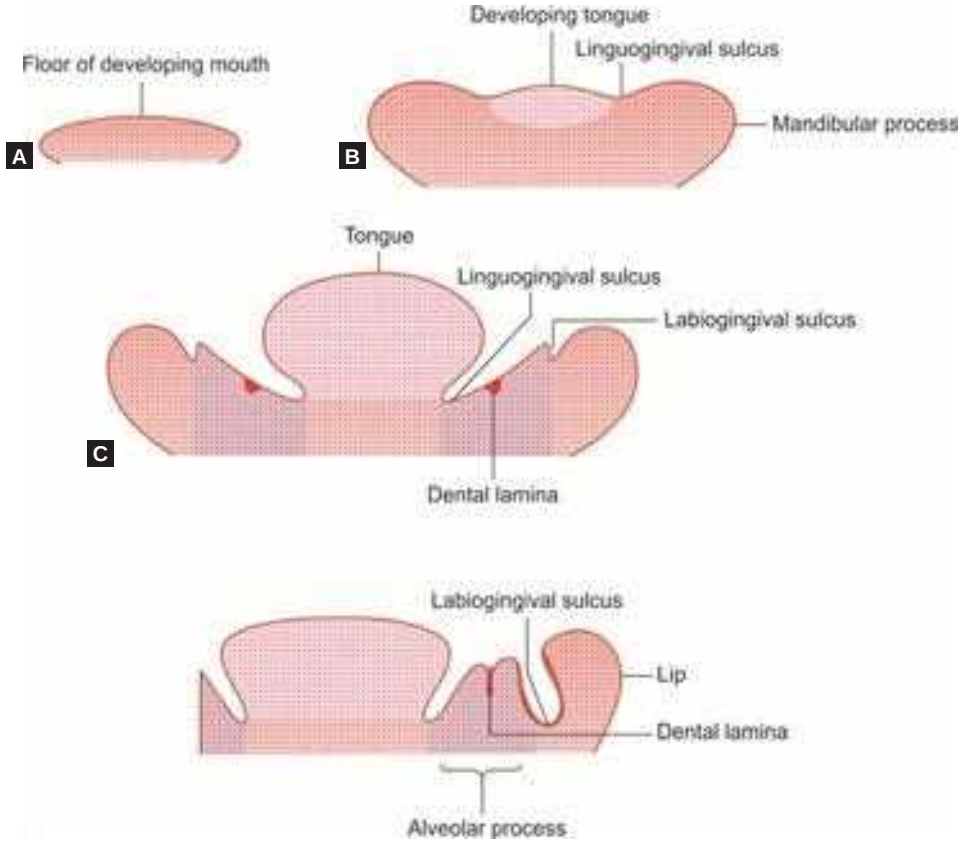


Fig. 12.2: (A) Floor of mouth formed by fused mandibular processes. (B) Linguogingival sulcus separates developing tongue from rest of mandibular processes. (C) Labiogingival sulcus separates alveolar process from lip (or cheek). The dental lamina, seen in the alveolar process, gives origin to teeth (Also see Fig. 12.3)

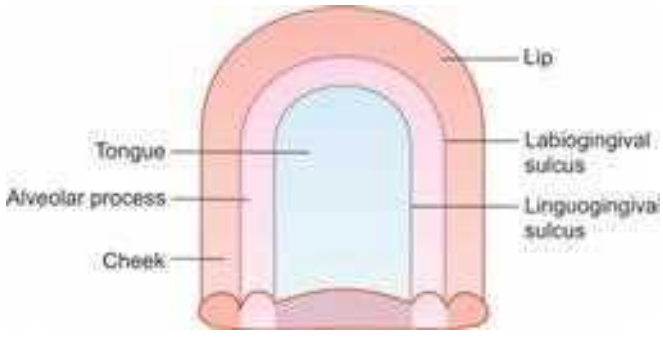


Fig. 12.3: Floor of mouth showing labiogingival and linguogingival sulci

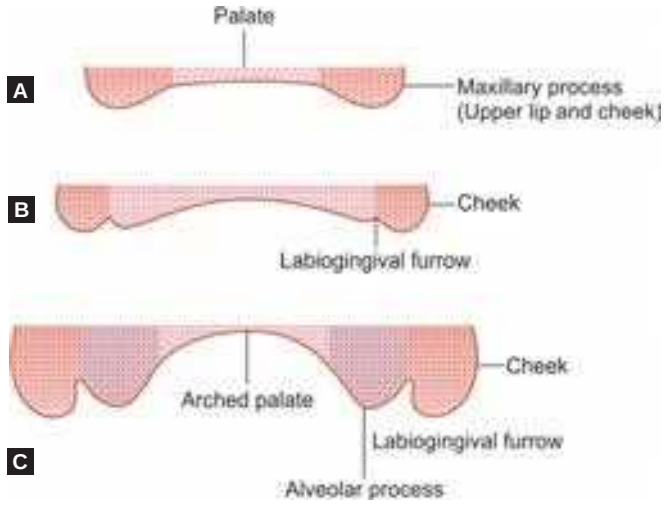


Fig. 12.4: Development of some structures seen in relation to the roof of the mouth. (A) Maxillary processes and palate. (B) Labio-gingival furrow separates upper lip (or upper part of cheek) from alveolar process (of upper jaw). (C) Medial margin of alveolar process becomes distinct because of upward arching of the palate

its lower end assumes a cup-shaped appearance (Fig. 12.6C). The cup comes to be occupied by a mass of mesenchyme called the *dental papilla* (According to some authorities, this mesenchyme is of neural crest origin). The enamel organ and the dental papilla together constitute the tooth germ. At this stage the developing tooth looks like a cap: it is, therefore, described as the *cap stage* of tooth development. The cells of the enamel organ that line the papilla become columnar. These are called *ameloblasts* (Fig. 12.6D).

- *Bell stage:* Mesodermal cells of the papilla that are adjacent to the ameloblasts arrange themselves as a continuous epithelium like layer. The cells of this layer are called *odontoblasts* (Fig. 12.6E). The ameloblasts and odontoblasts are separated by a basement membrane. The remaining cells of the papilla from the pulp of the tooth. The developing tooth now looks like a bell (*bell stage*).
- *Apposition stage:* Ameloblasts lay down enamel on the superficial (outer) surface of the basement membrane. The odontoblasts lay down dentine on its deeper surface. The process of laying down of enamel and of dentine is similar to that of formation of bone by osteoblasts. As layer after layer of enamel and dentine are laid down, the layer of ameloblasts and the layer of odontoblasts move away from each other (Fig. 12.7).
- After the enamel is fully formed the ameloblasts disappear leaving a thin membrane, the *dental cuticle*, over the enamel. The odontoblasts, however, continue to separate the dentine from the pulp throughout the life of the tooth.
- The alveolar parts of the maxillae and mandible are formed by ossification in the corresponding alveolar process. As ossification progresses, the roots of the teeth are surrounded by bone.
- The root of the tooth is established by continued growth into underlying mesenchyme. Odontoblasts in this region lay down dentine. As layers of dentine are deposited, the pulp space becomes progressively narrower and is gradually converted into a canal through which nerves and blood vessels pass into the tooth.
- In the region of the root there are no ameloblasts. The dentine is covered by mesenchymal cells that differentiate into *cementoblasts*. These cells lay down a layer of dense bone called the *cementum*. Still further to the outside, mesenchymal cells form the periodontal

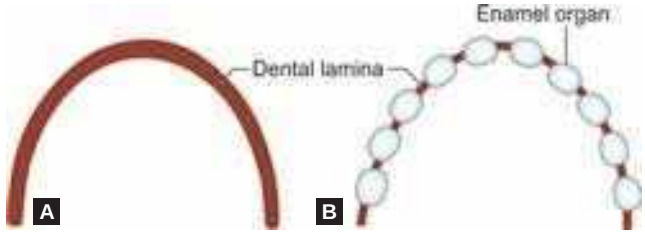


Fig. 12.5: Formation of enamel organs from dental lamina. (A) Dental lamina following the curve of the alveolar process (Compare with Fig. 12.3). (B) Enamel organs formed in relation to the dental lamina

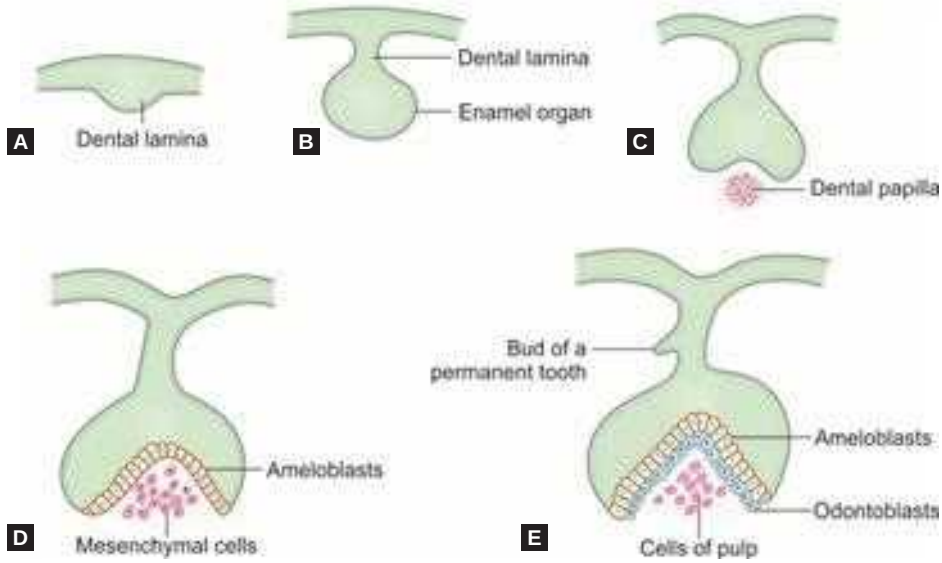


Fig. 12.6: Stages in the formation of a tooth germ. (A) Dental lamina formed by proliferation of ectoderm lining the alveolar process. (B) Deeper part of dental lamina enlarges to form enamel organ. (C) Mesodermal cells invaginate the enamel organ to form the papilla. (D) Layer of ameloblasts (ectoderm) formed from deepest cells of enamel organ. (E) Odontoblasts, derived from mesodermal cells, form a layer next to the ameloblasts

ligament which connects the root to the socket in the jaw bone.

- The permanent teeth are formed as follows:
  - The dental lamina gives off a series of buds, one of which lies on the medial side of each developing milk tooth (Figs 12.8 and 12.9). These buds form enamel organs exactly as described above. They give rise to the permanent incisors, canines and premolars.
  - The permanent molars are formed from buds that arise from the dental lamina posterior to the region of the last milk tooth.
- The dental lamina is established in the 6th week of intrauterine life. At birth the germs of all the temporary teeth, and of the permanent incisors, canines and first molars, show considerable development. The germs of the permanent premolars and of the second molars are rudimentary. The germ of the third molar is formed after birth. The developing tooth germs undergo calcification. All the temporary teeth and the permanent lower first molar begin to calcify before birth; the other permanent teeth begin to calcify at varying ages after birth.
- The eruption of a tooth is preceded by a major development of its root. The ages at which teeth erupt vary considerably. The average age of eruption of temporary tooth and permanent tooth and structural derivatives of tooth are summarized in Tables 12.1 to 12.3 respectively.

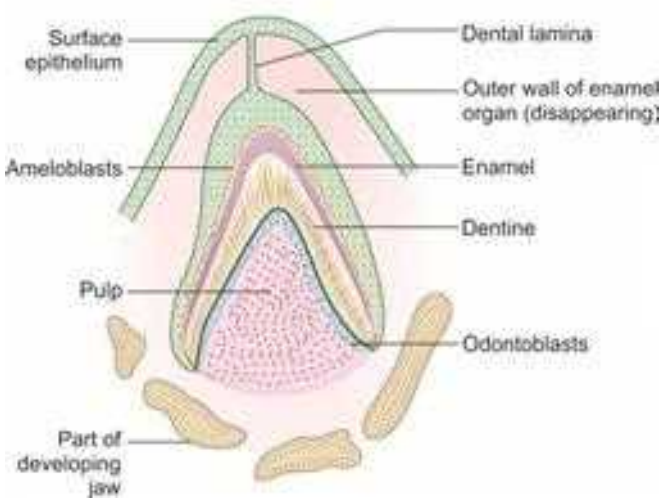


Fig. 12.7: Parts of a developing tooth. Ameloblasts lay down enamel. Odontoblasts lay down dentine. Ossification in relation to mesenchymal cells surrounding the developing tooth forms the jaw

Clinical correlation

Anomalies of teeth

- One or more teeth may be absent. Complete absence is called ***anodentia***.
- Supernumerary teeth may be present.
- Individual teeth may be abnormal. They may be too large or too small. They may have supernumerary cusps or roots. Alternatively, cusps or roots may be less than normal.
- Two (or more) teeth may be fused to each other (***gemination***).
- The alignment of the upper and lower teeth may be incorrect (***malocclusion***). This may be caused by one or more of the above anomalies or by defects of the jaws.
- Eruption of teeth may be precocious (i.e. too early). Lower incisors may be present at birth.
- Eruption of teeth may be delayed. The third molar frequently fails to erupt.
- Teeth may form in abnormal situations, e.g. in the ovary or in the hypophysis cerebri.
- There may be improper formation of the enamel or dentine of the tooth.

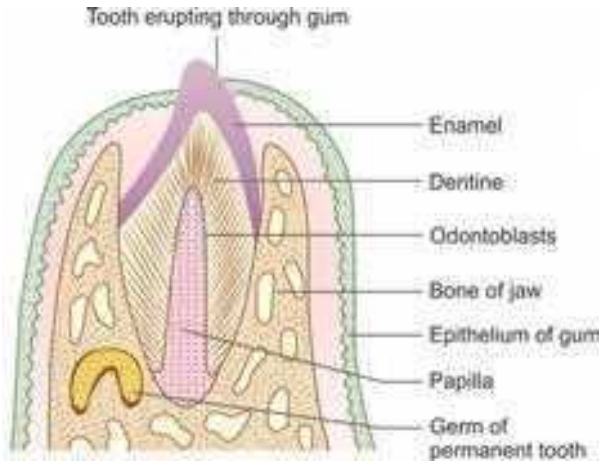


Fig. 12.8: Diagram of an erupting temporary tooth. Note its relationship to the jaw. Also observe germ of permanent tooth

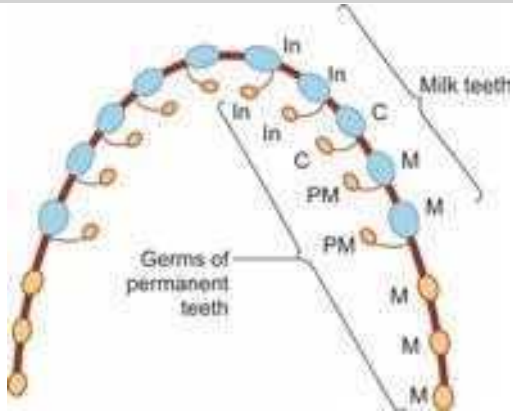


Fig. 12.9: Origin of germs of permanent teeth. Germs of permanent incisors, canines, and premolars are formed in relation to temporary teeth (as seen in Fig. 12.8). Permanent molars arise from the dental lamina behind the part that gives rise to temporary teeth

TABLE 12.1: Temporary or milk teeth—Time of eruption

Tooth	*Time of eruption*
Lower central incisors	6–9 months
Upper incisors	8–10 months
Lower lateral incisors	12–20 months
First molar	12–20 months
Canines	16–20 months
Second molars	20–39 months

TABLE 12.2: Permanent teeth—Time of eruption

Tooth	*Time of eruption*
First molar	6–7 years
Central incisors	6–8 years
Lateral incisors	7–9 years
Premolars	10–12 years
Canines	10–12 years
Second molars	11–13 years
Third molars	17–21 years

TABLE 12.3: Summary of the derivation of parts of tooth

Tissue	*Structures formed*
Ectoderm	Ameloblasts → Enamel
Mesoderm (of neural crest origin?)	Odontoblasts → Dentine
Mesenchyme around tooth	• Cementum • Periodontal ligament

PHARYNX

- Floor of pharynx is formed by fusion of ventral parts of pharyngeal arches and pouches. The floor contributes for the development of tongue, thyroid gland and lower respiratory tract.
- The pharynx is derived from the cranial most part of the foregut. We have already seen that the endodermal pouches are formed in relation to the lateral wall of this part of the foregut. The floor of the foregut gives rise to a midline diverticulum from which the entire respiratory system is developed (Chapter 14: Liver and Biliary Apparatus, Pancreas and Spleen; Respiratory System; Body Cavities and Diaphragm). Most of the endodermal pouches lose contact with the pharyngeal wall. The opening of the pharyngotympanic tube represents the site of origin of the tubotympanic recess. The site of the midline respiratory diverticulum is represented by the inlet of the larynx.
- With the establishment of the palate and mouth, the pharynx shows a subdivision into nasopharynx, oropharynx and laryngopharynx. The muscles forming the wall of the pharynx are derived from the third and subsequent pharyngeal arches.

TONGUE

- The tongue develops in relation to the pharyngeal arches (1st to 4th) in the floor of the developing mouth. It develops during 4th to 8th weeks. We have seen that each pharyngeal arch arises as a mesodermal thickening in the lateral wall of the foregut and that it grows ventrally to become continuous with the corresponding arch of the opposite side (Fig. 12.10).
- The medial most parts of the mandibular arches proliferate to form two *lingual swellings* (Fig. 12.11). The lingual swellings are partially separated from each other by another swelling that appears in the midline. This median swelling is called the *tuberculum impar*.
- Immediately behind the tuberculum impar, the epithelium proliferates to form a downgrowth (*thyroglossal duct*) from which the thyroid gland develops. The site of this downgrowth is subsequently marked by a depression called the *foramen cecum*.
- Another, midline swelling is seen in relation to the medial ends of the second, third and fourth arches. This swelling is called the *hypobranchial eminence* or *copula of His* (Fig. 12.11). The eminence soon shows a subdivision into a cranial part related to the second and third arches (called the *copula*) and a caudal part related to the fourth arch (Fig. 12.12A). The caudal part forms the epiglottis.
- The *anterior two thirds of the tongue* is formed by fusion of the tuberculum impar and the two lingual swellings. The anterior two thirds of the tongue is thus derived from the mandibular arch (Figs 12.12B and C). According to some, the tuberculum impar does not make a significant contribution to the tongue.

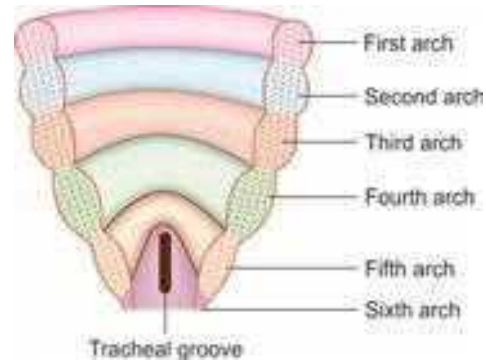


Fig. 12.10: Floor of primitive pharynx: Stage 1. Note that the right and left pharyngeal arches meet in the midline to form the floor of the pharynx

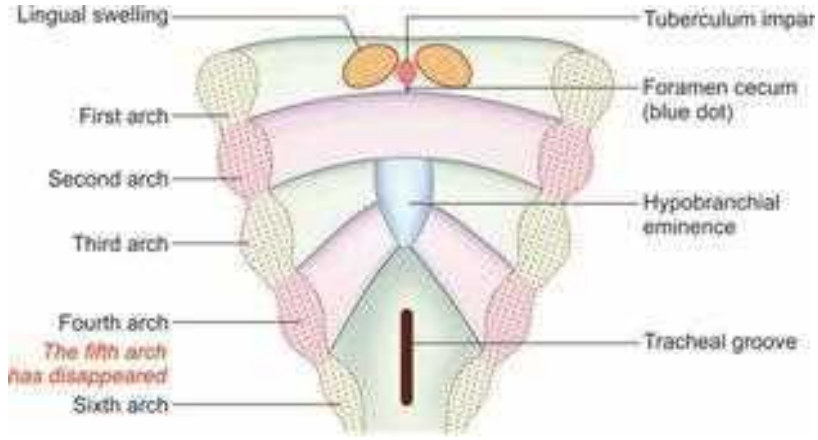


Fig. 12.11: Floor of primitive pharynx: Stage 2. The fifth pharyngeal arch has disappeared. Note the right and left lingual swellings, and the tuberculum impar formed in relation to the first arch; and the hypobranchial eminence formed in relation to the medial ends of the third and fourth arches

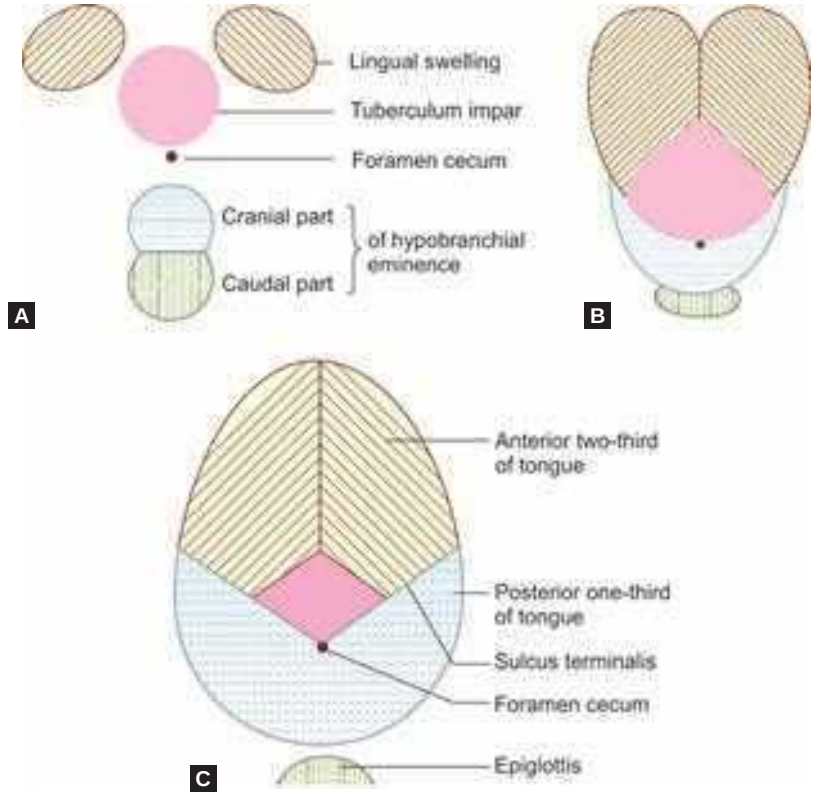


Fig. 12.12: Scheme to show the origin of different parts of the tongue

- The *posterior one-third of the tongue* is formed from the cranial part of the hypobranchial eminence (copula) (Fig. 12.12). In this situation, the second arch mesoderm gets buried below the surface. The third arch mesoderm grows over it to fuse with the mesoderm of the first arch (Fig. 12.13). The posterior one-third of the tongue is thus formed by third arch mesoderm.
- The *posterior most part of the tongue* is derived from the fourth arch (Fig. 12.13).
- The line of junction of anterior two thirds and posterior one-third of tongue is indicated by an inverted V-shaped *sulcus terminalis*. In keeping with its embryological origin, the anterior two thirds of the tongue is supplied by the lingual branch of the mandibular nerve, which

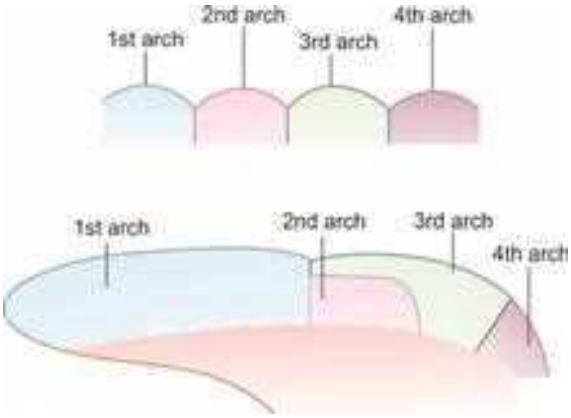


Fig. 12.13: Scheme to show how the second arch is buried by overgrowth of the third arch, during development of the tongue

is the posttrematic nerve of the first arch, and by the chorda tympani which is the pretrematic nerve of this arch. The posterior one-third of the tongue is supplied by the glossopharyngeal nerve, which is the nerve of the third arch. The most posterior part of the tongue is supplied by the superior laryngeal nerve, which is the nerve of the fourth arch.

- The components of tongue include mucous membrane, muscles and fibroareolar stroma. The mucosa of tongue is derived from endoderm of foregut.
- The musculature of the tongue is derived from the occipital myotomes. This explains its nerve supply by the hypoglossal nerve, which is the nerve of these myotomes.
- The fibroareolar stroma is derived from the pharyngeal arch mesoderm.
- The epithelium of the tongue is at first made up of a single layer of cells. Later it becomes stratified and papillae become evident. *Taste buds* are formed in relation to the terminal branches of the innervating nerve fibers. The circumvallate papillae of tongue develop from the cranial part of hypobranchial eminence and migrate to the anterior aspect of sulcus terminalis. They are supplied by glossopharyngeal nerve.
- The various components of the tongue and their embryonic origin and innervation are presented in Table 12.4.

Clinical correlation

Anomalies of the tongue

- The tongue may be too large (***macroglossia***) or too small (***microglossia***). Very rarely the tongue may be absent (***aglossia***).
- The tongue may be bifid because of nonfusion of the two lingual swellings.
- The apical part of the tongue may be anchored to the floor of the mouth by an overdeveloped frenulum. This condition is called ***ankyloglossia*** or tongue-tie. It interferes with speech. Occasionally, the tongue may be adherent, to the palate (***ankyloglossia superior***).
- A red, rhomboid-shaped smooth zone may be present on the tongue in front of the foramen cecum. It is considered to be the result of persistence of the tuberculum impar.
- Thyroid tissue may be present in the tongue either under the mucosa or within the muscles.
- Remnants of the thyroglossal duct may form cysts at the base of the tongue.
- The surface of the tongue may show fissures.

DERIVATIVES OF ORAL CAVITY

The derivatives of oral cavity are:

- Salivary glands—Appear as epithelial buds from oral cavity
  - Parotid gland: Ectodermal
  - Submandibular gland: Endodermal
  - Sublingual gland: Endodermal.

TABLE 12.4: Summary of the derivation of components of the tongue

Component	*Embryonic component*	*Sensory nerve General sensation*	*Sensory nerve Taste sensation*	*Motor nerve*
Mucosa of anterior 2/3rd (Oral part) Epithelium + connective tissue	1st arch	V—Mandibular Lingual branch	VII—Facial Chorda tympani branch	
Mucosa posterior 1/3rd (Pharyngeal part) Epithelium + Connective tissue	3rd arch	IX—Glossopharyngeal		
Posterior most near vallecula Epithelium+ Connective tissue	4th arch	X—Vagus Superior laryngeal nerve		
Musculature All intrinsic + All extrinsic except palatoglossus	Occipital myotomes (3–4 nos) 4th arch Palatoglossus			XII—Hypoglossal X—Vagus—pharyngeal branches
Papillae and taste buds	CV and foliate: IX—glossopharyngeal Fungiform and filiform: VII—Facial			

- Pituitary gland—Roof of stomatodeum contributes for adenohypophysis. The development of pituitary gland is discussed in Chapter 18.

SALIVARY GLANDS

The salivary glands develop as outgrowths of the buccal epithelium. The outgrowths are at first solid and are later canalized. They branch repeatedly to form the duct system. The terminal parts of the duct system develop into secretory acini.

As the salivary glands develop near the junctional area between the ectoderm of the stomatodeum and the endoderm of the foregut, it is difficult to determine whether they are ectodermal or endodermal.

Parotid Gland

It is the first salivary gland to appear (early 6th week). It arises from the oral ectoderm near angle of stomatodeum. It grows outward between maxillary process and mandibular arch in the form of ectodermal cords of cells. Proximal part canalizes and forms duct that opens into the mouth. The distal part extends into the cheek mesenchyme and reaches up to the developing ear where it branches and expands to form the secreting units/alveoli of gland. Fusion of maxillary process and mandibular arch results in shifting of opening of parotid duct into the vestibule opposite upper second molar. Capsule and connective tissue is formed from the surrounding mesoderm.

Submandibular Gland

It appears in the later part of 6th week. It appears as an endodermal bud or outgrowth in the floor of stomatodeum at the linguogingival sulcus. Canalization of outgrowth occurs to form duct, acini and ductules. Duct opens on sublingual papilla.

Sublingual Gland

It appears during 8th week as multiple endodermal buds from linguogingival sulcus. One or more of the salivary glands may sometimes be absent.

TIME TABLE OF SOME EVENTS DESCRIBED IN THIS CHAPTER

Time table of some events described in this chapter has been shown in Table 12.5.

TABLE 12.5: Time table of developmental events

Age	*Developmental events*
4 weeks	Tongue starts forming, i.e. two lateral lingual swelling and tuberculum impar become visible.
5 weeks	Hypobranchial eminence becomes visible.
6 weeks	Dental laminae of upper and lower jaws are established.
7 weeks	Salivary glands start developing.
8 weeks	Enamel organs are formed.
10 weeks	Enamel organ becomes cup-shaped.
6 months	Enamel and dentine have formed considerably. Formation of tongue is almost complete.
Just before birth	Cementum is formed.
After birth	Periodontal ligaments are formed before eruption of teeth.

REVIEW QUESTIONS

1. Explain development of tongue.
2. What are the stages in the development of tooth?
3. What is the time of eruption of temporary and permanent teeth?
4. Explain development of salivary glands.

Chapter 13

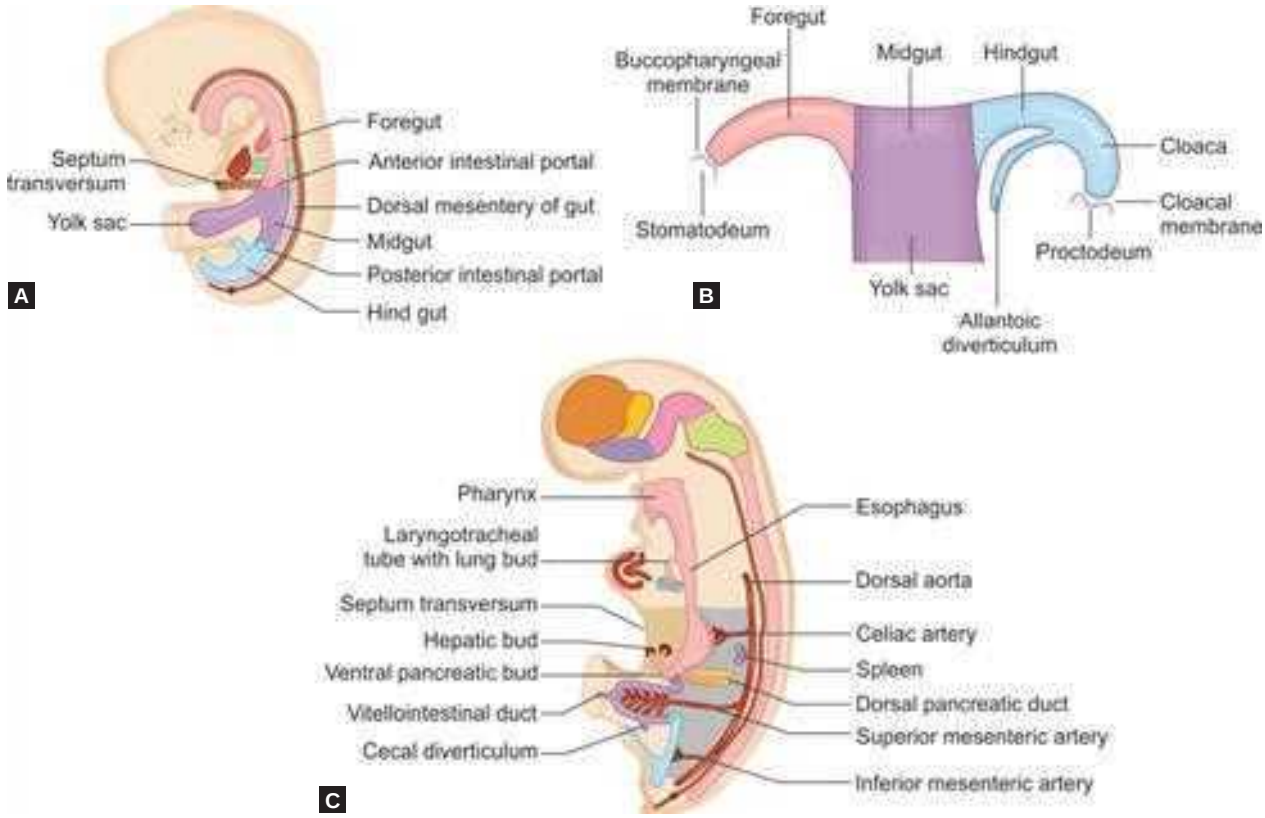
Alimentary System—II: Gastrointestinal Tract

HIGHLIGHTS

- *Endoderm*, which is at first in the form of a flat sheet, is converted into a tube by formation of head, tail and lateral folds of the embryonic disc. This tube is the gut.
- The gut consists of *foregut, midgut* and *hindgut*. The midgut is at first in wide communication with the yolk sac. Later it becomes tubular. Part of it forms a loop that is divisible into *prearterial* and *postarterial* segments.
- The most caudal part of the hindgut is the cloaca. It is partitioned to form the *primitive rectum* (dorsal) and the *primitive urogenital sinus*.
- The *esophagus* is derived from the foregut.
- The *stomach* is derived from the foregut.
- *Duodenum:* The superior part and the upper half of the descending part are derived from the foregut. The rest of the duodenum develops from the midgut.
- The *jejunum* and *ileum* are derived from the prearterial segment of the midgut loop.
- The postarterial segment of the midgut loop gives off a cecal bud. The *cecum* and *appendix* are formed by enlargement of this bud.
- The *ascending colon* develops from the postarterial segment of the midgut loop.
- After its formation, the gut undergoes *rotation*. As a result, the cecum and ascending colon come to lie on the right side; and the jejunum and ileum lie mainly in the left half of the abdominal cavity.

INTRODUCTION

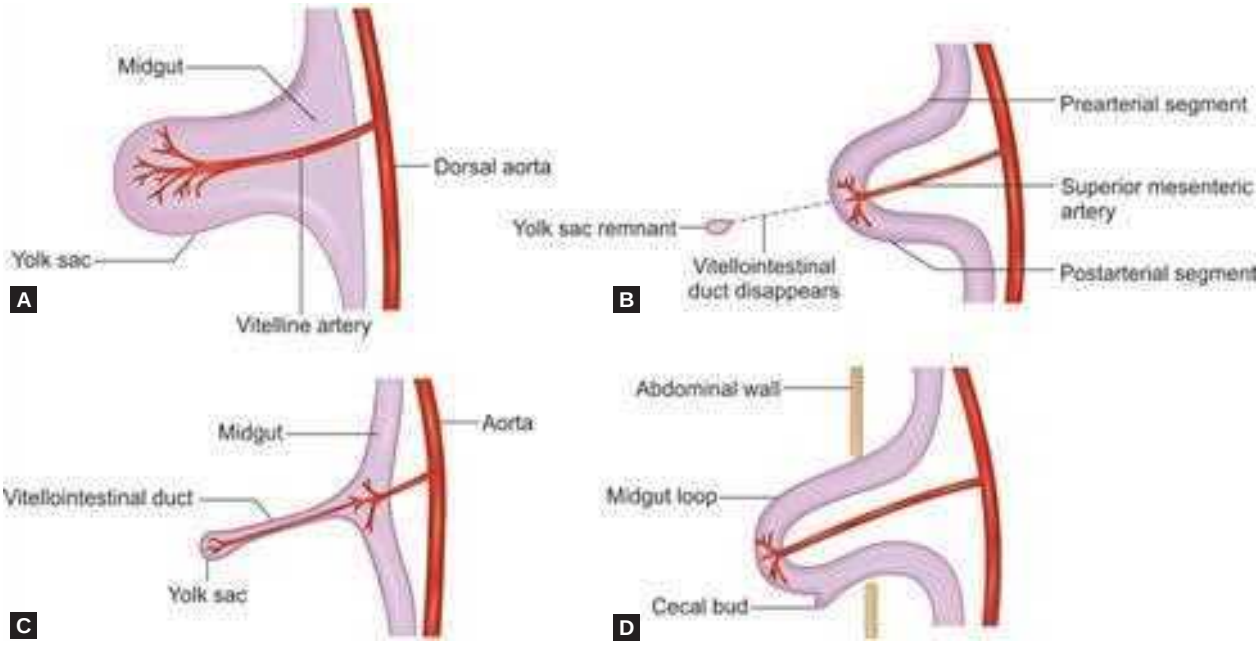
- With the establishment of the head and tail folds, part of the cavity of the *definitive yolk sac* is enclosed within the embryo to form the *primitive gut* (Figs 13.1A to C). The primitive gut is in free communication with the rest of the yolk sac. The part of the gut cranial to this communication is the *foregut*; the part caudal to the communication is the *hindgut*, while the intervening part is the *midgut* (Figs 13.1A to C). The communication between foregut and midgut is called *anterior intestinal portal* which is represented in the adult by the termination of bile duct in the second part of duodenum. The communication between the midgut and hindgut is called *posterior intestinal portal* which corresponds in the adult to the junction of right two-thirds with the left one-third of transverse colon.
- The foregut is in the head fold of the embryo. Cranially, the foregut is separated from the stomodeum by the buccopharyngeal membrane. The hindgut is in the tail fold of the embryo. Caudally, the hindgut is separated from the proctodeum by the *cloacal membrane*. At a later stage of development, the buccopharyngeal and cloacal membranes disappear, and the foregut and hindgut are in communication with stomodeum and proctodeum respectively (Fig. 13.1B). Thus, the gut communicates with the exterior. The midgut during early embryonic period communicates with the extraembryonic part of yolk sac via *vitellointestinal duct*. The vitellointestinal duct disappears by 5th week of development.
- The epithelial lining of various parts of the gastrointestinal tract is of endodermal origin. In the region of the mouth and the anal canal, however, some of the epithelium is



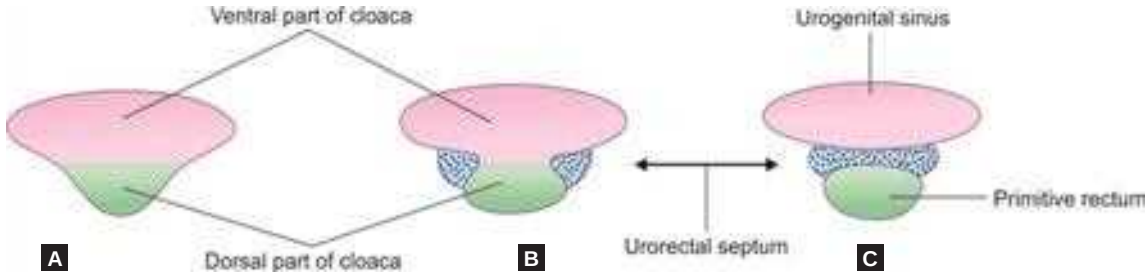
Figs 13.1A to C: Parts of the primitive gut

derived from the ectoderm of the stomodeum and of the proctodeum respectively.

- The gut is fixed to the ventral and dorsal body wall of the embryo by *ventral* and *dorsal mesenteries*.
- While the gut is being formed, the circulatory system of the embryo undergoes considerable development. A midline artery, the *dorsal aorta*, is established and comes to lie just dorsal to the gut (Figs 13.1C and 13.2). It gives off a series of branches to the gut. Those in the region of the midgut, initially, run right up to the yolk sac and are, therefore, called *vitelline arteries*. Subsequently, most of these ventral branches of the dorsal aorta disappear and only three of them remain; one for the foregut, one for the midgut and one for the hindgut. The artery of the abdominal part of the foregut is the *celiac*, that of the midgut is the *superior mesenteric* and that of the hindgut is the *inferior mesenteric*.
- The wide communication between the yolk sac and the midgut is gradually narrowed down (Fig. 13.2B) with the result the midgut becomes tubular. Thereafter, the midgut assumes the form of a loop (Fig. 13.2C). The superior mesenteric artery now runs in the mesentery of this loop to its apex. The loop can, therefore, be said to have a *proximal* or *prearterial* segment and a *distal* or *postarterial* segment. A bud (*cecal bud*) soon arises from the postarterial segment very near the apex of the loop (Fig. 13.2D).
- For a number of weeks, the midgut loop comes to lie outside the abdominal cavity of the embryo. It passes through the umbilical opening into a part of the extraembryonic coelom that persists in relation to the most proximal part of the umbilical cord. The loop is subsequently withdrawn into the abdominal cavity.
- While considering the formation of the allantoic diverticulum, it was seen that the diverticulum opens into the ventral aspect of the hindgut (Figs 13.1A to C). The part of the hindgut caudal to the attachment of the allantoic diverticulum is called the *cloaca*. The cloaca soon shows a subdivision into a broad ventral part and a narrow dorsal part (Figs 13.3A to C). These two parts are separated from each other by the formation of the *urorectal septum*, which is first formed in the angle between the allantois and the cloaca (Fig. 13.4B).
- The ventral subdivision of the cloaca is now called the *primitive urogenital sinus*, and gives origin to some parts of the urogenital system. The dorsal part is called the *primitive rectum*. It forms the rectum, and part of the anal canal. The urorectal septum grows toward the cloacal membrane and eventually fuses with it (Fig. 13.4B). The cloacal membrane is now divided into a



Figs 13.2A to D: Establishment of the midgut loop. (A) Midgut in wide communication with the yolk sac. Note vitelline artery passing from dorsal aorta to the yolk sac; (B) Yolk sac much smaller, and attached to midgut through a narrow vitellointestinal duct. The original vitelline artery gives branches to the midgut; (C) The midgut increases in length and forms a loop. The loop has a prearterial segment and a postarterial segment; (D) Midgut loop passes out of abdominal cavity. The cecal bud arises from the postarterial segment



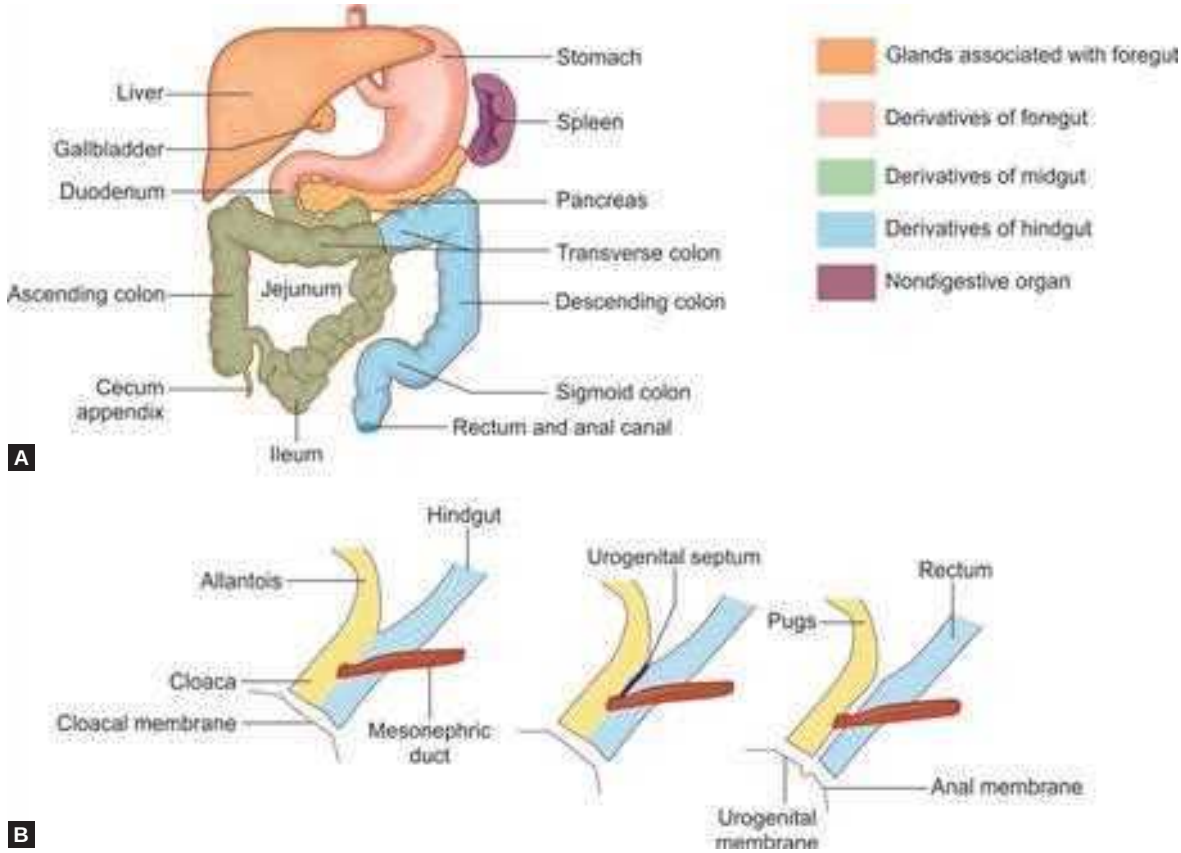
Figs 13.3A to C: Formation of urorectal septum as seen in transverse sections. This septum divides the cloaca into the primitive urogenital sinus and the primitive rectum

ventral *urogenital membrane*, related to the urogenital sinus, and a dorsal *anal membrane* related to the rectum.

- Mesoderm around the anal membrane becomes heaped up with the result that the anal membrane comes to lie at the bottom of a pit called the anal pit, or proctodeum. The anal pit contributes to the formation of the anal canal.
- Each one of the three segments of the primitive gut is divided into two parts. The derivatives of the gut are shown in Figures 13.1 and 13.4.

Derivatives of Foregut

- *Prelaryngeal part*:
  - Part of the floor of the mouth, including the tongue
  - Pharynx
  - Salivary glands
  - Various derivatives of the pharyngeal pouches, and the thyroid
  - Respiratory system.
- *Postlaryngeal part*:
  - Esophagus



Figs 13.4A and B: Derivatives of gut. Formation of urorectal septum as seen in longitudinal sections through the cloaca

- Stomach
- Duodenum: Whole of the superior (first) part and upper half of the descending (second) part (up to the major duodenal papilla)
- Liver and extrahepatic biliary system
- Pancreas.

Derivatives of Midgut

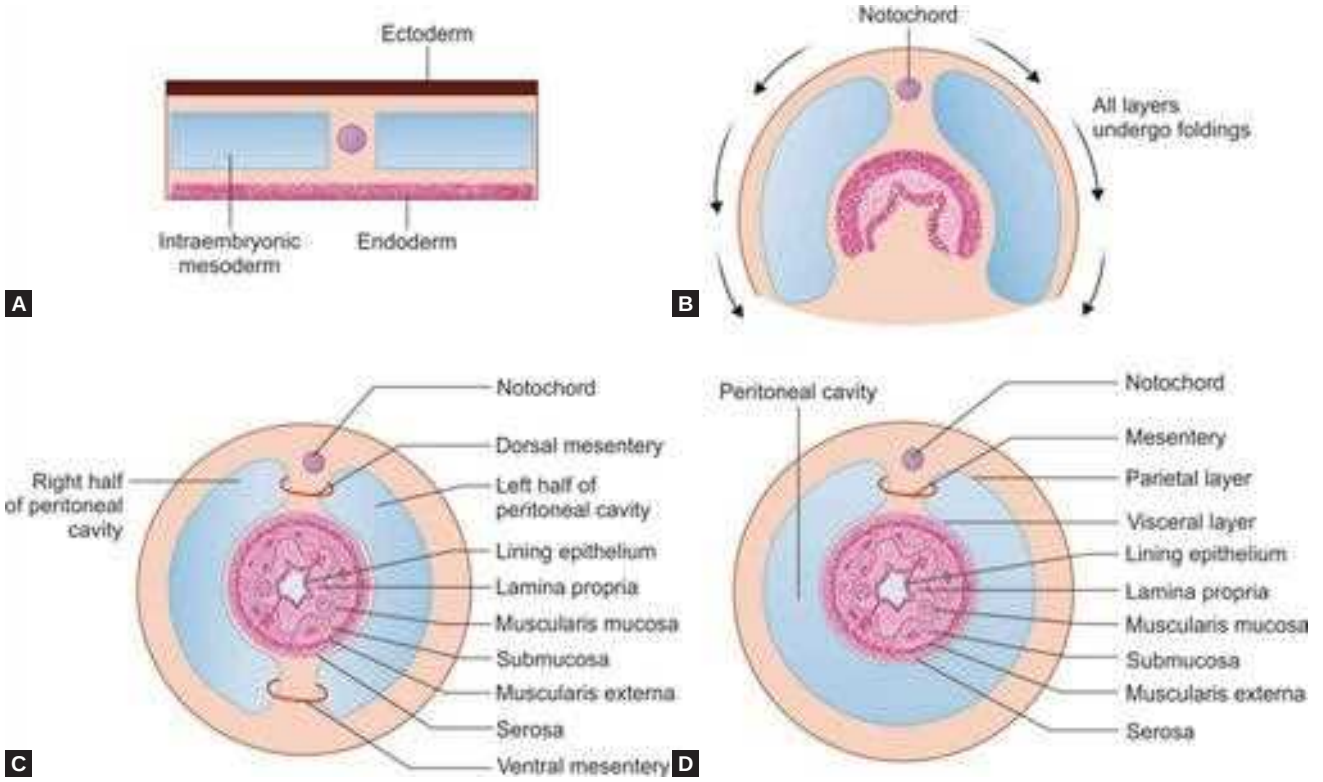
- *Prearterial segment*:
  - Duodenum: Descending (second) part distal to the major papilla; horizontal (third) and ascending (fourth) parts
  - Jejunum
  - Ileum except terminal part.
- *Postarterial segment*:
  - Terminal ileum
  - Cecum and appendix
  - Ascending colon
  - Right two-thirds of transverse colon.

Derivatives of Hindgut

- *Preallantoic part*:
  - Left one-third of transverse colon
  - Descending colon
  - Pelvic/sigmoid colon.
- *Postallantoic part*: It forms a dilated endodermal cloaca which is divided by urorectal septum into a dorsal part (primitive rectum) and a ventral part (primitive urogenital sinus).
  - Rectum
  - Upper part of anal canal
  - Parts of the urogenital system derived from the primitive urogenital sinus.
- At this stage, it might be noted that the endoderm of the foregut, midgut and hindgut gives rise only to the epithelial lining of the intestinal tract. Smooth muscle, connective tissue and peritoneum are derived from the splanchnic mesoderm (Figs 13.5 and 13.6).

Arteries of the Gut (Figs 13.1A to C)

- The celiac artery is the artery of the foregut. It supplies the gut from the lower part of the esophagus to the middle of the duodenum.
- The superior mesenteric artery is the artery of the midgut.



Figs 13.5A to D: Scheme to show how the gut is formed by lateral folding of the embryonic disc. (A) Embryonic disc before lateral folding; (B) The lateral edges of the disc grow in a ventral direction; (C and D) The edges pass medially to meet in the middle line. In this way, the layer of endoderm is converted into a tube which is the future gut. The ectoderm also meets in the midline and cuts off the coelom from the exterior

- The inferior mesenteric artery is the artery of the hindgut.

DERIVATION OF INDIVIDUAL PARTS OF ALIMENTARY TRACT

Foregut Development

Esophagus

- The esophagus is developed from the part of the foregut between the pharynx and the stomach.
- It is at first short but elongates with the formation of the neck, with the descent of the diaphragm, and with the enlargement of the pleural cavities.
- The musculature of the esophagus is derived from mesenchyme surrounding the foregut. Around the upper two-thirds of the esophagus, the mesenchyme forms striated muscle. Around the lower one-third, the muscle formed is smooth (as over the rest of the gut).

Stomach

- The stomach is first seen as a fusiform dilatation of the foregut just distal to the esophagus. Its dorsal border is attached to the posterior abdominal wall by a fold of peritoneum called the *dorsal mesogastrium*. Its ventral border is attached to the septum transversum by another fold of peritoneum called the *ventral mesogastrium* (Figs 13.7A and B; Figs 13.8 and 13.9A).
- Subsequently, the liver and the diaphragm are formed in the substance of the septum transversum (Fig. 13.9C).
- The ventral mesogastrium now passes from the stomach to the liver and from the liver to the diaphragm and anterior abdominal wall (Figs 13.7C and 13.9). The part

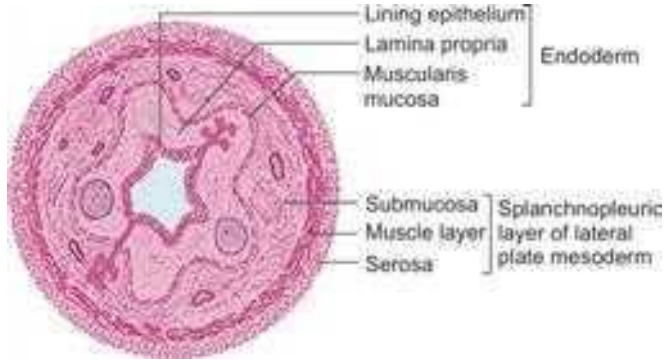
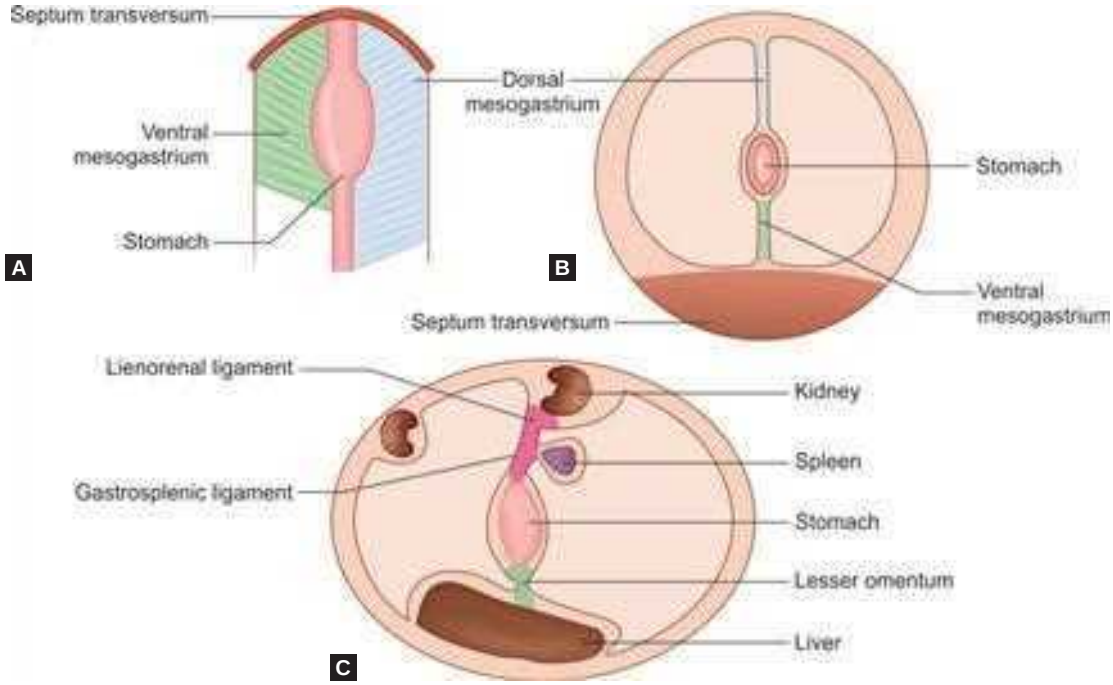


Fig. 13.6: Derivation of the coats of the gut



Figs 13.7A to C: (A) Side view of stomach showing the dorsal and ventral mesogastria; (B) Transverse section through "A" showing that the ventral mesogastrium connects the stomach to the septum transversum; (C) The most important remnant of the ventral mesogastrium is the lesser omentum. It passes from the stomach to the liver (which develops in the septum transversum). The spleen is formed in relation to the dorsal mesogastrium. Its formation divides this part of the dorsal mesogastrium into the gastrosplenic ligament and the lienorenal ligament

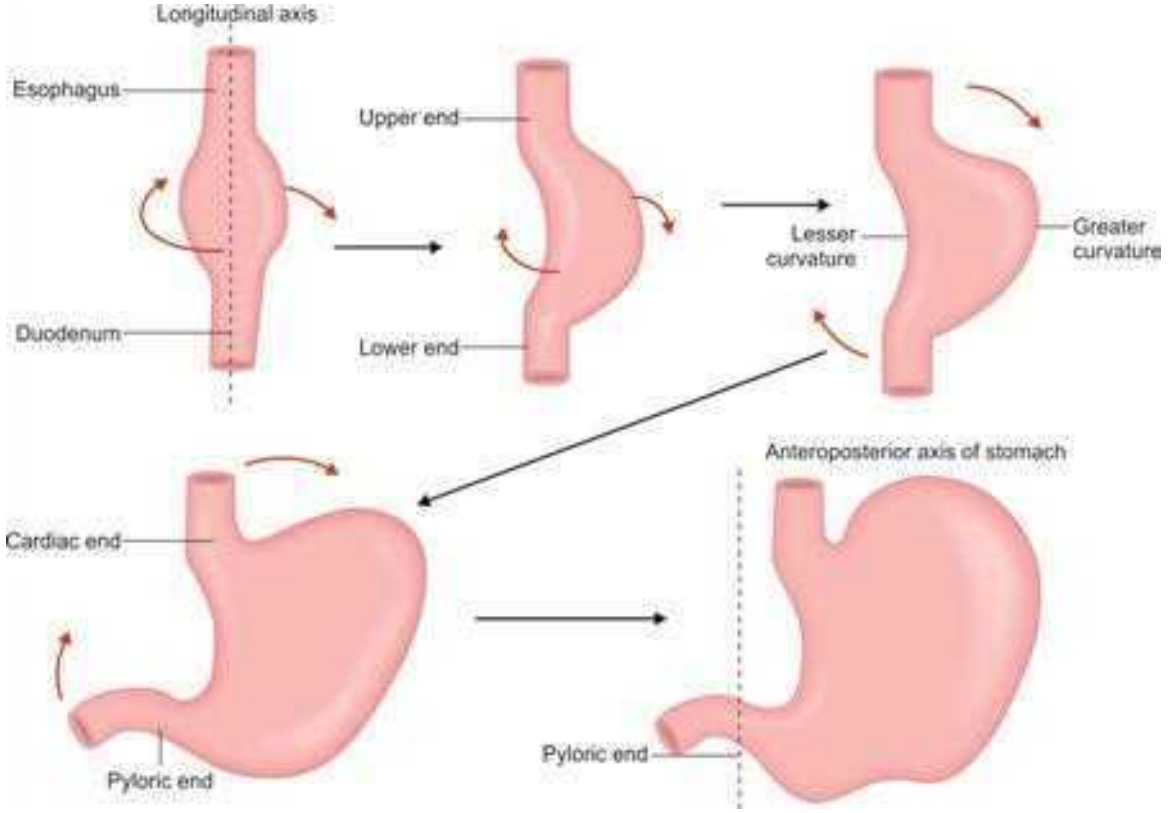
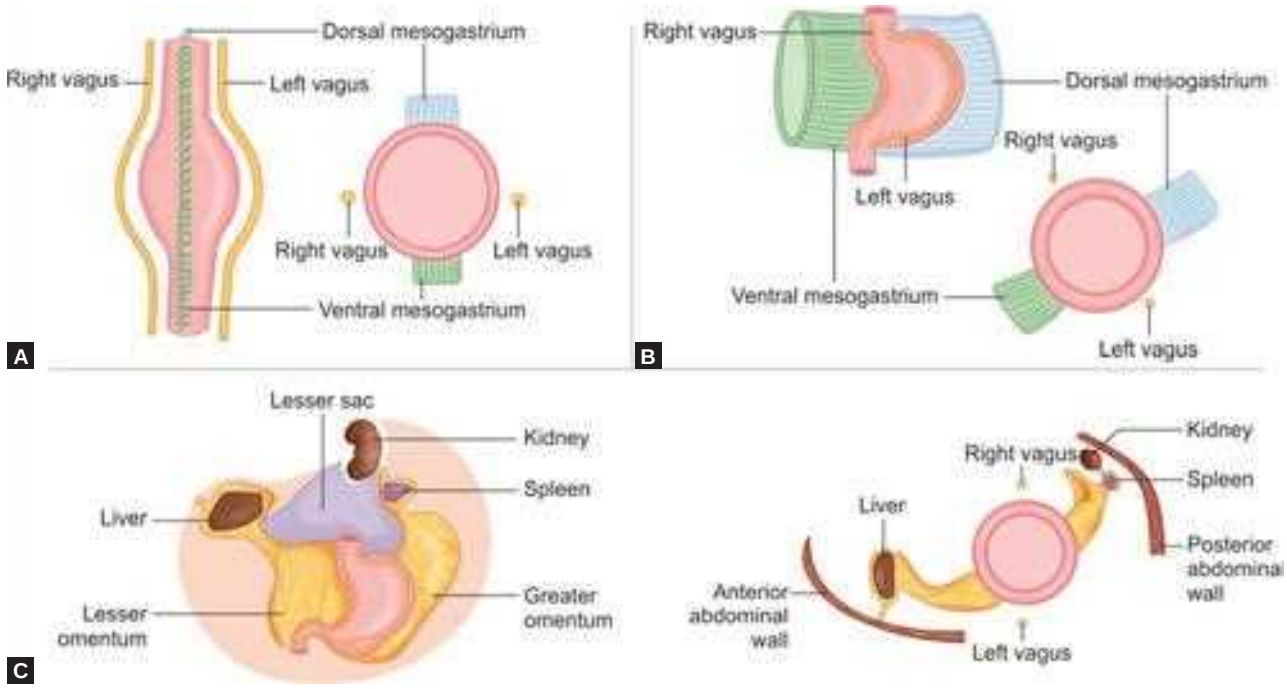


Fig. 13.8: Changes in the position and shape of stomach



Figs 13.9A to C: Changes in the relation of vagus nerve and the mesogastrium to the developing stomach

of the ventral mesogastrium between the liver and the stomach becomes the *lesser omentum*, while the part between the liver and the diaphragm (and anterior abdominal wall) gives rise to the coronary and *falciform ligaments*.

- Similarly, the dorsal mesogastrium is divided by the development of the spleen into a part between stomach and spleen (*gastrosplenic ligament*) and a part between spleen and posterior abdominal wall called the *lienorenal ligament* (Figs 13.7C and 13.9).
- The stomach undergoes differential growth resulting in considerable *alteration in its shape* and *orientation*. The original ventral border comes to face upward and to the right and becomes the *lesser curvature*. The dorsal border now points downward and to the left and becomes the *greater curvature*. The original left surface becomes its anterior surface and the original right surface becomes the posterior surface (Fig. 13.8).
- The rotation of the stomach can be explained as follows (Figs 13.8 and 13.9):
  - First rotation is 90° clockwise along longitudinal axis. This changes orientation of its surfaces and change in the position of vagus nerves.
  - Second rotation: It is in transverse/anteroposterior axis. This brings about changes in position of fundus and duodenum and in the position of ends of stomach.
- One surface of stomach grows faster than the other resulting in formation of greater and lesser curvatures.
- The rotation and differential growth of surfaces explains the change in relationship of right and left vagus nerves to posteroinferior and anterosuperior surfaces respectively.
- During rotation the cranial end tilts to the left and the caudal end to the right to assume the adult position.
- Rotation and disproportionate growth of the stomach alters the position of dorsal and ventral mesogastria.
- Rotation along longitudinal axis pulls dorsal mesogastrium to the left thus forming the *lesser sac/omental bursa* behind the stomach. During this rotation, the ventral mesogastrium is pulled to the right (Figs 13.9B and C). With the growth of stomach, the dorsal mesogastrium lengthens and the spleen develops between the layers of dorsal mesogastrium splitting it into gastrosplenic and lienorenal ligaments.
- Due to rotation along anteroposterior axis, the dorsal mesogastrium bulges downward and continues to grow to form a double-layered greater omentum.
- The lesser omentum and falciform ligament are formed from the ventral mesogastrium (a derivative of septum transversum).

Duodenum

- The superior (or first) part and the upper half of the descending (or second) part of the duodenum are derived from the foregut.
- The rest of the duodenum develops from the most proximal part of the midgut (Fig. 13.10A).

- The part of the gut that gives rise to the duodenum forms a loop attached to the posterior abdominal wall by a mesentery called *mesoduodenum* (Figs 13.10B and C). Later, this loop falls to the right. The mesoduodenum then fuses with the peritoneum of the posterior abdominal wall with the result that most of the duodenum becomes retroperitoneal (Figs 13.11A to C).
- The mesoduodenum persists in relation to a small part of the duodenum adjacent to the pylorus. This is the part seen in radiographs as the duodenal cap.
- In keeping with its development, the proximal part of the duodenum is supplied by branches of the celiac artery, and the distal part by branches of the superior mesenteric artery.

Midgut Development

The midgut elongates to form U-shaped intestinal loop that is suspended from the posterior abdominal wall by a short mesentery. Anteriorly, it communicates with the yolk sac by the narrow vitellointestinal duct. The superior mesenteric artery runs in the middle of midgut loop and divides it into a prearterial (cranial) and a postarterial (caudal) segment.

Jejunum and Ileum

The jejunum and most of the ileum are derived from the prearterial segment of the midgut loop. The terminal portion of the ileum is derived from the postarterial segment proximal to the cecal bud (Figs 13.12A and B).

Cecum and Appendix

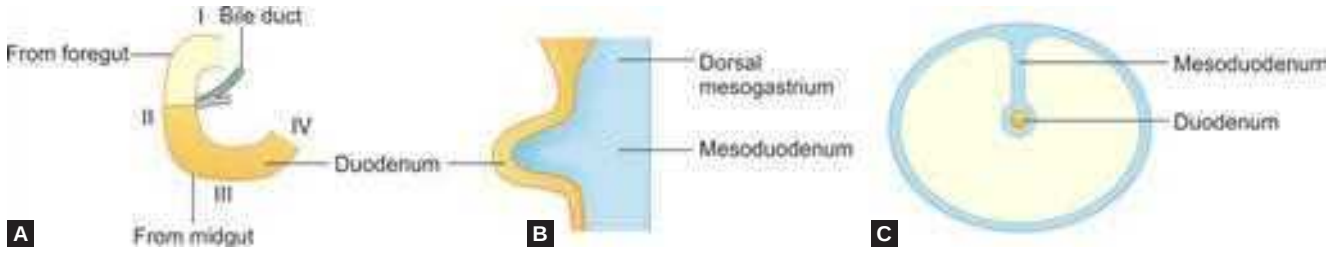
- The cecal bud is a diverticulum that arises from the postarterial segment of the midgut loop (Fig. 13.13). The cecum and appendix are formed by enlargement of this bud. The proximal part of the bud grows rapidly to form the cecum. Its distal part remains narrow and forms the appendix (Figs 13.14A to E).
- During greater part of fetal life, the appendix arises from the apex of the cecum (Figs 13.14A to F). Subsequently, the lateral (or right) wall of the cecum grows much more rapidly than the medial (or left) wall with the result the point of attachment of the appendix comes to lie on the medial side (Figs 13.14A to E).

Ascending Colon

It develops from the postarterial segment of the midgut loop (Figs 13.12A and B) distal to the cecal bud.

Hindgut Development

Refer to the description of hindgut and its subdivisions in the earlier part of this chapter and in Figures 13.4A and B. With the formation of tail fold and growth of the



Figs 13.10A to C: Development of the duodenum. (A) Part of the duodenum above the entry of the bile duct is derived from the foregut, and the part below this level is derived from the midgut; (B and C) At first the duodenum has a mesentery called the mesoduodenum. As seen in "B", this is continuous, cranially, with the dorsal mesogastrium. The mesoduodenum later disappears (Fig. 13.11)

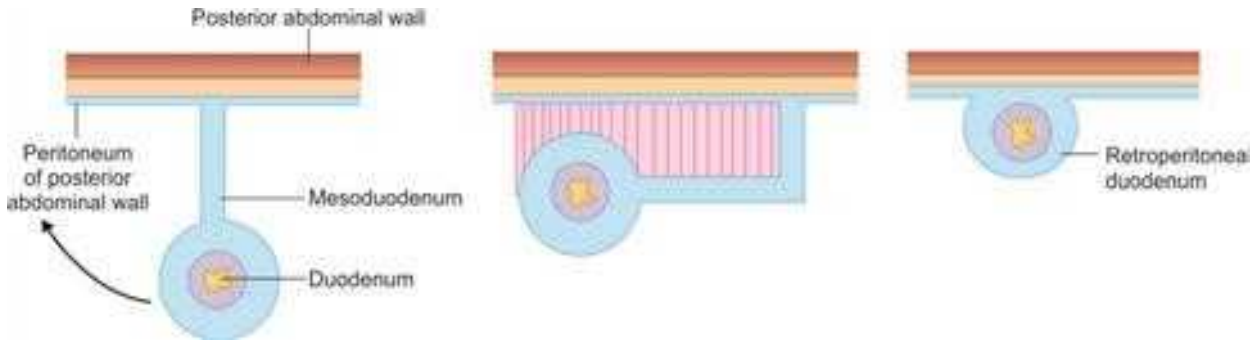
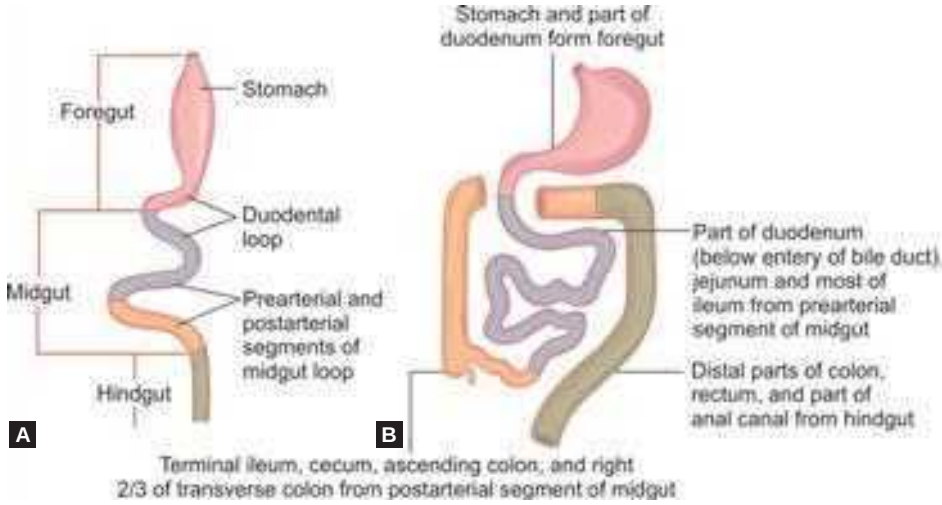


Fig. 13.11: Scheme to show how the mesoduodenum disappears. The duodenum then becomes retroperitoneal duodenum



Figs 13.12A and B: Derivation of various parts of the gut

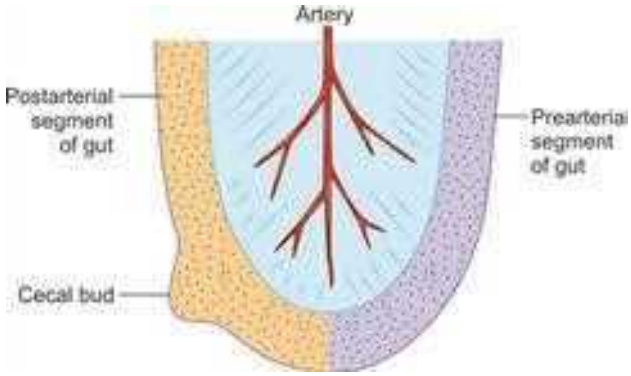
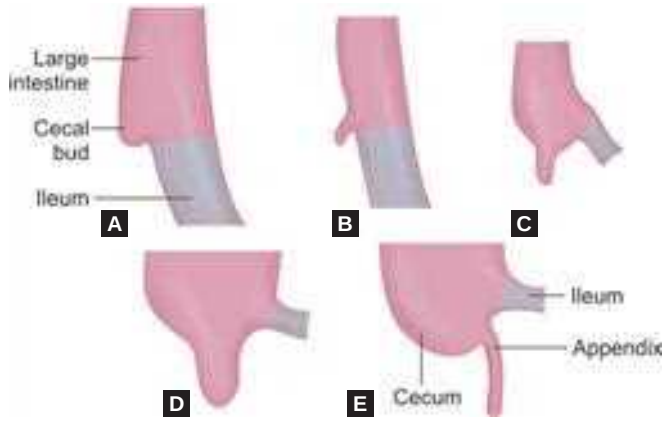


Fig. 13.13: Midgut loop. In this figure, the loop has been drawn to correspond with the orientation of the ileocecal region in postnatal life (Actually, the prearterial segment is cranial to the postarterial segment)



Figs 13.14A to E: Development of cecum and appendix. The orientation is as in Figure 13.13

embryo, the urorectal septum comes into contact with the cloacal membrane dividing it into the urogenital and anal membranes. Rupture of anal membrane creates anal opening for the hindgut dorsally. Rupture of urogenital membrane creates opening for the urogenital sinus ventrally. The point of contact of tip of urorectal septum and cloacal membrane becomes the perineal body.

Transverse Colon

The right two-thirds of the transverse colon develop from the postarterial segment of the midgut loop. The left one-third arises from the hindgut. This mode of origin is reflected in its arterial supply; the right two-thirds are supplied by the superior mesenteric artery and the left one-third by the inferior mesenteric.

Descending Colon

The descending colon develops from the hindgut.

Rectum

The rectum is derived from the primitive rectum, i.e. the dorsal subdivision of the cloaca. According to some authorities, the upper part of the rectum is derived from the hindgut proximal to the cloaca.

Anal Canal

It develops from two different components one endodermal and the other ectodermal. The upper two-thirds of it is derived from endoderm of the hindgut (primitive rectum). The lower one-third is derived from the ectodermal

proctodeum or anal pit (Figs 13.15A to C). The two parts are separated by anal membrane initially. Later this membrane ruptures and the line of junction of the endodermal and ectodermal parts is represented by the anal valves (*pectinate line*). The blood supply of the two parts also differs. The arteries supplying the anal canal are superior rectal artery (branch of superior mesenteric artery) and the inferior rectal artery (branch of internal pudendal artery, which is a branch of internal iliac artery). The venous drainage from endodermal part (superior rectal) is into the portal vein and from the ectodermal part (inferior rectal) is into the systemic veins. The nerve supply also differs. The endodermal part is innervated by autonomic nerves and the ectodermal part by somatic nerves. There is change in lining epithelium between the two parts. Above the pectinate line, the anal canal is lined by columnar epithelium and below that by stratified squamous epithelium.

Physiological Umbilical Hernia

During 3rd week of development, the prearterial segment of midgut loop elongates rapidly. There is rapid growth of liver during this period. Because of rapid elongation of midgut loop and rapid growth of liver and the developing mesonephric kidney, the abdominal cavity becomes too small to accommodate all the intestinal loops. The midgut loop enters the extraembryonic coelomic cavity in the umbilical cord during 6th week of development. This herniation of intestinal loop is called physiological umbilical hernia.

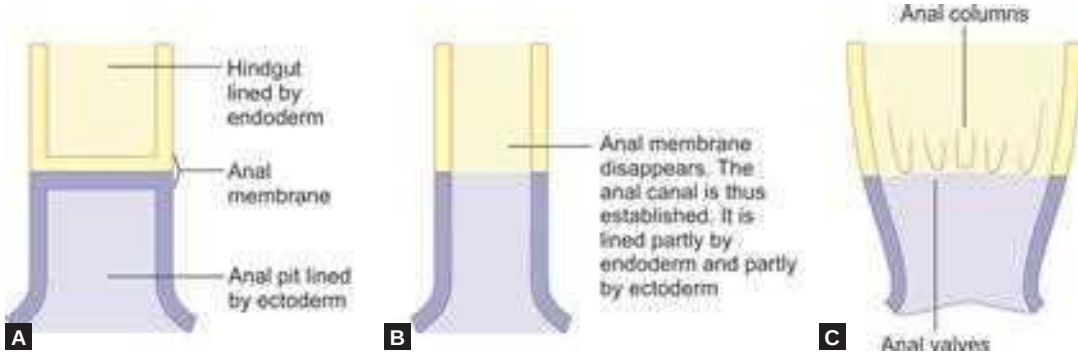
ROTATION OF THE GUT

After its formation, the midgut loop lies outside the abdominal cavity of the embryo, in a part of the extraembryonic coelom that persists near the umbilicus. The loop has a prearterial, or proximal, segment and a postarterial, or distal, segment (Fig. 13.16A). Along with growth in length the midgut loop rotates around the axis of superior mesenteric artery.

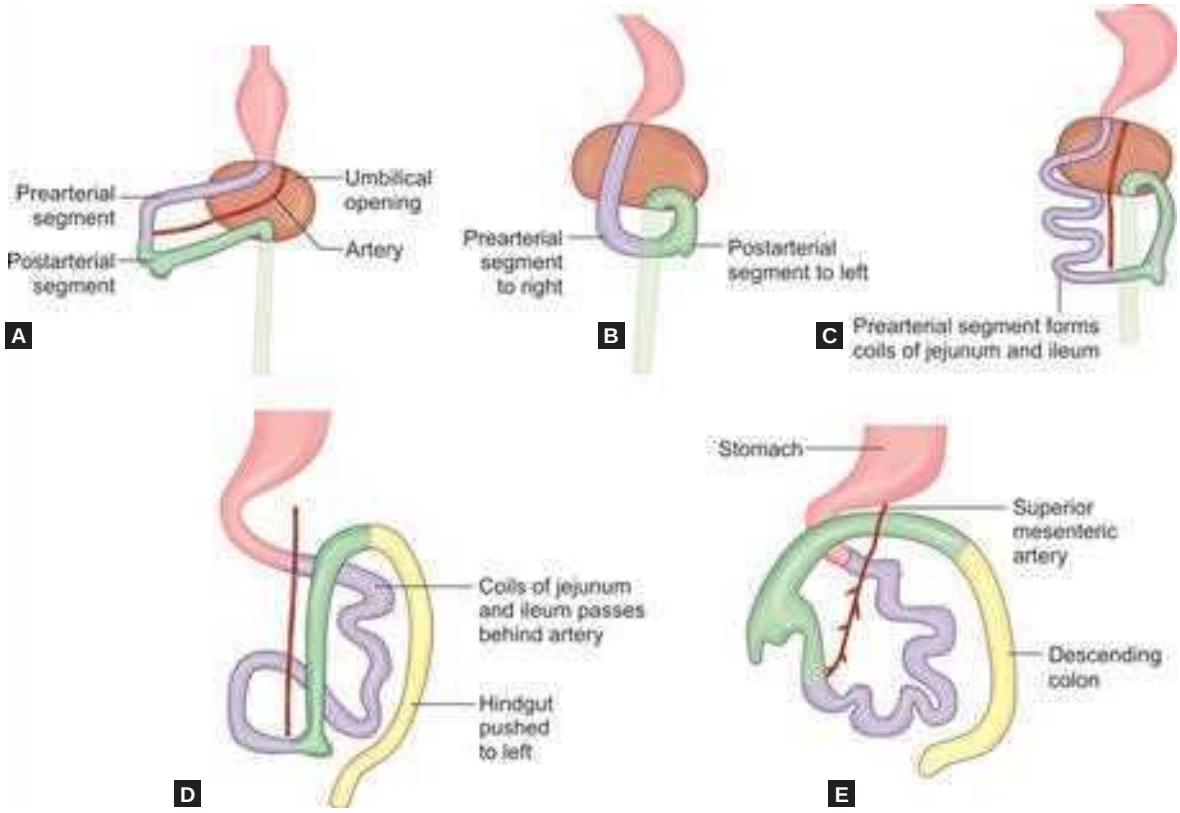
When we view the midgut loop from the ventral aspect, it makes a rotation of 270° in counterclockwise direction around the axis of superior mesenteric artery. During rotation also elongation of intestinal loop and coiling of jejunum and ileum takes place. Large intestine also shows elongation but without coiling.

The total rotation of midgut can be divided into three stages of each 90°. A 90° rotation occurs during the herniation and the remaining 180° during the return of intestinal loop into the abdominal cavity.

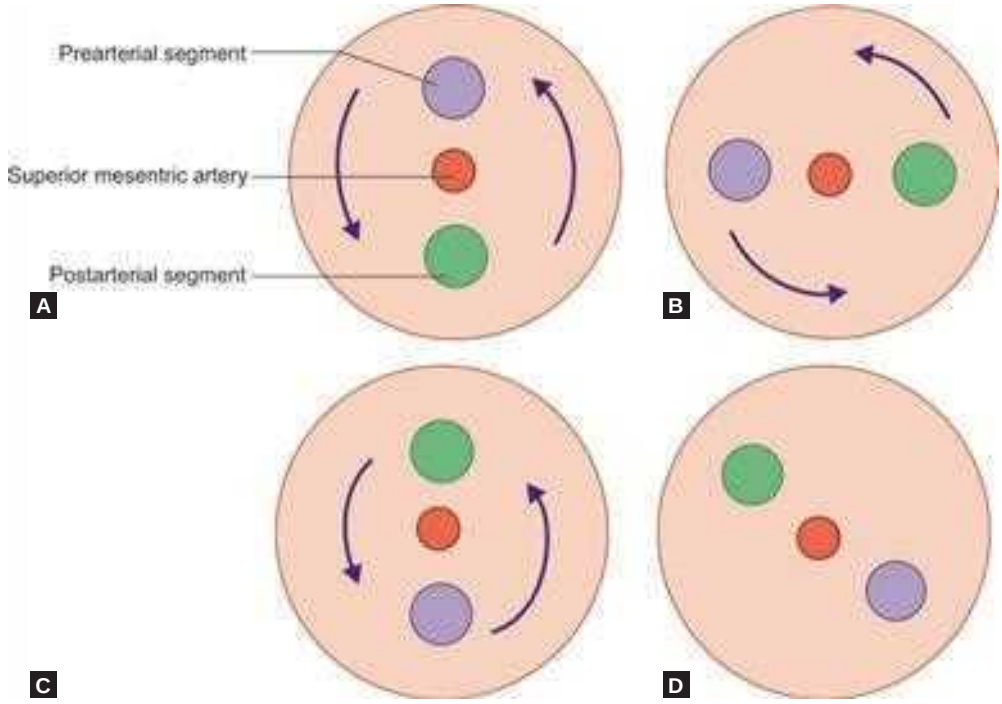
- *First stage rotation*: Initially, the loop lies in the sagittal plane outside the umbilical ring. Its proximal segment is cranial and ventral to the distal segment (Fig. 13.17A). The midgut loop now undergoes rotation. This rotation plays a very important part in establishing the definitive relationships of the various parts of the intestine. The steps in rotation must, therefore, be clearly understood. Viewed from the ventral side the loop undergoes an anticlockwise rotation by 90°, with the result that it now lies in the horizontal plane (Figs 13.16B and 13.17B). The prearterial segment comes to lie on the right side and the postarterial segment on the left of the superior mesenteric artery which forms the axis (compare Figs 13.16A and B).
- *Second stage rotation*: The prearterial segment now undergoes great increase in length to form the coils of the jejunum and ileum. These loops still lie outside the abdominal cavity, to the right side of the distal limb (Fig. 13.16C). During 10th week, the herniated intestinal loops return to the abdominal cavity. The reasons for return of the loop are the regression of mesonephric kidney, reduced growth of liver and expansion of abdominal cavity. The coils of jejunum and ileum (prearterial segment) now return to the abdominal cavity. As they do so, the midgut loop undergoes a further anticlockwise rotation of 90°. As a result, the coils of jejunum and ileum pass behind the superior mesenteric artery into



Figs 13.15A to C: (A) Anal membrane separates hindgut from anal pit; (B) Anal membrane disappears; (C) Scheme to show the parts of the anal canal in which the lining epithelium is derived from ectoderm or endoderm



Figs 13.16A to E: Stages in rotation of the gut. Study these figures carefully along with the corresponding description in the text. In Figure 13.16 E, note that the cecum moves to the right, and the transverse colon now lies in front of the superior mesenteric artery



Figs 13.17A to D: Scheme to show the orientation of the proximal and distal ends of the midgut loop at different phases of the rotation of the gut. Arrows indicate the direction of rotation. Compare with Figure 13.16

the left half of the abdominal cavity (Fig. 13.17D). The duodenum, therefore, comes to lie behind the artery and the coils of jejunum and ileum occupy the posterior and left part of the abdominal cavity.

- *Third stage rotation/retraction of herniated loop*: The postarterial segment of the midgut loop returns to the abdominal cavity. As it does so, it also rotates in an anticlockwise direction of 90° (Fig. 13.17E). With the result, the transverse colon lies anterior to the superior mesenteric artery, and the cecum comes to lie on the right side. At this stage re-wise reading the rotation of gut with Figures 13.16 and 13.17 for understanding the orientation of various parts of gut.
- At this stage, the cecum lies just below the liver, and an ascending colon cannot be demarcated. Gradually, the cecum descends to the iliac fossa, and the ascending, transverse and descending parts of the colon become distinct.

FIXATION OF THE GUT

- At first all parts of the small and large intestines have a mesentery by which they are suspended from the posterior abdominal wall. After the completion of rotation of the gut, the duodenum, the ascending colon, the descending colon and the rectum become retroperitoneal by fusion of their mesenteries with the posterior abdominal wall (as indicated in Figures 13.11A to C).
- The original mesentery persists as the mesentery of the small intestine, the transverse mesocolon and the pelvic mesocolon.

Clinical correlation

Developmental anomalies of the gut

Congenital obstruction

This may be due to a variety of causes.

- ***Atresia:*** The continuity of the lumen of the gut is interfered with as follows:
  - A segment of the gut may be missing (Fig. 13.18A)
  - A segment of the gut may be replaced by fibrous tissue (Fig. 13.18B)
  - A septum may block the lumen (Fig. 13.18C).
- ***Stenosis:*** The lumen may be abnormally narrow (Fig. 13.18D) (As a normal developmental process, there is epithelial occlusion of the lumen of gut in early stages of development. The gut later gets recanalized. Some cases of atresia, duplication and stenosis of gut may be due to abnormal recanalization).
- ***Nondevelopment of nerve plexuses*** in the wall of a part of the intestinal tract may result in difficulty in the passage of intestinal contents through the part. Such a defect in the lower part of the colon gives rise to a condition in which the colon proximal to the defective segment becomes greatly distended with its contents. This condition is called ***megacolon or Hirschsprung's disease*** (Fig. 13.19).
- ***Abnormal thickening of muscular wall:*** This is seen typically at the pyloric end of the stomach ***(congenital pyloric stenosis)*** (Fig. 13.20). The thickened muscle bulges into the lumen and narrows it. According to some authorities, the primary cause of this defect is the same as in megacolon.
- ***External pressure*** by abnormal peritoneal bands or abnormal blood vessels. Such bands are often seen in relation to the duodenum (Fig. 13.21). The duodenum may also be compressed by an annular pancreas (Fig. 13.22).
- ***Imperforate anus:*** This is caused by stenosis or atresia of the lower part of the rectum or the anal canal. Some varieties of this condition are shown in Figures 13.23 and 13.24.

Abnormal communications or fistulae

Parts of the gut may have abnormal communications with other cavities or with the surface of the body. These are most frequently seen in relation to the esophagus and the rectum, and are usually associated with atresia of the normal passage.

- ***Tracheoesophageal fistula:*** Atresia of the esophagus is often accompanied by abnormal communications between the esophagus and trachea as illustrated in Figures 13.24A to D.
- ***Incomplete septation of the cloaca:*** The rectum may communicate with the urinary bladder, urethra, or vagina (Figs 13.25A to C, E, F and H), or may open onto the perineum at an abnormal site (Figs 13.25D and G). These conditions are associated with imperforate anus.

Duplication

Varying lengths of the intestinal tract may be duplicated. The duplicate part may form only a small cyst, or may be of considerable length. It may or may not communicate with the rest of the intestine (Figs 13.26A and B).

Diverticula

These may arise from any part of the gut. They are most common near the duodenum (Fig. 13.27).

Vitellointestinal duct anomalies

- Meckel's diverticulum is a small persist part of vitellointestinal duct. It is seen along the antimesenteric border of ileum. It is seen in 2% of people and is 2 feet from ileocecal junction and 2.0 cm in length. Persistence of it is called ***Meckel's diverticulum*** or ***diverticulum ilei.*** It is of surgical importance as it may undergo inflammation giving rise to symptoms similar to those of appendicitis.
- Meckel's diverticulum is also of interest as pancreatic tissue or a gastric type of mucosa may be present in its wall (In such cases, ulceration and perforation can occur in the diverticulum).
- Occasionally, the whole of the vitellointestinal duct, or its distal part alone, may be patent. The former conditions lead to a ***fecal fistula*** at the umbilicus. The latter condition leads to formation of an ***umbilical sinus***.
- The vitellointestinal duct may be represented by cysts (enterocystoma or ***vitelline cyst***) or by ***fibrous cords*** (Figs 13.28A to F). Fibrous cords constitute a danger in later life as coils of intestine may get twisted round them leading to strangulation.
- Remnants of the vitellointestinal duct may also give rise to growths.

Errors of rotation

- ***Nonrotation of the midgut loop:*** In this condition, the small intestine lies toward the right side of the abdominal cavity, and the large intestine toward the left (Fig. 13.29A).
- ***Reversed rotation:*** The transverse colon crosses behind the superior mesenteric artery, and the duodenum crosses in front of it (Fig. 13.29B).
- ***Nonreturn of umbilical hernia:*** Sometimes, the coils of intestine that develop from the midgut loop remain outside the abdominal cavity. The child is born with loops of intestine hanging out of the umbilicus. This condition is called ***exomphalos*** or ***omphalocele*** (Fig. 13.30).
- ***Subhepatic cecum and appendix:*** This results due to failure of descent of cecum. Sometimes the descent of cecum is partial. Or it can be excessive descent resulting in its positional variations.

Congenital umbilical hernia

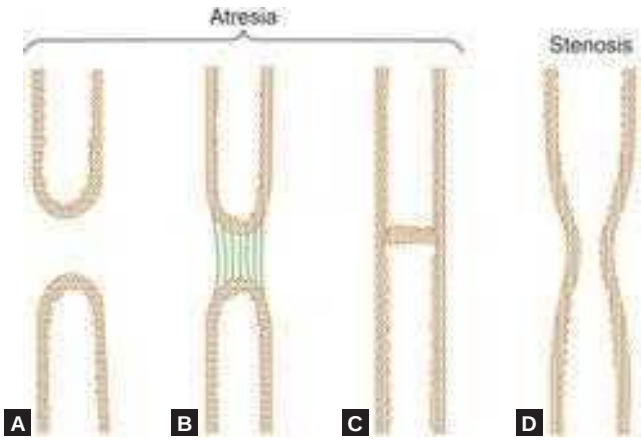
Loops of intestine and other abdominal contents may also be seen outside the abdominal cavity for an entirely different reason. In ***congenital umbilical hernia,*** the muscle layer and skin are absent in the region of the umbilicus, creating a defect in the abdominal wall through which abdominal contents can pass. Such contents are covered with peritoneum, but in exomphalos they are covered only by amnion.

Errors of fixation

- Parts of the intestine that are normally retroperitoneal may have a mesentery. Abnormal mobility of this part of the intestine may result in its twisting. This condition is called ***volvulus***. Twisting of blood vessels to the loop can lead to obstruction of its blood supply.
- Parts of the intestines, that normally have a mesentery, may be fixed by abnormal adhesions of peritoneum.
- The cecum may remain subhepatic, or may descend only to the lumbar region. Alternatively, it may descend into the pelvis (Figs 13.31A to C).

Situs inversus

In this condition, all abdominal and thoracic viscera are laterally transposed, i.e. all parts normally on the right side are seen on the left side, and vice versa. For example, the appendix and duodenum lie on the left side, and the stomach on the right side.



Figs 13.18A to D: Varieties of atresia and stenosis of the gut

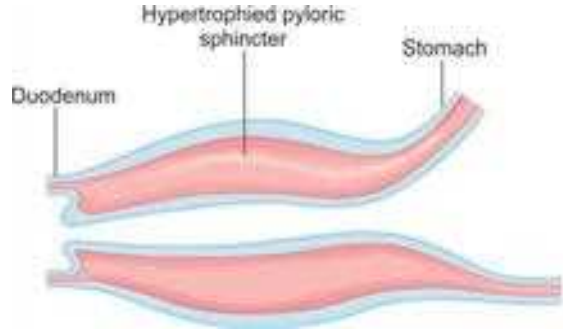


Fig. 13.20: Congenital pyloric stenosis

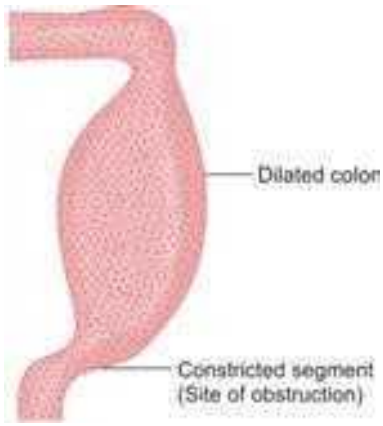


Fig. 13.19: Megacolon

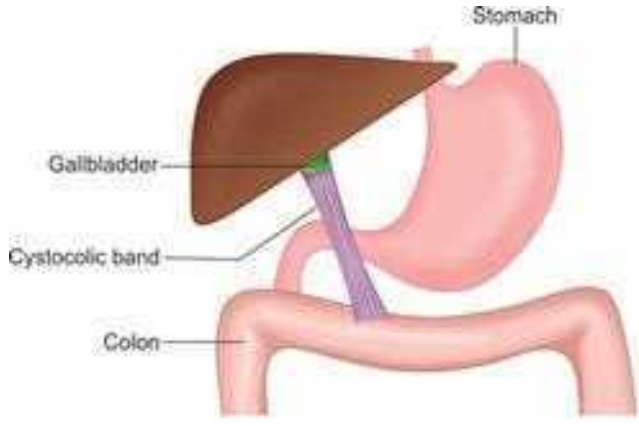


Fig. 13.21: Obstruction of duodenum by a cystocolic band passing from the gallbladder to the transverse colon

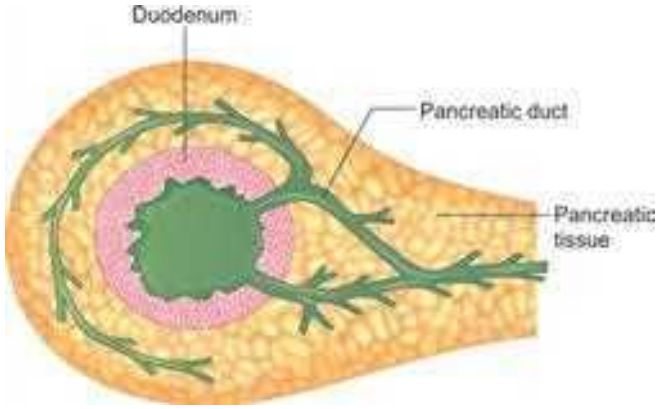
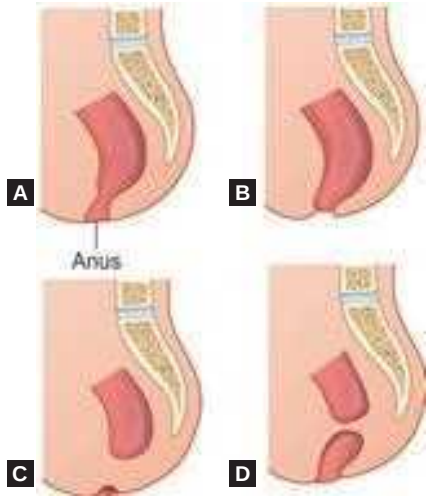
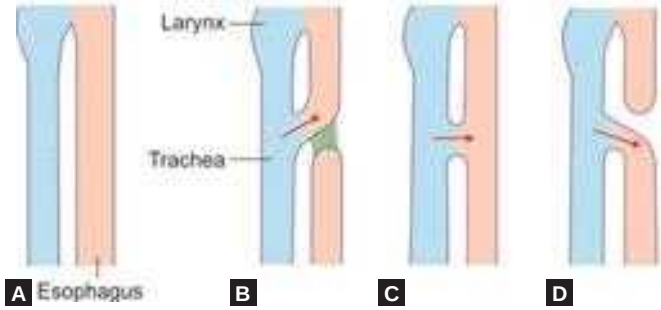


Fig. 13.22: Annular pancreas surrounding the duodenum



Figs 13.23A to D: Various types of imperforate anus. (A) Stenosis of anal canal; (B) Persistent anal membrane; (C) The proctodeum is represented by a solid mass of ectodermal cells and there is a big gap between it and the hindgut (rectum); (D) Upper and lower parts of rectum separated by a gap



Figs 13.24A to D: (A) Normal arrangement of trachea and esophagus; (B to D) Various forms of tracheoesophageal fistulae

TIME TABLE OF SOME EVENTS DESCRIBED IN THIS CHAPTER

Time table of some events described in this chapter is shown in Table 13.1.

TABLE 13.1: Time table of some developmental events

Age	*Developmental events*
16 days	Allantoic diverticulum starts appearing
3 weeks	Gut begins to acquire tubular form because of head and tail foldings. At the end of third week, the buccopharyngeal membrane ruptures
4 weeks	The fusiform shape of the stomach becomes visible
5 weeks	Stomach rotates and dilates. Intestinal loop begins to form. Cecal bud can be identified
6 weeks	Intestinal loop is well formed. Urorectal septum starts dividing the cloaca. Allantois and appendix become clearly visible. Stomach completes its rotation
7 weeks	Septation of cloaca into rectum and urogenital sinus is completed. Intestinal loop herniates out of the abdominal cavity
8 weeks	Intestinal loop rotates counterclockwise
9 weeks	Anal membrane breaks down
3 months	Head and tail foldings are completed. Herniated coils of intestine return to the abdominal cavity

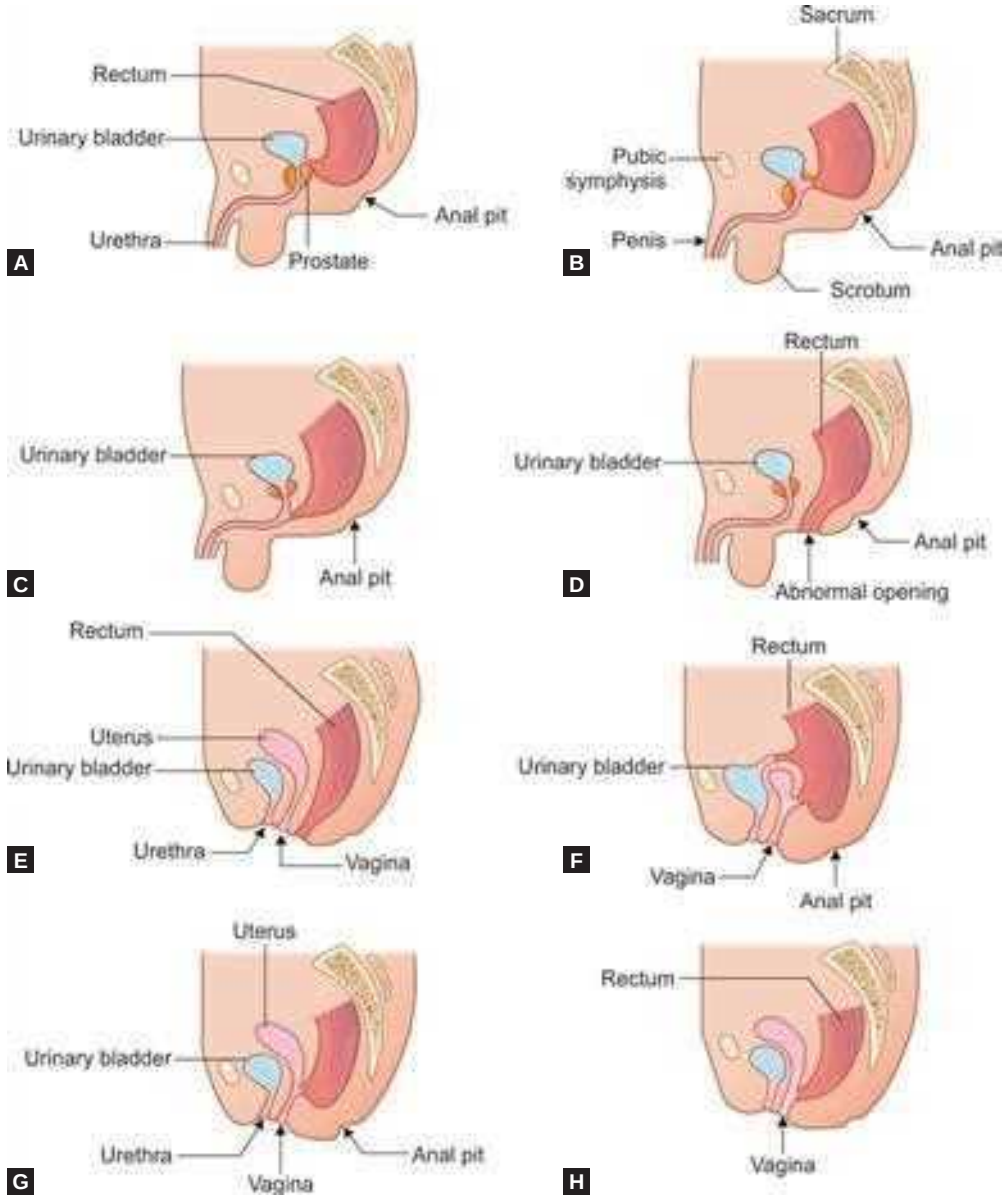
EMBRYOLOGICAL BASIS FOR CLINICAL CONDITIONS OR ANATOMICAL OBSERVATIONS

Case Scenario 1

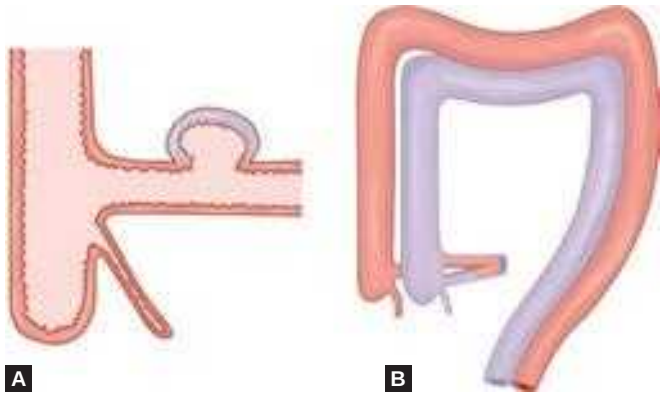
A 30-year-old 3rd gravida with previous normal obstetric history delivered a dead female (?) fetus of 32 weeks. Gestational age; 2.6 kg weight; 20 cm crown-rump length (CRL)—with multiple congenital anomalies. Observe the Figures 13.32A and B and give your observations and embryological explanation for each.

The observations as per the Figures 13.32A and B are as follows:

- Omphalocele: Failure of fusion of four ectomesodermal folds—defect in infraumbilical part of anterior abdominal wall with abdominal organs lying outside called omphalocele. The prognosis of this condition is bad as nearly 25% of infants die before birth and 50–80% present associated anomalies and nearly 10% present chromosomal anomalies. In the present case, associated anomalies were present. In the absence of associated defects and if the omphalocele contains only a herniated bowel, it can be corrected surgically.



Figs 13.25A to H: Various types of rectal fistulae in the male (A to D) and female (E to H). The fistula may be between rectum and urinary bladder (i.e. rectovesical) as in (A) and (F), between rectum and urethra (rectourethral) as in (B) and (C), and between rectum and vagina (rectovaginal) as in (G), (H) and (F). More than one type may be present at the same time (F). The rectum may open onto the perineum at an abnormal site (D) and (E). In these cases, the anal pit is formed at the normal site



Figs 13.26A and B: Degrees of duplication of the gut represented by a cyst on the terminal ileum as in (A), and by duplication of the entire colon and terminal ileum as in (B)

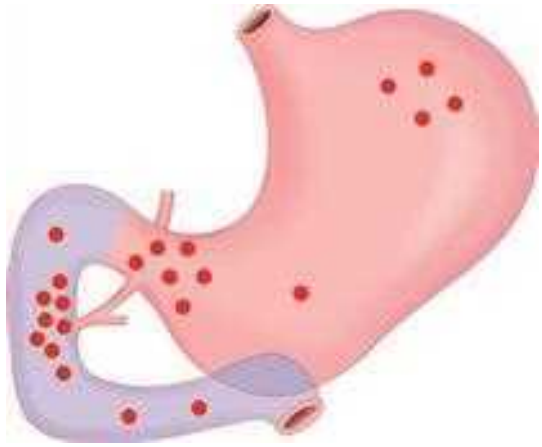
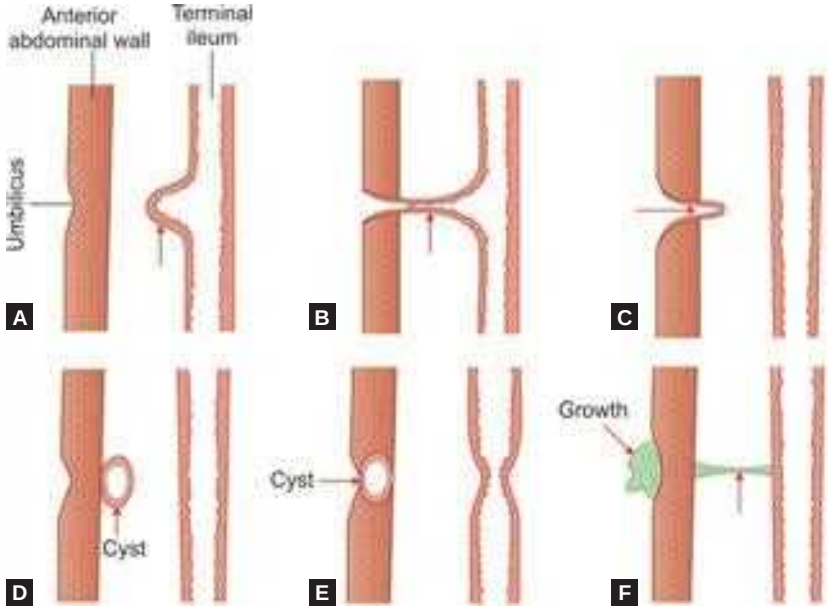
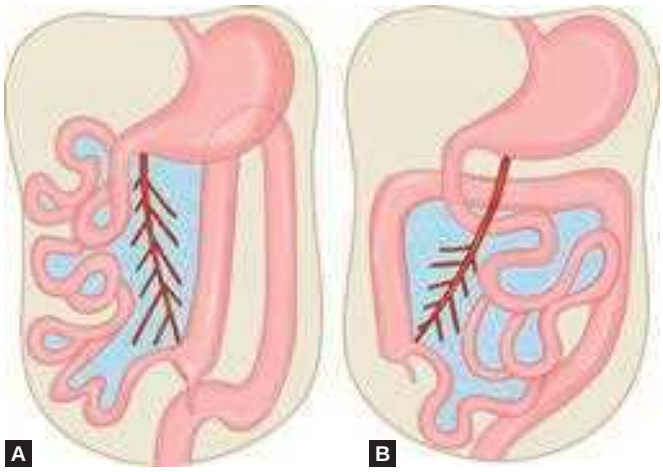


Fig. 13.27: Sites at which congenital diverticula may arise from stomach and duodenum



Figs 13.28A to F: Anomalies in relation to the vitellointestinal duct (see arrows). (A) Meckel's diverticulum; (B) Patent vitellointestinal duct; (C) Umbilical sinus; (D) Cyst attached to the abdominal wall. A cyst may also be seen attached to the gut, or embedded in the abdominal wall as shown in "E"; (E) Stenosis of gut at the site of attachment of duct; (F) Vitellointestinal duct represented by a fibrous cord. An umbilical growth arising from remnants of the duct is also shown



Figs 13.29A and B: Errors of rotation. (A) Nonrotation. Coils of small intestine lie in the right half of the abdomen, and colon in the left half; (B) Reversed rotation. The duodenum lies anterior to the superior mesenteric artery, and the colon crosses behind it

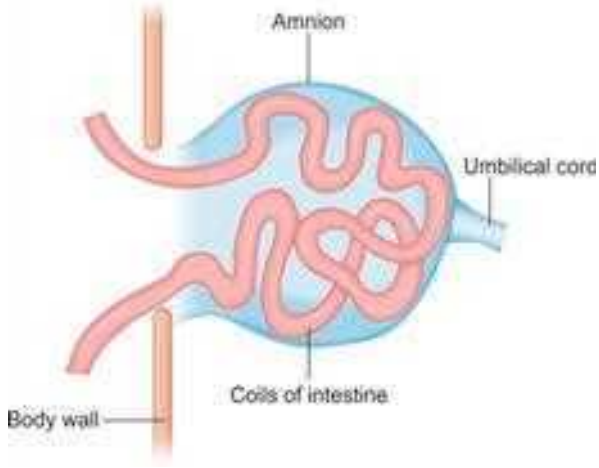
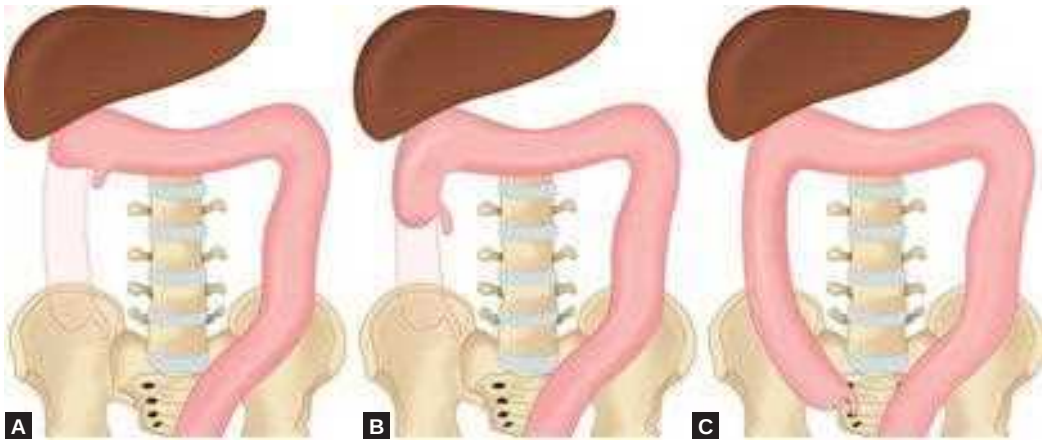
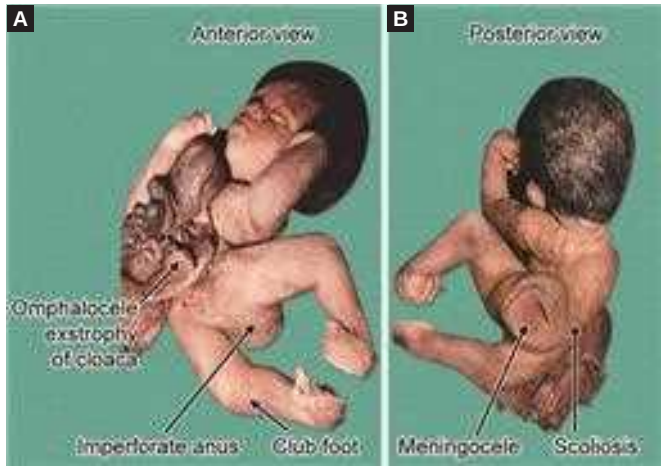


Fig. 13.30: Exomphalos. Coils of intestine derived from the midgut loop fail to return into the abdominal cavity



Figs 13.31A to C: Errors in descent of the cecum. (A) Subhepatic; (B) Lumbar; (C) Pelvic. The normal position is shown in dotted line in (A) and (B)



Figs 13.32A and B: A dead fetus with OEIS complex

- Abnormal closure of pelvic wall resulted in exstrophy of bladder/cloaca where the rectum and bladder will be lying outside. Absence of partitioning of endodermal cloaca and cloacal membrane resulted in this condition. Isolated bladder exstrophy can be managed by bladder reconstruction.
- Imperforate anus is due to failure of rupture of anal membrane to establish communication of anal canal to outside.
- Skeletal anomalies that were observed are curved spine called scoliosis and bilateral clubfoot.
- Neurological anomaly of lumbosacral meningocele was observed.
- Since these conditions presented multiple anomalies, it is called OEIS complex where each letter stands for a clinical condition:
  - Omphalocele
  - Exstrophy of bladder or cloaca
  - Imperforate anus
  - Skeletal anomalies.

This is a most serious form of ventral lower midline malformation with associated multisystem anomalies.

Prenatal diagnosis of this condition is by:

- Biochemical: Elevated serum alpha-fetoproteins
- Ultrasound:
  - Nonvisualization of the normally filled fetal bladder
  - Infraumbilical anterior abdominal wall defect, omphalocele
  - Renal anomalies
  - Neural tube defect—meningocele
  - Increased nuchal translucency.

REVIEW QUESTIONS

1. Write a short note on tracheoesophageal fistula.
2. What are the stages in the rotation of gut?
3. Describe the errors in rotation of gut.
4. Explain development of cecum and appendix.
5. Explain development of duodenum.
6. Name the derivatives of foregut.
7. Name the derivatives of midgut.
8. Name the derivatives of hindgut.

Chapter 14

Liver and Biliary Apparatus; Pancreas and Spleen; Respiratory System; Body Cavities and Diaphragm

HIGHLIGHTS (FIG. 14.1 AND FLOWCHART 14.1)

- *Liver* and *biliary apparatus* develop from endodermal *hepatic bud*. The hepatic bud arises as an outgrowth from the ventral wall of terminal part of foregut.
- *Pancreas* develops from two endodermal buds, the *dorsal and ventral pancreatic buds* that arise at the junction of foregut and midgut. Most of the pancreas develops from dorsal bud. The ventral bud forms lower part of head and uncinated process.
- *Spleen* develops in the mesoderm of dorsal mesogastrium.
- *Respiratory system* develops from a median endodermal diverticulum of foregut. At its caudal end the diverticulum divides into right and left *lung buds*.
- *Larynx* and *trachea* develop from the endodermal respiratory diverticulum cranial/proximal to its division.
- *Bronchial tree and alveoli of the lungs* develop from repeated division of the lung buds.
- *Pleural, pericardial and peritoneal cavities* develop from intraembryonic coelom. Before formation of head fold the intraembryonic coelom consists of right and left halves that are connected, across the midline, cranial to the prochordal plate.
- *Pericardial cavity* is derived from the median midline part of intraembryonic coelom. With the formation of head fold of embryo this cavity comes to lie ventral to the foregut.
- *Peritoneal cavity* is derived from the right and left limbs of intraembryonic coelom. After the formation of lateral folds of embryo the two limbs unite to form single cavity.
- *Pleural cavities* are formed from right and left pleuropericardial canals that connect pericardial and peritoneal cavities. Each pleuropericardial canal is invaginated by corresponding endodermal lung bud. Growth and expansion of lung bud leads to great enlargement of pleuropericardial canal and formation of pleural cavity.
- *Diaphragm* develops in relation to the septum transversum. It receives contribution from pleuroperitoneal membranes, the body wall and the mesenteries of the esophagus.

LIVER AND BILIARY APPARATUS

LIVER AND INTRAHEPATIC BILIARY APPARATUS

- *Developmental primordium*: Liver is the largest gland in the body (Fig. 14.2). It has number of functions including exocrine, endocrine, hematopoietic, metabolic and phagocytic. It develops from endodermal *hepatic bud* during 4th week of intrauterine life (IUL). It arises from the ventral margin of terminal part of foregut that forms upper half of second part of duodenum (Fig. 14.3A).
- *Direction of growth of hepatic bud*: Hepatic bud consists of rapidly proliferating endodermal cells that grow ventrally and cranially into the *ventral mesogastrium* (Fig. 14.3B) and through it into the *septum transversum* (Unsplit part of intraembryonic mesoderm between pericardial cavity and yolk sac cavity) (Fig. 14.3C).
- *Subdivisions of hepatic bud*: The hepatic bud elongates and divides into a larger cranial part *pars hepatica* that forms the liver, and a smaller caudal part *pars cystica*

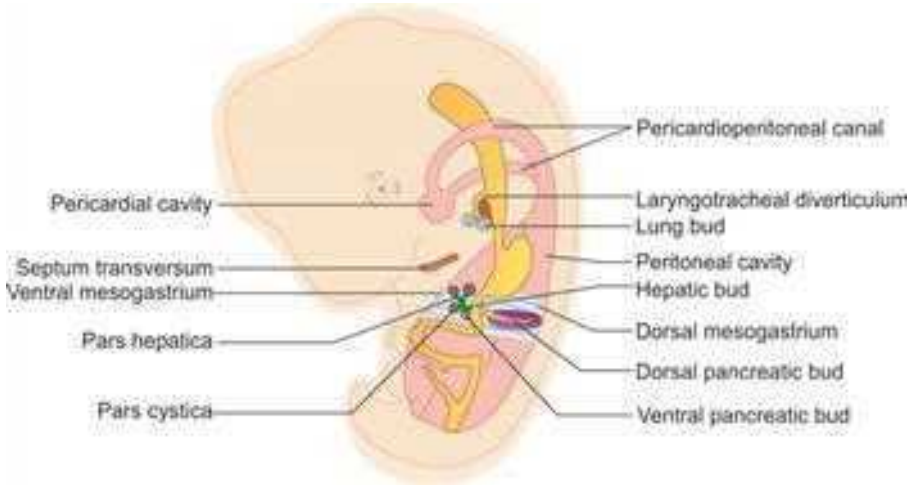


Fig. 14.1: Development of liver, biliary apparatus, pancreas, respiratory system, body cavities and diaphragm

that forms gallbladder and cystic duct (Figs 14.14.3B and C).

- *Division of pars hepatica*: It divides into right and left branches that become *right and left hepatic ducts*. The terminations of the hepatic ducts contribute for the two solid *right and left lobes of the liver* (Figs 14.3D and E). The two lobes of the liver are of equal size during early development but the size of left lobe reduces gradually (Table 14.1). In the 3rd month of intrauterine life (IUL), the weight of liver is one-tenth of total body weight of fetus and occupies most of the upper abdomen. In the 7th month of IUL it reduces to one-fifth of body weight.
- *Formation of hepatic architecture*:
  - From the terminal part of right and left branches of pars hepatica (hepatic ducts), when they reach septum transversum clusters of cells (hepatocytes) in the form of laminae arise, and break up into interlacing columns called *hepatic trabeculae* (Fig. 14.3E).
  - In between hepatic trabeculae the *hepatic sinusoids* develop in situ.
  - During this process of formation of hepatic trabeculae, the *vitelline and umbilical veins* that are running longitudinally in the septum transversum, break up and establish communication with the hepatic sinusoids (Fig. 14.4).
  - Within the substance of liver the hepatic ducts branch repeatedly and canalized to acquire a lumen to form *intrahepatic biliary passages* (Fig. 14.5).
  - The hepatic trabeculae differentiate into the components of parenchyma, i.e. *liver cells* and *cells lining intrahepatic biliary system* (Fig. 14.5). Septum transversum contributes for the *Kupffer cells, hematopoietic cells* and *connective tissue cells*.
  - Reorganization of cells of hepatic bud and the mesenchymal cells of connective tissue and blood vessels form the hepatic lobule with bile canaliculi, portal triad and sinusoids of liver (Fig. 14.5).
- *Formation of peritoneal folds in relation with the liver from septum transversum*: With the rapid growth of developing liver into the septum transversum, the mesoderm of septum transversum between the liver and foregut becomes the *lesser omentum*, and the part between liver and ventral abdominal wall becomes the *falciform, triangular and coronary ligaments*. Lesser omentum and falciform ligament together are called *ventral mesentery/ventral mesogastrium* (Fig. 14.6).

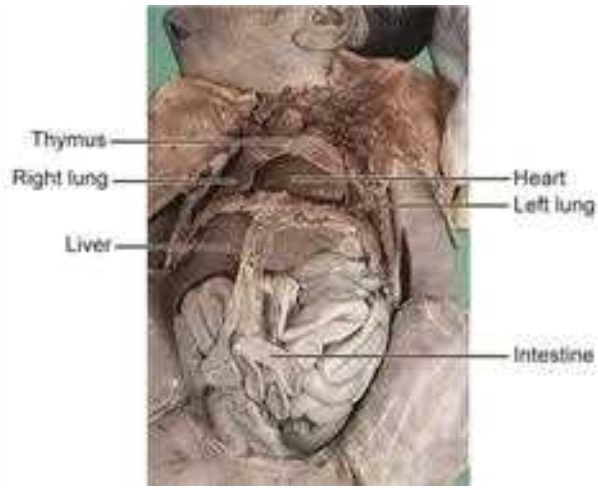
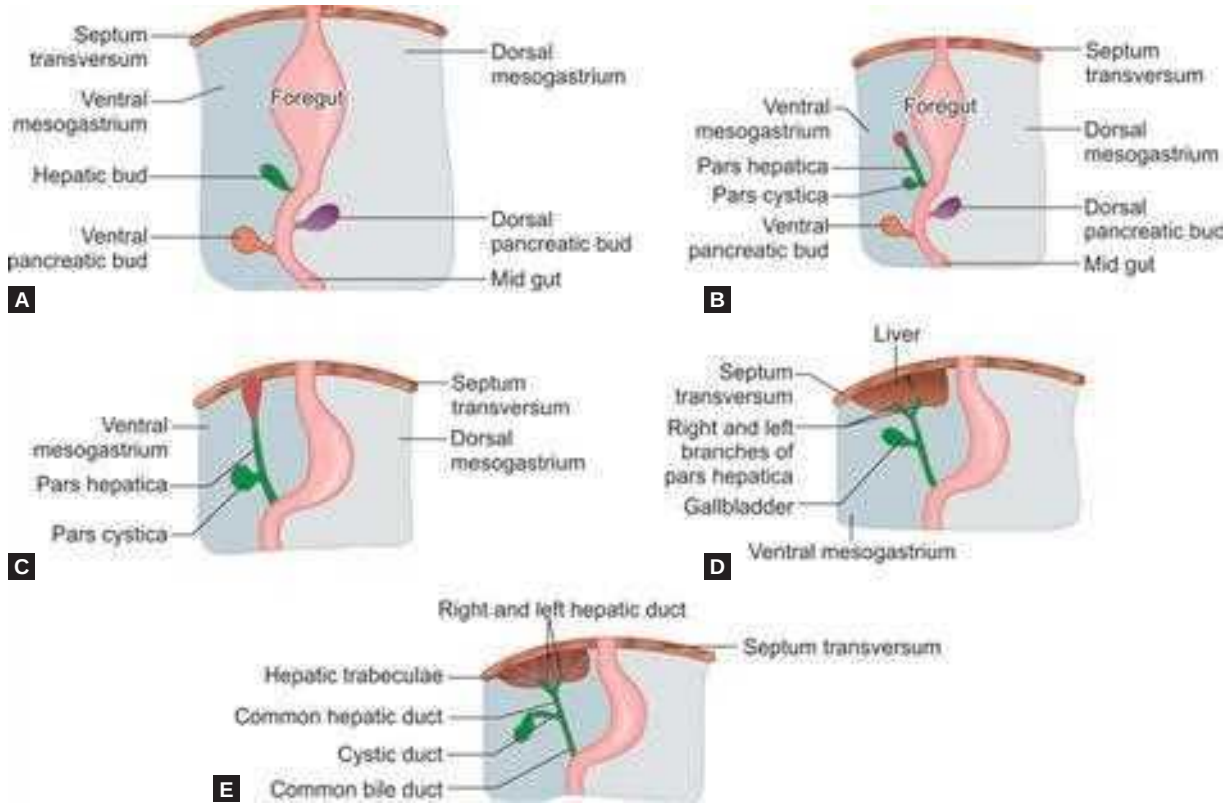


Fig. 14.2: Full term fetus showing thoracic and abdominal viscera

Functions of Fetal Liver

- *Hematopoiesis*: Begins in 6th week of IUL and continues up to birth. After birth this function is carried out by spleen and bone marrow.



Figs 14.3A to E: Development of liver: (A) Origin of hepatic bud from the ventral wall of terminal part of foregut; (B) Hepatic bud growing into the ventral mesogastrium and dividing into pars hepatica and pars cystica; (C) Pars hepatica growing toward septum transversum through ventral mesogastrium; (D) Division of pars hepatic into right and left parts forming the right and left lobes of liver; (E) Formation of sheets of hepatic cells

TABLE 14.1: Development of gross and microscopic components of adult liver

Adult component	*Developmental derivative*
Gross appearance	
Two lobes	Two terminal divisions of pars hepatica of hepatic bud in contact with septum transversum
Microscopic appearance	
Hepatic cells and intrahepatic biliary apparatus (parenchyma)	Hepatic bud (endoderm)
• Fibrous capsule of Glisson • Connective tissue cells • Kupffer's cells • Hematopoietic cells • Blood vessels	Septum transversum (Intraembryonic mesoderm)
Sinusoids	Absorption and breakdown of vitelline and umbilical veins in septum transversum between hepatic trabeculae

- *Bile secretion*: Secretion of bile by hepatocytes starts during 12th week of IUL. The bile is released into the foregut derived part of duodenum and then passes through the rest of intestine. The first stool passed by the new born is green in color due to the excretion of bile and it is called *meconium*.

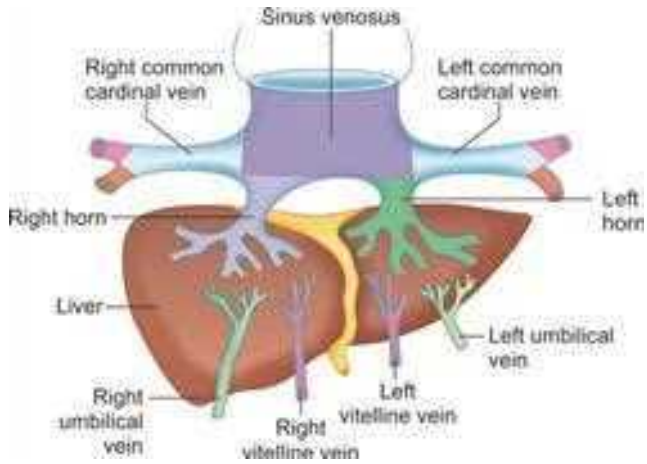


Fig. 14.4: Breaking up of umbilical and vitelline veins in septum transversum and their communication with hepatic sinusoids

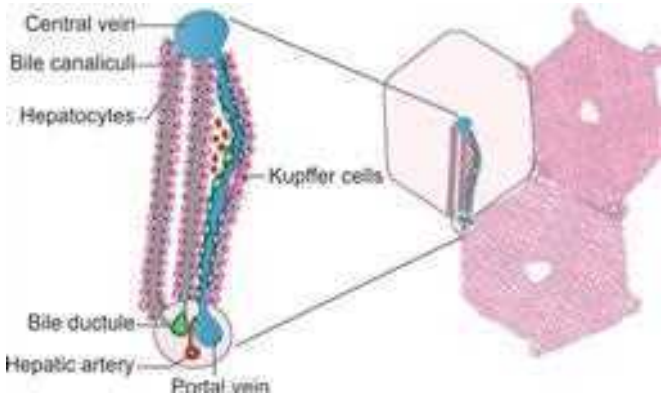


Fig. 14.5: Intrahepatic biliary apparatus. Reorganization of hepatic cells and blood vessels to form hepatic lobule, bile canaliculi, portal triad and sinusoids

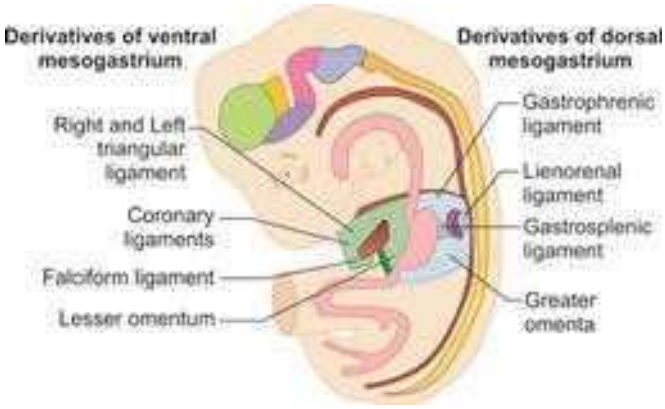


Fig. 14.6: Formation of peritoneal folds from septum transversum—Lesser omentum, falciform, coronary and triangular ligaments

Clinical correlation

Sagittal section of a 6-week embryo of 1.0 cm CRL showing the developing liver occupying greater part of abdominal cavity with the stomach and spleen (Fig. 14.7A). Transverse section of embryo at 6 weeks showing the developing liver (Fig. 14.7B).

Anomalies of liver are rare. Those that are reported in literature are (Fig. 14.8):

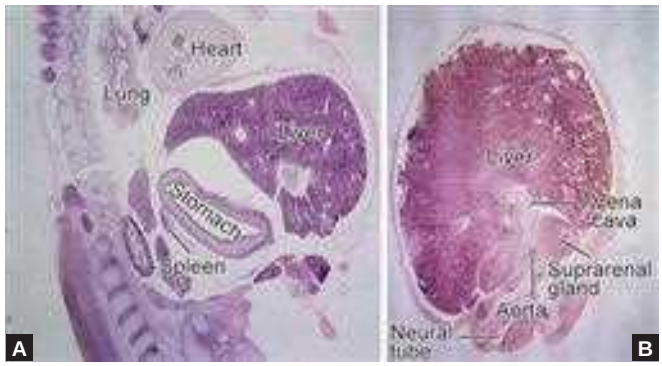
- Absence of quadrate lobe
- ***Reidel's lobe:*** tongue like extension of right lobe of liver (Fig. 14.8A).
- Anomalous lobulation (Fig. 14.8B)
- Accessory liver in falciform ligament (Fig. 14.8C)
- ***Polycystic liver:*** Failure of union of intrahepatic biliary canaliculi and ductules with extrahepatic bile ducts, results in the formation of cysts within the liver. It is usually associated with cysts in the kidney and pancreas.
- ***Intrahepatic biliary atresia:*** It is a serious anomaly and is not compatible with life unless a liver transplantation is undertaken.
- ***Rudimentary live***r.

Additional explanation

- ***Role of septum transversum in the development of hepatic bud:*** Septum transversum has an inductive effect on differentiation, proliferation, branching and formation of hepatocytes and cells lining the intrahepatic and extrahepatic biliary apparatus from the hepatic bud.
- ***Establishment of hepatic architecture:*** Columnar endodermal cells of foregut get transformed into pseudostratified cells in early hepatic bud, that later transforms into bipotential hepatoblast. The hepatoblasts reorganize when they come into contact with the mesenchyme of septum transversum and the breaking down umbilical and vitelline veins (to form sinusoids). They are bipotential, as they can differentiate into two types of cells. Those hepatoblasts close to the branches of portal vein (derived from vitelline veins) become cuboidal cells and along with the mesenchyme around periportal vein they form primordia for bile ducts, which acquire a lumen by reorganization of cells.
- ***Lobular arrangement:*** Initially the arrangement of hepatocytes was around the portal triad (interlobular bile ductule, branch of portal vein and hepatic artery) forming portal lobule. Later the hepatocytes change to an arrangement along sinusoids and around central vein forming the classical hepatic lobule.
- ***Massive growth of liver when compared to other organs:*** The reasons for massive growth of liver in the early part is due to oxygen rich blood supply through the left umbilical vein and ductus venosus, presence of large number of sinusoids and rapidly proliferating hemopoietic tissue. Further facilitated by herniation of midgut between 6th to 10th weeks of IUL. The reduction in weight in later part of IUL is due to the reduced hematopoietic activity, reduction of physiological hernia and to provide room for expanding and lengthening derivatives of the gut.
- ***Gradual decrease in size of left lobe when compared to right lobe:*** More space provided for enlargement of right lobe by early closure of pleuropericardial and pleuroperitoneal membranes on right side than left side. Right lobe is supplied by right branch of portal vein that conveys blood via superior mesenteric vein, from small intestine that contains products of digestion. Hence a nutritional advantage for the right lobe. Whereas, left branch of portal vein receives blood from large intestine via inferior mesenteric vein and splenic vein making left lobe at nutritionally disadvantageous position. Further, the left branch of portal vein is longer and narrower than the right branch and makes an angle with the trunk of portal vein making the left lobe at a nutritionally disadvantageous position. Because of these reasons the left lobe undergoes degeneration.
- ***Molecular mechanisms in liver development:*** Fibroblast growth factor (FGF2) from cardiac mesoderm and bone morphogenetic proteins (BMPs) from septum transversum.

GALLBLADDER AND EXTRAHEPATIC BILIARY PASSAGES (EXTRAHEPATIC BILIARY APPARATUS)

- *Gallbladder* and *cystic duct* develop from pars cystica of hepatic bud (Figs 14.3B to E).



Figs 14.7A and B: Serial section of 6 weeks embryo: (A) Sagittal section showing the developing liver, stomach and spleen; (B) Transverse section showing developing liver

- *Extrahepatic duct system of biliary apparatus*: The narrow portion of hepatic bud between pars cystica and duodenal part of foregut forms the *common bile duct*. The undivided part of pars hepatica distal to the origin of pars cystica forms the *common hepatic duct*. The right and left branches of pars hepatica become *right and left hepatic ducts* (Fig. 14.3E).
- The bile duct at first opens on the ventral aspect of developing duodenum. Due to the differential growth of duodenal wall, and rotation of duodenal loop, it opens on the dorsomedial aspect of duodenum along with ventral pancreatic bud (Fig. 14.18D).

Clinical correlation

Anomalies of the gallbladder

Anomalies of shape:

- ***Phrygian cap:*** Fundus may be folded on itself to form a cap-like structure (Fig. 14.9A).
- ***Hartmann's pouch:*** The wall of infundibulum may project downward as a pouch, which may be adherent to the cystic duct or even to the bile duct (Fig. 14.9B).

Anomalies of position:

- Transverse position on the under surface of right lobe, or under the left lobe (Fig. 14.10).
- ***Floating gallbladder:*** The gallbladder will be lined by peritoneum on all sides. It may be attached to the liver by a fold of peritoneum or it may be completely free (Fig. 14.11).
- ***Intrahepatic gallbladder:*** It may be embedded in the substance of liver (Fig. 14.12).

Duplication *(Fig. 14.13):*

- The lumen may be partially or completely divided by a septum, which may or may not extend into the cystic duct.
- The gallbladder may be completely or partially duplicated.

Other anomalies:

- ***Sessile gallbladder:*** The gallbladder may directly open into the bile duct instead of the cystic duct (Fig. 14.14).
- ***Agenesis:*** Absence of gallbladder
- Diverticula may arise from any part of the organ.

Anomalies of the extrahepatic duct system

Abnormal length: There is considerable variation in the level at which various ducts join each other, with the result that occasionally some of them may become abnormally long, or short (Figs 14.15A to D).

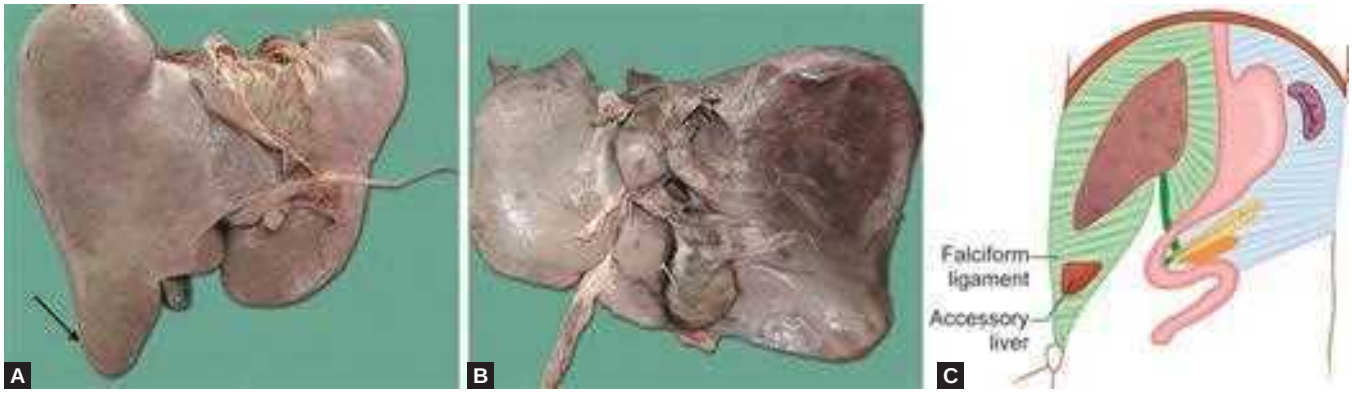
Abnormal mode of termination (Figs 14.15E to H):

- Cystic duct may join left side of common hepatic duct, passing either in front of it, or behind it, to reach its left side.
- Cystic duct may end in the right hepatic duct.
- Cystic duct may pass downward, anterior to the duodenum, before joining the common hepatic duct.
- Bile duct may open into the pyloric, or even the cardiac end of the stomach.

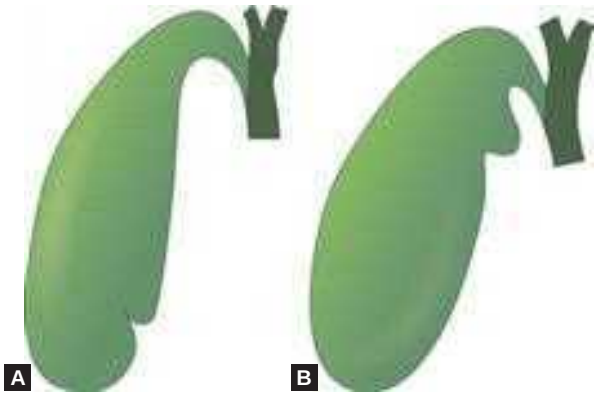
Atresia (Figs 14.16A to F): Parts of the duct system, and sometimes the whole of it, may be absent.

Duplication (Figs 14.17A to C):

- Parts of the duct system may be duplicated.
- Accessory ducts arising from the right lobe may terminate in the right hepatic duct, the cystic duct, the bile duct, or even directly into the gallbladder.



Figs 14.8A to C: Anomalies of liver: (A) Reidel's lobe; (B) Anomalous lobulation; (C) Accessory liver in falciform ligament



Figs 14.9A and B: Anomalies of shape of gallbladder (A) Phrygian cap; (B) Hartmann's pouch

Fig. 14.12: Intrahepatic gallbladder in a fetal lung in which the gallbladder is embedded in liver tissue

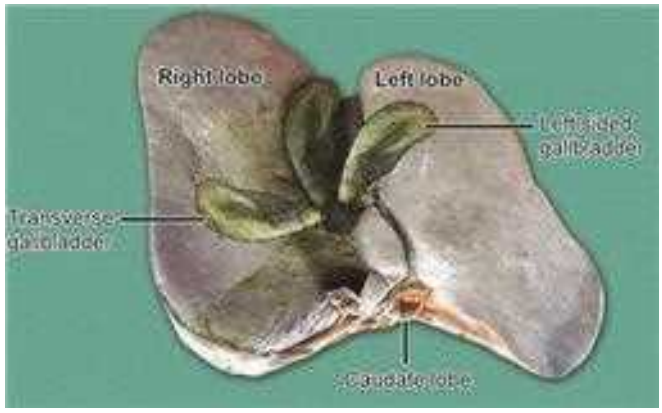


Fig. 14.10: Anomalies of position of gallbladder—Transverse position on the under surface of right lobe, or under the left lobe

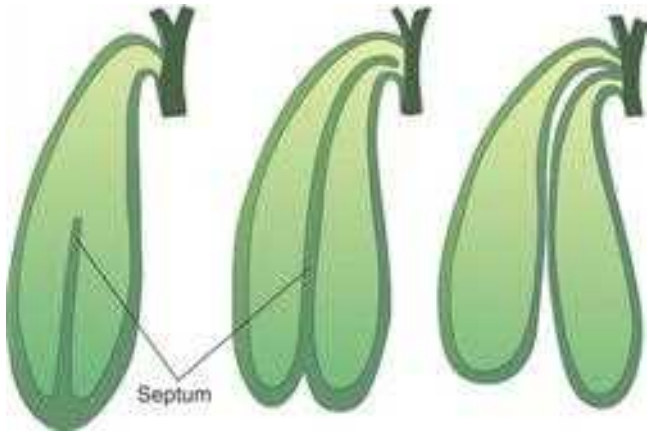


Fig. 14.13: Duplication of gallbladder in which the lumen is partly (A and B) or completely (C) divided

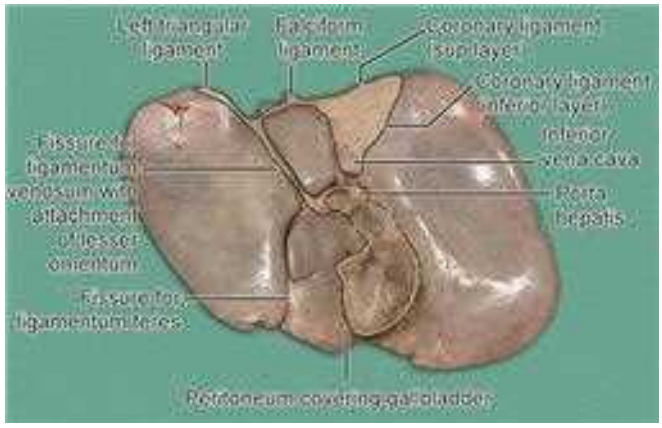


Fig. 14.11: Floating gallbladder in which the organ is covered all round by peritoneum

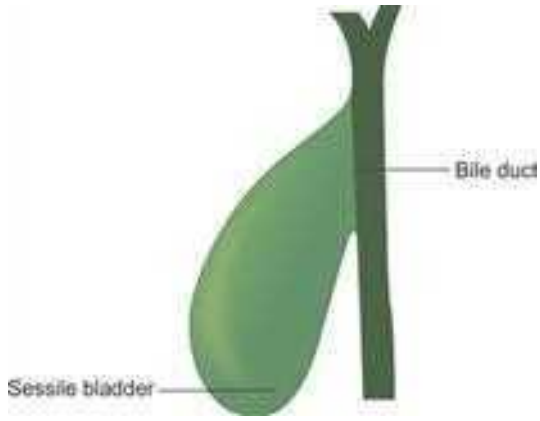
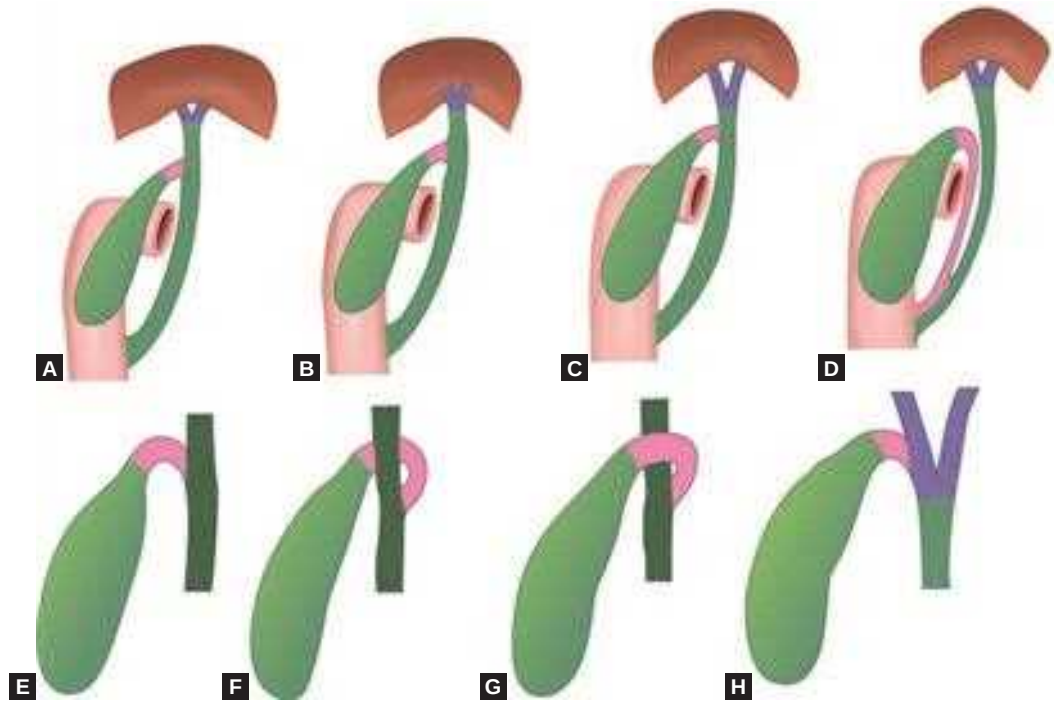
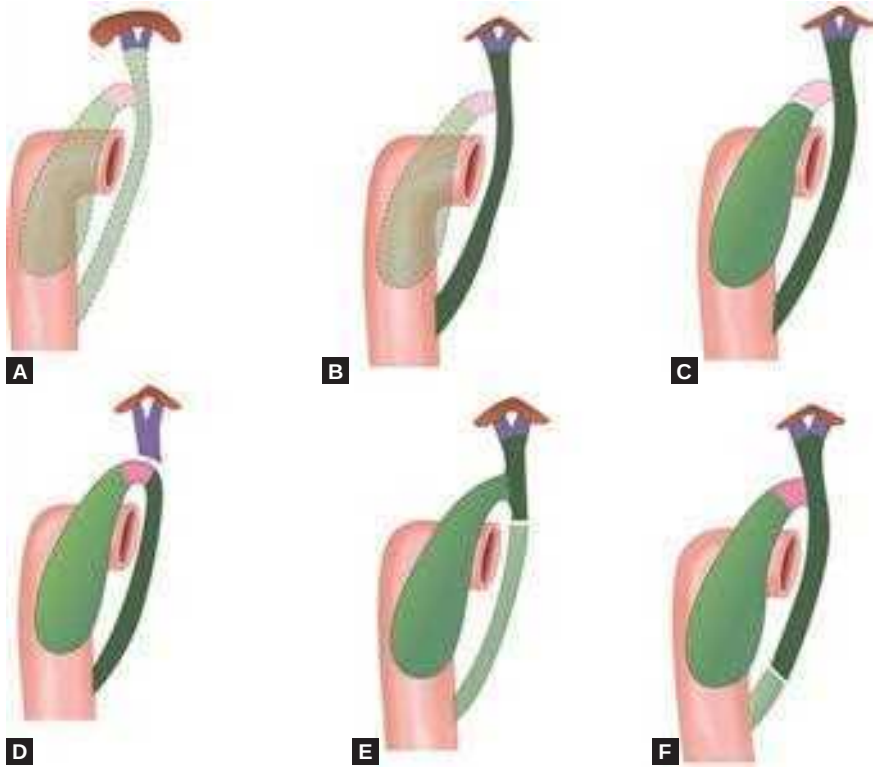


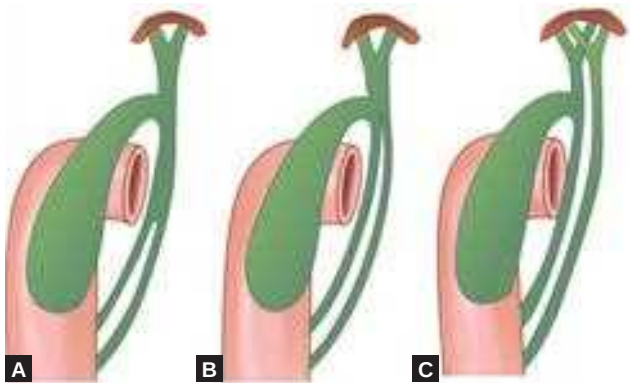
Fig. 14.14: Sessile gallbladder in which the gallbladder may open directly into the bile duct



Figs 14.15A to H: Anomalies of extrahepatic duct system: (A) Normal; (B) Right and left hepatic ducts join within liver substance; (C) Long hepatic ducts; (D) Very long cystic duct; (E) Normal; (F) Cystic duct passes behind common hepatic duct and joins its left side; (G) Cystic duct passes in front of common hepatic duct and joins its left side; (H) Cystic duct joining right hepatic duct



Figs 14.16A to F: Agenesis of parts of extrahepatic biliary tract. Missing parts indicated in light color: (A) Complete agenesis; (B) Gallbladder and cystic duct missing; (C) Cystic duct missing; (D) Hepatic duct missings; (E) Bile duct missing; (F) Terminal part of bile duct is missing



Figs 14.17A to C: Duplication of parts of extrahepatic biliary tract: (A) Partial duplication of bile duct; (B) Complete duplication of bile duct; (C) Complete duplication of bile duct, common hepatic duct and right and left hepatic ducts

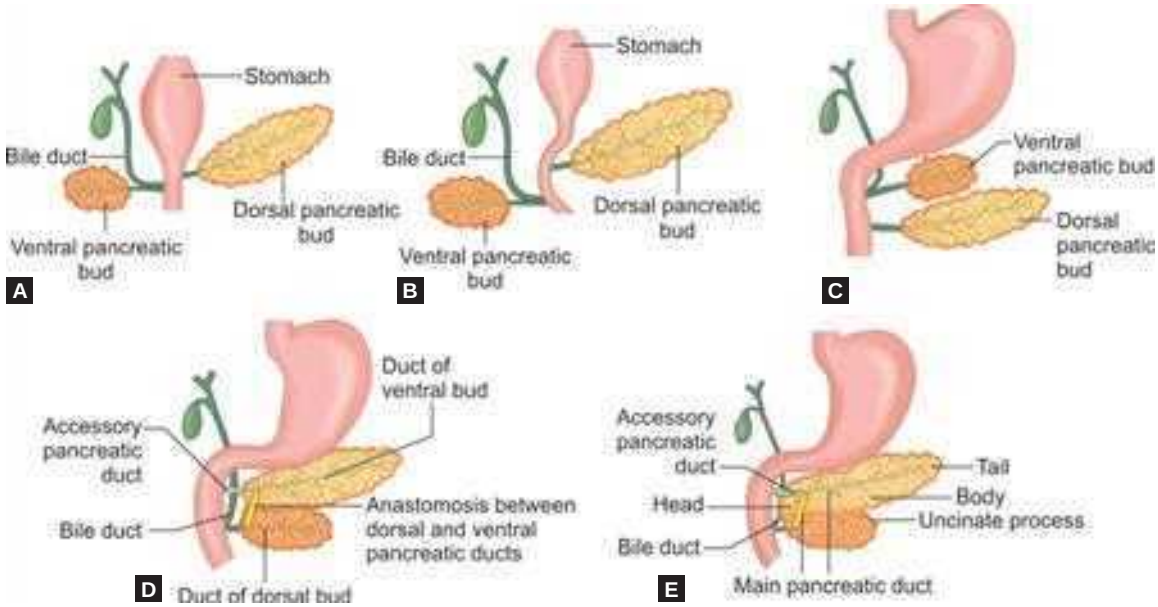
TABLE 14.2: Development of gross and microscopic components of adult pancreas

Adult component	*Developmental derivative*
Gross appearance	
Head	• *Upper part:* Dorsal pancreatic bud • *Lower part:* Ventral pancreatic bud
Uncinate process	Ventral pancreatic bud
Neck	Dorsal pancreatic bud
Body	Dorsal pancreatic bud
Tail	Dorsal pancreatic bud
Duct system	
Main pancreatic duct of Wirsung	Distal three-fourths of dorsal pancreatic duct + proximal one-fourth of ventral pancreatic duct + anastomosis between the two. The three components are: 1. Dorsal pancreatic duct distal to anastomosis between dorsal and ventral pancreatic ducts 2. Anastomosis between dorsal and ventral pancreatic ducts 3. Ventral pancreatic duct proximal to anastomosis between dorsal and ventral pancreatic ducts
Accessory pancreatic duct of Santorini	Proximal one-fourth of dorsal pancreatic duct proximal to anastomosis between dorsal and ventral pancreatic ducts.
Microscopic appearance	
Capsule, septa, connective tissue, and blood vessels	Adjacent mesoderm
Ducts	Reorganization of endodermal cells of pancreatic buds and adjacent mesoderm followed by canalization
Acini (Exocrine part)	Cell clusters at the terminal parts of duct system that reorganize and canalize
Islets of Langerhans (Endocrine part)	Groups of cells separated from the duct system

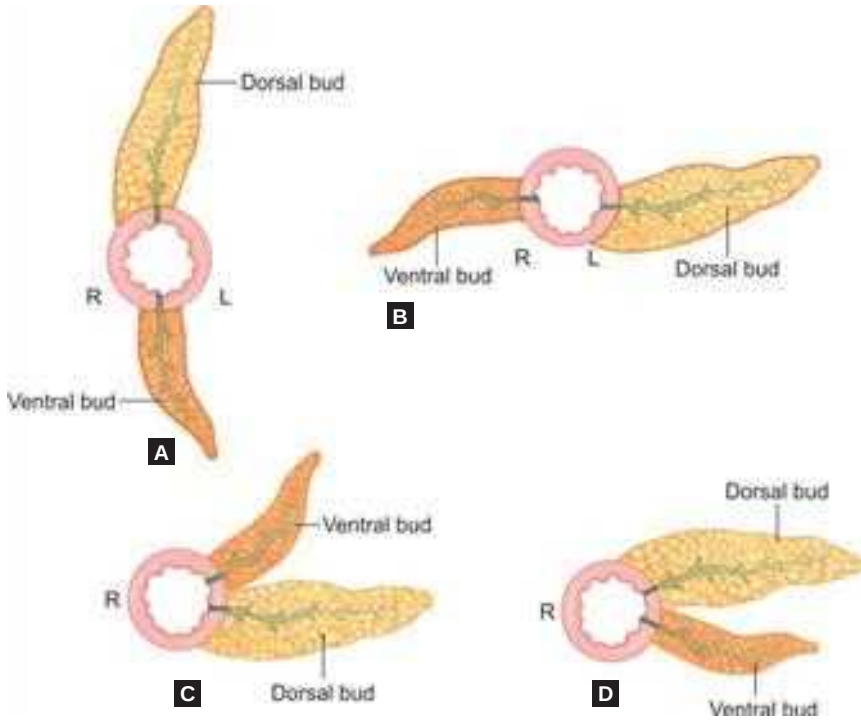
PANCREAS AND SPLEEN

PANCREAS

- *Developmental primordia*: Functionally the pancreas is both exocrine and endocrine gland with difference in microscopic structure of the two components. But the developmental primordium of the two structural and functional components is common. The pancreas develops from two endodermal buds, the *dorsal and ventral pancreatic buds* (Table 14.2).
- *Site of origin of pancreatic buds*: The dorsal and ventral pancreatic buds arise from the dorsal and ventral walls of terminal part of foregut (future second part of duodenum), caudal to hepatic bud (Fig. 14.18A) before rotation of midgut.
- *Dorsal pancreatic bud*:
  - First to appear (4th week) and larger in size when compared to ventral bud.
  - It is cephalic to the ventral bud.
  - It grows between the two layers of dorsal mesentery of duodenum.
- *Ventral pancreatic bud*:
  - It arises in close relation to hepatic bud, in the inferior angle between duodenum and hepatic bud.
  - It appears later and is smaller than the dorsal bud.
  - It grows between the two layers of ventral mesentery of duodenum.
- *Change in the position of pancreatic buds*:
  - Before rotation of duodenal loop the ventral pancreatic bud is on ventral aspect and the dorsal pancreatic bud is on dorsal aspect of duodenum (Fig. 14.19A). With the rotation of duodenal loop to the right, the ventral pancreatic bud along with primitive bile duct comes to the right, and the dorsal bud to the left of duodenum (Figs 14.18B and 14.19B).
  - Due to differential growth of wall of gut, the attachment of ventral pancreatic bud along with primitive bile duct (hepatic bud derivative) shifts to the left moving closer to the dorsal pancreatic bud (Figs 14.18C and 14.19C and D).
- *Fusion of buds*: Pancreatic tissue formed from ventral and dorsal pancreatic buds fuse to form one mass in 7th week of IUL (Figs 14.18E and 14.19D).
- *Derivatives of pancreatic buds*:
  - Ventral pancreatic bud forms lower part of head and uncinated process of pancreas (Fig. 14.18E).



Figs 14.18A to E: Development of pancreas: (A) Appearance of dorsal and ventral pancreatic buds before rotation of gut; (B) Rotation of ventral and dorsal pancreatic buds with rotation of duodenal loop; (C and D) Shifting of ventral pancreatic bud to the left along with bile duct; (E) Fusion of dorsal and ventral pancreatic buds



Figs 14.19A to D: Changes in relative position of pancreatic buds: (A) Initial position in which the ventral and dorsal buds lie in the direction indicated by their names; (B) Position after duodenal loop falls to the right. The ventral bud to the right and dorsal bud to the left; (C and D) Movement of ventral bud to the left lying close to dorsal bud with differential growth of duodenal wall

  - Dorsal pancreatic bud forms upper part of head, neck, body and tail of pancreas (Fig. 14.18E).
- *Duct system of pancreas*:
  - Initially the ducts of dorsal and ventral pancreatic buds are separate and they open separately into the duodenum (Fig. 14.20A).
  - Opening of dorsal pancreatic duct is 2.0 cm proximal to the opening of ventral pancreatic duct.
  - Ventral pancreatic duct and bile duct (hepatic bud derivative) have a common opening in the duodenum.
  - The ducts of dorsal and ventral pancreatic buds anastomose establishing a cross-communication between the two (Fig. 14.20B).
  - The *main pancreatic duct* is formed in its distal part, by the duct of dorsal bud, in its middle part by the oblique cross-communication between ducts of two buds and in its proximal part by the duct of ventral bud. The main pancreatic duct, therefore, opens into the duodenum at the major duodenal papilla, along with the bile duct (Fig. 14.20C).
  - The *accessory pancreatic duct* is formed by the proximal part of dorsal pancreatic duct (between the anastomosis and duodenum). It remains narrow and opens into the minor duodenal papilla 2.0 cm proximal to major duodenal papilla (Fig. 14.20C).

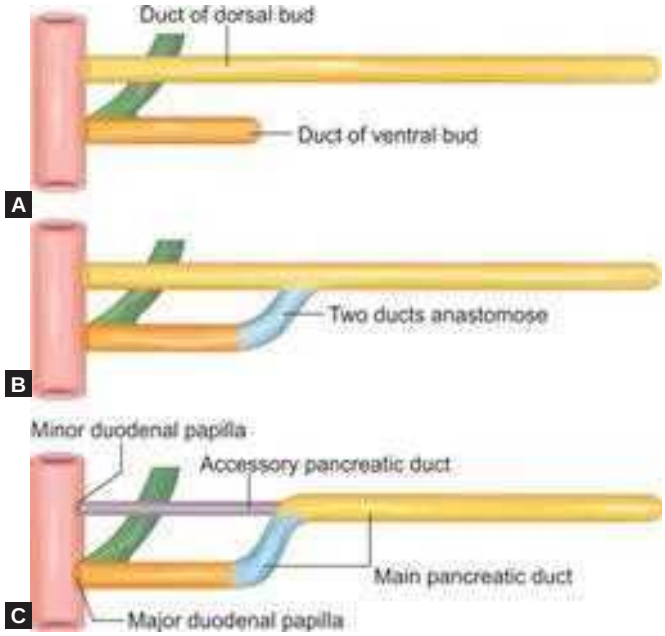


Fig. 14.20: Formation of duct system of pancreas. Distal part of main pancreatic duct is derived from the distal part of duct of dorsal bud, communication between dorsal and ventral ducts and from the duct of ventral bud. Accessory pancreatic duct is derived from the proximal part of dorsal pancreatic duct

  - Repeated branching of the major and minor pancreatic ducts forms the interlobular and intralobular ducts and ductules (Fig. 14.21).
- *Parenchyma (Fig. 14.21)*:
  - The parenchyma develops from branching of endodermal pancreatic buds into the surrounding mesoderm.
  - The parenchyma of pancreas consists of exocrine and endocrine secreting units.
  - The exocrine part of pancreas, consisting of acinar secreting units, develops from proliferation and reorganization of cells at the terminations (ductules) of duct system.
  - The endocrine part, i.e. Islets of Langerhan's, develops from separation of groups of cells from the terminations of duct system.
- *Retroperitoneal location of entire pancreas except tail*: Though initially both buds were suspended in the respective mesogastria, due to their migration they occupy a position posterior to the peritoneum except the tail of pancreas which lies in the lienorenal ligament.

Functions of Fetal Pancreas

- Its exocrine function begins after birth.
- Endocrine function begins in fetal and in early embryonic period. By 7th week α-cells start secreting glucagon and by 10th week insulin production by β-cells begins.

Clinical correlation

Anomalies of the pancreas

- ***Annular pancreas:*** Pancreatic tissue surrounds the duodenum completely and may obstruct it (Fig. 14.22).
- ***Divided pancreas (pancreas divisum):*** Failure of fusion of parts of pancreas derived from dorsal and ventral pancreatic buds with each other (Fig. 14.23A).
- ***Accessory pancreatic tissue:*** It may be found in stomach, duodenum, jejunum, Meckel's diverticulum, gallbladder and spleen.
- ***Inversion of pancreatic ducts:*** Embryonic arrangement of the ducts persists and the greater part of the pancreas is drained through the minor duodenal papilla (Fig. 14.23C).

SPLEEN

- *Developmental primordia*: Spleen is a lymphoid organ. It develops from mesoderm in the dorsal mesogastrium, close to the developing stomach (Fig. 14.7A).
- *Spleniculi*: It develops as a collection of mesenchymal cells to form small lobular masses of splenic tissue (Spleniculi) in the dorsal mesogastrium (Fig. 14.24A). These lobules later fuse to form single mass of spleen.

Presence of splenic notches along the upper border of adult spleen indicates lobulated development of spleen (Fig. 14.25).

- As the mesenchymal cells proliferate, the splenic mass projects in the left layer of dorsal mesogastrium (Fig. 14.24B).
- Because of the splenic projection the dorsal mesogastrium is divided into an anterior part extending from the stomach to the spleen the *gastrosplenic ligament,* and a posterior part that extends from the spleen to the posterior abdominal wall the *lienorenal ligament (Fig. 14.24B)*.
- The posterior layer of dorsal mesogastrium fuses with the posterior abdominal wall (Fig. 14.24C). As a result of this fusion and change in orientation of the stomach, the posterior part of dorsal mesogastrium between spleen and posterior abdominal wall now shifts its position. It extends between spleen and left kidney forming the *lienorenal ligament*.
- As a consequence of this fusion and change in orientation of stomach, the spleen comes to lie on the left side and takes part in forming left boundary of the lesser sac of peritoneum (Fig. 14.24D).
- Capsule, septa and connective tissue framework including reticular fibers develop from mesoderm. The mesenchymal cells differentiate into lymphoblasts and other blood forming cells.

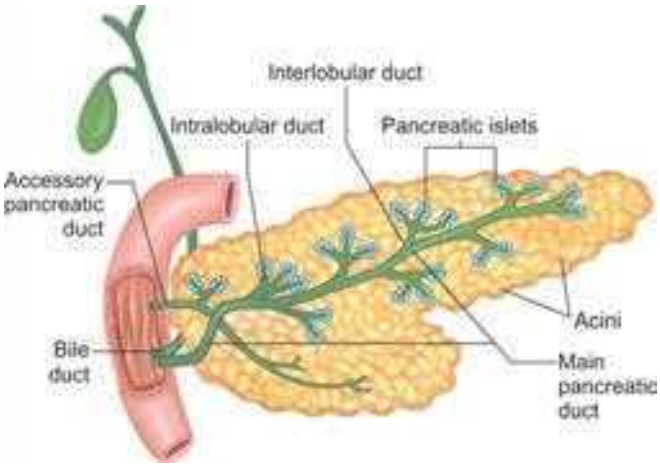
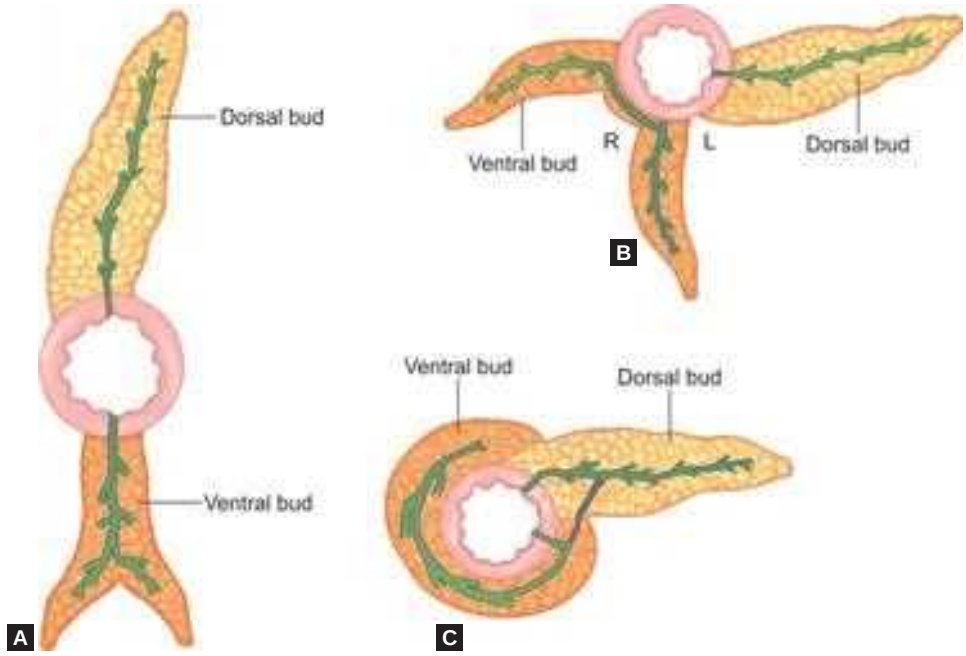


Fig. 14.21: Development of exocrine and endocrine components of pancreas. Major and minor ducts, interlobular and intralocular ducts, ductules, exocrine and endocrine units of pancreas are shown

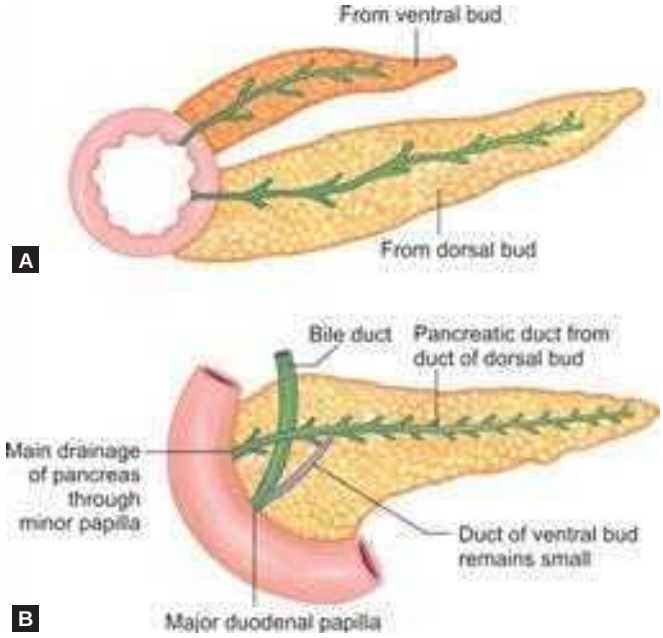
Clinical correlation

Anomalies of spleen

- The spleen may be lobulated (Fig. 14.25).
- Agenesis
- Accessory spleen may be seen
  - At the hilum of spleen
  - In the gastrosplenic ligament
  - In the lienorenal ligament
  - Within the pancreas
  - Along the splenic artery
- Situs inversus—the spleen on the right side of the abdomen. The liver and pancreas are also reversed.



Figs 14.22A to C: Anomalies of pancreas—Annular pancreas. Pancreatic tissue is completely surrounding the duodenum



Figs 14.23A and B: Anomalies of pancreas: (A) Divided pancreas. The parts of pancreas arising from dorsal and ventral buds remain separate; (B) Inversion of pancreatic ducts where main pancreatic duct is formed entirely by the duct of dorsal pancreatic bud, and opens at the minor duodenal papilla. The duct of ventral bud is small

BODY CAVITIES AND DIAPHRAGM

BODY CAVITIES

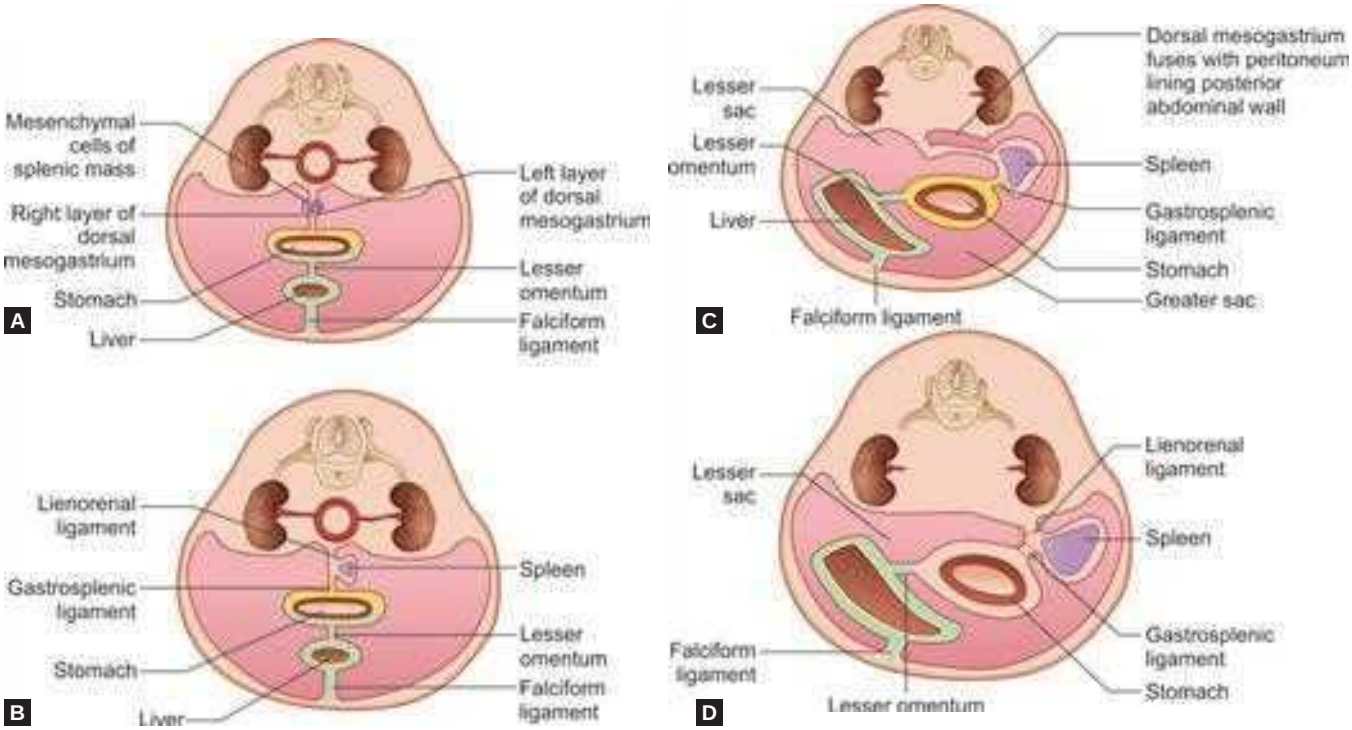
Subdivisions and Developmental Primordium of Body Cavities

There are three body cavities. They are pericardial, pleural and peritoneal cavities. They are derivatives of the *intraembryonic coelom*. The pericardial cavity develops in relation to heart, the pleural cavity in relation to lungs and the peritoneal cavity in relation to the abdominal viscera.

Formation of Intraembryonic Coelom

In the Chapter 5 the subdivisions of intraembryonic mesoderm and a brief description of formation of intraembryonic coelom were presented. For understanding the intraembryonic coelom it is described in detail in this chapter. Refer Figures 5.5 to 5.8 of Chapter 5: Further Development of Embryonic Disc.

- *Appearance of lateral plate mesoderm*: In the 3rd week of development intraembryonic mesoderm (Fig. 5.5) develops from the primitive streak and separates ectoderm from endoderm except at prochordal plate, cloacal membrane and in the midline caudal to prochordal plate that is occupied by notochord. The intraembryonic mesoderm subdivides into three components. They are *paraxial mesoderm*, *intermediate mesoderm* and *lateral plate mesoderm* from medial to lateral on either side of the developing notochord (Fig. 5.6).
- *Appearance of intraembryonic coelom*: Small cavities appear in the lateral plate mesoderm that coalesce to form one large horse-shoe shaped or inverted U-shaped cavity, the *intraembryonic coelom* (IEC) during the 4th week of IUL (Figs 5.7 and 14.26).
- *Communication between intraembryonic coelom (IEC) with extraembryonic coelom (EEC)*: To begin with the intraembryonic coelom is a closed cavity (Figs 5.7 and 14.26) but soon it communicates with extraembryonic coelom to provide nutrition to the differentiating germ layers of embryo by a process of diffusion of fluid in the chorionic sac (Figs 5.8 and 14.27).
- *Splitting of lateral plate mesoderm*: With the formation of intraembryonic coelom, the lateral plate mesoderm is split into two layers a parietal and a visceral. The parietal (somatopleuric) layer that is in contact with ectoderm and continuous with somatopleuric extraembryonic mesoderm over the amnion. The visceral (splanchnopleuric) layer is adjacent to endoderm and is continuous with splanchnopleuric layer of extraembryonic mesoderm covering yolk sac (Fig. 5.8).
- *Formation of serous cavities from intraembryonic coelom*:
  - The IEC gives rise to the serous cavities of the body, i.e. pericardial, pleural and peritoneal cavities (Figs 14.28 to 14.30). The IEC is in two halves, one on either side of the midline and joined together cranial to prochordal plate (Figs 14.28 to 14.30).
  - Before the formation of head fold of the embryo the IEC has a narrow midline portion and two lateral parts. The midline part lies caudal to septum transversum and cranial to prochordal plate near the cranial end of the embryonic disc (Figs 14.28 to 14.30). From this part of the coelom the *pericardial cavity* is formed. The two lateral limbs of the coelom form the *peritoneal cavities* (Fig. 14.30). At this stage there is no pleural cavity as the lung buds are not developed.
  - For some time, the pericardial and peritoneal cavities are connected to each other by a pair of narrow *pericardio-peritoneal canals* (Fig. 14.30). These canals are contributed by the cranial part of each limb of inverted U-shaped IEC. The pericardio-peritoneal canals undergo great enlargement to form the *pleural cavities* when the lung buds come in



Figs 14.24A to D: Development of spleen: (A) Spleen appears in dorsal mesogastrium; (B) Spleen bulges into the left layer of dorsal mesogastrium. Dorsal mesogastrium division into gastrosplenic and lienorenal ligaments; (C) Fusion of dorsal mesogastrium with peritoneum of posterior abdominal wall, changing relationship of dorsal mesogastrium and lesser sac of peritoneum; (D) Change in orientation of stomach and spleen in relation to lesser sac and formation of gastrosplenic and lienorenal ligaments from dorsal mesogastrium

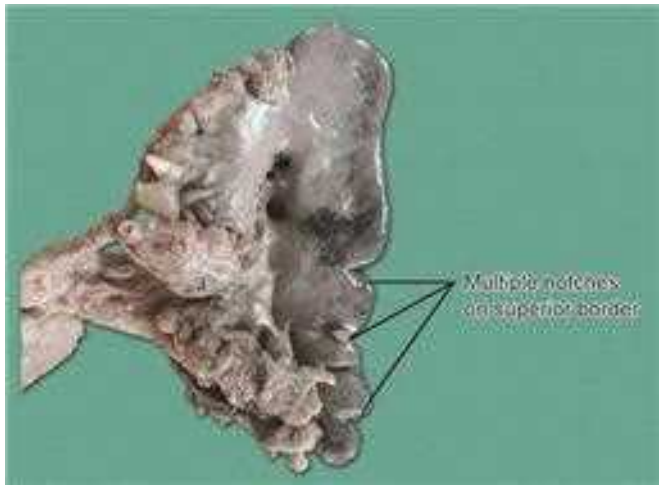


Fig. 14.25: Multiple notches along superior border of spleen indicting persistence of fetal lobulation

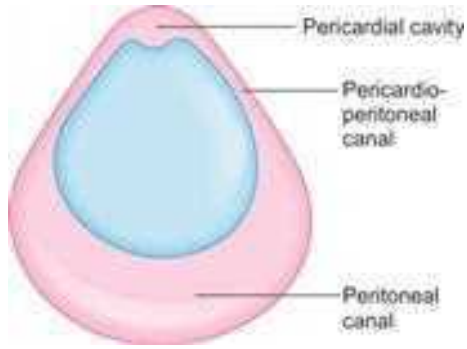


Fig. 14.26: Intraembryonic coelom and its subdivisions

contact with them. Later the two primitive peritoneal cavities (Caudal parts of each limb of inverted U-shaped IEC) fuse to form single *peritoneal cavity* (Figs 14.31 and 14.32).

- *Partitioning of cavities:*
  - The pericardio-peritoneal canal lies lateral to esophagus part of foregut and dorsal to septum transversum (Fig. 14.33).

- Partitions develop to separate definitive pericardial, pleural and peritoneal cavities from one another. Appearance of a cranial and a caudal partition in each pericardio-peritoneal canal separates it from pericardial and peritoneal cavities.

The partitioning of pericardio-peritoneal canal is described in detail in the development of pleural cavities.

- *Derivatives of parietal and visceral layers of IEC:*
  - The parietal and visceral layers of pericardium, pleura and peritoneum are formed from parietal/somatopleuric and visceral/splanchnopleuric layers of intraembryonic mesoderm respectively.
  - The mesodermal cells lining the cavities differentiate into a flattened epithelial lining called mesothelium. The mesothelium gives the peritoneum, pleura and pericardium their smooth surfaces.

Pericardial Cavity

- The midline part of intraembryonic coelom that lies near the cranial end of embryonic disc forms the pericardial cavity (Figs 14.28 to 14.30).
- Before the formation of head fold the primitive pericardial cavity lies between the septum transversum (cranially) and prochordal plate (caudally) (Figs 14.28 to 14.30). Between septum transversum and prochordal plate is the cardiogenic area where the primitive heart tubes develop (Figs 14.28 to 30). The heart tubes are in the floor of the developing pericardial cavity (Figs 14.28 to 14.30).
- During the formation of head fold the heart tube and pericardial cavity undergo 180° rotation and with the result the heart tube occupies the roof of pericardial cavity.

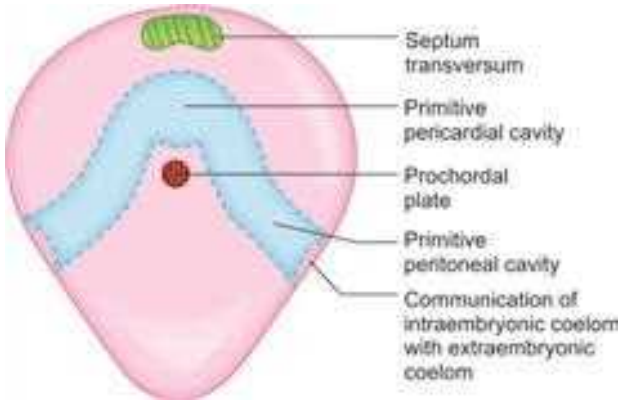


Fig. 14.27: Subdivisions of intraembryonic coelom and its communication with extraembryonic coelom

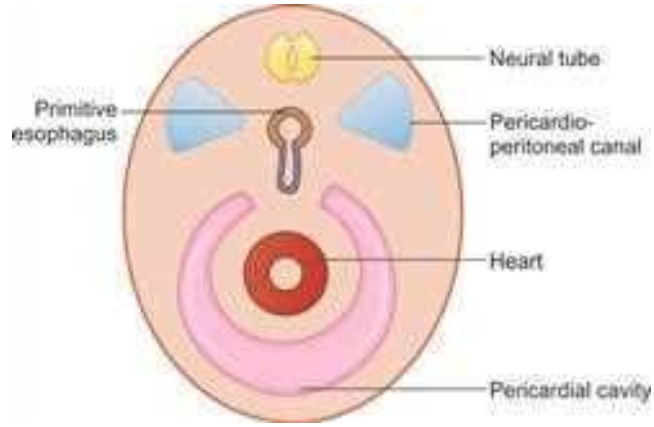


Fig. 14.29: Pericardial and pleuroperitoneal canals in relation to developing heart and foregut

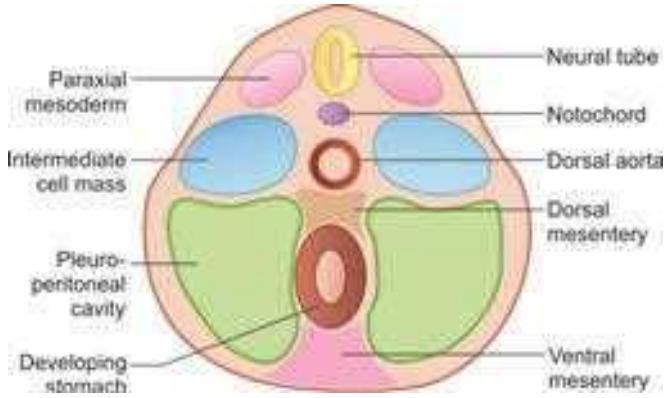


Fig. 14.28: Transverse section at the level of septum transversum in a 4-week embryo showing the components of intraembryonic mesoderm and pleuroperitoneal canal

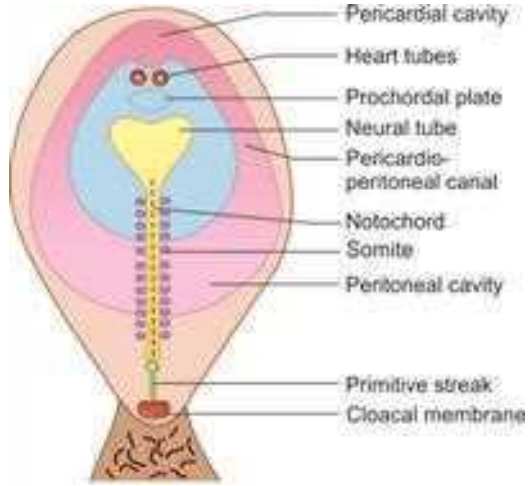


Fig. 14.30: Subdivisions of intraembryonic coelom and their location before head fold

- After the formation of head fold, the pericardial cavity and heart tube occupy a position ventral to the developing foregut, caudal to stomodeum and cranial to the septum transversum (Fig. 14.31).

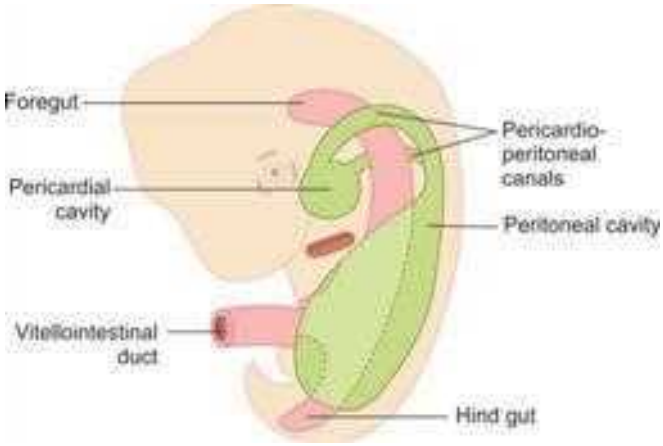


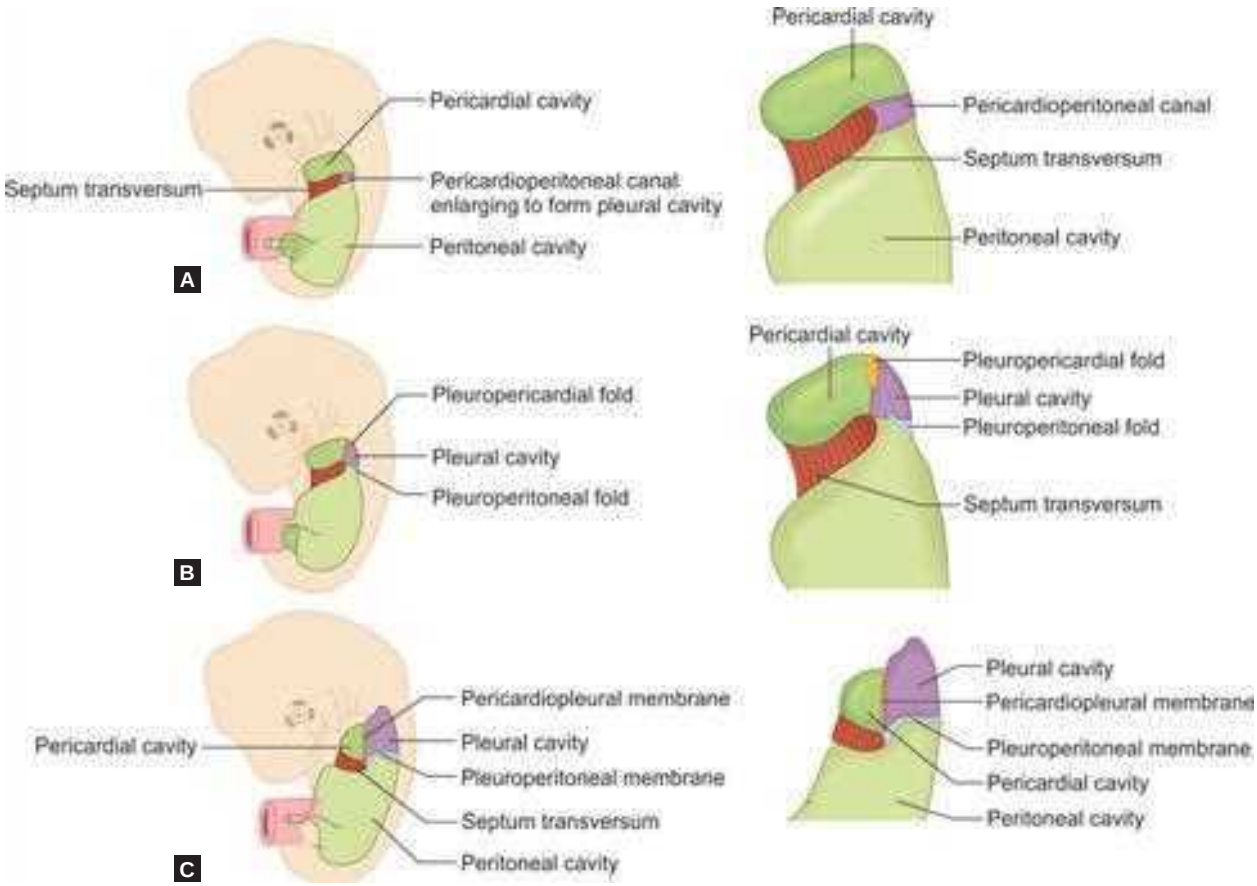
Fig. 14.31: Lateral view of embryo after head fold showing parts of intraembryonic coelom and their relationship to the gut

- The fibrous pericardium and parietal layer of serous pericardium develop from somatopleuric layer of intraembryonic mesoderm. The visceral layer of pericardium develops from splanchnopleuric layer of intraembryonic mesoderm.

The development of pericardial cavity is closely related to the development of heart. Hence, it will be described in detail in the Chapter 15: Cardiovascular System.

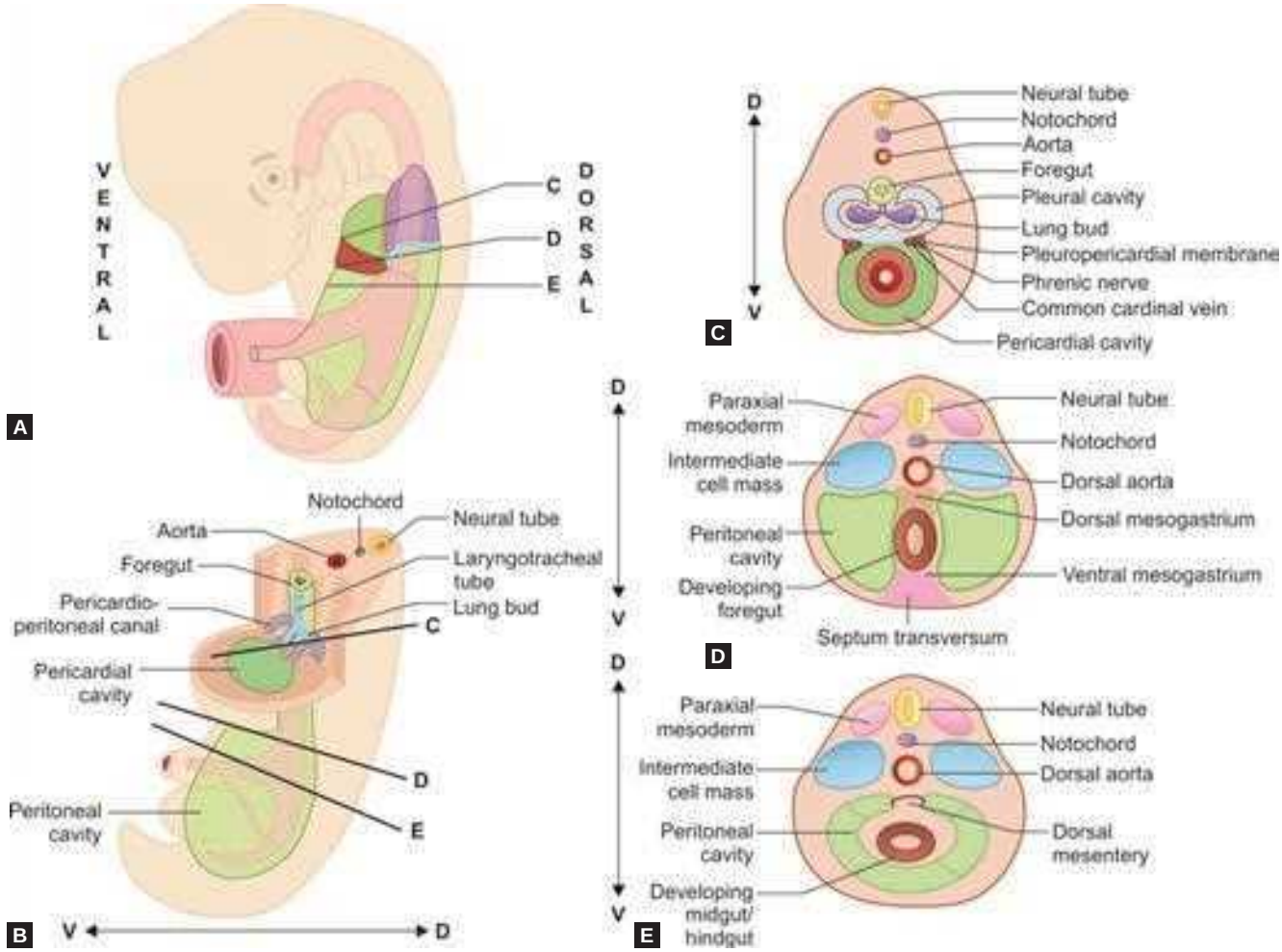
Pleural Cavity

- The right and left pleural cavities develop from right and left pericardio-peritoneal canals (Fig. 14.31), i.e. cranial part of each limb of inverted U-shaped IEC.
- *Partitioning of pleural, pericardial and peritoneal cavities*:
  - Partitions develop to separate definitive pericardial, pleural and peritoneal cavities from one another
  - With the growth of lung bud into the pericardio-peritoneal canal, the canal enlarges to form pleural cavity (Fig. 14.32A).



Figs 14.32A to C: Formation of pleural cavity and its separation from pericardial and peritoneal cavities: (A) Enlargement of pericardio-peritoneal canal to form pleural cavity; (B) communication between pleural cavity and pericardial and peritoneal cavities by pericardio-pleural and pleuro-peritoneal openings and formation of pleuro-pericardial and pleuro-peritoneal folds; (C) Separation of pleural cavity from the pericardial and peritoneal cavities by formation of pericardio-pleural and pleuro-peritoneal membranes

- A pair of membranous ridges appears in the cranial and caudal parts of lateral wall of pericardio-peritoneal canal (Fig. 14.32B). They are:
  - A cranial *pericardio-pleural fold* above the developing lung bud. It separates the pericardial cavity from the pleural cavities as they enlarge. The folds become the *pleuropericardial membranes* (Fig. 14.32C). The pleuropericardial fold contains the common cardinal vein and phrenic nerve.
  - A caudal *pleuroperitoneal fold* below the developing lung bud. This fold enlarges and forms *pleuroperitoneal membrane* (Fig. 14.32C).
- *Position of pericardioperitoneal canals*: The two pericardio-peritoneal canals connecting pericardial and peritoneal cavities lie dorsal to septum transversum and on either side of dorsal mesentery of esophagus part of foregut. With the formation of head fold, the pericardial cavity migrates to a position ventral to the foregut. The two pericardio-peritoneal canals wind backward on either side of the foregut (Figs 14.33A and B).
- *Invagination of lung buds into the pericardio-peritoneal canals*: The two lung buds originating from the ventral aspect of foregut now invaginate the pericardio-peritoneal canals (Fig. 14.33C) from the medial side.

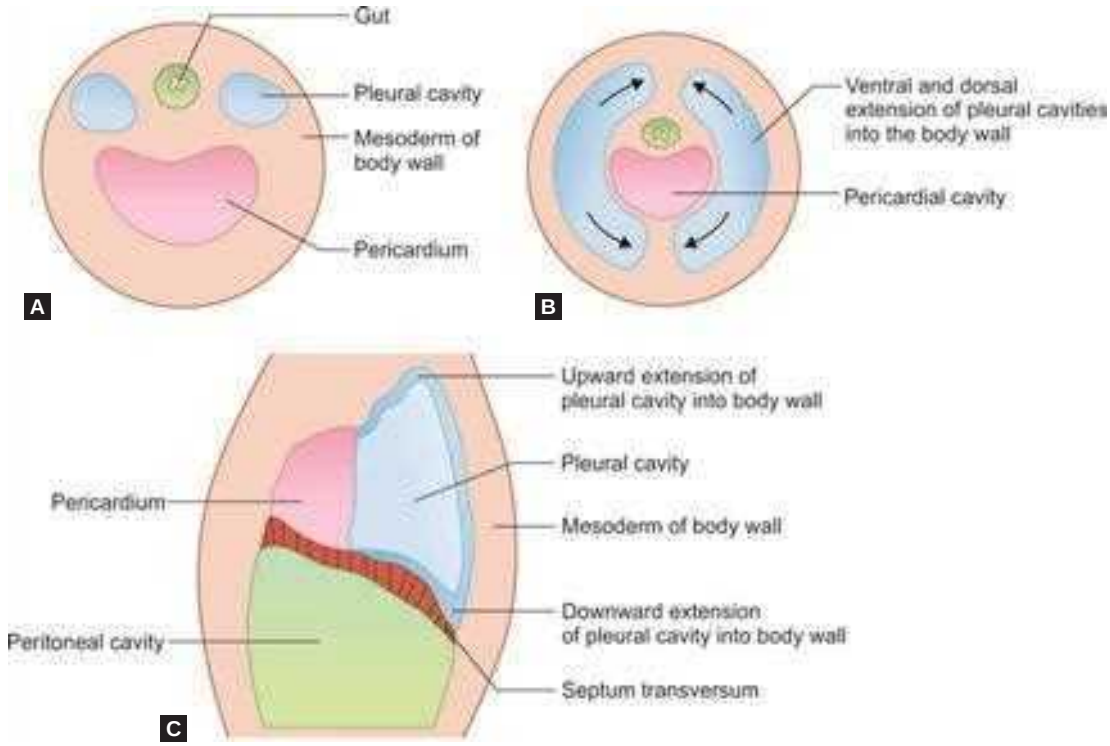


Figs 14.33A to E: Sagittal and transverse sections of embryo showing relationship of coelomic cavities with the developing gut, lung buds and septum transversum: (A) Sagittal section showing coelomic cavities and primitive gut tube; (B) Sagittal section showing pericardioperitoneal canals on either side of esophagus on the dorsolateral aspect of septum transversum. Developing lung buds are seen projecting into the pericardioperitoneal canal; (C) Transverse section showing the lung buds projecting into the pleural cavity, pericardiopleural membrane containing phrenic nerve and common cardinal vein; (D) Transverse section of cranial part of abdomen showing right and left halves of peritoneal cavity and dorsal and ventral mesogastria in relation to foregut; (E) Transverse section of caudal part of abdomen showing the fused peritoneal cavity and dorsal mesentery in relation to midgut/ hindgut In the figure the lines V - D represents ventral (V) to dorsal (D) aspect of each section.

- *Formation of pleural cavity and its communication with other coelomic cavities*: As the lung buds enlarge to form the lungs, the pericardio-peritoneal canals balloon out to form the pleural cavities (Figs 14.32A and 14.33C). Each developing pleural cavity now communicates with the pericardial cavity through the *pericardio-pleural opening* and with the peritoneal cavity through the *pleuroperitoneal opening* (Fig. 14.32B).
- *Closure of communications between coelomic cavities*: In subsequent development, these openings are closed by the formation of the *pericardio-pleural* and the *pleuro-peritoneal membranes*, respectively (Fig. 14.32C). These two membranes are continuous with the septum transversum. The *pericardio-pleural membrane* forms the lateral boundary for the *pericardio-pleural opening* and contains the common cardinal vein and phrenic nerve (Fig. 14.33C). The *pleuro-peritoneal membrane*, an extension from the body wall closes the *pleuro-peritoneal opening* and helps in completing the development of diaphragm.
- *Extension of pleural cavities into the body wall*: The pleural cavities are at first dorsolateral to the pericardium (Fig. 14.34A). With the expansion of lungs and descent of heart, the pleural cavities extend into the mesoderm of the body wall (which is expanding at the same time), and gradually come to lie lateral, and to some extent ventral, to the pericardium (Fig. 14.34B). The pleural cavities also extend downward into the mesoderm that forms the posterior abdominal wall, and upward toward the neck (Fig. 14.34C).
- *Splitting of mesoderm of body wall*: With the expansion of the pleural cavity the mesoderm of the body wall is split into two parts. An outer part that forms the wall of the thorax, and an inner part over the pericardial cavity. The latter is called the *pleuropericardial membrane*. The phrenic nerve runs through this membrane. Later this membrane forms the *fibrous pericardium*. This explains the course of phrenic nerve over the pericardium.

Peritoneal Cavity

- Peritoneal cavity is the largest of the coelomic cavities. It is formed from the distal parts of two limbs of the horseshoe or inverted *U*-shaped intraembryonic coelom.
- The closure of pleuroperitoneal openings by pleuroperitoneal membranes separates the peritoneal cavity from the pleural cavities.
- The caudal parts of the two limbs of horse-shoe-shaped IEC were separate initially. With laterals folding of the embryo, the two parts fuse to form single large peritoneal



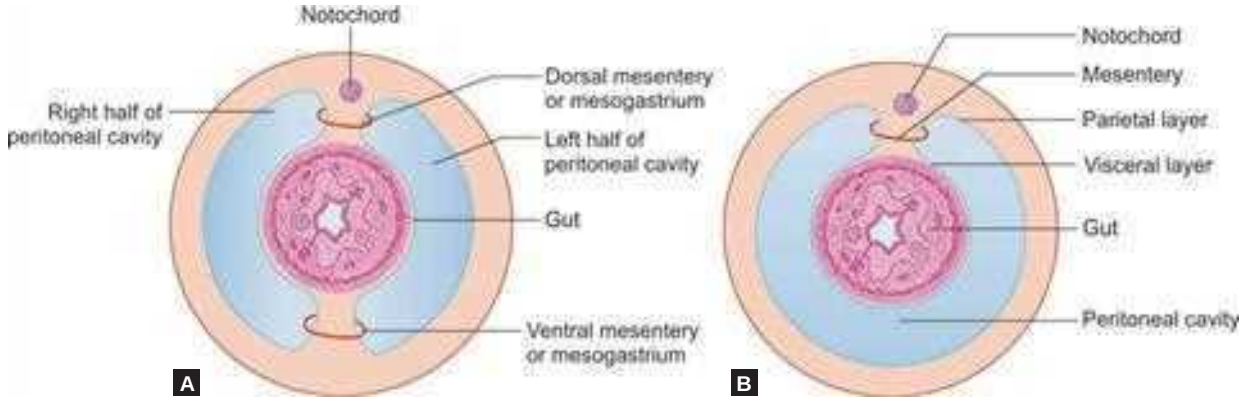
Figs 14.34A to C: Expansion of pleural cavities into the body wall: (A) Pleural cavities on dorsolateral aspect of pericardium; (B) Ventral and dorsal extension of pleural cavities into the body wall; (C) Upward and downward extension of pleural cavities into the body wall

cavity. But, the cranial part of peritoneal cavity remains in two halves (Figs 14.33A and B and 14.35A and B).

- The peritoneal cavity is in communication with the extraembryonic coelom at the umbilicus up to 10th week of IUL (Fig. 14.32). Later with the reduction of physiological hernia and return of midgut loop into the abdomen from the umbilicus the peritoneal cavity loses communication with the extraembryonic coelom.
- The splanchnopleuric intraembryonic mesoderm forms the *visceral layer* of peritoneum. The *parietal layer* of peritoneum derived from somatopleuric layer of intraembryonic mesoderm lines the body wall.
- *Mesenteries of the gut*:
  - The line of reflection of parietal peritoneum to visceral peritoneum forms the *mesenteries* for various organs of gastrointestinal tract.
  - Mesentery is a double layer of visceral peritoneum that connects the primitive gut to the body wall. It contains nerves and vessels.
  - There is a *dorsal mesentery* and a *ventral mesentery* in relation to the dorsal and ventral margins of the primitive gut.
  - The dorsal and ventral mesenteries divided the peritoneal cavity into right and left halves.
  - The mesentery connecting the primitive gut to the anterior abdominal wall is called *ventral mesentery*. The ventral mesentery soon disappears except the part connecting the distal part of foregut, i.e. caudal part of esophagus, stomach and proximal duodenum to the anterior abdominal wall which is known as *ventral mesogastrium* (Fig. 14.33D). With the extension of developing hepatic bud into the ventral mesogastrium, it splits into a part connecting foregut with liver, the *lesser omentum* and a part connecting liver with anterior abdominal wall, the *falciform ligament* (Fig. 14.24).
  - Because of fusion of two halves of the caudal part of horse-shoe-shaped IEC the lower part of peritoneal cavity is single and the midgut and hindgut do not have ventral mesentery (Fig. 14.33E).
  - The mesentery connecting the primitive gut to the posterior abdominal wall is called *dorsal mesentery* (Fig. 14.33E). Its attachment is at first in the midline.
  - As a result of changes, involving the rotation of the gut, and as a result of some parts of the gut becoming retroperitoneal, the line of attachment of the dorsal mesentery becomes complicated. The peritoneal cavity, therefore, comes to be subdivided into a number of pockets that are partially separated by folds of peritoneum (Fig. 14.36).

Development of the Lesser Sac/Omental Bursa

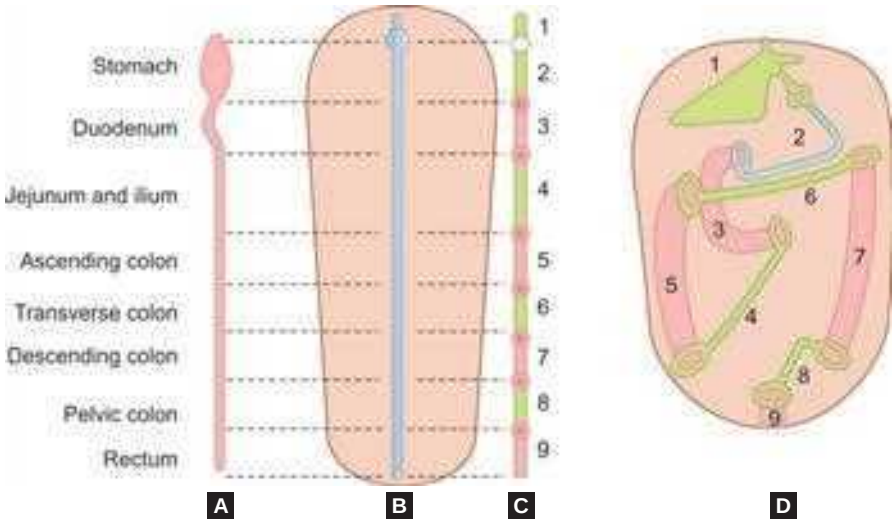
- Lesser sac is the part of peritoneal cavity that lies behind the stomach and lesser omentum.
- It communicates with the greater sac of peritoneal cavity through a small opening called *Foramen of Winslow* or *epiploic foramen* that lies behind the right free margin of lesser omentum.
- The development of lesser sac is closely related to the development of stomach.
- Three distinct processes are involved in the formation of the lesser sac of peritoneum. They are:
  - *Formation of pneumoenteric recesses in dorsal mesogastrium and formation of major part of lesser sac*:
    - *Cavities in dorsal mesogastrium*: The dorsal mesogastrium that connects the stomach to the posterior wall of the abdomen is, initially, a thick membrane (Fig. 14.37A). Two small cavities appear in this membrane. These are the *right* and *left pneumatoenteric recesses* (Fig. 14.37B).
    - *Formation of part of lesser sac behind stomach from right pneumoentreic recess*: The left recess



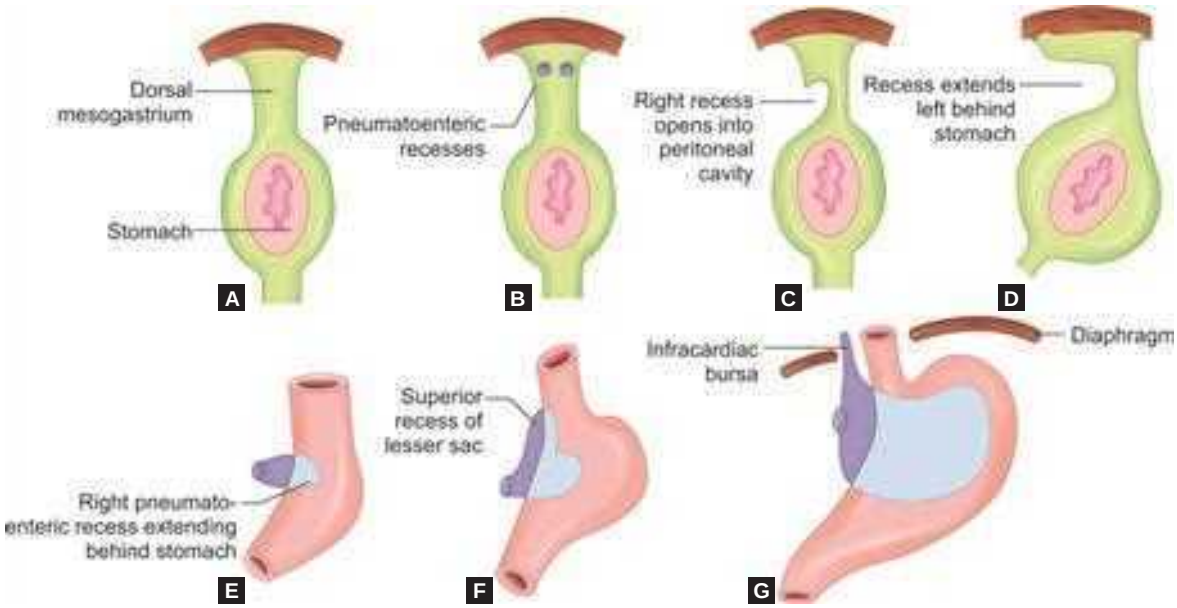
Figs 14.35A and B: Peritoneal cavity before (A) and after (B) lateral folding of embryo

soon disappears. The right recess opens into the peritoneal cavity (Fig. 14.37C). The cavity of right recess now enlarges considerably and extends to the left to form the part of the *lesser sac* that lies *behind the stomach* (Figs 14.37D and E).

- *Cranial extension of right pneumoenteric recess behind liver*: The cavity of right pneumoenteric recess also extends cranially on the right side of the esophagus, *behind the liver* and below the diaphragm. This extension is the *superior recess of lesser sac* (Fig. 14.37F).



Figs 14.36A to D: Peritoneal relations of gut. In (A) the gut is shown as a simple midline tube. In (B) the dorsal wall of the abdomen is shown to indicate the midline attachment of the dorsal mesentery. The esophagus and rectum are seen passing through the wall. In (C) it is shown that alternate segments, i.e. 3, 5, 7 and 9 (odd numbers) become retroperitoneal while the segments 2, 4, 6 and 8 (even numbers) retain their mesentery and (D) Shows the ultimate disposition of these segments on the posterior abdominal wall. (1) Represents the ventral mesogastrium (2) The dorsal mesogastrium (3) The duodenum (4) The mesentery of the jejunum and ileum (5) The ascending colon (6) The transverse mesocolon (7) The descending colon (8) The pelvic mesocolon and (9) The rectum

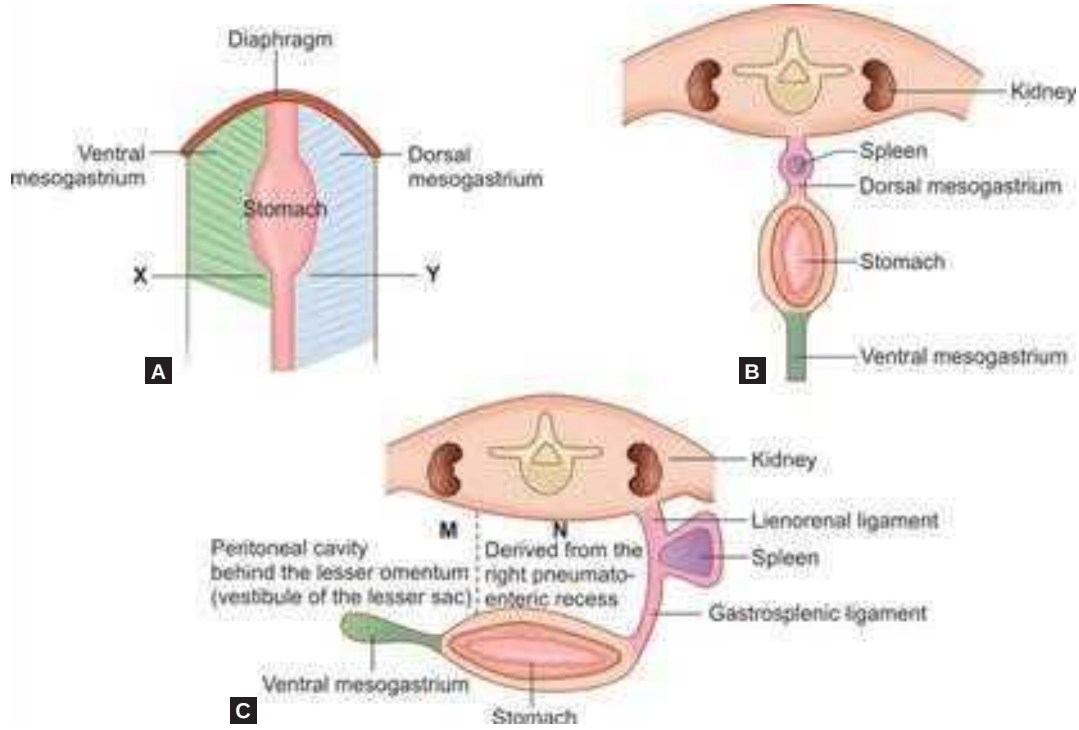


Figs 14.37A to G: Development of lesser sac: (A and B) Formation of right and left pneumoenteric receeses in dorsal mesogastrium; (C) Disappearance of left recess and opening of right into the peritoneal cavity; (D and E) Extension of right pneumoenteric recess to the left behind the stomach; (F) Cranial extension of pneumoenteric recess to form superior recess; (G) Extensions of the right pneumoenteric recess above the level of the diaphragm to form infracardiac bursa

- *Cranial most extension of right pneumoenteric recess above diaphragm to form infracardiac bursa*: Subsequently, with the establishment of the diaphragm, the uppermost part of the cranial extension of right pneumoenteric recess comes to lie above the diaphragm, where it gives rise to the *infracardiac bursa* (Fig. 14.37G).

– *Formation of a part of lesser sac behind lesser omentum*:
  - *Formation of lesser omentum from ventral mesogastrium*: While the right pneumoenteric recess extends to the left, the stomach makes a counterclockwise rotation around its longitudinal axis changing its orientation. The posterior border of stomach (to which the dorsal mesogastrium was attached), now faces to the left. This border forms the *greater curvature*. The ventral border (to which the ventral mesogastrium was attached), now comes to face to the right and forms the *lesser curvature* (Figs 14.38A and B). The ventral mesogastrium may now be called the *lesser omentum*.
  - *Extension of peritoneal cavity behind lesser omentum*: As a result of this change in the orientation of the stomach, a part of the peritoneal cavity comes to lie behind the lesser omentum (M in Fig. 14.38C). This part of the peritoneal cavity now forms *vestibule of the lesser sac*. It is continuous with the part of lesser sac lying behind the stomach (derived from the right pneumoenteric recess Fig. 14.38C).

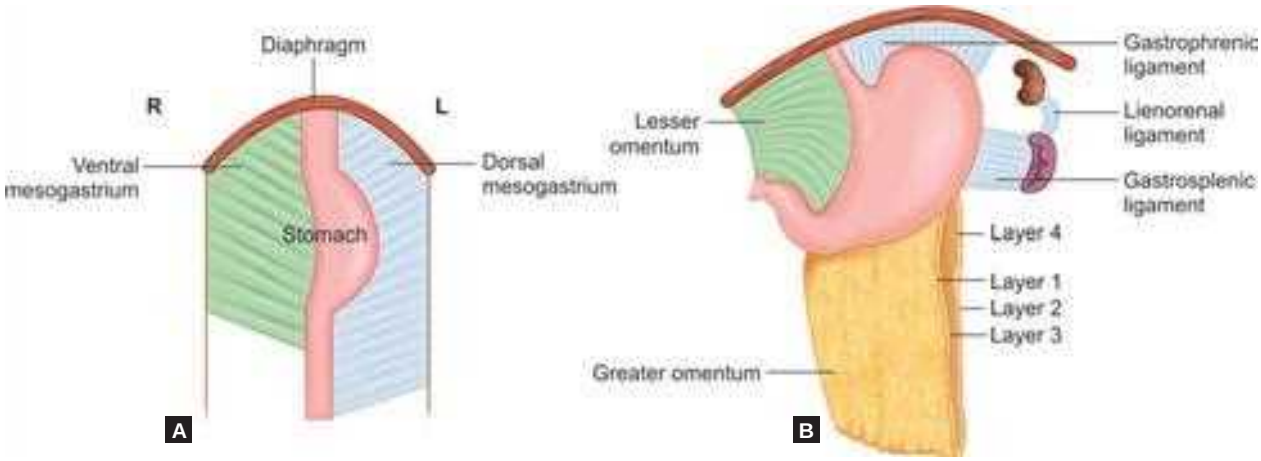
– *Divisions of dorsal mesogastrium and formation of lower part of lesser sac*: With the altered orientation of stomach (Fig. 14.39A) and the development of spleen, the dorsal mesogastrium, which is attached to the greater curvature is subdivided into three parts (Fig. 14.39A).
  - The part extending from the stomach to the diaphragm is the gastrophrenic ligaments, from stomach to spleen is the *gastrosplenic ligament* and from the spleen to the left kidney is the *lienorenal ligament*. The gastrosplenic and lienorenal ligaments, therefore, form the left margin of the lesser sac and the part of lesser sac extending between these ligaments is the *splenic recess of lesser sac*.
  - The part extending from the lower border of the stomach to the posterior abdominal wall (Figs 14.39C and 14.40A) forms the *greater omentum*. The greater omentum undergoes enlargement. With the result it comes to increasingly project



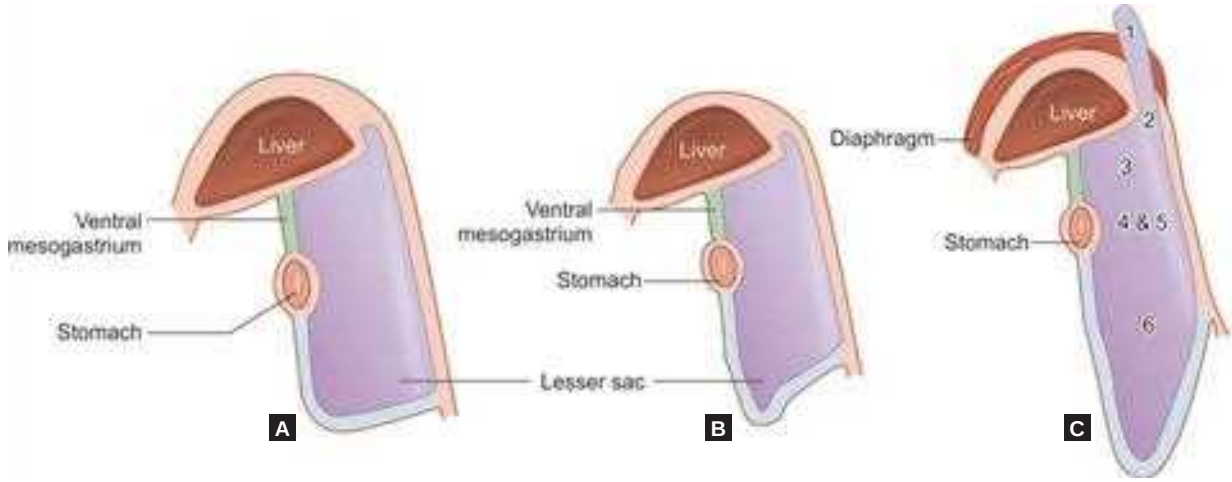
Figs 14.38A to C: Schemes to explain formation of the lesser sac: (A) Shows the dorsal and ventral mesogastria. Note that the ventral mesogastrium has a free border facing downward and forward; (B) The appearance if a section is cut in the plane XY in A; (C) Due to rotation of gut original ventral border of the stomach comes to lie on the right side. Two parts of the lesser sac—one derived from the right pneumoenteric recess and the part that comes to lie behind the ventral mesogastrium (lesser omentum)

below the level of the stomach, and becomes folded on itself. The space within this fold forms the *inferior recess of the lesser sac* (Fig. 14.40B).

- The parts of lesser sac can be summarized as follows (Fig. 14.40C):
  - *Infracardiac bursa*: Cranial extension of right pneumoenteric recess above the diaphragm.
  - *Superior recess*: Cranial extension of right pneumoenteric recess behind liver and on right side of esophagus.
  - *Vestibule of lesser sac*: Part of right pneumoenteric recess behind lesser omentum.
  - *Part behind stomach*: Part of right pneumoenteric recess.
  - *Splenic recess of lesser sac*: Part of right pneumoenteric recess between gastrosplenic and lienorenal ligaments.
  - *Inferior recess of lesser sac*: Extension of right pneumoenteric recess between layers of greater omentum.



Figs 14.39A and B: Parts of the dorsal mesogastrium and their attachments to the stomach and to the posterior abdominal wall: (A) Attachment of dorsal and ventral mesogastria and (B) Formation of gastrophrenic, gastrosplenic and lienorenal ligaments and greater omentum from dorsal mesogastrium. Elongation of greater omentum is shown with formation of four layers (1, 2, 3 and 4 from anterior to posterior) and cavity of lesser sac between the layers 3 and 4



Figs 14.40A to C: Development of the lesser sac: Downward extension of the sac by elongation and folding of the greater omentum. The derivation of the parts numbered in (C) is (1) from cranial extension of right pneumoenteric recess above diaphragm (Infracardiac bursa), (2) cranial extension of right pneumoenteric recess on right side of esophagus (superior recess), (3) part of peritoneal cavity that comes to lie behind ventral mesogastrium, (4 and 5) right pneumoenteric recess in between gastrosplenic and lienorenal ligaments, i.e. splenic recess and (6) cavity produced by elongation and folding of greater omentum on itself (inferior recess)

TABLE 14.3: Development of various peritoneal folds

Peritoneal fold in adult	*Embryological origin*	*Extent*	*Contents*
Gastrosplenic ligament	Dorsal mesogastrium	Stomach and spleen	Short gastric vessels and left gastroepiploic vessels
Gastrophrenic ligament	Dorsal mesogastrium	Stomach to diaphragm	Left inferior phrenic vessels
Lienorenal ligament	Dorsal mesogastrium	Left kidney to spleen	Splenic artery, tail of pancreas
Lesser omentum	Ventral mesogastrium	Stomach and first part of duodenum to liver	• Along lesser curvature of stomach—right and left gastric vessels, gastric lymph nodes and gastric nerves • Right free margin—hepatic artery, portal vein and bile duct, hepatic plexus of nerves and lymph vessels
Falciform ligament	Ventral mesogastrium	Liver to anterior abdominal wall	Ligamentum teres hepatis (Left umbilical vein), paraumbilical veins
Coronary ligament (Superior and inferior layers)	Ventral mesogastrium	Liver to diaphragm	—
Triangular ligaments	Ventral mesogastrium	Liver to diaphragm	—
Greater omentum	Dorsal mesogatrium	Between greater curvature of stomach to transverse colon	Right and left gastroepiploic vessels
Transverse mesocolon	Dorsal mesentery	Transverse colon to posterior abdominal wall	Middle colic vessels
Mesentery of small intestine	Dorsal mesentery	Between posterior abdominal wall and small intestine (Jejunum and ileum)	Superior mesenteric vessels, lymphatics, autonomic nerve plexus, lymph nodes and fat
Sigmoid mesocolon	Dorsal mesentery	Sigmoid colon to posterior abdominal wall	Sigmoid vessels, superior rectal vessels
Mesoappendix	Dorsal mesentery	Mesentery of ileum to appendix	Appendicular vessels

DIAPHRAGM

- Diaphragm is the dome-shaped musculotendinous partition that separates the thoracic and abdominal cavities.
- The pericardial and pleural cavities are above (or cranial to) it, whereas the peritoneal cavity is below (caudal to) it. The development of the diaphragm is, therefore, intimately related to the development of these cavities.
- Diaphragm is *mesodermal* in origin. *Four mesodermal components* contribute for its development (Table.14.4). The components are:
  - *Septum transversum* is the most important component and it contributes for the unpaired anterior and median part that includes central tendon, esophageal and vena caval openings. The liver develops in the caudal part of septum transversum. Its cranial part helps to form the diaphragm.
  - *Pleuroperitoneal membranes* form the paired dorsolateral part of diaphragm.

TABLE 14.4: Developmental components of adult diaphragm

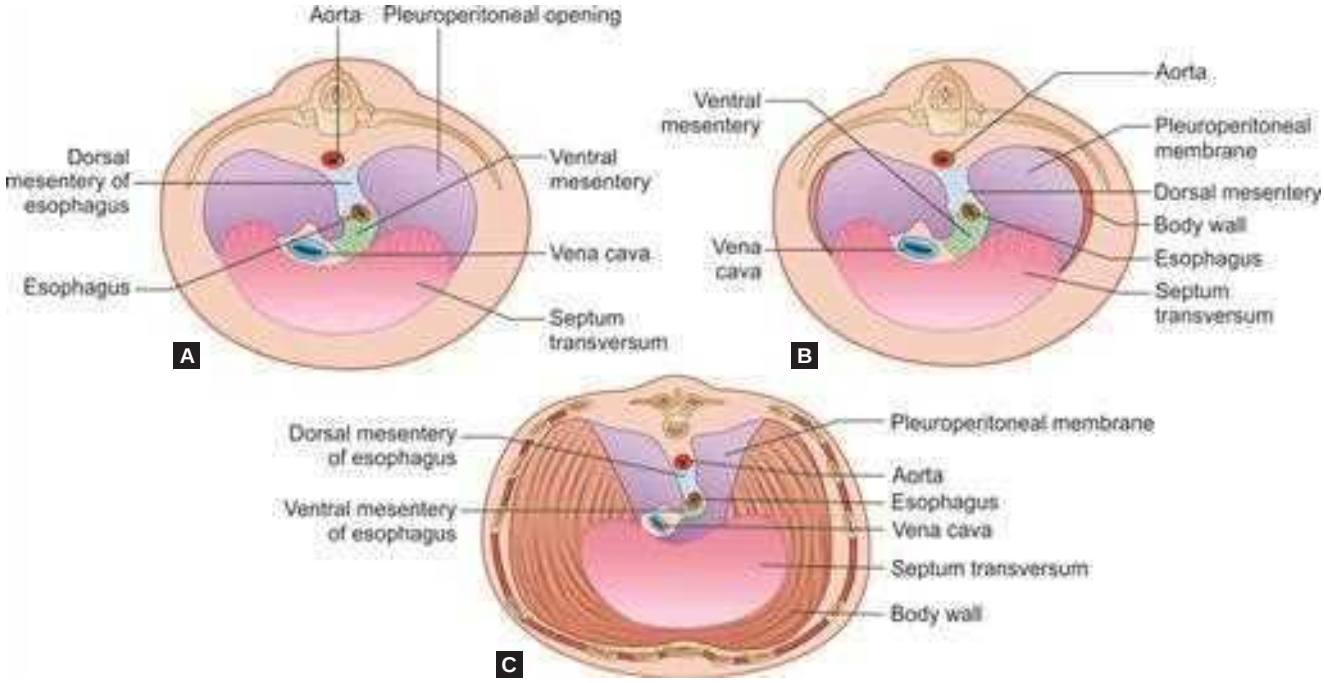
Components of diaphragm	*Embryological origin*
Central tendon	Septum transversum (Unpaired)
Right and left crus	Dorsal mesentery of esophagus (Unpaired)
Large, peripheral (posterolateral) part—costal part	Muscular (mesodermal) ingrowth from lateral body wall external to the part derived from pleuroperitoneal membranes
Small peripheral part—right and left	Pleuroperitoneal membranes (Paired)

  - *Ventral and dorsal mesenteries of esophagus* form the irregular dorsal median part that fuses with septum transversum and pleuroperitoneal membranes.
  - *Mesoderm of body wall* including mesoderm around dorsal aorta that fuses with pleuroperitoneal membranes.
- *Fusion of mesodermal components to form diaphragm*: The formation of the septum transversum was

considered in detail in Chapter 5: Further Development of Embryonic Disc.

- Septum transversum is the unsplit part of intraembryonic mesoderm in the cranial part of embryo. It develops during 3rd week of IUL and lies opposite C3, C4 and C5 somites.
- After the formation of head fold, during 4th week of IUL the septum transversum migrates in a ventro-caudal direction, and forms the thick incomplete partition between thoracic and abdominal cavities, leaving large gaps that has to be closed by other components that contribute components for diaphragm development (Figs 14.33A and B).
- Dorsal to septum transversum is the esophagus and its mesenteries. The ventral margin of esophagus is connected to the dorsal border of septum transversum by ventral mesentery (derived from septum transversum). The dorsal margin of esophagus is attached to posterior abdominal wall by dorsal mesentery (mesoesophagus) (Fig. 14.41A).
- Dorso-lateral to the septum transversum, the pleural and peritoneal cavities communicate through the pleuroperitoneal canals that lie one on either side of the esophagus. Dorsal margin of septum transversum forms the anterior boundary for the pleuroperitoneal openings for some time (Fig. 14.42A).
- The partition between the thorax and the abdomen is completed when the pleuroperitoneal canals are closed by the formation of the pleuroperitoneal membranes (Fig. 14.41A).
- During 6th week septum transversum expands and fuses with ventral and dorsal mesenteries of esophagus and pleuroperitoneal membranes (Figs 14.41B and 14.42B). Dorsal mesentery of esophagus (mesoesophagus) contributes for the median portion containing the two crura of the diaphragm.
- During 9th–12th weeks of IUL the pleural cavities increase in size. Simultaneously, the diaphragm also enlarges, and this enlargement takes place at the expense of the lateral body wall (Figs 14.41C and 14.42C). Splitting of body wall tissue contributes for the peripheral parts of diaphragm outside the parts contributed by pleuroperitoneal membranes. With the result the thorax as a whole also expands and extensions of pleural cavity into body wall forms costodiaphragmatic recess of pleura (Fig. 14.34C).

- *Various views on proportion of contribution by each developmental component*: There is, however, considerable controversy as to how much of the diaphragm is formed from each of the constituents. According to some workers, the septum transversum forms only the central tendon, while according to others, it gives rise to almost the whole of the costal and sternal parts of the diaphragm. The crura of the diaphragm are



Figs 14.41A to C: Development of diaphragm: (A) Pleuroperitoneal canals, septum transversum and mesenteries of esophagus; (B) Closure of pleuroperitoneal canal their closure; (C) Expansion from body wall

formed from the mesoderm of the posterior abdominal wall (mesoesophagus/dorsal mesentery of esophagus), as a result of the downward extension of the pleural cavities into this region.

- *Embryological components of adult diaphragm*:
  - Septum transversum—Central tendon of diaphragm
  - Pleuroperitoneal membranes—Small peripheral part
  - Mesoderm of body wall—Large peripheral part
  - Dorsal mesentery of esophagus—Crura of diaphragm.
- *Diaphragm innervation*:
  - During 4th week of IUL septum transversum lies at the level of C3, C4 and C5 somites. The ventral rami of spinal nerves from C3, C4 and C5 spinal segments grow into the septum transversum and fuse to form the phrenic nerve.
  - The phrenic nerves enter septum transversum through pleuropericardial fold and later pleuropericardial membrane. This membrane forms the *fibrous pericardium*. Thus, the phrenic nerves course through the fibrous pericardium. Phrenic nerve contributes for sole motor and sensory supply to the central part of diaphragm.
  - The peripheral part of diaphragm develops from the lateral body wall. Lower intercostal nerves provide sensory supply to this part of diaphragm derived from lateral body wall. The sensory innervation of the peripheral parts of the diaphragm by the intercostal nerves is an evidence for the contribution made by the body wall to the muscle.
- *Descent of diaphragm*:
  - At first (4th week of IUL) the septum transversum is at cervical 3rd–5th segments.
  - Later (6th weeks of IUL) the diaphragm descends to the thoracoabdominal junction opposite T7–T12 vertebra.
  - At 8th week of IUL the dorsal part of diaphragm reaches the level of T12/L1 vertebra due to rapid growth of dorsal part of body of embryo when compared to ventral part.
  - When the diaphragm descends, it carries its nerve supply (phrenic nerve) with it.
  - The factors responsible for descent of diaphragm are:
    - Elongation of neck
    - Descent of heart
    - Expansion of pleural cavities.

Clinical correlation

Anomalies of diaphragm

- ***Diaphragmatic hernias*:** Failure of development of parts of diaphragm resulting in gaps in the muscle. Abdominal contents may pass through these gaps to produce diaphragmatic hernias. Diaphragmatic hernias may be (Fig. 14.43):
  - *Posterolateral*: Due to failure of closure of pleuroperitoneal canal.
  - *Posterior*: Due to failure of development of crura.
  - *Retrosternal*: Due to the presence of abnormally large gap between sternal and costal parts of diaphragm.
- ***Accessory diaphragm*:** It is rare and when present it partially subdivides the lung into two parts.
- ***Congenital eventration of diaphragm*:** Diaphragm may be thin and aponeurotic and may bulge upward into the thorax. The bulging may be unilateral or may be confined to a smaller area.

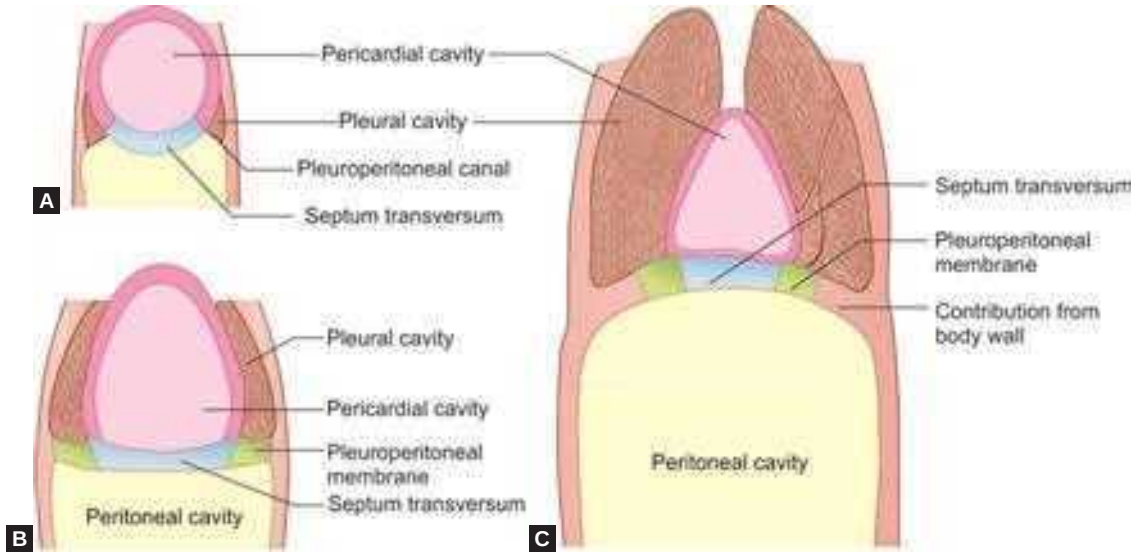


Fig. 14.42: Development of the diaphragm. Showing developmental components and how expansion of the pleural cavities into the body wall causes the wall to form part of the diaphragm

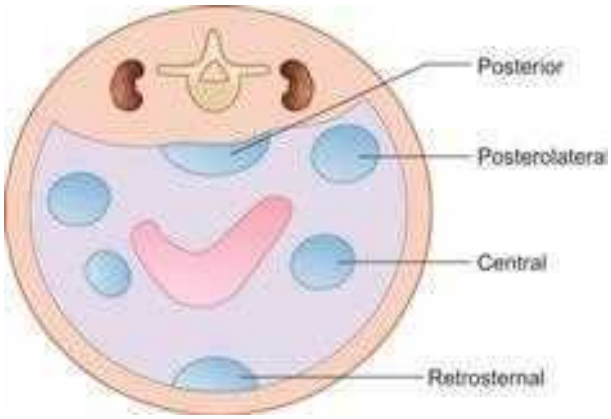


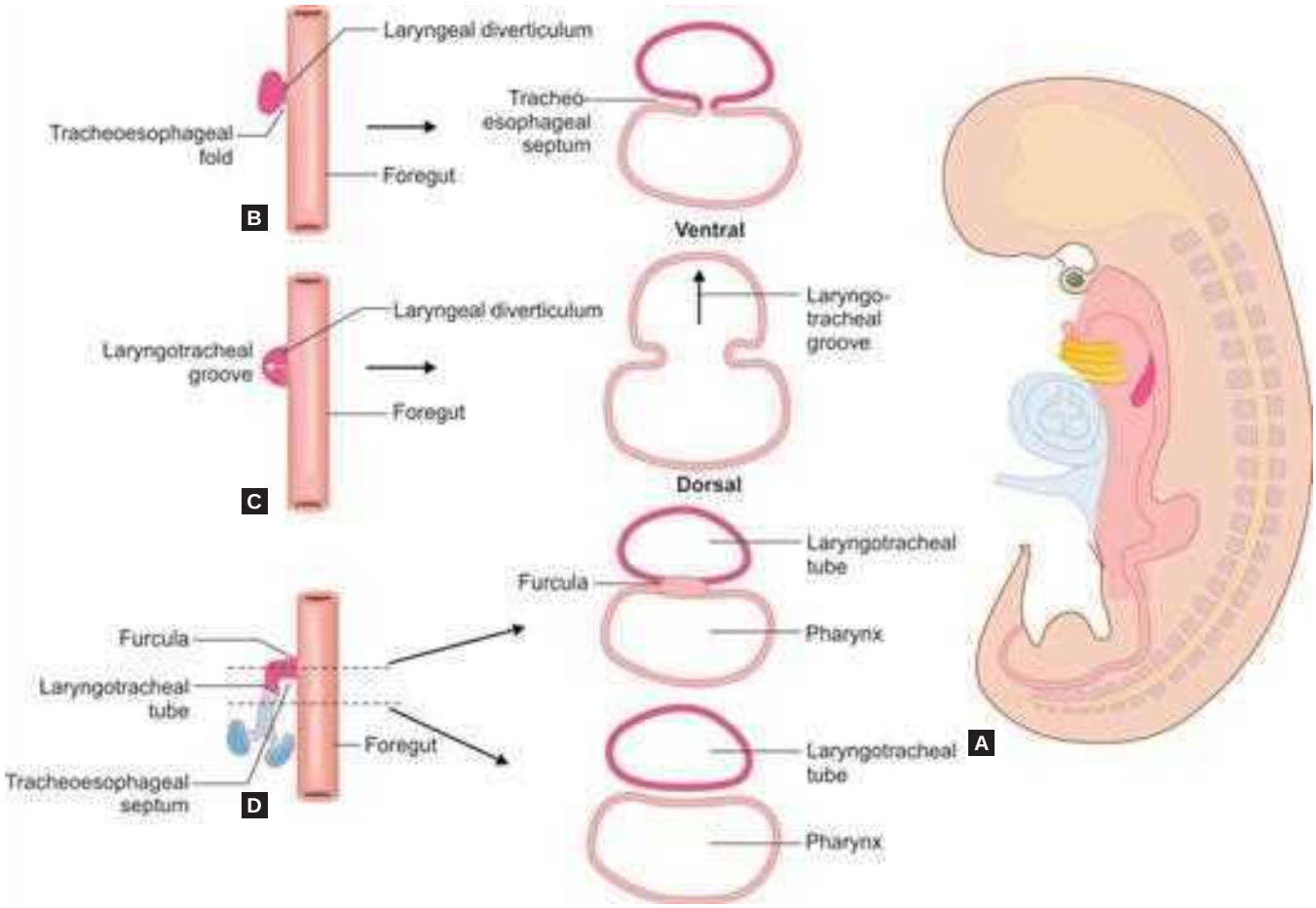
Fig. 14.43: Sites of congenital defects in diaphragm. Abdominal contents may pass through these gaps to produce diaphragmatic hernias

RESPIRATORY SYSTEM

- *Subdivisions of respiratory system*:
  - *Anatomically* it is divided into upper and lower respiratory tracts.
    - *Upper respiratory tract* includes nostrils, nasal cavities, paranasal air sinuses, pharynx and larynx.
    - *Lower respiratory tract* consists of trachea, bronchi, terminal bronchioles, respiratory bronchioles and alveoli.
  - *Functionally* it is divided into conducting part and respiratory part.
    - *Conducting part* includes entire upper respiratory tract and lower respiratory part up to terminal bronchioles and it facilitates conduction, cleaning, warming and moistening of air.
    - *Respiratory part* includes respiratory bronchioles, alveolar ducts and alveoli and is concerned with exchange of gases.

The development of various components of upper respiratory tract up to pharynx is described in the chapters of face, nose and palate and in alimentary system—Part 1. In the present chapter development of various components of respiratory system from larynx to alveoli will be dealt.

- *Developmental primordia of respiratory system*:
  - The respiratory system is *endodermal* in origin.
  - It develops from a median *endodermal* diverticulum of the foregut (*respiratory diverticulum*) (Figs 14.33B and C and 14.44A) and the adjacent *splanchnopleuric intraembryonic mesoderm*.
  - The lining epithelium of larynx, trachea, bronchi, bronchioles and alveoli are *endodermal* in origin.
  - The connective tissue, cartilages and muscles in relation to the various parts of respiratory system are derived from *splanchnopleuric mesoderm* surrounding the foregut.
- Development of respiratory (laryngotracheal) diverticulum:
  - Development of respiratory system is first seen as a midline evagination known as *respiratory diverticulum* or *laryngotracheal diverticulum* from the ventral wall of pharyngeal part of foregut (Fig. 14.44A).
  - Respiratory or laryngotracheal diverticulum is caudal to hypobranchial eminence (epiglottis) and is in the floor of developing pharynx (Figs 14.45A and 14.46).
  - It extends in caudal direction and elongates (Figs 14.44A to D and 14.46).
- *Derivatives of respiratory diverticulum:*
  - The free caudal end of the respiratory diverticulum grows downward to enter the thorax where it becomes bifid to form the right and left *bronchial/lung buds* (Figs 14.47 and 14.48A).
  - The part of diverticulum cranial to the bifurcation is laryngotracheal tube and it forms the *larynx* and *trachea*, while the *lung buds* form the *bronchi and lung parenchyma* (Figs 14.44D and 14.46).
- *Separation of laryngotracheal tube from the foregut:*
  - The groove separating the ventral pharyngeal wall and the proximal part of respiratory diverticulum is the *laryngotracheal groove*. It appears during 4th week of IUL (Figs 14.44C, 14.45A and 14.48A).
  - The laryngotracheal groove is flanked by sixth pharyngeal arch on either side (Fig. 14.45A).
  - As the laryngotracheal groove deepens, the *laryngotracheal tube* and its continuation, the *respiratory diverticulum* are formed (Figs 14.44D and 14.46).
  - At the caudal part of respiratory diverticulum two lateral folds known as *tracheooesophageal folds* grow medially from the margins of tracheoesophageal groove, and fuse to form *tracheoesophageal septum* (Figs 14.44B and D).
  - The tracheoesophageal septum is coronally oriented and extends caudocranially. The caudal part of septum separates trachea ventrally from the esophagus dorsally (Figs 14.44B and D).
  - The respiratory primordium maintains its communication with the pharynx through laryngeal orifice or inlet of larynx. The cranial part of tracheoesophageal septum is arrested to form a sagittal slit the *inlet of larynx* or *furcula of His* through which the laryngotracheal tube communicates with the pharynx (Figs 14.44D and 14.49A).

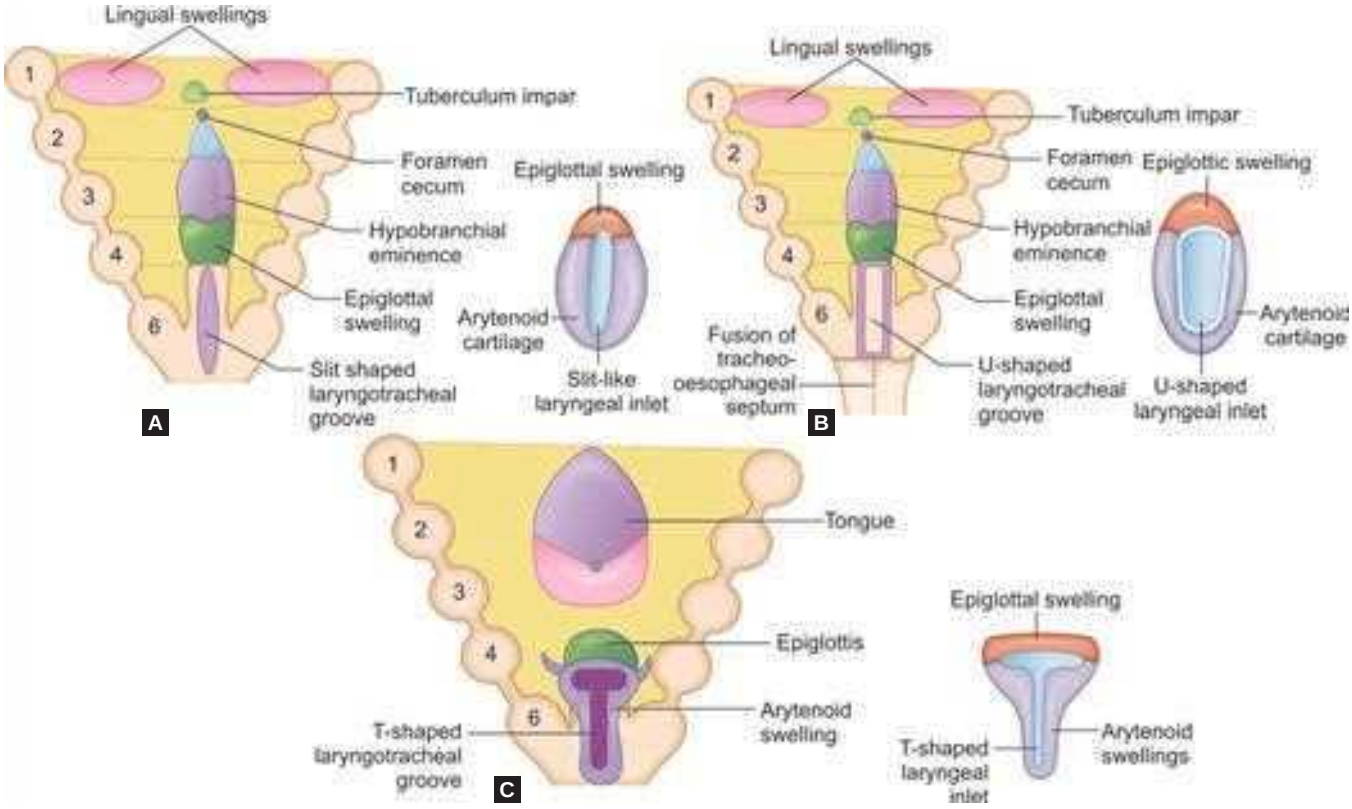


Figs 14.44A to D: (A) Development of laryngotracheal or respiratory diverticulum. A median endodermal respiratory diverticulum from the ventral wall of foregut; (B to D) B to D are developing respiratory diverticulum and its separation from the foregut and its subdivisions

- *Invagination of lung bud into the pleural cavity:*
  - Each lung bud invaginates into the pericardio-peritoneal canal (Fig. 14.33B).
  - The right and left pericardioperitoneal canals form right and left *pleural cavities* (Figs 14.48B and 14.50).

LARYNX

- The larynx develops from the cranial most part of the laryngotracheal tube.
- *Inlet of larynx*:
  - The communication between the laryngotracheal tube and the pharynx persists as a slit like orifice, the *inlet of the larynx* or *furcula of His* (Figs 14.45A and 14.49A).
  - The laryngeal inlet is surrounded by the mesenchyme of 4th and 6th pharyngeal arches. This mesenchyme proliferates to form thyroid, cricoid, and arytenoid cartilages (Figs 14.45A and 14.49A and B).
  - The slit-like laryngeal inlet becomes *U*-shaped in the beginning, and is bounded at its cephalic end by caudal part of hypobranchial eminence (4th arch derivative) and on each side by a mucous fold derived from 6th arch, in which a pair of arytenoid swellings develop (Figs 14.45B and C).
  - Because of proliferation of mesenchyme of 4th and 6th arches the *U*-shaped laryngeal inlet now changes into a *T*-shaped orifice. The horizontal limb of T is caudal to hypobranchial eminence and the vertical limb is between arytenoid swellings. Reorganization of cartilages results in characteristic adult shape of laryngeal inlet. The caudal part of hypobranchial eminence (4th arch) forms epiglottis and cuneiform cartilages. The upper part of arytenoid swelling forms the arytenoid and corniculate cartilages, whereas the lower part of it forms the cricoid cartilage (Figs 14.45C and 14.49B and C).



Figs 14.45A to C: Development of inlet of larynx: (A) slit-shaped laryngotracheal groove caudal to hypobranchial eminence; (B) U-shaped laryngotracheal groove; (C) T-shaped laryngotracheal groove

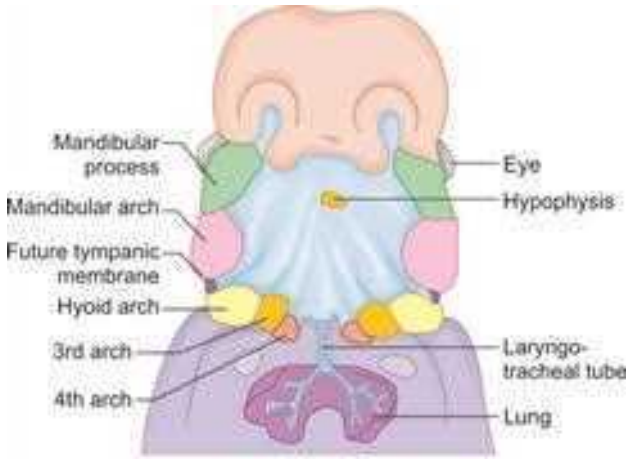


Fig. 14.46: Developing larynx, trachea and lung and the pharyngeal arches and stomodeum

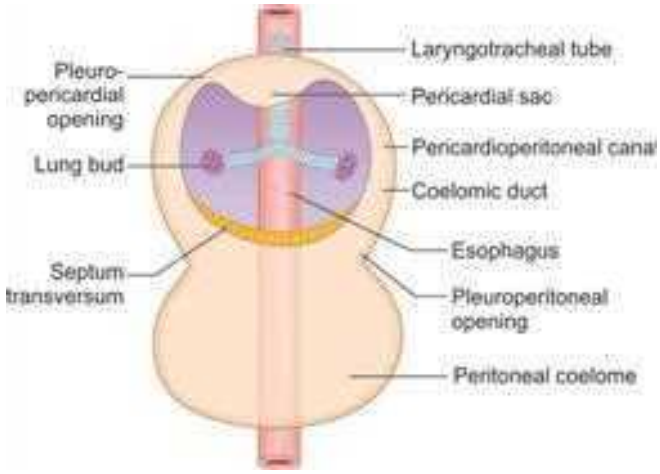


Fig. 14.47: Developing lung buds coming in contact with pleuropericardial canal developing pharynx

- *Developmental components of larynx*:
  - *Lining epithelium*: The internal lining of larynx is derived from *endoderm*.
  - *Cartilages*: The epiglottis, thyroid and cuneiform cartilages are derived from *4th arch*. Cricoid, arytenoid and corniculate cartilages are derivatives of *6th arch* (Figs 14.49A to C).
  - *Muscles*: The laryngeal muscles are derived from branchial mesoderm as indicated by their nerve supply (All intrinsic muscles by *recurrent laryngeal*

nerve (6th arch) except cricothyroid which is supplied by external laryngeal branch of *superior laryngeal nerve* (4th arch).

- *Interior of larynx*: Initially there will be rapid proliferation of epithelium of larynx occluding its lumen. Later vacuolation and recanalization of epithelium produces two lateral recesses known as *ventricles of larynx*. These recesses are bounded by endodermal folds that differentiate to form an upper pair of vestibular folds and a lower pair of vocal folds. The vocal folds are at the junction of 4th and 6th arches. Hence, the sensory innervation of mucosa of larynx above the vocal folds is from internal laryngeal branch of vagus (4th arch) whereas that part below the vocal folds is from recurrent laryngeal branch of vagus (6th arch) (Fig. 14.49C).

Clinical correlation

Anomalies of larynx

- ***Laryngocele:*** In this condition, the laryngeal saccule is abnormally large. It may extend beyond the larynx proper, and may even form a swelling in the neck.
- ***Congenital stenosis or atresia:*** There may be stenosis or atresia of the larynx.
- The entire larynx, or part of it (e.g. vocal cords), may be duplicated.
- ***Laryngoptosis:*** The larynx lies low down in the neck. Part of it may be behind the sternum. One or more of the laryngeal cartilages may be absent.

TRACHEA

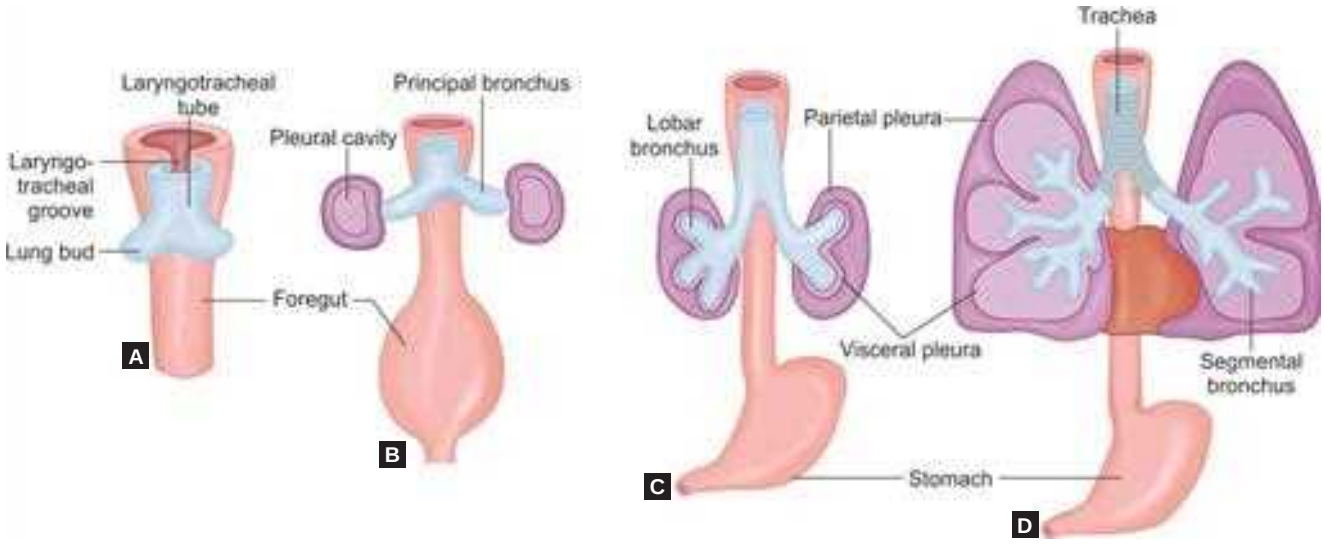
- The trachea develops from the intermediate part of laryngotracheal tube that lies between the points of its bifurcation into *bronchial or lung buds* and the larynx. With the caudocranial extension of tracheoesophageal septum, the trachea elongates. At birth the bifurcation of trachea lies at the level of lower border of 4th thoracic vertebra.
- *Developmental components of trachea*:
  - The endoderm of laryngotracheal diverticulum forms the lining epithelium and glands of trachea.
  - The cartilage, muscle, and connective tissue of trachea develop from splanchnopleuric mesoderm surrounding laryngotracheal tube.

EXTRAPULMONARY BRONCHI

- The laryngotracheal diverticulum divides during 5th week of IUL to form the *right and left principal bronchi*.
- The right principal bronchus is larger than the left and is in line with the trachea. The left division comes to lie more transversely (Fig. 14.48B).

INTRAPULMONARY BRONCHI AND LUNGS

- *Formation of pleural cavity and pleura:*
  - With the subsequent growth of the respiratory diverticulum in caudal and lateral direction, the lung buds come in contact with the respective *pericardioperitoneal canal* and bulges into it (Figs 14.47, 14.48B and C and 14.50).
  - The pericardioperitoneal canals are narrow, and lie on either side of the foregut. The canals are filled with gradually expanding bronchial/lung buds (Fig. 14.51).



Figs 14.48A to D: Respiratory diverticulum growth and development: (A) Bifid respiratory diverticulum to form the right and left bronchial/lung buds. (B) Formation of principal bronchi and contact of lung buds with pleural cavity; (C) Formation of lobar bronchi and expanding lung within pleural cavity; (D) Formation of segmental bronchi

- *Pleuropericardial* and *pleuroperitoneal folds* separate the pericardioperitoneal canals from the pericardial and peritoneal cavities, respectively. This results in formation of primitive *pleural cavity* from that part of pleuropericardial canal lying in contact with the dividing and expanding lung bud (Fig. 14.51).
- The splanchnopleuric layer of intraembryonic mesoderm in contact with the dividing and expanding lung bud becomes the *visceral pleura* and the somatopleuric layer of intraembryonic mesoderm covering the inner aspect of body wall forms the *parietal pleura*. The space between the parietal and visceral pleura forms the *pleural cavity* (Figs 14.48C and D).

• *Subdivisions of intrapulmonary bronchi*:
- When it comes in contact with the developing pericardioperitoneal canal (pleural cavity) the right and left principal bronchi subdivide into secondary/*lobar bronchi* (Figs 14.48C and 14.52).
  - The left principal bronchus shows two subdivisions (upper and lower) that represent the two lobar bronchi of the left lung.
  - The right division divides into three lobar bronchi (superior, middle, and inferior) of right lung.
- As the pleura lines the surface of each lobe separately, the lobes come to be separated by fissures (Figs 14.48C and D and 14.52).
- The substance of the lung is formed by further subdivisions of the lobar bronchi.
  - Each lobar bronchus further subdivides to form *segmental bronchi* in the 7th week of IUL.
- Each segmental bronchus and its surrounding mesenchyme constitute the *bronchopulmonary segment*. Each lung contains 10 bronchopulmonary segments (Figs 14.48D and 14.52).

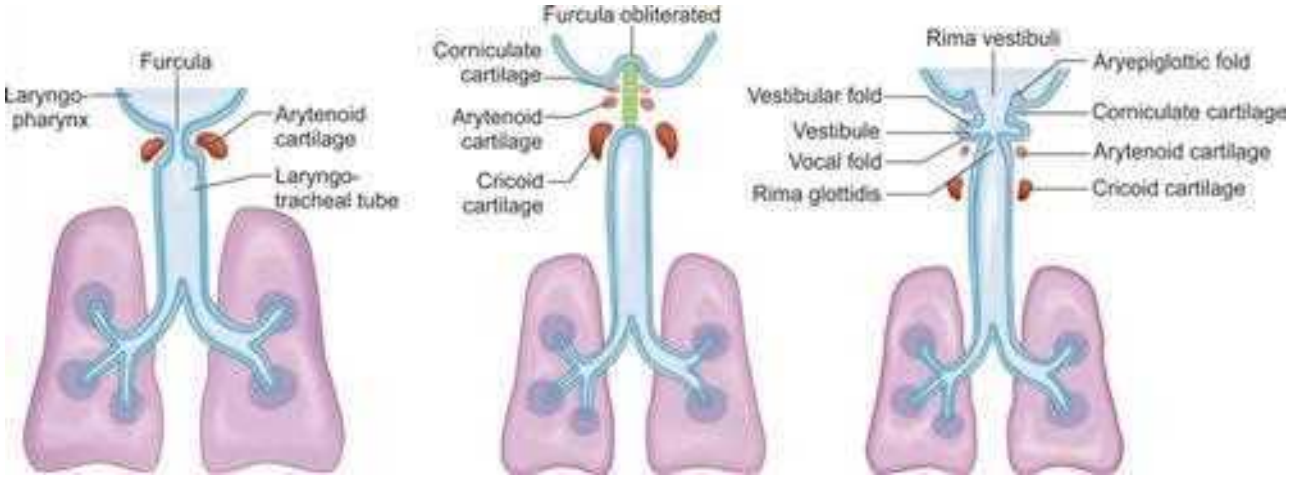


Fig. 14.49: Development of inlet and cartilages of larynx from 4th and 6th pharyngeal arches

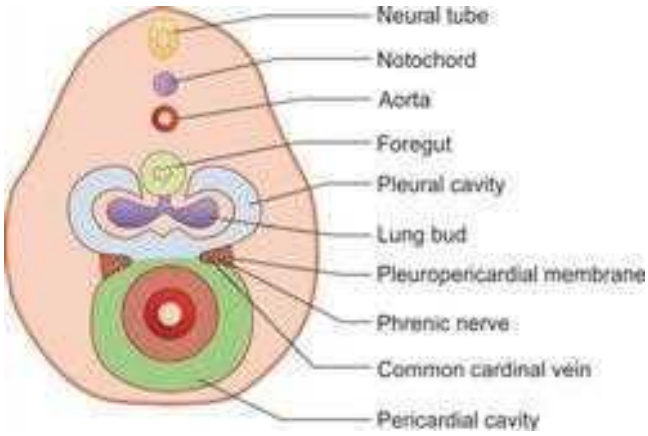


Fig. 14.50: Invagination of lung bud into the pleural cavity. Formation of pleuropericardial membrane and the location of phrenic nerve and common cardinal vein in it

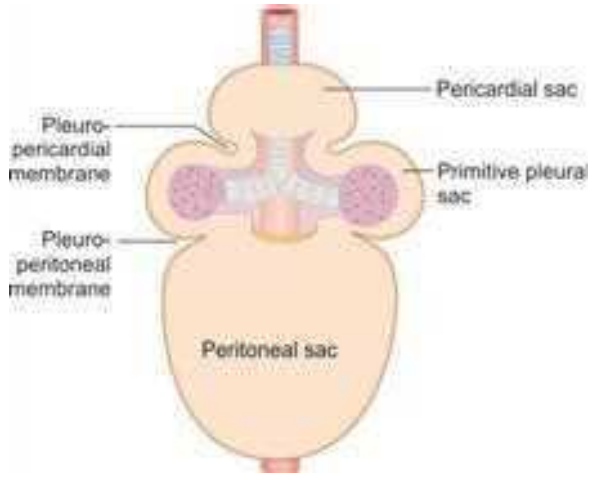


Fig. 14.51: The three coelomic cavities with pleuropericardial and pleuroperitoneal membranes separating them

- The total number of divisions of each main bronchus is about 17 by 7th month of IUL, and six more after birth to attain final shape of adult lung.
- Divisions and subdivisions of each segmental bronchus form the *bronchioles*, *terminal bronchioles, respiratory bronchioles, alveolar ducts and alveolar sacs, and alveoli*. After the establishment of the bronchial tree, alveoli are formed by expansion of the terminal parts of the tree.

Clinical correlation

Anomalies of the trachea

- ***Agenesis of trachea:*** Very rarely trachea may be absent. The bronchi to the lungs may arise from the blind bifurcation (Fig. 14.53B) or from the esophagus (Fig. 14.53C).
- ***Tracheoesophageal fistula (TEF):*** There is abnormal communication between esophagus and trachea. This is associated with esophageal atresia. This occurs due to defective formation of tracheoesophageal septum, resulting in communication between trachea and esophagus. The various types of TEF are:
  - Atresia of distal esophagus with communication between proximal esophagus and trachea (Fig. 14.53D)
  - Both proximal and distal parts of esophagus connected to trachea by a single passage (Fig. 14.53E).
- A ***diverticulum*** may arise from the trachea.
- ***Accessory/displaced bronchi*** may arise from the trachea above its bifurcation or even from the esophagus. Such a bronchus:
  - May be blind (Fig. 14.54A).
  - May supply a mass of lung tissue (accessory lobe) which is not a normal part of the lungs (Fig. 14.54B).
  - May replace a normal bronchus (e.g. apical) in one of the lungs (Fig. 14.54C).

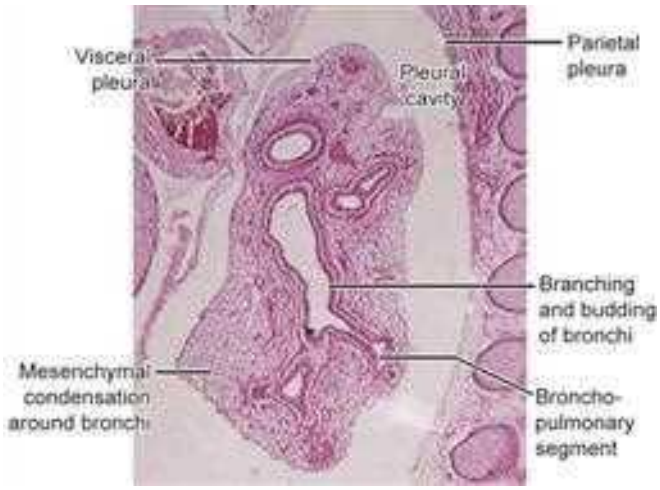


Fig. 14.52: Sagittal section of a developing lung at 6 weeks gestation branching bronchial tree and mesenchymal condensation to form lobes of lung

- *Parenchyma of lung*:
  - The parts of the lung parenchyma, developing from the endoderm of lobar bronchi, are separated from one another by s mesoderm.
  - The endoderm of respiratory diverticulum forms the lining epithelium of the bronchial tree.
  - The mesoderm forms the cartilages, smooth muscle and connective tissue, basis of lung and also gives rise to the pleura. As the pleura lines the surface of each lobe separately, the lobes come to be separated by fissures.

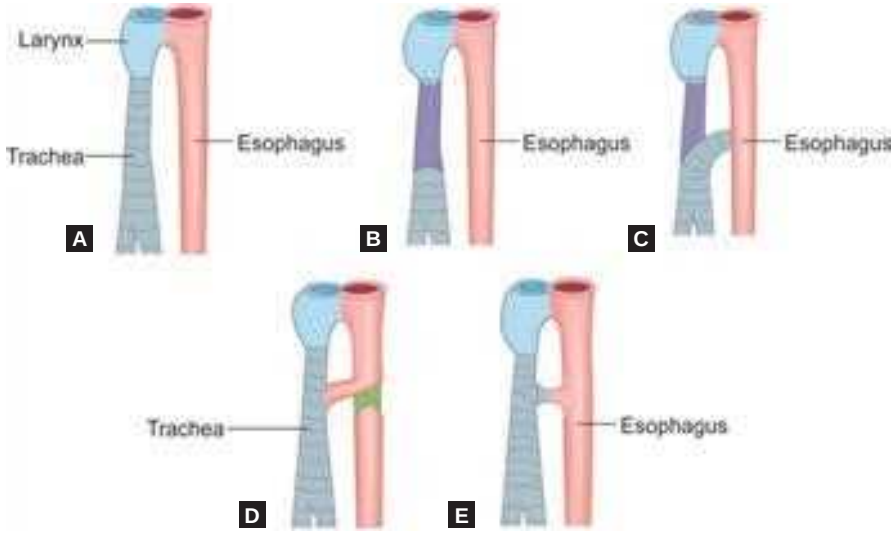
Development of various segments of respiratory system is represented in Flowchart 14.2.

Maturation of lung: There are four stages in the maturation of lung. The ramifications of the bronchial tree pass through these stages. They are represented in Table 14.5 and Figure 14.55.

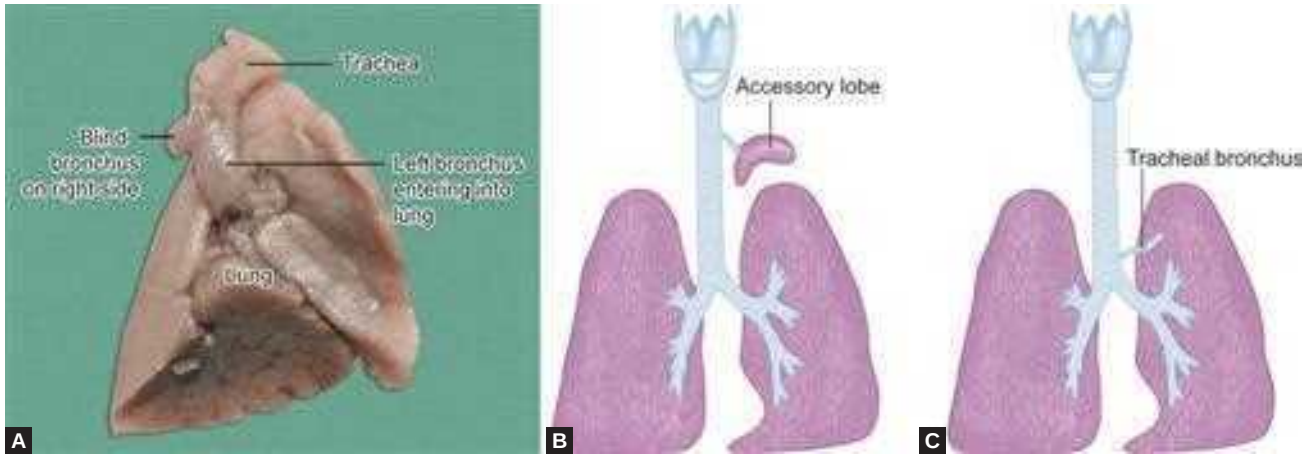
- During fetal life all subdivisions of the bronchial tree are lined by cuboidal epithelium that undergoes changes with the maturation of lung (Table 14.5). Within the respiratory passages some of the cells become specialized to for production of surfactant. This substance is rich in phospholipids and forms a thin layer over alveoli and reduces surface tension.
- *Pulmonary surfactant*: Before birth the respiratory passages are full of fluid derived from amniotic fluid which also contains surfactant. When the new born begins to breathe, the fluid is rapidly absorbed and partly expelled. The surfactant remains as a thin layer lining the alveoli. This prevents collapse of alveoli during expiration. In premature babies, a deficiency of surfactant may cause difficulty in expansion of the lung and can be a cause of death of the baby.
- *Viable age of fetus*: The pulmonary circulation is established early in fetal life. However, most of the blood is at first short circuited through foramen ovale and ductus arteriosus. The amount of blood circulating through the lungs progressively increase, and by the 7th month of IUL the circulation is rich enough to provide adequate oxygen for sustaining life. Hence an infant born, thereafter, is viable (i.e. it can live).

Molecular mechanism in respiratory system development

- Appearance of lung bud is dependent on increase in retinoic acid in adjacent mesoderm
- Transcription factor TBX4 is expressed in the endoderm of foregut that develops into respiratory diverticulum.
- ***Branching of bronchial tree:*** Interaction between the endoderm derived epithelium and splanchnopleuric mesoderm derived mesenchyme (epithelial-mesenchymal interaction) under the influence of fibroblast growth factors.



Figs 14.53A to E: Anomalies of trachea: (A) Normal; (B) Agenesis of trachea—bronchi from blind bifurcation; (C) Agenesis of trachea—bronchi from esophagus; (D) Atresia of distal esophagus—communication between proximal esophagus and trachea; (E) Proximal and distal esophagus communicating with trachea by a single passage



Figs 14.54A to C: Varieties of tracheal bronchi: (A) Blind bronchus; (B) Supplying accessory lobe; (C) Replacing apical bronchus

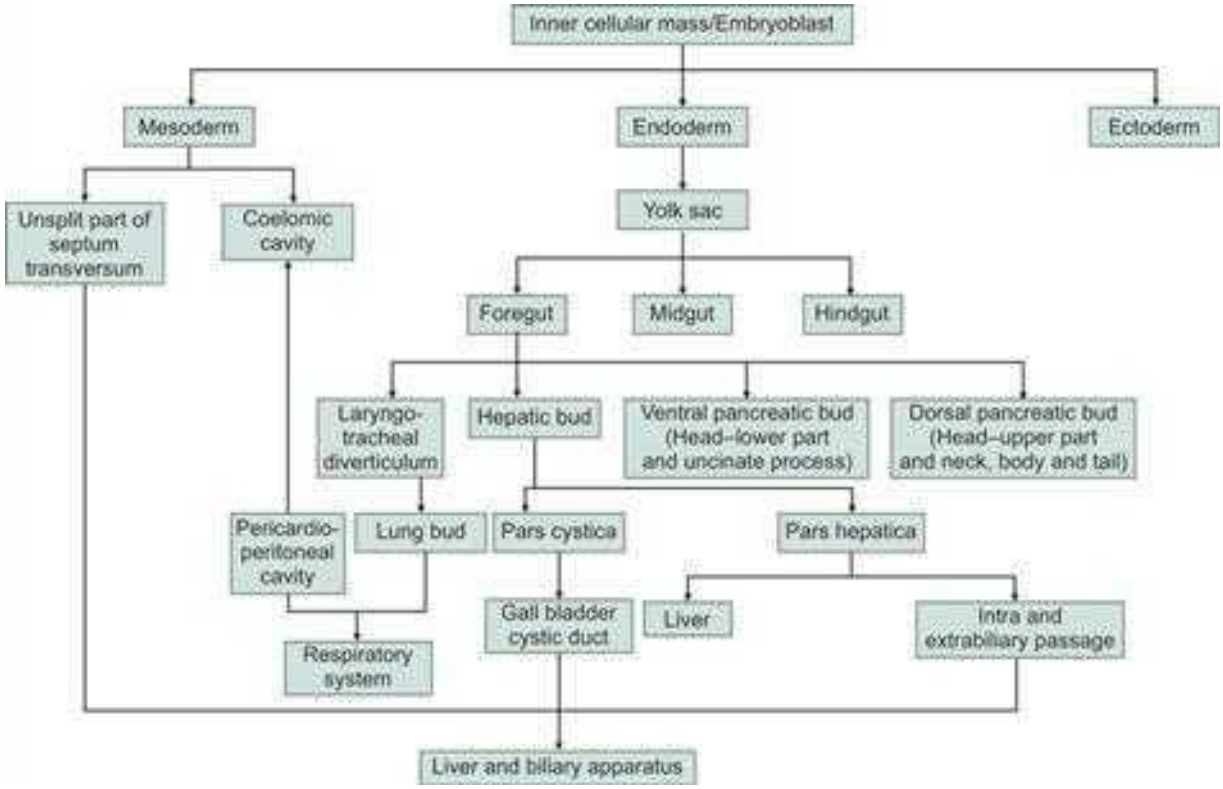
- ***Adult position of lungs:*** With the branching of bronchial tree and expansion of terminal parts of tree, the lungs assume the adult position and bifurcation of trachea is fixed at 4th thoracic vertebra.
- ***Factors influencing lung development:***
  - Adequate thoracic space facilitating lung growth
  - Fetal respiratory movements
  - Adequate amniotic fluid volume.
- ***Developmental relationship between lungs and kidneys:*** Urine excreted from fetal kidneys maintains the amniotic fluid volume which is essential for normal fetal lung development. In renal agenesis reduced bronchial breathing results in pulmonary hypoplasia.

Clinical correlation

***Anomalies of the lungs*:**

- ***Respiratory distress syndrome (RDS) or hyaline membrane disease (HMD):*** Insufficient production of surfactant leading to collapse of lung in expiration. This requires prenatal treatment of mothers with glucocorticoids and use of artificial surfactants in the premature new born.
- ***Agenesis and hypoplasia:*** The whole of one lung, or one of its lobes (and associated bronchi), may fail to develop, or may remain underdeveloped.

Flowchart 14.1: Overview of development of liver, biliary apparatus and pancreas zygote



- ***Abnormalities of lobes:***
  - *Absence of fissures* that is normally present leads to a reduction in the number of lobes. Absence of the transverse fissure of the right lung results in a right lung with only two lobes (Fig. 14.56A).
  - *Presence of abnormal fissures*:
    - A transverse fissure may be present on the left side with the result that the left lung has three lobes (Fig. 14.56B).
    - The medial basal segment (cardiac lobe) of the left lung may be separated by a fissure from the rest of the lower lobe (Fig. 14.56D).
    - The superior segment of the lower lobe may be similarly separated (Fig. 14.56C).
    - A part of the upper lobe of the right lung may come to lie medial to the azygos vein. This part is called the azygos lobe (Fig. 14.57). In this condition the azygos vein is suspended from the wall of the thorax by a fold of parietal pleura (mesoazygos).
- ***Accessory lobes*** are usually connected to bronchi that are not part of the normal bronchial tree. Such bronchi may arise from the:
  - Trachea above its bifurcation (upper accessory lobe) (Fig. 14.18B).
  - Esophagus (lower accessory lobe, Fig. 14.22). Occasionally, the lobe may not have any bronchus.
- ***Sequestration of lung tissue:*** An area of embryonic lung tissue may separate from the tracheobronchial tree (sequestration = separation). Such tissue may form a complete lobe (lobar sequestration), which may have an independent pleural covering. In other cases the sequestrated tissue may lie within a lobe (intralobar sequestration). The sequestrated lung tissue derives its blood supply from an abnormal branch of the aorta. The condition is most frequently seen in the lower lobe of the left lung.
- ***Lung hernia:*** Part of a lung may herniate: (a) through the inlet of the thorax, (b) through a defect in the thoracic wall, (c) into the mediastinum, or (d) into the opposite pleural cavity.
- ***Ectopic lung:*** Either the entire lung or a lobe of it arises from trachea or esophagus. This is due to the development of respiratory buds from the foregut in addition to the main respiratory system or in place of normal lung (Fig. 14.58).
- ***Congenital cysts of lung:*** Due to dilatation of terminal bronchi. These can be multiple and give honeycomb appearance of lung on X-ray.

Flowchart 14.2: Development of various segments of respiratory system

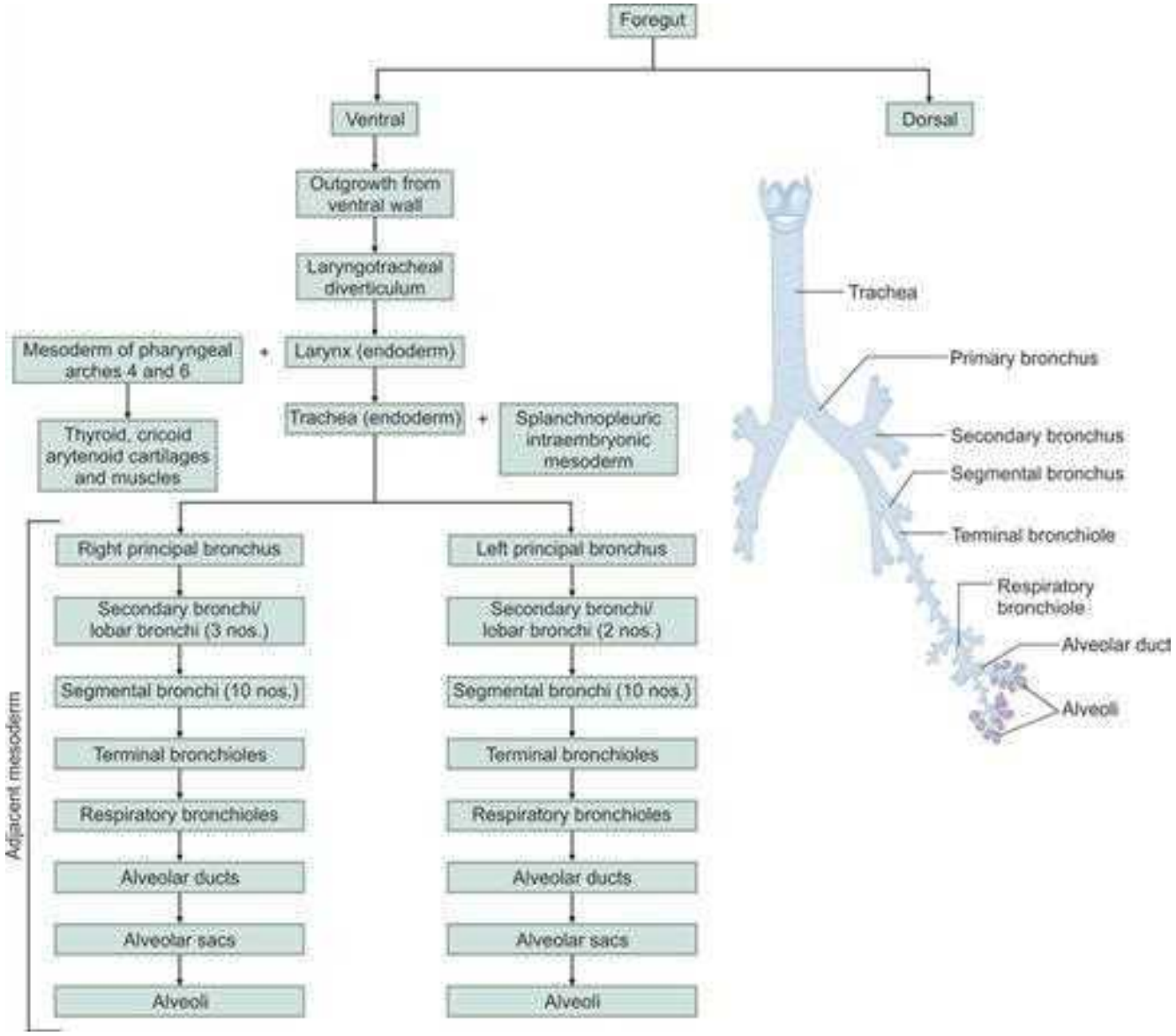


TABLE 14.5: Stages in the maturation of lung—morphological changes in bronchial tree and its functional importance

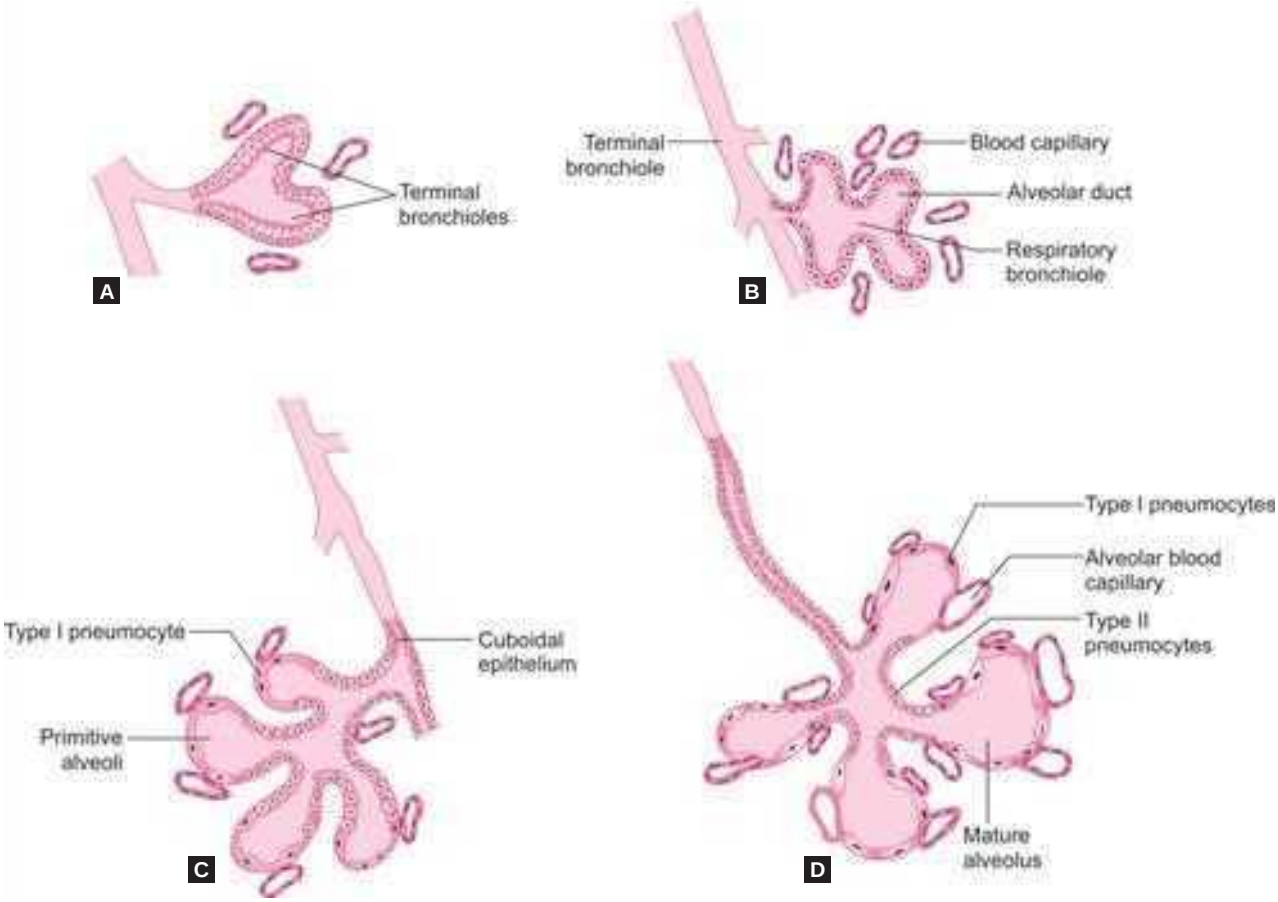
Stage of lung and age of embryo/fetus/new born	*Changes in the morphology of bronchial tree and its functional importance*
Pseudoglandular stage 6–16 weeks (2nd–4th month of IUL) (Fig. 14.55A)	• The lung is similar to exocrine gland giving the appearance of tubuloacinar mucus gland. • Bronchi divide up to terminal bronchiole. Respiratory portion of lung not developed. • Proximal part of bronchial tree is lined by columnar epithelium and the distal part by cuboidal epithelium. • Adjacent mesenchymal cells around the epithelium differentiate into smooth muscle, cartilage and connective tissue cells. • Respiration is not possible; hence, premature fetuses born at this stage cannot survive.
Canalicular stage 17–26 weeks (5th–7th month of IUL) (Fig. 14.55B)	• Three generations of branching of bronchial tree occurs. • Respiratory bronchioles, alveolar ducts and few alveolar sacs are formed. • Vascularization of lung tissue due to increase in capillary network in relation to distal air spaces, i.e. future alveoli. • Some of the cuboidal respiratory epithelial cells change into simple squamous type I pneumocytes. Remaining cuboidal cells are specialized and form type II pneumocytes that act as *stem cells* and produce the surfactant. • Fetus born at this stage can survive if intensive care is provided.

Contd...

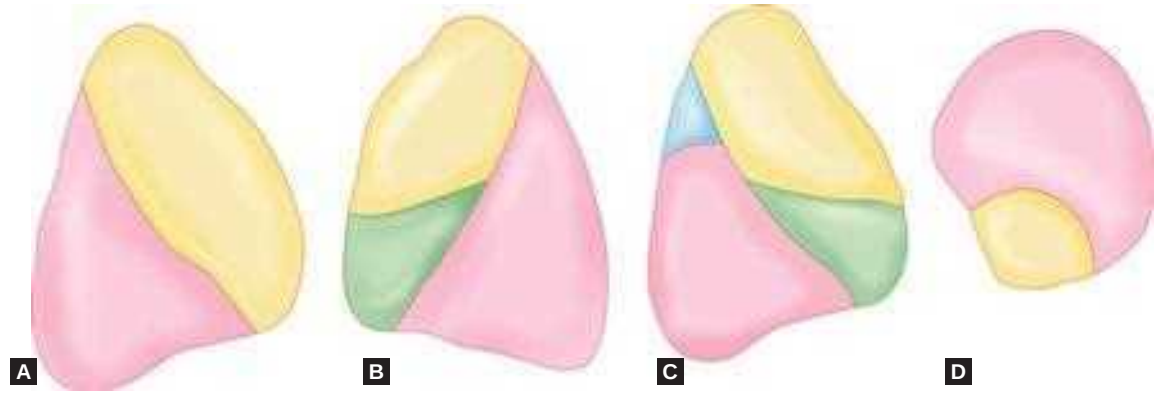
Contd...

Stage of lung and age of embryo/fetus/new born	***Changes in the morphology of bronchial tree and its functional importance***
Saccular stage 27 weeks to full term (7th month of IUL to delivery) (Fig. 14.55C)	• More number of primitive alveoli develops. • Change in lining epithelium of alveolar sacs to type I pneumocytes with change of cuboidal cells lining bronchioles into thin, flat cells. • Gas exchange between blood and air possible in primitive alveoli • Type II cells secrete phospholipid rich surfactant that lowers surface tension and prevent collapse of alveoli during expiration. • Intimate contact between epithelium of alveolar sac and capillaries (*blood-air barrier*) develops to permit gaseous exchange. • The fetus born at this stage is viable.
Alveolar stage From birth to 8 years of postnatal life (Fig. 14.55D)	• Division of respiratory bronchioles forming alveolar ducts and definitive alveoli takes place. • Definitive alveoli will be formed and their number is more. • The amount of surfactant production increases. • Rapid exchange of gases between alveolar epithelium and capillary endothelium occurs.

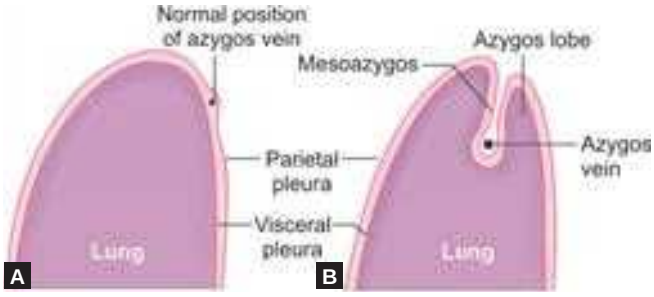
Abbreviation: IUL, intrauterine life



Figs 14.55A to D: Stages in maturation of lung: (A) Pseudoglandular stage; (B) Canalicular stage; (C) Saccular stage; (D) Alveolar stage



Figs 14.56A to D: Abnormal lobes of lungs: (A) Right lung with only two lobes; (B) Left lung with three lobes; (C) Apical segment of lower lobe is separate; (D) Separate medial basal segment



Figs 14.57A and B: (A) Normal relationship of azygos vein to the lung; (B) Azygos lobe of lung

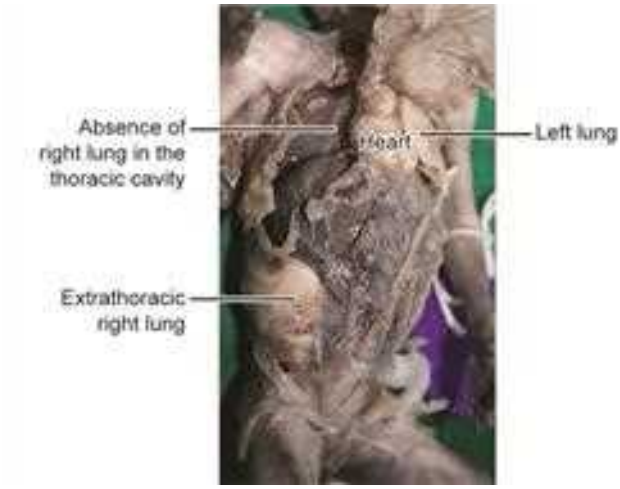


Fig. 14.58: Fetus with extrathoracic lung in abdominal cavity

EMBRYOLOGICAL BASIS FOR CLINICAL CONDITIONS OR ANATOMICAL OBSERVATIONS

Case Scenario 1

A baby was born at 26th week of gestation and was having difficulty in breathing. Can you explain the embryological basis for this condition and how this baby can be treated to avoid mortality?

- This condition is called neonatal respiratory distress syndrome (RDS) or hyaline membrane disease (HMD).
- The alveoli of a baby born before 28 weeks of gestation (premature birth/preterm delivery) are not fully developed and cannot produce sufficient phospholipid rich fluid called surfactant.
- The pulmonary surfactant is produced by type II alveolar epithelial cells from 26th week to birth with a gradual increase in quantity with increasing maturity of alveoli (Fig. 14.55)
- The surfactant lowers surface tension at air-blood interface and prevents collapse of alveoli during expiration. Insufficient production of surfactant raises the air-blood surface membrane tension leading to collapse of alveoli during expiration and thus difficulty in breathing.
- The mortality rate is nearly 20% for these infants if left untreated. The treatment requires a specialized neonatal

intensive care for breathing support from a ventilator and artificial surfactants.

Case Scenario 2

At autopsy of a dead aborted fetus there was only trachea, left bronchus and left lung in the thoracic cavity. Right bronchus was seen as a blind diverticulum. Right lung was found in the abdominal cavity with the right bronchus extending from esophagus. What is the embryological basis for this condition?

- This is a case of an extrathoracic lung or ectopic lung. It is a congenital abnormality in the development of lung (Fig. 14.58).
- The laryngotracheal diverticulum originating from the ventral wall of developing foregut and divides during 5th week of IUL to form the *right and left principal bronchi.*
- The principal bronchi when they come in contact with the developing pericardioperitoneal canals (pleural cavity) each undergoes subdivisions to form intrapulmonary bronchial tree.
- The contact between the endodermal respiratory diverticulum and its cartilaginous, muscular and connective tissue components of splanchnopleuric mesodermal origin is under the influence of retinoic acid produced by the adjacent mesoderm and its upregulation of TBX4 transcription factor.
- In the present case due to the reduced production of retinoic acid in the mesoderm of thoracic region adjacent to right principal bronchus prevented the contact, growth and expansion of right principal bronchus. Hence, right principal bronchus ended as a blind diverticulum.
- Additional respiratory diverticulum originated from the esophagus part of foregut and has come into contact with the retinoic acid rich splanchnic mesoderm in the right side of abdominal cavity and has grown and expanded in to the lung. Hence, it resulted in extrathoracic lung of esophageal origin.

Case Scenario 3

A mother brought her newborn baby to the pediatrician with a complaint of feeding difficulty as the baby is not swallowing the milk and when tried to feed there were episodes of choking. The pediatrician attempted to introduce a nasogastric tube but could not. He ordered for an X-ray chest which showed absence of stomach bubble. When enquired about antenatal history it was found about to be a case of polyhydramnios.

- It is a case of esophageal atresia (Fig. 14.53)
- Because of esophageal atresia, during intrauterine life the fetus could not swallow amniotic fluid and it resulted *in polyhydramnios*.
- Because of esophageal atresia the new born is not able to swallow the milk and it resulted in choking and inability to pass the nasogastric tube.
- Absence of stomach bubble on X-ray suggests the agenesis of esophagus.
- Treatment is to reconnect the two ends of esophagus.

Case Scenario 4

What is the embryological basis for difference in innervation of mucosa and muscles of larynx by different nerves?

- The laryngeal muscles are derived from branchial mesoderm as indicated by their nerve supply. All intrinsic muscles of larynx are supplied by recurrent laryngeal nerve (nerve of 6th arch) except cricothyroid which is supplied by external laryngeal branch of superior laryngeal nerve (nerve of 4th arch).
- The vocal folds are formed at the junction of 4th and 6th arches. Hence, the sensory innervation of mucosa of larynx above the vocal folds is from internal laryngeal branch of superior laryngeal nerve vagus (4th arch) whereas that part below the vocal folds is from recurrent laryngeal branch of vagus (6th arch) (Fig. 14.49C).

REVIEW QUESTIONS

1. Explain development of liver.
2. Write a short note on septum transversum.
3. Explain development of extrahepatic biliary apparatus.
4. Explain development of pancreas.
5. Explain development of spleen.
6. Explain development of lesser sac.
7. Explain development of peritoneal folds.
8. Explain development of diaphragm.
9. Explain development of larynx.
10. Describe the stages in the maturation of lung.

Chapter 15

Cardiovascular System

HIGHLIGHTS

The *heart* develops from splanchnopleuric mesoderm related to that part of the intraembryonic coelom that forms the pericardial cavity. This mesoderm is the *cardiogenic area*.

- Two *endothelial heart tubes* (right and left) appear and fuse to form one tube. This tube has a venous end, and an arterial end.
- A series of dilatations appear on this tube. These are (1) *bulbus cordis,* (2) *ventricle,* (3) *atrium* and (4) *sinus venosus*.
- Further subdivisions are named as follows. The bulbus cordis consists of a proximal one-third (which is dilated), a middle one-third called the *conus*, and a distal one-third called the *truncus arteriosus*. The narrow part connecting atrium and ventricle is the *AV canal*. The sinus venosus has right and left horns.
- The right and left atria of the heart are formed by partition of the primitive atrium. This partition is formed by the *septum primum* and the *septum secundum*. A valvular passage, the *foramen ovale*, is present between these two septa. It allows flow of blood from right atrium to left atrium.
- The dilated proximal one-third of the bulbus cordis, the conus, and the primitive ventricle unite to form one chamber. This is partitioned to form right and left ventricles. This partition is made up of the following: (1) *Interventricular septum* that grows upward from the floor of the primitive ventricle; (2) A *bulbar septum* that divides the conus into two parts; (3) The gap left between these two is filled by proliferation of AV cushions that are formed in the AV canal.
- The truncus arteriosus is continuous with the *aortic sac*. This sac has right and left horns. Each horn is continuous with six *pharyngeal* (or *aortic*) *arch arteries*. These arteries join the dorsal aorta (right or left). The first, second and fifth arch arteries disappear. The caudal parts of the right and left dorsal aortae fuse to form one median vessel.
- The *ascending aorta* and *pulmonary trunk* are formed from the truncus arteriosus.
- The *arch of aorta* is formed by the aortic sac, its left horn, and the left fourth arch artery.
- The *descending aorta* is formed partly from the left dorsal aorta, and partly from the fused median vessel.
- The *brachiocephalic artery* is formed from the right horn of the aortic sac.
- The *common carotid artery* is derived from part of the third arch artery.
- The *pulmonary artery* is derived from the sixth arch artery.
- The *arteries to the gut* are formed from ventral splanchnic branches of the dorsal aorta
- The *renal, suprarenal* and *gonadal arteries* are formed from lateral splanchnic branches of the dorsal aorta.
- Arteries to the body wall and limbs are derived from dorsolateral (somatic intersegmental) branches of the aorta.
- The *left subclavian artery* is derived from part of the seventh cervical intersegmental artery.
- The *right subclavian artery* is formed from seventh cervical intersegmental artery and partly from the right fourth arch artery.
- The *portal vein* is derived from right and left vitelline veins and anastomoses between them.
- The *superior vena cava* is derived from part of the right anterior cardinal vein, and from the right common cardinal vein.
- The *inferior vena cava* receives contributions from several veins (and anastomoses between them). These are the right posterior cardinal vein, the right subcardinal vein, the right supracardinal vein, and the right hepatocardiac channel.

PART 1: HEART

INTRODUCTION

Need for transport system to maintain nutrition and development of embryo: The need for a transport system for the embryo is its changing demands at different stages of development, the source and adequacy of available nutritional supply.

Nutrition of the Embryo at Various Stages of Development

Before implantation (1st week): During the zygote and morula stage, as the conceptus is small the meagre store of deutoplasm available with the ovum provides the nutrition. During blastocyst stage, it receives nutrients by simple diffusion from the uterine gland secretions. The waste products diffuse out in the opposite direction.

During implantation (2nd week): The implanting blastocyst as it is eroding the endometrial wall there is breaking down of endometrial cells in the path of blastocyst. The breakdown products are absorbed by diffusion.

After implantation (3rd week): The nutrition is provided by establishment of uteroplacental circulation where the lacunar spaces in syncytiotrophoblast facilitate diffusion.

The transport of nutrition from 1st week to 3rd week is by an indirect method of diffusion through amnion, extraembryonic coelom, primary yolk sac and secondary yolk sac. But this nutrition is not sufficient. Because of increased functional demands of growing embryo, development of a separate blood vascular system is necessary.

COMPONENTS OF BLOOD VASCULAR SYSTEM

- Development of separate blood vascular system starts at the beginning of the 3rd week. It is by formation of blood cells and blood vessels in the extraembryonic mesoderm of yolk sac, connecting stalk and chorion. In the intraembryonic mesoderm, they develop 2 days later.
- The internal surfaces of the heart and of all blood vessels are lined by a layer of flattened cells called *endothelium*. The endothelium is supported, on the outside, by varying amount of muscle, and connective tissue. All the above mentioned components of the heart and blood vessels are of mesodermal origin.

FORMATION OF BLOOD CELLS AND VESSELS

The first blood cells are formed by induction of mesodermal cells to hemangioblasts (common precursor for blood cells and vessels). Later definitive hemopoietic stem cells develop in the bone marrow. The blood vessels develop by two processes, i.e. vasculogenesis and angiogenesis.

Vasculogenesis (Fig. 15.1)

- It is the process of differentiation of angioblasts into endothelial cells and formation of primitive vascular network.
- It is development of vessels from the aggregations of blood cells, i.e. blood islands.

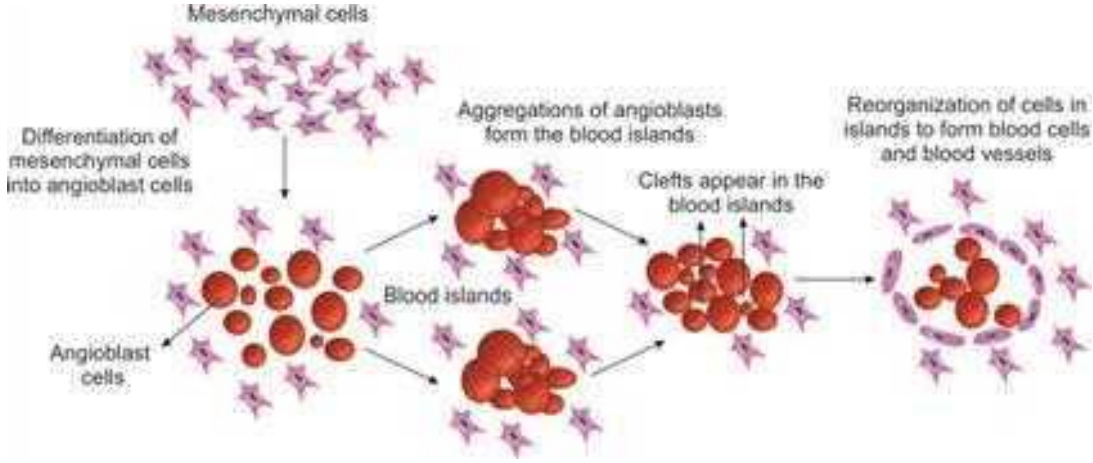


Fig. 15.1: Vasculogenesis

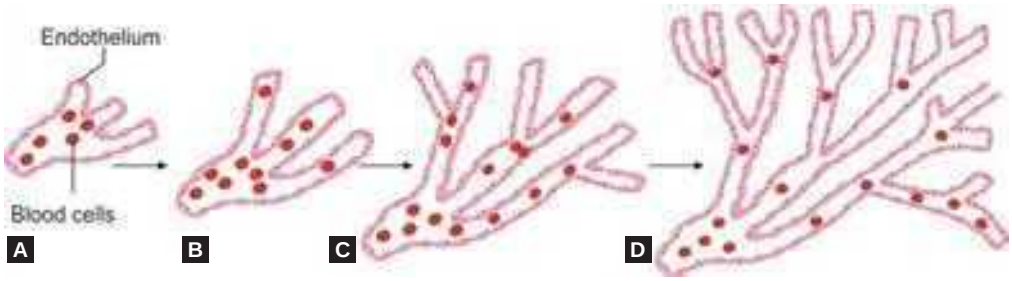


Fig. 15.2: Angiogenesis-stages in formation of blood vessels

- The mesenchymal cells differentiate into angioblast cells.
- Aggregations of angioblasts form the blood islands.
- Later, clefts appear in the blood islands.
- The clefts communicate with one another forming capillary plexus.
- Reorganization of cells in islands leads to the formation of blood cells and blood vessels.

Angiogenesis (Fig. 15.2)

- It is the process of sprouting of new vessels from existing ones.
- Very early in the life of the embryo mesenchyme differentiates, over the yolk sac, in the connecting stalk, and in the body of the embryo itself, to form small masses of *angioblastic tissue*. This angioblastic tissue gives rise to *endothelium* and also to *blood cells*.
- The first blood vessels are derived from this endothelium. The vessels rapidly proliferate in number and become interconnected to form a vascular system.
- Soon thereafter, a primitive heart begins to pump blood through this network of vessels with the result that nutrition from the placenta and yolk sac can be made available to the growing embryo.
- Angiogenesis begins first in the yolk sac wall during 3rd week. Erythrocytes produced in the yolk sac have nuclei.
- Inside the embryo it begins in 5th week. Erythrocytes produced in the embryo do not have nuclei.
- Hematopoiesis inside in the embryo occurs first in the liver, then later in the spleen, thymus, and bone marrow.

Molecular regulation of angiogenesis and vasculogenesis

Molecular regularization of vasculogenesis is by fibroblast growth factor 2 (FGF2) and vasculoendothelial growth factor (VEGF). Sprouting of new vessels from existing one is by VEGF and maturation and modeling of vasculature is by platelet-derived growth factor (PDGF) and transforming growth factor-B (TGF-B).

EXTRAEMBRYONIC BLOOD VASCULAR SYSTEM

In the early part of 3rd week of development, the extraembryonic blood cells and blood vessels develop in the following regions by a process similar to the development of blood vessels in general described above.

- Wall of yolk sac
- Connecting stalk
- Chorion.

Those developing in the wall of yolk sac become the *vitelline* vessels. Those developing in the connecting stalk and chorion become the *umbilical* vessels (Fig. 15.3).

INTRAEMBRYONIC BLOOD VASCULAR SYSTEM

In the later part of 3rd week of development, the intraembryonic blood cells and blood vessels are formed

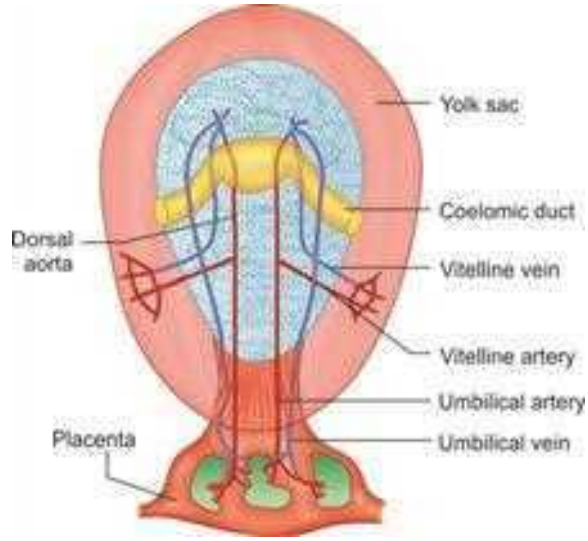


Fig. 15.3: Conceptus showing intra- and extraembryonic blood vessels

in the intraembryonic mesoderm and establish connection with the extraembryonic vessels. Before the formation of embryonic folds, two longitudinal vessels (*dorsal aortae*) develop in the flattened embryonic disc on either side of notochord and along the dorsal wall of yolk sac (Figs 15.3 and 15.4).

The heart (like all blood vessels) is mesodermal in origin. It is formed from splanchnopleuric mesoderm lying immediately cranial to the prochordal plate. This mesoderm constitutes the cardiogenic area. The *primitive heart tubes* develop in the cardiogenic area.

The heart is, therefore, the first organ of the body to start functioning. We have seen that the pericardial cavity is formed from the cranial, midline, part of the intraembryonic coelom (Figs 5.8 and 5.10). With the formation of the coelom, the intraembryonic mesoderm of the region splits into a somatopleuric layer adjoining the ectoderm (in roof of pericardial cavity), and a splanchnopleuric layer adjoining the endoderm (Fig. 5.8) and forming the floor of the pericardial cavity.

The dorsal aortae at the cephalic end of embryonic disc invade the cardiogenic area and join the primitive heart tubes. At the caudal end of the embryonic disc, the dorsal aortae extend into the connecting stalk as *umbilical arteries*. Some blood vessels sprout from each dorsal aorta into the yolk sac that forms the *vitelline arteries* (Figs 15.3 and 15.4).

The *umbilical veins* develop in relation to somatopleuric layer of intraembryonic coelom. The *vitelline veins* develop in the splanchnopleuric layer of intraembryonic coelom. *Cardinal veins* develop in the body wall of embryo. Umbilical and vitelline veins pass through the septum transversum and join the cranial end of each primitive heart tube. Cardinal veins also join the cranial ends of primitive heart tubes (Figs 15.3 and 15.4).

DEVELOPMENT OF HEART

Cardiac Progenitor Cells and Primary and Secondary Heart Fields

Cardiac progenitor cells appear at the caudal epiblast, lateral to primitive streak during 16th–18th days of development. They migrate cranially in an orderly sequence through the primitive streak into the splanchnopleuric layer of intraembryonic mesoderm where they form a horseshoe-shaped *primary heart field* along the cranial end of embryonic disc rostral to buccopharyngeal membrane and neural folds (Fig. 15.5). A sequence specification of cardiac progenitor cells known as laterality sequencing, i.e. atria, left ventricle and part of right ventricle from lateral to medial is produced by primary heart field.

By 21st day, a secondary *heart field* appears in the splanchnopleuric mesoderm ventral to posterior pharynx (space between internal nares and soft palate). Now, there is change in the laterality sequencing because of spiraling of pulmonary trunk and aorta and their exits from the chambers of heart. Now, they are arranged as part of right ventricle and the outflow tract of heart (conus cordis and truncus arteriosus).

Cardiogenic Area

Pharyngeal endoderm underlying primary heart field induces formation of cardiogenic area where horseshoe-shaped blood islands are formed by angiogenic clusters, which later get canalized to form endothelial lined heart tubes that are surrounded by myoblasts. Bilateral blood islands close to midline or paranotochordal region form the dorsal aortae.

The heart develops from angioblastic tissue that arises from this splanchnopleuric mesoderm, which is,

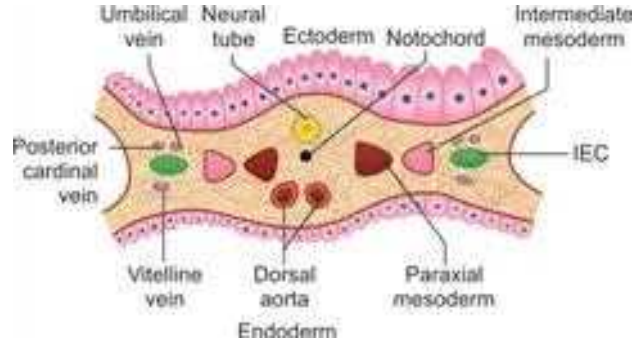


Fig. 15.4: Transverse section of embryonic area showing dorsal aortae, vitelline, umbilical and posterior cardinal veins

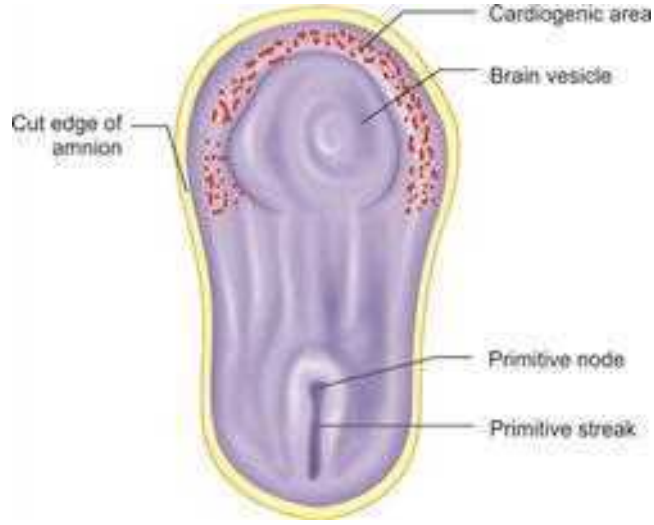


Fig. 15.5: Embryonic disc showing horseshoe-shaped cardiogenic area

therefore, called the cardiogenic area. This area is between the dorsal wall of yolk sac and the floor (splanchnopleuric layer) of pericardial cavity (which is derived from part of the intraembryonic coelom). With the establishment of the head fold, the splanchnopleuric mesoderm and the developing heart come to lie dorsal to the pericardial cavity, and ventral to the foregut (Fig. 5.12).

The endothelial heart tube is derived from the splanchnopleuric mesoderm related to the pericardial cavity (Fig. 15.6A). After formation of the head fold, this tube lies dorsal to the pericardial cavity and ventral to the foregut (Fig. 15.6B). The tube invaginates into the pericardial sac on its dorsal side. As it does so, the splanchnopleuric mesoderm lining the dorsal side of the pericardial cavity proliferates to form a thick layer called the *myoepicardial mantle* (or *epimyocardial mantle*) (Figs 15.6C and D). When the invagination is complete, the myoepicardial mantle completely surrounds the heart tube. It gives rise to the cardiac muscle (myocardium) and also to the visceral layer of pericardium (epicardium). The parietal layer of pericardium is derived from somatopleuric mesoderm.

Molecular basis for laterality sequencing

The molecular basis for laterality sequencing is the accumulation of the signaling molecules the serotonin [5-hydroxytryptamine (5-HT)], nodal and fibroblast growth factor 8 (FGF8) on left side and monoamine oxidase (MAO) on right side. The PITX2 is the master gene for left sidedness.

For a good understanding of the relationship between developing heart tube and the pericardial cavity, students are advised to go through the Figures 5.7 to 5.10.

The heart is at first seen in the form of right and left endothelial heart tubes (Figs 15.7A to E) that soon fuse with each other. The single tube thus formed shows a series of dilatations (Fig. 15.8). These are:

- Bulbus cordis
- Ventricle (we will refer to it as the primitive ventricle)
- Atrium (we will refer to it as the primitive atrium or atrial chamber)
- Sinus venosus.

The sinus venosus and atrium are connected by *sinoatrial orifice*. The atrium and ventricle are in communication through *atrioventricular canal* (*AV canal*).

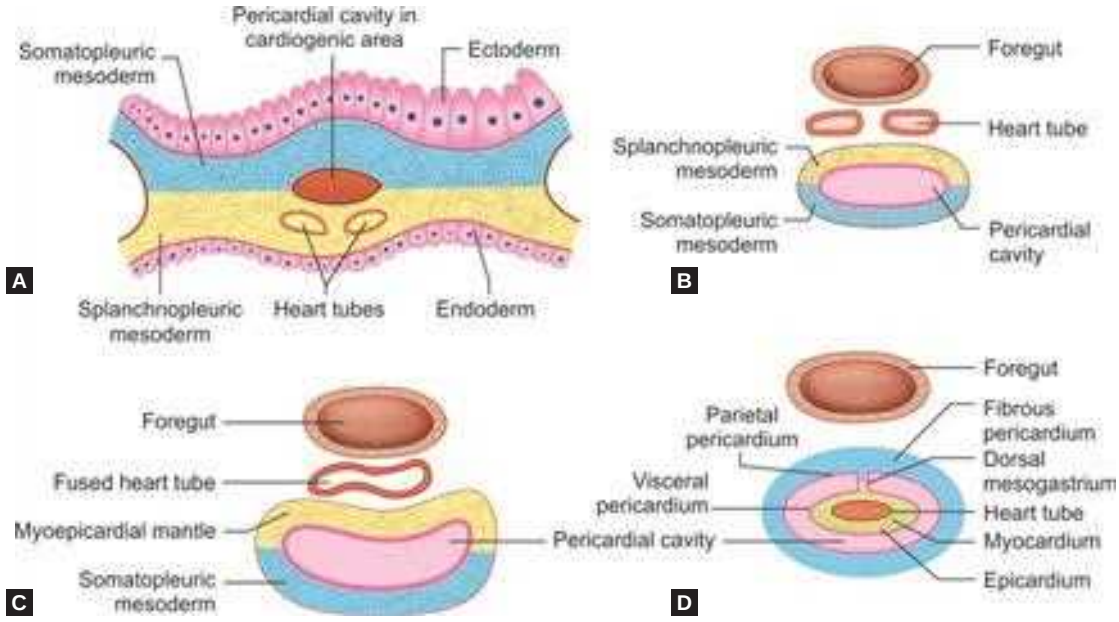
The fate of the various parts of the heart tube is summarized in Figure 15.9. Cardiac septations to form definitive heart occur between 5th and 8th weeks. Simultaneous development of all the chambers takes place though they are considered separately for easy understanding.

DEVELOPMENT OF VARIOUS CHAMBERS OF THE HEART

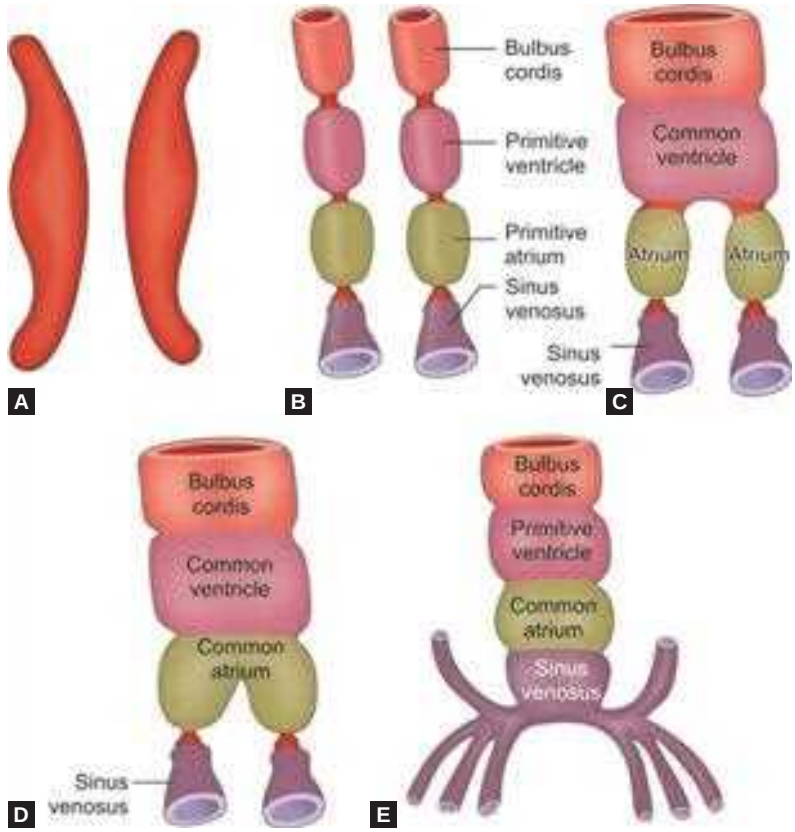
Development of Atria

Sinus Venosus and Its Absorption into the Right Atrium

- *Parts and tributaries*: This is the caudal most (venous end) part of primitive heart tube. It presents a body with two prolongations that are referred to as its *right* and *left horns*. One *vitelline vein* (from the yolk sac), one *umbilical vein* (from the placenta) and one *common*



Figs 15.6A to D: Relationship of heart tubes to pericardial cavity. (A) Before formation of head fold; (B) After formation of head fold; (C and D) Show the process of invagination of the pericardial cavity by the single heart tube



Figs 15.7A to E: (A) Right and left heart tubes; (B to E) Progressive fusion of tubes from cranial to caudal end. Fusion of sinus venosus is partial

cardinal vein/duct of Cuvier (from the body wall) join each horn of the sinus venosus (Fig. 15.8). Initially both the horns of sinus venosus are of equal size. Due to left to right shunts, most of the blood drains to right horn of sinus venosus.

- *Communication with atrium*: The sinus venosus and the primitive atrial chamber are at first connected by a wide opening. It is known as *sinoatrial orifice*. However, they become partially separated by *grooves* that appear on the lateral wall of the heart tube, at the junction of these two chambers (Figs 15.10A to E).
- *Regression of left horn and its tributaries*: The *right groove* remains shallow but the *left* one becomes very deep (Figs 15.11A to C) with the result that the left part of the sinus venosus becomes completely separated from the atrial chamber. Its blood now enters the atrium through the right half of the sinus. Simultaneously, the left horn of the sinus venosus and its tributaries become much reduced in size, and the left horn now appears to be just another tributary of the right half of the sinus venosus (Fig. 15.11C). The left horn becomes part of the *coronary sinus* (Fig. 15.12).
- *Change in size and orientation of sinoatrial orifice*: Initially the sinoatrial orifice is larger in size, transverse in orientation and median in position. Gradually the

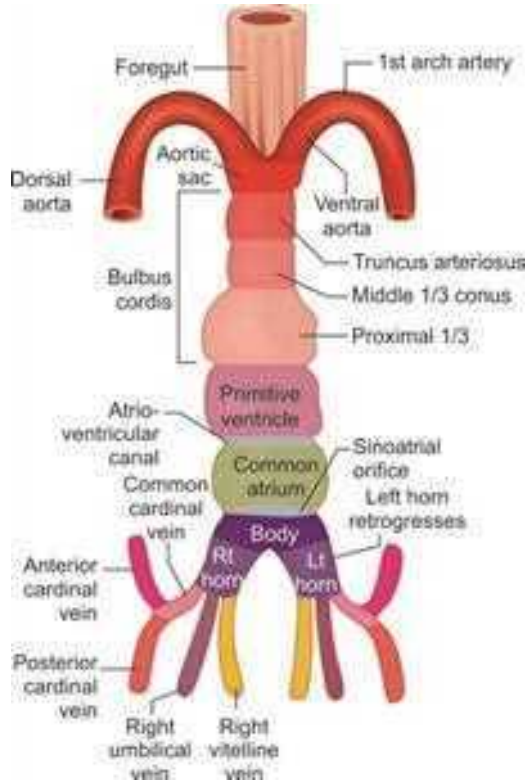


Fig. 15.8: Subdivisions of fused heart tube

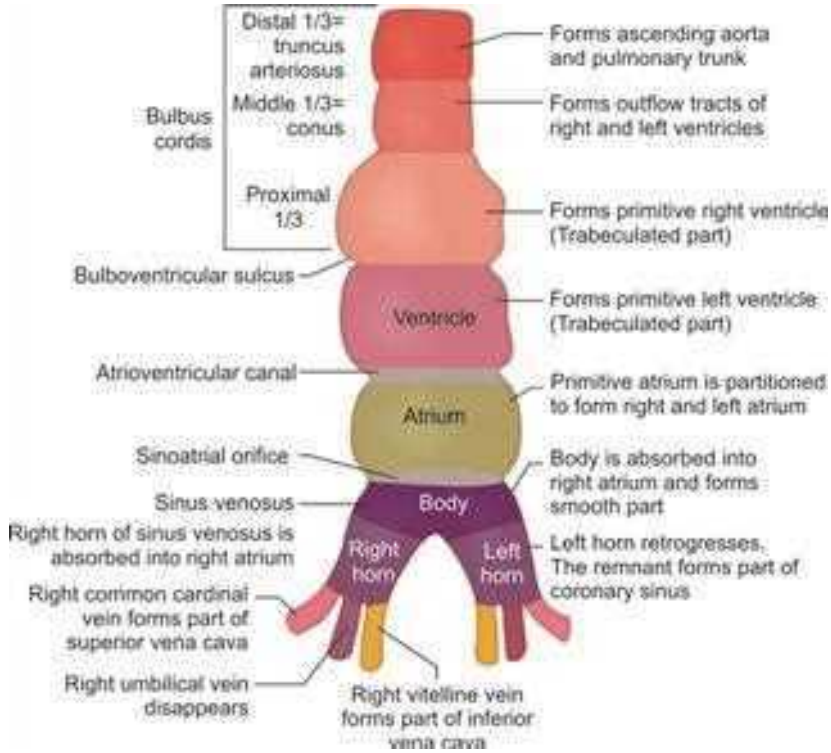
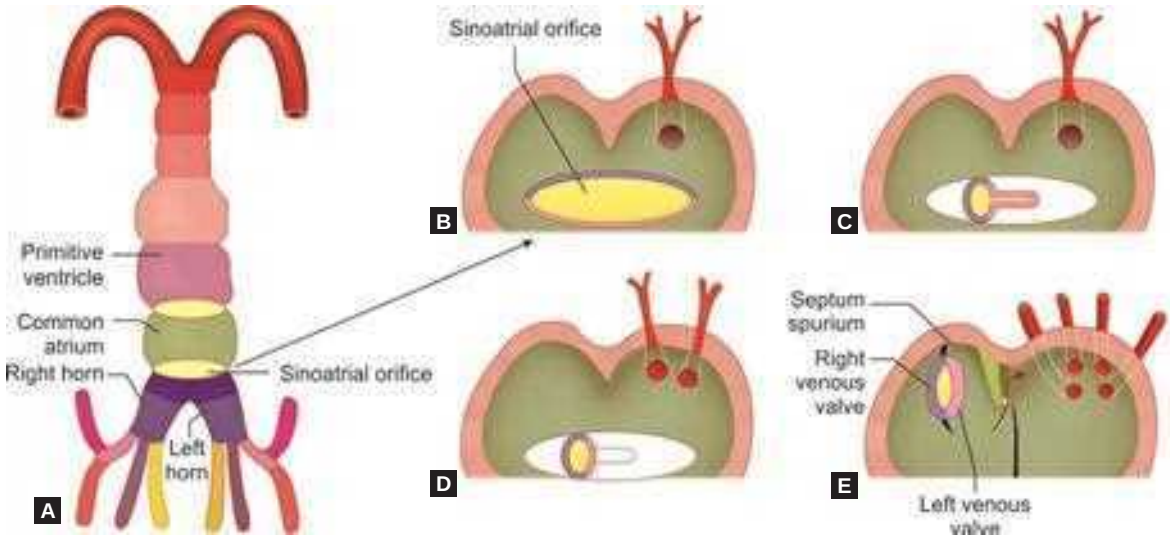


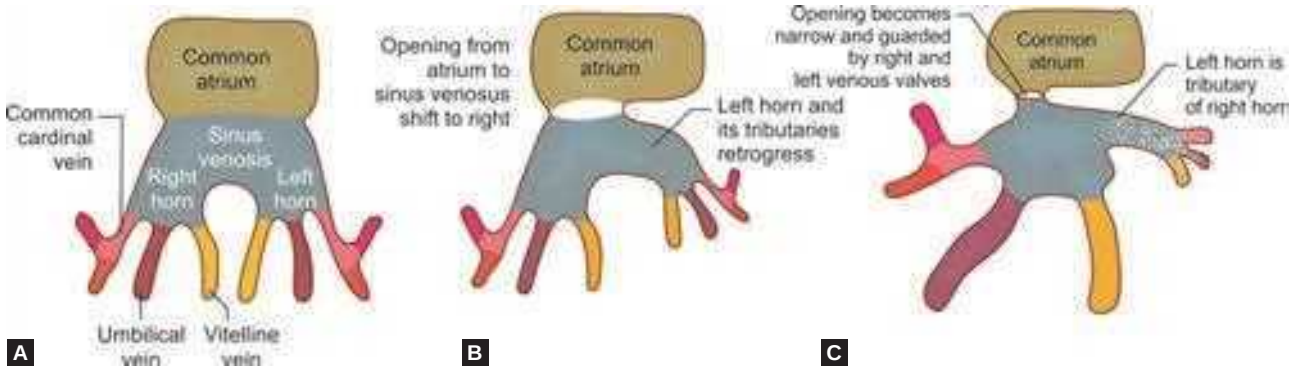
Fig. 15.9: Main subdivisions of the heart tube and their fate



Figs 15.10A to E: (A) Sinoatrial orifice in heart tube. (B to E) Changes in the sinoatrial orifice. Note that firstly, the centrally placed orifice (B) shifts to the right (C). Secondly, the orifice that is at first transversely orientated becomes vertical (D). Dotted lines in (D) indicate the outline of the opening in the previous figure to show how the change occurs. (E) The right and left venous valves guarding sinoatrial orifice

opening becomes narrow and shifts to the right and onto the dorsal aspect of primitive atrium. It changes its orientation from transverse to oval and finally to vertical with a narrow slit. The slit has right and left margins called the *right* and *left venous valves*. Cranially these two valves fuse to form a structure called the *septum spurium* (Figs 15.10 and 15.13). Caudally it forms the sinus septum.

- *Fate of tributaries of sinus venosus*: Each common cardinal vein receives the venous blood from cranial and caudal parts of embryo through anterior and posterior cardinal veins respectively. The right common



Figs 15.11A to C: Retrogression of the left horn of sinus venosus

cardinal vein becomes part of the *superior vena cava*. The vitelline veins receive blood from the yolk sac. The right vitelline vein forms the terminal part of the *inferior vena cava* (Figs 15.12 and 15.14).

- *Fate of right and left venous valves*: The right margin of the original sinoatrial orifice (i.e. the right venous valve) expands very greatly and divides into three parts by two muscular bands, the (1) *superior* and (2) *inferior limbic bands*. The three parts of right venous valve are the (1) *crista terminalis* (Figs 15.12A and B), (2) *valve of the inferior vena cava* and (3) *valve of the coronary sinus*. The left venous valve gets incorporated into the development of septum secundum.

The embryonic parts and adult derivatives of sinus venosus are presented in Table 15.1.

Atrioventricular Canal

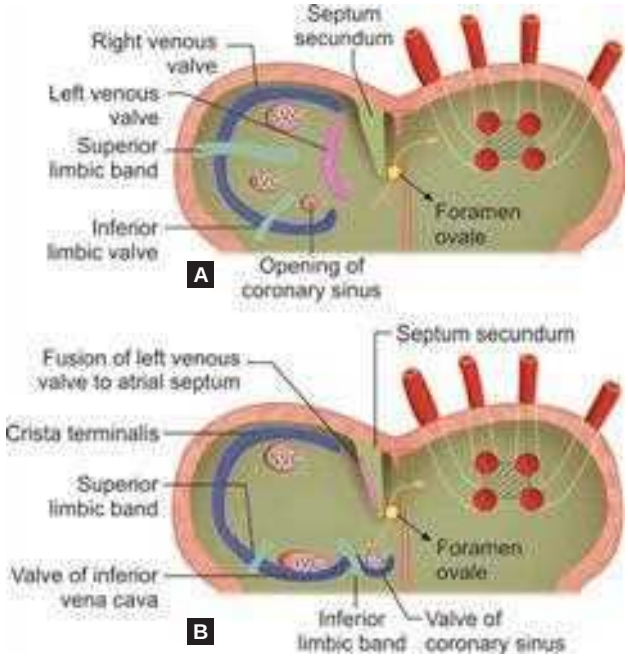
This is the communication between common atrial chamber and ventricle.

The AV canal divides into right and left halves as follows:

- The AV canal is circular in shape initially and later becomes transverse.
- Two thickenings, the AV endocardial cushions appear on the dorsal and ventral walls of AV canal by proliferation of subendocardial mesenchymal cells around right and left AV canals.
- They grow toward each other and fuse. The fused cushions form the *septum intermedium* (Fig. 15.16).
- The AV endocardial cushions take part in the formation of interatrial and interventricular septa. They are involved in many congenital heart diseases.

Formation of Interatrial Septum

The atrial chamber undergoes division into right and left halves by formation of two septa (that later fuse) (Figs 15.13A to D).



Figs 15.12A and B: Sinus venosus—Fate of right and left venous valves. The right venous valve expands greatly and forms the crista terminalis, the valve of the inferior vena cava and the valve of the coronary sinus. The left venous valve remains small and fuses with the interatrial septum. Formation of interatrial septum. Incorporation of pulmonary veins into the left atrium

Appearance of septum primum: Notching of roof and ventral wall of primitive atrium because of pressure of bulbus cordis results formation of a sickle-shaped fold from the roof and dorsal wall of primitive atrium. This fold is called *septum primum*. It is located exactly in the midline and is to the left of septum spurium. It grows downward toward AV canal and septum intermedium. It ultimately fuses with the septum intermedium (Fig. 15.13A).

However, note the following carefully. Throughout fetal life oxygenated blood reaches the right atrium from the placenta. This blood has to reach the left atrium, and for

TABLE 15.1: Sinus venosus—embryonic parts and adult derivatives

Embryonic part	*Change*	*Adult structure*
Right horn	Enlargement and incorporation into right atrium	Sinus venarum
Left horn and body	Reduces in size	Coronary sinus
Right duct of Cuvier/ common cardinal vein	-	Superior vena cava (intrapericardial part)
Left duct of Cuvier/ common cardinal vein	Reduction in size	Oblique vein of left atrium
Cross communication between right and left anterior cardinal veins	New establishment	Left brachiocephalic vein
Right anterior cardinal vein caudal to cross communication	-	Superior vena cava (extrapericardial part)
Left anterior cardinal vein caudal to cross communication	Obliteration	Left superior intercostal vein Ligament of left vena cava
Suprahepatic part of right vitelline vein	Common hepatic vein	Inferior vena cava (terminal part)
Cephalic part of right posterior cardinal vein	-	Arch of azygos vein

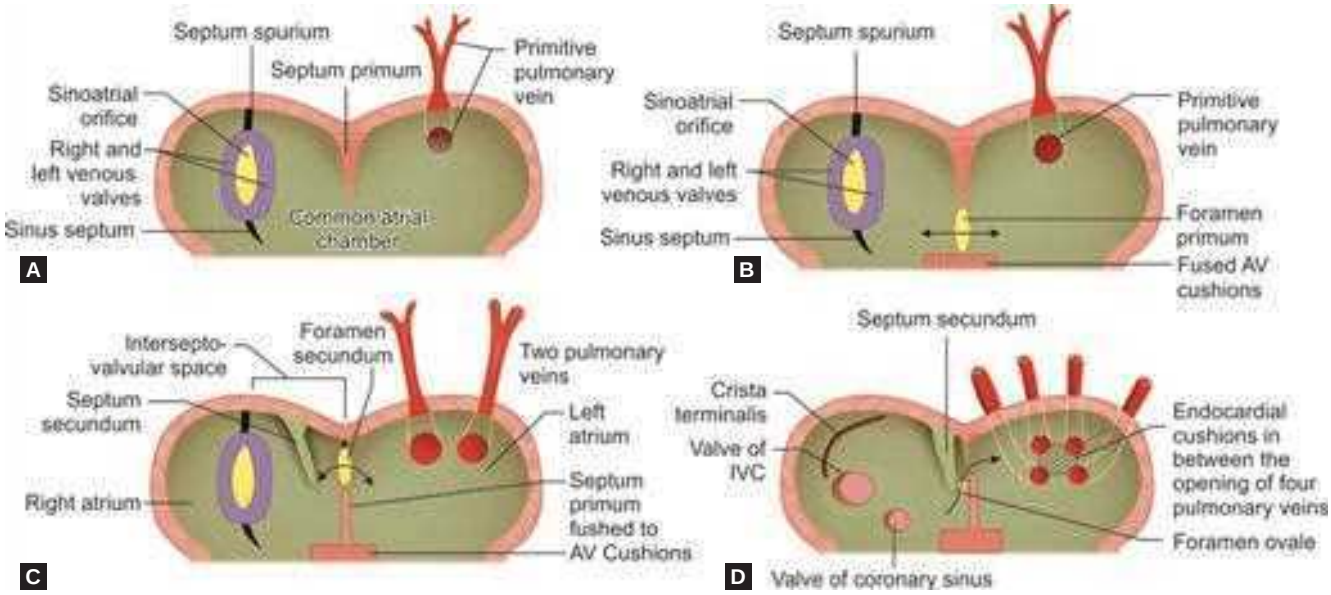
this purpose a communication between right and left atria is essential.

Appearance of ostium primum: Before the septum primum reaches and fuses with the septum intermedium, blood flows through the gap between them. This gap is the *foramen primum* (Fig. 15.13B).

Appearance of ostium secondum: Before the foramen primum can be closed, it is essential that another path for flow of blood be created. This is achieved by breaking down of the upper part of the septum primum. The new gap is the *foramen secundum*. The septum primum now has a free upper edge (Fig. 15.13C).

Appearance of septum secundum: The *septum secundum* grows down from the roof of the atrial chamber, to the right of the septum primum. As it grows, it comes to overlap the free upper edge of the septum primum. The left venous valve and the cephalic attachment of septum primum get incorporated into the septum secondum.

Appearance of foramen ovale: Once the two septa overlap blood has to flow through the interval between the septa. This gap is the *foramen ovale*. It is an oblique valvular passage that allows blood to flow from right to left, but not from left to right (Fig. 15.13D).This is patent throughout



Figs 15.13A to D: Sinoatrial orifice and venous valves in right atrium. Formation of interatrial septum. (A) Septum primum appears; (B) Septum primum grows toward fused AV cushions. The gap between them is the foramen primum; (C) Septum primum fuses with AV cushions. At the same time, the upper part of the septum primum degenerates to form the foramen secundum. The septum secundum is formed to the right of the septum primum; (D) Septum secundum overlaps the free edge of septum primum. Blood now flows from left to right through the oblique cleft between the two septa. Incorporation of pulmonary veins into the left atrium

fetal life. The caudal end of septum secondum is sickle-shaped (concave ventrocaudally).

Obliteration of foramen ovale: After birth of the baby, the left atrium starts receiving oxygenated blood from the lungs, the pressure of this chamber becomes greater than that of right atrium and there is no need for flow of blood from right atrium to left atrium. The foramen ovale is, therefore, obliterated by fusion of the septum primum and septum secundum. In terms of adult anatomy, the *annulus ovalis* represents the lower free edge of the septum secundum while the *fossa ovalis* represents the septum primum.

Development of Right Atrium

With the understanding of the sinus venosus and its parts, partitioning of AV canal and formation of interatrial septum, the development of right atrium can be summarized.

The main right atrium is derived from three sources. They are:

1. *Right half of the primitive atrium*: It forms the rough trabeculated part (atrium proper) in front of crista terminalis including right auricle.
2. *Absorption of right horn of sinus venosus into the right half of primitive atrium*: This forms the smooth part (sinus venarum) behind crista terminalis develops from absorption of right horn of sinus venosus into the right atrium by great enlargement of the sinoatrial orifice (Figs 15.12 and 15.14).
3. *Absorption of right half of AV canal*: Most ventral smooth part.

After absorption of the sinus venosus into the right atrium, the coronary sinus and the venae cavae are seen opening into the atrium. The expanded right venous valve that is partitioned by the two limbic bands becomes the crista terminalis, valve of Inferior vena cava and valve of coronary sinus. The left venous valve fuses with atrial septum. Note that the crista terminalis lies at the junction of the part of the right atrium derived from the sinus venosus (*sinus venarum*) and the atrium proper.

Absorption of Pulmonary Veins into the Left Atrium

- At the time when the septum primum is just beginning to form, a single pulmonary vein opens into the left half of the primitive atrium (Figs 15.10A to E). When traced away from the heart (Figs 15.13 and 15), the vein divides into a right and a left branch each of which again bifurcates, to drain the corresponding lung bud.
- Gradually, the parts of the pulmonary veins nearest to the left atrium are absorbed into the atrium, with the result that four separate veins, two from each side, come to open into it (Figs 15.13 to 15).

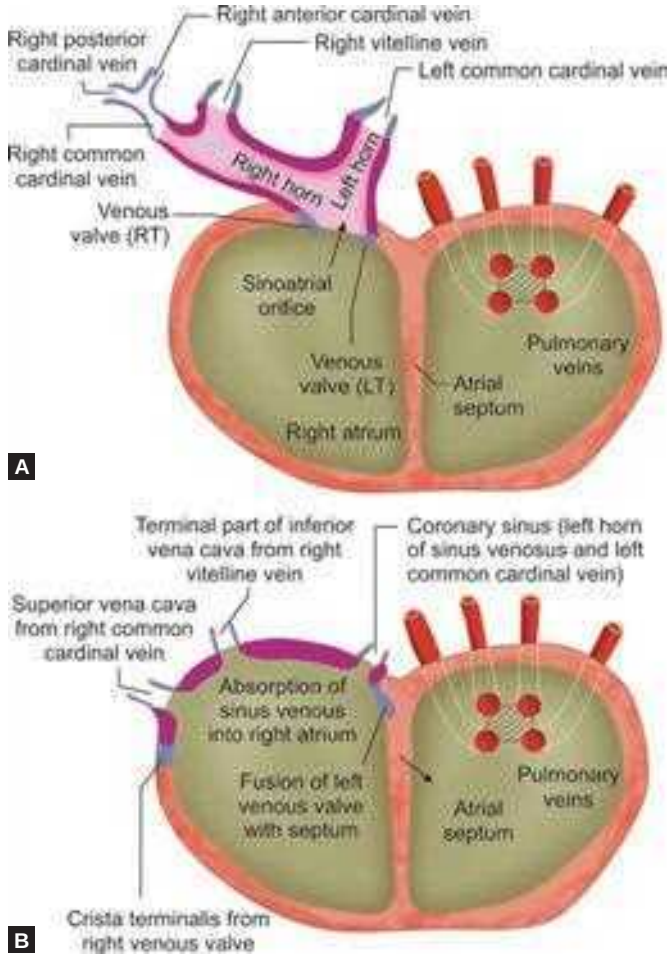
Development of Left Atrium

The left atrium is derived from the following three components (Figs 15.13 to 15.16):

1. *Left half of the primitive atrial chamber*: This contributes for the rough anterior part and left auricle.
2. *Left half of the AV canal*: It forms the most anterior smooth part.
3. *Absorbed proximal parts of the pulmonary veins*: It forms the posterior smooth part between the openings of pulmonary veins that form the anterior boundary for oblique sinus of pericardium.

Development of Ventricles

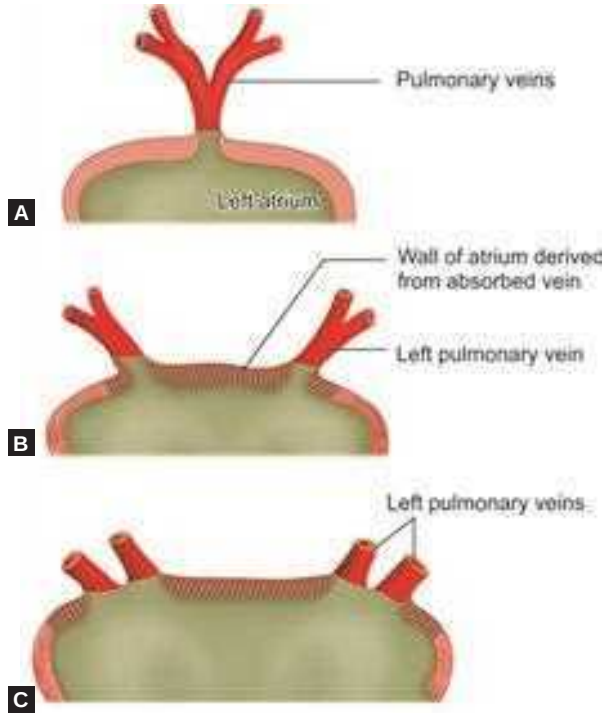
The right and left ventricles are formed by partitioning of primitive ventricle and incorporation of bulbus cordis.



Figs 15.14A and B: Partitioned atrial chambers and sinoatrial orifice and pulmonary veins in partitioned atria

Bulbus Cordis

The *bulbus cordis* is the cranial most part (arterial end) of the developing heart tube. It is divisible into three parts, i.e. (1) proximal, (2) middle and (3) distal. The proximal one-third is dilated and does not have any special name; the middle one-third is called the conus, and the distal one-third is called the truncus arteriosus (Fig. 15.8).



Figs 15.15A to C: (A to C) Absorption of pulmonary veins into the left atrium. At first only one vein from the lungs enters the left atrium. The proximal part of the vein is gradually absorbed and is incorporated into the wall of the atrium. As a result of continued absorption of tributaries, four veins (two right and two left) finally open into the atrium

- The proximal one-third of the bulbus cordis merges with the cavity of the primitive ventricle and forms the *bulboventricular chamber*. It takes part in forming the trabeculated part of the right ventricle (Figs 15.9 and 15.17).
- The middle part (*conus cordis*) forms the outflow part of both the ventricles. Two septa are formed in relation to the walls of this part. They are the *proximal* and *distal bulbar septa*. The proximal bulbar septum contributes for the formation of *interventricular septum*. The distal bulbar septum separates the conus into *aortic vestibule* and *conus arteriosus/infundibulum* (Figs 15.18A to C)
- The distal part (*truncus arteriosus*) undergoes division by spiral *aortopulmonary septum* into *ascending aorta* and *plumonary trunk*. The spiral septum is formed by union of right superior and left inferior *truncus swellings* or *cushions*. Orientation and fusion of these cushions takes place in such a manner that at its lower end, the pulmonary trunk lies ventral to the aorta, but as it is traced upward it comes to lie on its left side. This is because of the difference in the orientation of the spiral septum (Figs 15.18A to C) at different levels.
  - Proximal part—coronal orientation and continuous with distal bulbar septum with the result, the pulmonary trunk is anterior and the aorta is posterior.
  - Intermediate part—anteroposterior orientation with pulmonary trunk on left and aorta on right.
  - Distal part—again coronal orientation with aorta anterior and pulmonary trunk posterior.
- The truncus arteriosus is continuous distally with the aortic sac. The aortic sac is continuous with right and left pharyngeal arch arteries. These arteries arch backward to become continuous with the right and left dorsal aortae.

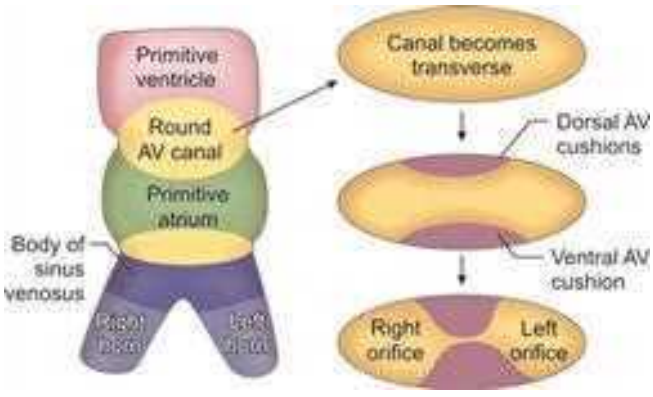


Fig. 15.16: Division of AV canal into right and left orifices

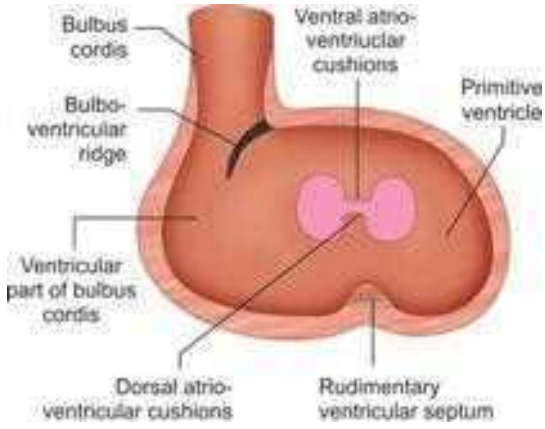
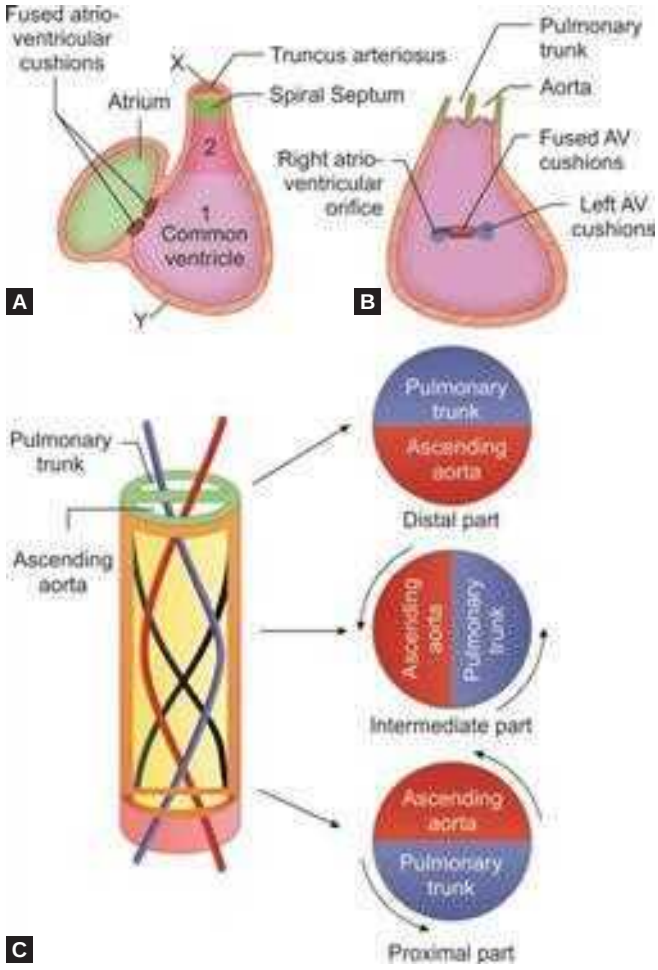


Fig. 15.17: Formation of bulboventricular cavity



Figs 15.18A to C: (A) Two parts of the ventricular chamber. Part 1 lies anterior to the atrioventricular orifice. Part 2 is conical and lies higher up; (B) This is a section across the ventricle in the plane XY, shown in (A). Sections in the plane indicated by the arrow in (A) are shown in Figure 15.19; (C) Aorticopulmonary/ spiral septum

From Figures 15.18A to C, note that the bulboventricular cavity consists of:

- A dilated lower part (1, in figure) that communicates with the atria; and
- A conical upper part (2, in figure) communicating with the truncus arteriosus.
- Part "1" is derived from the proximal one-third of the bulbus cordis and the primitive ventricle, while part "2" is from the conus.

Formation of Interventricular Septum

The ventricular cavity formed after the conus and proximal one-third of bulbus cordis has merged into the primitive ventricle and has to be subdivided into right and left halves in such a way that each half communicates with the corresponding atrium.

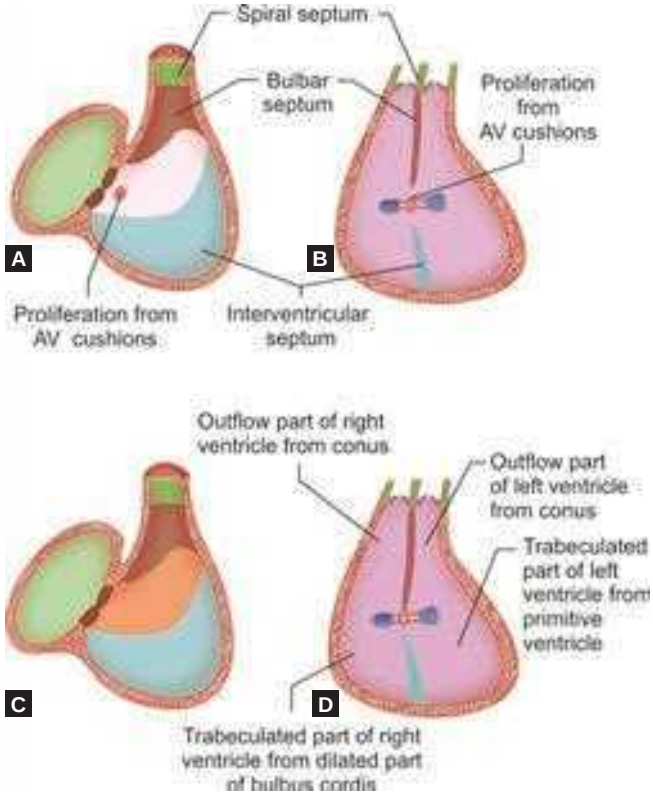
The right ventricle opens into the pulmonary trunk and the left ventricle into the aorta. This subdivision takes place as follows by the formation of interventricular septum.

The interventricular septum consists of three parts that develop from different sources. They are (1) *muscular*, (2) *bulbar* and (3) *membranous* parts.

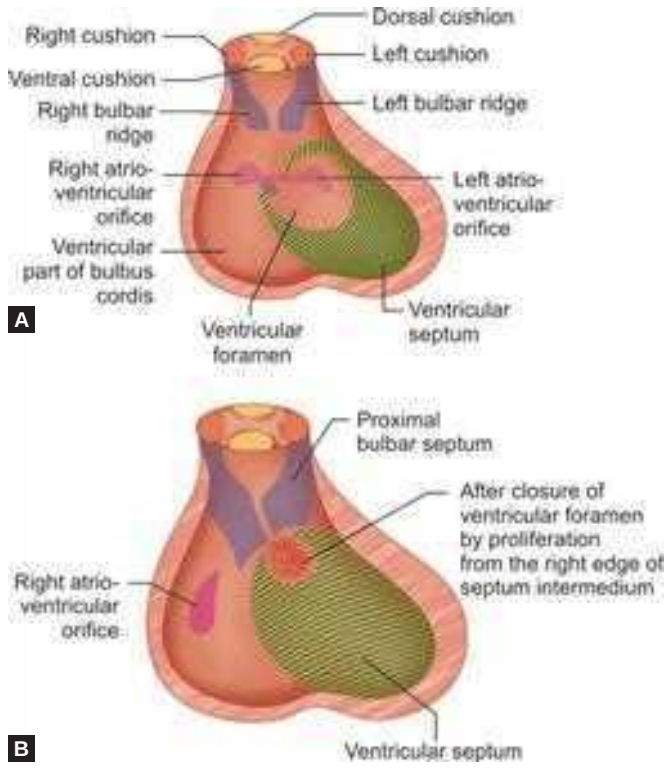
Muscular part: A septum, called the *interventricular septum*, grows upward *from the floor of the bulboventricular cavity* and divides the lower dilated part of this cavity into right and left halves (Fig. 15.19A). Passive dilatation of bulboventricular cavities on either side of the septum and hemodynamic forces are responsible for its formation. It meets the fused AV cushions (*septum intermedium*) and partially fuses with them (Figs 15.19A to D and 15.20A and B). On the external surface of the heart, the site of formation of the interventricular septum corresponds to the *bulboventricular sulcus* (Fig. 15.23A). The cephalic margin of septum is free, concave and twisted. It presents a *dorsal* and a *ventral horn*. The dorsal horn fuses with right edge of dorsal AV cushion and the ventral horn fuses with the left edge of ventral AV cushion. An interventricular foramen appears between the two ventricles at the upper margin of interventricular septum. The closure interventricular foramen is facilitated by septum intermedium and proximal bulbar septum (Figs 15.20A and B).

Bulbar part: Two ridges, termed the *right* and *left bulbar ridges*, arise in the wall of the bulboventricular cavity (in the part derived from the conus). The right ridge arises from the dorsal and right wall and is cephalic to right AV orifice. The left ridge arises from the ventral and left wall. These ridges grow toward each other and fuse to form a *bulbar septum* (Figs 15.19A and B, and Fig. 15.20). The right ridge is in line with the dorsal horn and the left ridge with the ventral horn of muscular part of AV septum. Fusion of right and left bulbar ridges forms the proximal bulbar septum. The bulbar septum grows downward toward the muscular part of interventricular septum but does not quite reach it, with the result that a gap is still left between the two.

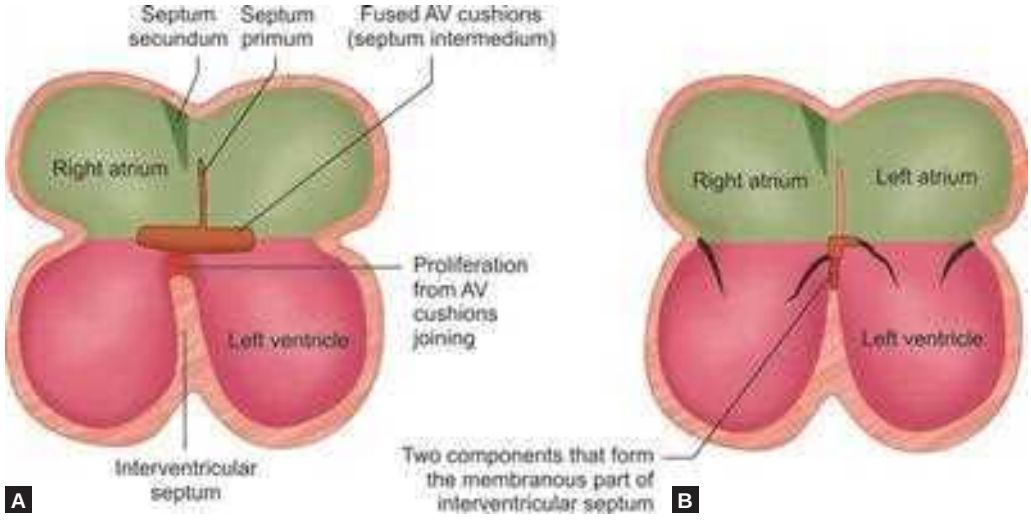
Membranous part: The gap between the upper edge of the interventricular septum, and the lower edge of the bulbar septum, is filled by proliferation of tissue from the right side of the AV cushions (Figs 15.19 and 15.20) and the right and left bulbar ridges. The *membranous part of the interventricular septum* is divisible into an anterior part, which separates the right and left ventricles, and a posterior part which separates the left ventricle from the right atrium (also called *AV septum*). The anterior part is derived from the proliferation of tissue from the endocardial cushions as described above. The derivation of the posterior part is shown in Figures 15.21A and B. It will be seen that the interatrial and interventricular septa do not meet the



Figs 15.19A to D: Two stages in the formation of the ventricular septum. (B) and (D) correspond to (A) and (C) respectively. (A) Bulbar septum grows down from above, and interventricular septum grows upward from below; (C and D) The gap between the bulbar septum and the interventricular septum is filled in by proliferation from AV cushions. For explanation of orientation of these figures, see legend to Figures 15.18A and B



Figs 15.20A and B: (A and B) Interior of bulboventricular cavity showing the cephalic margin of interventricular septum and its two horns and the proximal bulbar septum formation



Figs 15.21A and B: In Figure (A) note that the interatrial and interventricular septa do not meet the atrioventricular (AV) cushions in the same plane. In Figure (B) note that the membranous part of the interventricular septum is made up of the original. AV cushion between the attachment of the interatrial and interventricular septa, and of the endocardial proliferation from these cushions. The first part separates the left ventricle from the right atrium while the second part separates the two ventricles. The tricuspid valve is attached to the membranous septum at the junction of these parts. These figures are sections in the plane indicated by an arrow in Figure 15.18A

AV cushions in the same line. As a result, a part of these cushions separates the left ventricle from the right atrium. This part of the AV cushions forms the posterior part of the membranous septum.

The interventricular septum is probably formed more by downward enlargement of the right and left ventricular cavities on either side of the septum, rather than by active growth of the septum itself.

Right and Left Ventricles

They are formed by:

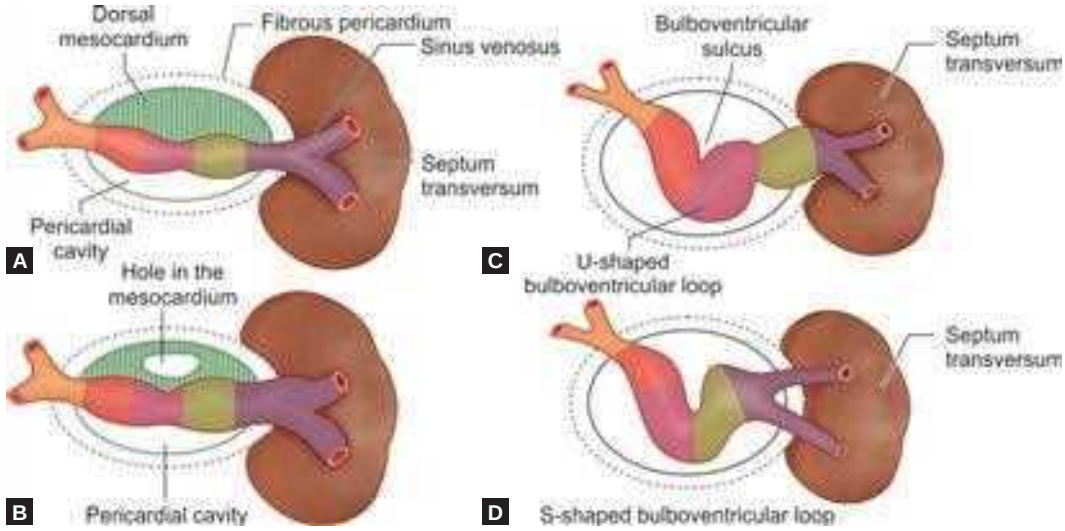
- The inflow (rough) parts of both ventricles are formed by corresponding parts of primitive ventricle.
- The outflow parts (smooth parts), i.e. infundibulum of right ventricle and aortic vestibule of left ventricle are formed by the middle one-third of the bulbus cordis only, i.e. the conus. The conus forms the outflow tracts (smooth parts) of both the right and left ventricles.

EXTERIOR OF THE HEART

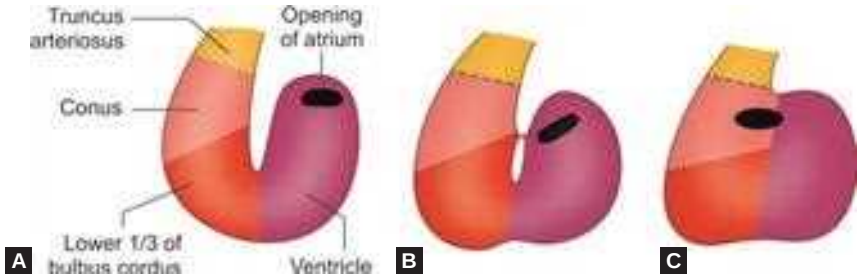
- The heart tube is, for some time, is placed longitudinally and suspended from the dorsal wall of the pericardial cavity by two layers of pericardium that constitute the *dorsal mesocardium* (Fig. 15.22A).
- This mesocardium soon disappears and the heart tube lies free within the pericardial sac, suspended by its two ends (Figs 22B and C).
- However, at this stage, the caudal part of the heart tube (atrium, sinus venosus) is embedded within the substance of the septum transversum.
- The part of the heart tube lying within the pericardial cavity is thus made up of bulbus cordis and ventricle. This part of the tube grows rapidly and, therefore, becomes folded on itself to form a "U"-shaped *bulboventricular loop* (Fig. 15.22C). Now, the primitive atrium is to the left and dorsal to primitive ventricle.
- Subsequently, as the atrium and sinus venosus are freed from the septum transversum, they come to lie behind and above the ventricle, and the heart tube is now "S"-shaped (Fig. 15.22D).
- At this stage, the bulbus cordis, and ventricle, are separated by a deep *bulboventricular sulcus* (Figs 15.22D and 23). This sulcus gradually becomes shallower so that the conus, the proximal part of the bulbus cordis, and the ventricle, come to form one chamber (Figs 15.23A to C), which communicates with the truncus arteriosus.
- The atrial chamber which lies behind the upper part of the ventricle, and of the truncus arteriosus, expands; and as it does so parts of it come to project forward on either side of the truncus. The sinus venosus moves away from the septum transversum and occupies a position dorsal to primitive atrium. As a result of these changes, the exterior of the heart assumes its definitive shape (Figs 15.24A to D).

VALVES OF THE HEART

- The *mitral* and *tricuspid* valves are formed by proliferation of connective tissue under the endocardium of the left and right AV canals.
- The *pulmonary* and *aortic* valves are derived from *endocardial cushions* that are formed at the junction



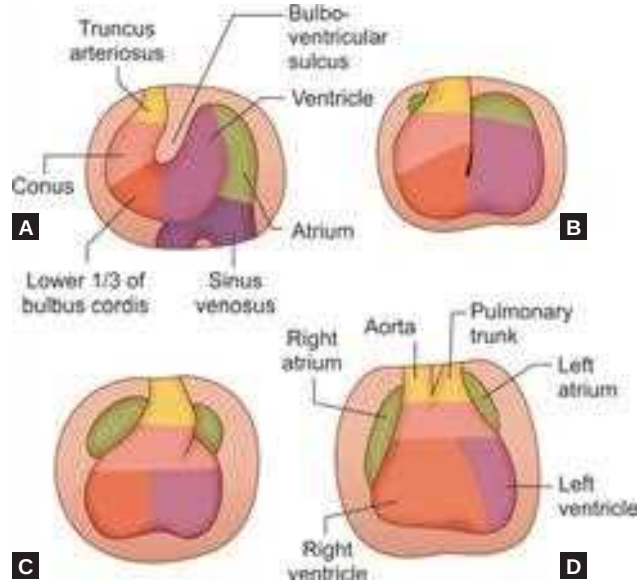
Figs 15.22A to D: Schemes to show the following. (A) Heart tube suspended by mesocardium; (B) Appearance of a hole in mesocardium; (C) Disappearance of mesocardium resulting in formation of transverse sinus of pericardium; In Figures (B) to (D) note gradual freeing of heart tube from septum transversum, and folding of heart tube



Figs 15.23A to C: (A to C) Scheme to show incorporation of conus (and proximal dilated part of bulbus cordis) into the ventricle by disappearance of the bulboventricular sulcus. Note that the opening of atrium into ventricle gradually shifts to the center of the posterior wall of the common bulboventricular chamber. The part labeled "conus" includes the dilated part of the bulbus cordis

of the truncus arteriosus and the conus. Two cushions, right and left, appear in the wall of the conus. They grow and fuse with each other (Figs 15.25A to D).

- With the separation of the aortic and pulmonary openings, the right and left cushions are each subdivided into two parts, one part going to each orifice (Figs 15.25A to D).
- Simultaneously, two more cushions, anterior and posterior appear.
- As a result, the aortic and pulmonary openings each have three cushions, from which three cusps of the corresponding valve develop.
- The pulmonary valve is at first ventral to the aortic valve (Figs 15.25A to D).
- Subsequently, there is a rotation so that the pulmonary valve comes to lie ventral and to the left of the aortic valve (Figs 15.25A to D).
- It is only after this rotation that the cusps acquire their definitive relationships (pulmonary trunk: 1 posterior, 2 anterior; aorta: 1 anterior, 2 posterior).



Figs 15.24A to D: (A to D) Stages in establishment of external form of the heart

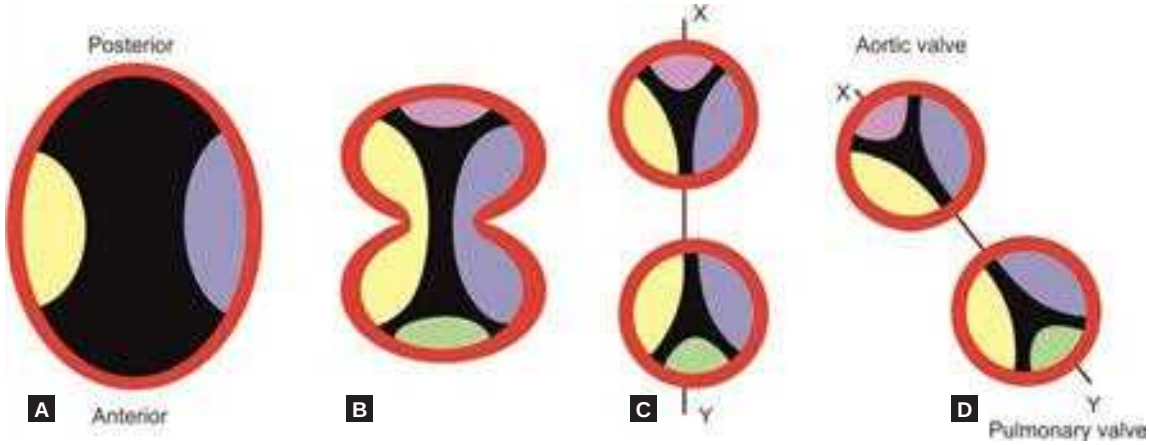
CONDUCTING SYSTEM OF THE HEART

- At the stage when there are two heart tubes, a pacemaker (which later forms the *sinoatrial node*) lies in the caudal part of the left tube.
- After fusion of the two tubes, it lies in the sinus venosus.
- When the sinus venosus is incorporated into the right atrium, it comes to lie near the opening of the superior vena cava.
- The AV node and the AV bundle form in the left wall of the sinus venosus, and in the AV canal.
- After the sinus venosus is absorbed into the right atrium, the AV node comes to lie near the interatrial septum.

PERICARDIAL CAVITY

We have already noted several important facts about the development of the pericardial cavity, and these may be briefly recapitulated as follows:

- The pericardial cavity is a derivative of the part of the intraembryonic coelom that lies in the midline, cranial to the prechordal plate (Fig. 5.8).
- After the formation of the head fold, the pericardial cavity comes to lie on the ventral side of the body of the embryo (Fig. 5.10).
- The heart tube invaginates the pericardial sac from the dorsal aspect (Figs 15.6A to D).
- The parietal layer of the serous pericardium, and the fibrous pericardium, are derived from the somatopleuric mesoderm lining the ventral side of the pericardial cavity (Figs 15.6A to D).
- The visceral serous pericardium is derived from the splanchnopleuric mesoderm lining the dorsal side of the pericardial cavity (Fig. 15.6D).
- The heart tube is initially suspended within the pericardial cavity by the dorsal mesocardium, which



Figs 15.25A to D: Formation of aortic and pulmonary valves. Note that the vessels undergo an anticlockwise rotation [compare axis XY in (C) and (D)]. It is only after this rotation that the cusps of the aortic and pulmonary valves acquire their definitive position

soon disappears (Figs 15.22A and B). We may now consider certain additional facts.

- After disappearance of the dorsal mesocardium, the visceral and parietal layers of pericardium are in continuity only at the arterial and venous ends of the heart tube (Figs 15.26A, B, D and E).
- With the folding of the heart tube, the arterial and venous ends come closer to each other. The space between them becomes the *transverse sinus of pericardium* (Figs 15.26C and F).
- A number of blood vessels are formed at the two ends of the heart tube. At the arterial end, these are the aorta and the pulmonary trunk. At the venous end, they are the superior vena cava, inferior vena cava, and four pulmonary veins (Fig. 15.27A).
- The definitive reflections of the pericardium are formed merely by rearrangement of these vessels as shown in Figure 15.27B. Rearrangement of the veins at the venous end results in the formation of an isolated pouch of pericardium, in relation to the four pulmonary veins. This is the *oblique sinus of pericardium*.

Clinical correlation

Congenital anomalies of the heart

Anomalies of position

- ***Dextrocardia***: The chambers and blood vessels of the heart are reversed from side to side, i.e. all structures that normally lie on the right side are on the left, and vice versa (Fig. 15.28). This may be a part of the condition called ***situs inversus***, in which all organs are transposed. When dextrocardia is not a part of situs inversus, it is usually accompanied by anomalies of the chambers of the heart, and of the great vessels.
- ***Ectopia cordis***: The heart lies exposed, on the front of the chest, and can be seen from the outside, due to defective development of the chest wall.

Atresia or stenosis

Any of the orifices of the heart may have too narrow an opening (stenosis), or none at all (atresia). The aortic and pulmonary passages may also show supravalvular, or subvalvular, stenosis (Figs 15.30A to C). Alternatively, the openings may be too large as a result of which the valves become incompetent.

In pulmonary stenosis, the foramen ovale and the ductus arteriosus remain patent. In aortic stenosis also, the ductus arteriosus is patent and blood flows into the aorta through it.

Abnormal growth

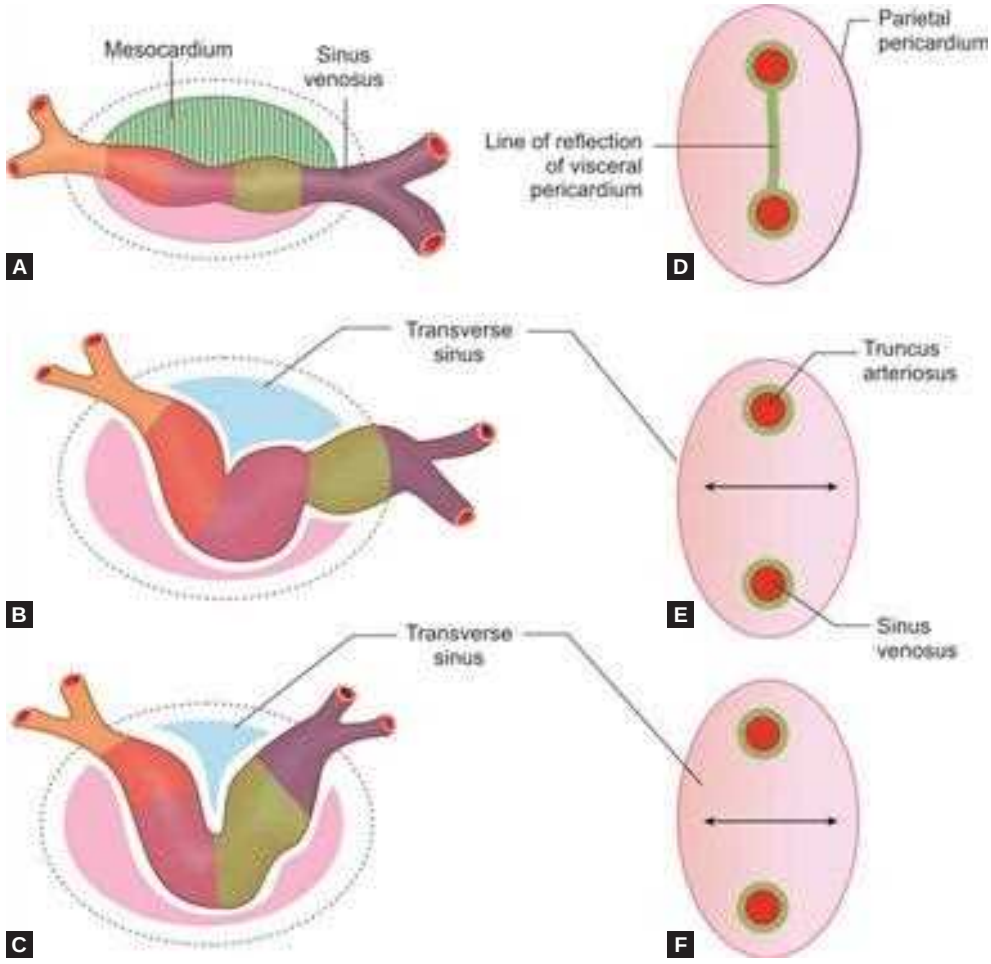
There may be accessory cusps in the valves. Congenital tumors may be formed. The left atrium may be partially subdivided by a transverse septum. The myocardium may be poorly developed (***hypoplasia***).

Defective formation of septa

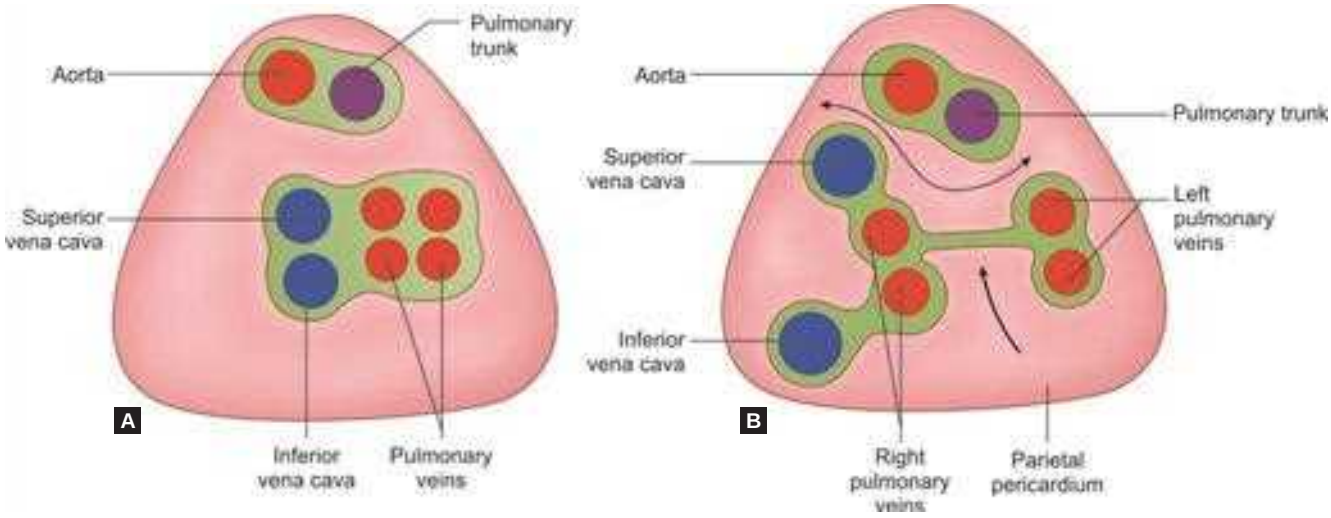
This results in the formation of abnormal passages.

Interatrial septal defects may be of three types:

- The septum primum may fail to reach the AV endocardial cushions, as a result of which the foramen primum persists (Fig. 15.31A). This ***ostium primum defect*** can also be caused by defective formation of AV endocardial cushions.
- The septum secundum may fail to develop as a result of which the foramen secundum remains wide open (***ostium secundum defect***; Fig. 15.31B).
- The septum primum and secundum may develop normally but the oblique valvular passage between them may remain patent (***patent foramen ovale***; Fig. 15.31C). The patency is significant only if there is shunt of blood through it. In many cases, a probe can be passed through the oblique slit (***probe patency***) but there is no shunt.
- Occasionally, there is premature closure of the foramen ovale (i.e. before birth). As a result, the right atrium and ventricle undergo great hypertrophy, while the left side of the heart is underdeveloped.
- ***Interventricular septal defects*** may be seen either in the membranous or in the muscular part of the septum (Fig. 15.31D). They are the most common congenital anomalies of the heart.
- ***Defects of the spiral septum***: The spiral septum may not be formed at all. This condition is called patent truncus arteriosus (Fig. 15.29). Partial absence of the septum leads to communications (shunts) between the aorta and the pulmonary trunk.



Figs 15.26A to F: Schemes showing the relationship of the heart tube to the pericardial sac. (A), (B) and (C) are lateral views while (D), (E) and (F) show the dorsal aspect of the interior of the pericardial sac at corresponding stages. Disappearance of the mesocardium leads to formation of the transverse sinus of pericardium. Note that with the folding of the heart tube, the arterial and venous ends of the heart tube are brought closer together, and the transverse sinus comes to lie between them



Figs 15.27A and B: (A) Scheme to show that the oblique sinus of pericardium is established by rearrangement of veins entering the heart. The sinus is indicated by the lower arrow in (B), the upper arrow indicates the transverse sinus

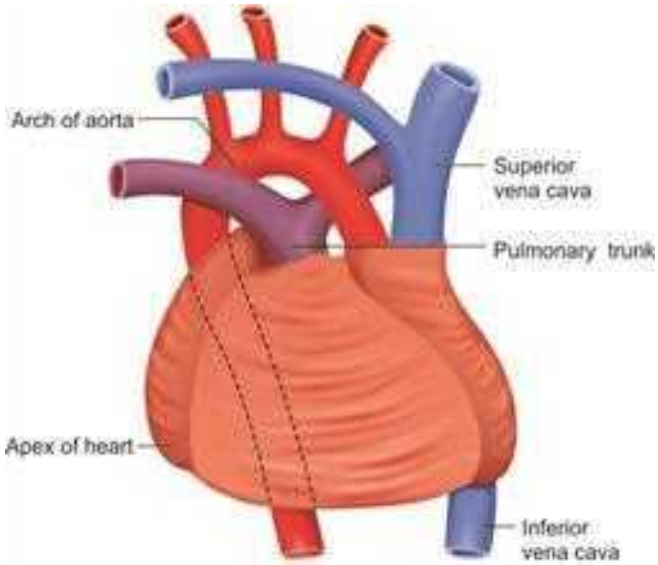


Fig. 15.28: Dextrocardia. The chambers and large blood vessels show right left reversal

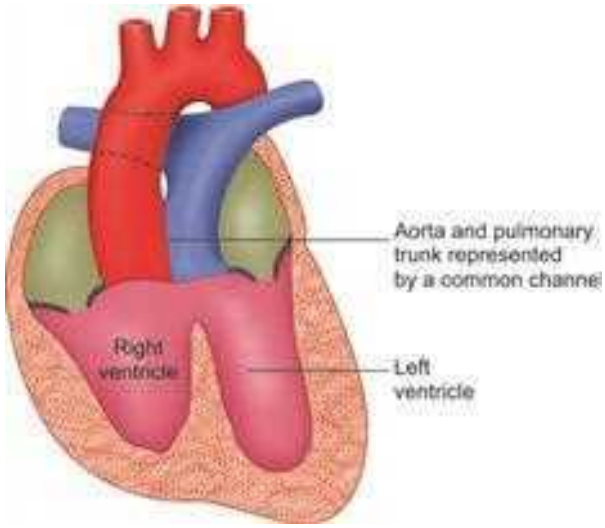
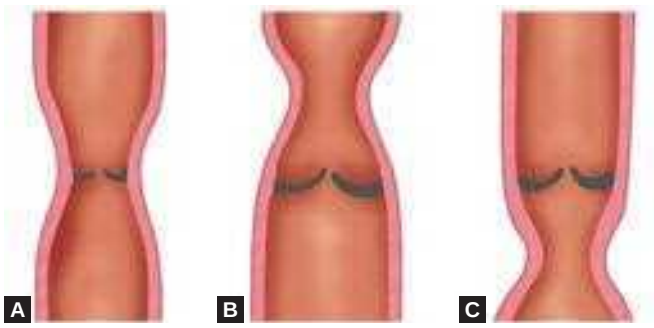


Fig. 15.29: Patent truncus arteriosus. The ascending aorta and pulmonary trunk are represented by a single channel that opens into both ventricles



Figs 15.30A to C: Types of aortic stenosis. (A) Valvular; (B) Supravalvular; (C) Infravalvular

- ***Atrioventricular canal defect or persistent AV canal***: Defective formation of the AV cushions may lead to a condition in which all four chambers of the heart may intercommunicate. The interatrial and interventricular septa are incomplete (as the normal contributions to these septa from the endocardial cushions are lacking).
  If fusion of endocardial cushions is too far to the right, it causes ***tricuspid atresia***. As such cushions are not in alignment with the interventricular septum, the upper part of the latter is defective. With tricuspid atresia, there is increased pressure in the right atrium, as a result of which the foramen ovale fails to close.
  Defective formation of septa, if marked, can lead to a two-chambered heart (***cor biloculare***) in which there is one common ventricle and one common atrium. Alternatively, a three-chambered heart (***cor triloculare***) may be seen; it may consist of a single ventricle with two atria or of a single atrium with two ventricles (***cor triloculare biventriculare***).

Combined defects

Two or more of the defects may coexist. One classically recognized condition of this type is known as ***Fallot's tetralogy***. It consists of (Fig. 15.32):

- Interventricular septal defect
- Aorta overriding the free upper edge of the ventricular septum
- Pulmonary stenosis
- Hypertrophy of the right ventricle.

Other defects

- The pericardium may be partially or completely absent.
- It may be congenital defects in the conducting system of the heart.

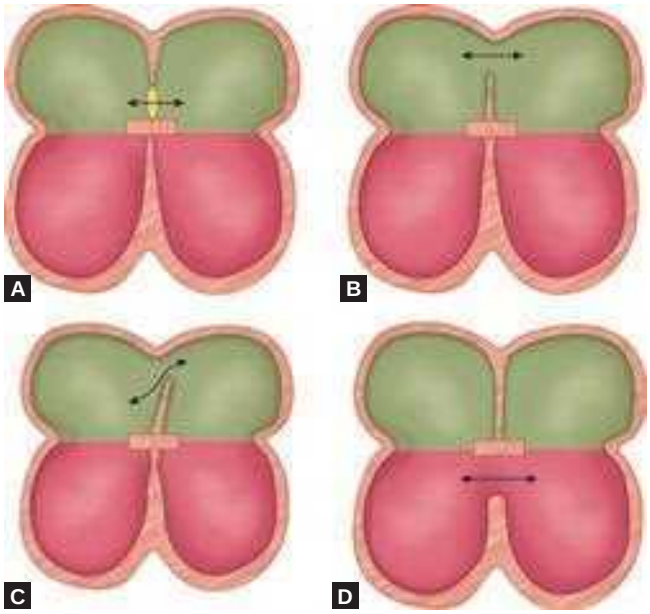
Anomalies of relationship of chambers to great vessels

- ***Transposition of great vessels***: The aorta arises from the right ventricle and the pulmonary trunk from the left ventricle.
- ***Taussig-Bing syndrome***: The aorta arises from the right ventricle; and the pulmonary trunk overrides both the right and left ventricles, there being an interventricular septal defect.
- The superior or inferior vena cava may end in the left atrium.
- The pulmonary veins may end in the right atrium or in one of its tributaries.

PART 2: ARTERIES

PHARYNGEAL ARCH ARTERIES AND THEIR FATE

- The first arteries to appear in the embryo are the right and left *primitive aortae*. They are continuous with the two endocardial heart tubes.
- Each primitive aorta consists of three parts (Fig. 15.33A):
  1. A portion lying ventral to the foregut (*ventral aorta*)
  2. An arched portion lying in the first pharyngeal arch
  3. A dorsal portion lying dorsal to the gut (*dorsal aorta*)
- After the fusion of the two endocardial tubes, the two ventral aortae partially fuse to form the *aortic sac*, the unfused parts remaining as the *right and left horns* of the sac (Fig. 15.33B).



Figs 15.31A to D: Septal defects. (A) Septum primum defect; (B) Septum secundum defect; (C) Patent foramen ovale; (D) Interventricular septum defect

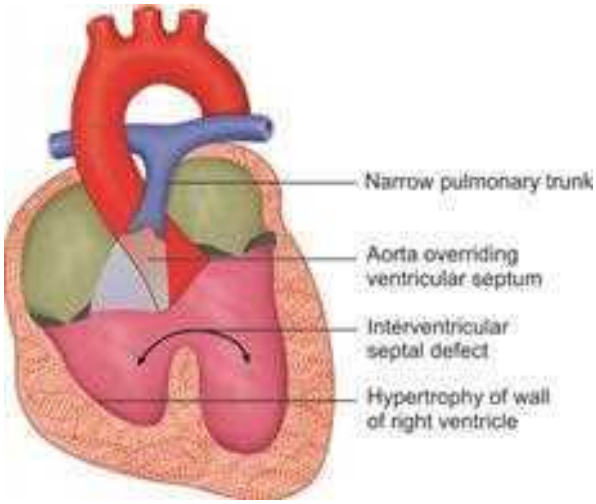
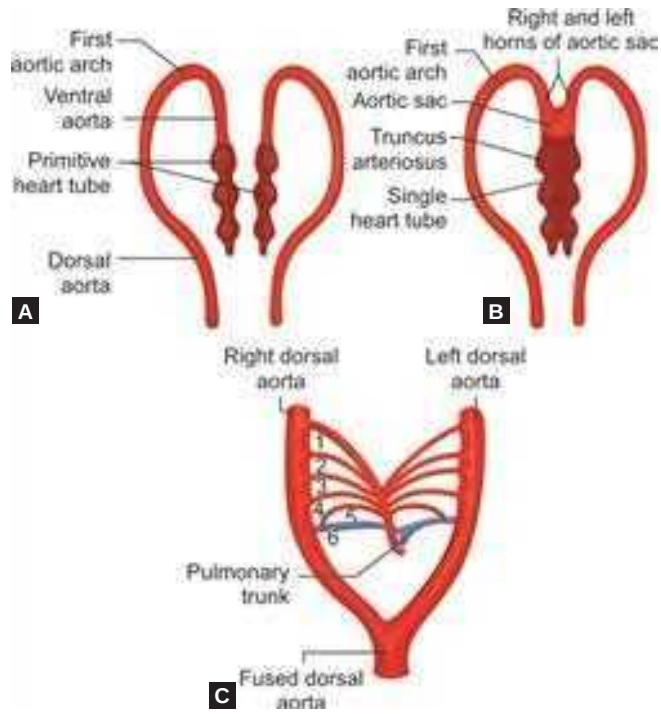


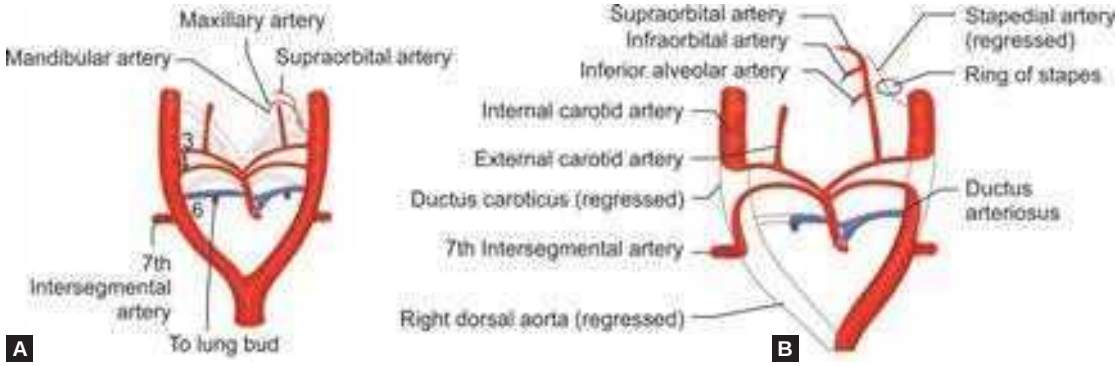
Fig. 15.32: Four features that constitute Fallot's tetralogy

- Successive arterial arches now appear in the second to sixth pharyngeal arches, each being connected ventrally to the right or left horn of the aortic sac and dorsally to the dorsal aorta (Fig. 15.33C).
- The major arteries of the head and neck, and of the thorax, are derived from these arches as follows:
  - The greater part of the first and second arch arteries disappear (Fig. 15.34A). In adult life, the first arch artery is represented by the *maxillary artery*. The second arch artery persists for some part of fetal life as the *stapedial artery*: it may contribute to the formation of the *external carotid artery*.



Figs 15.33A to C: Relation of first aortic arch to heart tubes. (A) Before fusion of heart tubes; (B) After fusion; (C) Aortic arches. Each arch connects the aortic sac to the dorsal aorta. Note that actually all arches are never present at the same time. The first and second arches have retrogressed by the time the sixth appears

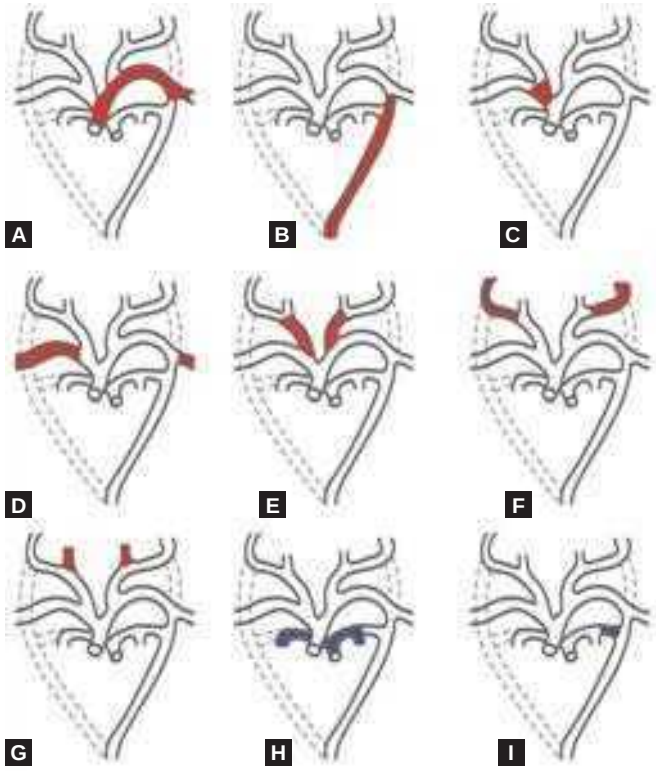
  - The fifth arch artery also disappears (Fig. 15.34A).
  - Now, the aortic sac is connected only with the arteries of the third, fourth and sixth arches.
  - The third and fourth arch arteries open into the ventral part, and the sixth arch artery into the dorsal part, of the aortic sac (Fig. 15.34B).
  - The spiral septum that is formed in the truncus arteriosus extends into the aortic sac; and fuses with its posterior wall in such a way that blood from the pulmonary trunk passes only into the sixth arch artery, while that from the ascending aorta passes into the third and fourth arch arteries (Fig. 15.34B).
- Several changes now take place in the arterial arches to produce the adult pattern as follows:
  - The two dorsal aortae grow cranially, beyond the point of attachment of the first arch artery (Fig. 15.34B).
  - The portion of the dorsal aorta, between the attachment of the third and fourth arch arteries (*ductus caroticus*), disappears on both sides (Fig. 15.34B).
  - The portion of the right dorsal aorta, between the point of attachment of the fourth arch artery and the



Figs 15.34A and B: Fate of aortic arches: (A) Disappearance of first, second and fifth arches; (B) Disappearance of ductus caroticus (on both sides), and of part of right dorsal aorta. Part of the right sixth arch also disappears

point of fusion of the two dorsal aortae, disappears (Fig. 15.34B).
- Each sixth arch artery gives off an artery to the developing lung bud. On the right side, the portion of the sixth arch artery between this bud and the dorsal aorta disappears. On the left side, this part remains patent and forms the *ductus arteriosus*. The ductus arteriosus carries most of the blood from the right ventricle to the dorsal aorta. It is obliterated after birth and is then seen as the *ligamentum arteriosum*.
- Each third arch artery gives off a bud that grows cranially to form the *external carotid artery* (Fig. 15.35G).
- The dorsal aortae give off a series of lateral intersegmental branches to the body wall. One of these, the seventh cervical intersegmental artery supplies the upper limb bud. It comes to be attached to the dorsal aorta near the attachment of the fourth arch artery (Fig. 15.34B).

- The development of the main arteries can now be summarized as follows:
  - The *ascending aorta* and the *pulmonary trunk* are formed from the truncus arteriosus (Fig. 15.34B).
  - The *arch of the aorta* is derived from the ventral part of the aortic sac, its left horn, and the left fourth arch artery (Fig. 15.35A).
  - The *descending aorta* is derived from the left dorsal aorta, below the attachment of fourth arch artery, along with the fused median vessel (Fig. 15.35B).
  - The *brachiocephalic artery* is formed by the right horn of the aortic sac (Fig. 15.35C).
  - The proximal part of the *right subclavian artery* (Fig. 15.35D) is derived from the right fourth arch artery, and the remaining part of the artery being derived from the seventh cervical intersegmental artery.
  - *Left subclavian artery* is derived entirely from the seventh cervical intersegmental artery, which arises from the dorsal aorta opposite the attachment of the fourth arch artery (Fig. 15.35D).
  - The *common carotid artery* is derived, on either side, from part of the third arch artery, proximal to the external carotid bud (Fig. 15.35E).
  - The *internal carotid artery* is formed by the portion of the third arch artery distal to the bud, along with the original dorsal aorta cranial to the attachment of the third arch artery (Fig. 15.35F).
  - As the right third and fourth arch arteries arise from the right horn of the aortic sac, the common carotid and subclavian arteries become branches of the brachiocephalic artery.
  - As already mentioned, the *external carotid artery* arises as a bud from the third arch artery (Fig. 15.35G).
  - The *pulmonary arteries* are derived from the part of the sixth arch arteries lying between the pulmonary trunk and the branches to the lung buds (Fig. 15.35H).
  - As already stated, the part of the left sixth arch artery, between the branch to the lung bud and the aorta, forms the *ductus arteriosus* (Fig. 15.35I).
- Relationship of the main nerves of the head and neck to the arteries:
  This can be explained on the basis of the development of the arteries.
  - The nerves of the pharyngeal arches are, at first, lateral to the corresponding arteries.
  - The nerves of the first, second and third arches (V, VII and IX) retain their lateral positions.
  - The disappearance of the ductus caroticus enables the nerve of the fourth arch (*superior laryngeal*) to move medially, and it comes to lie deep to the main arteries of the neck.



Figs 15.35A to I: (A) The arch of the aorta is derived from the aortic sac, its left horn, and the left 4th arch artery; (B) The descending aorta is derived from the left dorsal aorta, and fused dorsal aortae; (C) The brachiocephalic artery is derived from the right horn of the aortic sac; (D) The right subclavian artery is derived from the right 4th arch artery and from the right 7th cervical intersegmental artery. The left subclavian artery is formed only from the left 7th cervical intersegmental artery; (E) The common carotid artery is derived from the proximal part of the 3rd arch artery; (F) The internal carotid artery is derived from distal part of the 3rd arch artery and dorsal aorta (cranial—most part); (G) The external carotid artery arises as a bud from the 3rd arch artery; (H) The pulmonary arteries arise from the 6th arch arteries; (I) The ductus arteriosus is derived from part of the left 6th arch artery

– The nerve of the sixth arch (*recurrent laryngeal*) is at first caudal to the artery of this arch (Fig. 15.36A). With the disappearance of part of the sixth arch artery, on the right side, the nerve moves cranially and comes into relationship with the right fourth arch artery (subclavian) (Fig. 15.36B). On the left side, it retains its relationship to that part of the sixth arch which forms the ductus arteriosus. With the elongation of the neck, and the descent of the heart, these nerves are dragged downward and, therefore, have to follow a recurrent course back to the larynx.

Adult derivatives of truncus arteriosus, aortic sac and aortic arches are presented in Table 15.2.

Clinical correlation

Anomalous development of pharyngeal arch arteries

We have seen that the development of the normal arterial pattern is dependent upon the disappearance of some parts of the pharyngeal arch arteries. Occasionally, this process is disturbed in that:

- Some parts that normally disappear may persist.
- Some parts that normally persist may disappear.

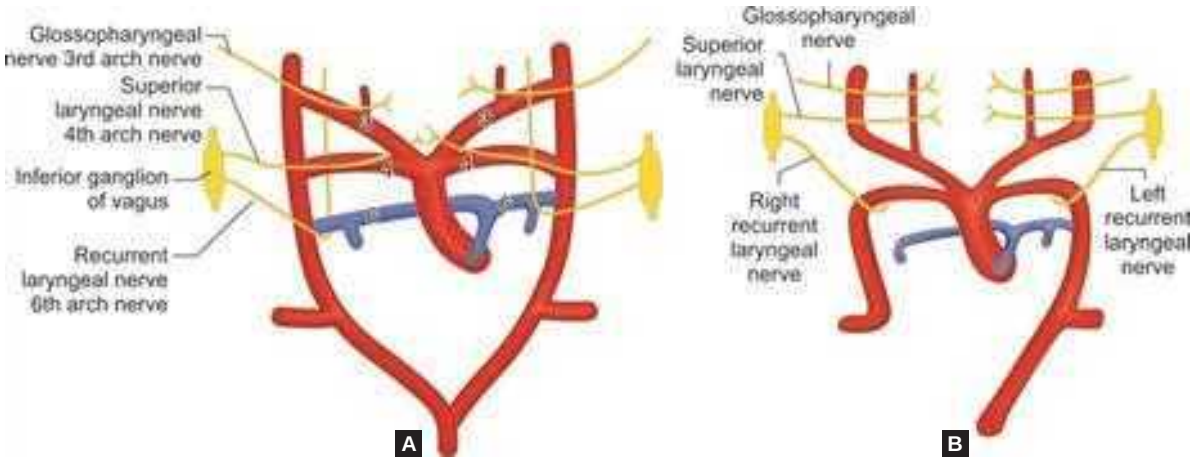
As a result, several anomalies may be produced. Some of these are as follows:

- Double aortic arch (Fig. 15.37A): The arterial ring can compress the trachea and esophagus.
- Right aortic arch (Fig. 15.37B).
- The ductus arteriosus, which is normally occluded soon after birth, may remain patent (***patent ductus arteriosus***).
- The ***right subclavian artery*** may arise as the last branch of the aortic arch (Fig. 15.37C). Such an artery runs to the right behind the esophagus (Fig. 15.38). Along with the aorta this artery forms an arterial ring enclosing the trachea and esophagus. The ring may press upon and obstruct these tubes. In this abnormality, the right recurrent laryngeal nerve does not hook around the subclavian artery. It passes directly to the larynx. An arterial ring can also be formed if the dorsal aorta persists on both sides.
- The ductus caroticus may persist. As a result, the left internal carotid arises directly from the aortic arch, and the right internal carotid from the subclavian (Fig. 15.37D).
- Interrupted aortic arch: A segment of the aortic arch may be missing. The ascending aorta ends by supplying the left common carotid artery. The left subclavian artery arises from the distal segment which receives blood through a patent ductus arteriosus.
- The aorta may show a localized narrowing of its lumen, leading to partial or even complete obstruction to blood flow. This condition is called ***coarctation of the aorta***. Coarctation is most frequently seen near the attachment of the ductus arteriosus to the aorta. It may be (1) proximal to the attachment (***preductal***) (Fig. 15.37E) or (2) distal to the attachment of the ductus (***postductal***) (Fig. 15.37F) in which case the right ventricle supplies the distal part of the body through the ductus arteriosus. When coarctation is postductal, numerous anastomoses are established between branches of the aorta taking origin above the constriction and those arising below this level. Coarctation is said to be a result of the process of obliteration of the ductus arteriosus extending into the aorta. It can also occur as an abnormality in the vessel wall.
- Some other anomalies and the mode of origin of the branches of the arch of the aorta are illustrated in Figure 15.37G.

DEVELOPMENT OF OTHER ARTERIES

The primitive dorsal aortae give off three groups of branches (Fig. 15.39). These are as follows:

- The ventral splanchnic arteries supply the gut. Most of these arteries disappear but three arteries, the *celiac, superior mesenteric* and *inferior mesenteric* remain to



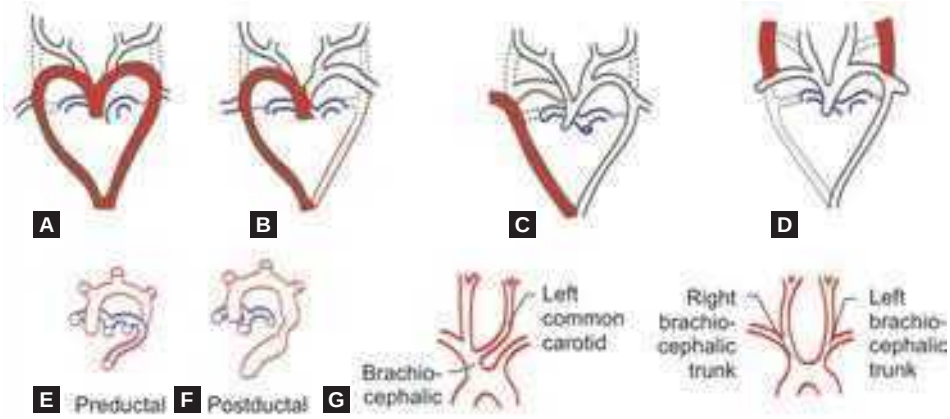
Figs 15.36A and B: (A and B) Relationship of the vagus and recurrent laryngeal nerves to the aortic arches. For explanation see text

TABLE 15.2: Adult derivatives of truncus arteriosus, aortic sac and aortic arches

Adult derivatives	*Embryological structures*
Ascending aorta	Truncus arteriosus
Arch of aorta	Aortic sac, left horn of aortic sac and left 4th arch artery
Descending aorta	Left dorsal aorta and fused dorsal aorta
Brachiocephalic artery	Right horn of aortic sac
Right subclavian artery	Right 4th arch artery and 7th cervical intersegmental artery
Left subclavian artery	Left 7th cervical intersegmental artery
Common carotid artery	Proximal part of 3rd arch artery
Internal carotid artery	Distal part of 3rd arch artery and cervical part of dorsal aorta
External carotid artery	As a bud from 3rd arch artery
Pulmonary trunk	Truncus arteriosus
Pulmonary artery	Part of 6th arch artery
Ductus arteriosus	Part of left 6th arch artery between lung bud and aorta

supply the infradiaphragmatic part of the foregut, the midgut, and the hindgut respectively. Other remnants of these vessels are the *bronchial* and *esophageal* arteries.

- The *lateral or intermediate splanchnic arteries* supply structures developing from the intermediate mesoderm. These persist as the *renal, suprarenal, phrenic*, and *spermatic or ovarian arteries*.
- The *dorsolateral* (*somatic intersegmental*) branches run between two adjacent segments. They retain their original intersegmental arrangement in the thoracic and lumbar regions where they can be recognized as the *intercostal* and *lumbar* arteries.
  - Each dorsolateral artery divides into a dorsal and a ventral division. The ventral division gives off a lateral branch that is most conspicuous in the region of the limb buds. The dorsal division runs dorsally and supplies the muscles of the back. Each dorsal division gives off a spinal branch that runs medially to supply the spinal cord.
  - The branches of the dorsolateral arteries of successive segments become interconnected by the formation of longitudinal anastomoses.
  - In the neck, the dorsal branches are connected by anastomoses that are formed in three situations (Fig. 15.40).
    1. *Precostal*, in front of the necks of the ribs (or costal elements). The precostal anastomoses persist as *the thyrocervical trunk, ascending cervical* and *superior intercostal* arteries.
    2. *Postcostal*, between the costal elements and the transverse processes. The postcostal anastomoses form the greater part of the *vertebral artery*.
    3. *Post-transverse*, behind the transverse processes. The post-transverse anastomoses remain as the *deep cervical* artery.
  - The ventral divisions of the somatic intersegmental arteries are interconnected by anastomoses that are formed on the ventral aspect of the body wall, near the midline (Fig. 15.41). These form the *internal thoracic*, *superior epigastric* and *inferior epigastric* arteries.
- At this stage, special mention must be made of the *seventh cervical intersegmental artery*. The main stem of this artery becomes the *subclavian* artery. Like other dorsolateral arteries, it divides into dorsal, ventral and lateral divisions. The dorsal division forms the *stem of the vertebral* artery. The lateral division grows into the upper



Figs 15.37A to G: Anomalies associated with the development of aortic arches. (A) Double aortic arch; (B) Right aortic arch; (C) Anomalous right subclavian artery; (D) Persistent ductus caroticus; (E) Coarctation of aorta (preductal); (F) Coarctation of aorta (postductal); (G) Anomalies in pattern of branches. Left common carotid artery arising from brachiocephalic artery. Left subclavian and left common carotid arising by a common stem (left brachiocephalic)

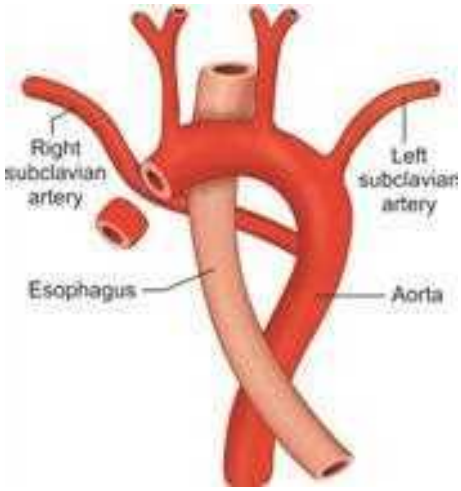


Fig. 15.38: Relationship of abnormal right subclavian artery to the esophagus and to the arch of the aorta

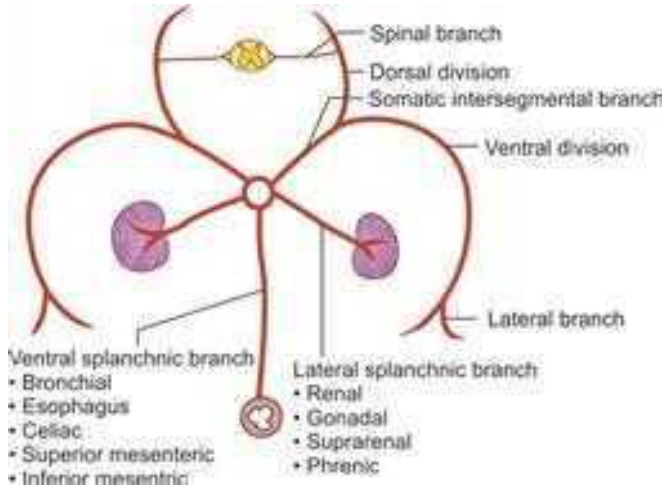


Fig. 15.39: Basic branching pattern of the embryonic dorsal aorta

limb forming the *axillary and brachial* arteries. The ventral division forms the *stem of the internal thoracic* (mammary) artery.

Development of Vertebral Artery (Figs 15.42A and B)

- The first part of the artery, from its origin to the point of entry into the foramen transversarium of the sixth cervical vertebra, is formed by the dorsal division of the seventh cervical intersegmental artery.
- The vertical part (second part), lying in the foramina transversaria, is formed from the postcostal anastomoses between the first to sixth cervical intersegmental arteries.
- The horizontal (third) part, running transversely on the arch of the atlas, is derived from the spinal branch of the first cervical intersegmental artery.

Development of Internal Thoracic Artery (Fig. 15.41)

- The main stem of the artery is formed by the ventral division of the seventh cervical intersegmental artery.
- The vertical part of the artery (including its superior epigastric branch) is derived from the ventral

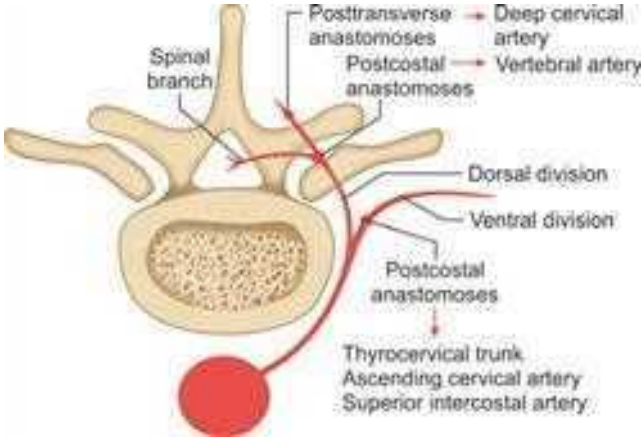


Fig. 15.40: Sites of vertical anastomoses between branches of dorsal aorta. The fate of the anastomoses is also shown

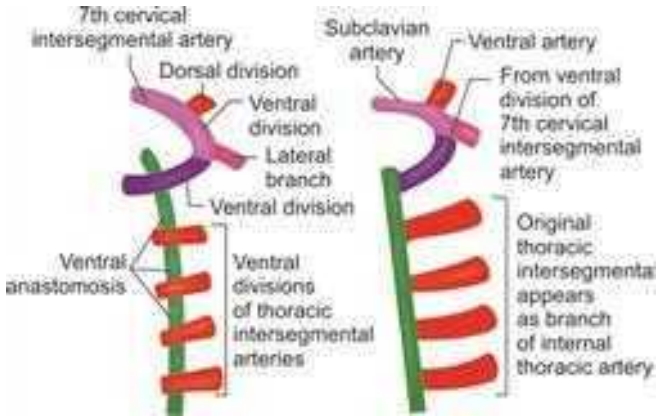
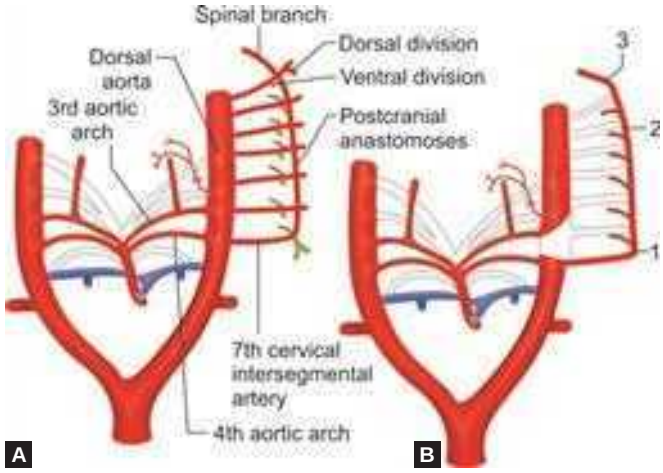


Fig. 15.41: Development of the internal thoracic artery



Figs 15.42A and B: (A and B) Development of the vertebral artery. In (B) the part labeled 1 is derived from the dorsal division of the seventh cervical intersegmental artery; part 2 from the postcostal anastomoses; and part 3 from the spinal branch of the first cervical intersegmental artery

anastomoses between the ventral divisions of the thoracic intersegmental arteries (intercostal arteries).

Umbilical Artery

- Before the fusion of the two dorsal aortae, the umbilical arteries appear as continuations of their distal ends (Fig. 15.43A).
- After fusion of the dorsal aortae, they appear as lateral branches of the single dorsal aorta (Fig. 15.43B).
- Subsequently, each umbilical artery gets linked up with that part of the fifth lumbar intersegmental artery which forms the internal iliac artery (Fig. 15.43C).
- The part of the umbilical artery, between the aorta and the anastomosis with the internal iliac, disappears so that the umbilical artery is now seen as a branch of the internal iliac (Figs 15.43D and E).
- In postnatal life, the proximal part of the umbilical artery becomes the *superior vesical artery*, while its distal part is obliterated to form the medial umbilical ligament.

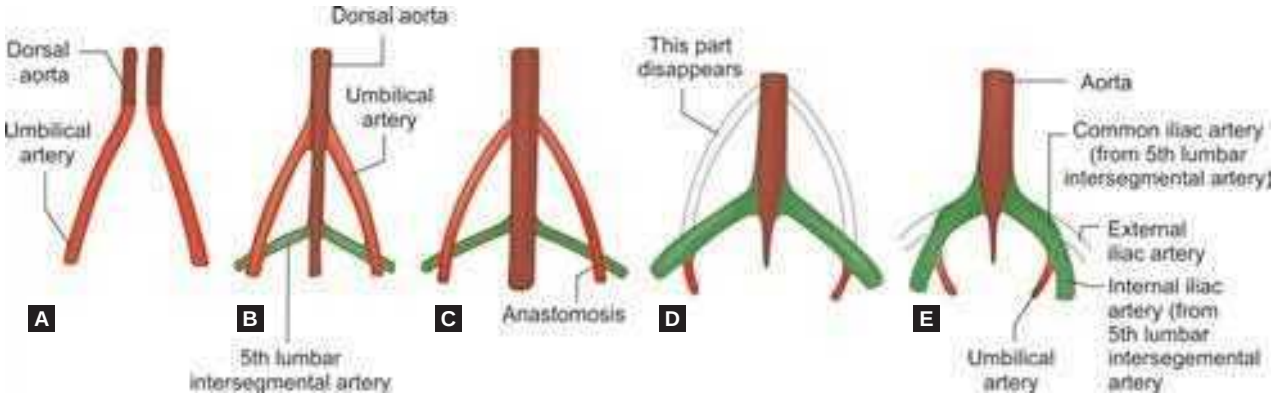
Development of Arteries of Limbs

The limbs are supplied by lateral branches of the somatic intersegmental arteries that belong to the segments from which the limb buds take origin. These vessels form an arterial plexus.

However, each limb soon comes to have *one axis artery* that runs along the central axis of the limb. Other arteries that are formed as branches of the axis artery or as new formations later take over a considerable part of the arterial supply, as a result of which much of the original axis artery may disappear.

Axis Artery of the Upper Limb

- It is formed by the *seventh cervical intersegmental artery* (Fig. 15.44).
- It runs along the ventral axial line and terminates in palmar capillary plexus in hand.
- It persists as the:
  - Axillary artery
  - Brachial artery
  - Anterior interosseous artery
  - Deep palmar arch.
- A median artery develops from anterior interosseous artery and grows distally to communicate with palmar capillary plexus.
- The digital arteries of hand develop from the palmar capillary plexus.
- The radial and ulnar arteries appear late in development.
- The *left subclavian artery* represents the main stem of the seventh cervical intersegmental artery, and the proximal part of its lateral division. This explains the origin of the



Figs 15.43A to E: Development of the umbilical artery. (A) Umbilical arteries are seen as continuations of the right and left dorsal aortae, before their fusion; (B) After fusion of dorsal aortae, the umbilical arteries appear as lateral branches of the aorta. They cross the 5th lumbar intersegmental artery; (C) Umbilical arteries establish anastomoses with the 5th lumbar intersegmental artery; (D) The part of the umbilical artery between the dorsal aorta and the 5th lumbar intersegmental artery disappears; and the umbilical artery is now seen as a branch of the latter; (E) The 5th lumbar intersegmental artery forms the common iliac and internal iliac arteries; and the umbilical is now seen as a branch of the internal iliac

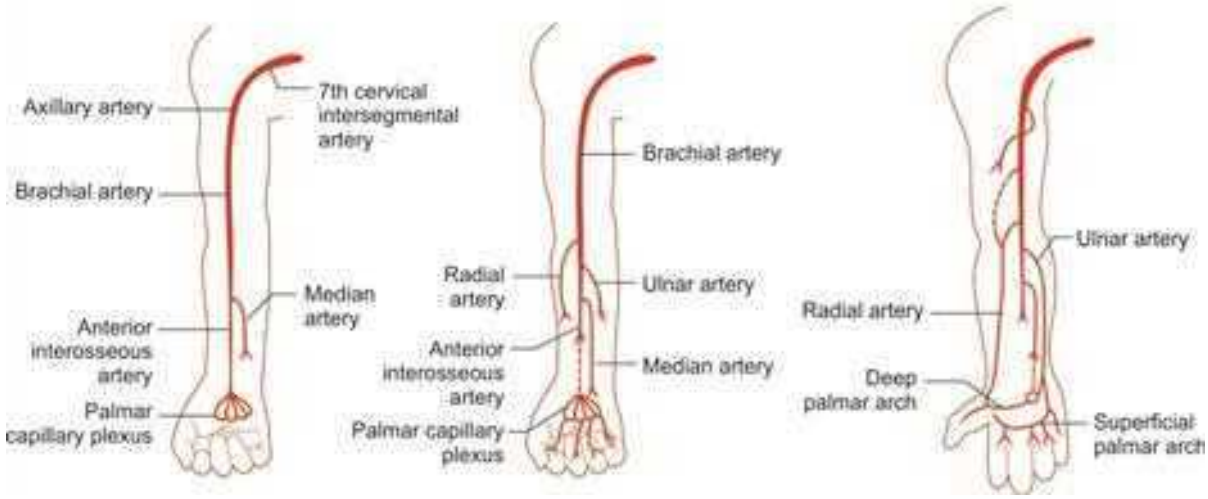


Fig. 15.44: Development of arteries of upper limb

vertebral (dorsal division) and internal thoracic (ventral division) arteries from it.

- The distal part of the *right subclavian artery* has a similar origin, but its proximal part is derived from the right fourth aortic arch.

Axis Artery of the Lower Limb

- It is derived from the *fifth lumbar intersegmental* artery.
- It is seen as a branch of the internal iliac and runs on the dorsal aspect of the limb.
- The femoral artery is a new vessel formed on the ventral aspect of the thigh.
- Proximally it gets linked above with the external iliac (which is a branch of the axis artery), and below with the popliteal artery.
- In the adult, the original axis artery is represented by (Fig. 15.45):
  - Inferior gluteal artery
  - A small artery accompanying the sciatic nerve
  - Part of the popliteal artery above the level of the popliteus muscle
  - Distal part of the peroneal artery
  - Part of the plantar arch.

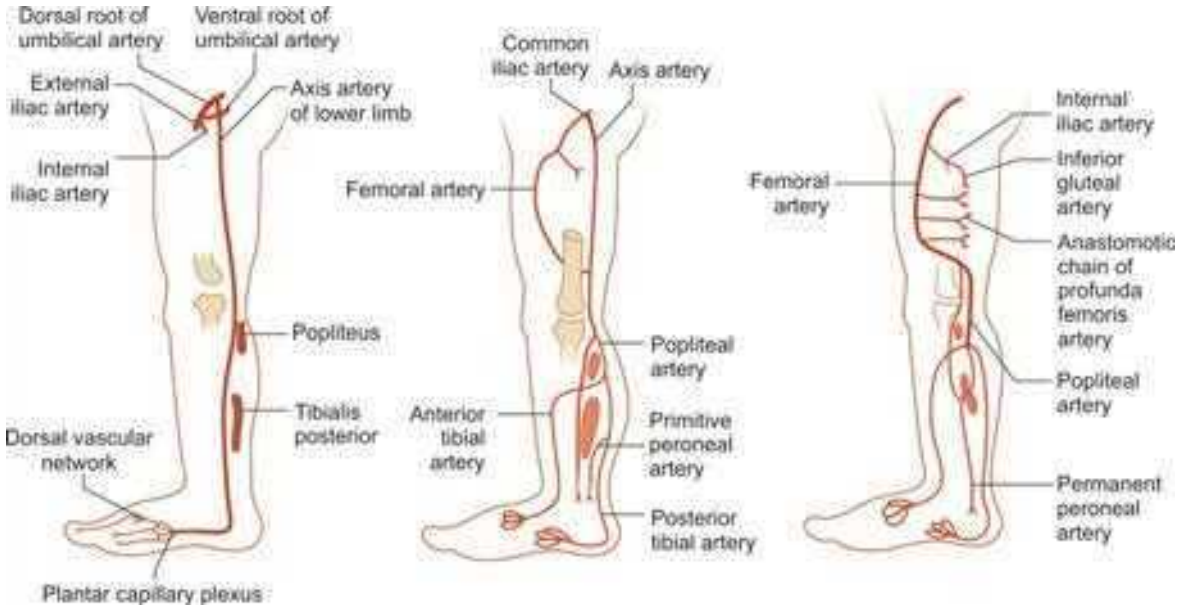


Fig. 15.45: Development of arteries of lower limb

PART 3: VEINS

INTRODUCTION

The main veins of the embryo are three sets/pairs of longitudinally directed veins—categorized into two groups (Fig. 15.46). All drain into sinus venosus.

1. Visceral veins
   - Vitelline/omphalomesenteric veins
   - Umbilical veins—placenta
2. Somatic veins
   - Cardinal veins.

Interconnections between the veins lead to establishment of shortest hemodynamic route by regression and/or enlargement of some veins. This results in the formation of major system of veins of adult.

- Portal system
- Caval system
- Azygos system.

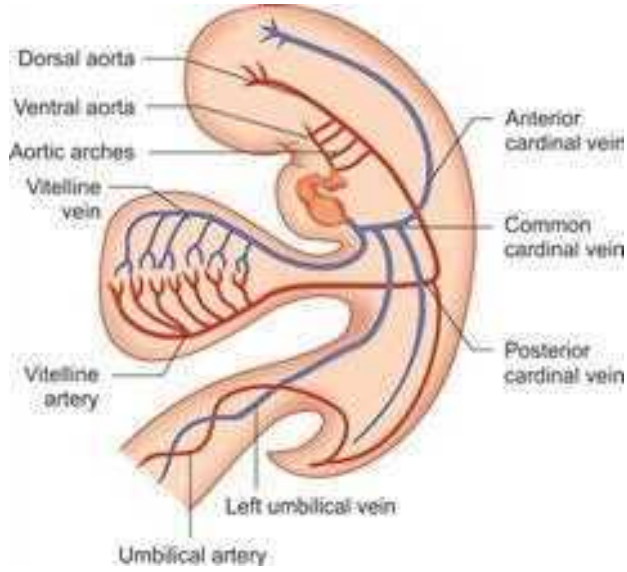


Fig. 15.46: Primitive veins of the embryo

VISCERAL VEINS

These are as follows:

1. Right and left *vitelline veins* from the yolk sac. These are also called *omphalomesenteric veins*.
2. Right and left *umbilical veins* from the placenta.

The umbilical and vitelline veins open into the corresponding horn of the sinus venosus (Fig. 15.47A). The parts of these veins that are nearest to the heart are embedded in the septum transversum. These veins undergo considerable changes as follows:

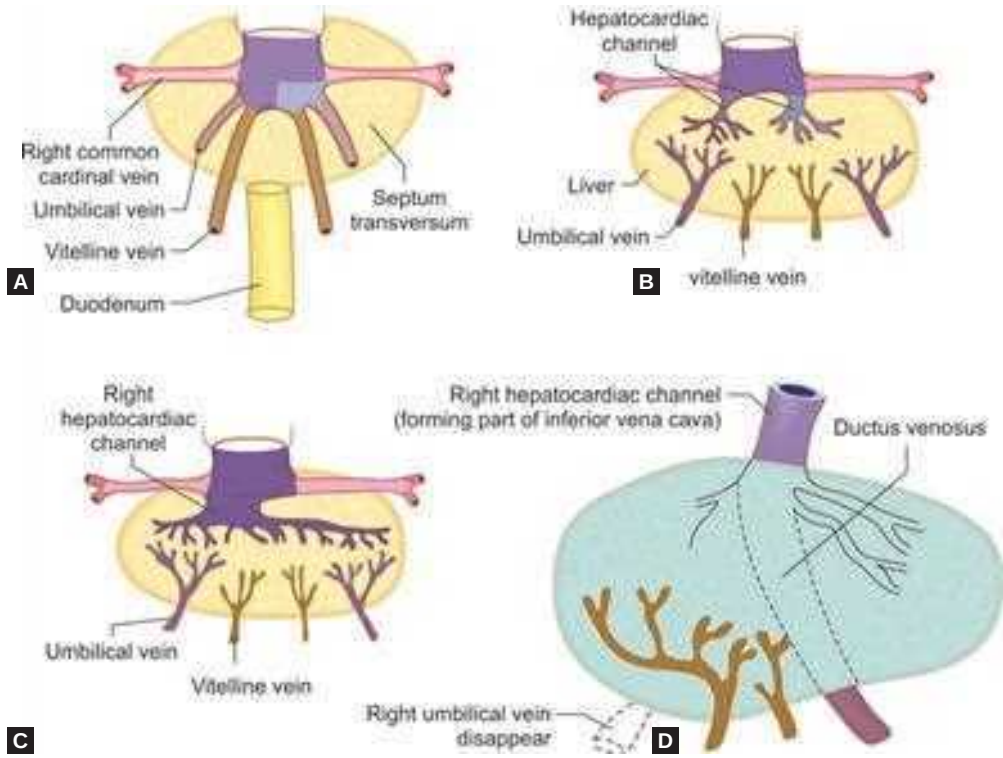
- With the appearance of hepatic bud in septum transversum, the vitelline veins can be divided into three parts
  1. Infrahepatic part
  2. Intrahepatic part
  3. Suprahepatic part.
- With the development of the liver, in the septum transversum, the proximal parts of the vitelline and umbilical veins become broken up into numerous small channels that contribute to the sinusoids of the liver.

These sinusoids drain into the sinus venosus, through the persisting terminal parts of the vitelline veins that are now called the right and left *hepatocardiac channels* (Fig. 15.47B). The proximal parts of the umbilical veins lose their communications with the sinus venosus.

- Meanwhile, the left horn of the sinus venosus undergoes retrogression and as result the left hepatocardiac channel disappears. All blood from the umbilical and vitelline veins now enters the sinus venosus through the right hepatocardiac channel (also called *common hepatic vein*). This vessel later forms the cranial most part of the inferior vena cava (Fig. 15.47C).
- The right umbilical vein disappears, and all blood from the placenta now reaches the developing liver through the left vein (*Note*: The left vein is "left") (Fig. 15.47D). In order to facilitate the passage of this blood through the liver, some of the sinusoids enlarge to create a direct passage connecting the left umbilical vein to the right hepatocardiac channel. This passage is called the *ductus venosus*.
- While these changes are occurring within the liver, the parts of the right and left vitelline veins that lie outside the substance of the liver undergo alterations leading to the formation of the *portal vein*.

Development of Portal Vein

- The proximal (infrahepatic) parts of the two vitelline veins lie on the right and left sides of the developing duodenum (Fig. 15.48A).
- The veins soon become interconnected by three transverse anastomoses, two of which lie ventral to the duodenum. The third anastomosis lies dorsal to the duodenum, and is between the two ventral anastomoses (Fig. 15.48B). These anastomoses form a figure of eight around the U-shaped duodenum.
  - Cephalic ventral anastomosis
  - Middle dorsal anastomosis
  - Caudal ventral anastomosis.
- The superior mesenteric and splenic veins (which develop independently) join the left vitelline vein, a short distance caudal to the dorsal anastomosis.
- Some parts of the vitelline veins now disappear. The portal vein and its right and left divisions are derived from the veins that remain (Fig. 15.48C).



Figs 15.47A to D: Umbilical and vitelline veins. (A) Note the umbilical and vitelline veins passing through the septum transversum to reach the sinus venosus; (B) Growth of liver cells within the septum transversum breaks up part of the umbilical and vitelline veins into capillaries. Blood reaching the liver through the umbilical and vitelline veins now goes to the heart through the right and left hepatocardiac channels; (C) Left hepatocardiac channel disappears; (D) Right hepatocardiac channel (which later forms part of the inferior vena cava) now drains the liver. Right umbilical veins disappear. All blood from the placenta now reaches the liver through the left umbilical vein. Formation of ductus venosus short circuits, this blood to the right hepatocardiac channel

The veins that disappear are:

- Part of the right vitelline vein caudal to the dorsal anastomosis
- Part of the left vitelline vein caudal to the entry of the superior mesenteric and splenic veins
- Caudal ventral anastomosis
- Left vitelline vein between dorsal anastomosis and cranial ventral anastomosis.

The veins that persist to form the *stem of the portal vein* are (Fig. 15.48C):

- The left vitelline vein between the entry of the superior mesenteric and splenic veins and the dorsal anastomosis (1, in Fig. 15.48C)
- The dorsal anastomosis itself (2, in Fig. 15.48C)
- The right vitelline vein between the dorsal anastomosis and the cranial ventral anastomosis (3, in Fig. 15.48C).

Left branch of portal vein is formed by:

- The cranial ventral anastomosis
- A part of the left vitelline vein cranial to cranial ventral anastomosis (4, in Fig. 15.48C).

Right branch of portal vein is formed by:

- Right vitelline vein cranial to cranial ventral anastomosis (5, in Fig. 15.48C).

Intrahepatic part of vitelline veins breaks down into a network of capillary plexus due to the growing hepatic laminae of cells. These join the hepatic sinusoids that develop in situ between hepatic laminae. The sinusoids that carry the blood of these branches to the liver substance constitute the *venae advehentes* (intrahepatic branches of portal vein). Those sinusoids that drain this blood to the inferior vena cava are called the *venae revehentes* (tributaries of the hepatic veins).

The left umbilical vein now ends in the left branch of the portal vein (left end of cephalic ventral anastomosis; Fig. 15.48D), while the ductus venosus connects the left branch of the portal vein to the inferior vena cava (right hepatocardiac channel).

The differences between vitelline, umbilical and cardinal veins are summarized in Table 15.3.

SOMATIC VEINS

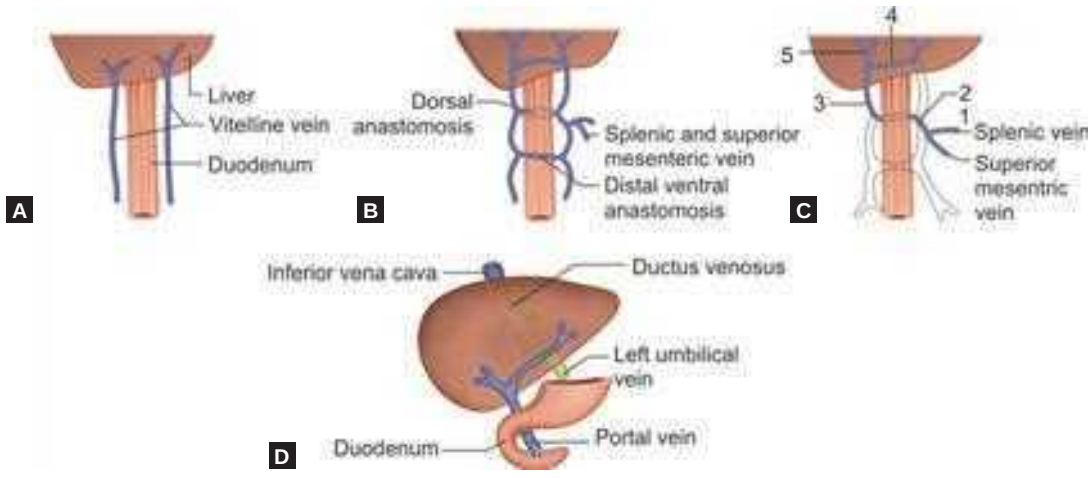
The earliest somatic veins are:

- Right and left *anterior cardinal veins* that drain the cranial part of the embryo, including the brain.
- Right and left *posterior cardinal veins* that drain the caudal part of the embryo.

The anterior and posterior cardinal veins of each side join to form the corresponding *common cardinal vein* (or *duct of Cuvier*), which opens into the corresponding horns of the sinus venosus (Fig. 15.49A).

Fate of Anterior Cardinal and Common Cardinal Veins

The anterior cardinal veins are joined by the subclavian veins that drain the forelimbs (Fig. 15.49B). Soon thereafter, the anterior cardinal veins become interconnected by a transverse anastomosis (Fig. 15.49C), proximal to their junction with the subclavian veins. The part of the left anterior cardinal vein caudal to this anastomosis retrogresses, and so does the left common cardinal (Fig. 15.49D).



Figs 15.48A to D: Development of the portal vein. (A) Right and left vitelline veins; (B) Vitelline veins joined by three transverse anastomoses: cranial ventral, caudal ventral and middle dorsal; (C) Some of the veins disappear. The portal vein is formed from: 1. Part of left vitelline vein. 2. Dorsal anastomosis. 3. Part of right vitelline vein. 4. The cranial ventral anastomosis becomes the left branch of the portal vein. 5. Right vitelline cranial to cranial ventral anastomosis forms right branch

Superior vena cava is derived from (Fig. 15.49E):

- Right anterior cardinal vein, caudal to the transverse anastomosis with the left anterior cardinal
- Right common cardinal vein. Note that the right horn of the sinus venosus forms part of the right atrium, and thus the superior vena cava comes to open into this chamber.

Right brachiocephalic vein is derived from the right anterior cardinal vein, between the point of its junction with the subclavian vein and the point of its junction with the transverse anastomosis (Fig. 15.49F).

Left brachiocephalic vein is derived from (Fig. 15.49G):

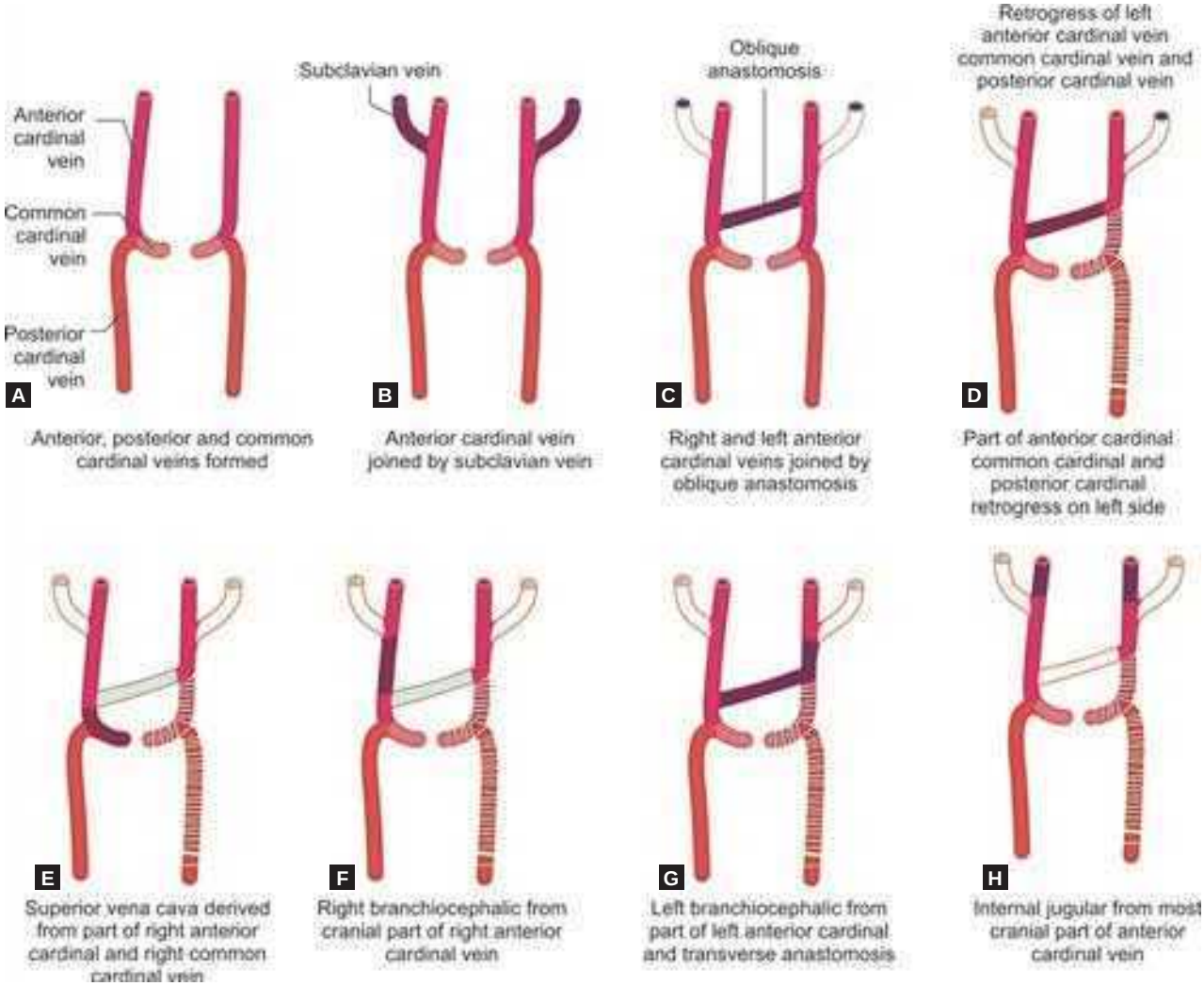
- The part of the left anterior cardinal vein corresponding to the right brachiocephalic vein
- The transverse intercardinal anastomosis.

Internal jugular veins develop from the parts of the anterior cardinal veins cranial to their junction with the subclavian veins (Fig. 15.49H).

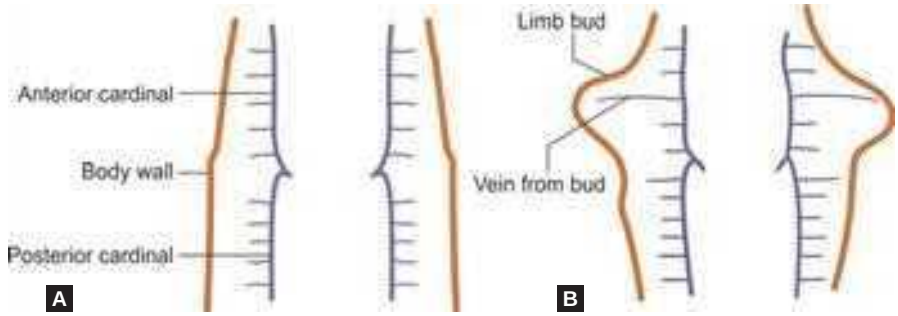
External jugular veins arise as secondary channels and are not derived from the anterior cardinal veins.

TABLE 15.3: Differences between three systems of veins

Vitelline veins	*Umbilical veins*	*Cardinal veins*
Drains from yolk sac	Drains from placenta through umbilical cord	Drains from body wall
Traverses through splanchnopleuric layer of coelom	Traverses through somatopleuric layer of coelom	Common cardinal vein passes along lateral wall of coelomic ducts
Terminates in floor of sinus venosus medial to umbilical vein and close to the gut	Terminates in floor of sinus venosus lateral to vitelline vein	Opens into the respective horn of sinus venosus
Contains poorly oxygenated blood	Contains well oxygenated blood	Contains poorly oxygenated blood



Figs 15.49A to H: Fate of anterior cardinal veins, and the development of major veins draining the upper part of the body



Figs 15.50A and B: Formation of the vein for the forelimb bud. In Figure (A), we see veins from the body wall draining into the anterior and posterior cardinal veins. In Figure (B), we see that one of these veins lying at the level of the limb bud enlarges to drain the limb

The anterior and posterior cardinal veins receive a series of *intersegmental veins* from the body wall (corresponding to the intersegmental branches of the dorsal aortae. The *subclavian veins* are formed by considerable enlargement of one of these veins in the region of the upper limb bud (Figs 15.50A and B).

Retrogressing veins of left side: Caudal part of the anterior cardinal vein and the whole of the common cardinal vein of the left side undergo retrogression. The greater part of the posterior cardinal vein of this side disappears, but a small part adjoining the common cardinal vein persists as a small vein. The left horn of the sinus venosus undergoes considerable retrogression and is reduced to a tributary of the right horn. These retrogressing veins of the left side persist into adult life as the left superior intercostal vein and the coronary sinus which are derived as follows:

The *left superior intercostal vein* is formed by (Fig. 15.51):

- The left anterior cardinal vein caudal to the transverse anastomosis
- The most cranial part of the left posterior cardinal vein. The second and third intercostal veins drain into this vein.

Coronary sinus: The medial part of the *coronary sinus* is derived from the left horn of the sinus venosus (Fig. 15.52). The lateral part of the coronary sinus is derived from the proximal part of the left common cardinal vein. The remaining part of the left common cardinal vein persists as the *oblique vein of the left atrium*.

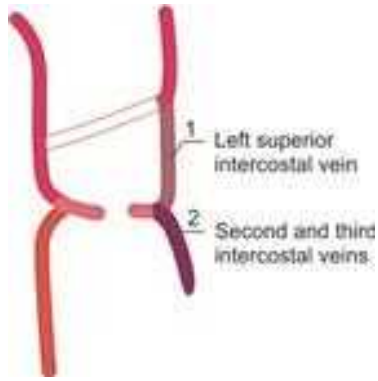
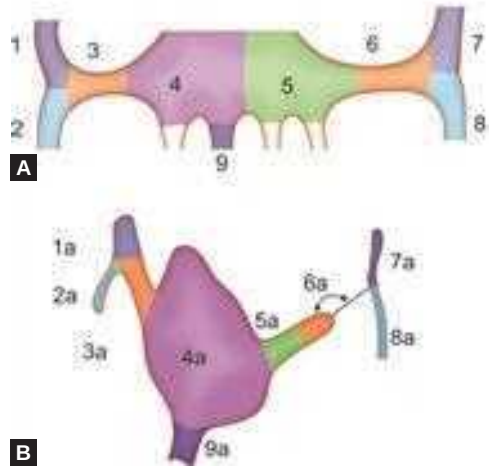


Fig. 15.51: Development of the left superior intercostal vein. The part labeled 1 is derived from the anterior cardinal vein; and part 2 from posterior cardinal vein. Note the intercostal veins draining into it

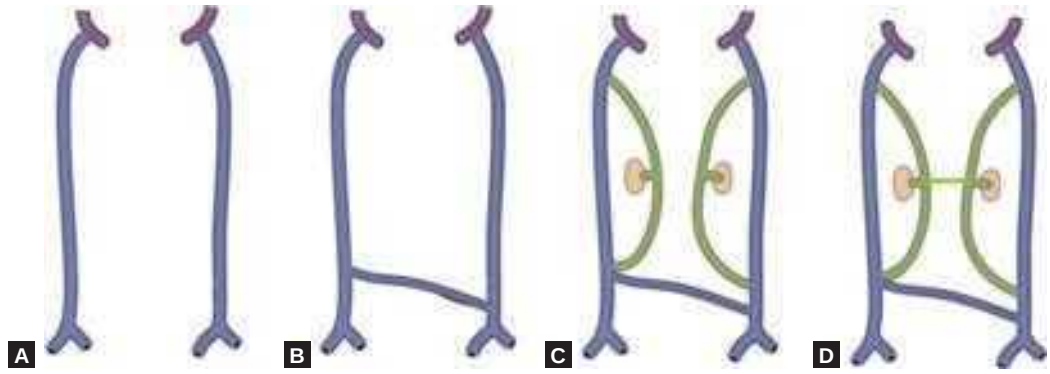


Figs 15.52A and B: (A) Derivation of the coronary sinus and related structures. 1 and 7 = right and left anterior cardinal veins; 2 and 8 = posterior cardinal veins; 3 and 6 = common cardinal veins; 4 and 5 = right and left horns of sinus venosus; 9 = right vitelline vein. The fate of these structures is shown in (B) 1a + 3a = superior vena cava; 2a = terminal part of the azygos vein; 4a = part of right atrium; 5a and proximal half of 6a = coronary sinus; distal half of 6a = oblique vein of left atrium; 7a + 8a = left superior intercostal vein; 9a = inferior vena cava

VEINS OF THE ABDOMEN

The inferior vena cava, the veins of the kidneys, gonads and suprarenals, and the veins draining the walls of the thorax and abdomen, are derived from a series of longitudinal venous channels that appear in the embryo. Some of these are as follows:

- *Posterior cardinal veins*: At their cranial ends, these veins join the anterior cardinal veins to form the common cardinal veins. Near their caudal ends, they receive the



Figs 15.53A to D: (A) Posterior cardinal veins; (B) Formation of transverse anastomosis; (C) Formation of subcardinal veins. Note that they drain the developing kidney; (D) The two subcardinal veins become interconnected

veins of the lower limb bud (external iliac) and of the pelvis (internal iliac) (Fig. 15.53A). The caudal ends of the two posterior cardinal veins become interconnected by a transverse anastomosis (Fig. 15.53B).

- *Subcardinal veins* (green in Figs 15.54A to D) are formed in relation to the mesonephros. Cranially and caudally they communicate with the posterior cardinal veins. The subcardinals receive the veins from the developing kidneys.

At the level of the renal veins, the two subcardinals become connected by a transverse *intersubcardinal anastomosis* (Figs 15.53C and D).

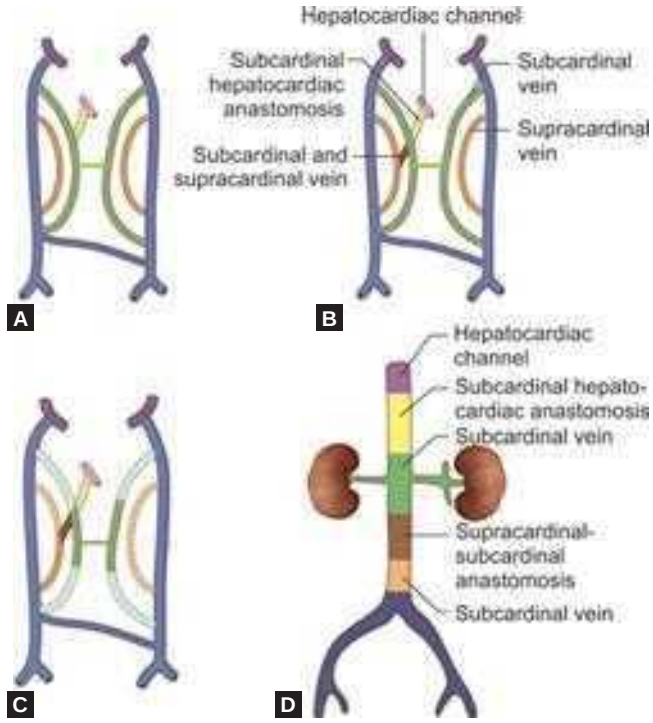
The cranial part of the right subcardinal vein also establishes an anastomosis with the right hepatocardiac channel (Fig. 15.54A).

- *Supracardinal veins* (also called thoracolumbar veins) (red in Figs 15.54A to D) communicate cranially and caudally with posterior cardinal veins. They also communicate with the subcardinal veins through anastomoses which join the subcardinals just below the renal veins (Fig. 15.54B).

Many parts of these longitudinal venous channels disappear (Fig. 15.54C). The veins that remain give rise to the inferior vena cava, renal veins, veins of gonads and the suprarenal veins as follows:

Inferior vena cava is derived from the following in caudal to cranial sequence (Fig. 15.54D):

- *Lowest part of the right posterior cardinal vein* (between its junction with the supracardinal, and the anastomosis between the two posterior cardinals).
- *Lower part of the right supracardinal vein* (between its junction with the posterior cardinal, and the supracardinal-subcardinal anastomosis).
- *Right supracardinal-subcardinal anastomosis.*
- *Right subcardinal vein* (between the supracardinal-subcardinal anastomosis and the anastomosis between the subcardinal vein and the right hepatocardiac channel). This is the *renal segment* of the vena cava.

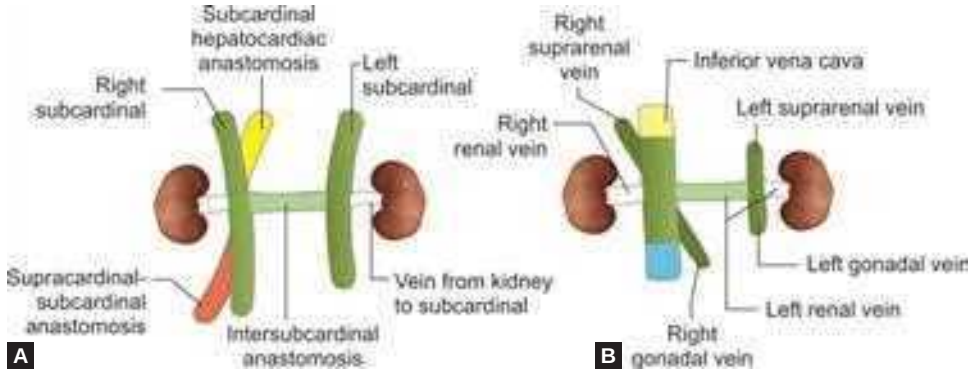


Figs 15.54A to D: (A to D) Development of the inferior vena cava. Subcardinal veins are green; supracardinal veins are orange; the subcardinal-hepatocardiac anastomosis is yellow; the hepatocardiac channel itself is purple; and the supracardinal-subcardinal anastomosis is brown. The inferior vena cava receives contributions from each of these components as indicated by the color in (D)

- *Subcardinal-hepatocardiac anastomosis.*
- *Right hepatocardiac channel.* (This is the *hepatic segment* of the vena cava).

Common iliac veins:

- *Right common iliac vein* is derived from the most caudal part of the right posterior cardinal vein.
- *Left common iliac vein* represents the anastomosis between the two posterior cardinal veins.



Figs 15.55A and B: (A and B) Formation of renal, suprarenal and gonadal veins. The right renal vein is formed as a tributary of the right subcardinal vein. The left renal vein is derived from vein draining left kidney into left subcardinal vein; part of left subcardinal vein itself; and intersubcardinal anastomosis. On each side, the suprarenal veins and gonadal veins represent remnants of the subcardinal veins. From (B), it is seen why these veins drain, on the right side into the inferior vena cava; and on the left side into the left renal vein

Renal veins:

- The *right renal vein* is a mesonephric vein that originally drains into the subcardinal vein (Fig. 15.55A). It opens into that part of the vena cava that` is derived from the subcardinal vein (Fig. 15.55B).
- The *left renal vein* is derived from:
  - The mesonephric vein that originally drains into the left subcardinal vein (Fig. 15.55A)
  - A small part of the left subcardinal vein
  - The intersubcardinal anastomosis. As this anastomosis lies in front of the aorta, the left renal vein has a similar relationship (Fig. 15.55B).

The *suprarenal veins* are remnants of the part of the subcardinal veins above the intersubcardinal anastomosis. It is clear from Figure 15.49B that the termination of the right suprarenal vein in the inferior vena cava, and that of the left suprarenal vein in the left renal vein, is because of their developmental origin.

The *testicular or ovarian veins* are remnants of the parts of the subcardinal veins below the intersubcardinal anastomosis. The reason for the difference in the manner of termination of the veins of the two sides is obvious from Figure 15.55B.

AZYGOS SYSTEM OF VEINS

The veins draining the body wall at first drain into the posterior cardinal vein (Fig. 15.56A). Their drainage is soon transferred to longitudinal venous channels called the *veins of the azygos line* (or medial sympathetic line) (Fig. 15.56B).

Cranially these channels drain into the posterior cardinal veins. The channels of the two sides are brought into communication with each other by vessels that run dorsal to the aorta (Fig. 15.56B).

With the retrogression of the left common cardinal vein, the left azygos line loses its communication with the posterior cardinal, and the blood of this channel now drains into the right azygos line through the postaortic anastomoses. The development of the azygos system of veins can now be summarized as follows (Fig. 15.56C).

- The *azygos vein* is formed from:
  - The vein of the right azygos line; and
  - The most cranial part of the right posterior cardinal vein through which it opens into the superior vena cava (formed from the right common cardinal).
- The vertical parts of the *hemiazygos* and the *accessory hemiazygos* veins represent the left azygos line. Their horizontal parts are formed by the postaortic anastomoses between the azygos lines of the two sides.
- The second and third left intercostal veins retain their connection with the left posterior cardinal vein, and are drained through the left superior intercostal vein.
- The abdominal parts of the veins of the azygos line are represented by the ascending lumbar veins.

Clinical correlation

Anomalies of veins

Minor anomalies in the mode of formation of various veins are extremely common. Anomalies of major veins are, however, rare. Some of these are as follows:

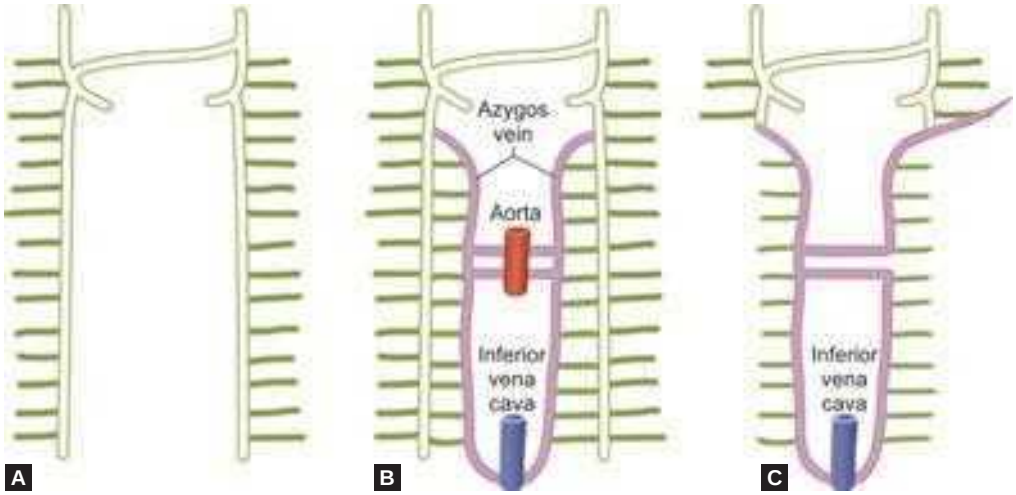
- ***Left superior vena cava***: This is due to the failure of the left anterior and common cardinal veins to retrogress. The left superior vena cava opens into the right atrium through a large coronary sinus. In this condition, the normal (right) superior vena cava may be reduced in size or may even be absent (Fig. 15.57).
- ***Double inferior vena cava (Figs 15.58A to D)***: Generally, the vena cava is double only below the level of the renal veins. Both channels may be present on the right side (Fig. 15.58B). This is caused by persistence of both the subcardinal and supracardinal veins below the level of the kidneys. There may be an additional channel on the left side (Figs 15.58C and D).

- ***Left inferior vena cava***: The infrarenal part of the vena cava may be present on the left side only (Fig. 15.58E).
- ***Azygos continuation of inferior vena cava***: The hepatic segment of the inferior vena cava may be absent. This is due to nondevelopment of the anastomosis between the right subcardinal vein and the right hepatocardiac channel. In such cases the upper part of the inferior vena cava follows the course of the azygos vein and opens into the superior vena cava. The hepatic veins open into the right atrium at the usual site of the inferior vena cava (Figs 15.58F and G).
- ***Preureteric vena cava***: The inferior vena cava normally lies posterior to the right ureter. Sometimes, it may be anterior to the right ureter. The ureter then hooks around the left side of the vena cava. This anomaly is caused when the infrarenal part of the vena cava develops from the subcardinal vein (which lies anterior to the ureter), instead of the supracardinal vein (which lies posterior to the ureter).

PART 4: FETAL CIRCULATION

The circulation in the fetus is essentially the same as in the adult except for the following (compare Fig. 15.59 with Fig. 15.60).

- *Placenta*: The source of oxygenated blood is not the lung but the placenta. Gaseous exchange takes place here.
- *Umbilical vein*: Oxygenated blood from the placenta comes to the fetus through the umbilical vein, which joins the left branch of the portal vein. A small portion of this blood passes through the substance of the liver to the inferior vena cava, but the greater part passes direct to the inferior vena cava through the ductus venosus (Fig. 15.47D).
- *Ductus venosus*: It is for bypassing hepatic circulation. A sphincter mechanism in the ductus venosus controls blood flow.



Figs 15.56A to C: (A) Veins from the body wall draining into anterior and posterior cardinal veins; (B) With the formation of the azygos venous channel (Az), most of the veins of the body wall now drain into it; (C) Shows the ultimate arrangement. Note that veins from the 1st intercostal space drain into the innominate veins directly (anterior cardinal). The veins of the left 2nd and 3rd spaces drain into the left superior intercostal vein which is formed partly by the anterior cardinal and partly by the posterior cardinal veins. On the right side, the veins of these spaces drain into the part of the azygos vein representing the terminal part of the right posterior cardinal

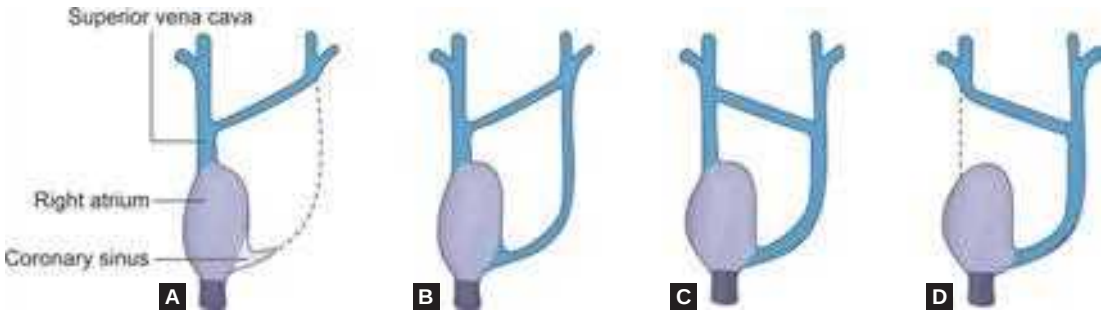
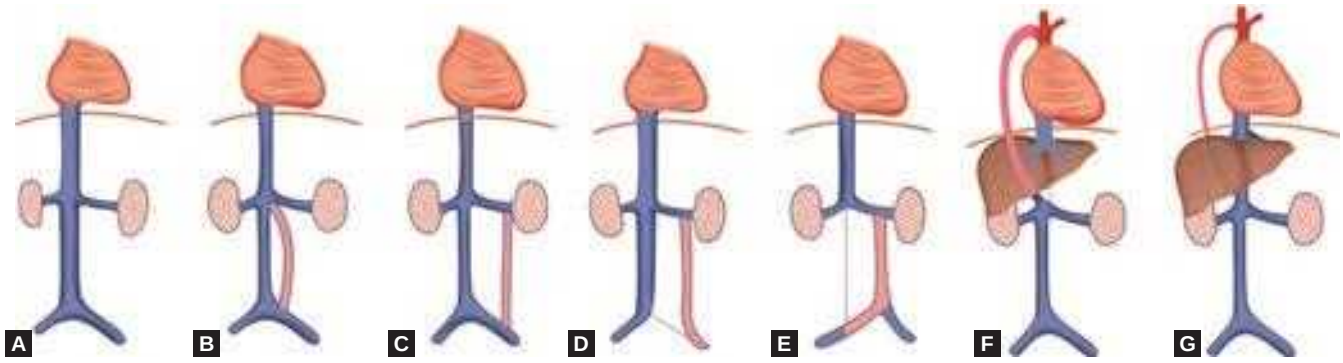


Fig. 15.57: Types of left superior vena cava. The normal pattern is shown in (A)



Figs 15.58A to G: Anomalies of the inferior vena cava. (A) Shows the normal pattern while (B), (C) and (D) show various types of duplication of the infrarenal segment. In (E) the normal infrarenal segment is absent and is replaced by a vessel on the left side; (F) Shows absence of the hepatic segment of the vena cava, the blood flow taking place along a much enlarged vena azygos; (G) Shows the corresponding normal pattern

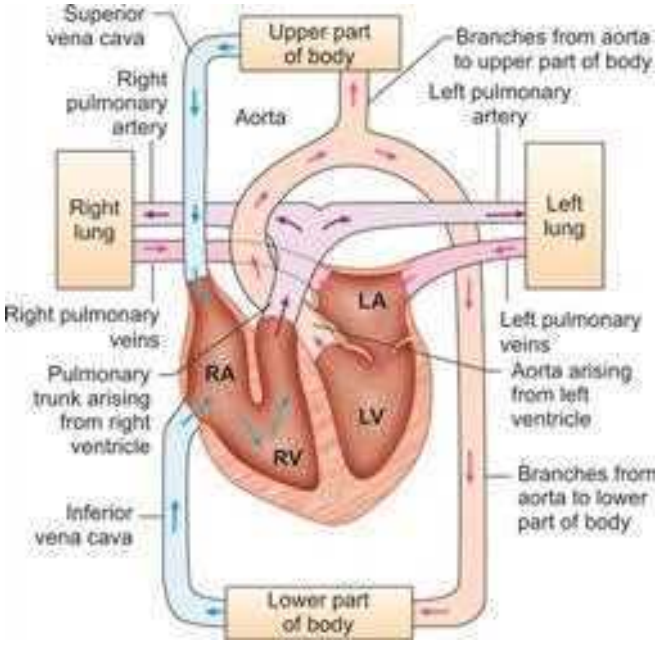


Fig. 15.59: Scheme of the circulation in the human adult

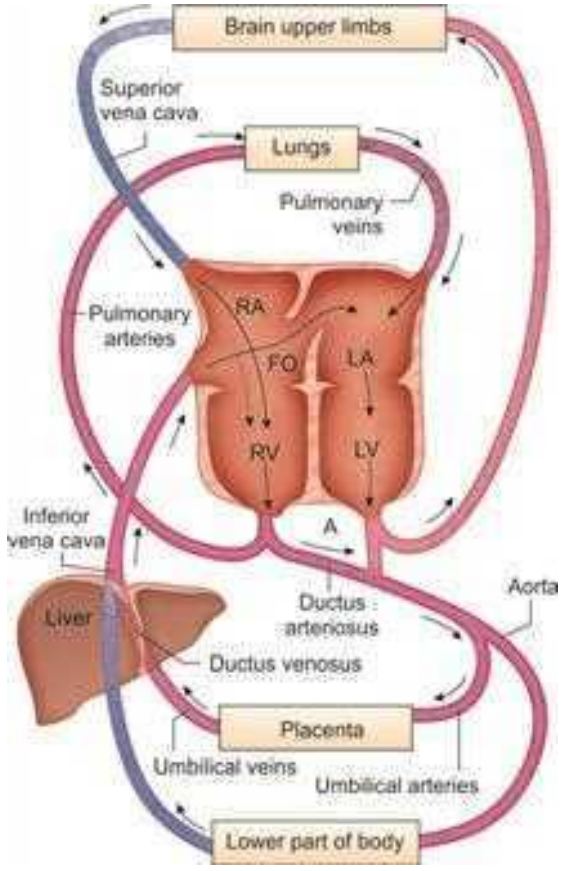


Fig. 15.60: Scheme of the fetal circulation. The degree of deoxygenation is shown by the intensity of shading. RA = right atrium; LA = left atrium; RV = right ventricle; LV = left ventricle; FO = foramen ovale

- *Foramen ovale*: It connects the two atria. The oxygen-rich blood reaching the right atrium through the inferior vena cava is directed by the valve of the inferior vena cava toward the foramen ovale. Here it is divided into two portions by the lower edge of the septum secundum (*crista dividens*):
  1. Most of it passes through the foramen ovale into the left atrium.
  2. The rest of it gets mixed up with the blood returning to the right atrium through the superior vena cava, and passes into the right ventricle.
- *Ductus arteriosus*: It is for bypassing pulmonary circulation. From the right ventricle, the blood (mostly deoxygenated) enters the pulmonary trunk. Only a small portion of this blood reaches the lungs, and passes through it to the left atrium. The greater part is short-circuited by the ductus arteriosus into the aorta.

- *Blood supply to fetus*:
  - We have seen that the left atrium receives blood from two sources the oxygenated blood from the right atrium and a small amount of deoxygenated blood from the lungs.
  - The blood in the left atrium is, therefore, fairly rich in oxygen. This blood passes into the left ventricle and then into the aorta. Some of this oxygen-rich blood passes into the carotid and subclavian arteries to supply the brain, the head and neck, and the upper extremities. The rest of it gets mixed up with poorly oxygenated blood from the ductus arteriosus.
  - The parts of the body that are supplied by branches of the aorta arising distal to its junction with the ductus arteriosus, therefore, receive blood with only moderate oxygen content.
- *Umbilical arteries*: They carry deoxygenated blood from fetus. Much of the blood of the aorta is carried by the umbilical arteries to the placenta where it is again oxygenated and returned to the heart.
- *Fetal circulation—peculiarities*:
  - Three times blood shunts along its course at:
    - Ductus venosus—to direct blood to inferior vena cava by passing liver without losing oxygen content
    - Foramen ovale—to equalize distribution to each half of heart and more oxygenated blood to upper half vital organs
    - Ductus arteriosus—to direct blood to placenta for oxygenation by passing lungs
  - More oxygenated blood for upper limb. Hence the length of upper limbs is more than lower limbs in the fetus.
  - Sphincteric action at the junction of left umbilical vein and ductus venosus regulates oxygen content of inferior vena cava and excessive load on heart
  - Admixture of oxygenated and deoxygenated blood takes place in the liver, terminal part of inferior vena cava, both atria and distal part of arch of aorta.
  - Transseptal blood flow throughout fetal life through ostium primum and foramen ovale.

CHANGES IN THE CIRCULATION AT BIRTH

Soon after birth, several changes take place in the fetal blood vessels. These lead to the establishment of the adult type of circulation. The changes are as follows:

- *Contraction of thick muscle wall*: The muscle in the wall of the umbilical arteries contracts immediately after birth, and occludes their lumen. This prevents loss of fetal blood into the placenta.

TABLE 15.4: Postnatal occlusion of vessels, their remnants and reasons for their closure

Vessel	*Remnant*	*Reasons for closure*
Umbilical arteries	Proximal part—superior vesical artery Distal part—fibrosed—medial umbilical ligament	Contraction of thick muscle wall
Left umbilical vein	Ligamentum teres of the liver	
Ductus venosus	Ligamentum venosum	• Functional closure within minutes after birth • Structural closure occurs within 3–7 days • Closure of ductus venosus is caused by strong contraction of its muscle wall
Ductus arteriosus	Ligamentum arteriosum	Smooth muscle contraction At birth, opposite direction of blood flow from aorta to pulmonary artery supplies more oxygenated blood than before that causes smooth muscle contraction

- The lumen of the umbilical veins and the ductus venosus is also occluded, but this takes place a few minutes after birth, so that all fetal blood that is in the placenta has time to drain back to the fetus.
- The ductus arteriosus is occluded, so that all blood from the right ventricle now goes to the lungs, where it is oxygenated. Initial closure of the ductus arteriosus is caused by contraction of muscle in the vessel wall. Later intima proliferation obliterates the lumen.
- The pulmonary vessels increase in size and, consequently, a much larger volume of blood reaches the left atrium from the lungs. As a result, the pressure inside the left atrium is greatly increased. Simultaneously, the pressure in the right atrium is diminished because blood from the placenta no longer reaches it. The net result of these pressure changes is that the pressure in the left atrium now exceeds that in the right atrium causing the valve of the foramen ovale to close.

The vessels that are occluded soon after birth are, in due course, replaced by fibrous tissue, and form the ligaments as shown in Table 15.4.

PART 5: LYMPHATIC SYSTEM

- The first signs of the lymphatic system are seen in the form of a number of endothelium-lined *lymph sacs*. Traditionally, these sacs have been considered to be

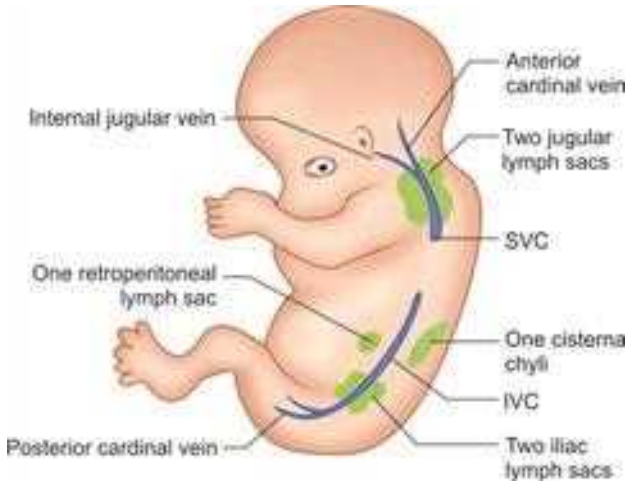


Fig. 15.61: Various lymph sacs

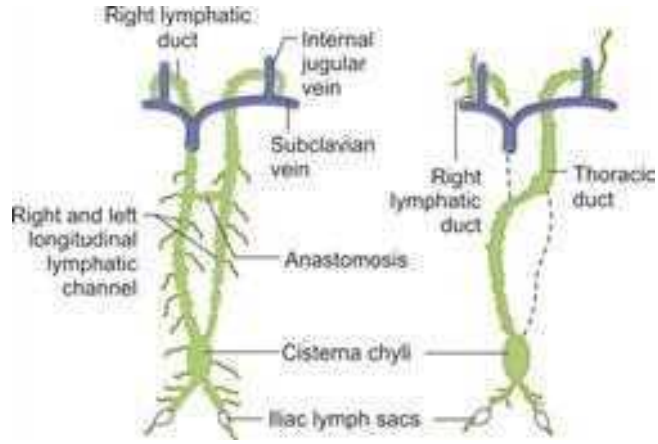


Fig. 15.62: Development of thoracic duct and right lymphatic duct

outgrowths from veins. However, they are now regarded to be predominantly independent formations from mesenchyme.

- There are six major lymph sacs that can be recognized. The right and left *jugular sacs* lie near the junction of the posterior cardinal and subclavian veins (i.e. at the future junction between the internal jugular and subclavian veins). The right and left *posterior (or iliac) sacs* lie around the corresponding common iliac vein. The *retroperitoneal sac* (unpaired) lies in relation to the root of the mesentery. The sixth sac (again unpaired) is the *cisterna chyli*. It lies in the midline some distance caudal to the retroperitoneal sac (Fig. 15.61).
- Lymphatic vessels are formed either by extension from the sacs or may form *de novo*, and extend into various tissues. Ultimately all the sacs except the cisterna chyli are invaded by connective tissue and lymphocytes, and are converted into groups of lymph nodes.
- The *thoracic duct* is derived from right and left channels that connect the cisterna chyli to the corresponding jugular sac. The two channels anastomose across the midline. The thoracic duct is formed from the caudal part of the right channel, the anastomosis between the right and left channels, and the cranial part of the left channel. The cranial part of the right channel becomes the *right lymphatic duct* (Fig. 15.62).

TIME TABLE OF SOME EVENTS DESCRIBED IN THIS CHAPTER

Time table of some events described in this chapter is shown in Table 15.5.

TABLE 15.5: Time table of some developmental events

Age	*Developmental events*
3rd week	Blood and vessels forming cells (angioblastic islands) appear. The cardiogenic area, heart tubes and pericardium are formed
4th week	Heart and pericardium lie ventral to foregut Subdivisions of heart tube are visible Heart begins to beat (becomes functional) Heart septa begin to form Aortic arches begin to establish in cranial to caudal sequence Most of the first aortic arch disappears at the end of 4th week Veins start forming
5th week	The spiral septum is formed Formation of aortic arches is complete Lymphatic sacs form The cardinal, umbilical and vitelline veins are formed Conduction system of heart forms
6th week	Coronary circulation is becoming established Atrioventricular valves and papillary muscles are forming
7th week	Heart septa are completely formed

Note: The heart is most susceptible to teratogens between 3rd and 6th weeks. It can be affected up to the 8th week.

EMBRYOLOGICAL BASIS FOR CLINICAL CONDITIONS OR ANATOMICAL OBSERVATIONS

Case Scenario 1

A prenatal transabdominal fetal ultrasound presented an abnormal image of fetal four-chambered heart showing a gap (marked with arrow) below the typical crossing as shown in Figure 15.63. What could be the cause for this gap and

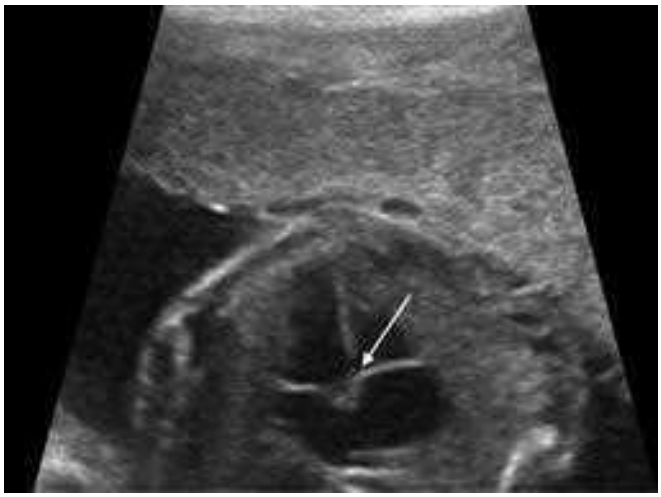


Fig. 15.63: Fetal ultrasound showing ventricular septal defect
Image Courtesy: Dr Ganesh Kumar and Dr Sasikala

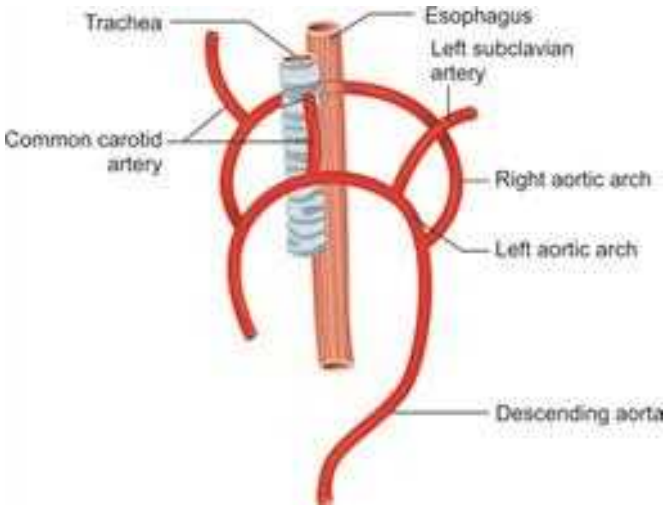


Fig. 15.64: Double aortic arch around trachea and esophagus

what structures forming the crossing. Give embryological explanation.

- A four-chambered ultrasound view of fetal heart is shown in Figure 15.63.
- The four-chambered heart is because of the formation of interatrial septum above, interventricular septum below and AV endocardial cushions laterally.
- Fusion of these structures forms the typical crossing seen in ultrasound.
- In this fetus, there is ventricular septal defect in the membranous part of interventricular septum forming patent interventricular foramen.
- Ventricular septal defects are the most common cardiac malformations of which defective membranous part is the most serious defect.

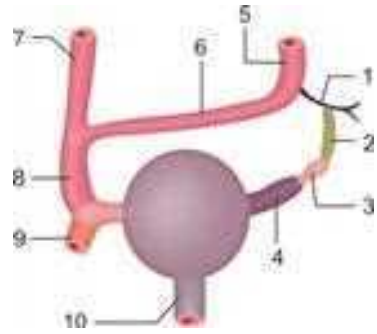


Fig. 15.65: Sinus venosus—its embryological components and adult derivatives

Case Scenario 2

Give the embryological explanation for the Figure 15.64.

- It is a diagram describing double aortic arch around trachea and esophagus.
- A vascular ring is surrounding the trachea and esophagus.
- In normal development, the right dorsal aorta between the origin of 7th cervical intersegmental artery and its fusion with left dorsal aorta disappears. In the present case, it persisted.
- The right aortic arch was passing behind the trachea and esophagus (foregut derivatives) and the left aortic arch was passing in front of them forming a vascular ring.
- The vascular ring compresses these structures and produces symptoms of dyspnea (difficulty in breathing) and dysphagia (difficulty in swallowing).

Case Scenario 3

Observe the diagram (Fig. 15.65) and name the embryological components and adult derivatives of the numbered structures.

1. Left anterior cardinal vein (caudal part)—left superior intercostal vein
2. Left anterior cardinal vein (caudal part)—ligament of left vena cava
3. Left common cardinal vein—oblique vein of left atrium
4. Left horn and body of sinus venosus—coronary sinus
5. Left anterior cardinal vein—part of left brachiocephalic vein
6. Anterior intercardinal anastomosis—part of left brachiocephalic vein
7. Right anterior cardinal vein—right brachiocephalic vein
8. Right anterior cardinal vein (caudal part) and right common cardinal vein—superior vena cava (extra-pericardial and intrapericardial parts respectively).
9. Right posterior cardinal vein (cephalic part)—arch of azygos vein
10. Suprahepatic part of right vitelline vein (common hepatic vein)—inferior vena cava (terminal part).

REVIEW QUESTIONS

1. Describe sinus venosus.
2. Explain development of right atrium.
3. Explain development of left atrium.
4. Write notes on interatrial septum.
5. Write notes on interventricular septum.
6. Explain development of pericardium.
7. Explain development of arch of aorta.
8. Explain development of subclavian artery.
9. Describe ductus arteriosus.
10. Explain development of vertebral artery.
11. Explain development of internal thoracic artery.
12. Describe axis artery of upper limb.
13. Describe axis artery of lower limb.
14. Explain development of portal vein.
15. Describe coronary sinus.
16. Explain development of inferior vena cava.
17. Describe fetal circulation.
18. Explain development of thoracic duct.

Chapter 16

Urogenital System

HIGHLIGHTS

- The *urogenital system* is derived from the *intermediate mesoderm*, and the *primitive urogenital sinus* (UGS) which is a part of the cloaca.
- The primitive UGS divides into the *vesicourethral canal* and *the definitive UGS*.
- The vesicourethral canal divides into the *urinary bladder* and the *primitive urethra*.
- The definitive UGS has a *pelvic part* and a *phallic part*.
- The *kidneys* develop from two sources. The excretory tubules (nephrons) are derived from the *metanephros* (= lowest part of nephrogenic cord which is derived from intermediate mesoderm). The collecting part is formed by ramification of the *ureteric bud* (which arises from the mesonephric duct).
- The *ureter* arises from the ureteric bud.
- The urinary bladder is derived from the cranial part of the vesicourethral canal (endoderm). The epithelium of the trigone is derived from absorbed mesonephric ducts.
- The *female urethra* is derived from the primitive urethra and the pelvic part of the UGS.
- In the male, the *prostatic urethra* corresponds to the female urethra. The *membranous urethra* is derived from the pelvic part of UGS and the *penile urethra* from the phallic part of the UGS. The terminal part is ectodermal.
- The *prostate* is formed by buds arising from the caudal part of the vesicourethral canal and the pelvic part of the UGS.
- The *uterine tubes* are derived from paramesonephric ducts (mesoderm).
- The *uterus* is formed from the uterovaginal canal (fused right and left paramesonephric ducts).
- *External genitalia* are formed from swellings that appear around the urogenital membrane.
- *Gonads* (testis and ovary) are derived from coelomic epithelium covering the nephrogenic cord. Ova and spermatozoa arise from *primordial germ cells* that arise in the region of the yolk sac. The testis is formed in the lumbar region, and later descends to the scrotum.
- The *duct system of the testis* is derived from mesonephric tubules and from the mesonephric duct.

INTRODUCTION

Urinary and genital systems are closely associated in their development, topography and function. Two embryonic structures that play an important role in the development of the urogenital system are the intermediate mesoderm and the cloaca. These are briefly considered below.

Intermediate Mesoderm

Intraembryonic mesoderm is subdivided into three parts (Fig. 16.1):

1. Paraxial mesoderm which becomes segmented to form the somites.
2. Lateral plate mesoderm in which the intraembryonic coelom appears.
3. Intermediate mesoderm lying between the two.

Before head fold the intermediate mesoderm is between paraxial mesoderm and coelomic cavity in lateral plate mesoderm. After the folding of the embryonic disc and the formation of the peritoneal cavity, the intermediate mesoderm forms a bulging on the posterior abdominal wall lateral to the attachment of the dorsal mesentery of

the gut. It is known as *urogenital ridge*. Its surface is covered by the epithelium lining the peritoneal cavity (*coelomic epithelium*) (Fig. 16.2A). The urogenital ridge is divided into two parts. The medial part is called genital ridge that gives rise to the genital system and a lateral part the *nephrogenic cord* that forms the urinary system.

The nephrogenic cord extends from the cervical region to the sacral region of the embryo. At varying stages of development, a number of important structures are formed in relation to the nephrogenic cord on each side. These are (Fig. 16.2B):

- *Excretory renal tubules* (para-, meso- and metanephric) associated with the development of the kidney.
- The *nephric duct* which is formed in relation to the developing excretory tubules. At later stages, this becomes the *mesonephric duct*.
- The *paramesonephric duct*, which is formed lateral to the nephric duct.
- The *gonad (testis or ovary)*, which develops from the coelomic epithelium lining the medial side of the nephrogenic cord.

Cloaca

The part of hindgut caudal to allantois is called cloaca. It is divided by the urorectal septum (in the angle between allantois and cloaca) into dorsal *primitive rectum* and ventral *primitive urogenital sinus* (Figs 13.3 and 13.4).

In further development, the primitive urogenital sinus is subdivided into a cranial part, called the *vesicourethral canal*, and a caudal part, called the *definitive urogenital sinus*. The openings of the mesonephric ducts (see below) lie at the junction of these two subdivisions (Figs 16.3 and 16.4A).

Still later, the definitive urogenital sinus shows a division into a cranial, *pelvic part*, and a caudal *phallic part* (Fig. 16.4B).

The urogenital system is derived from the various structures that develop in the intermediate mesoderm, and from the various subdivisions of the cloaca, as described below.

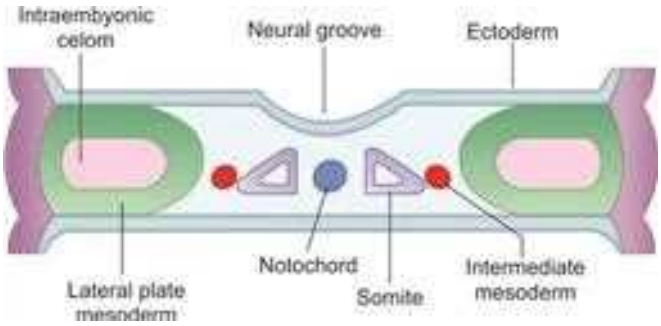
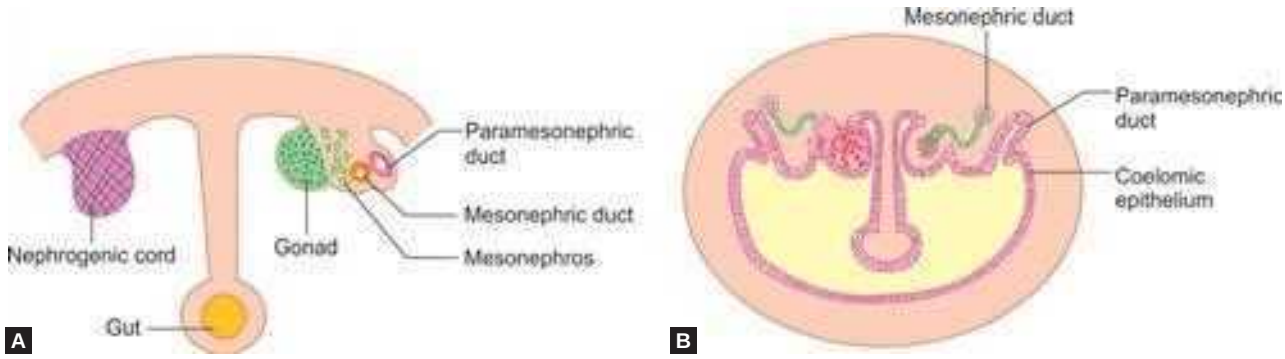


Fig. 16.1: Location of intermediate mesoderm

DEVELOPMENT OF KIDNEYS

- The definitive human kidney arises from two distinct sources.
  1. *The secretory part*, i.e. excretory tubules (or *nephrons*) are derived from the lowest part of the nephrogenic cord. This part is the metanephros, the cells of which form the *metanephric blastema*.
  2. *The collecting part* of the kidney is derived from a diverticulum called the ureteric bud which arises from the lower part of the mesonephric duct (Figs 16.5A and B).
- Some of the features of the development of the kidney in the human embryo can be appreciated only if the evolutionary history of the organ is kept in mind. The vertebrate kidney has passed through three stages of evolution (Fig. 16.6).
  1. The most primitive kidney is called the *pronephros*. It is the functioning kidney in some cyclostomes and teleost fishes. The pronephros is formed in relation to the cervical region of the nephrogenic cord. This is followed by appearance of the mesonephros in the thoracolumbar region, and finally by formation of



Figs 16.2A and B: (A) Nephrogenic cord and (B) structures that develop in it

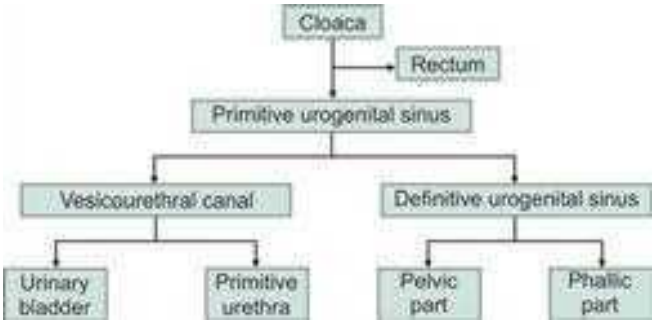
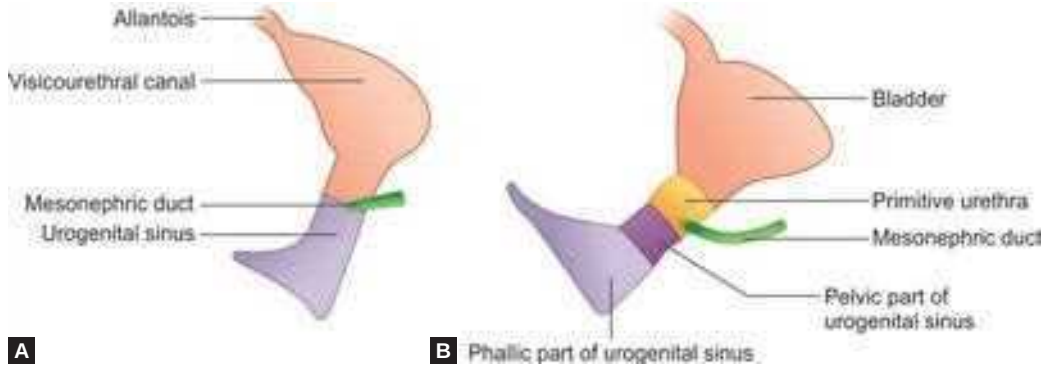


Fig. 16.3: Subdivisions of the cloaca. Also see Figure 16.4

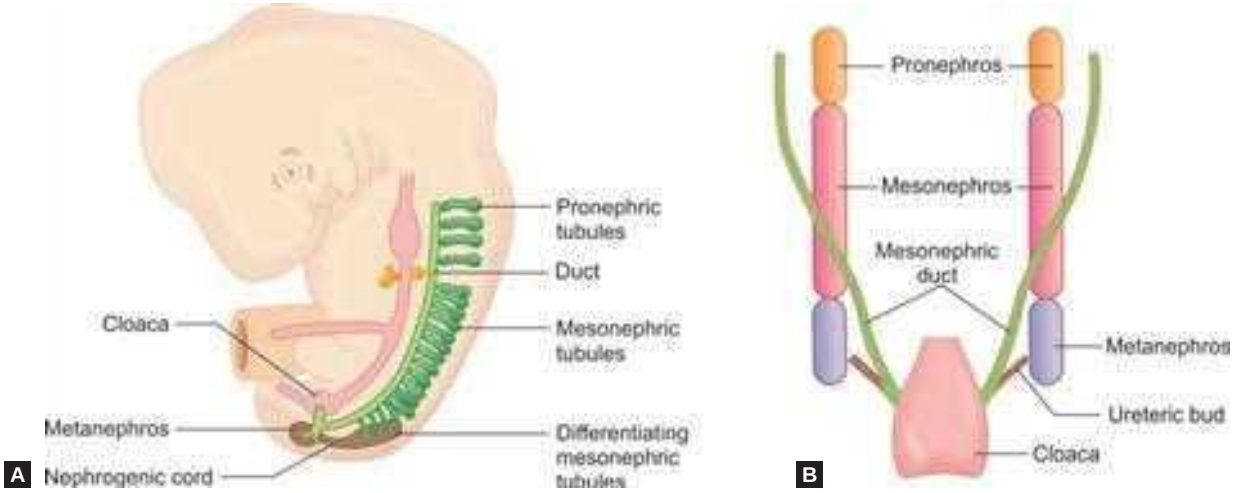
the metanephros in the sacral region (Figs 16.5 and 16.6). The human pronephros is nonfunctional, and disappears soon after its formation. A *nephric duct* formed in relation to the pronephros and ending in the cloaca, however, persists.

2. The pronephros has been succeeded in higher vertebrates by the *mesonephros* that is the functioning kidney of most anamniotes (Amphbia and some fishes). The mesonephros consists of a series of excretory tubules that develop in the thoracolumbar region. These tubules drain into the nephric duct which may now be called the *mesonephric duct*. Most of the mesonephric tubules disappear, but some of them are modified and take part in forming the duct system of the testis (Fig. 16.6).
3. The kidney of amniotes (including man) is called the *metanephros*. It begins in the sacral region. It is seen as an unsegmented mass called *metanephric blastema*.

- During the development of the human embryo, the evolutionary history of the kidney repeats itself being a classic example of the saying that *ontogeny repeats phylogeny*.
- As the ureteric bud grows cranially toward the metanephric blastema, its growing end becomes dilated to form an *ampulla*. The ampulla divides repeatedly. The first three to five generations of branches fuse to form the



Figs 16.4A and B: Subdivisions of the primitive urogenital sinus. Also see Figure 16.3



Figs 16.5A and B: (A) Lateral view of embryo showing pronephros, mesonephros and metanephros; (B) The mesonephric duct opening into the cloaca and giving off the ureteric bud

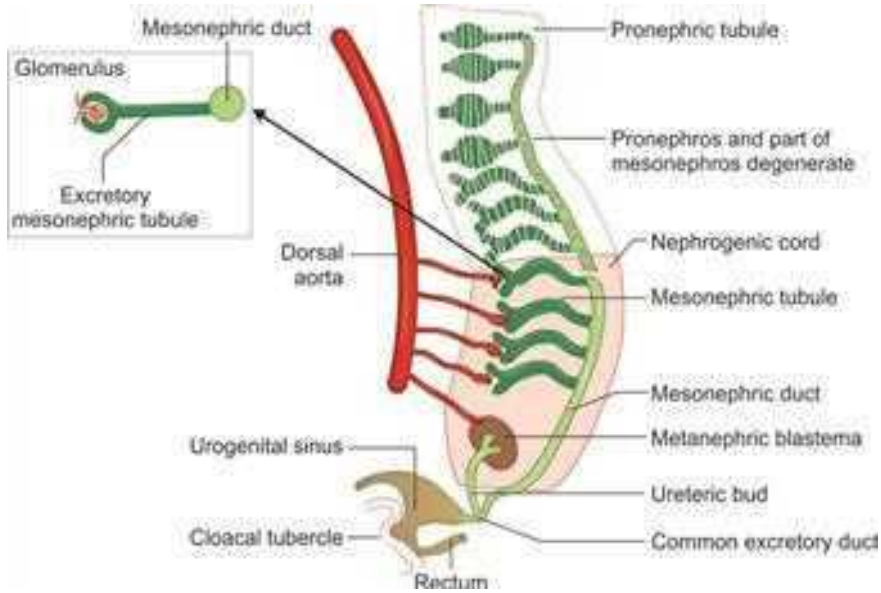
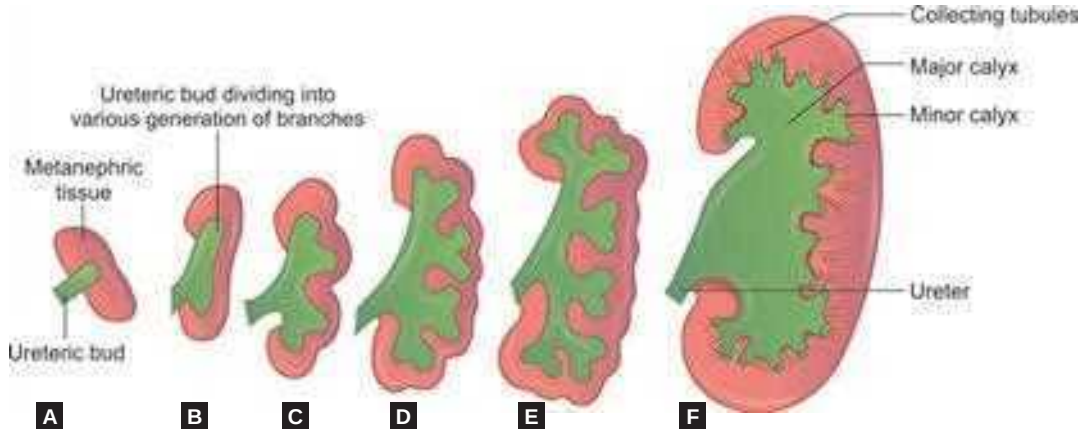


Fig. 16.6: Some details of developing pronephros, mesonephros and metanephros. The pronephros and pronephric duct degenerate soon after formation. The proximal part of the mesonephros shows segmentation (in craniocaudal sequence). The segments contain functional excretory tubules that drain into the mesonephric duct. Most of these tubules disappear by the time the metanephros forms the definitive kidney



Figs 16.7A to F: Formation of the collecting system of the kidney, from ramifications of the ureteric bud

pelvis of the kidney. The next divisions become the *major calyces* while further divisions form the *minor calyces* and *collecting tubules* (Figs 16.7A to F). The number of collecting tubules formed is one to three million.

- The cells of the metanephric blastema in contact with an ampulla undergo differentiation to form a *nephron*. This differentiation is induced by the ampulla. Loosely arranged cells of the metanepric blastema form solid clumps in relation to the ampulla. Each solid clump is converted into a vesicle. The vesicle soon becomes pear-shaped and opens into the ampulla. The vesicle now becomes an S-shaped tube. Its distal end comes to be invaginated by a tuft of capillaries which form a *glomerulus*. The various parts of the nephron are derived from this S-shaped tube. The various stages in the formation of a nephron are shown in Figure 16.8.

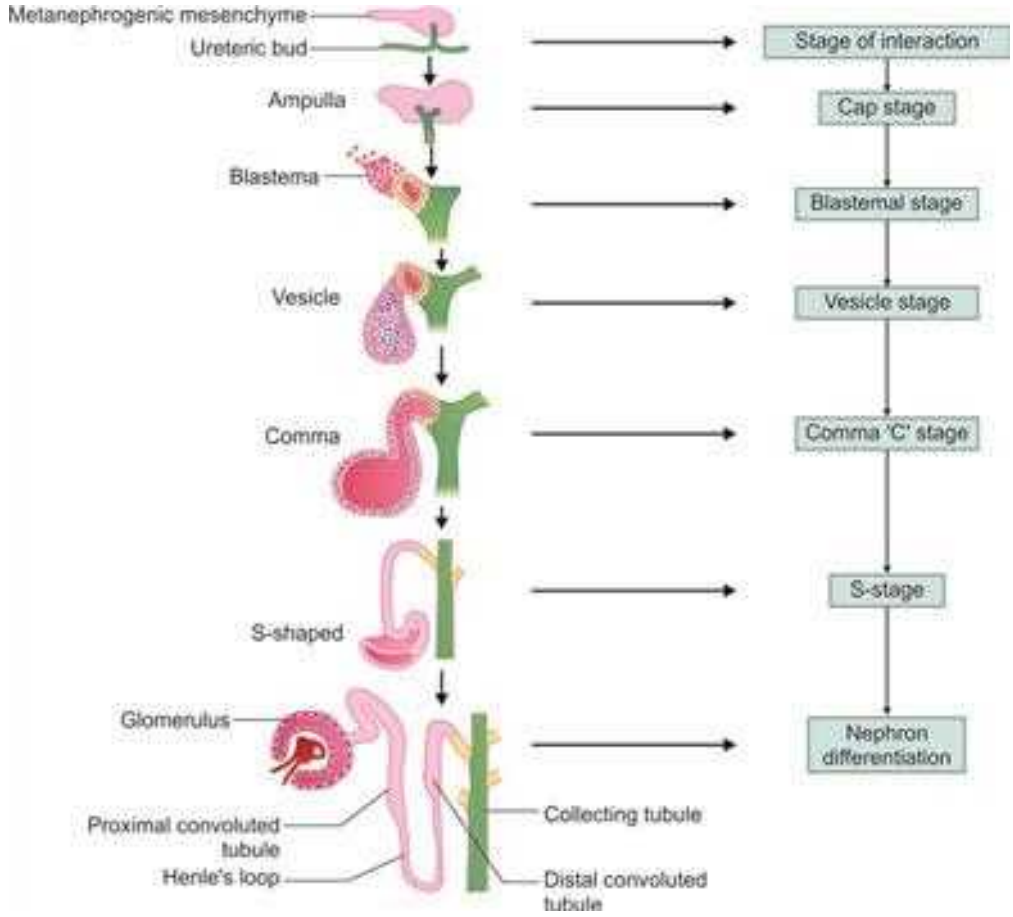


Fig. 16.8: Scheme to show stages in the development of the nephron

Ascent of the Kidney

- The definitive human kidney is derived from the metanephros and lies in the sacral region in the initial stages of development. In subsequent development of the embryo, differential growth of the abdominal wall causes the kidney to ascend to the lumbar region (Fig. 16.9).
- The metanephros, at first, receives its blood supply from the lateral sacral arteries, but with its ascent, higher branches of the aorta take over the supply. The definitive renal artery represents the lateral splanchnic branch of the aorta at the level of the second lumbar segment.
- During ascent, the kidneys pass through the fork like interval between the right and left umbilical arteries. If the arteries come in the way of ascent, the kidney may remain in the sacral region.

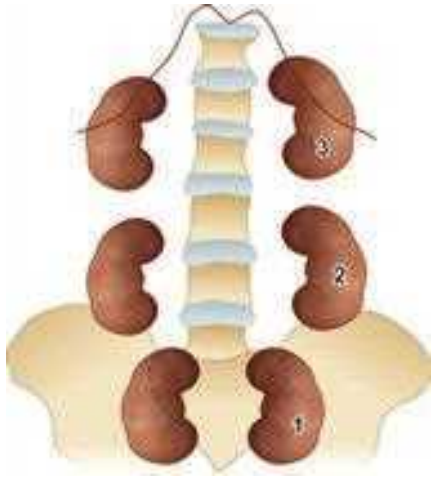


Fig. 16.9: Ascent of the kidney

Rotation of the Kidney

The hilum of the kidney, at first, faces anteriorly. The organ gradually rotates so that the hilum comes to face medially.

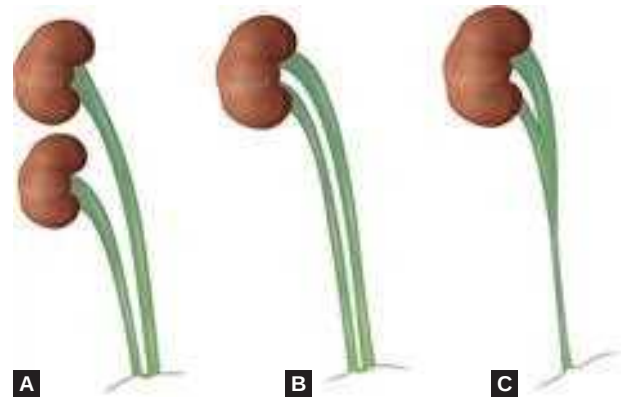
Clinical correlation

Anomalies of kidneys

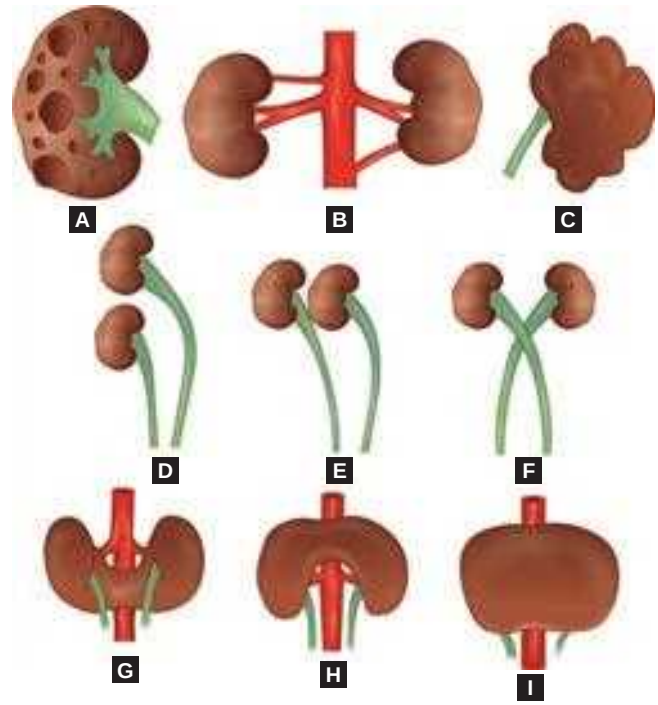
- One or both kidneys may be absent (***agenesis***). The kidney may be underdeveloped (***hypoplasia***) or overdeveloped (***hyperplasia***). Adrenal tissue may be present within the substance of the kidney.
- Distention of the pelvis with urine (***hydronephrosis***) may occur as a result of obstruction in the urinary passages.
- ***Duplication***: There may be an extra kidney on one side. It may be separate, or may be fused to the normal kidney (Figs 16.10A to C).
- ***Anomalies of shape***:
  - *Horseshoe kidney:* The lower poles of the two kidneys (or sometimes the upper poles) may be fused. The connecting isthmus may lie either in front of, or behind, the aorta and inferior vena cava (Figs 16.11G and H). A horseshoe kidney does not ascend higher than the level of the inferior mesenteric artery as the latter prevents its higher ascent.
  - *Pancake kidney:* The two kidneys may form one mass, lying in the midline or on one side (Fig. 16.11I). The two kidneys may lie on one side, one above the other, the adjacent poles being fused.
  - *Lobulated kidney:* The fetal kidney is normally lobulated. This lobulation may persist (Fig. 16.11C).
- ***Anomalies of position***:
  - The kidneys may fail to ascend. They then lie in the sacral region.
  - The ascent of the kidneys may be incomplete as a result of which they may lie opposite the lower lumbar vertebrae.
  - The kidneys may ascend too far, and may even be present within the thoracic cavity.
  - Both kidneys may lie on one side of the midline. They may lie one above the other or side by side (Figs 16.11D and E). The ureter of the displaced kidney crosses to the opposite side across the midline.
  - Both kidneys may be displaced to the opposite side. The two ureters then cross each other in the midline (Fig. 16.11F).
- ***Abnormal rotation***:
  - *Nonrotation*: The hilum is directed forward.
  - *Incomplete rotation*: The hilum is directed anteromedially.
  - *Reverse rotation*: The hilum is directed anterolaterally.
- ***Congenital polycystic kidney***: Failure of the excretory tubules of the metanephros to establish contact with the collecting tubules, leads to the formation of cysts. Isolated cysts are commonly seen, but sometimes the whole kidney is a mass of such cysts (Fig. 16.11A). The cysts press upon normal renal tissue and destroy it. An alternative recent view about the formation of cysts in the kidney is that they are derived from abnormally developed collecting tubules.
- ***Aberrant renal arteries***: The kidney may receive its blood supply partially or entirely, from arteries arising at an abnormal level (Fig. 16.11B). In the case of nonascent, or of incomplete ascent, the aberrant arteries may constitute the only supply to the organ. An aberrant artery may be the only source of arterial blood to a segment of the kidney. It may press upon the ureter and cause obstruction, leading to hydronephrosis.
- ***Multiple anomalies:*** Two or more of the above anomalies may coexist. Anomalies of position are frequently associated with those of rotation.

ABSORPTION OF LOWER PARTS OF MESONEPHRIC DUCTS INTO CLOACA

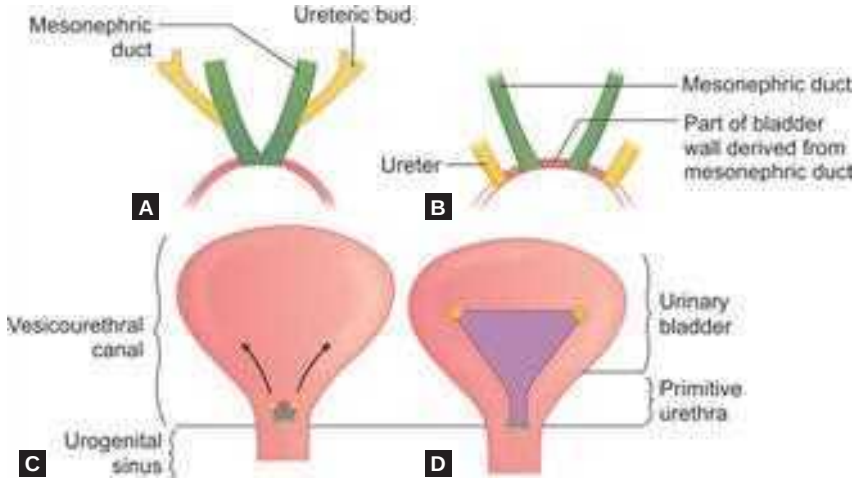
The lower ends of the mesonephric ducts open into that part of the cloaca that forms the urogenital sinus. The ureteric buds arise from the mesonephric ducts, a little cranial to the



Figs 16.10A to C: Anomalies of ureters. Also see Figure 16.11



Figs 16.11A to I: Anomalies of the kidney. (A) Congenital polycystic kidney; (B) Aberrant renal arteries; (C) Lobulated kidney; (D to F) Transposition of kidney; (G and H) Horseshoe kidney; (I) Pancake kidney



Figs 16.12A to D: (A) Mesonephric duct opens into primitive urogenital sinus; (B) As the sinus grows the proximal parts of mesonephric ducts are absorbed so that the mesonephric ducts and ureters now open separately; (C) The openings are at first close together; (D) Further absorption of ureters causes their opening to shift upward and laterally. The shaded area is derived from absorbed parts of ureters and mesonephric ducts, and is mesodermal. It forms the trigone of the bladder and the posterior wall of part of the urethra

cloaca (Fig. 16.12A). The parts of the mesonephric ducts, caudal to the origin of the ureteric buds, are absorbed into the vesicourethral canal; with the result the mesonephric ducts and the ureteric buds now have separate openings into the cloaca (Fig. 16.12B). These openings are at first close together (Fig. 16.12C). However, the openings of the ureteric buds move cranially and laterally due to continued absorption of the buds. The triangular area (on the dorsal wall of the vesicourethral canal) between the openings of the ureteric buds and those of the mesonephric ducts is derived from the absorbed ducts and is, therefore, of mesodermal origin (Fig. 16.12D).

DEVELOPMENT OF THE URETER

The ureter is derived from the part of the ureteric bud that lies between the pelvis of the kidney, and the vesicourethral canal.

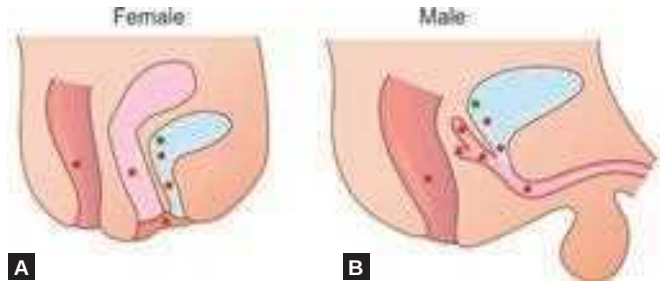
Clinical correlation

Anomalies of the ureter

- The ureter may be partially or completely duplicated (Figs 16.10A to C). This condition may, or may not, be associated with duplication of the kidney. Very rarely, there may be more than two ureters on one, or both, sides. Of the two ureters, one may open into the urinary bladder while the other may open at an abnormal site (see below).
- Instead of opening into the urinary bladder, the ureter may end in the prostatic urethra, ductus deferens, seminal vesicles, or rectum, in the male (Fig. 16.13B); and in the urethra, vagina, vestibule or rectum in the female (Fig. 16.13A).
- The upper end of the ureter may be blind, i.e. it is not connected to the kidney.
- The ureter may be dilated (***hydroureter***) because of obstruction to urine flow.
- The ureter may have valves or diverticula.
- The right ureter may pass behind the inferior vena cava. It then hooks around the left side of the vena cava; this may result in kinking and obstruction of the ureter. The real defect is in the development of the vena cava as described in Chapter 15.

DEVELOPMENT OF THE URINARY BLADDER

- The part of primitive urogenital sinus above the openings of the mesonephric ducts is called vesicourethral canal. It presents a wider upper part and a narrower lower part. The allantois opens into the apex of the upper wider part of vesicourethral canal. The allantois extends from



Figs 16.13A and B: Abnormal sites at which the ureter may open

the apex of the urinary bladder. The urinary bladder develops from this dilated part of vesicourethral canal including the proximal part of allantois. The lower narrow part of vesicourethral canal becomes the primitive urethra.

- With the absorption of mesonephric ducts and ureteric buds into the posterior wall of vesicourethral canal, the trigone of bladder will be formed. The trigone is mesodermal in origin.
- The epithelium of the urinary bladder develops from the cranial part of the vesicourethral canal (endoderm). The epithelium of the trigone of the bladder is derived from the absorbed mesonephric ducts (mesoderm) (However, it is later overgrown by the surrounding endodermal cells).
- The muscular and serous walls of the organ are derived from splanchnopleuric mesoderm.
- The developing bladder is continuous cranially with the allantois. It is uncertain whether the allantois contributes to the formation of the bladder. The allantois atrophies, and is seen in postnatal life as a fibrous band, the urachus (median umbilical ligament), extending from the apex of the bladder to the umbilicus.

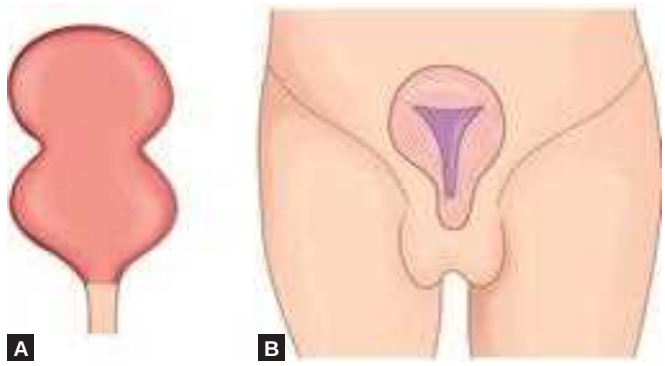
Clinical correlation

Anomalies of the urinary bladder

- The urinary bladder may be absent, or may be duplicated. The sphincter vesicae may be absent.
- The lumen of the urinary bladder may be divided into compartments by septa.
- The bladder may be divided into upper and lower compartments (***hourglass bladder***) because of a constriction in the middle of the organ (Figs 16.14A).
- The bladder may communicate with the rectum (Fig. 13.25A).
- ***Ectopia vesicae***: The lower part of the anterior abdominal wall, as well as the ventral wall of the bladder, may be missing. As a result, the cavity of the bladder may be exposed on the surface of the body (Fig. 16.14B). This defect is usually associated with epispadias. Ectopia vesicae is caused by failure of mesoderm to migrate into the lower abdominal wall (between umbilicus and genital tubercle). Failure of migration may be due to excessive development of the cloacal membrane. The ectoderm of the anterior abdominal wall and the endoderm of the ventral wall of the urinary bladder remain unsupported and thin. Their rupture leads to the exposure of the cavity of the urinary bladder.
- ***Congenital diverticula*** may be present. These are found at the junction of the trigone with the rest of the bladder.

DEVELOPMENT OF THE FEMALE URETHRA

The female urethra is derived from the caudal part of the *vesicourethral canal* (endoderm). We have seen that the posterior wall of this canal is derived from the mesonephric ducts and is, therefore, mesodermal in origin. The female urethra may receive a slight contribution from the pelvic part of the urogenital sinus (Figs 16.15A to E).



Figs 16.14A and B: Anomalies of the bladder. (A) Hourglass bladder; (B) Ectopia vesicae. The ureteric openings and the trigone are seen on the surface of the body

DEVELOPMENT OF THE MALE URETHRA

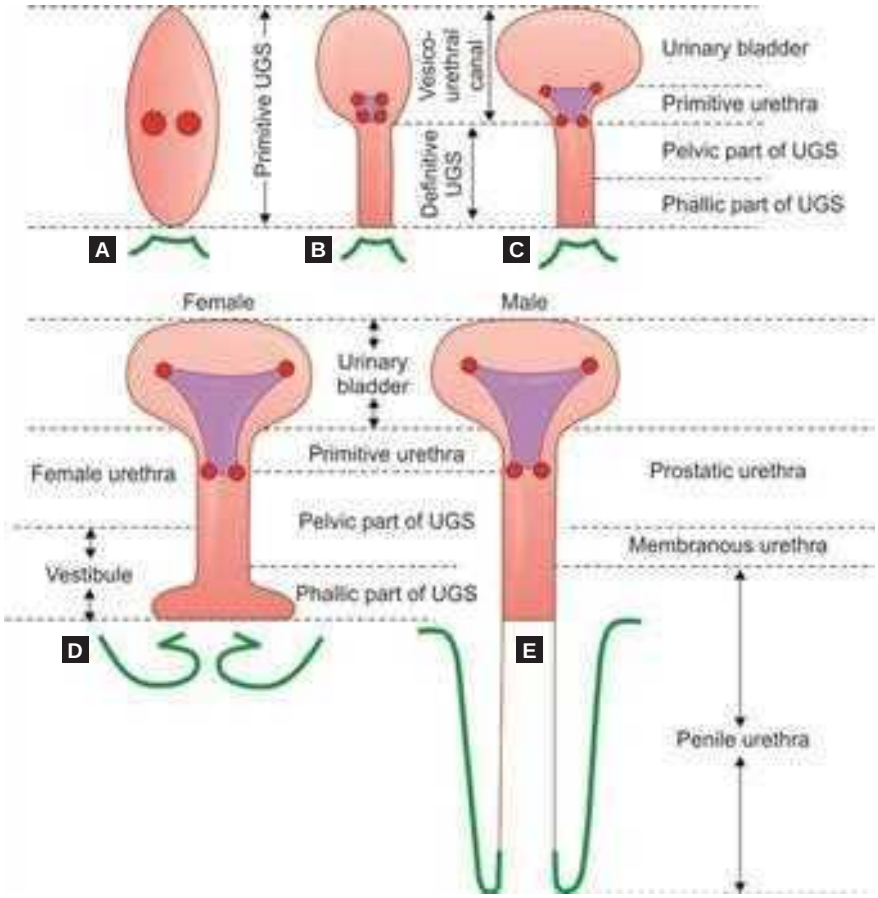
- The part of the male urethra extending from the urinary bladder up to the openings of the ejaculatory ducts (original openings of mesonephric ducts) is derived from the caudal part of the vesicourethral canal (endoderm). The posterior wall of this part is derived from absorbed mesonephric ducts (mesoderm). (It may later be overgrown by endoderm).
- The rest of the prostatic urethra, and the membranous urethra, are derived from the pelvic part of the definitive urogenital sinus.
- The penile part of the urethra (except the terminal part) is derived from the epithelium of the phallic part of the definitive urogenital sinus (see "Development of Penis").
- The terminal part of the penile urethra that lies in the glans is derived from ectoderm (Figs 16.15A to E).

From the above, it will be clear that the female urethra corresponds to the prostatic part of the male urethra.

Clinical correlation

Anomalies of the urethra

- There may be obstruction to the urethra at its junction with the bladder.
- The urethra may show diverticula.
- It may be duplicated in whole or in part.
- The urethra may have abnormal communications with the rectum (Figs 13.25B and C), the vagina (Fig. 16.16) or the ureter (Figs 16.13A and B).
- Hypospadias and epispadias.



Figs 16.15A to E: Development of urethra: (A) Primitive urogenital sinus (UGS) showing opening of mesonephric ducts; (B) Primitive UGS divided into vesicourethral canal and definitive UGS. Mesonephric ducts and ureters open separately at the junction of the two parts; (C) Vesicourethral canal subdivided into urinary bladder and primitive urethra. The definitive urogenital sinus (UGS) divides into pelvic and phallic parts; (D) The female urethra is formed from the primitive urethra and from part of the pelvic portion of UGS. The rest of the pelvic part of UGS forms the vestibule; (E) In the male, the prostatic urethra is formed in the same way as the female urethra. The membranous urethra is derived from the pelvic part of UGS. The penile urethra is derived from the phallic part of UGS. Red circles = openings of mesonephric ducts and ureters. Blue = part derived from mesoderm. Green = ectoderm

DEVELOPMENT OF THE PROSTATE

This gland develops from a large number of buds that arise from the *epithelium of the prostatic urethra*, i.e. from the caudal part of the vesicourethral canal, and from the pelvic part of the definitive urogenital sinus. These buds form the *secretory epithelium* of the gland. The buds that arise from the mesodermal part of the prostatic urethra (i.e. posterior wall, above the openings of the ejaculatory ducts) form the *inner glandular zone* of the prostate. Buds arising from the rest of the prostatic urethra (endoderm) form the *outer glandular zone* (Figs 16.17A).

The outer zone differentiates earlier than the inner zone. In later life, the outer zone is frequently the site of carcinomatous change, while the inner zone is affected in senile hypertrophy of the organ. The muscle and connective tissue of the gland are derived from the surrounding

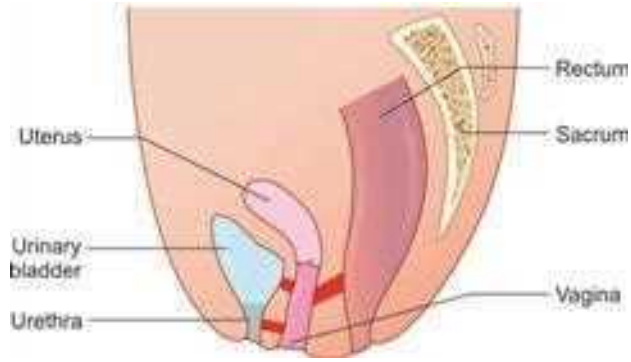
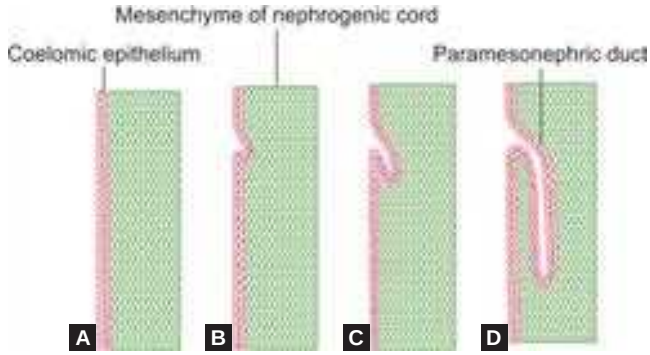
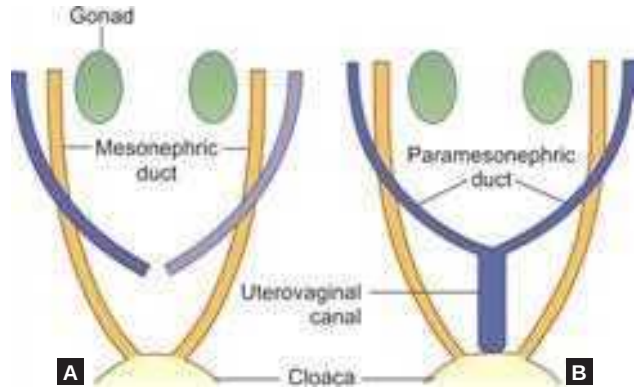


Fig. 16.16: Vaginal fistulae are abnormal communications between vagina and surrounding cavities. The fistulae are shown in solid black. They may connect the vagina to the rectum (rectovaginal fistula); to the urinary bladder (vesicovaginal fistula) or to the urethra (ureterovaginal fistula)

Figs 16.17A and B: Mesodermal and endodermal derivatives of the prostate. The glands of the median lobe, which open onto the posterior wall of the prostatic urethra (above the opening of the ejaculatory ducts), are mesodermal. (A) Transverse section above the level of the opening of ejaculatory ducts; (B) Sagittal section



Figs 16.18A to D: Formation of paramesonephric ducts by invagination of coelomic epithelium



Figs 16.19A and B: Formation of uterovaginal canal by fusion of the caudal parts of paramesonephric ducts

mesenchyme which also forms the capsule of the gland. The secretory elements of the prostate are rudimentary at birth. They undergo considerable development at puberty. The organ undergoes progressive atrophy in old age, but in some men it undergoes benign hypertrophy. The prostate may, rarely, be absent.

Female Homologues of Prostate

Endodermal buds, similar to those that form the prostate in the male, are also seen in the female. The buds that arise from the caudal part of the vesicourethral canal give rise to the *urethral glands*, whereas the buds arising from the urogenital sinus form the *paraurethral glands of Skene*.

PARAMESONEPHRIC DUCTS

Paramesonephric ducts are present in the intermediate mesoderm. They are formed by invagination of coelomic epithelium (Figs 16.18A to D). They lie lateral to the mesonephric ducts in the cranial part of the nephrogenic cord (Fig. 16.19A). When traced caudally, they cross to the medial side of the mesonephric ducts. Here the ducts of the two sides meet and fuse in the middle line to form the *uterovaginal canal* (or *uterine canal*) (Fig. 16.19B). The caudal end of this canal comes in contact with the dorsal wall of the definitive urogenital sinus. In the female, this part of the sinus gives rise to the vestibule. In the female, the paramesonephric ducts give origin to the *uterine tubes*, the *uterus*, and *part of the vagina* (Fig. 16.20A).

DEVELOPMENT OF UTERUS AND UTERINE TUBES

The epithelium of the uterus develops from the fused paramesonephric ducts (uterovaginal canal: 1, in Figure 16.20A). The myometrium is derived from surrounding mesoderm (3, in Figure 16.20A). As the thickness of the myometrium increases, the unfused horizontal parts of the two paramesonephric ducts come to be partially embedded within its substance, and help to form the fundus of the uterus (2, in Figure 16.20A). The cervix can soon be recognized as a separate region. In the fetus, the cervical part is larger than the body of the uterus.

The uterine tubes develop from the unfused parts of the paramesonephric ducts. The original points of invagination of the ducts into the coelomic epithelium remain as the abdominal openings of the tubes. Fimbriae are formed in this situation.

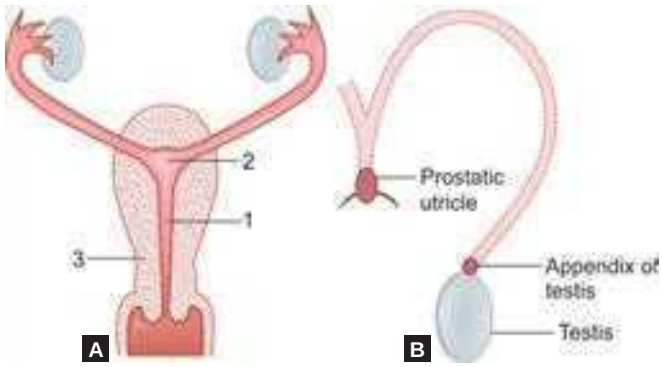
Clinical correlation

Anomalies of the uterus

- The uterus may be in the form of two horns (bicornuate, Fig.16.21A) or completely or partially separated (septate, Fig.16.21B). Complete duplication of uterus and cervix is referred to as ***uterus didelphys*** (Fig.16.21F).
- The uterus is in two horns, the cervix is separated and the vagina is single and is known as ***uterus bicornis*** and ***bicolis*** (Fig. 16.21C).
- The entire uterus may be absent.
- Uterus may be slightly indented in the middle and is known as ***arcuate uterus*** (Fig.16.21D).
- Uterus and vagina both may be separated in to two and is known as ***subseptate uterus*** (Fig.16.21E).
- The uterus may remain rudimentary.
- There may be atresia of the lumen either in the body or in the cervix.

Anomalies of the uterine tubes

- The uterine tubes may be absent, on one or both sides.
- The tubes may be partially, or completely, duplicated on one or both sides.
- There may be atresia of the tubes.



Figs 16.20A and B: Fate of paramesonephric ducts. (A) In the female, they form the uterine tubes, the uterus, and part of the vagina; (B) In the male, most of the duct disappears. Remnants are seen as the appendix of the testis and the prostatic utricle. 1: uterovaginal canal; 2: fundus of uterus; 3: myometrium

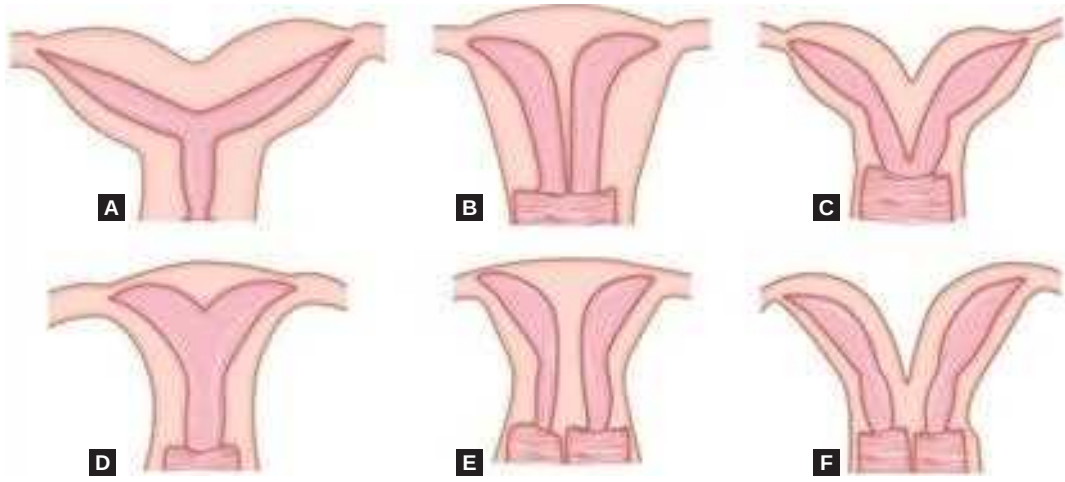
DEVELOPMENT OF VAGINA

- The lower end of the uterovaginal canal comes in close contact with the dorsal wall of the phallic part of the urogenital sinus (Fig. 16.22A). However, the uterovaginal canal and the urogenital sinus are soon separated from each other by the formation of a solid plate of cells called the *vaginal plate*. The vagina is formed by the development of a lumen within the vaginal plate (Fig. 16.22D). The vaginal plate is formed as follows:
  - Endodermal cells of the urogenital sinus proliferate to form two swellings called the *sinovaginal bulbs* (Fig. 16.22B). These bulbs soon fuse to form one mass.
  - Most of the vaginal plate is formed from these sinovaginal bulbs (Fig. 16.22C).
  - The part of the vaginal plate near the future cervix is derived from mesodermal cells of the uterovaginal canal.
  - The hymen is situated at the junction of the lower end of the vaginal plate with the urogenital sinus. Both surfaces of the hymen are lined by endoderm.

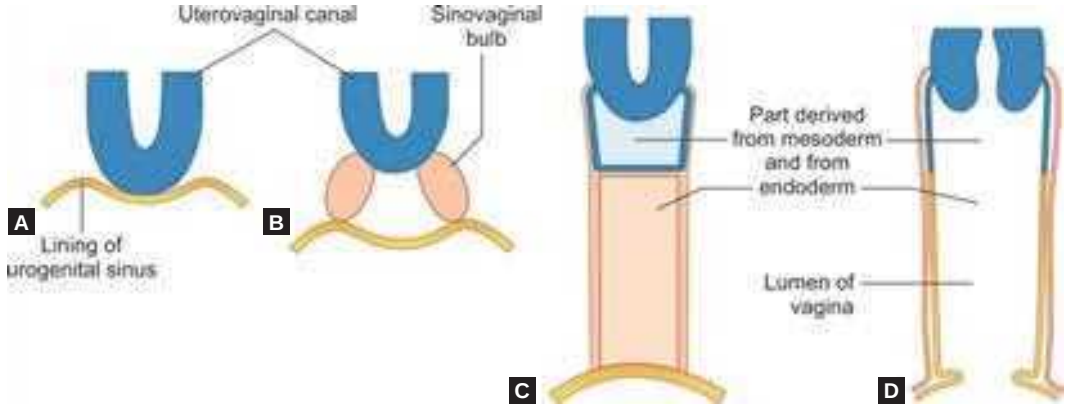
Clinical correlation

Anomalies of vagina

- The vagina may be duplicated. This condition is usually associated with duplication of the uterus (Fig. 16.21A).
- The lumen may be subdivided longitudinally, or transversely, by a septum.
- The vagina may be absent. This condition may or may not be associated with absence of the uterus.
- The hymen may be imperforated.
- The vagina may have abnormal communications with the rectum (***rectovaginal fistula***) or with the urinary bladder (***vesicovaginal fistula***) (Fig. 16.16).



Figs 16.21A to F: Anomalies of the uterus: (A) Bicornuate uterus; (B) Septate uterus; (C) Bicornis bicollis uterus; (D) Uterus arcuatus; (E) Subseptate uterus; (F) Didelphys uterus



Figs 16.22A to D: (A) Uterovaginal canal (mesoderm) in contact with lining of urogenital sinus (UGS) (endoderm); (B) Sinovaginal bulbs are formed by proliferation of endodermal lining; (C) Solid vaginal plate derived partly from mesoderm of uterovaginal canal and partly from endoderm of sinovaginal bulbs; (D) Vagina formed by canalization of vaginal plate

Paramesonephric Ducts in Male

The paramesonephric ducts remain rudimentary in the male. The greater part of each duct eventually disappears (Fig. 16.20B). The cranial end of each duct persists as a small rounded body attached to the testis (*appendix of testis*) that may occasionally give rise to cysts.

It has generally been considered that the *prostatic utricle* represents the uterovaginal canal and is, therefore, a homologue of the uterus. However, it is now believed to correspond mainly to the vagina (and possibly part of the uterus).

DEVELOPMENT OF EXTERNAL GENITALIA

Introduction

With the formation of the urorectal septum, the cloacal membrane comes to be subdivided into a ventral, urogenital membrane, and a caudal anal membrane (Figs 16.23). The urogenital membrane becomes elongated in a craniocaudal direction. The mesoderm on either side of it is soon heaped up to form two longitudinal elevations called the *primitive urethral folds* (Figs 16.23 and 16.24). In addition to these folds, three other elevations of mesoderm are soon apparent. These are:

- The *genital tubercle* which is situated in the midline between the urogenital membrane and the lower part of the anterior abdominal wall; and
- The right and left *genital swellings* (Fig. 16.23).

Development of Female External Genitalia (Figs 16.23 to 16.25)

- The genital tubercle becomes cylindrical and forms the *clitoris*.
- The genital swellings enlarge to form the *labia majora*. Their posterior ends fuse across the midline to form the posterior commissure.
- The urogenital membrane breaks down, so that continuity is established between the urogenital sinus (which forms the vestibule) and the exterior. The primitive urethral folds now form the *labia minora*. It will be obvious that they are lined on the outside by ectoderm and on the inside by endoderm (Figs 16.24 and 16.25).

Clinical correlation

Anomalies of female external genitalia

- The clitoris may be absent, may be bifid, or may be double. It may be enlarged in hermaphroditism.
- The labia minora may show partial fusion.
- The urethra may open on the anterior wall of the vagina; this is the female equivalent of male hypospadias.

Development of Male External Genitalia (Figs 16.23, 16.24 and 16.26)

- The genital tubercle becomes cylindrical and is now called the *phallus*. It undergoes great enlargement to form the *penis*. As the phallus grows, the *glans* becomes distinguishable by the appearance of a *coronary sulcus*.

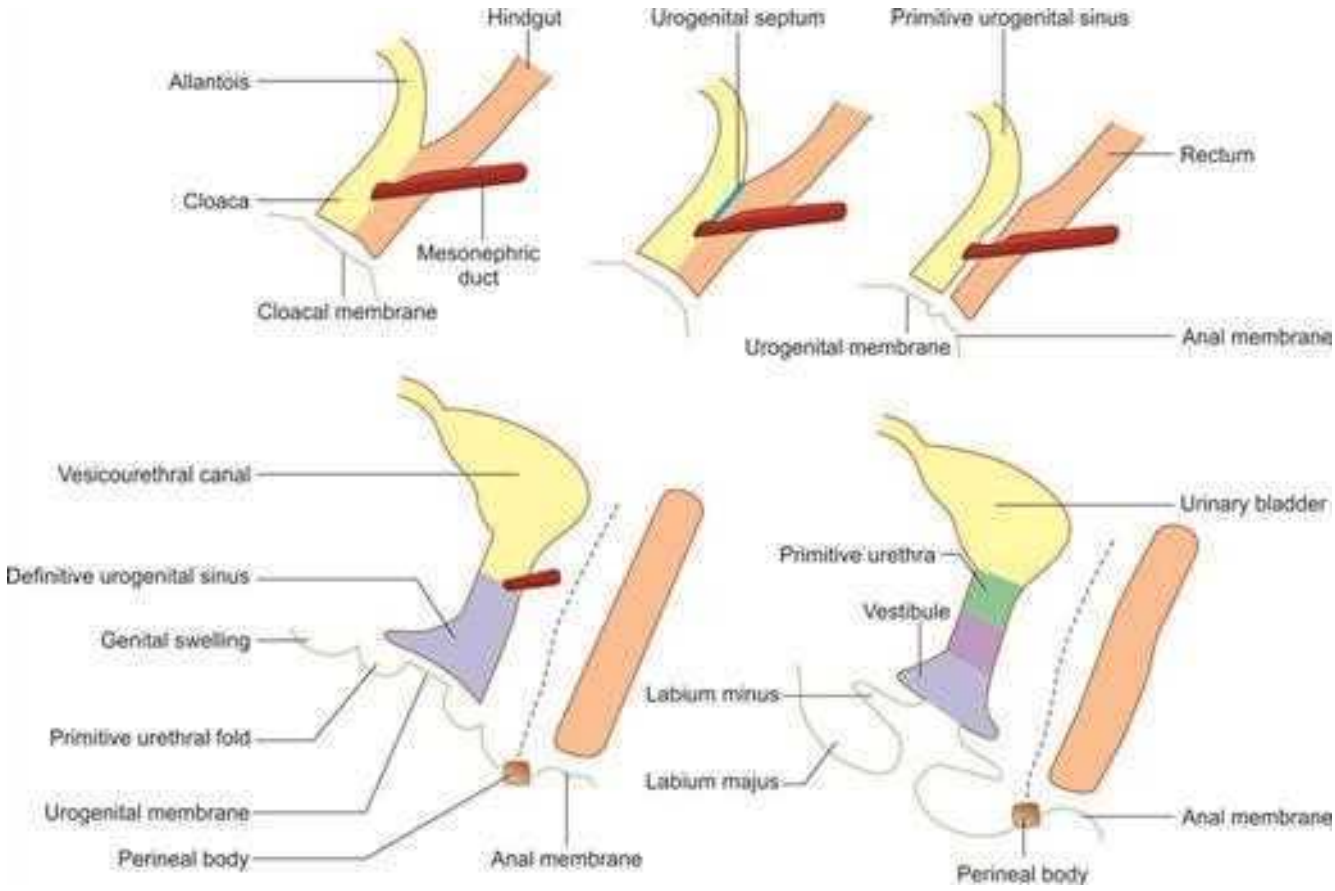


Fig. 16.23: Subdivisions of primitive urogenital sinus and cloacal membrane

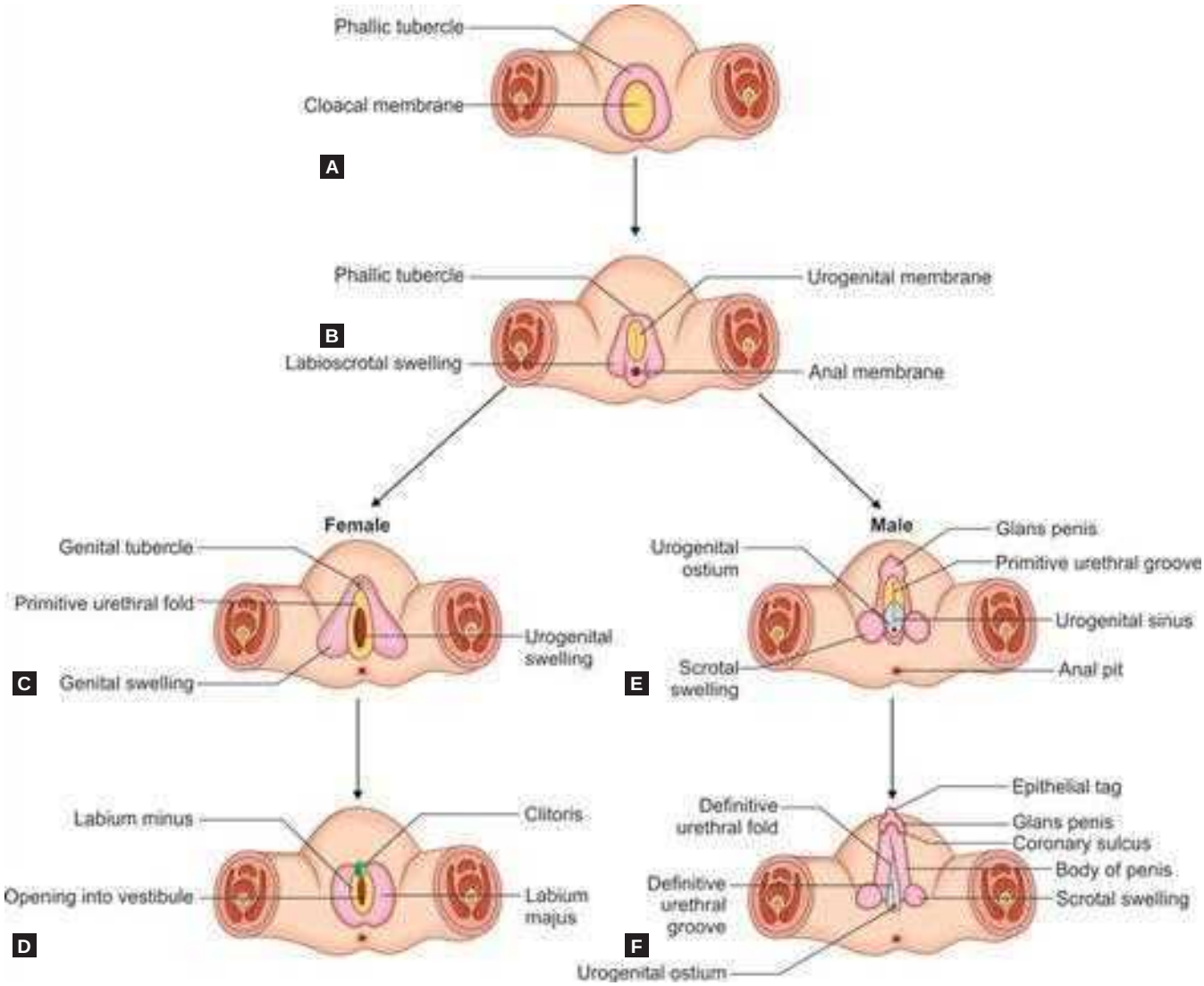
Still later, the prepuce is formed by reduplication of the ectoderm covering the distal part of the phallus (Figs 16.23, 16.24 and 16.26).

- We have seen that the urogenital membrane lies in a linear groove, flanked on either side by the primitive urethral folds (Figs 16.27A to C). As the phallus grows, this groove elongates and extends onto its under surface (Fig. 16.27B). This groove is lined by ectoderm and is called the *primitive urethral groove*.
- From Figures 16.27C and D, it will be clear that the phallus is closely related to the endodermal lining of the phallic part of the urogenital sinus. The endodermal cells of this lining proliferate, and grow into the phallus, in the form of a solid plate of cells called the *urethral plate* (Fig. 16.27C). The cells of the urethral plate are in contact with the ectodermal cells lining the primitive urethral groove.
- The urogenital membrane soon breaks down, so that the urogenital sinus (phallic part) opens to the outside, in the caudal part of the primitive urethral groove (Fig. 16.27D). At the same time, the cells forming the core of the urethral plate degenerate, along with the ectodermal cells lining the primitive urethral groove. In this way, a deeper groove (called the *definitive urethral groove*) lined by endodermal cells, is now formed on the under surface of the phallus (Figs 16.27E and F). At the base of the phallus, this groove is continuous with the cavity of the urogenital sinus (Fig. 16.27F). The margins of this groove are called the *definitive urethral folds*.

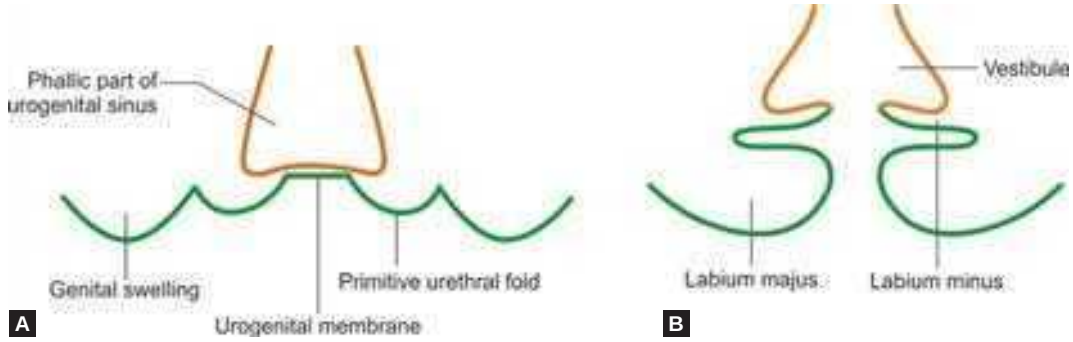
These folds now approach and fuse with each other. The fusion begins posteriorly in the region of the urogenital sinus and extends forward onto the phallus (Figs 16.27G and H). The penile urethra is formed as a result of this fusion. It will now be apparent that the wall of the penile urethra is made up of the:

1. Original endodermal lining of the phallic part of the urogenital sinus.
2. The endodermal cells of the urethral plate.

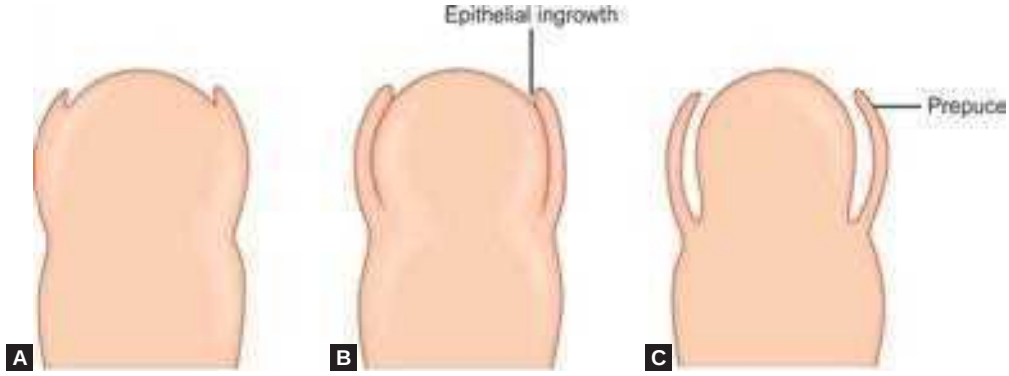
The penile urethra formed in this way extends only up to the glans penis. The distal most part of the urethra is of ectodermal origin, and is formed by canalization of a solid mass of ectodermal cells (Figs 16.27G and H).



Figs 16.24A to F: Development of external genitalia. A and B at different stage. C and D in female. E and F in male. (A) Cloacal membrane; (B) Cloacal membrane divides into urogenital membrane and anal membrane; (C) Right and left genital swellings, and a median genital tubercle appear; (D) Urogenital membrane breaks down. Its edges form the primitive urethral folds; (E) Genital tubercle becomes the glans penis.primitive urethral folds and groove are seen; (F) Formation of definitive urethral groove and urethral folds are formed.The genital swellings fuse to form the scrotum



Figs 16.25A and B: Development of female external genitalia. Ectoderm is shown in solid line and endoderm in dotted line. Compare with Figure 16.23



Figs 16.26A to C: Formation of the prepuce of the penis

- The genital swellings fuse with each other, in the midline, to form the scrotal sac into which the testes later descend.

Prenatal Diagnosis of Sex

The sex of a baby can be determined before birth by ultrasound examination. The penis can be seen in a male child. In this connection, it has to be noted that in fetuses about 3–4 months old, the genital tubercle is equally developed in both the male and female. Ultrasound examination at this stage can be misleading as the clitoris can be mistaken for a penis.

Clinical correlation

anomalies of male external genitalia

- The entire penis may be absent. Alternatively, the corpora cavernosa, or the prepuce, may be missing. The opening of the prepuce may be too narrow to allow retraction (***phimosis***).
- The penis may be double or bifid.
- Rarely, the penis may lie posterior to the scrotum.
- The urethral folds may fail to fuse, partially, or completely. When failure to fuse is complete, the scrotum is in two halves and the genitals look like those of the female (Fig. 16.28A). If the defect is confined to the anterior part of the phallus, the urethra opens on the under surface of the penis. This condition is called hypospadias (Fig. 16.28B).
- The urethra sometimes opens on the dorsal aspect of the penis. The condition is called ***epispadias***, and is usually associated with ectopic vesicae. In such cases, it is believed that the genital tubercle is formed caudal to the urogenital membrane instead of being ventral to it. When the membrane ruptures, the urogenital sinus opens cranial to the developing penis. Other anomalies of the penile urethra have been described earlier.

Primordial Germ Cells (Fig. 16.29)

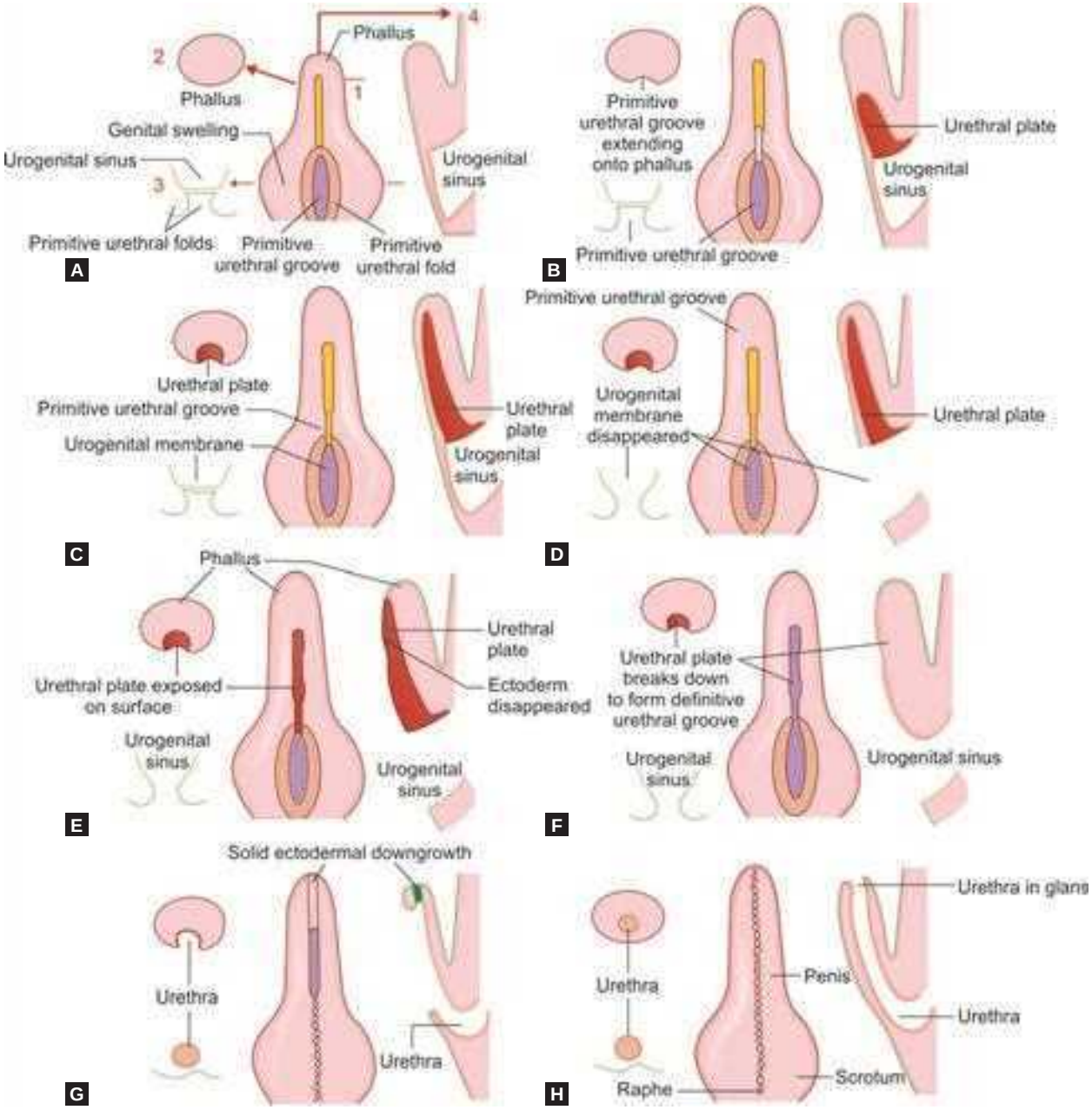
The cells of the ovaries and the testes, from which germ cells are formed, are believed to be segregated early in the life of the embryo. They probably differentiate in the wall of the yolk sac and migrate to the region of the developing gonads. All spermatozoa and ova that are formed throughout the life of the individual are believed to arise from these *primordial germ cells*. Migration of primordial germ cells into them is essential for development of the gonads. These cells have an inducing effect on the gonad.

DEVELOPMENT OF TESTES

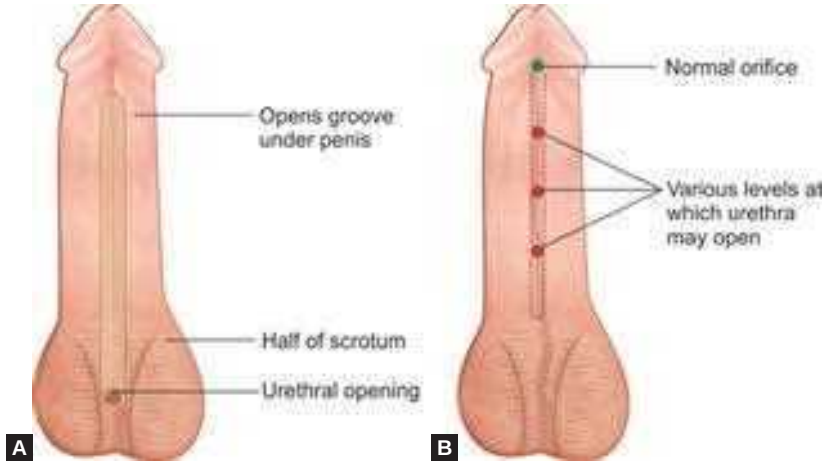
- Each testis develops from the coelomic epithelium that covers the medial side of the mesonephros, of the corresponding side (Figs 16.30 and 16.31). In the region where the testis is to develop, this germinal epithelium becomes thickened. This thickening is called *the genital ridge*.
- The cells of the germinal epithelium proliferate and form a number of solid *sex cords* that grow into the underlying mesenchyme. They reach deep into the gonad and are called *medullary cords*. They are soon canalized to form the *seminiferous tubules*. Meanwhile, the primordial germ cells migrate to the region of the developing testis and get incorporated in the seminiferous tubules.
- The *interstitial cells* of the testis are derived from sex cords that are not canalized. Some of them are also derived from the surrounding mesenchyme.
- The mesenchymal cells, surrounding the developing testis, form a dense layer of fibrous tissue. This is the *tunica albuginea*. It completely separates the sex cords from the germinal epithelium and, thereafter, this epithelium can make no further contribution to testicular tissue.

Duct System of Testes

We have seen, above, that the testis develops in close proximity to the mesonephros, and the mesonephric duct.



Figs 16.27A to H: Stages in the development of male genitalia and of penile urethra. In each set (A to H), the central Figure (1) shows the genital region from the ventral aspect; (2) and (3) are transverse sections at the levels indicated; and (4) is a median section through the region. In sections, ectoderm is depicted in black line, and endoderm is red. Mesoderm is green. (A) Note the following. The phallus is formed by enlargement of the genital tubercle. Caudal to the phallus there is a median, longitudinal depression, the primitive urethral groove (PUG) bounded by primitive urethral folds (PUFs). Lateral to these folds we see the genital swellings (GS). In the depth of the PUG, there is the urogenital membrane which separates the groove from the urogenital sinus (UGS); (B) The phallus has enlarged. The PUG is beginning to extend onto it. A solid mass of endodermal cells derived from the UGS, extends into the phallus. This mass is the urethral plate (UP); (C) The PUG is now fully formed. The UP has enlarged and extends deeper into the phallus; (D) The urogenital membrane has broken down so that endoderm of the UGS can now be seen from outside; (E) Ectoderm overlying the UP has disappeared. As a result, endoderm of the plate is seen on the surface; (F) Cells in the center of the UP now break down and convert the plate into a groove that is seen on the surface. This is the definitive urethral groove, and the folds forming its edges are the definitive urethral folds; (G) The definitive urethral folds grow toward each other and fuse to form a median raphe. In this way the definitive urethral groove is converted into a tube, which is the urethra. This process of fusion starts caudally and progresses cranially; (H) In this Figure and in "G" note that the urethra formed as described above does not extend into the glans. The part of the urethra lying in the glans is derived from ectoderm which first forms a solid cord that is later canalized



Figs 16.28A and B: (A) Cleft scrotum; (B) Hypospadias. The urethra opens onto the ventral aspect of the penis

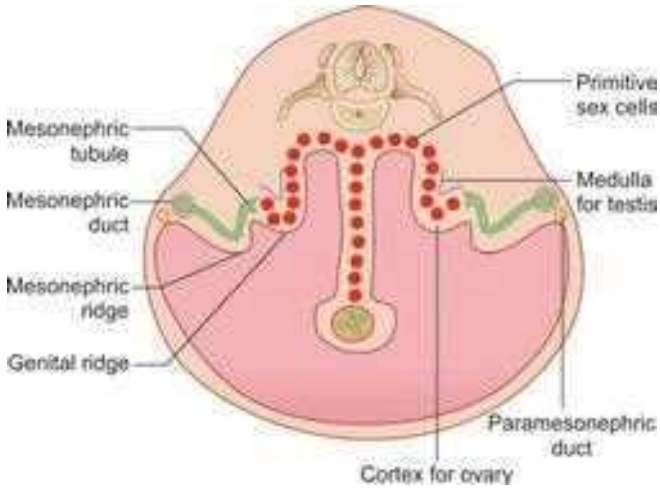


Fig. 16.29: Migration of primordial germ cells from the neighborhood of the yolk sac to the developing gonad

We have also seen that most of the mesonephric tubules degenerate. Some of them that lie near the testis persist and, along with the mesonephric duct, form the duct system of the testis (Figs 16.32A to C). The ends of the seminiferous tubules anastomose with one another to form the *rete testes*. The rete testes, in turn, establish contact with persisting mesonephric tubules which form the *vasa efferentia*.

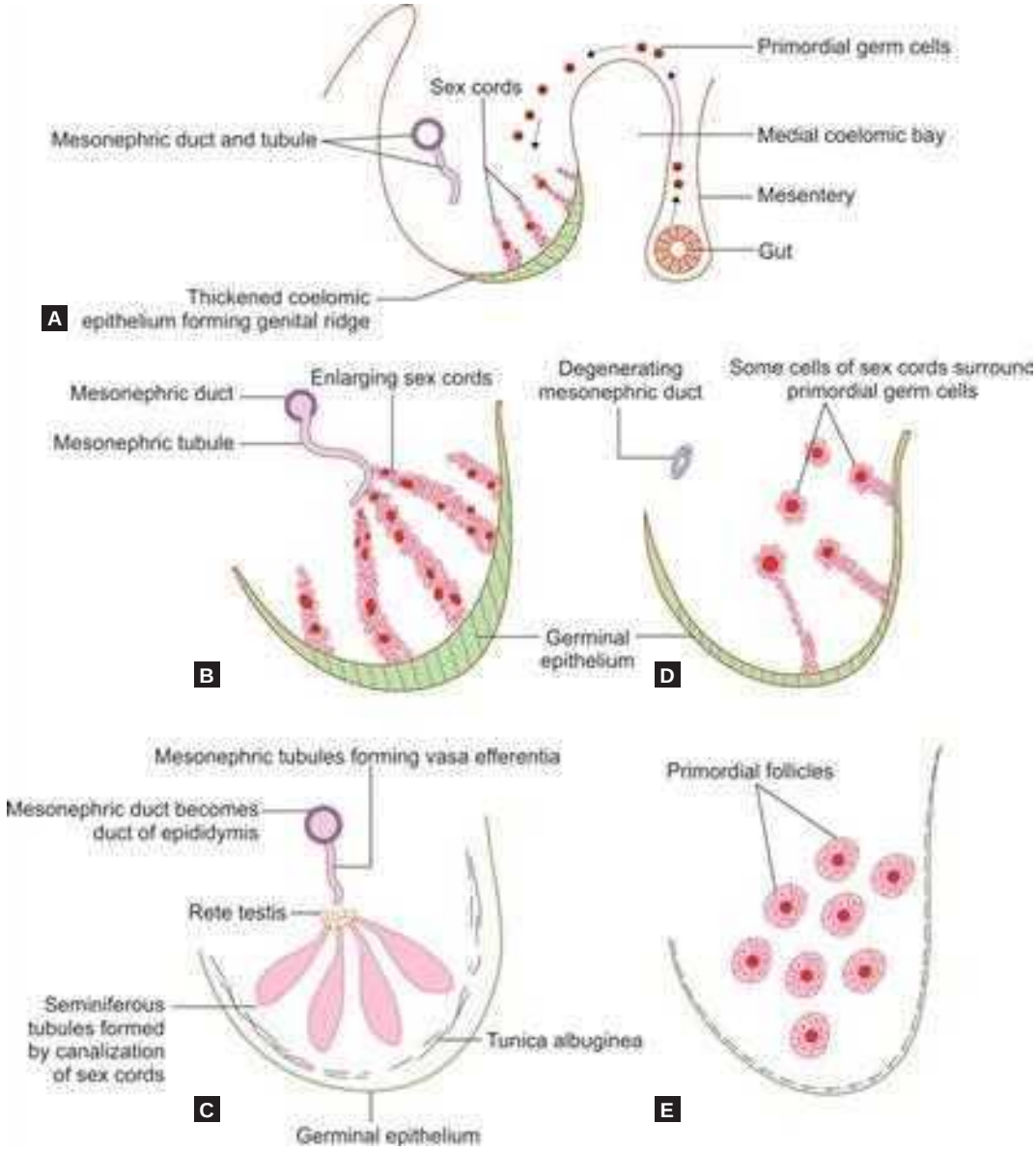
The cranial part of the mesonephric duct becomes highly coiled on itself to form the *epididymis* while its distal part becomes the *ductus deferens*. The *seminal vesicle* arises, on either side, as a diverticulum from the lower end of the mesonephric duct. The part of the mesonephric duct between its opening into the prostatic urethra, and the origin of this diverticulum, forms the *ejaculatory duct*.

Descent of Testes

The testes develop in relation to the lumbar region of the posterior abdominal wall (Fig. 16.33). During fetal life, they gradually descend to the scrotum. They reach the iliac fossa during the 3rd month, and lie at the site of the deep inguinal ring up to the 7th month of intrauterine life. They pass through the inguinal canal during the 7th month (Figs 16.34 and 16.35), and are normally in the scrotum by the end of the 8th month (Fig. 16.36).

The descent of the testes is caused or assisted by several factors. These are:

- Differential growth of the body wall.
- *Formation of inguinal bursa*: About the 6th month of intrauterine life, the various layers of the abdominal wall, of each side, show an outpouching toward the scrotum (Fig. 16.37). This pouch progressively increases in size, and depth, and eventually reaches the bottom of the scrotal sac. The descending testis enters this pouch to reach the scrotum. Note that the pouch is formed before the testis enters it. The cavity of the inguinal bursa becomes the *inguinal canal*, while the various layers of its wall form the coverings of the testis and spermatic cord.
- The *gubernaculum*: This is a band of mesenchyme which extends from the lower pole of the testis to the scrotum. For many years, it was believed that descent of the testis was caused by shortening of the gubernaculum. However, we now know that this is not possible because the gubernaculum does not contain any contractile tissue. According to some authorities, the gubernaculum does not reach the scrotum but reaches the bottom of the inguinal bursa. In spite of this, the gubernaculum



Figs 16.30A to E: Development of gonads. (A) Indifferent stage; (B and C) Testis; (D and E) Ovary

does play an important part in the descent of the testis as follows:

- When the embryo increases in size, the gubernaculum does not undergo a corresponding increase in length. There is thus a relative shortening of the gubernaculum and, as a result, the testis assumes a progressively lower position.
- The gubernaculum helps to dilate the inguinal bursa. It provides a continuous pathway for the descending testis.

- *Processus vaginalis*: This is a diverticulum of the peritoneal cavity. It actively grows into the gubernacular mesenchyme of the inguinal canal and of the scrotum (Fig. 16.37). While descending the testis invaginates the processus vaginalis from behind. After the descent of the testis is completed, the processus vaginalis loses all connection with the peritoneal cavity and becomes the *tunica vaginalis* (Figs 16.38A to C).
- The descent of the testis is greatly influenced by hormones secreted by the pars anterior of the hypophysis cerebri.

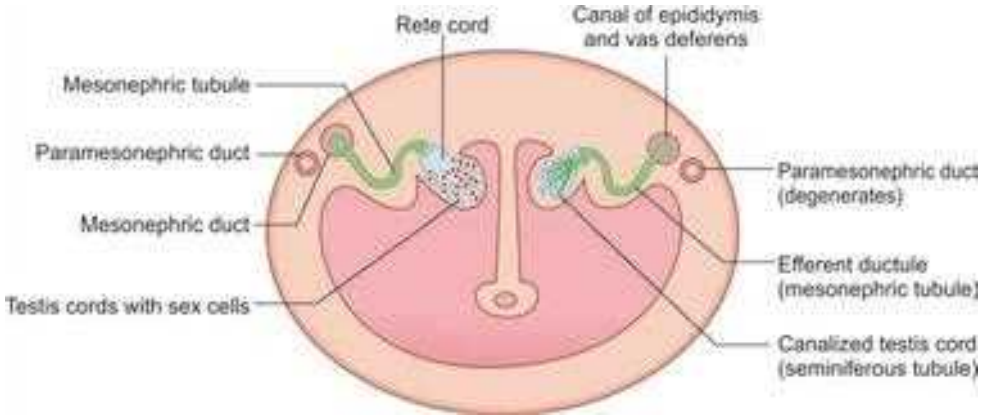
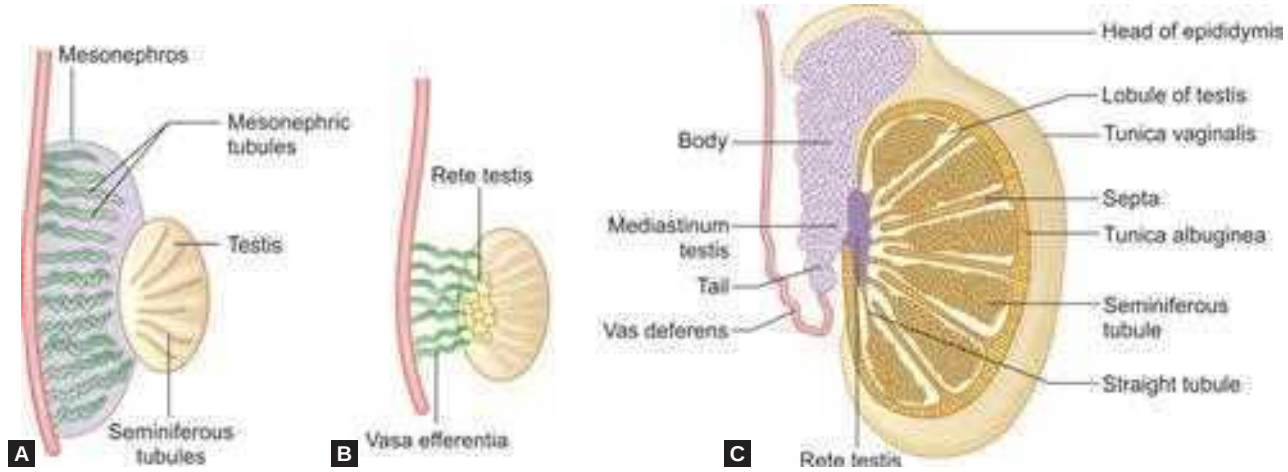


Fig. 16.31: Development of testis



Figs 16.32A to C: Development of duct system of the testis. Structures derived from sex cords are shown in gray

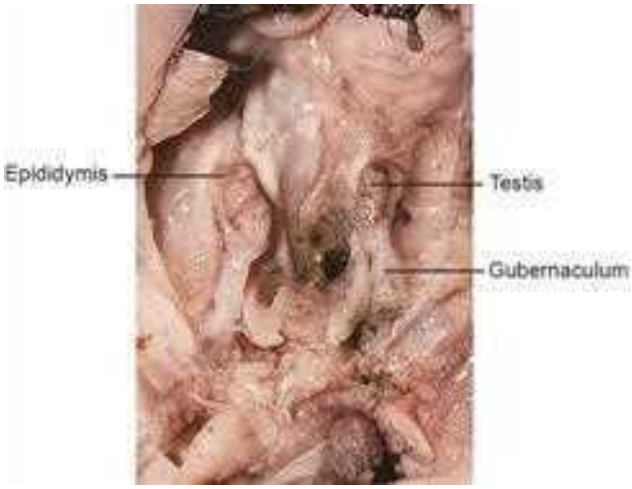


Fig. 16.33: Developing testis in lumbar region

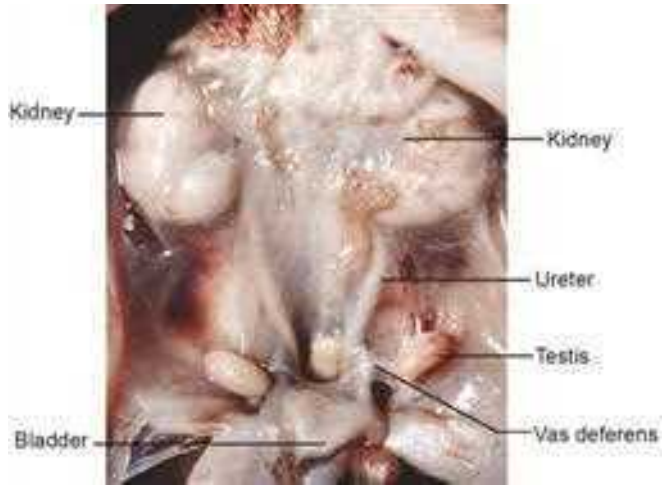


Fig. 16.34: Testis in inguinal canal

Fig. 16.35: Testis in upper scrotum

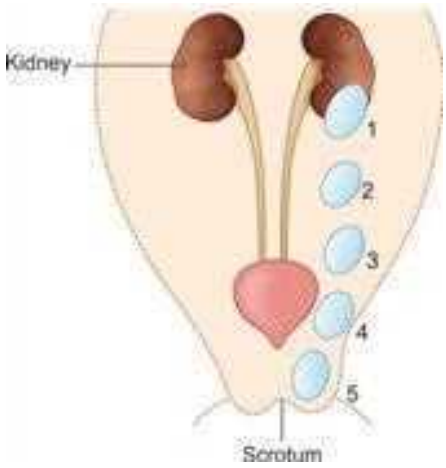


Fig. 16.36: Descent of the testis (from the lumbar region to the scrotum)

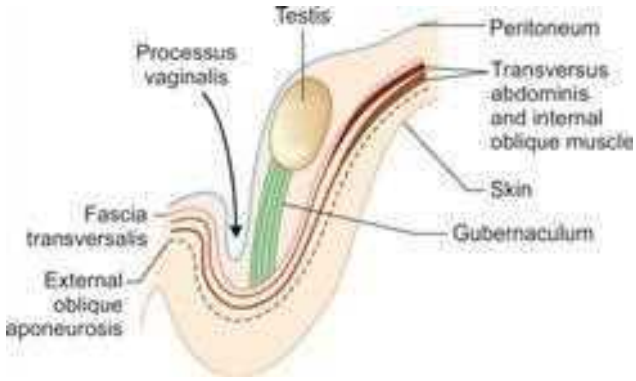
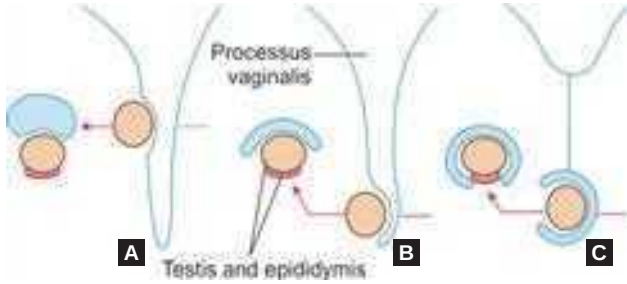


Fig. 16.37: The gubernaculum, which helps in descent of the testis



Figs 16.38A to C: Relation of descending testis to processus vaginalis. Note that as the testis descends it progressively invaginates the processus vaginalis

Vestigial Structures in the Region of the Testis

A number of vestigial structures are to be seen in the neighborhood of the testis. Their importance lies in the fact that any one of them may enlarge to form a cyst. These structures are:

- Appendix of testis (also called hydatid of Morgagni)
- Appendix of epididymis
- Superior aberrant ductules
- Inferior aberrant ductules
- Paradidymis.

DEVELOPMENT OF THE OVARY

- The early stages in the development of the ovary are exactly the same as in the testis (Figs 16.30A, C and E).
- The coelomic epithelium on the medial side of the mesonephros becomes thickened to form genital ridges.
- Cords of cells (sex cords or medullary cords) proliferate from this germinal epithelium, and grow into the underlying mesoderm.
- Primordial germ cells that are formed in relation to the yolk sac migrate to the region of the developing ovary, and give rise to oocytes.
- The sex cords become broken up into small masses. The cells of each mass surround one primordial germ cell, or oocyte, to form a *primordial follicle*.
- According to some authorities, the original (medullary) sex cords undergo regression in the ovary, and are replaced by a new set of *cortical cords* arising from coelomic epithelium. Follicular cells are derived from these cortical cords.
- *Interstitial gland cells* differentiate from mesenchyme of the gonad.
- As no tunica albuginea is formed, the germinal epithelium may contribute to the ovary even in postnatal life.

Descent of the Ovary

The ovary descends from the lumbar region, where it is first formed, to the true pelvis. A gubernaculum forms, as in the male, and extends from the ovary to the labium majus. It becomes attached to the developing uterus at its junction with the uterine tube. The part of the gubernaculum that persists between the ovary and the uterus becomes the (round) *ligament of the ovary*. The part between the uterus and the labium majus becomes the *round ligament of the uterus*.

Clinical correlation

Anomalies of testis

- The testis may be absent on one or both sides.
- The testis may be duplicated.
- The two testes may be fused together.

Anomalies of descent (cryptorchidism): Descent of the testis may fail to occur, or may be incomplete. The organ may lie in the lumbar region, in the iliac fossa, in the inguinal canal, or in the upper part of the scrotum. Some interesting facts about this condition are as follows:

- The testis may complete its descent after birth.
- Spermatogenesis often fails to occur in an undescended testis.
- An undescended testis is more likely to develop a malignant tumor than a normal testis. The condition can be surgically corrected.

Abnormal positions (ectopia): The testis may lie (Fig. 16.39):

- Under the skin of the lower part of the abdomen.
- Under the skin of the front of the thigh.
- In the femoral canal.
- Under the skin of the penis.
- In the perineum behind the scrotum.

Also see hermaphroditism.

anomalies of duct system of testis

- The seminiferous tubules may fail to establish connection with the vasa efferentia.
- The ductus deferens may be absent, in whole or in part, on one or both sides.
- The ductus deferens may have no connection with the epididymis.

anomalies of the processus vaginalis

We have seen that the part of the processus vaginalis, that extends from the deep inguinal ring up to the tunica vaginalis, normally disappears. This may persist in whole, or in part. Abdominal contents may enter it to produce various forms of ***inguinal hernia***. Alternatively, fluid may accumulate in it producing the condition called ***hydrocele***. Various forms of hernia and of hydrocele are shown in Figures 16.40A to G.

Fig. 16.39: Ectopic positions of the testis. a = under skin of the abdomen; b = over front of thigh; c = in femoral canal; d = under skin of penis; e = in perineum

Clinical correlation

Anomalies of ovary

- The ovary may be absent on one or both sides.
- The ovary may be duplicated.
- The ovary may descend into the inguinal canal or even into the labium majus.
- Adrenal or thyroid tissue may be present in the ovary. The ovary sometimes contains cells that are capable of differentiating into various tissues like bone, cartilage, hair, etc. and the growth of these cell rests can give rise to a peculiar tumor called a ***teratoma***.

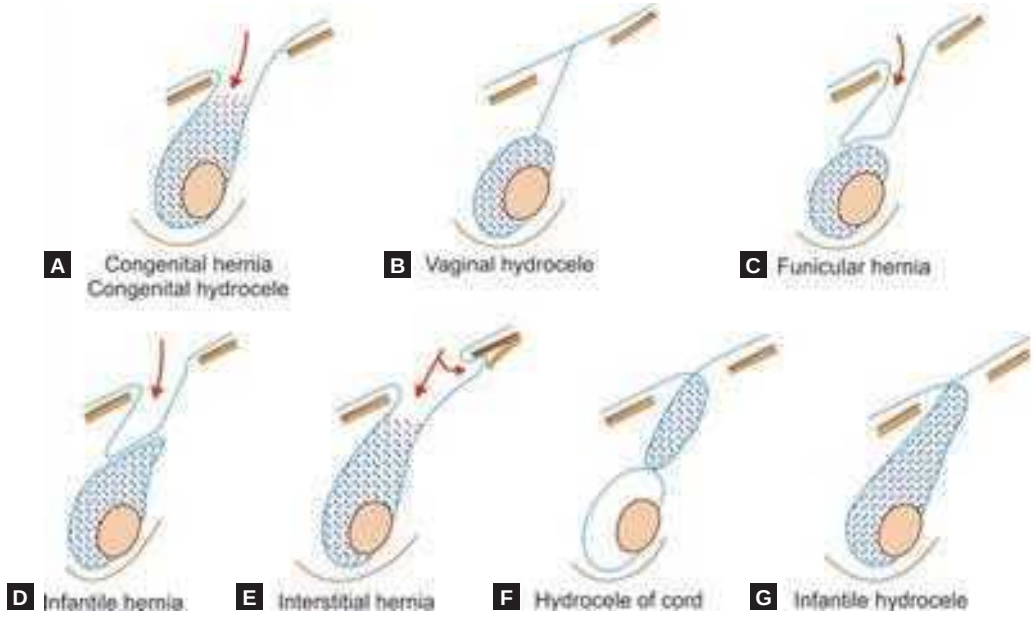
FATE OF MESONEPHRIC DUCT AND TUBULES IN THE MALE

The mesonephric ducts give rise to the following structures (Figs 16.41 and 16.42):

- Ureteric buds from which the ureters, pelvis, calyces and collecting tubules of the kidneys are derived
- Trigone of the urinary bladder
- Posterior wall of the part of the prostatic urethra, cranial to the openings of the ejaculatory ducts
- Epididymis
- Ductus deferens
- Seminal vesicles
- Ejaculatory ducts
- Mesodermal part of prostate
- *Appendix of epididymis*: This is a small rounded structure attached to the head of the epididymis (Fig. 16.40A). It represents the cranial end of the mesonephric duct. Occasionally, it may give rise to a cyst. This is not to be confused with the appendix of the testis, which is a remnant of the paramesonephric duct.

Remnants of Mesonephric Tubules

We have seen that most of the mesonephric tubules disappear. Some persist to form the *vasa efferentia*. Other mesonephric tubules persist to form some vestigial structures that are seen near the testes. Their only importance is that they sometimes give rise to cysts. These remnants are as follows:



Figs 16.40A to G: Anomalies of processus vaginalis. Abnormal persistence of the processus vaginalis can lead to hernia (passage into it of abdominal contents, indicated by arrows); or hydrocele (collection of fluid, shown as dots). Various types of hernia and hydrocele are shown

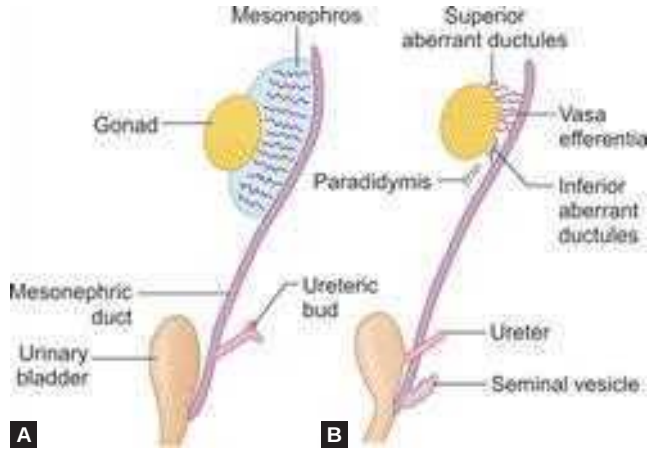
- The *superior aberrant ductules* (or *epigenital tubules*) lie cranial to the vasa efferentia. They are connected to the testis but not to the epididymis.
- The *inferior aberrant ductules* lie caudal to the vasa efferentia. They are connected only to the epididymis.
- The *paradidymis* consists of tubules that lie between the testis and the epididymis (*paragenital tubules*) but are not connected to either of them.

FATE OF MESONEPHRIC DUCTS AND TUBULES IN THE FEMALE

As in the male, the mesonephric ducts give rise to the ureteric bud from which the ureter, pelvis, calyces and collecting tubules of the kidneys are derived, and give rise to the trigone of the bladder. The posterior wall of the female urethra is also derived from them.

The mesonephric ducts and tubules do not establish any connection with the developing ovary. However, they give rise to some vestigial structures seen in the broad ligament near the ovary. These are (Fig. 16.42B):

- *Epoophoron*: This consists of a longitudinal duct running parallel to the uterine tube, and a number of transverse ductules that open into the longitudinal duct. It corresponds to the epididymis and vasa efferentia of the male (Note that the word "epoophoron" means "above egg basket": ep = above, oo = egg, and phoron = basket). In some cases, the longitudinal duct is unusually long.

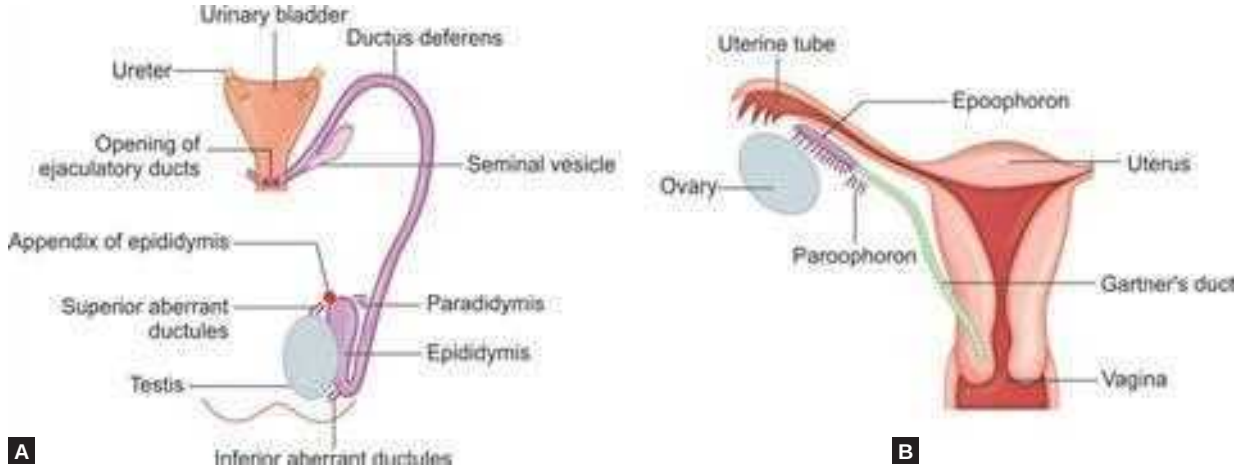


Figs 16.41A and B: (A) Mesonephric duct, early stage; (B) Mesonephric duct in the male, before descent of the testis

It runs along the side of the uterus, and lower down, becomes embedded in the wall of the cervix. It, however, never opens into the uterine lumen. It is the equivalent of the male ductus deferens and is also called Gartner's duct.

- *Paroophoron*: This consists of small blind tubules lying between the ovary and the uterus, and is the female equivalent of the paradidymis. The word paroophoron means "near egg basket".

Male and female homologues derived from undifferentiated genital system are presented in Table.16.1.



Figs 16.42A and B: Some structures derived from the mesonephric ducts. (A) In the male, these are the epididymis, the ductus deferens, the seminal vesicles and ejaculatory ducts. The appendix of the epididymis is a vestigial remnant; (B) In the female, most of the duct disappears, some remnants are seen as the epoophoron. For complete list of derivatives of the mesonephric ducts see text

CONTROL OF DIFFERENTIATION OF GENITAL ORGANS

From the account of the development of the gonads and genitalia, it is seen that these organs are derived from the same primordia in both sexes. The male and female genital systems are identical till the beginning of 7th week of intrauterine life. The factors that determine whether these organs will develop as in the male or as in the female are as follows:

- The most important factor is the chromosomal sex of the individual, which is determined at the time of fertilization. We have already seen that individuals with two X-chromosomes are female, while those with one X-chromosome and one Y-chromosome are male.
- The Y-chromosome bears a gene that is responsible for production of a *testis determining factor*. This factor plays a vital role in causing the developing gonad to

TABLE 16.1: Male and female homologues derived from undifferentiated genital system

Embryonic structure	*Male derivative*	*Female derivative*
Indifferent gonad	Testis	Ovary
Primordial germ cells	Spermatozoa	Ova
Cortex—sex cords	Seminiferous tubules (spermatogonia and Sertoli cells)	Ovarian follicles
Medulla	Rete testis	Rete ovarii
Mesonephric tubules	Ductuli efferentes (vasa efferentia)	Epoophoron
	Paradidymis	Paroophoron
Mesonephric (Wolffian) duct	Appendix of epididymis	Appendix of ovary
	Epididymis	Duct of epoophoron
	Ductus deferens	
	Ejaculatory ducts	
	Seminal vesicle	
	Ureter, pelvis, calyces and collecting tubules	
Paramesonephric (Müllerian) ducts	Appendix of testis (hydatid of Morgagni)	Paratubal cyst
		Uterine tube
	Prostatic utricle	Uterus
		Upper part of vagina

Contd...

Contd...

Embryonic structure	*Male derivative*	*Female derivative*
Gubernaculum	Gubernaculum testis	Round ligament of ovary and round ligament of uterus
Urogenital sinus	Urinary bladder Urethra Prostate gland Bulbourethral glands	Urinary bladder Urethra Vagina Urethral and paraurethral glands Greater vestibular glands
Müllerian eminence (sinus tubercle)	Seminal colliculus	Hymen
Phallus/genital tubercle	Body and glans penis Corpora cavernosa and corpus spongiosum	Body and glans of clitoris
Urogenital fold	Ventral aspect of penis and penile raphe	Labia minora
Labioscrotal swellings	Scrotum and scrotal raphe	Labia majora, mons pubis

become a testis. Apart from a direct action on the gonad, this factor influences other genes that play a role in the process. Under the influence of these genes, supporting (Sertoli) cells are formed from cells of the sex cords and interstitial (Leydig) cells are formed from mesenchymal cells of the gonadal ridge.

- Once the testis is formed, interstitial cells in it begin to produce testosterone (under the influence of gonadotropins produced in the placenta). This testosterone influences the differentiation of genital ducts, and external genitalia. By the end of 18th week of intrauterine life, fetal interstitial cells disappear to reappear only at the time of puberty.
- Supporting cells in the fetal testis produce a *Müllerian inhibiting substance*. This substance causes regression of paramesonephric ducts. The sertoli cells also secrete an *androgen binding factor* that helps in formation of spermatozoa from spermatogonia. As the Y-chromosome is missing in a female fetus, none of the processes described above take place. The estrogens (derived from maternal and placental sources) influence the formation of internal and external genital organs.

Clinical correlation

Hermaphroditism

Abnormal development of the gonad and the genitalia gives rise to various types of hermaphroditism. A hermaphrodite is really a person who is both a male and a female at the same time. Such a person has never been known to exist. However, persons having both testes and ovaries have been reported and such individuals are referred to as ***true hermaphrodites***. The word ***pseudohermaphrodite*** is used for a person whose external genitalia look like those of one sex, whereas the gonad is of the other sex.

Some forms of hermaphroditism are as follows:

True hermaphroditism

The person has at least one testis and one ovary in the body. The external genitalia may be male, or female, or midway between the two. The chromosomal sex may be either male or female.

Pseudohermaphroditism

Gonads are of one sex, while genitalia (internal, external or both) are of opposite sex. A patient having a testis is described as a ***male hermaphrodite***; and one having an ovary is described as a ***female hermaphrodite***.

Female pseudohermaphroditism is caused by excess of androgens produced by the fetal suprarenal gland (adrenogenital syndrome). It may also be caused by administration of progestins to the mother during pregnancy.

TIME TABLE OF SOME EVENTS DESCRIBED IN THIS CHAPTER

Time table of some events in the development of urogenital sinus is shown in Table 16.2.

TABLE 16.2: Time table of some developmental events

Age	*Developmental events*
3rd week	Formation of intermediate mesoderm. External genitalia begin to form
4th week	• Pronephric tubules begin to form and have regressed by the end of the same week • Mesonephric tubules start forming • Urorectal septum begins to form
5th week	The metanephros is formed
6th week	• Mesonephros is well developed • The cloacal membrane divides into the urogenital and the anal membrane
7th week	Urogenital sinus is established
3rd month	• Urethral folds fuse with each other • At the end of the month, prostate begins to develop
12th week	The definitive kidney (metanephros) becomes functional
5th month	Vagina gets canalized

Note: The external genitalia are most susceptible to teratogens between the 7th and 9th weeks; but they can be affected later in pregnancy as well.

REVIEW QUESTIONS

1. Explain development of kidney.
2. Explain developmental anomalies of kidney.
3. Write a note on primitive urogenital sinus.
4. Explain development of urinary bladder.
5. Explain development of male urethra.
6. Explain development of female urethra.
7. Explain development of prostate.
8. Write a note on paramesonephric duct.
9. Write a note on mesonephric duct.
10. Describe remnants of paramesonephric duct in males.
11. Write a note on primordial germ cells.
12. Describe descent of testis.
13. Describe the fate of mesonephric duct and tubules in males.
14. Describe the fate of mesonephric duct and tubules in females.

Chapter 17

Nervous System

HIGHLIGHTS

- Nervous system develops from the specialized ectoderm overlying the notochord known as *neurectoderm*.
- Neurectoderm overlying the notochord becomes thickened to form the *neural plate*.
- Neural plate is converted to *neural groove*, and then to *neural tube*.
- Neural tube has an enlarged cranial part that forms the *brain*, and a narrow caudal part that becomes the *spinal cord*. Neural tube presents a *central cavity* (lumen) that contains cerebrospinal fluid and a peripheral *wall* that forms *nervous tissue*.
- The cranial part of neural tube shows three dilatations: *prosencephalon*, *mesencephalon* and *rhombencephalon*.
- The prosencephalon divides into diencephalon and telencephalon. The telencephalon forms most of the *cerebral hemisphere* including the *corpus striatum*. The *lateral ventricle* is the cavity of the telencephalon. The diencephalon forms the thalamus, hypothalamus and related structures. Its cavity is the *third ventricle*.
- The mesencephalon forms the *midbrain*. Its cavity forms the *cerebral aqueduct*.
- The rhombencephalon divides into *metencephalon* and *myelencephalon*. The metencephalon forms the *pons*. It also forms the *cerebellum*. The myelencephalon forms the medulla *oblongata*. The fourth *ventricle* is the cavity of the rhombencephalon.
- The *neural crest cells* are made up of specialized surface ectodermal cells that lie along the lateral edges of the neural plate and later along the dorsolateral aspect of neural tube. Its most important derivatives are cells of *sensory ganglia, parasympathetic ganglia* and of *sympathetic ganglia*. It also forms the cells of the *adrenal medulla* and *Schwann cells* that form *myelin* and *neurilemmal sheaths* for peripheral nerve fibers.
- The wall of neural tube at first has a single layer of cells. They multiply and form three layers/zones. They are ependymal/matrix, *mantle* and *marginal* from the lumen to periphery. *Neurons* develop in the mantle layer which forms *the gray matter* and the processes of neurons occupy the marginal layer that becomes the *white matter*. The mantle layer is divided into a ventral part, the *basal lamina/floor plate* and a dorsal part, the *alar lamina/roof plate*. These are separated by a groove, the *sulcus limitans*.
- In the spinal cord, the alar lamina forms the *posterior/dorsal gray column*, and the basal lamina forms the *anterior/ventral gray column*. The marginal layer becomes the *white matter*.
- In the medulla, pons and midbrain, *efferent cranial nerve nuclei* develop in the basal lamina and *afferent nuclei* in the alar lamina.
- The alar lamina of the myelencephalon also forms the *olivary nuclei* (which migrate ventrally), and the *pontine nuclei* (which migrate into the pons). The alar lamina of the metencephalon contributes for the development of *cerebellum*. The alar lamina of the mesencephalon forms the *colliculi*, the *red nucleus* and the *substantia nigra*.

INTRODUCTION

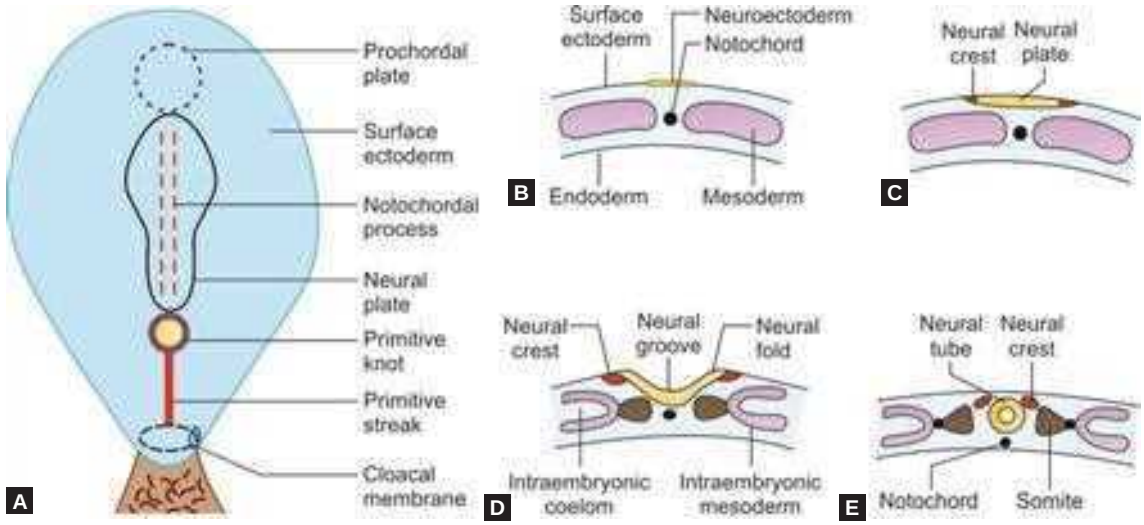
The formation of neural tissue has been considered in Chapter 7, where we have seen that the ependymal (or neuroepithelial) cells of the neural tube give rise to neurons and to neuroglia. We have also studied the formation of myelin sheath. We shall now consider the development of individual parts of the nervous system.

NEURAL TUBE AND ITS SUBDIVISIONS

Apart from its blood vessels and some neuroglial elements (microglia), the whole of the nervous system is derived from surface ectoderm covering the embryo. While the embryo is still in the form of a three-layered (prelaminar) embryonic disc, the surface ectoderm on the dorsal aspect of notochord differentiates. That part of the ectoderm destined to give origin to the brain and spinal cord, can be distinguished as a specialized part called *neurectoderm*.

Neurulation: It is the process of formation of neural tube by conversion of neural plate. It extends from pre-somite to post time period of development as described below.

- *Neurectoderm and neural plate stage (Pre-somite period, 16th–19th day):* Neurectoderm is situated on the dorsal (amniotic) aspect of the embryonic disc in the midline. It overlies the notochordal process (Figs 17.1A and B). Under the inductive effect of notochord, the neurectoderm soon becomes thickened to form the *neural plate* (Figs 17.1A and C). Rapid longitudinal growth along the embryonic axis stretches the neural plate, which extends from the primitive knot caudally to the prochordal plate/buccopharyngeal membrane rostrally. The neural plate at this stage is slipper shaped with broad cranial and narrow caudal ends that contribute for the formation of brain and spinal cord (Fig. 17.1A) respectively.
- *Neural groove and folds stage (Somite period, 20th/21st day):* The neural plate becomes depressed along the midline with raised margins (edges) on either side. This results in the formation of *neural groove* and *folds* on the dorsal aspect of the neural plate (Fig. 17.1D). This groove becomes progressively deeper. The surface ectodermal cells at the edges of neural plate differentiate into neural crest cells.
- *Neural tube stage (Somite period, 22nd–25th day):* The two edges of the neural plate (neural folds) come nearer to each other, and eventually fuse, thus converting the neural groove into the cylindrical *neural tube* (Fig. 17.1E). The neural tube gets separated from the surface ectoderm.
  - *Openings of neural tube*: The stages in the formation of the neural tube do not proceed simultaneously along the entire length of neural plate. The middle part is the first to become tubular around 21st/22nd day (4th somite stage). For some time, the neural tube is open cranially and caudally. These temporary openings are called the *anterior neuropore* and *posterior neuropore,* respectively. These openings facilitate communication between neural tube and amniotic cavity. The amniotic fluid circulates through the developing neural tube through these openings, providing nutrition to the developing neurectodermal cells, before the establishment of circulatory system.
  - *Closure of neuropores:* Fusion of two edges of the neural plate extends cranially, and caudally starting



Figs 17.1A to E: Formation of neural tube. (A) Dorsal view of pear-shaped embryonic disc with slipper-shaped neural plate; (B to E) Transverse sections of embryo showing various stages in the development of neural tube. (B) Embryonic disc before formation of neural plate; (C) Neural plate formed by thickening of ectoderm; (D) Neural plate is converted to a groove; (E) The groove is converted to a tube

Note: Neural crest cells lie along the edges of the neural plate (C), on neural groove (D). After formation of the neural tube, the neural crest cells lie dorsal to it (E)

TABLE 17.1: Subdivisions and adult derivatives of neural tube

Neural tube subdivisions	*Primary brain vesicles*	*Secondary brain vesicles*	*Parts of adult brain*	*Cavities*
Brain	Prosencephalon (Forebrain)	Telencephalon	Cerebral hemispheres a. Cerebral cortex b. Corpus striatum i. Caudate nucleus ii. Lentiform nucleus	Lateral ventricles
		Diencephalon	a. Thalamus b. Hypothalamus c. Epithalamus	Third ventricle
	Mesencephalon (Midbrain)	Mesencephalon	Midbrain	Cerebral aqueduct
	Rhombencephalon (Hindbrain)	Metencephalon	a. Pons b. cerebellum	Fourth ventricle
		Myelencephalon	Medulla oblongata	
Spinal cord	Spinal cord	Spinal cord	Spinal cord	Central canal

in its middle. Ultimately, neuropores disappear leaving a closed neural tube.

- Anterior neuropore closes around 25th day (Beginning of 4th week–20th somite stage). The location of anterior neuropore is represented in the adult as *lamina terminalis* that forms the anterior boundary for the 3rd ventricle.
- Posterior neuropore closes on 28th day (end of 4th week–25th somite stage). In the adult, the posterior neuropore represents the *terminal ventricle,* present in the proximal 5–6 mm of filum terminale. Non-closure of neural tube results in *neural tube defects.*

– *Differentiation of neural tube:* The neural tube presents a central cavity and a peripheral wall. The cavity represents the ventricles of brain and central canal of spinal cord. From the wall of the neural tube, various cells of central and peripheral nervous system develop.

– *Separation of neural crest cells:* With the closure of neural tube and restoration of surface ectoderm, the neural crest cells form bilateral masses along the dorsolateral aspect of the neural tube deep to surface ectoderm.

– *Differential rate of growth and subdivisions of neural tube (Post-somite period––from 5th week):* The parts of the brain that are developed from each of these divisions of the neural tube are shown in Figure 17.2 and Table 17.1.

– Even before the neural tube has completely closed, it is divisible into an enlarged cranial part that forms *brain* and a caudal tubular part that forms *spinal cord* (Fig. 17.2A). The spinal cord to begin with, is short, but gradually increases in length as the embryo grows. Later, it is further subdivided into three primary and then into five secondary brain vesicles.

– The cavity of the developing brain soon shows three dilatations (Fig. 17.2B). Craniocaudally, these are the *prosencephalon, mesencephalon*, and *rhombencephalon*.

– The *prosencephalon* becomes subdivided into *telencephalon* and *diencephalon* (Fig. 17.2C). The telencephalon consists of right and left telencephalic vesicles.

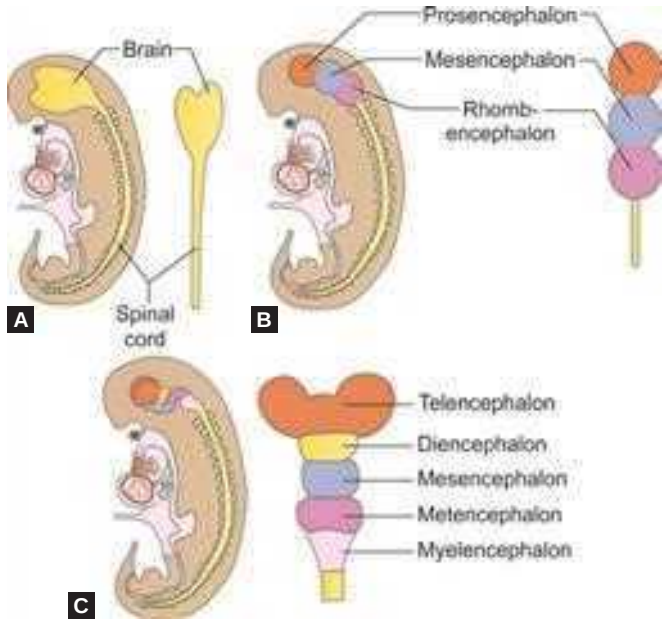


Fig. 17.2: Primary brain vesicles and their subdivisions (Secondary brain vesicles) in developing embryo

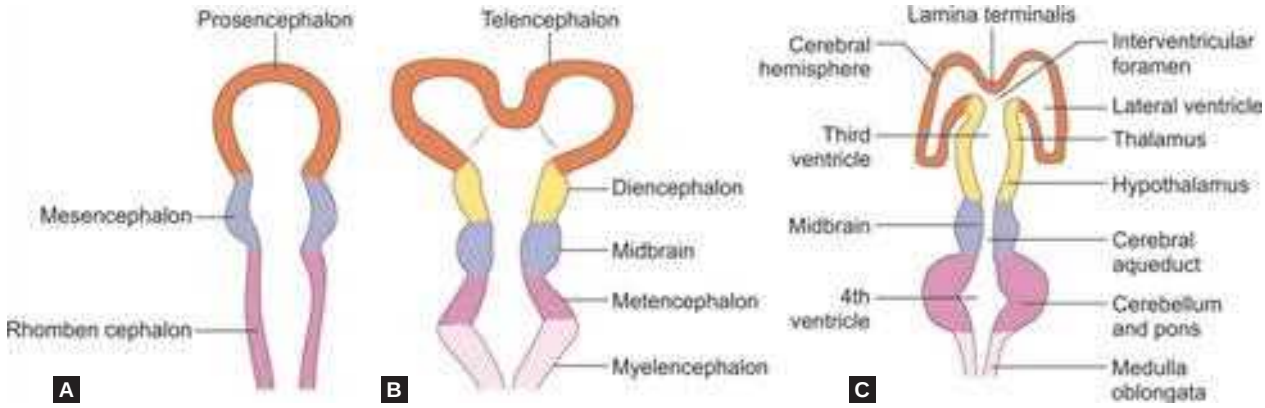


Fig. 17.3: Development of ventricles of brain

- The mesencephalon contributes for the development of midbrain (Figs 17.2B and C).
- The *rhombencephalon* also becomes subdivided into a cranial part, the *metencephalon*, and a caudal part, the *myelencephalon*. The metencephalon forms the pons and cerebellum. The myelencephalon forms the medulla oblongata (Fig. 17.2C).

- *Cavities of brain vesicles:* Each of the subdivisions of the developing brain encloses a part of the original cavity of the neural tube (Fig. 17.3). The cavities in relation with the various brain vesicles form the ventricular system of adult brain.
  - The cavity of each telencephalic vesicle becomes the *lateral ventricle*.
  - The cavity of the diencephalon (along with the central part of the telencephalon), becomes *third ventricle*. The two lateral ventricles communicate with the third ventricle through *interventricular foramen of Monro*.
  - The cavity of the mesencephalon remains narrow, and forms the *aqueduct of Sylvius* through which the third ventricle communicates with fourth ventricle.
  - The cavity of rhombencephalon forms the *fourth ventricle*.
  - The cavity of spinal cord, the *central canal* is the downward continuation of fourth ventricle. The central canal presents a terminal dilatation at its lower end called *terminal ventricle*.
- *Formation and circulation of cerebrospinal fluid:* The cerebrospinal fluid (CSF) is formed in the ventricles; mainly in the lateral ventricle by choroid plexuses (infoldings of blood vessels of the pia mater covered by a thin coat of ependymal cells that projection into the ventricles of the brain and secretes the cerebrospinal fluid). CSF flows from lateral ventricles to third ventricle through interventricular foramen and from third ventricle to fourth ventricle through cerebral aqueduct. It leaves the ventricular system through three foramina (a median foramen of Magendie and two lateral foramina of Luschka) into the subarachnoid space around brain and spinal cord (Fig. 17.4).
- The prosencephalon, mesencephalon and rhombencephalon are at first arranged craniocaudally (Fig. 17.5A). Their relative position is greatly altered by the appearance of a number of flexures. These are:
  - *Cervical flexure:* at the junction of the myelencephalic part of rhombencephalon and the spinal cord (Fig. 17.5B). It is concave ventrally. It makes a 90° angle between hindbrain and spinal cord.
  - *Mesencephalic flexure* (or cephalic flexure): in the region of the midbrain (Fig. 17.5C) and is concave ventrally.
  - *Pontine flexure:* It is at the middle of rhombencephalon, dividing it into the cranial metencephalon and the caudal myelencephalon (Fig. 17.5D). It is convex ventrally. This flexure changes the shape of this part of neural tube. Its cavity becomes the diamond-shaped fourth

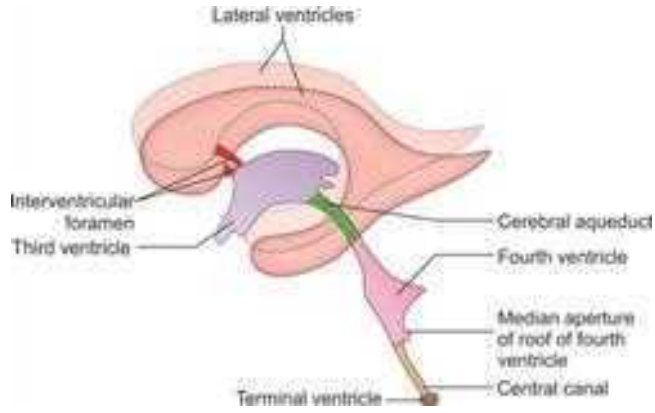
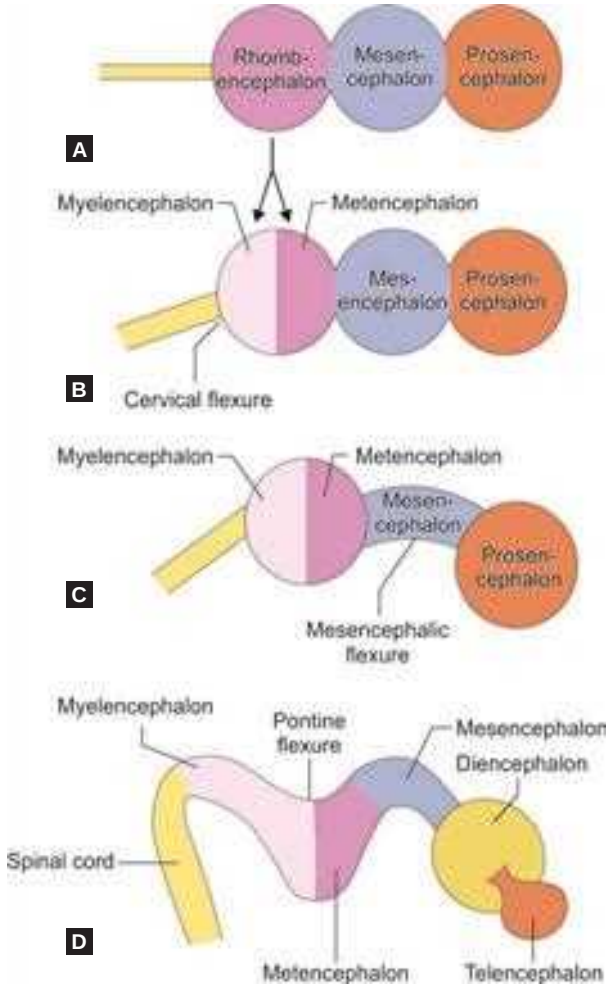


Fig. 17.4: Ventricles of brain



Figs 17.5A to D: Flexures of neural tube. (A) Neural tube before formation of flexures; (B) Cervical flexure; (C) Mesencephalic flexure; (D) Pontine flexure

ventricle. This cavity is widest at the point of folding and narrows cranially and caudally.

- *Telencephalic flexure:* It occurs much later, between the telencephalon and diencephalon.
- These flexures and differential growth of vesicles of brain lead to the orientation of the various parts of the brain as in the adult (Fig. 17.6).

NEURAL CREST CELLS

At the time of formation of neural plate, some cells at the junction of neural plate and the surface ectoderm become specialized (on either side) to form the primordia of the neural crest (Figs 17.1C and D).

With the separation of the neural tube from the surface ectoderm, the cells of the neural crest appear as groups of cells lying along the dorsolateral sides of the neural tube (Fig. 17.1E).

The neural crest cells soon become free (by losing the property of cell to cell adhesiveness). They migrate to distance places throughout the body. In subsequent development, several important structures are derived from the neural crest cells. The cells are divided into a dorsal mass and a ventral mass. Various derivatives of neural crest cells are as follows:

- Dorsal Mass
  - Neuroblasts
    - Pseudounipolar neurons of the posterior (dorsal) nerve root ganglia of spinal nerves
    - Neurons of the sensory ganglia of the fifth, seventh, eighth, ninth and tenth cranial nerves
  - Spongioblasts
    - Capsular/satellite cells of all sensory ganglia
    - Schwann cells that form the neurilemma and myelin sheaths of all peripheral nerves
  - Pluripotent cells
    - Mesenchyme of dental papilla, odontoblasts and dentine
    - *Melanoblasts*: Pigment cells of the skin
    - Cartilage cells of branchial arches
    - Leptomeninges (Pia mater and arachnoid mater)
- Ventral Mass
  - Sympathoblasts (Small cells)
    - Neurons of the sympathetic ganglia
    - Neurons of peripheral parasympathetic ganglia of cranial nerves (3rd, 7th, 9th, 10th)
  - Chromaffin cells (Large cells)
    - Suprarenal medulla
    - Para-aortic body
    - Argentaffin cells
    - Enterochromaffin cells/APUD cells
- Other structures believed to arise from the neural crest are as follows:
  - Bones of the face and part of the vault of skull (frontal, parietal, squamous temporal, part of the sphenoid, maxilla, zygomatic, nasal, vomer, palatine and mandible)
  - Dermis, smooth muscle and fat of face and ventral aspect of neck
  - Muscles of the ciliary body
  - Sclera and choroids of eye
  - Substantia propria and posterior epithelium of cornea
  - Connective tissues of thyroid, parathyroid, thymus and salivary glands

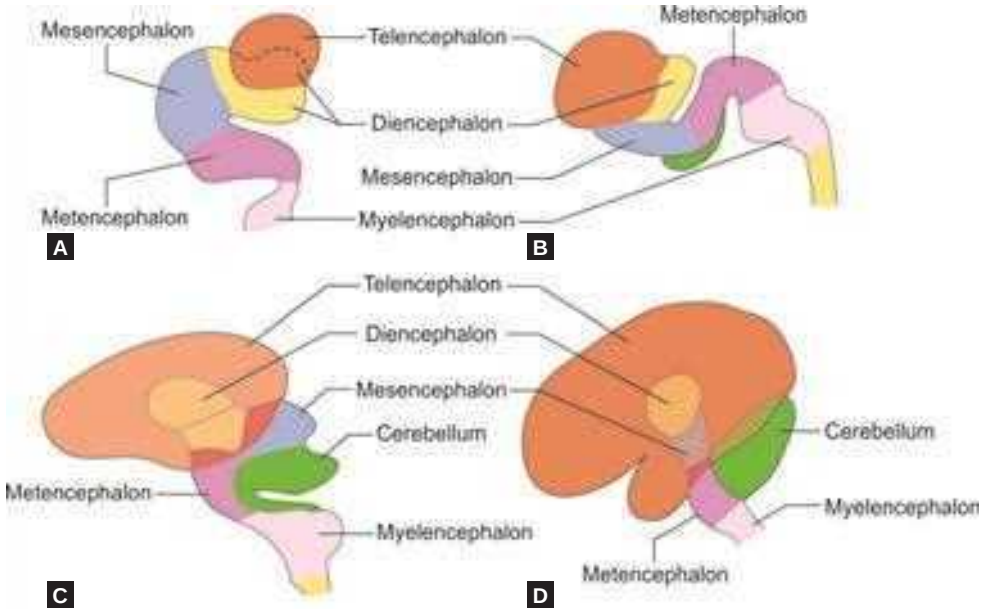


Fig. 17.6: Development of external form of the human brain. Note progressive overlapping of diencephalon and mesencephalon by the expanding telencephalon

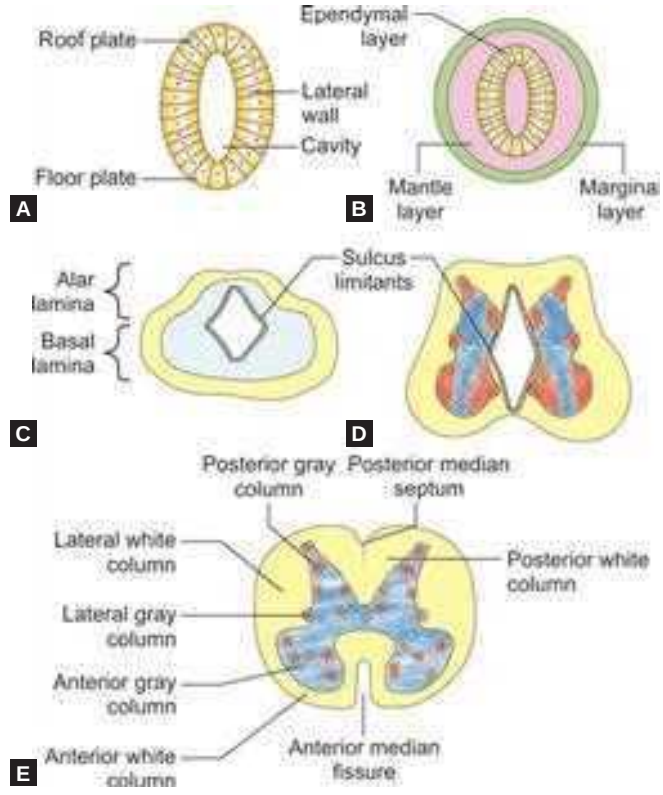
- Derivatives of the first, second and third pharyngeal cartilages
- C cells of the thyroid gland
- Cardiac semilunar valves, and conotruncal septum (spiral septum plus bulbar septum)
- Smooth muscle of blood vessels of the face and of forebrain.

SPINAL CORD

The spinal cord develops from caudal cylindrical part of the neural tube. When this part of the neural tube is first formed, its cavity is in the form of a dorsoventral cleft. The lateral walls are thick, but the roof (dorsal) and the floor (ventral) are thin (Fig. 17.7A). The wall of the tube is subdivided into the ependymal or matrix cell layer, the mantle layer and the marginal layer (Fig. 17.7B) as already described in Chapter 7.

The mantle zone grows faster in the ventral part of the neural tube, and becomes thicker, than in the dorsal part. As a result, the ventral part of the lumen of the neural tube becomes compressed. The line separating the compressed ventral part, from the dorsal part, is called the *sulcus limitans* (Fig. 17.7C). With its formation, the lateral wall of the developing spinal cord can be divided into a *dorsal* or *alar lamina*, and a *ventral* or *basal lamina*. This division is of considerable functional importance. The cells of basal lamina are *motor/efferent* in function and that of alar lamina are *sensory/afferent*. The alar and basal laminae are also called the alar (roof) and basal (floor) plates, respectively.

With continued growth in thickness of the mantle layer, the spinal cord gradually acquires its definitive form (Figs



Figs 17.7A to E: Development of spinal cord. (A) Single layered neural tube; (B) Establishment of ependymal, mantle and marginal layers; (C and D) Division of mantle layer into alar and basal laminae; (E) Establishment of ventral and dorsal gray columns. The dorsal part of the cavity of the neural tube disappears. The ventral part persists as the central canal

17.7D and E). With growth of the alar lamina, the dorsal part of the cavity within the cord becomes obliterated: the *posterior median septum* is formed in this situation. The ventral part of the cavity remains as the *central canal*. Further enlargement of the basal lamina causes it to project forwards on either side of the midline, leaving a furrow, the *anterior median fissure*, between the projecting basal laminae of the two sides.

The nerve cells that develop in the mantle zone of the basal lamina become the *neurons of the anterior gray column* (Fig. 17.8). The axons of these cells grow out of the ventrolateral angle of the spinal cord to form the anterior/*ventral/motor nerve roots* of the spinal nerves. The nerve cells that develop in the mantle layer of the alar lamina form the *neurons of the posterior gray column*. These are sensory neurons of the second order. Their axons travel predominantly upwards in the marginal layer to form the *ascending tracts* of the spinal cord. Many of these cells form *interneurons*.

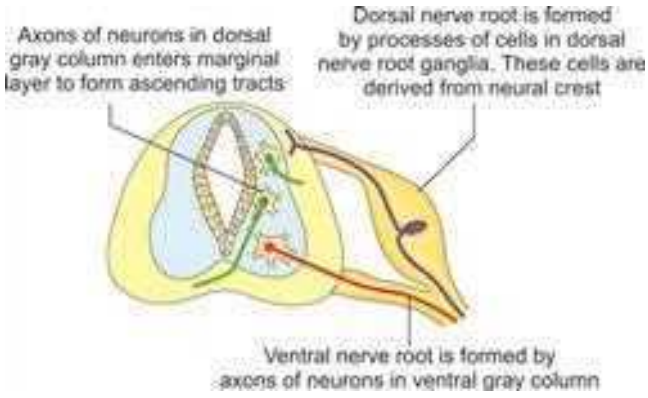


Fig. 17.8: Development of ventral and dorsal spinal nerve roots

The *posterior/dorsal/sensory nerve roots* are formed by the axons of cells that develop from the neural crest (Figs 17.8 and 17.9). Groups of these cells (pseudounipolar neurons) collect on the dorsolateral aspect of the developing spinal cord to form the *dorsal nerve root ganglia* (or *spinal ganglia*). The axons of these cells divide into two processes. The central processes migrate towards the spinal cord, and establish contact with the dorsolateral aspect of the latter, thus forming the *dorsal nerve roots*. These axons finally synapse with neurons of the posterior gray column developing in the alar lamina. The peripheral processes of the cells of the dorsal nerve root ganglia grow outwards to form the sensory components of the spinal nerves.

As stated above, the axons of neurons in the posterior gray column enter the marginal layer, to form the *ascending*

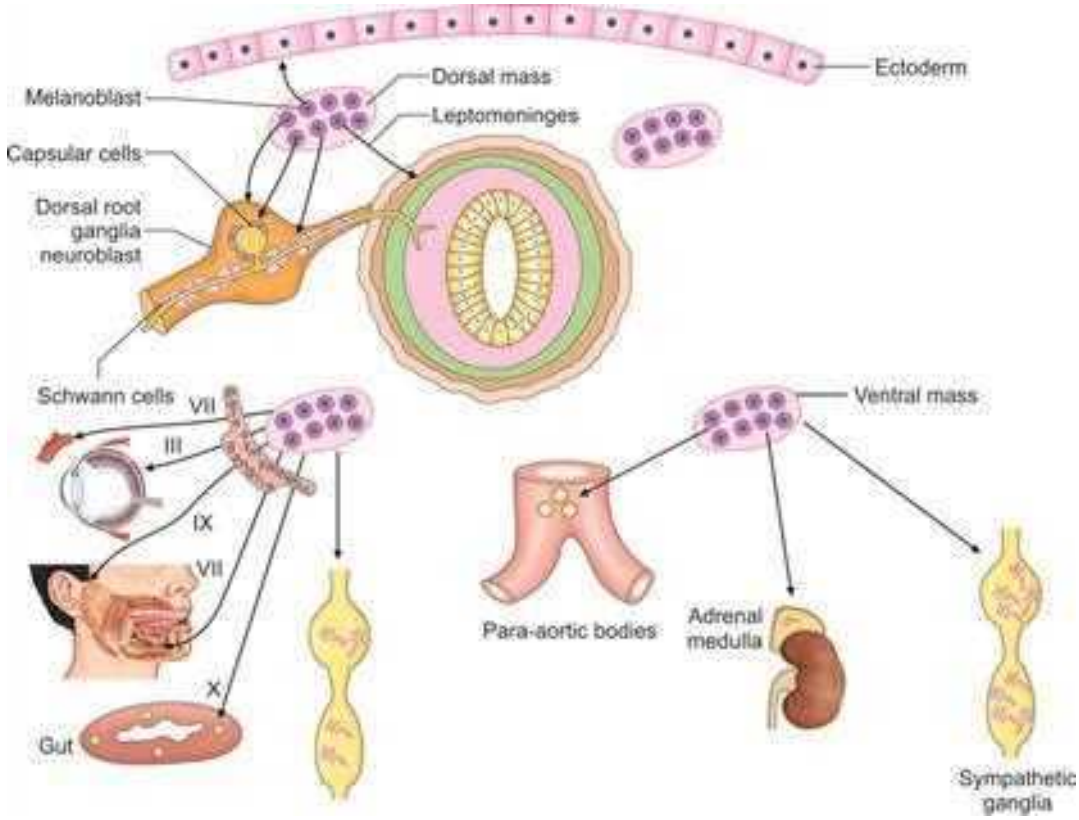


Fig. 17.9: Traditionally recognized derivatives of the neural crest. Some additional derivatives are now recognized (as mentioned in the text)

tracts of the spinal cord. At the same time, axons of cells developing in various parts of the brain grow downwards to enter the marginal layer of the spinal cord and form its *descending tracts*. These ascending and descending tracts form the white *matter* of the spinal cord. As the mantle layer takes on the shape of the anterior and posterior *gray columns*, the white matter becomes subdivided into anterior, lateral and posterior *white columns*.

Positional changes in spinal cord*:*

- The spinal cord up to 3rd month of intrauterine life extends throughout the length of the developing vertebral canal (Fig. 17.10A). The length of spinal cord and vertebral canal are equal. The spinal nerves run horizontally from their segment of origin to exit through the corresponding intervertebral foramina.
- Subsequently, due to the differential growth of spinal cord and vertebral column (the vertebral column becomes much longer than the spinal cord) at birth, the lower end of the cord is at the level of the third lumbar vertebra (Figs 17.10B and C). This process of recession of the spinal cord continues after birth as a result of which, in the adult, the cord usually ends at the level of the lower border of the first lumbar vertebra (Fig. 17.10D).
- One effect of this recession (of the cord) is that the intervertebral foramina no longer lie at the level at which the corresponding spinal nerves emerge from the spinal cord (Fig. 17.11). The nerves have, therefore, to follow an oblique downward course to reach the foramina. This obliquity is least for the cervical nerves, and greatest for the sacral and coccygeal nerves.

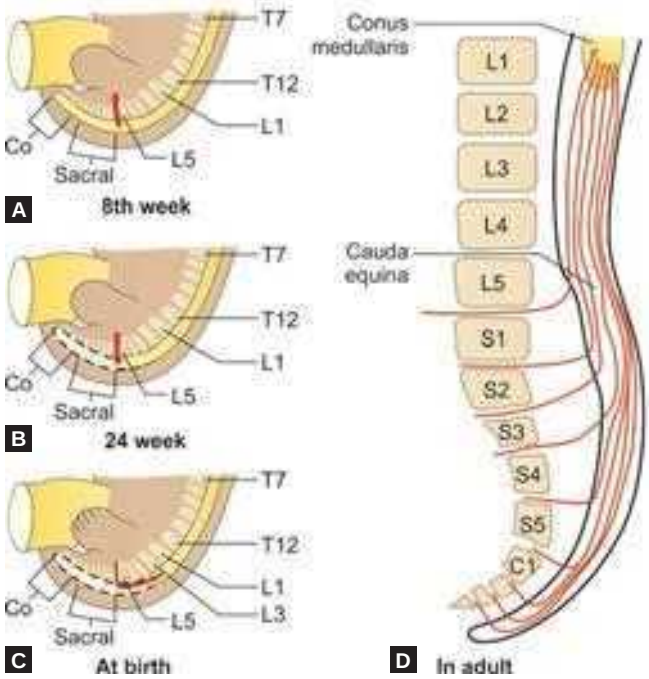


Fig. 17.10: Recession of spinal cord. Note that the lower end of the cord gradually move cranially, relative to the vertebrae

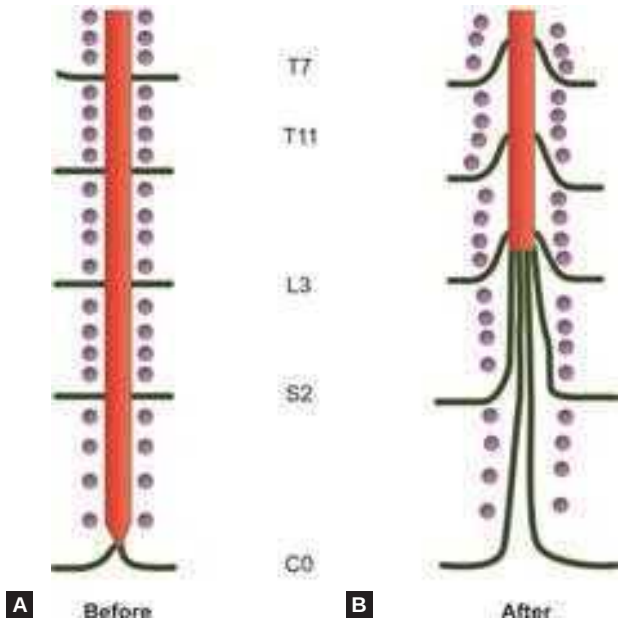


Fig. 17.11: Effect of recession of spinal cord on course of spinal nerves. (A) Shows the condition before recession begins. Spinal nerves pass horizontally from the spinal cord to their exit from the vertebral canal; (B) Shows the condition after recession has occurred. The nerves now have to run obliquely downwards to reach the points of exit. The obliquity is greatest in the case of the lowest nerves

Functional columns in gray matter of spinal cord: The axons of cells of basal lamina leaving the spinal cord as ventral roots join with the peripheral processes of pseudounipolar neurons of dorsal root ganglia to form mixed spinal nerves. The cells of basal and alar lamina are arranged as two longitudinal functional columns each. They are divided into somatic and visceral columns. Visceral column is close to sulcus limitans (Fig. 17.12). These four functional columns in spinal cord are concerned with general sensations only.

- *Afferent columns in alar lamina:* They receive the central processes of pseudounipolar neurons present in dorsal root ganglia. The two functional columns in alar lamina are:
  - *General somatic afferent:* It extends throughout the spinal cord and receives exteroceptive and proprioceptive information.
  - *General visceral afferent:* It is restricted to thoracolumbar and sacral regions of spinal cord and receives information from viscera and blood vessels.

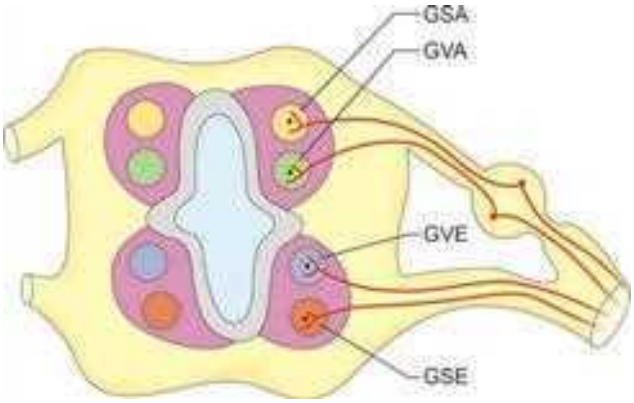


Fig. 17.12: Transverse section of spinal cord showing functional columns in gray matter

Abbreviations: GSA, general somatic afferent; GSE, general somatic efferent; GVE, general visceral efferent; GVA, general visceral afferent

- *Efferent columns in basal lamina:* The efferent columns in basal lamina give origin to the motor nerve fibers. The two functional columns in basal lamina are:
  - *General visceral efferent:* It is restricted to thoracolumbar and sacral regions of spinal cord. The neurons of this column provide preganglionic sympathetic fibers that synapse in the peripheral nervous system ganglia for supply to cardiac muscle, smooth muscle in the walls of viscera, glands and blood vessels.
  - *General somatic efferent:* It extends throughout the spinal cord and provides fibers that innervate skeletal muscles.

BRAINSTEM

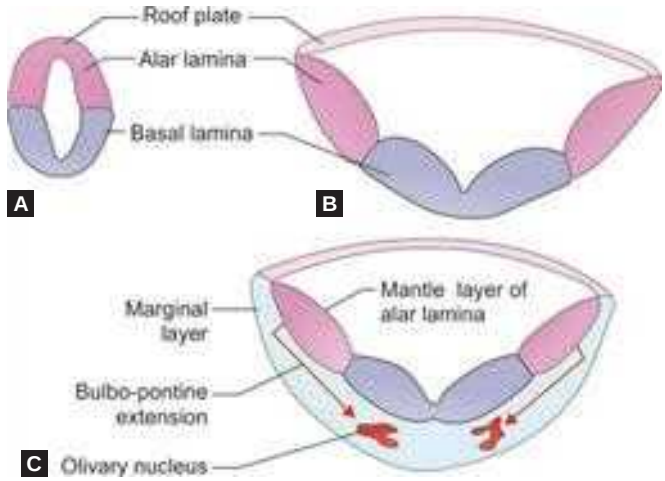
The medulla oblongata, pons and midbrain together constitute brainstem.

Medulla Oblongata

The medulla oblongata develops from the myelencephalon. The caudal closed part of the medulla is similar to that of the spinal cord and has a central canal. The appearance of the sulcus limitans divides each lateral wall into a dorsal or alar lamina, and a ventral or basal lamina (Fig. 17.13A). Rostral/cranial part of medulla is an open part where the thin roof *plate* becomes greatly widened as a result of which the alar laminae come to lay dorsolateral to the basal laminae. Thus, both these laminae are now in the floor of the developing fourth ventricle (Fig. 17.13B).

Cells developing in the lateral part of each alar lamina migrate ventrally, and reach the marginal layer overlying the ventrolateral aspect of the basal lamina. These cells constitute the caudal part of the *bulb-pontine extension*, and develop into the *olivary nuclei* (Figs 17.13C and 17.14).

The remaining cells of the alar lamina develop into the sensory nuclei of the cranial nerves related to the medulla. The motor nuclei of these nerves are derived from the basal lamina (Figs 17.15, 17.16A and B).



Figs 17.13A to C: (A) Development of medulla oblongata; (B) Note the great widening of the roof plate; (C) Note the bulbo-pontine extension and the olivary nuclei

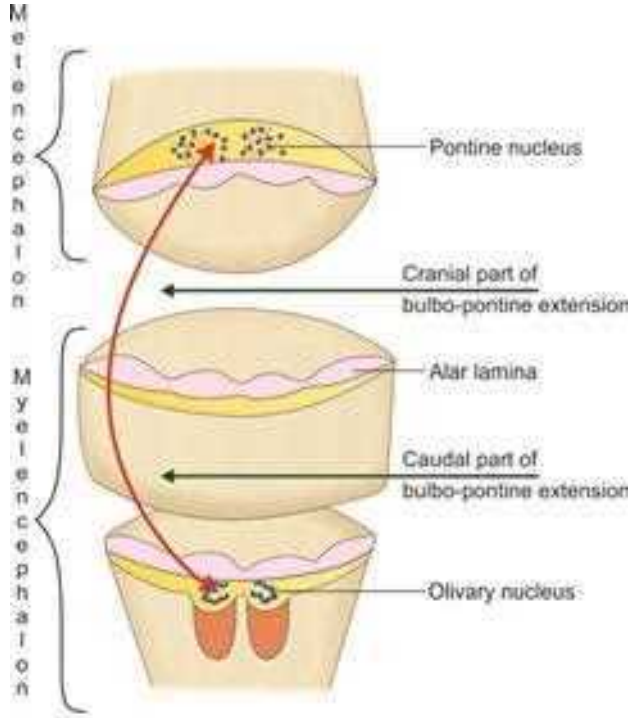


Fig. 17.14: Scheme to show the cranial and caudal parts of the bulbo-pontine extension. The caudal part lies in the medulla and forms the olivary nuclei, while the cranial part lies in the pons and forms the pontine nuclei

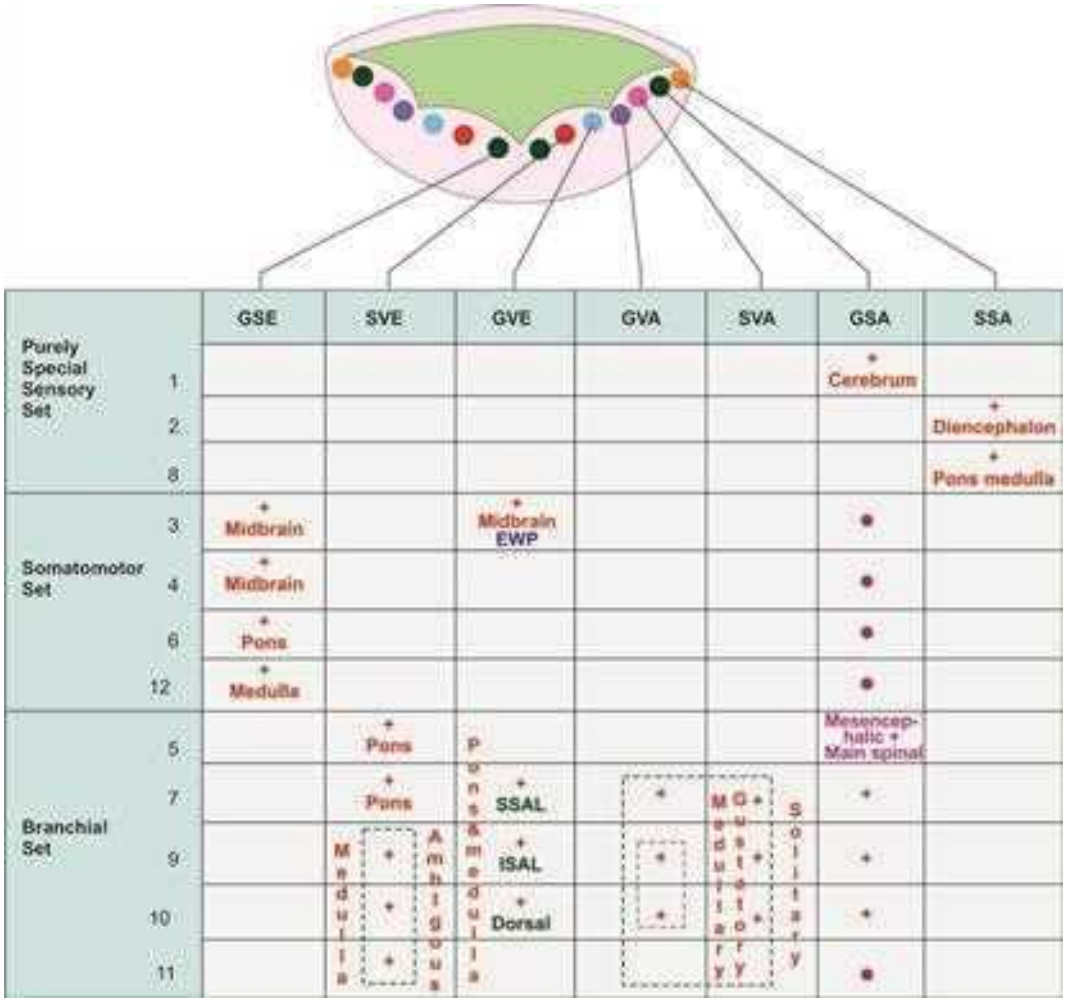
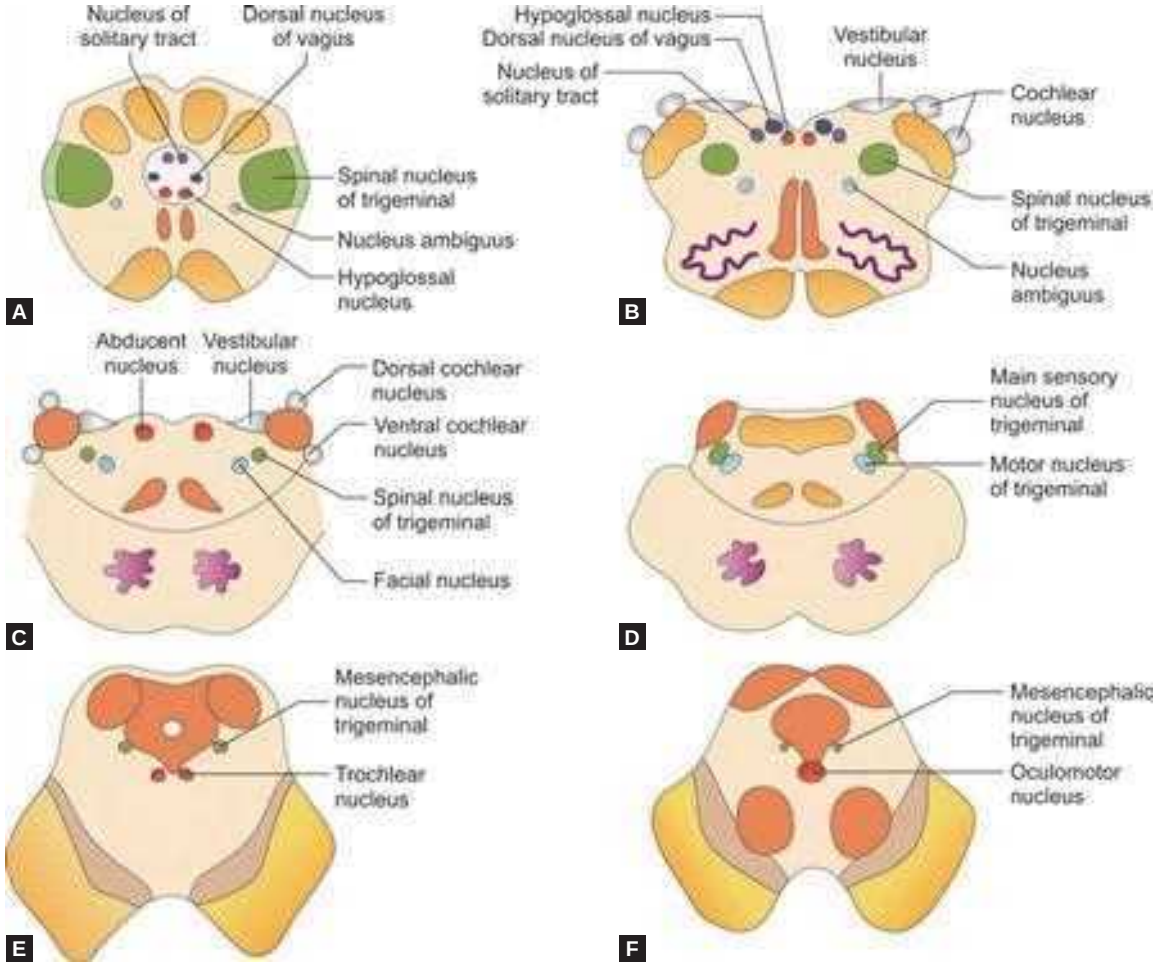


Fig. 17.15: Functional classification of cranial nerve nuclei. The upper figure shows the arrangement of nuclear columns in the brainstem of the embryo. The lower figure shows the nuclei derived from each column. Numbers indicate cranial nerves connected to the nuclei

Abbreviations: GSA, general somatic afferent; GSE, general somatic efferent; GVE, general visceral efferent; SSA, special somatic afferent; SVE, special visceral efferent; GVA, general visceral afferent; SVA, special visceral afferent

Functional columns of nuclei in brainstem: The nerve cells of the alar and basal laminae in the brainstem are at first grouped in accordance with their function and are arranged as illustrated in Figure 17.15 and presented in Table 17.2. These nuclei are arranged in seven longitudinal columns instead of four columns of spinal cord. The alar lamina contains four columns and the basal lamina contains three columns. The arrangement of nuclei in relation to sulcus limitans is that the visceral group is closer to sulcus than the somatic and in each group the general group is closer to sulcus limitans than the special.

- *Afferent columns in alar lamina:* The four functional columns in alar lamina are:
  - *General visceral afferent (GVA):* concerned with visceral sensations conveyed through 9th and 10th cranial nerves.
  - *Special visceral afferent (SVA):* concerned with taste sensation conveyed through 7th, 9th and 10th cranial nerves from the tongue, pharynx and soft palate.
  - *General somatic afferent (GSA):* contains mesencephalic, chief and spinal nuclei of trigeminal nerve concerned with proprioception, touch, and pain and temperature sensations respectively from the head and neck region.



Figs 17.16A to F: Location of cranial nerve nuclei as seen in transverse sections at various levels of the brainstem; (A and B) at the level of medulla; (C and D) at the level of pons; (E and F) at the level of midbrain

TABLE 17.2: Brainstem nuclei derived from alar and basal lamina

Brainstem part	*Alar lamina*	*Basal lamina*
Midbrain	Colliculi Substantia nigra Red nucleus Mesencephalic nucleus of trigeminal nerve (GSA)	Oculomotor nucleus (GSE) Edinger-Westphal nucleus (GVE) Trochlear nucleus (GSE)
Pons	Pontine nuclei Vestibular nuclei (SSA) Cochlear nuclei (SSA) Main sensory nucleus of trigeminal nerve (GSA) Nucleus of spinal tract of trigeminal nerve (GSA) Nucleus of tractus solitarius (SVA and GVA)	Motor nucleus of trigeminal nerve (SVE) Motor nucleus of facial nerve (SVE) Nucleus of abducent nerve (GSE) Superior salivatory nucleus (GVE) Lacrimatory nucleus (GVE)
Medulla	Inferior olivary nuclei Vestibular nuclei (SSA) Nucleus of spinal tract of trigeminal nerve (GSA) Nucleus of tractus solitarius (SVA and GVA) Part of dorsal nucleus of vagus (GVA)	Part of dorsal nucleus of vagus (GVE) Inferior salivatory nucleus (GVE) Nucleus ambiguus (SVE) Hypoglossal nucleus (GSE)

Abbreviations: GSA, general somatic afferent; GSE, general somatic efferent; GVE, general visceral efferent; SSA, special somatic afferent; SVE, special visceral efferent; GVA, general visceral afferent; SVA, special visceral afferent

  - *Special somatic afferent (SSA):* contains vestibular and cochlear nuclei concerned with equilibrium and hearing.
- *Efferent columns of basal lamina:* The three functional columns in basal lamina are:
  - *General somatic efferent (GSE):* contains nuclei of cranial nerves (3rd, 4th, 6th and 12th) that supply the extraocular muscles of eyeball and musculature of tongue
  - *Special visceral efferent/Branchial efferent (SVE):* contains the nuclei of cranial nerves (5th, 7th, 9th, 10th and 11th) that supply to musculature derived from pharyngeal arches
  - *General visceral efferent (GVE):* contains the cranial nerve nuclei of origin for preganglionic neurons of peripheral parasympathetic ganglia.
    - *Edinger-Westphal nucleus (3rd cranial nerve):* Ciliary ganglion
    - *Superior salivatory nucleus (7th cranial nerve):* Pterygopalatine and submandibular ganglia
    - *Inferior salivatory nucleus (9th cranial nerve):* Otic ganglion
    - *Dorsal nucleus of vagus (10th cranial nerve):* for supply of thoracic and abdominal viscera

Subsequently, some of these nuclei migrate ventrally, from their primitive position in the floor of the fourth ventricle. Their ultimate position is indicated in Figure 17.16. The *gracile* and *cuneate* nuclei are derived from the lowermost part of the somatic afferent column. The *white matter* of the medulla is predominantly extraneous in origin, being composed of fibers constituting the ascending and descending tracts that pass through the medulla.

Pons

The pons arises from the ventral part of the metencephalon. It also receives a contribution from the alar lamina of the myelencephalon, in the form of the cranial part of the bulbopontine extension (Figs 17.16C and D and 17.17). This extension comes to lie ventral to the metencephalon, and gives rise to the *pontine nuclei*. Axons of cells in these nuclei grow transversely to form the *middle cerebellar peduncle*.

As in the myelencephalon, the roof of the metencephalon becomes thin and broad (Fig. 17.16). The alar and basal laminae are thus orientated as in the medulla.

The lateral part of each alar lamina (often called the *rhombic lip*) becomes specialized to form the cerebellum. The ventral part of the alar lamina gives origin to the sensory cranial nerve nuclei, and the basal lamina to the motor cranial nerve nuclei, of the pons (Figs 17.15, 17.16C and D).

The nuclei derived from the basal and alar laminae lie in the dorsal or tegmental part of the pons. The ventral part of the pons is constituted by:

- Cells of the bulbopontine extension (derived from the alar lamina of the myelencephalon), that form the pontine nuclei. Axons of the cells in these nuclei grow transversely and form the *middle cerebellar peduncle*.
- Corticobulbar and corticospinal fibers that descend from the cerebral cortex, pass through this region on their way to the medulla and spinal cord. Some fibers from the cerebral cortex terminate in relation to the pontine nuclei. These are the corticopontine fibers.

Midbrain

The midbrain is developed from the mesencephalon. The cavity of the mesencephalon remains narrow and forms the aqueduct. As described in the case of the spinal cord, the mantle layer becomes subdivided into a dorsal or alar lamina and a ventral or basal lamina by the appearance of the sulcus limitans (Table 17.2). The nuclei which develop from the basal lamina are oculomotor nerve nucleus, trochlear nerve nucleus and Edinger-Westphal nucleus.

The alar lamina gives rise to the cells of the colliculi. At first, these form one mass which later becomes subdivided by a transverse fissure into a pair of superior and a pair of inferior colliculi. Some cells of the alar lamina migrate ventrally to form the *red nucleus* and the *substantia nigra* (Table 17.2) (Figs 17.16E and F).

The marginal layer of the ventral part of the mesencephalon is invaded by downward growing fibers of the corticospinal, corticobulbar and corticopontine

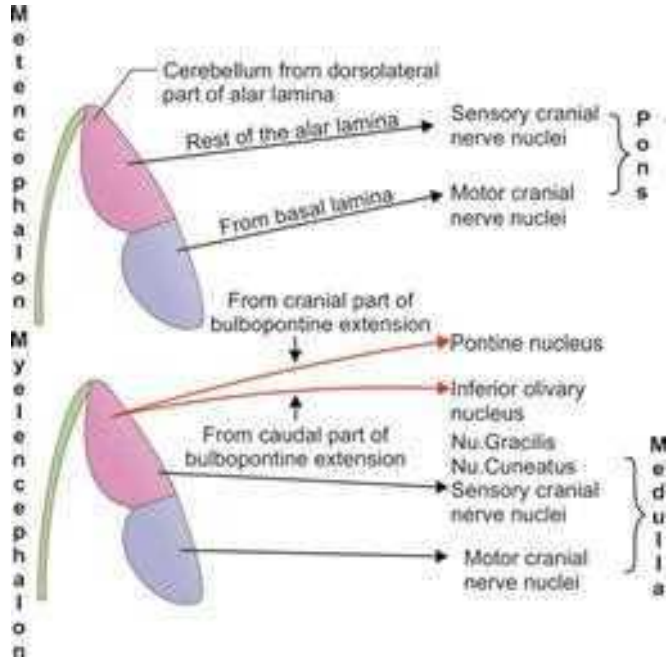


Fig. 17.17: Scheme of the development of medulla and pons

pathways. This region, thus, becomes greatly expanded, and forms the *basis pedunculi* (crus cerebri).

CEREBELLUM

The cerebellum develops from the dorsolateral part of the alar lamina of metencephalon (Fig. 17.18A). There are at first two primordia of the cerebellum, right and left. These extend medially in the roof plate of the metencephalon to eventually fuse across the midline (Figs 17.18B to D). As the cerebellum increases in size, fissures appear on its surface. The lateral lobes and vermis can soon be distinguished, as a result of differential growth.

The developing cerebellum can be divided into an *intraventricular part* that bulges into the cavity of the developing fourth ventricle, and an *extraventricular part* that is seen as a bulging on the surface (Fig. 17.18C). At first, the intraventricular part is the larger of the two, but at a later stage, the extraventricular part becomes much larger than the intraventricular part and constitutes almost the whole of the organ (Figs 17.18D to F).

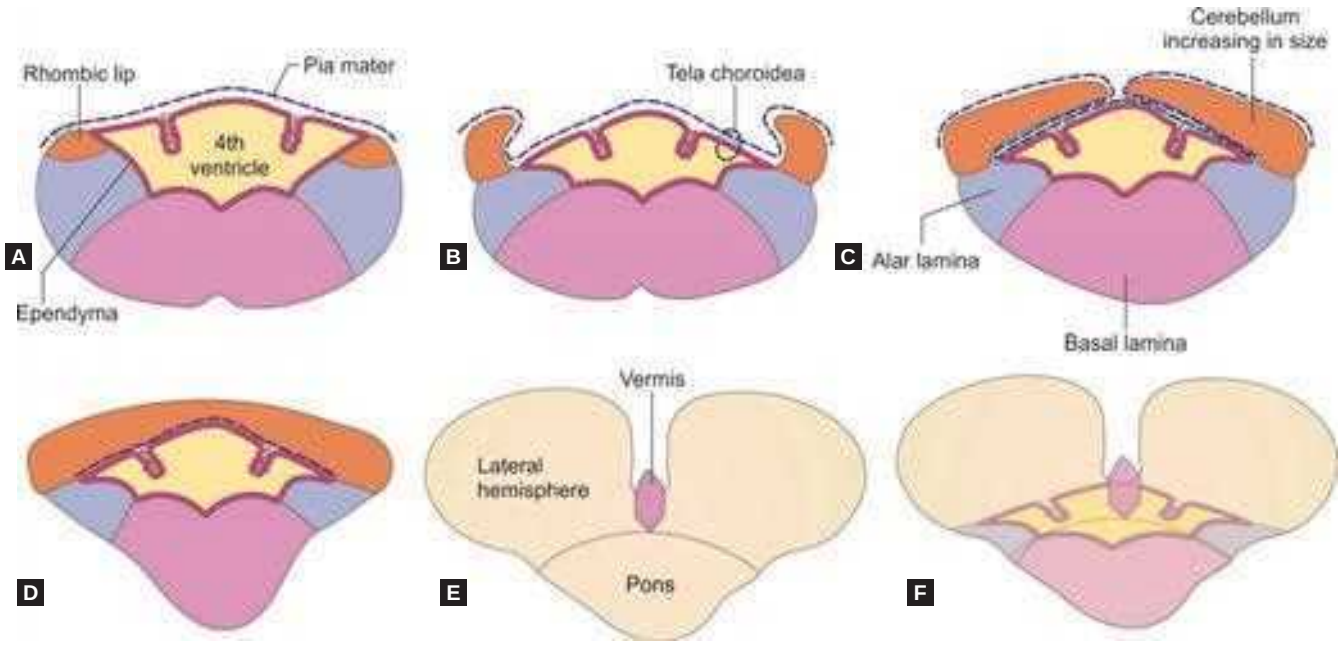
The cerebellum, at first, consists of the usual matrix cell (neuroepithelial), mantle and marginal layers (Fig. 17.19A). Some cells of the mantle layer migrate into the marginal layer to form the external granular layer in the marginal zone. The cells of the external granular layer actively proliferate, expand and results in folding of the surface leading to the formation of folia. Further proliferation of the neuroepithelial cells and their migration to the deeper part of the marginal layer forms internal granular layer, which is the definitive granular cortex, consisting of the Purkinje and Golgi neurons. Inward migration of small nerve cells and glial cells from external granular layer towards internal granular layer to intermingle with those cells already present in this layer occurs (Fig. 17.19B). The cells of the mantle layer that are not migrating into the cortex develop into the *dentate, emboliform, globose* and *fastigial nuclei* (Fig. 17.19C).

Posterolateral sulcus is the first fissure to appear and separates the flocculonodular lobe from rest of the cerebellum (Fig. 17.19D). During 3rd month, fissura prima, fissura seconda and post-pyramidal fissures appear that separate various lobes of corpus cerebelli (Fig. 17.19E).

The *superior cerebellar peduncle* is formed chiefly by the axons growing out of the dentate nucleus. The *middle cerebellar peduncle* is formed by axons growing into the cerebellum from the cells of the pontine nuclei, while the *inferior cerebellar peduncle* is formed by fibers that grow into the cerebellum from the spinal cord and medulla.

CEREBRAL HEMISPHERE

The cerebrum is a derivative of the prosencephalon (Figs 17.20A and E). Prosencephalon is divisible into a median diencephalon and two lateral telencephalic vesicles (Figs 17.2C, 17.20B and F). The telencephalic vesicles give origin, on either side, to the *cerebral cortex* and the *corpus striatum*. The diencephalon gives rise to the *thalamus, hypothalamus* and related structures.



Figs 17.18A to F: Some stages in the development of the cerebellum: (A) Cerebellar rudiments appear from alar lamina of metencephalon; (B) They grow into the roof plate of the metencephalon to meet in the midline; (C) Cerebellum enlarges and bulges out of the fourth ventricle; (D to F) Lateral hemispheres and vermis can be distinguished

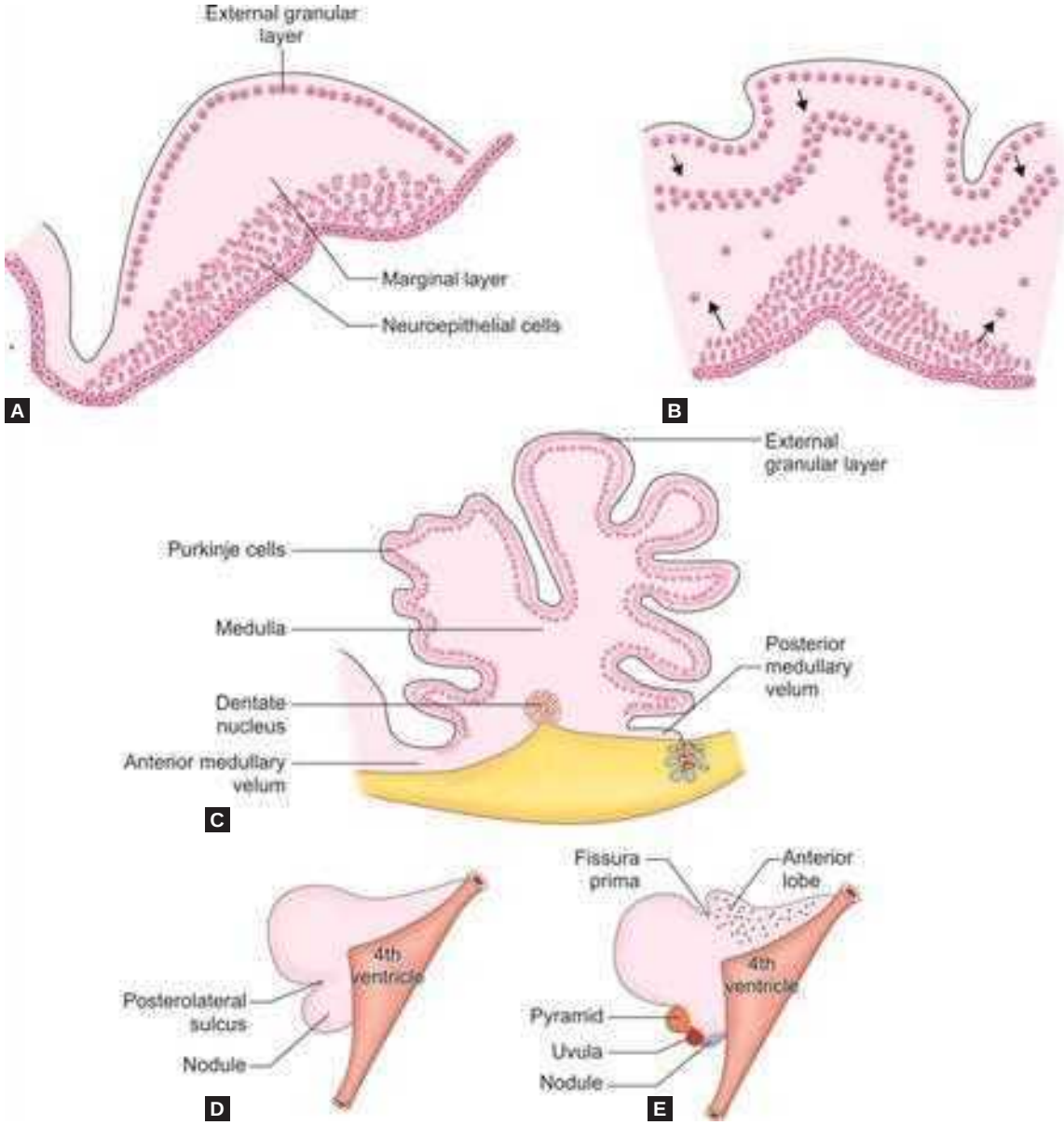
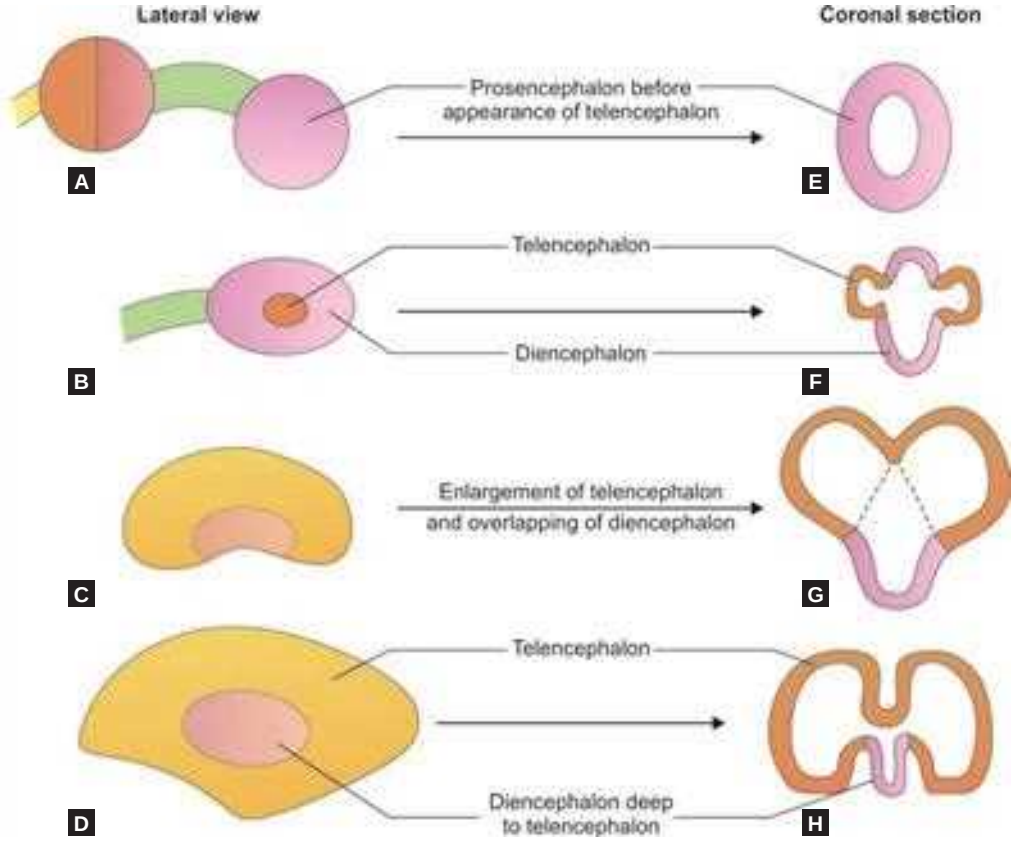


Fig. 17.19: Histogenesis of cerebellum

The telencephalic vesicles are at first small (Figs 17.20B and F), but rapidly increase in size extending upwards, forwards and backwards (Figs 17.20C, G and 17.21). As a result of these extensions, the telencephalon comes to completely cover the lateral surface of the diencephalon (Figs 17.20D and H) and eventually fuses with it (Fig. 17.21). Thus, the cerebral cortex and corpus striatum will come to lie lateral to the thalamus and hypothalamus.

With further upward, forward and backward extension of the telencephalic vesicles, the vesicles of the two sides come into apposition with each other above, in front of, and behind the diencephalon (Figs 17.20H and 17.21).

Formation of lateral and third ventricles: The cavity of the diencephalon forms the *third ventricle*, while the cavities of the two telencephalic vesicles form the *lateral ventricles* (Fig. 17.3). Each lateral ventricle is at first a spherical space within the telencephalic vesicle (Fig. 17.22A). With the forward and backward growth of the vesicle, the ventricle becomes elongated anteroposteriorly (Fig. 17.22B). The posterior end of the telencephalic vesicle now grows downwards and forwards, to form the temporal lobe, and the cavity within it becomes the inferior horn (Figs 17.22C and D). The ventricle thus becomes C-shaped. Finally, as a result of backward growth, the occipital pole of the hemisphere becomes



Figs 17.20A to H: Development of the cerebral hemisphere. This series of figures shows the changes in the relative size and position of the diencephalon and the telencephalic vesicles. Figures (A), (B), (C) and (D) are lateral views. Figures (E), (F), (G) and (H) are corresponding coronal sections along the axes indicated. (A and E) Prosencephalon before appearance of telencephalic vesicles; (B and F) Telencephalic vesicles appear; (C and G) Telencephalic vesicles enlarge and partially cover diencephalon; (D and H) Telencephalon much larger than diencephalon and completely overlapping it

established, the part of the ventricle within it becoming the posterior horn (Fig. 17.22E).

With the enlargement of the telencephalic vesicles, their medial walls become opposed to each other (Fig. 17.20H). In this way, a groove bounded by the two medial surfaces is formed, these surfaces being continuous with each other in the floor of the groove. Floor of this groove forms the roof of the third ventricle. Just above the floor of this groove, the medial wall is invaginated laterally into the cavity of the lateral ventricle. The cavity of the invagination is the choroid fissure (Figs 17.22, 17.23A and B). A fold of pia mater extends into this fissure and forms the telachoroidea (Fig. 17.23C). A bunch of capillaries is formed within this fold and forms the choroid plexus (Fig. 17.23D). The original wall of the ventricle lining the choroid plexus remains very thin and forms the ependymal covering of the plexus (Fig. 17.23). Note that the telachoroidea is in intimate relationship to both lateral ventricles and also to the roof of the third ventricle (Fig. 17.23). With the establishment of

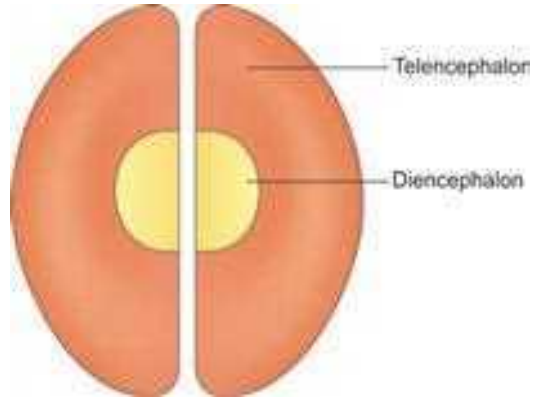


Fig. 17.21: Two telencephalic vesicles come to be apposed to each other in front of and behind the diencephalon

the temporal pole and the formation of the inferior horn of the lateral ventricle, the choroid fissure becomes C-shaped (Fig. 17.22). The inferior part of the fissure now invaginates into the inferior horn of the lateral ventricle (Fig. 17.22E).

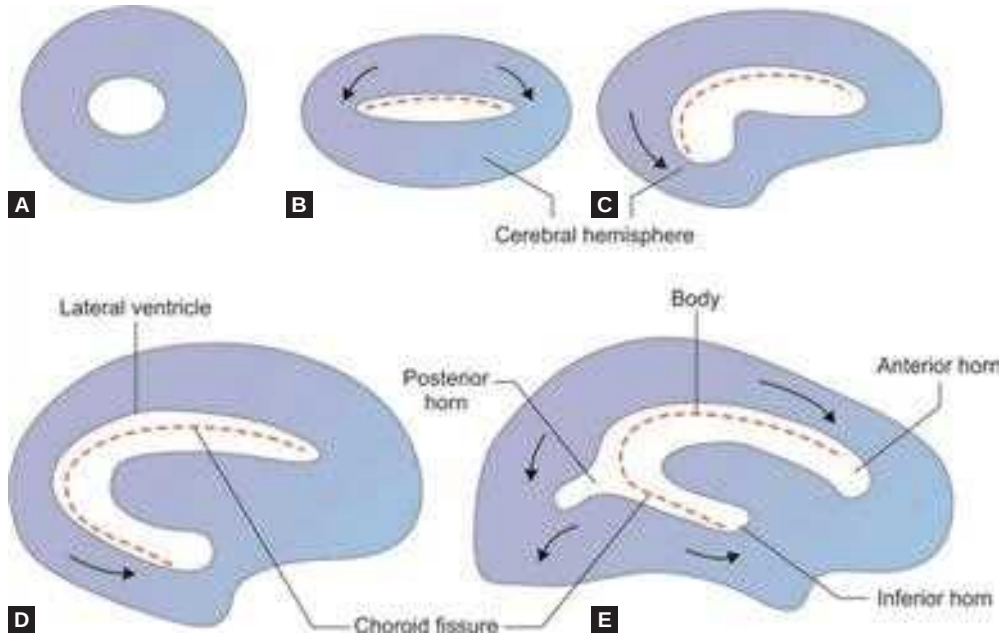


Fig. 17.22: Establishment of the form of the cerebral hemisphere and of the lateral ventricle. Arrows indicate direction of growth. The choroid fissure is shown in dotted line

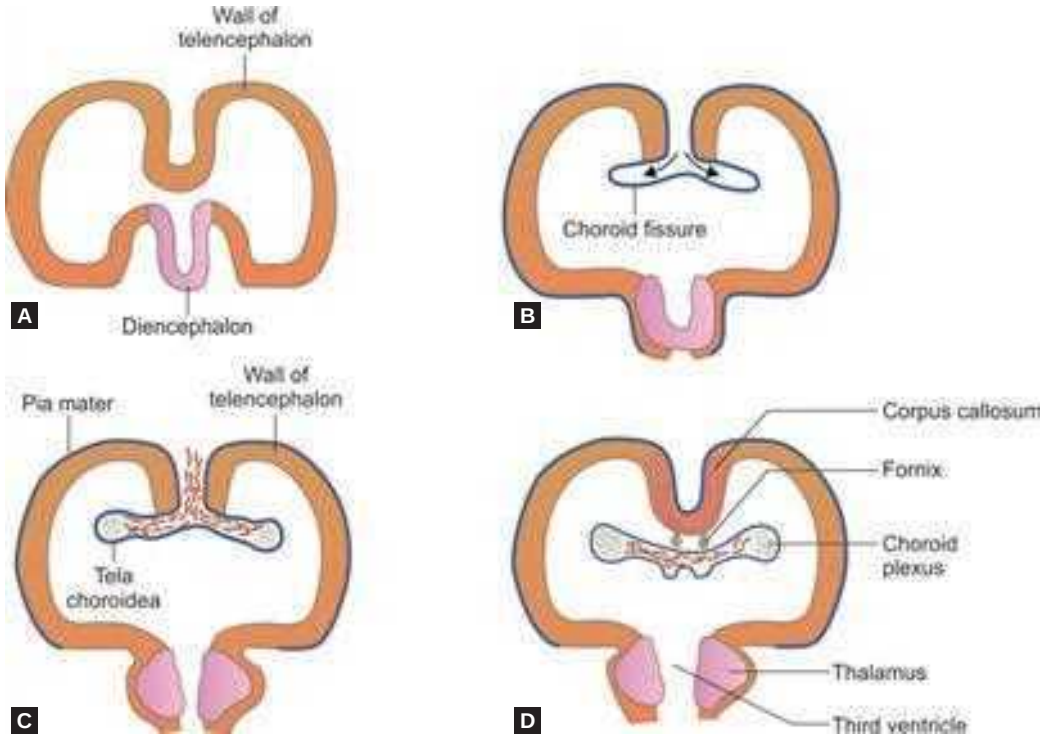


Fig. 17.23: Formation of the choroid fissure, telachoroidea (fold of pia mater) and choroid plexus (bunch of capillaries). The wall of the telencephalon remains thin at this site

Thalamus and Hypothalamus

The thalamus and hypothalamus develop from the diencephalon. After the establishment of the telencephalon, the lateral wall of the diencephalon becomes thickened. It is soon subdivided into three regions by the appearance of two grooves, called the *epithalamic and hypothalamic sulci* (Fig. 17.24A).

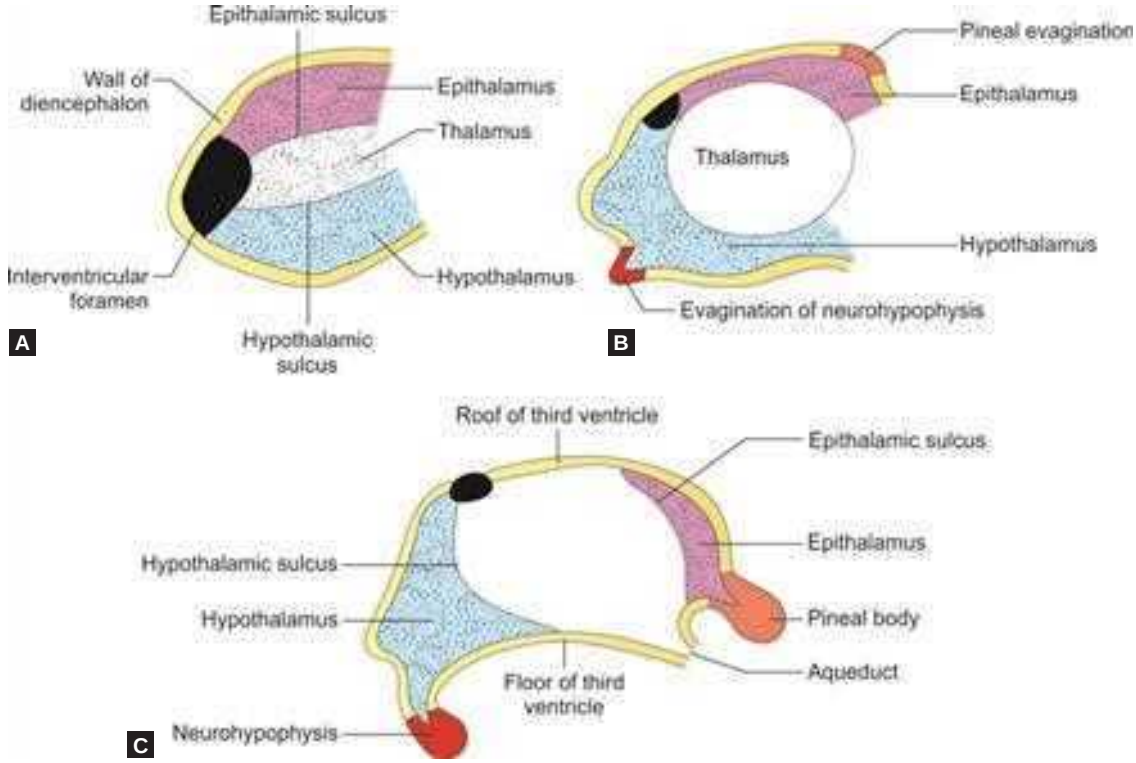


Fig. 17.24: Development of thalamus and hypothalamus. The appearance of the epithalamic and hypothalamic sulci divides the diencephalon into thalamus, epithalamus and hypothalamus. The pineal body is formed in relation to the epithalamus, and the neurohypophysis in relation to the hypothalamus

The central part, lying between these two sulci, enlarges to form the *thalamus* (Figs 17.24B and C). The part above the epithalamic sulcus remains relatively small and forms the *epithalamus*, which is represented by the habenular nuclei and the pineal body. The part below the hypothalamic sulcus forms the *hypothalamus*.

The various nuclei of the thalamus and hypothalamus are formed by multiplication of cells in the mantle layer of the wall of the diencephalon.

Corpus Striatum

The corpus striatum is a derivative of the telencephalon. Early in its development, each telencephalic vesicle can be subdivided into a basal part which is thick, and a superior part which is thin (Figs 17.25A and B). Some of the cells, in the mantle layer of the thick basal part, migrate into the overlying marginal layer to form part of the cerebral cortex. The remaining cells of the mantle layer of this region form the corpus striatum.

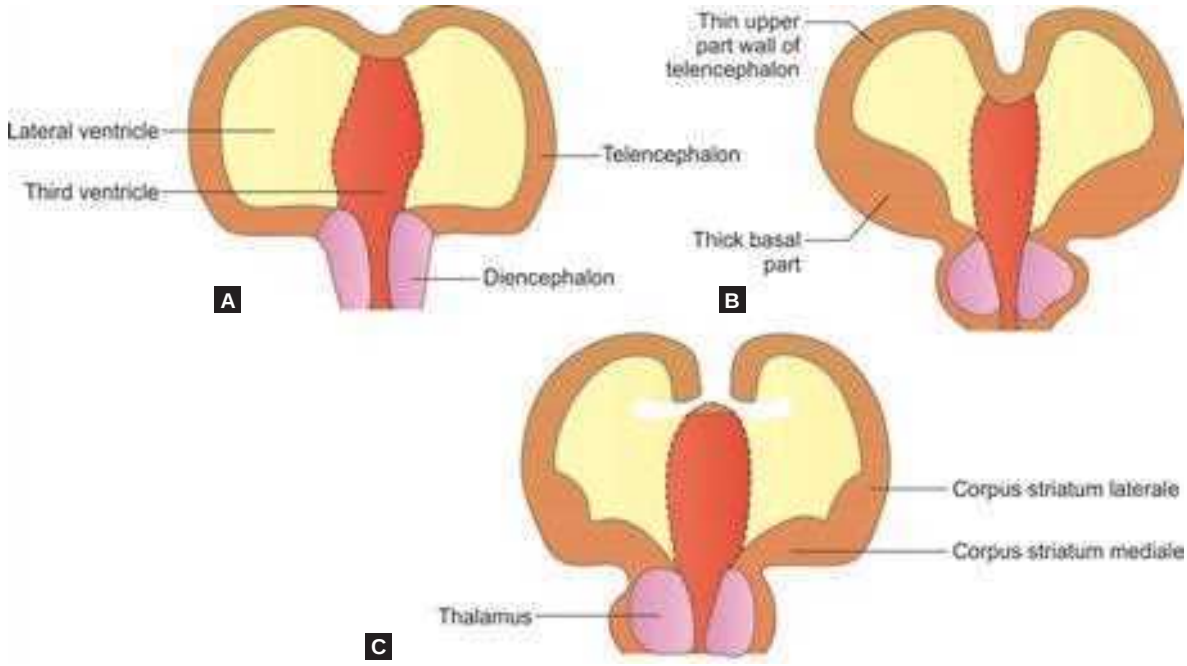
The developing corpus striatum soon becomes subdivided into medial and lateral subdivisions, which increase in thickness (Fig. 17.25C). Meanwhile, the cerebral cortex is developing and numerous axons, which are growing downwards from it or growing towards it, pass through the region of the corpus striatum and divide it into a deeper and a superficial part. These fibers constitute the internal *capsule* (Fig. 17.26).

The part of the corpus striatum that comes to lie deep to the internal capsule becomes the *caudate nucleus*, and the superficial part becomes the lentiform *nucleus* (Figs 17.27 and 17.28). The lentiform nucleus later becomes subdivided into the *putamen*, and the *globus pallidus*.

Cerebral Cortex

The cerebral cortex is formed by migration of cells from the mantle layer into the overlying marginal layer. These cells divide, and subdivide, leading to considerable thickening of the cortex. By full term, several layers of cells can be recognized. Simultaneously, there is considerable side-to-side expansion of the cortex as a result of which its surface area is greatly increased.

As the surface expansion is at a greater rate than that of the hemisphere as a whole, the cortex becomes folded on itself. Sulci and gyri are formed as a result of this folding. The region of the insula is relatively slow in growth, and is gradually overgrown by adjacent areas, which form the opercula of the insula.



Figs 17.25A to C: Early development of corpus striatum as seen in coronal sections: (A) Telencephalon before appearance of corpus striatum; (B) Wall of basal part thickened; (C) Thickening divides into medial and lateral parts

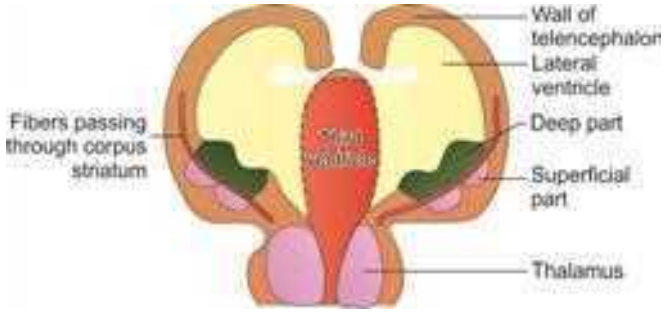


Fig. 17.26: Wall of telencephalon at a stage somewhat later than that shown in Figure 17.32C. The region of the developing corpus striatum is divided (longitudinally) into deep and superficial parts (by nerve fibers growing downwards through it)

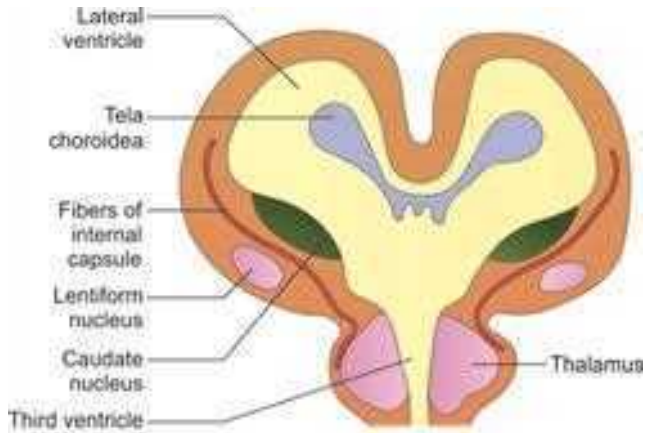


Fig. 17.27: Deep part of corpus striatum becomes the caudate nucleus. Superficial part becomes the lentiform nucleus. Note relation of these to the thalamus developing in the diencephalon

From a developmental point of view, the cerebral cortex consists of:

- Hippocampal cortex
- Pyriform cortex
- Neocortex.

The neocortex is the most important part. It undergoes very great expansion and forms the whole of the cerebral cortex seen on the superolateral and medial surfaces of the cerebral hemisphere, and the cortex of the inferior surface excluding the pyriform area (Figs 17.29 and 17.30).

The hippocampal cortex forms the hippocampus, and the indusium griseum. The pyriform cortex gives rise to the part of the cerebral cortex that receives olfactory sensations. It forms the uncus, the anterior part of the *parahippocampal gyrus*, and the *anterior perforated substance*.

The developing telencephalon has a medial wall (apposed to its counterpart of the opposite side), a superolateral wall, and a basal striatal region (Fig. 17.25C). The hippocampal cortex develops in the medial wall, the pyriform cortex in the marginal layer superficial to the corpus striatum, and the neocortex in the superolateral region (Fig. 17.30).

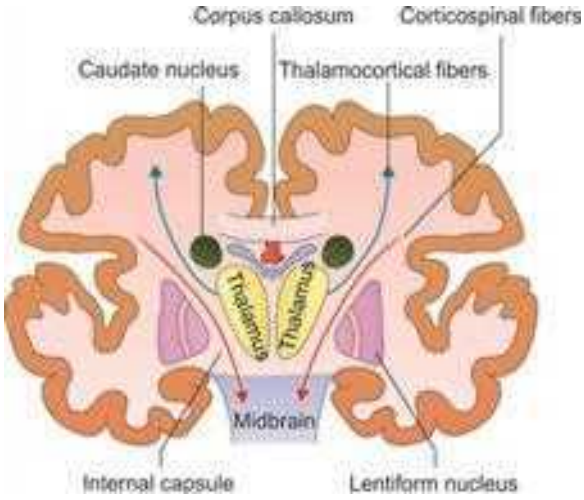


Fig. 17.28: With enlargement of the telencephalon, the lentiform nucleus comes to lie lateral to the thalamus. The internal capsule passes through the interval between the lentiform nucleus laterally and the caudate nucleus and thalamus medially

From Figures 17.29 and 17.30 it will be seen that the hippocampal cortex is closely related to the choroid fissure. With the establishment of the inferior horn of the lateral ventricle, the hippocampal cortex follows the curve of the choroid fissure and thus, assumes a ring-like configuration (Fig. 17.29B). However, the superior part of the hippocampal cortex is soon separated from the fissure by the formation of the corpus callosum. This part of the cortex remains rudimentary and forms the *indusium griseum*. The lower part of the hippocampal cortex undergoes relatively greater development and becomes the hippocampus and the *dentate gyrus* (Fig. 17.29C). With the expansion of the neocortex, these structures are pushed into the cavity of the inferior horn of the lateral ventricle (Fig. 17.30B).

White Matter of Cerebrum

The bulk of the cerebrum is constituted by its white matter. This is made up of:

- Axons of cortical cells that grow towards other areas of the cortex, either in the same or in the opposite hemisphere.
- Axons of cortical cells that grow downwards through the hemisphere, on their way to the brainstem and spinal cord.
- Axons that connect the thalamus, hypothalamus and basal ganglia to one another and to the cortex.
- Axons that grow into the hemisphere from the brainstem and spinal cord.

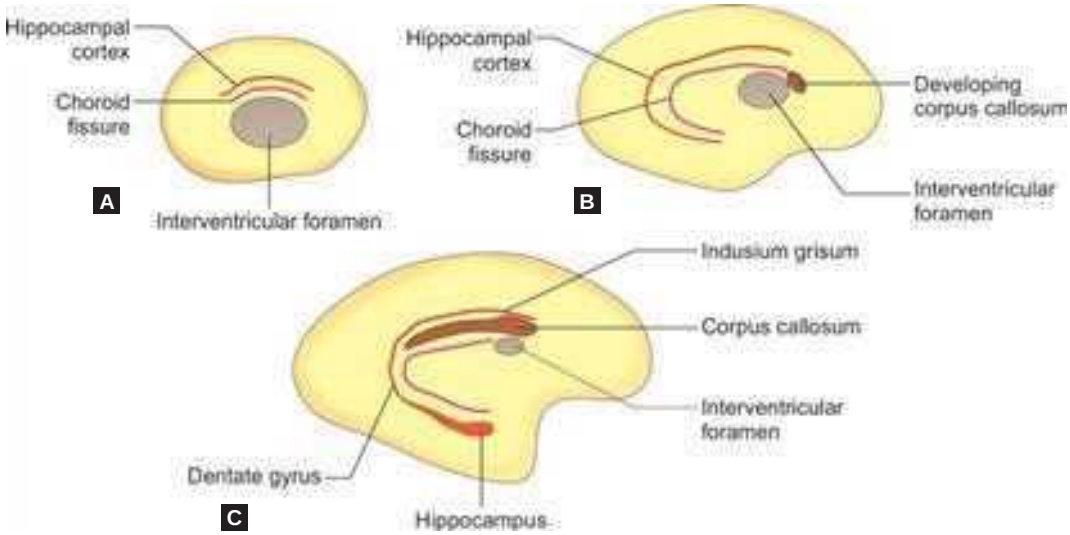


Fig. 17.29: Three stages in formation of the hippocampal cortex

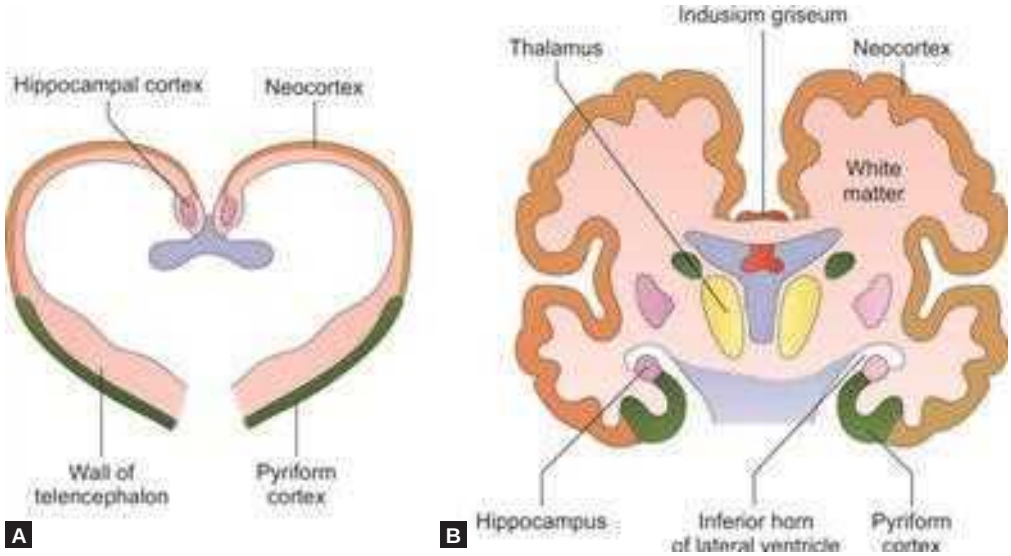


Fig. 17.30: Development of the cerebral cortex. Most of the cerebral cortex is derived from the neocortex. The hippocampal cortex forms the hippocampus and the indusium griseum

Cerebral Commissures

The part of the wall of the neural tube that closes the cranial end of the prosencephalon is called the lamina terminalis (Figs 17.31 and 17.32A). After the appearance of the telencephalic vesicles, the lamina terminalis lies in the anterior wall of the third ventricle. Any neuron growing from one hemisphere to the other must pass through this lamina (Fig. 17.31). To facilitate this passage, the lamina terminalis becomes thickened to form the *commissural plate* (Fig. 17.32B). Three principal commissural fibers pass through lamina terminalis.

- The first commissural fibers to develop form the *anterior commissure*.
- The second is the *hippocampal commissure/fornix*.
- The *corpus callosum* appears later and is the largest commissure. It, at first, lies anterior to the diencephalon, but because of rapid increase in its size, it extends backwards and roofs over this region (Figs 17.32B and C). The part of lamina terminalis that stretches between corpus callosum and fornix persists as *septum pellucidum*.
- Other commissures that appear are the *optic chiasma*, the *habenular commissure* and the *posterior commissure*.

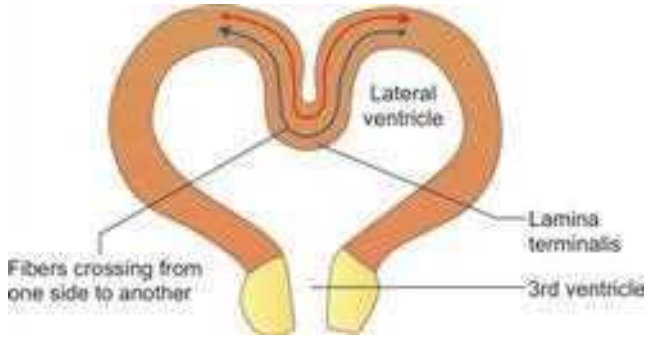


Fig. 17.31: Diagram to show how the lamina terminalis serves as a path for nerve fibers passing from one cerebral hemisphere to the other

Clinical correlation

Anomalies of brain and spinal cord

Neural tube defects (NTDS)

These are a group of conditions where due to non-approximation of neural folds, they result in an opening in the spinal cord or brain or both from the early human development.

There are two types of NTDs: open and closed.

Open NTDs are more common. It results when the brain and/or spinal cord are exposed at birth through a defect in the skull or vertebrae. Examples are anencephaly and ***spina bifida.***

Closed NTDs are rare and occur when the spinal defect is covered by skin. It is due to malformation of fat/bone/membranes.

Outward bulging of neural tube and covering membranes

As a result of non-fusion of the neural tube, or of overlying bones (e.g. spina bifida), neural tissue may lie outside the cranial cavity or vertebral canal. When this happens in the region of the brain, the condition is called ***encephalocele*** (Fig. 17.33A), and when it occurs in the spinal region, it is called myelocele (Figs 17.33B).

When the condition is due to non-closure of the neural tube, the nervous tissue is exposed on the surface. Failure of closure of anterior and posterior neuropore results in conditions called ***anencephaly*** (Figs 17.33C and D) and ***spina bifida*** (Fig. 17.34), respectively. Failure of closure of entire neural tube results in a condition called ***rachischisis*** (Fig. 17.33E).

- ***Anencephaly (Fig. 17.33C and D):*** Failure of closure of anterior neuropore results in exposure of brain substance to the surface as an irregular degenerated mass. It is characterized by absence of vault of skull exposing the brain that gets degenerated and malformed. There will be absence of swallowing reflex in the fetus and hydramnios in the last trimester. Antenatal diagnosis of this condition is by ultrasonography and estimation of alpha fetoproteins in amniotic fluid. Prevention is by administration of folic acid before and during pregnancy.
- ***Rachischisis (Figs 17.33E and 17.34A):*** Failure of closure of neural groove results in exposure of neural tissue onto the surface.
- ***Spina bifida (Figs 17.34B to E and 17.35)***
  When the neural tube has closed and the outward bulging of spinal cord or its coverings is a result of a defect of the overlying bones. There are different types of spina bifida depending on the contents of the bulging.
  - Spina bifida occulta: where the spinal cord is normal (Fig. 17.34B)
  - Meningocele: pia and arachnoid protrude through the gap in the bifid spine forming a cystic swelling covered with skin (Figs 17.34C and 17.35A)
  - *Meningomyelocele:* the cystic swelling includes spinal cord (Figs 17.34D and 17.35B). Meningomyelocele sometimes is associated with downward projection of some part of cerebellum and medulla through foramen magnum and a resulting obstruction leading to hydrocephalus. Such combined malformation is called *Arnold-Chiari malformation*.
  - *Anterior spina-bifida:* rare anomaly. Two halves of vertebral body fails to fuse and the spinal meninges protrude ventrally through the gap (Fig. 17.34E).
- ***Hydrocephalus (Fig. 17.35C)***
  - This condition occurs due to accumulation of abnormal quantity of cerebrospinal fluid (CSF) in the ventricular system of brain. This can be due to blockage to the circulation of CSF or its excessive production. The pressure of the fluid causes degeneration of nervous tissue. The ventricles become very large and the infant is born with a large head. The enlargement of central canal and associated abnormal cavities near central canal is called ***syringomyelia***.
  - *Dandy-Walker syndrome:* a form of hydrocephalus resulting from blockage of median and lateral apertures of the fourth ventricle. Enlargement is predominantly in the posterior cranial fossa and the cerebellum is abnormal.

AUTONOMIC NERVOUS SYSTEM

Sympathetic Neurons

Any sympathetic pathway consists of two neurons, i.e. a preganglionic and a postganglionic neuron.

- The preganglionic neurons develop in the mantle layer of the thoracolumbar region of the spinal cord (segments T1 to L2 or L3). These cells are located near the sulcus limitans, and form the lateral horn of the cord (Fig. 17.36). The axons growing out from them are myelinated. They pass into the ventral nerve roots to enter the spinal nerves. After a very short course through the spinal nerves, they leave them and grow towards the postganglionic neurons. The postganglionic neurons form the various ganglia of the sympathetic trunk. Some postganglionic neurons come to lie near the viscera, and form visceral sympathetic ganglia. The preganglionic fibers meant for them do not relay in the sympathetic trunk but pass through branches of the trunk to reach the visceral ganglia.
- The axons of the postganglionic neurons grow towards the various viscera of the body, to innervate them. Some of them enter spinal nerves and are distributed through them to blood vessels, hair and sweat glands. Postganglionic neurons are generally believed to be derived from cells of the neural crest.

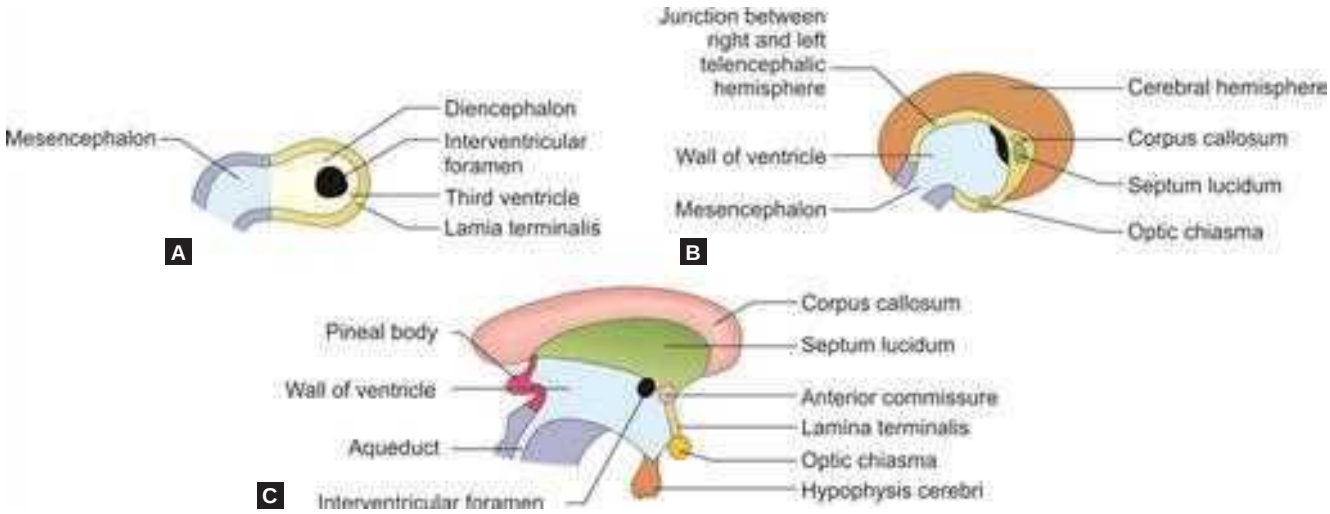
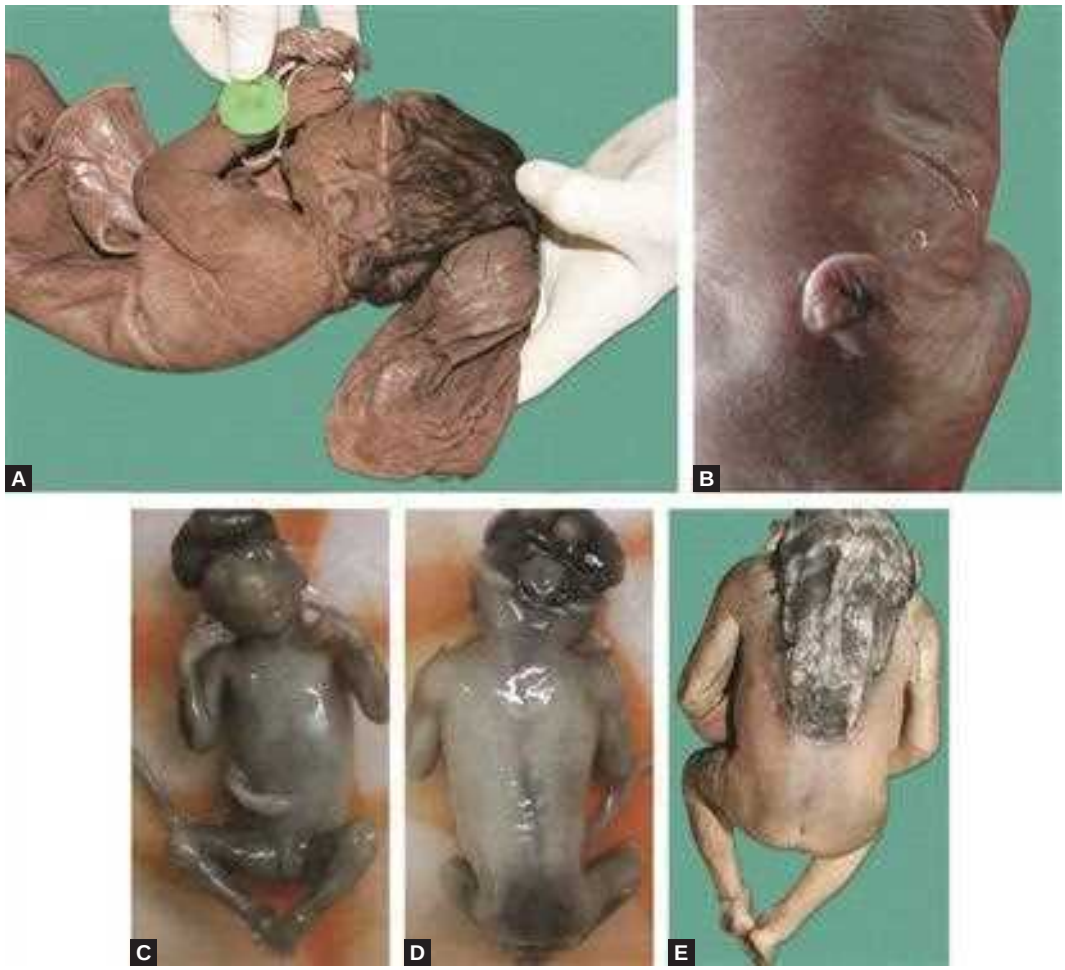
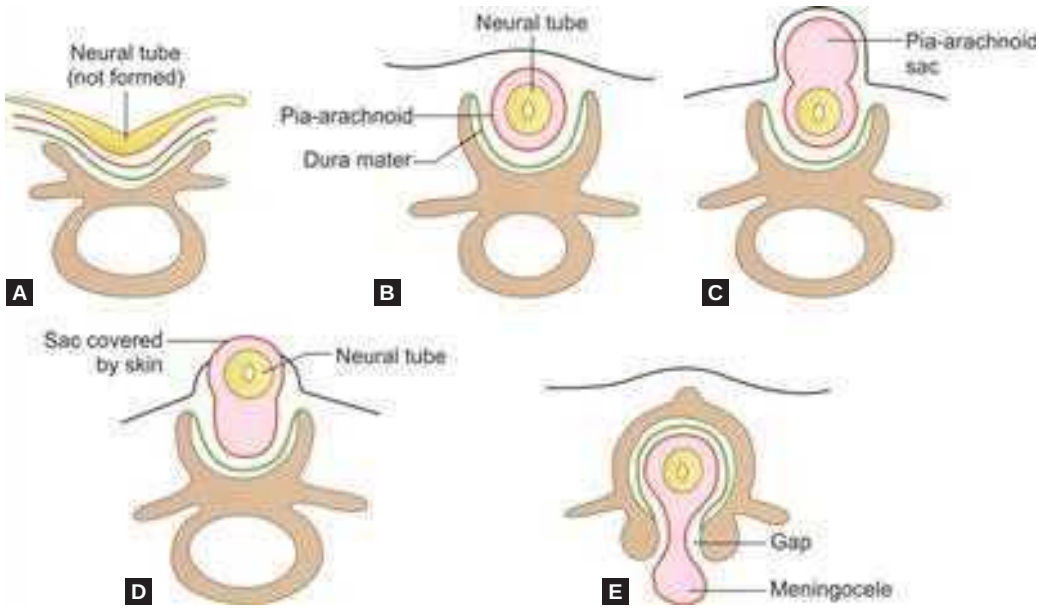


Fig. 17.32: Development of the corpus callosum and other commissures



Figs 17.33A to E: Aborted fetuses with neural tube anomalies. (A) Encephalocele; (B) Myelocele; (C and D) Anencephaly; (E) Rachischisis



Figs 17.34A to E: Anomalies of neural tube due to defective formation of vertebra, i.e. spina bifida. (A) Rachischisis; (B) Spina bifida occulta; (C) Spina bifida with meningocele; (D) Spina bifida with meningomyelocele; (E) Anterior spina bifida

Figs 17.35A to C: (A) Spina bifida: Meningocele; (B) Spina bifida with meningomyelocele; (C) Hydrocephalus

Parasympathetic Neurons

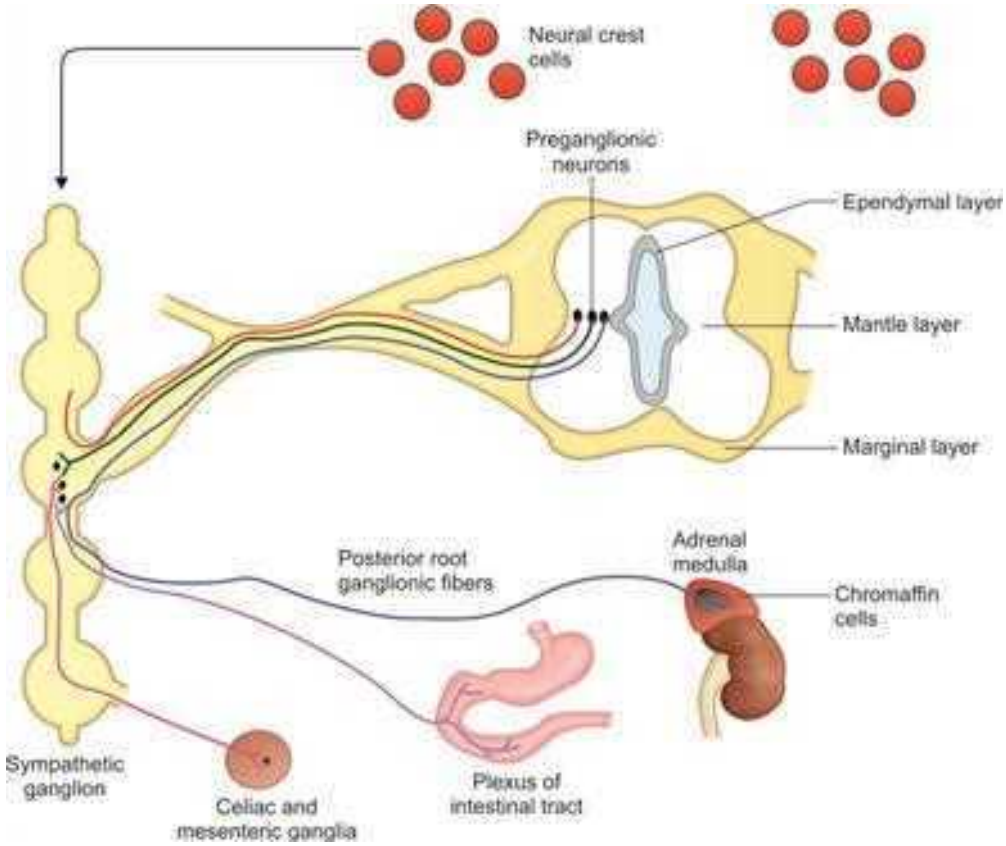
As in sympathetic pathways, parasympathetic pathways also consist of two neurons (preganglionic and postganglionic).

Preganglionic Neurons

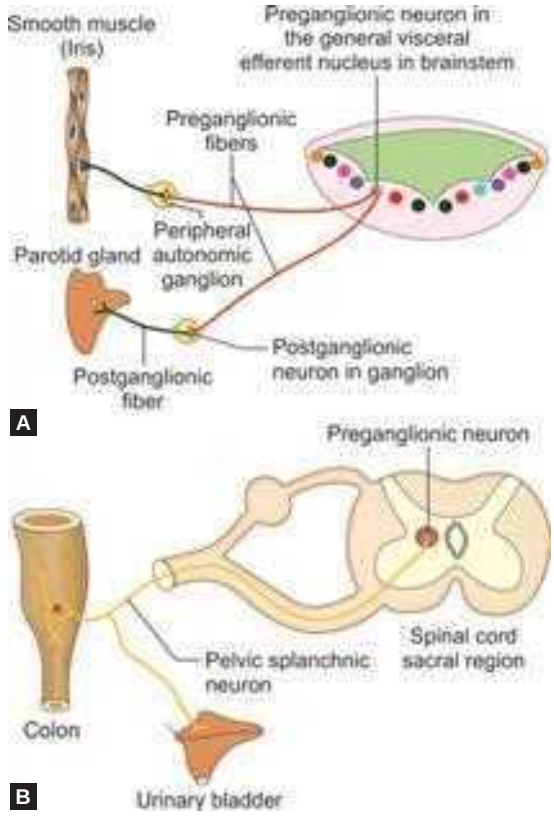
The preganglionic neurons of the parasympathetic system are formed in two distinct situations.

Cranial Parasympathetic Outflow

- These neurons are formed in relation to the general visceral efferent nuclear column of the brainstem (Fig. 17.37A). They give rise to the Edinger-Westphal nucleus, salivatory and lacrimatory nuclei, and the dorsal nucleus of the vagus.
- The preganglionic parasympathetic fibers taking origin from the Edinger-Westphal nucleus run in the oculomotor nerve to reach the ciliary ganglion.
- The superior salivatory and lacrimatory nuclei give origin to preganglionic fibers, which run in the facial nerve to reach the sphenopalatine and submandibular ganglia.



Figs 17.36A and B: Development of preganglionic and postganglionic sympathetic neurons. (A) Cranial outflow; (B) Sacral outflow



Figs 17.37A and B: Development of preganglionic and postganglionic parasympathetic neurons. (A) Cranial outflow. (B) Sacral outflow

- The inferior salivatory nucleus gives origin to the fibers which are related to the glossopharyngeal nerve and terminate in the otic ganglion.
- The dorsal nucleus of the vagus gives preganglionic parasympathetic fibers that terminate in various ganglia situated in the walls of viscera supplied by the vagus nerve.

Sacral Parasympathetic Outflow

The preganglionic neurons are formed in the mantle layer of the sacral part of the spinal cord (S2–S4). These cells lie near the sulcus limitans. Their axons constitute the preganglionic parasympathetic fibers, which terminate by synapsing with postganglionic neurons situated in the walls of pelvic viscera and hindgut (Fig. 17.37B).

Postganglionic Neurons

- Postganglionic parasympathetic neurons are derived from the neural crest cells.
- In the cranial region, the postganglionic parasympathetic neurons form the ciliary, otic, submandibular and sphenopalatine ganglia. Ganglia are also present in various viscera supplied by the vagus nerve.
- Postganglionic parasympathetic neurons are also present in various ganglia that lie in relation to the hindgut and pelvic viscera. These neurons receive preganglionic fibers of the sacral outflow.
- It should be noted that the entire length of the gut (from esophagus to anal canal) is populated by postganglionic parasympathetic neurons which are of neural crest origin. The neural crest cells within the gut form the enteric nervous system.

Molecular and genetic basis of neural tube formation

- Varieties of signals are required for induction of surface ectodermal cells to differentiate into neurectoderm.
- Signalling molecules of transforming growth factor B (TGF-B) family and inactivation of morphogenetic protein BMP4 (forms surface ectoderm) is required for formation of neuroectoderm.
- Certain genes, such as PAX3, sonic hedgehog are responsible for the closure of neural tube along with dietary cholesterol and folic acid (Vitamin B_{12}).
- Change in the expression of cell adhesion molecules by cells that are destined to become neurectodermal cells which start producing N-cadherin and N-CAM adhesion molecules instead of E-cadherin cause separation of neural tube form the surface ectoderm.

TIME TABLE OF SOME EVENTS IN NERVOUS SYSTEM DEVELOPMENT

Time table of some events described in this chapter has been shown in Table 17.3.

TABLE 17.3: Timetable of developmental events

Age	*Developmental events*
3rd week	Neural tube begins to form.
4th week	Neural folds begin to fuse. Primordia of sensory ganglia (spinal and cranial) are formed.
25th day	Closure of anterior neuropore.
28th day	Closure of posterior neuropore. The most cranial pair of cervical spinal ganglia develops.
5th week	Formation of brain vesicle. Sympathetic ganglia are formed. Cerebral hemispheres begin to form.
8th week	Cerebellum starts forming.
10th week	Corpus callosum forms.
12th week	Cerebellar cortex and Purkinje cells are formed.
15th week	The dentate nucleus is seen.
4th month	Myelination of nerve fibers begins.
Late fetal life	Sulci and gyri appear over cerebral hemispheres.

EMBRYOLOGICAL EXPLANATION FOR CLINICAL CONDITIONS OR ANATOMICAL OBSERVATIONS OF NERVOUS SYSTEM

Case Scenario 1

A stillborn baby was delivered with absence of cranial vault and exposure of brain substance which was seen as an irregular degenerated mass. The face appeared abnormal. When the mother is enquired about the obstetric history, she stated about the diagnosis of hydramnios by obstetrician. Explain the reason for hydramnios. State whether this condition can be diagnosed prenatally and if so what investigation has to be advised to the mother. What are the chances for survival of the baby? What preventive measures can be advised for future pregnancies?

- This condition is called anencephaly. In this condition, there is absence of major portion of brain, skull and scalp. It is a type of neural tube defect. Failure of closure of anterior neuropore results in exposure of brain substance to the surface as an irregular degenerated mass. Non-fusion of neural tube is associated with non-closure of cranium (cranium bifidum) and hence the cranial vault is absent. The characteristic appearance of the fetus is the protruding eyes, and the chin is continuous with neck due to absence of neck.
- The amniotic cavity is filled with clear, watery fluid known as amniotic fluid. It is produced by amniotic cells and maternal blood. At 38 weeks, it is about 500–100 mL in quantity. From 5th month of pregnancy, the fetus swallows about 400 mL of amniotic fluid per day. The swallowed fluid is absorbed through fetal gut and passes into maternal blood. Children born with disorder usually lack telencephalon. Because of the absence of brain, the swallowing reflex does not develop in anencephalic fetus resulting in excessive accumulation of amniotic fluid, i.e. hydramnios.
- This anomaly can be diagnosed prenatally by a detailed ultrasonography during 18–20 weeks of pregnancy. Biochemical tests include estimation of alpha-fetoproteins in the blood or in the amniotic fluid (obtained by transabdominal amniocentesis). There will be elevated alpha-fetoproteins in neural tube defects. These investigations should be suggested to the mother for future pregnancies. If the diagnosis of anencephaly is confirmed, termination of pregnancy is advised.
- Infants with this disorder do not survive. These fetuses are stillborn or die within few hours after birth.
- Folic acid supplementation before and during pregnancy reduces the chances of neural tube defects.

Case Scenario 2

A neonate was presented to the neonatologist with a soft bulging in the lumbosacral region and a large head, with symptoms of dyspnea, dysphagia and noisy breathing. A diminished gag reflex was noted on testing. Based on physical examination and radiological investigations, a diagnosis of Arnold-Chiari malformation was arrived. Give the embryological explanation for this condition.

- Arnold-Chiari malformation is a congenital deformity characterized by meningomyelocele, caudal displacement of medulla and tonsils of cerebellum through foramen magnum and hydrocephalus.
- The soft bulging is due to a posterior gap in vertebrae resulting from non-fusion of laminae to form vertebral spine. This condition is known as spina bifida.
- The soft bulging may contain only meninges (meningocele) or meninges and spinal cord (meningomyelocele). In the present case, it is meningomyelocele covered with skin.
- The large head is due to the accumulation of CSF in the ventricles of brain and is known as hydrocephalus. In the present case, the radiological investigation probably suggested herniation of tonsils of cerebellum and medulla oblongata through foramen magnum into the vertebral canal. This herniation must have led to the blockage of CSF due to obstruction at the foramen magnum resulting in hydrocephalus.
- Due to the compression of medulla and stretching of cranial nerves IX, X, XI, XII that are attached to the medulla, the symptoms of dyspnea, dysphagia, and diminished gag reflex, etc. have resulted.

REVIEW QUESTIONS

1. Write a short note on neural crest cells.
2. Describe the brainstem nuclei derived from alar lamina.
3. Describe the brainstem nuclei derived from basal lamina.
4. Explain the development of cerebellum.
5. Write a short note on spina bifida.

Chapter 18

Endocrine Glands

HIGHLIGHTS

- Endocrine gland is a ductless gland. Its cells secrete the substance called hormone, which is directly poured into the blood and transported to the target organ through circulation where it exerts its physiological function.
- The major endocrine glands of the body are *pituitary, pineal, adrenal, thyroid* and *parathyroid*. The others are islets of *Langerhans* in pancreas, *gonads* and *hypothalamus*.
- The endocrine glands develop from all three germ layers.
- They produce steroid hormones. Those developing from ectoderm or endoderm secrete amines.
- The pituitary gland develops from two different components. The *adenohypophysis* develops from a diverticulum extending upward from the roof of stomodeum called Rathke's pouch. The *neurohypophysis* develops from a downgrowth called infundibular process arising from the floor of the 3rd ventricle.
- The *pineal gland* develops as a diverticulum from the roof of the 3rd ventricle (diencephalon).
- The adrenal gland develops from two different sources. The *adrenal cortex* is derived from coelomic epithelium. The cells of the *adrenal medulla* are derived from the neural crest.

INTRODUCTION

The development of endocrine glands from the epithelial surface is described in Chapter 7, Figure 7.1.

CLASSIFICATION OF ENDOCRINE GLANDS

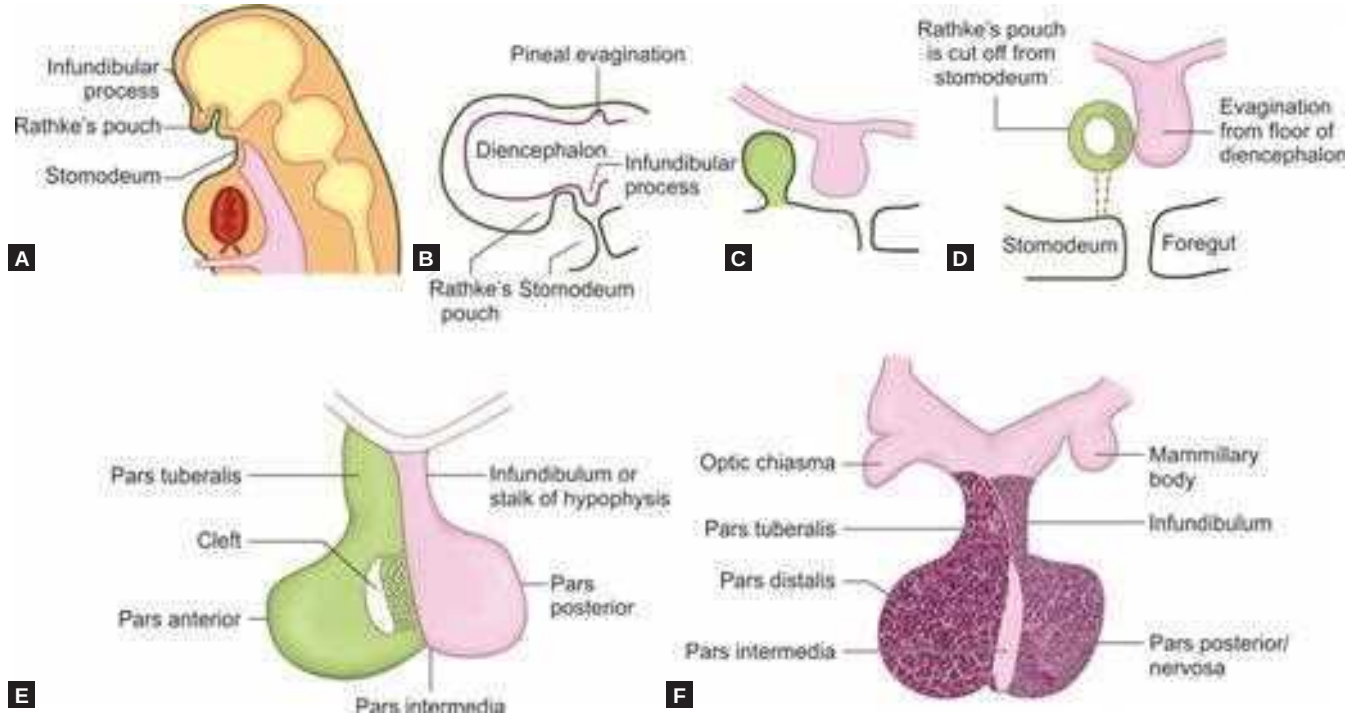
1. *Based on location*:
   a. Head region, e.g. hypothalamus, pineal gland, pituitary gland
   b. Neck region, e.g. thyroid gland, parathyroid gland
   c. Abdominal region, e.g. pancreas, adrenal, testis/ovary.
2. *Based on number*:
   a. Unilateral, e.g. pituitary, pineal gland, pancreas
   b. Bilateral, e.g. thyroid, adrenal, parathyroid gland.
3. *Based on secretion*:
   a. Telecrine—The hormones act at a distance place by traveling through circulation, e.g. thyroid gland, adrenal gland, pituitary gland, pancreas, etc.
   b. Paracrine—The hormones act on adjacent cells in the region where they are produced, e.g. gastric G cells, somatostatin secreting δ-cells that act on adjacent α- and β-cells in islets of Langerhans.
4. *Based on germ layer of origin*:
   a. Surface ectoderm—adenohypophysis
   b. Neuroectoderm—hypothalamus, neurohypophysis, pineal
   c. Neural crest cells—adrenal medulla
   d. Endoderm—pancreas
   e. Mesoderm—testis, ovary.

In this chapter, we will consider the development of three glands, i.e. (1) pituitary gland, (2) pineal gland and (3) adrenal gland as their development is closely connected with that of the nervous system. The development of gonads is considered in urogenital system. The development of thyroid and parathyroid in pharyngeal system and pancreas in development of organs associated with digestive system.

HYPOPHYSIS CEREBRI OR PITUITARY GLAND

The hypophysis cerebri or pituitary gland is developed from two separate sources, i.e. surface ectoderm and neuroectoderm.

- The adenohypophysis/*anterior pituitary* develops from an ectodermal diverticulum that grows upward from the roof of the stomodeum (primitive mouth), just in front of the buccopharyngeal membrane. The diverticulum is called *Rathke's pouch* (Fig. 18.1A).
- The Rathke's pouch appears in the 3rd week of intrauterine life. It extends upward toward the developing diencephalic floor. It loses contact with the surface epithelium by 2nd month and is cut off from the stomodeum (Figs 18.1B to D). The cavity of the pouch persists after birth as an *intraglandular cleft*.
- The *neurohypophysis/posterior pituitary* develops from a downgrowth from the floor of the 3rd ventricle (diencephalon) in the region of the infundibulum (Fig. 18.1C) during 6th week. This downgrowth (*infundibular process*) comes into relationship with the posterior aspect of Rathke's pouch and fuses with it (Figs 18.1D and E). This contact between the two is critical in the development of pituitary.
- The anterior wall of Rathke's pouch proliferates greatly to form the pars anterior (*pars distalis*) of the hypophysis. The posterior wall remains thin and forms the *pars intermedia*. The original cleft of Rathke's pouch separates these two parts. Some cells of the anterior part grow upward along the infundibular stalk to form the *tuberal part* of the hypophysis (Fig. 18.1E).
- The infundibular process forms *infundibular stalk* and *posterior lobe* (pars nervosa) of neurohypophysis. The cavity of infundibulum persists as infundibular recess of 3rd ventricle. Though posterior lobe is neuroectodermal in origin it does not contain nerve cells. Most of the cells are neuroglial and it is invaded by nerve fibers originating from hypothalamic nuclei.
- Rathke's pouch constricts at its base and gets separated from the stomodeum to come closer to neurohypophysis. With the formation of the mouth and pharynx, the original site of attachment of Rathke's pouch



Figs 18.1A to F: Development of hypophysis cerebri. (A) Rathke's pouch and infundibular process; (B and C) Approximation of the two developmental components of pituitary gland; (D) Rathke's pouch separating from stomodeum; (E) Closely placed developmental components and their subdivisions; (F) Microscopic appearance of pars anterior, intermedia and posterior

to the stomodeum, comes to lie in the roof of the nasopharynx corresponding to the junction of nasal septum and palate.

- The acidophil cells are formed in pars anterior during 3rd month of intrauterine life followed by other cells. During development of pituitary six different cells develop from Rathke's pouch and they form the pars anterior and pars intermedia. Though the posterior lobe is neuroectodermal in origin it does not contains neurons. The cells of posterior lobe are neuroglial (pituicytes) and are traversed by nerve fibers from hypothalamus (Fig. 18.1F).

Clinical correlation

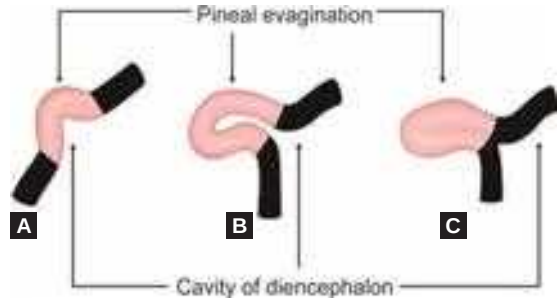
- ***Craniopharyngiomas:*** The original track of Rathke's pouch is called craniopharyngeal canal. Detachment of Rathke's pouch from the roof of stomodeum forms the adenohypophysis. Remnants of Rathke's pouch may sometimes give rise to peculiar type of brain tumors called ***craniopharyngiomas*** that are seen in relation to the sphenoid bone and the roof of the nasopharynx. They occur most commonly in children but also in men and women in their 50s and 60s.
- ***Pharyngeal hypophysis:*** Accessory anterior lobe tissue seen in relation to the posterior wall of the pharynx.
- ***Pituitary agenesis/hypoplasia:*** Rarely the hypophysis may fail to develop (agenesis) or may be underdeveloped (hypoplasia).
- ***Pharyngeal hypophysis:*** The gland may be located in the roof of nasopharynx.

PINEAL GLAND

The pineal gland (or pineal body) arises as an evagination of the roof of the diencephalon (Figs 18.1B and 18.2A). The outgrowth is at first hollow but later becomes solid (Figs 18.2B and C). The specific cells of the pineal body are believed to be modified neuroglial cells. For long considered to be a vestigial structure of no importance, the pineal gland is now known to secrete a number of hormones that have a regulatory influence on many other endocrine glands.

ADRENAL GLAND

- The adrenal gland consists of a superficial cortex and a deeper medulla. The cells of the *cortex* arise from coelomic epithelium (mesoderm). The cells of medulla are derived from the neural crest cells (ectoderm).
- The adrenal gland begins to develop in the 5th week of intrauterine life.
- The cells of the cortex arise from the coelomic epithelium that lies in the angle between the developing gonad and the attachment of the mesentery (Fig. 18.3A). The cells



Figs 18.2A to C: Development of pineal gland

arising from the coelomic epithelium may be divided into two groups:

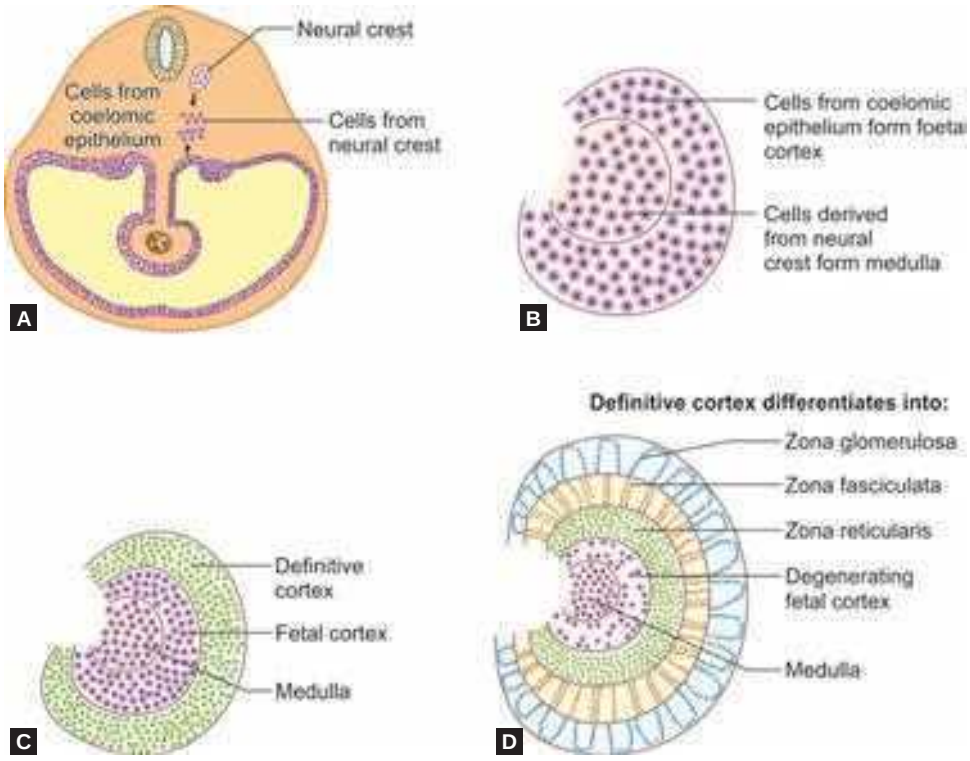
1. The cells that are formed first are large and are acidophils. They surround the cells of the medulla, and form the *fetal cortex* (Figs 18.3B to D). The fetal cortex disappears after birth.
2. Subsequently, the coelomic epithelium gives origin to smaller cells that surround the fetal cortex. These smaller cells form the *definitive cortex* (Figs 18.3C and D). According to some authorities, the cells of the fetal cortex are incorporated into the reticular zone of the definitive cortex.

- The differentiation of cortical zones (Fig. 18.3D) begins during the late fetal period. The zona glomerulosa and zona fasciculata are present at birth but the zona reticularis becomes recognizable at the end of the third year.
- The suprarenal of the human fetus is almost of the same size as that of the adult. It is quite large as compared to the fetal kidney. The size of the gland (particularly of fetal cortex) becomes smaller during the first year of postnatal life.
- The cells of the medulla are derived from the neural crest. They are similar to the postganglionic sympathetic neurons (cells of sympathetic ganglia). Preganglionic sympathetic neurons terminate in relation to them. These cells migrate to the region of the developing cortical cells and come to be surrounded by them.

Clinical correlation

Anomalies of adrenal

- Adrenal cortical tissue may be present at various ectopic sites. The entire adrenal may be ectopic and may lie deep to the capsule of the kidney. It may be fused to the liver or the kidney.
- There may be congenital hyperplasia (over development) of the cortex. In the male, this leads to the ***adrenogenital syndrome marked*** by very early development of secondary sexual characters. In the female, it may cause enlargement of the clitoris and the child may be mistaken for a male ***(pseudohermaphroditism).***



Figs 18.3A to D: Stages in the development of adrenal gland. (A) Contributions from coelomic epithelium and neural crest cells; (B) Formation of fetal cortex and medulla; (C) Formation of definitive cortex; (D) Differentiation of various zones of definitive cortex

CHROMAFFIN TISSUE

Chromaffin tissue is made up of cells similar to those of the adrenal medulla, and like the latter, is derived from the cells of the neural crest. This tissue is to be seen in relation to the abdominal aorta where it forms the para-aortic bodies (see Fig. 17.7). It is also seen in relation to sympathetic ganglia and plexuses and along the splanchnic nerves (see Fig. 17.7).

Molecular and genetic basis of pituitary, pineal and adrenal development

- Expression of transcription factors and growth factors in a tightly regulated pattern is responsible for the formation of Rathke's pouch, its orientation with posterior lobe, cell differentiation of anterior and posterior lobes and the hormonal production by the gland. Dysregulation of expression of these factors leads to congenital anomalies of pituitary and hormonal imbalance.
- Home box transcription factor Pax6 and intermediate filament vimentin are required for proper development of pineal gland.
- WT1 gene and transcription factors SF-1 and DAX-1 regulate the development of adrenal gland.

TIME TABLE OF SOME EVENTS DESCRIBED IN THIS CHAPTER

Timetable of some events described in this chpater is shown in Table 18.1.

TABLE 18.1: Timetable of some developmental events

Age	*Developmental events*
3rd week	Infundibular diverticulum develops in the floor of 3rd ventricle
4th week	Rathke's pouch projects from the roof of stomodeum
5th week	Adrenal gland begins to develop
8th week	Rathke's pouch loses its connection with the oral cavity

EMBRYOLOGICAL EXPLANATION FOR CLINICAL CONDITIONS OR ANATOMICAL OBSERVATIONS IN EYEBALL

Case Scenario 1

A 20-year-male was admitted to the neurology department with a history of chronic headache of generalized nature with episodes of nausea and vomiting of more than 2 years duration. Neurological examination presented bilateral papilledema suggestive of increased intracranial pressure. Computed tomography (CT) picture presented mild dilatation of inferior horns of both lateral ventricles. Magnetic resonance imaging (MRI) confirmed the diagnosis of craniopharyngioma. Tumor was removed by neurosurgeon and histopathological examination of extracted tumor confirmed the diagnosis. Explain the embryological basis for this clinical presentation.

- Craniopharyngioma is a benign tumor with malignant presentation.
- It accounts for 2.5–4% of all intracranial tumors.
- The tumor can be present at birth, but it may not be symptomatic until childhood or adulthood. It is believed to be primarily a congenital illness.
- It arises from the remnants of Rathke's pouch. Wnt/beta-catenin signaling pathway defect due to beta-catenin gene mutation can produce this condition.
- Ultrasonography during fetal period and CT and MRI scans at later age are the useful diagnostic tools.
- It occurs in sella turcica region of cranial cavity. Early diagnosis of this tumor helps in prevention of sequelae, i.e. raised intracranial tension and pressure effects on surrounding structures like optic chiasma, temporal lobe, and hypothalamus producing visual, neurological and hormonal disturbances.

REVIEW QUESTIONS

1. Explain the development of pituitary gland.
2. Explain the development of adrenal gland.

Chapter 19

Development of Eye

HIGHLIGHTS

- The visual system consists of eyeball, eyelids, extraocular muscles of eyeball and lacrimal apparatus.
- It includes walls/layers, refractive media and optic nerve.
- Developmental primordia of eyeball are optic vesicle, lens placode and the mesoderm surrounding the optic vesicle.
- *Optic vesicle* is an outgrowth of the prosencephalon. The optic vesicle is converted into the optic cup. Retina, the nervous layer of eyeball develops from the optic cup.
- *Lens placode* is a thickening of surface ectoderm close to optic vesicle from which the *lens* develops. Lens placode is converted to lens vesicle.
- Other coats of the eyeball (*choroid, sclera*) are derived from mesoderm surrounding the optic vesicle. The epithelium covering the superficial surface of the cornea is derived from surface ectoderm.
- The *eyelids* are formed by reduplication of surface ectoderm above and below the cornea.
- The *lacrimal sac* and *nasolacrimal duct* are derived from ectoderm buried in the naso-optic furrow.

INTRODUCTION

The various components of the eyeball are derived from the following primordia:

- Neuroectodermal outgrowth of the prosencephalon called the *optic vesicle* that forms retina, iris, and optic nerve.
- Specialized area of surface ectoderm (called the *lens placode*) that gives rise to the lens and corneal epithelium.
- Mesoderm surrounding the optic vesicle that forms fibrous and vascular coats of eye.
- Migrating neural crest cells that contribute for choroid, sclera and corneal epithelium.

FORMATION OF THE OPTIC VESICLE

- The region of the neural plate (Fig. 19.1A) destined to form the diencephalic part of prosencephalon shows a linear thickened area on either side (Fig. 19.1B) around 22nd day of development (8th somite stage).
- This area soon becomes depressed to form the *optic sulcus* (Fig. 19.1C).
- Meanwhile, the neural plate becomes converted into the prosencephalic vesicle. As the optic sulcus deepens, the wall of the prosencephalon overlying the sulcus bulges outwards to form the *optic vesicle* (Fig. 19.1D).
- The proximal part of the optic vesicle becomes constricted, and elongated, to form the *optic stalk* (Fig. 19.2C).

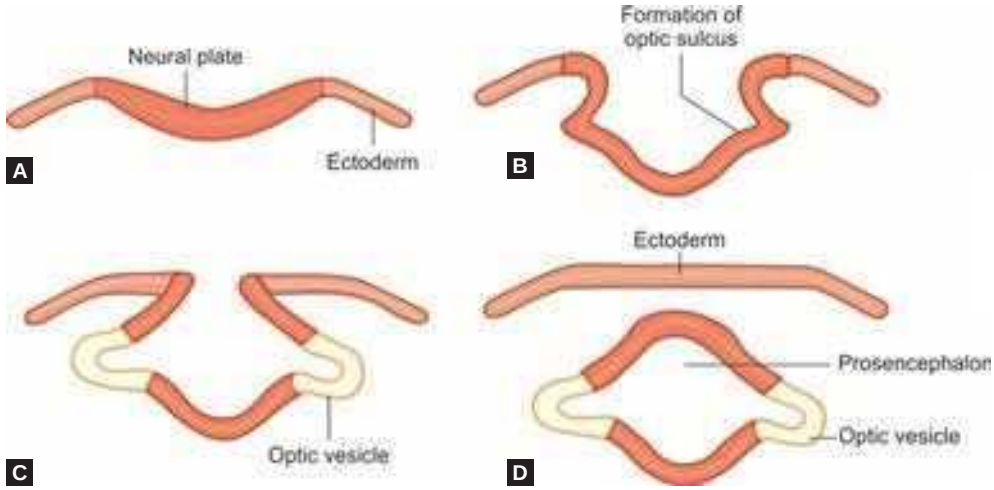
FORMATION OF LENS VESICLE

- As the optic vesicle grows laterally, it comes into relation with the surface ectoderm. An area of this surface ectoderm, overlying the optic vesicle, becomes thickened to form the *lens placode* (Fig. 19.2A).

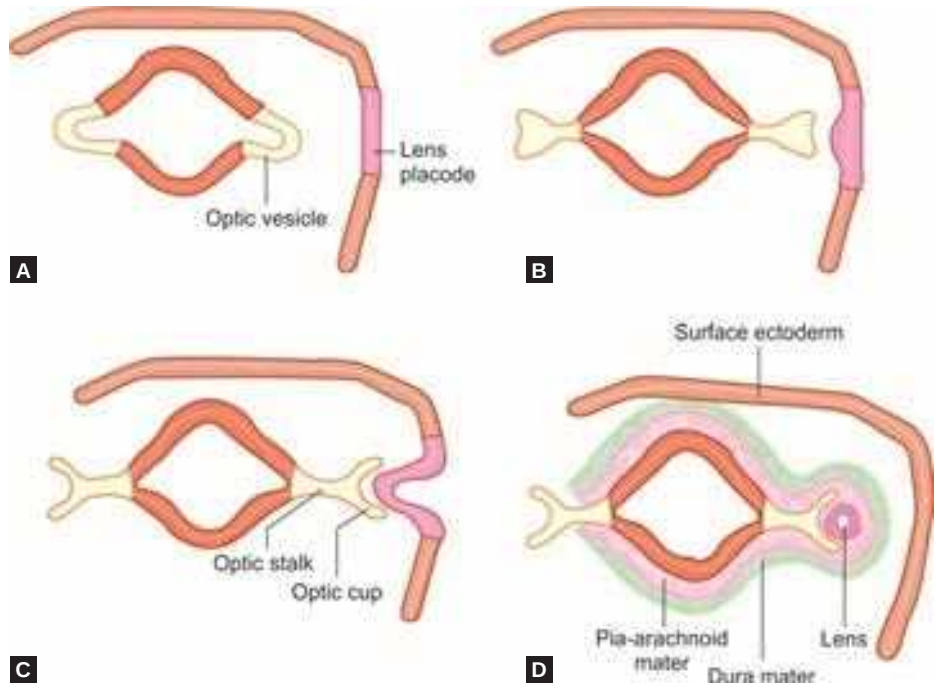
- The lens placode soon sinks below the surface to form *lens pit.* The ends of lens pit come closer and form *lens vesicle* (Figs 19.2B and C).
- The lens vesicle becomes completely separated from the surface ectoderm (Fig. 19.2D) by 33rd day of development.
- The proximal part of optic vesicle elongates into a narrow structure called optic stalk.

FORMATION OF THE OPTIC CUP

- While the lens vesicle is forming, the optic vesicle becomes converted into a double-layered *optic cup* (Fig. 19.2C).
- The optic cup is formed not because of the invagination of the developing lens into the optic vesicle. The conversion of the optic vesicle, to the optic cup, is a result of differential growth of the wall of the vesicle.



Figs 19.1A to D: Formation of the optic vesicle



Figs 19.2A to D: (A) Formation of lens placode; (B) Deepening of lens placode and optic cup; (C) Formation of the optic stalk and its invagination into the optic cup; (D) Lens vesicle and optic cup, meningeal coverings for developing optic cup and lens vesicle

- The margins of the cup grow over the upper and lateral sides of the lens to enclose it. However, such overgrowth does not take place on the inferior aspect of the lens, as a result of which the wall of the cup shows a deficiency in this situation. This deficiency extends for some distance along the inferior surface of the optic stalk and is called the *choroidal* or *fetal fissure* (Fig. 19.3).
- The vascular mesoderm in choroid fissure forms the hyaloid vessels that supply optic cup and lens vesicle. The distal part of hyaloid vessels degenerate whereas the proximal parts persists as central artery and vein of retina.
- The developing neural tube is surrounded by mesoderm, which subsequently condenses to form the meninges. An extension of this mesoderm covers the optic vesicle. Later, this mesoderm differentiates to form a *superficial fibrous layer* corresponding to the dura mater, and a *deeper vascular layer* corresponding to the pia-arachnoid (Figs 19.2D and 19.4A).

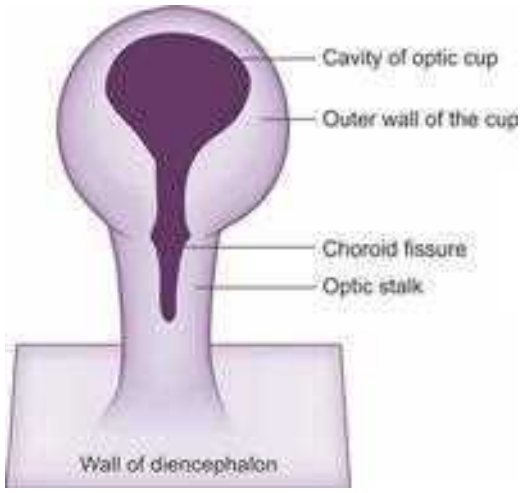


Fig. 19.3: Optic cup and stalk seen from below to show the choroidal fissure

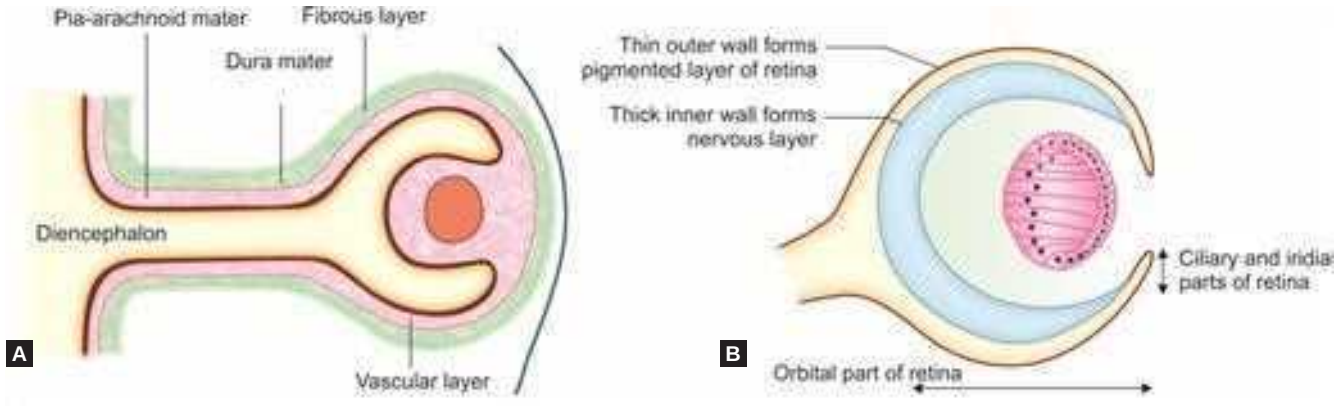
- With the formation of the optic cup, part of the inner vascular layer is carried into the cup through the choroidal fissure (Fig. 19.4A). With the closure of fissure, the mesenchyme that is inside the optic cup gives rise to hyaloid system of vessels.
- The outer fibrous layer surrounding anterior part of optic cup forms *cornea*. The corresponding vascular layer forms the iridopupillary membrane which attaches to anterior part of optic cup and forms *iris*.

DERIVATION OF PARTS OF THE EYEBALL

The derivation of the various parts of the eyeball can now be summarized as follows (Table. 19.1):

Lens

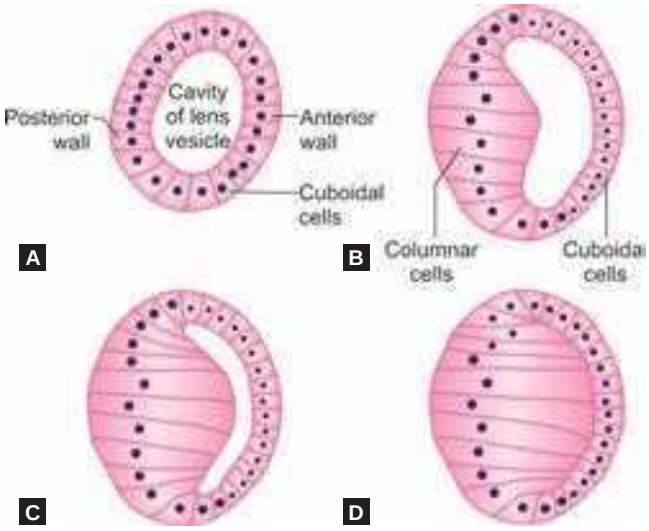
- The lens is formed from the lens vesicle.
- The lens vesicle is at first lined by a single layer of cuboidal cells (Fig. 19.5A).
- The cells in the anterior wall of the vesicle remain cuboidal. Those in the posterior wall gradually become elongated and become columnar (Figs 19.5B to D). As they do so the cavity of the vesicle is encroached upon and is eventually obliterated.
- The elongated cells of the posterior wall lose their nuclei and are converted into the primary fibers of the lens. The anterior layer remains as the epithelium covering this aspect of the lens (Figs 19.4B and 19.5D).
- The cells at the equatorial region of lens form the secondary lens fibers that continue to form from the anterior epithelium during childhood, thus increasing the diameter of the lens.
- While the secondary lens fibers are forming the primary lens, fibers become harder.



Figs 19.4A and B: (A) Developing optic cup surrounded by extensions of pia-arachnoid and dura mater; (B) Subdivisions of the optic cup

TABLE 19.1: Summary of derivation of various parts of the eyeball

Part	*Derived from*
Lens	Surface ectoderm
Retina	Neuroectoderm (optic cup)
Vitreous	Mesoderm (infiltrated by neural crest cells?)
Ciliary body	Mesoderm
Ciliary muscle	Mesenchymal cells covering the developing ciliary body (neural crest?)
Sphincter and dilator pupillae muscles	Neuroectoderm of optic cup
Iris	Mesoderm
Muscles of iris	Neuroectoderm (from optic cup)
Sclera	Mesoderm (infiltrated by neural crest cells?)
Cornea	Surface epithelium by ectoderm. Bowman's membrane, substantia propria, Descemet's membrane and inner epithelium by neural crest
Conjunctiva	Surface ectoderm
Blood vessels	Mesoderm
Optic nerve	Neuroectoderm. Its coverings (pia, arachnoid and dura) are derived from mesoderm



Figs 19.5A to D: Stages in the formation of lens of the eye

- In the beginning, the lens is supplied by hyaloid artery. Later with the degeneration of distal part of artery, the lens is deprived of its blood supply and becomes an avascular structure.

Retina

The *retina* is derived from the layers of the optic cup.

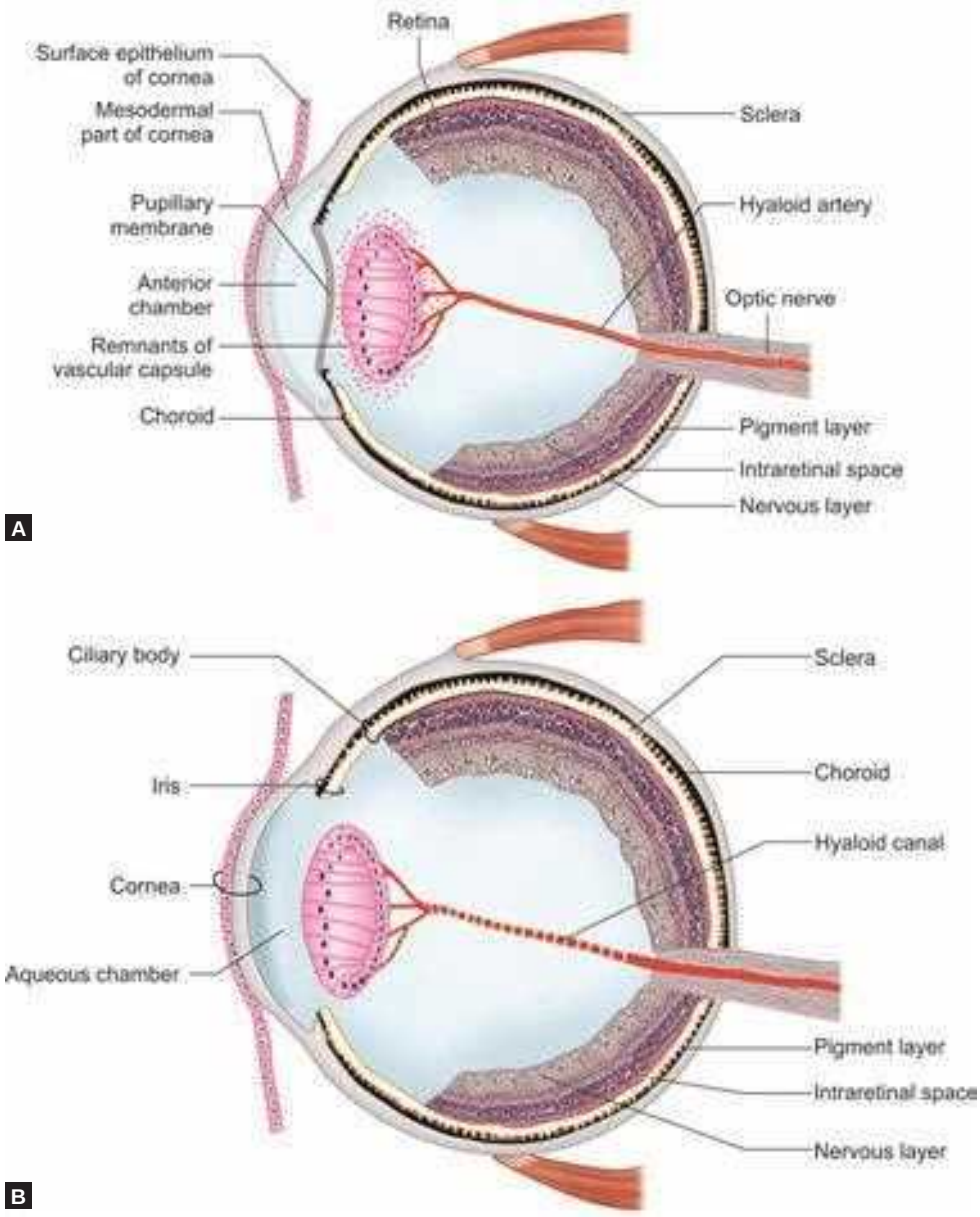
- The optic cup is divisible into anterior and posterior parts.
  - The larger posterior part becomes thick and forms the retina proper (*optical part of retina*) (Fig. 19.4B).
  - The anterior part remains thin and forms an epithelial covering for the ciliary body and iris (*ciliary and iridial parts of retina*) (Fig. 19.4B).
- There are two layers in the retina.
  - The outer wall of the posterior part of the optic cup remains thin. Its cells form the *pigmented layer* of the retina (Figs 19.6A and B).
  - The inner wall of the cup is called the *nervous layer* that differentiates into matrix, mantle and marginal layers as in the neural tube. After giving origin to the cells of the mantle layer, the cells of the matrix layer form the *rods and cones*. The cells of the mantle layer form the *bipolar cells*, the *ganglion cells*, other neurons of the retina, and also the supporting elements. The axons of the ganglion cells grow into the marginal layer to form the layer of nerve fibers. These fibers grow into the optic stalk by passing through the choroidal fissure. The optic stalk, is thus, convened into the *optic nerve* (Figs 19.6A and B).
  - The space between the pigmented and nervous layers is called *intraretinal space* that represents the original cavity of optic cup (Figs 19.6A and B). With the proliferation of cells of inner layer, this space gets obliterated before birth, and the rod and cone cells come in contact with pigment epithelium.

Vitreous

- The *vitreous* is believed to be derived partly from ectoderm and partly from mesoderm.
- The ectodermal component is derived mainly from the optic cup but the lens vesicle may also contribute to it.
- The mesodermal component comes into the optic cup through the choroidal fissure.

Choroid

- The *choroid* is formed from the inner vascular layer of mesoderm that surrounds the optic cup (Figs 19.4A and 19.6A and B).
- According to some authorities, this mesoderm contains cells derived from the neural crest.
- Anteriorly, the choroid is continuous with ciliary body and posteriorly it is continuous with pia-arachnoid around optic nerve.



Figs 19.6A and B: (A) Derivation of coats of the eyeball. Note pupillary membrane and hyaloid artery. See the layers of retina, remnants of vascular capsule; (B) The hyaloid artery and pupillary membrane have disappeared. Position of artery can be seen as the hyaloid canal

Ciliary Body and Iris

- The mesodermal basis of the *ciliary body* and *iris* is derived from a forward prolongation of the mesoderm forming the choroid.
- The inner surface of this mesoderm comes to be lined by two layers of epithelium (pigment and nervous layers) derived from the ciliary and iridial parts of the retina. The two layers of epithelium correspond to the two layers of the optic cup (Fig. 19.6B).
- The *ciliary muscle* has been generally regarded as mesodermal but the present view is that they are of neural crest origin. The connective tissue of ciliary body is derived from mesoderm. The *musculature of the iris* (sphincter and dilator pupillae) is of ectodermal origin (neuroectodermal cells of optic cup).

- The extension of iris does not extend up to the center leaving a gap known as the *pupil*. The color of the iris depends on the concentration of pigments in pigment-containing cells (chromatophores) in the iris. The iris is blue if the melanin pigment is concentrated on the posterior surface of the iris and is brown if it is distributed in the stroma.

Sclera

- The *sclera* is formed from the posterior part of the outer fibrous layer of mesoderm surrounding the optic cup, and corresponds to the dura (Figs 19.4A and 19.6A and B).
- Some authorities believe that (like the choroid) the mesoderm forming the sclera is infiltrated by cells from the neural crest.

Cornea

- The Bowman's layer, substantia propria, Descemet's membrane and inner endothelium of the *cornea* are derived from the neural crest and are formed by the same layer that forms the sclera.
- The superficial surface epithelium of the cornea is derived from the surface ectoderm (Figs 19.6A and B).

Anterior and Posterior Chambers of Eye

- The *anterior and posterior chambers* of the eye (aqueous chamber) are formed by a splitting of the mesoderm in the region, and correspond to the subarachnoid space of the brain.
- The cavity of the anterior chamber is formed by vacuolization of mesoderm present anterior to the lens.
- Vacuolization splits the mesenchyme into outer (anterior) and inner (posterior) layers.
- The outer layer becomes continuous with the sclera and with the substantia propria of the cornea.
- The inner layer lies in front of lens and iris and is termed the pupillary membrane (Fig. 19.6A).
- The mesodermal cells lining the cavity give origin to a flattened mesothelium.

Blood Vessels

- The *blood vessels of the eyeball* are formed in the mesodermal layer that is a continuation of the pia-arachnoid.
- Part of this mesoderm that gets invaginated into the optic cup, forms the retinal vessels. The central artery and vein of the retina at first lie in the choroidal fissure, but come to be buried in the fibers of the developing optic nerve. As the choroidal fissure extends for some distance along the optic stalk, the central artery of the retina runs through the substance of the distal part of the optic nerve.
- Initially, the lens is completely surrounded by a vascular capsule. The posterior part of the capsule is supplied by the hyaloid artery (Fig. 19.6A). This artery is a continuation of the central artery of the retina and passes through the vitreous. Later in fetal life, the vascular capsule and the hyaloid artery disappear, but the hyaloid canal in the vitreous (through which the artery passes) persists (Fig. 19.6B).
- The anterior part of the vascular capsule of the lens, comes to be lined posteriorly by the iridial part of the retina, and forms the iris (Figs 19.6A and B). The pupil is for some time closed by a part of this vascular tissue, which is termed the *iridopupillary membrane*. This membrane normally disappears before birth.

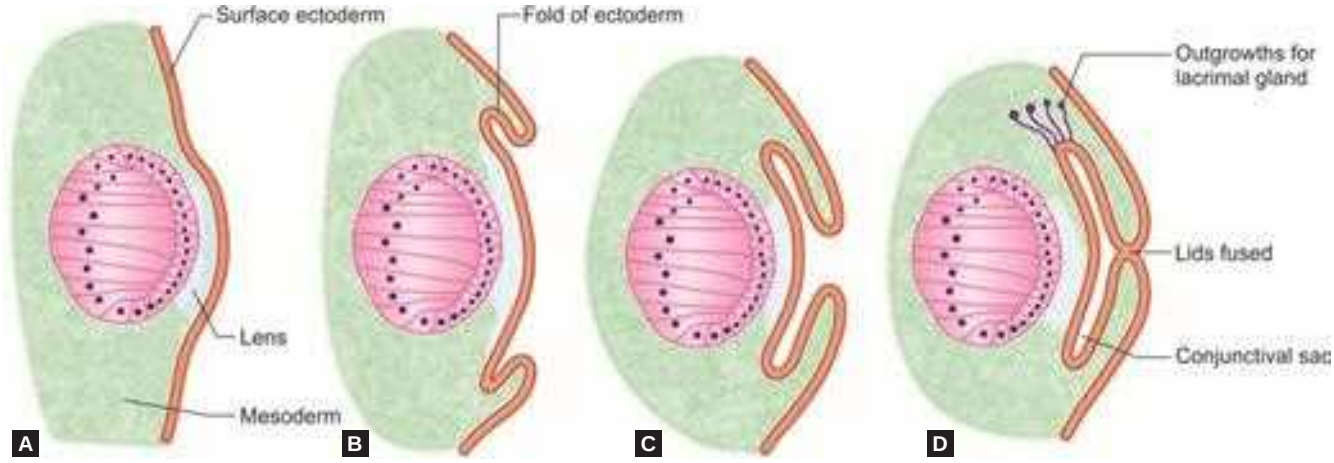
ACCESSORY STRUCTURES OF EYEBALL

Eyelids

- The eyelids are formed by reduplication of the surface ectoderm above and below the cornea (Figs 19.7A and B).
- The ectodermal folds formed contain some mesoderm that gives rise to muscle and to the tarsal plates.
- As the folds enlarge, their margins approach each other. Ultimately, they meet and fuse together.
- The lids, thus, cut off a space called the *conjunctival sac*. The conjunctiva is, thus, of ectodermal origin.
- The lids remain united with each other until the 7th month of intrauterine life.
- The eyelashes and glands in the eyelids develop from surface ectoderm.

Lacrimal Apparatus

- The *lacrimal gland* is formed from a number of buds that arise from the upper angle of the conjunctival sac (Fig. 19.7D).
- The *lacrimal sac* and *nasolacrimal duct* are derived from the ectoderm of the naso-optic (or nasolacrimal) furrow (Fig. 19.8). This furrow lies along the line of junction of the maxillary process and the lateral nasal process, and extends from the medial angle of the eye to the region of the developing mouth (Fig. 19.9). The ectoderm of



Figs 19.7A to D: Formation of eyelids, conjunctival sac and lacrimal gland

Fig. 19.8: Nasolacrimal (naso-optic) furrow

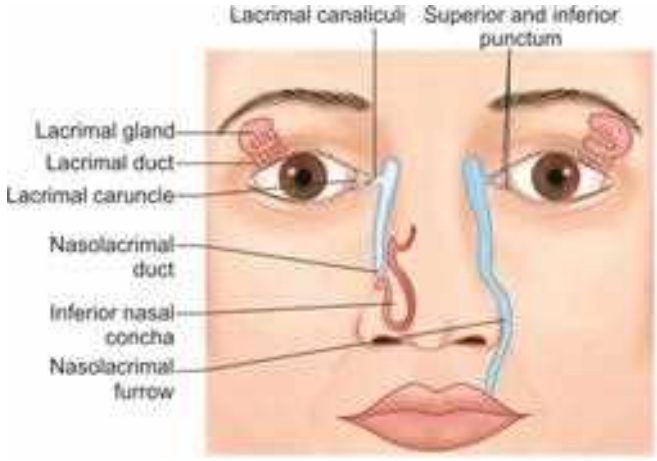


Fig. 19.9: Position of nasolacrimal furrow and of nasolacrimal duct projected onto an adult human face

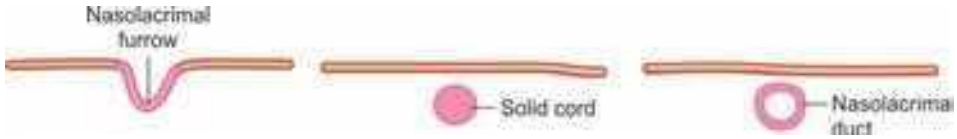


Fig. 19.10: Formation of nasolacrimal duct

the furrow becomes buried to form a solid cord that is subsequently canalized (Fig. 19.10). The upper part of this cord forms the *lacrimal sac*. The lower part, acquires a secondary connection to the nasal cavity, and forms the *nasolacrimal duct*.

- The *lacrimal canaliculi* are formed by canalization of ectodermal buds that arise from the margin of each eyelid near its medial end and grow to the lacrimal sac.

Extraocular Muscles of Eyeball

- They are derived from preoccipital myotomes that are supplied by 3rd, 4th and 6th cranial nerves.

Clinical correlation

Anomalies of the eyeball

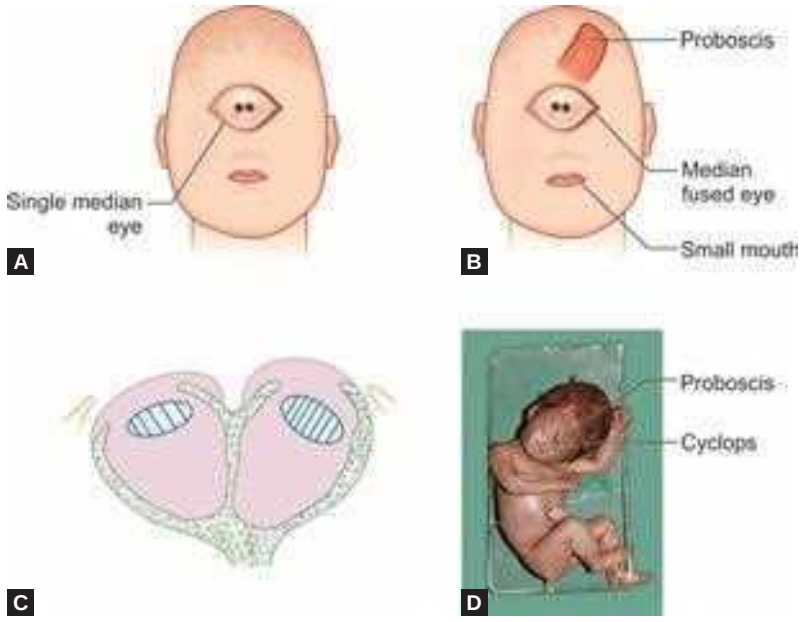
- ***Anophthalmos:*** The entire eyeball may fail to develop.
- ***Microphthalmos:*** The entire eyeball may remain very small.
- ***Cyclopia:*** The two eyes may fuse completely to form one median eye (Figs 19.11A, C and D).
- ***Synophthalmos:*** The two eyes may fuse partially (Fig. 19.11B). There will be associated under development of prosencephalon (holoprosencephaly) and frontonasal process (proboscis).
- The optic vesicle may not be invaginated by the lens and may remain as a cyst.
- ***Coloboma:*** It results from failure of the choroidal fissure to obliterate completely. It may lead to deficiencies (clefts) in various layers of the eyeball including the iris, ciliary body and choroid (Fig. 19.12B).
- The cornea may be absent. It may show anomalies of size and shape, and may also show congenital opacities.
- The sclera may be thin with the result that the pigment of the choroid can be seen through it (***blue sclera***).
- In addition to various types of coloboma, the iris may show anomalies of its histological structure. Very rarely, the sphincter or dilator pupillae muscle may be absent. The pupil may be abnormal in position, shape or size.
- The lens may, very rarely, be absent or may be very small. It may be abnormal in position or shape. It may show congenital opacities (***cataract***). Congenital cataract may be due to parathyroid deficiency, to avitaminosis or to the infection, German measles (acquired during early pregnancy). Cataract may be genetically determined.
- The hyaloid artery and the vascular capsule of the lens, or their remnants, may persist. When the capsule persists on the anterior aspect of the lens, it may completely occlude the pupil (***persistent pupillary membrane***).
- The various layers of the eye may show anomalies of pigmentation. There may either be too little pigment as in ***albinism***, or too much.
- The retina may show various congenital anomalies in its structure. These may involve the macula, and may result in visual defects, including those of color vision.
- ***Retinal detachment*** takes place along the intraretinal space, which is the plane of cleavage between pigment epithelium and nervous layers of retina.

Clinical correlation

Anomalies of accessory structures of eye

Anomalies of eyelids and related structures

- The eyelids may very rarely be absent. In these cases, there is no conjunctival sac. The conjunctiva and the cornea are replaced by skin.
- ***Coloboma of eyelid:*** Part of the eyelid may be missing (Fig. 19.12A).
- The palpebral fissure may be abnormally wide or narrow. It may be abnormal in orientation and shape. The two lids may be completely, or partially, fused with each other.
- There may be abnormal folds of skin in relation to the lids. Similar folds, e.g. ***epicanthus*** (Fig. 19.12C) may be a normal feature in certain races.
- The lid margins may be turned inwards (***entropion***) (Fig. 19.13B) or outwards (***ectropion***) (Fig. 19.13C) and rarely, the whole lid may be everted. Normal lid margins are shown in Fig. 19.3A.



Figs 19.11A to D: (A) Synophthalmos (fused median eye); (B) Synophthalmos with proboscis above the median eye; (C) A section through synophthalmos; (D) A fetus with cyclops and proboscis

- The levator palpebrae superioris may fail to develop. This leads to drooping of the lids (***ptosis***).
- The eyelashes, and eyebrows, may be missing, or may be duplicated. The eyelashes may be abnormal in direction.

Anomalies of the lacrimal apparatus

- The lacrimal gland may be absent or nonfunctional. The gland may be ectopic in position.
- The lacrimal passages may be absent in whole or in part, or there may be atresia of some part.
- The lacrimal duct may be represented by an open furrow on the face, due to nonobliteration of the naso-optic furrow (see oblique facial cleft, Fig. 11.2A).
- There may be supernumerary puncta, or canaliculi.

Molecular and genetic basis of eyeball development

- The proteins Wnt, BMP, TGF-β and FGF (fibroblast growth factor) are responsible for optic vesicle and PAX6 for lens vesicle differentiation.
- Inhibition of sonic hedgehog (SHH) and expansion of PAX2 expression causes failure of separation of eyes resulting in cyclops. Overexpression of SHH causes a loss of eye structures.
- Vitamin A deficiency during embryonic development can result in anterior segment defects (of cornea and eyelid).

TIME TABLE OF SOME IMPORTANT EVENTS DESCRIBED IN THIS CHAPTER

Time table of some events described in this chapter is shown in Table 19.2.

TABLE 19.2: Timetable of some developmental events

Age	*Developmental event*
22nd day	Appearance of optic sulcus (over the neural plate)
4th week	Optic vesicle comes in contact with surface ectoderm. Lens placode is forming
5th week	Eye primordium is completely surrounded by loose mesenchyme
6th week	Choroid fissure is formed. Lens vesicle is seen
7th week	A solid lens is formed

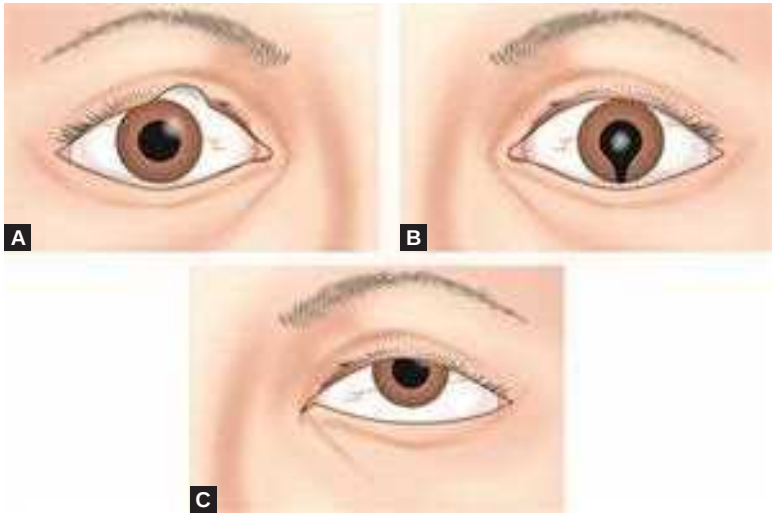
Note: The eyeball is most susceptible to teratogens during the 4th to 8th week, and can get affected till the end of pregnancy.

EMBRYOLOGICAL EXPLANATION FOR CLINICAL CONDITIONS OR ANATOMICAL OBSERVATIONS IN EYEBALL

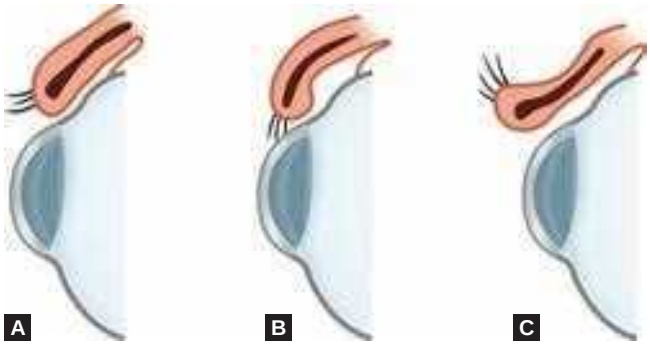
Case Scenario 1

A newly delivered baby presented cleft in the lower part of iris. What is the name given to this condition? Explain the embryological basis and name the structures that are involved.

This condition is called coloboma of the iris. As the optic cup is approaching the lens vesicle to enclose it, there will be a gap in the inferior surface of optic cup extending onto the optic stalk. This gap is called choroid fissure or fetal fissure. Through this fissure, the hyaloid artery reaches the



Figs 19.12A to C: (A) Coloboma of upper eyelid; (B) Coloboma of iris; (C) Epicanthal fold (Mangolian eye slant)



Figs 19.13A to C: (A) Normal lid; (B) Entropion; (C) Ectropion

posterior pole of eye. In normal development, the distal part of hyaloid artery degenerates and the choroid fissure fuses. Failure of fusion of choroid fissure results in coloboma. The nonfusion can occur at any part along the length of the choroid fissure. If the failure is in the distal part, it results in coloboma of iris. If it occurs in proximal part, it can cause coloboma of retina/choroid/optic nerve depending on the location of defect. Mutation of *PAX2* gene is responsible for this condition. It can be also associated with renal defects and is called renal coloboma syndrome.

Case Scenario 2

A newly born dead fetus presented with absence of eyes and nose. But on close observation, a median oblique cleft with a tubular appendage was noticed in the middle of forehead region. What is the name given to this condition? Explain the embryological basis and name the structures that are involved. Give the causes for this abnormality. Can it be diagnosed prenatally and if identified what medical advice to be given to the parents?

It is a case of severe craniofacial malformation. This condition is called holoprosencephaly with synophtholmos and proboscis. Holoprosencephaly is a structural malformation of the brain. There will be partial or total nonseparation of prosencephalon (forebrain). Majority of cases of holoprosencephaly are associated with a single completely fused eye (cyclops) in a single orbit or partially fused eyes (synophtholmos), absence of nose (arhinia) and a tubular appendage called proboscis. The cause for this condition can be genetic or there are environmental causes like maternal diabetes, alcohol use, etc. It can be diagnosed by fetal ultrasound or fetal MRI. This condition is not compatible with life and majority die in utero. If diagnosed prenatally, the parents should be advised termination of pregnancy to avoid mental agony.

REVIEW QUESTIONS

1. Write a short note on optic cup.
2. Explain the development of lens.
3. Explain the development of lacrimal apparatus.

Chapter 20

Development of the Ear

HIGHLIGHTS

- *Membranous labyrinth (internal ear)* is derived from a thickening of surface ectoderm called the *otic placode*. The otic placode is converted into the *otic vesicle* and then to different parts of the labyrinth.
- *Bony labyrinth* is formed from mesenchyme surrounding the membranous labyrinth.
- *Middle ear* and *auditory tube* develop from the tubotympanic recess (from 1st and 2nd pharyngeal pouches).
- *Ear ossicles:* The *malleus* and *incus* are derived from Meckel's cartilage. The *stapes* is derived from the cartilage of the 2nd pharyngeal arch.
- *External acoustic meatus* is derived from the first ectodermal cleft.
- *Auricle* is formed from swellings that appear around the cleft.

INTRODUCTION

The three morphological subdivisions of the ear are (1) external ear, (2) middle ear and (3) internal ear. Each one has a separate developmental origin (Table 20.1).

- Neuroectoderm of hindbrain region forms the *membranous labyrinth*. This forms the otic placode and otic vesicle. The otic vesicle differentiates to form vestibular and cochlear parts of internal ear.
- The mesenchyme surrounding the membranous labyrinth forms the *bony labyrinth* that forms otic capsule, which later gets chondrified leaving a space (periotic space) between membranous labyrinth and bony labyrinth.
- The dorsal part of 1st pharyngeal pouch and part of 2nd pharyngeal pouch (endoderm) form tubotympanic recess that gives rise to *pharyngotympanic tube* and *middle ear cavity*.
- The *ear ossicles* and *muscles of middle ear* develop from mesoderm of 1st and 2nd arches.
- The *external acoustic meatus* develops from ectodermal cleft of 1st arch.
- The *auricle* develops from mesodermal thickenings around 1st ectodermal cleft.
- The *tympanic membrane* is tridermal in origin as it develops from all the three germ layers.

INTERNAL EAR

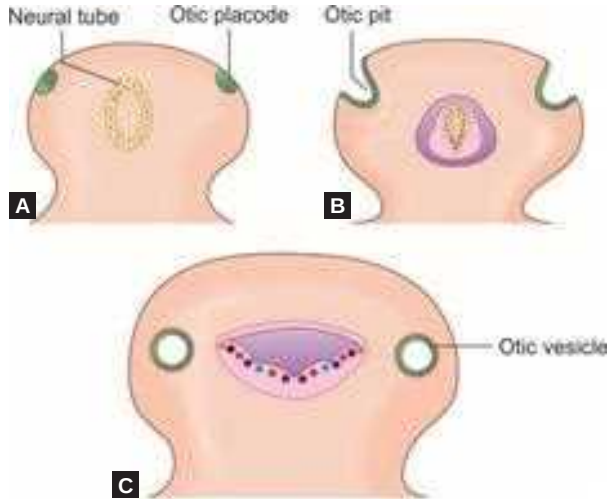
The internal ear is the first part to develop among the three parts of the ear. The developmental components of internal ear are membranous labyrinth and bony labyrinth.

Membranous Labyrinth

- It is derived from a specialized area of surface ectoderm on either side of the developing myelencephalic part of rhombencephalon. This area is first apparent as a thickening called the *otic placode* (Fig. 20.1A).
- The otic placode soon becomes depressed below the surface to form the *otic pit* (Fig. 20.1B).
- The otic pit then becomes rounded to form the *otic vesicle/otocyst* which separates from the surface ectoderm (Fig. 20.1C).
- The otic vesicle is at first an oval structure. By differential growth of various parts of its wall, it gives rise to the structures comprising the *membranous labyrinth*.

TABLE 20.1: Summary of derivation of various parts of the ear

Part	*Embryologically derived from*	*Parts formed*
Membranous labyrinth (Otocyst)	Neuroectoderm	Dorsal part—vestibular part: a. Utricle b. Semicircular ducts c. Endolymphatic duct Ventral part—cochlear part: a. Saccule b. Ductus reuniens c. Cochlear duct
Bony labyrinth (Otic capsule)	Mesenchyme around otocyst	a. Vestibule—around utricle and saccule b. Semicircular canals—around semicircular ducts c. Scala tympani—below cochlear duct d. Scala vestibuli—above cochlear duct
Ganglia of vestibulocochlear nerve	Neural crest cells	Vestibular ganglion—equilibrium Cochlear ganglion—hearing
Bones (Ear ossicles)	Malleus, incus—1st pharyngeal arch mesoderm Stapes—2nd pharyngeal arch mesoderm	Bones of the ear
Muscles	Tensor tympani—1st arch mesoderm Stapedius—2nd arch mesoderm	Intrinsic muscles of ear
Tubotympanic recess	Dorsal part of 1st and part of 2nd pharyngeal pouches (endodermal)	a. Pharyngotympanic/auditory tube b. Tympanic antrum c. Mastoid antrum and air cells
External acoustic meatus	1st ectodermal cleft	External auditory canal
Tympanic membrane	Apposition of endodermal tubotympanic recess and ectodermal external acoustic meatus with intervening mesoderm	Layers: a. Outer cuticular—ectodermal b. Middle fibrous—mesodermal c. Inner mucous—endodermal
Auricle	Mesodermal condensations of 1st and 2nd arches around 1st ectodermal cleft	1st arch: a. Tragus b. Crus of helix c. Helix 2nd arch: a. Antihelix b. Antitragus c. lobule

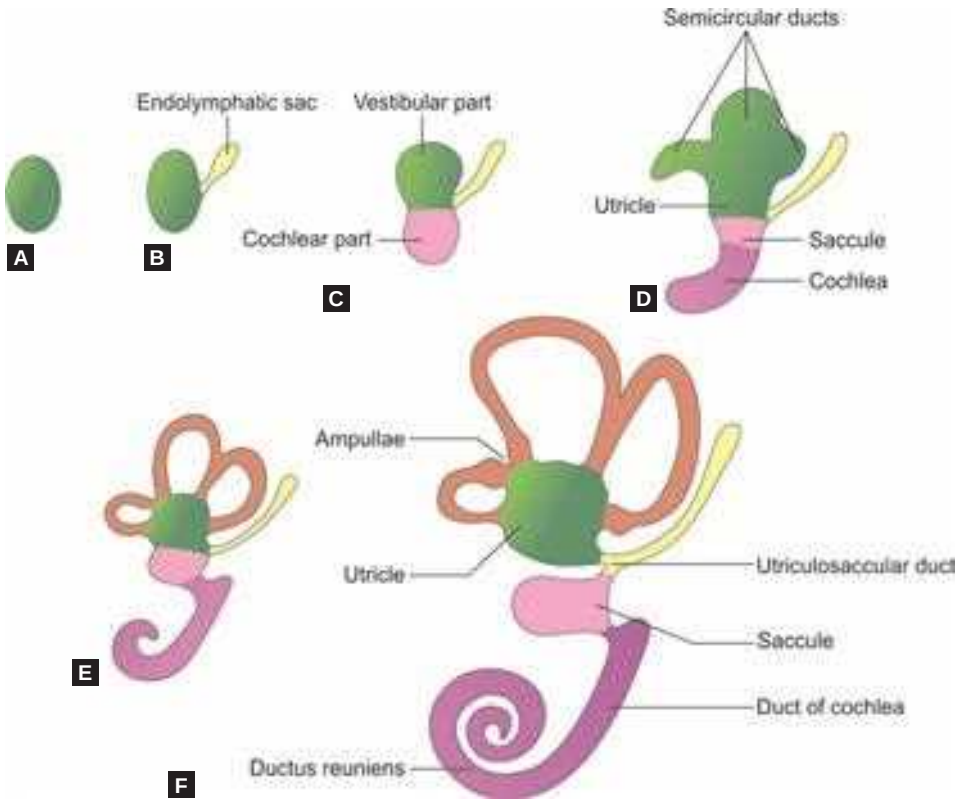


Figs 20.1A to C: Formation of membranous labyrinth. (A) Otic placode; (B) Otic pit; (C) Otic vesicle surrounded by mesenchyme

- The otic vesicle divides into a vestibular (dorsal) and cochlear (ventral) components.
- The ventral cochlear part gives rise to the saccule, cochlear duct (organ of Corti) and comes into contact with the spiral ganglion of vestibulocochlear nerve (Figs 20.2 and 20.3) derived from neural crest cells. The details are illustrated in Figures 20.2 and 20.3.
- The dorsal part gives rise to the utricle, semicircular ducts, endolymphatic duct and sac and comes into contact with the vestibular ganglion of vestibulocochlear nerve (Figs 20.2 and 3) derived from neural crest cells. The details are illustrated in Figures 20.2 and 20.3.
- Localized areas of epithelium of the membranous labyrinth undergo differentiation to form specialized sensory end organs of equilibrium and hearing. The sensory end organs of equilibrium are *cristae* of semicircular *ducts and macula* of utricle. The sensory organs of hearing are *macula* of saccule and *organ of Corti* of cochlea. These are innervated by peripheral processes of the cells of the vestibular and cochlear ganglia respectively. These ganglia are derived from the neural crest cells. Its cells are peculiar in that they remain bipolar throughout life (Fig. 20.4).

Bony Labyrinth

- The bony labyrinth is formed from the mesenchyme surrounding the membranous labyrinth (Fig. 20.5A). This mesenchyme becomes condensed to form the *otic capsule*. The mesenchymal condensation is soon converted into cartilage.



Figs 20.2A to F: Gradual transformation of a rounded otic vesicle to the highly complicated form of the membranous labyrinth. (A) Otocyst; (B) Formation of endolymphatic sac; (C) Division of otocyst into vestibular and cochlear parts; (D) Division of vestibular part into utricle and semicircular ducts and cochlear part into saccule and cochlear duct; (E and F) Further differentiation of components

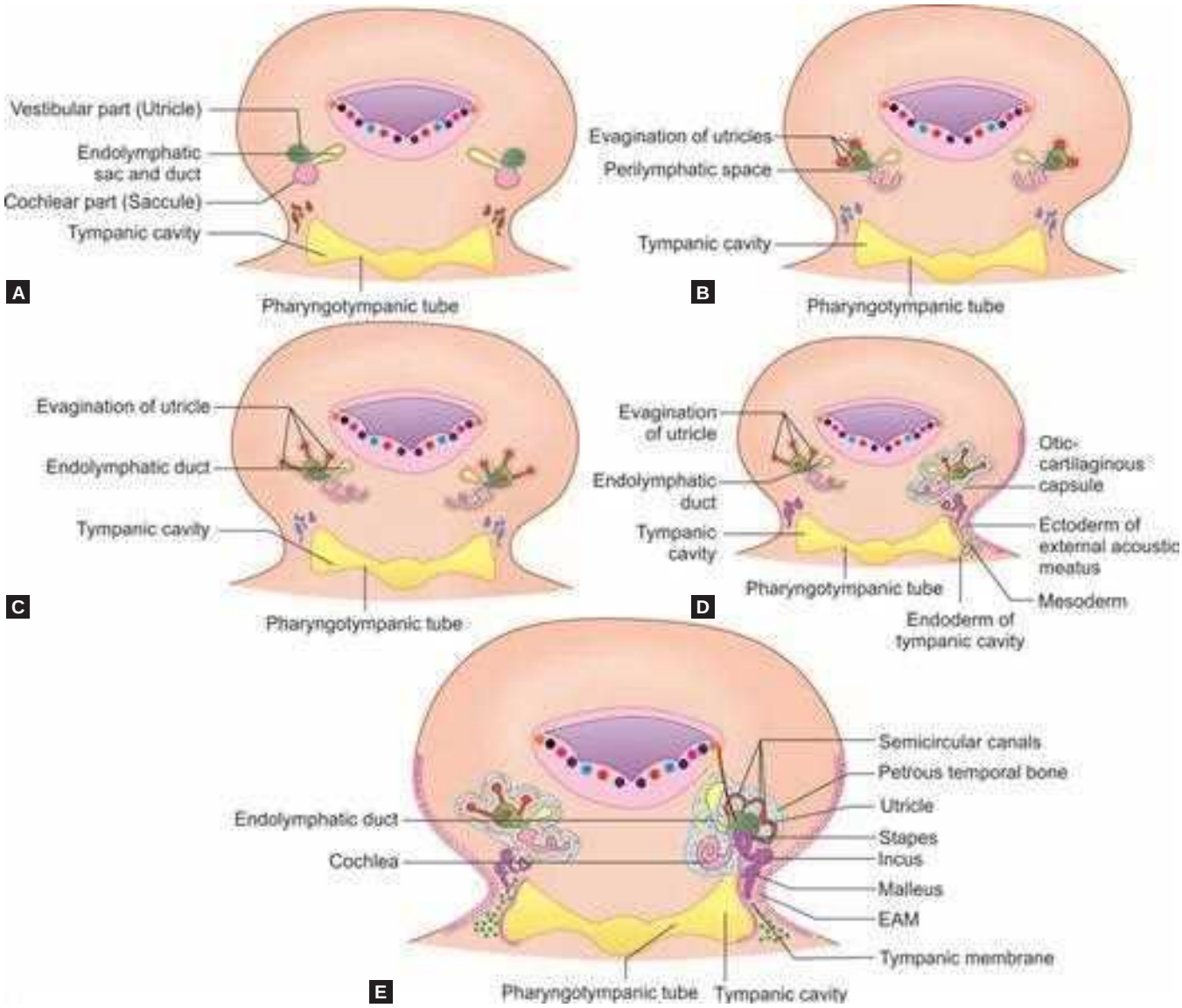
- Between this cartilage and the membranous labyrinth, there is a layer of loose periotic tissue (Fig. 20.5B). The spaces of the bony labyrinth are created by the disappearance of this periotic tissue. The membranous labyrinth is filled with a fluid called *endolymph*, while the periotic spaces surrounding it are filled with *perilymph* (Fig. 20.5C).
- The periotic tissue, around the utricle and saccule, disappears to form a space called the *vestibule* (Fig. 20.6). The periotic tissue, around the semicircular ducts, also disappears to form the *semicircular canals*. Two distinct spaces are formed, one on either side of the cochlear duct. These are the *scala tympani* and the *scala vestibuli*. The scala vestibuli communicates with the vestibule while the scala tympani grows toward the tympanic cavity, from which it remains separated by a membrane (Fig. 20.6).
- The cartilaginous labyrinth is subsequently ossified to form the bony labyrinth (Fig. 20.5D).

MIDDLE EAR

- The epithelial lining of the middle ear and of the pharyngotympanic tube is derived from the *tubotympanic recess*. This recess develops from the dorsal part of the 1st pharyngeal pouch, and also receives a contribution from the second pouch (Figs 20.7A and B). The tympanic antrum and mastoid air cells are formed by extensions from the middle ear.
- The *malleus* and *incus* are derived from the dorsal end of Meckel's cartilage, while the *stapes* is formed from the dorsal end of the cartilage of the 2nd pharyngeal arch (Reichert's cartilage) (Figs 20.8A and B).
- The ossicles are at first outside the mucous membrane of the developing middle ear. Later, they invaginate the mucous membrane, which covers them throughout life (Figs 20.8C to E). The ossicles of the ear fully ossify in the 4th month of intrauterine life. They are the first bones in the body to do so.
- The *tensor tympani* muscle is derived from the mesoderm of the 1st pharyngeal arch and the *stapedius* from that of the 2nd arch.

EXTERNAL EAR

- The *external acoustic meatus* is derived from the dorsal part of the first ectodermal cleft (Fig. 20.9A).



Figs 20.3A to E: Development of various components of internal ear and middle ear. (A) Formation of cochlear and vestibular parts, tympanic cavity and pharyngotympanic tube; formation of mesenchymal condensation around the membranous labyrinth; (B) Evaginations of utricle to form semicircular ducts; (C) Coiling of cochlea; (D) Cartilaginous otic capsule, approximation of external acoustic meatus and tubotympanic recess with intervening mesoderm; (E) Formation of ear ossicles, tympanic membrane, petrous temporal bone and connections with vestibular and cochlear nerves

- However, its deeper part is formed by proliferation of its lining epithelium, which grows toward the middle ear (Fig. 20.9B).
- This proliferation is at first solid (meatal plug), but is later canalized (Fig. 20.9C).
- The *auricle*, or pinna, is formed from about six mesodermal thickenings (called tubercles or hillocks) that appear on the mandibular and hyoid arches, around the opening of the dorsal part of the first ectodermal cleft (i.e. around the opening of the external acoustic meatus) (Figs 20.10A to E).

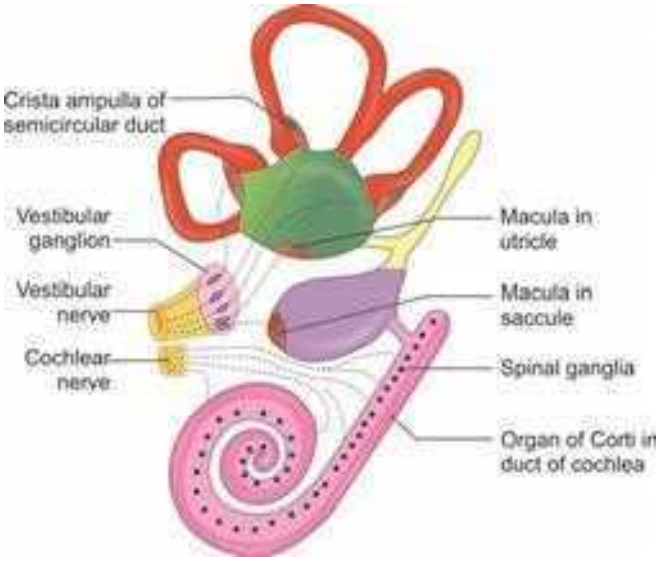
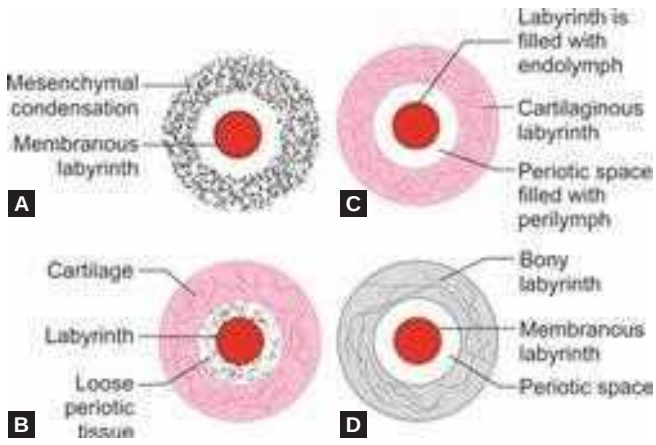


Fig. 20.4: Specialized sensory areas developing in the internal ear and their nerve supply



Figs 20.5A to D: Establishment of the basic structure of the bony labyrinth

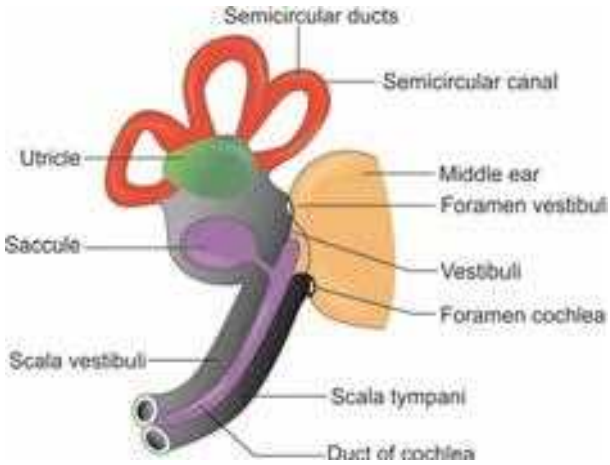
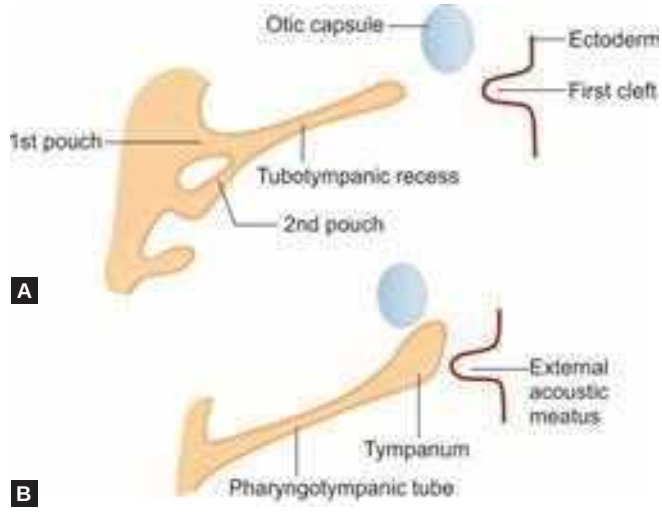
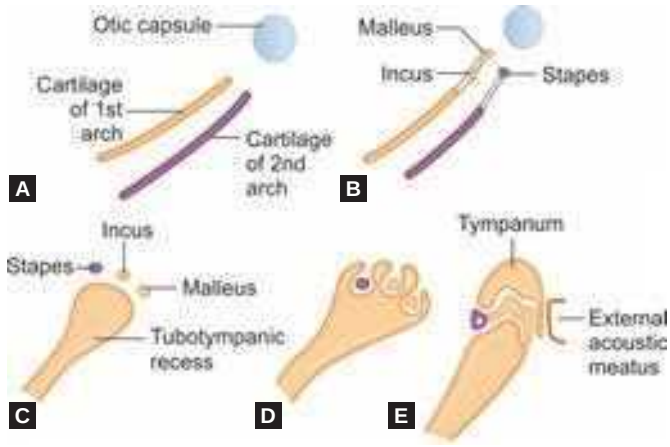


Fig. 20.6: Some parts of bony labyrinth (black) and of membranous labyrinth (pink)



Figs 20.7A and B: Development of the middle ear (tympanum). (A) Formation of tubotympanic recess, and (B) its subdivision into the tympanum and the pharyngotympanic tube



Figs 20.8A to E: The ossicles of the middle ear develop from the first and second pharyngeal arches. Ossicles of the ear gradually invaginate into the tympanum

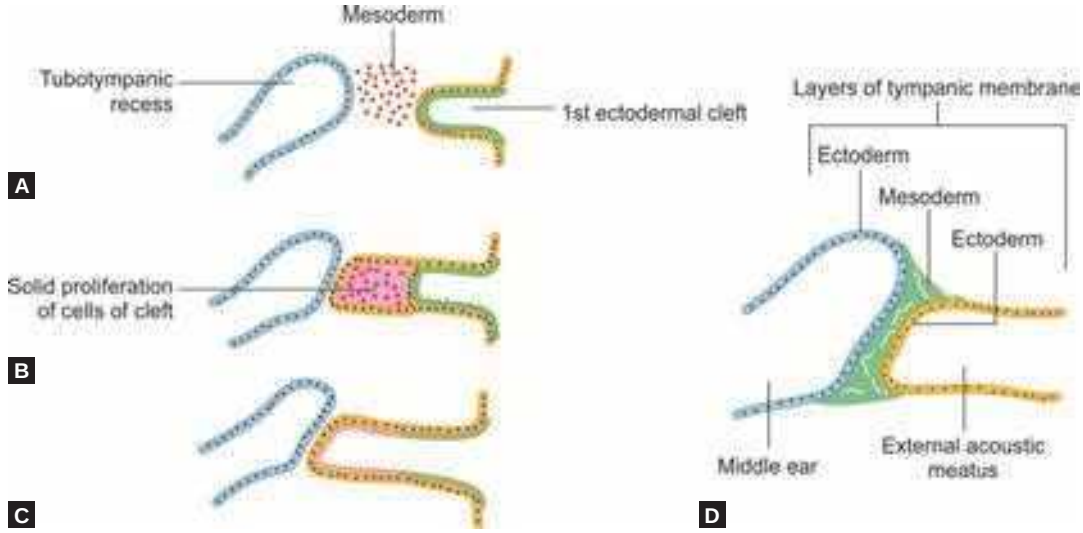
The mandibular arch forms only the tragus and a small area around it, the rest of the auricle being formed from the hyoid arch. This is consistent with the fact that the auricular muscles are supplied by the facial nerve.

Tympanic Membrane

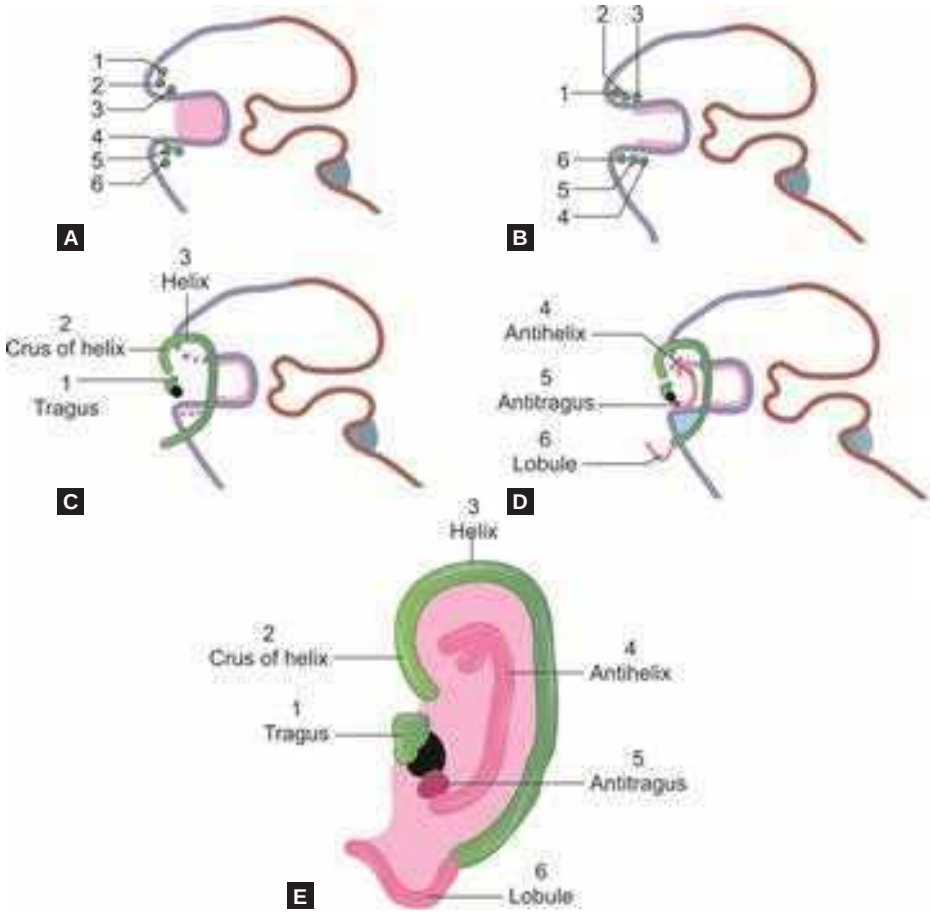
This is formed by apposition of the tubotympanic recess and the first ectodermal cleft, these two forming the inner (endodermal) and outer (ectodermal) epithelial linings of the membrane. The intervening mesoderm forms the connective tissue basis (Fig. 20.9D).

Two points worth noting are as follows:

- The handle of the malleus grows into the connective tissue from above.



Figs 20.9A to D: Development of external acoustic meatus. The solid mass of ectodermal cells seen in (B) has been canalized as seen in (C); (D) Layers of tympanic membrane



Figs 20.10A to E: Development of the auricle. (A) First ectodermal cleft around which the auricle develops; (B) Small swellings or hillocks appear; (C to E) Hillocks gradually fuse with one another to form the various parts of external ear

The chorda tympani nerve is at first outside the membrane but comes to lie within its layers, because of upward extension of the membrane.

Molecular and genetic basis of ear development

- The proteins Wnt and bone morphogenetic protein (BMP) of surrounding region are important for the formation of otic placode.
- Retinoic acid plays an important role in the anteroposterior differentiation of otic vesicle.
- Wnt and Shh are required for the formation of semicircular canals and cochlear duct.
- Defects in Noggin and Pax2 genes result in sensory neural deafness that plays a role in formation of cochlea.

Clinical correlation

Anomalies of the Ear

Anomalies of the auricle

- The development of the auricle may get arrested at any stage. As a result of this, it may be totally, or partially, absent; it may be represented by isolated nodules; or it may be very small. Alternatively it may be very large.
- The migration of the auricle from its primitive caudoventral position may remain incomplete. This migration occurs as a result of the growth of the maxillary and mandibular processes. This explains the association of caudoventral displacement of the auricle with ***mandibulofacial dysostosis***.

Anomalies of the external auditory meatus

- There may be stenosis or atresia of the meatus over its whole length, or over part of it. The lumen may be closed by fibrous tissue, by cartilage, or by bone.
- The normal curvature of the meatus may be accentuated as a result of which the tympanic membrane cannot be fully seen from the outside.

Anomalies of the middle ear

- The ossicles may be malformed. They may show abnormal fusion to one another or to the wall of the middle ear. The stapes may be fused to the margins of the fenestra vestibuli.
- The facial nerve may bulge into the middle ear and may follow an abnormal course.
- The stapedial artery may persist.

Anomalies of the internal ear

- Various parts of the membranous labyrinth may remain underdeveloped. In some cases, the cochlea alone is affected. These anomalies lead to congenital deafness.

TIME TABLE OF SOME EVENTS DESCRIBED IN THIS CHAPTER

Time table of some events described in this chapter is shown in Table 20.2.

TABLE 20.2: Time table of some developmental events

Age	*Developmental events*
22nd day	Otic placode is seen
5th week	Tubotympanic recess develops
6th week	Auricle starts forming. The cochlea and semicircular canals starts forming
7th week	The mesenchymal condensations for the three ear ossicles appear
8th week	The cochlea and semicircular canal assume their definitive external form
10th week	Scala vestibuli and scala tympani appear
7th month	External acoustic meatus gets canalized

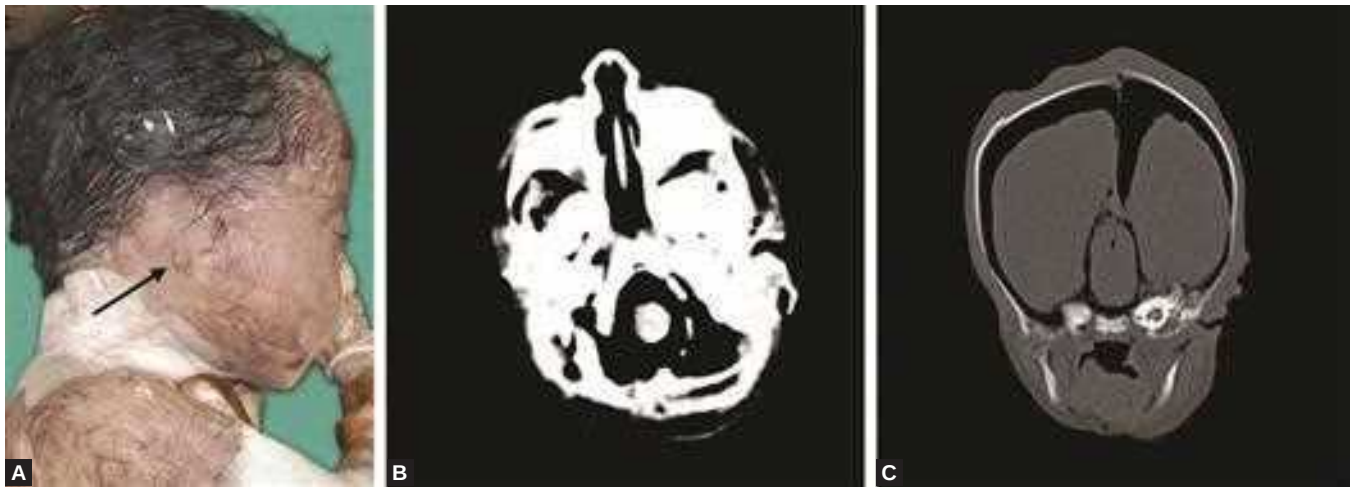
Note: The ear is most sensitive to teratogens during the 4th to 9th weeks, and can be affected up to the 12th week.

EMBRYOLOGICAL EXPLANATION FOR CLINICAL CONDITIONS OR ANATOMICAL OBSERVATIONS IN EAR

Case Scenario 1

A female fetus aborted at 8 months age presented a small peanut-like tag of soft tissue representing the right auricle (Fig. 20.11A) and a normal left auricle. When the head of the fetus is subjected to computed tomography (CT) scan, it revealed normal auricle and external auditory canal on left side and absence of auricle and auditory canal on right side (Fig. 20.11B). Middle ear cavity and ossicles were not visualized on both sides (Fig. 20.11C). Internal auditory canal, cochlea, semicircular canals, mastoid air cells and VII and VIII nerve complexes are normal on both sides. What is the name given to this condition? Explain the embryological basis and causes for this condition.

- It is a case of bilateral middle ear atresia with absence of middle ear cavity and ear ossicles with right-sided microtia.
- Microtia is a congenital abnormality in which auricle of external ear is small and deformed. Atresia is absence or closure of external auditory canal.
- Microtia and atresia of external auditory meatus usually occur together. Usually they are unilateral.
- During the early part of fetal development (5th week), complex processes are involved in the formation and in the movement of the cells for correct position of ear. For unknown reasons, this process is interrupted and results in absence (anotia) or a small (microtia) external ear with associated atresia of external auditory canal.
- Canalization of ectodermal cells of 1st ectodermal cleft results in formation of external acoustic meatus.



Figs 20.11A to C: Aborted fetus with bilateral middle ear atresia and right-sided microtia. (A) Peanut-like tag of right auricle (arrow); (B) CT image showing absence of right auricle and external auditory canal; (C) CT image showing absence of middle ear cavity and ossicles on both sides

- Formation of tubotympanic recess (endodermal) from dorsal part of 1st and part of 2nd pharyngeal pouches forms middle ear cavity.
- The mesoderm in between the ends of 1st ectodermal cleft and tubotympanic recess forms the ear ossicles.
- On the right side absence of ectodermal, endodermal and mesodermal components of 1st and 2nd pharyngeal arches resulted in microtia, atresia of external auditory canal, absence of ear ossicles and middle ear cavity.
- On the left side absence of endodermal and mesodermal components of 1st and 2nd pharyngeal arches resulted in absence of middle ear cavity and ossicles.
- On both sided there is no defect in the formation of internal ear components that are derived from neuroectoderm of hindbrain region.
- The cause for this abnormality can be genetic, environmental exposure to radiation, infections or due to the use of certain medicines taken by the mother.

REVIEW QUESTIONS

1. Explain the development of tympanic membrane.
2. Explain the development of external ear.
3. Explain the development of ear ossicles.
4. Describe otocyst.

Chapter 21

Clinical Applications of Embryology

GESTATIONAL PERIOD

Gestation

The process or period of development inside the womb between conception and birth is called gestation. The length of gestation is 280 days/40 weeks/9 months ± 7 days. Gestation (Latin) is synonymous with *menstrual age*. It begins with 1st day of last menstruation prior to conception. If the date of onset of menstrual bleeding in the last menstrual period (LMP) or cycle is known the expected date of delivery (EDD) can be calculated as shown in the following example:

Calculation of EDD from LMP:

LMP	10.09.2016
GP	07.09.0000

EDD	17.06.2017

Age of embryo: Menstrual age versus fertilization age.

Menstrual age (LMP) or *gestational age* is based on LMP.

Fertilization age is fetal developmental age and is 14 days less than the gestational age as fertilization takes place 14 days before the onset of next menstrual cycle.

Fertilization age = Menstrual age – 14 days

Gestational Period—Stages

- Germinal period: 1st–3rd week
  - Fertilization to differentiation of germ layers
- Embryonic period: 4th–8th week
  - Changes in shape/external appearance of embryo
  - Individual differentiation of germ layers
  - Formation of tissues and organs
- Fetal period: 3rd month—term
  - Rapid growth of fetus
  - Complete development of placenta

GROWTH OF THE EMBRYO

After its formation, the embryonic disc undergoes folding. This folding leads to major changes in body form (during the 4th–8th weeks of fetal life). The embryo acquires the external features of a human being. All organ systems are formed. Changes in external form of the embryo occur during the 4th–8th weeks. At the end of the embryonic period, the embryo can be recognized as human even though its size crown-rump length (CRL) is only about 30 mm.

Criteria used for estimation of age of an embryo are different for embryonic and fetal periods.

This is important for understanding the age of the embryo, i.e. gestational sac in the early stages and the effect of teratogens during this period.

For embryonic period, it is divided into the three stages. They are:

1. *Presomite*: 15th–20th day of development
   - Primitive streak
   - Notochord
   - Intraembryonic mesoderm (IEM)
2. *Somite*: 20th–30th day of development
   - Mesodermal segments
3. *Postsomite*: 31st–55th day. By measuring the length and weight of fetus by transabdominal ultrasound.

The importance of having a knowledge of the timing of embryological events, thus, becomes obvious. In the early stages of development, the age of an embryo is reckoned in days. Later, when events are less dramatic, age can be expressed in weeks or months. However, the exact age of

an embryo is not always known. An estimate can be made by observing the size of the embryo (expressed as CRL), or some other features like the number of somites. In most textbooks of embryology, there are numerous references to the timing of embryonic events (most commonly in terms of CRL). The disadvantage of doing so is that it adds yet one more complication to the understanding of an already intricate subject. Because of this reason, references to the timetable of development have been kept to the minimum in the main text.

DETERMINING THE AGE OF AN EMBRYO

Gestational Age—Importance

It is important to know the age of a developing embryo because this can affect:

- Clinical procedures—like amniocentesis or chorionic villus sampling.
- Obstetric care:
  - Post-term delivery
  - Cesarean section.
- Interpretation of antenatal tests:
  - Alpha-fetoproteins
  - Fetal diseases in pregnancy
  - Fetal growth assessment
  - Fetal heart rate.

The exact age of a living embryo can be found out only when the date of conception (fertilization) is known. This is almost impossible because fertilization is an internal event. Usually, the age has to be determined indirectly.

Estimation of gestational age from menstrual history may be unreliable as the expectant mother may not know the exact date of the last normal menstrual period, or her menstrual cycles may be irregular.

The somites begin to be seen in embryos about 21 days old. Embryos younger than this are called *presomite embryos* and their age is reckoned in days. Once the somites appear, the age is described in terms of the number of somites present, e.g. one-somite stage, four-somite stage, etc. When the embryo is about 30 days old, it is large enough to be measured.

However, the measurement of the length of an aborted embryo is not as simple as it sounds, as the embryo is bent on itself and cannot be straightened without fear of damage to it. Hence, instead of measuring its full length we measure what is called the CRL (Fig. 21.1). CRL is measured from the vertex of the skull to the midpoint between the apices of the buttocks.

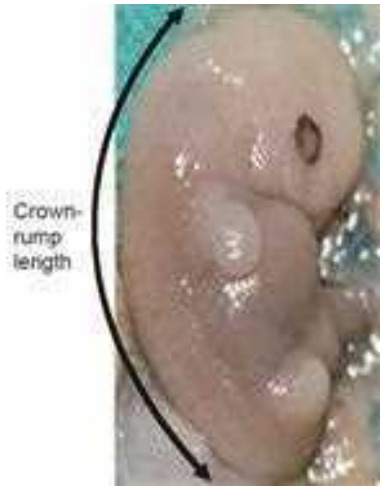


Fig. 21.1: Measurement of crown-rump length (CRL) of embryo

At the present time, it is possible to measure the CRL of an embryo accurately, within the womb of the mother, with the help of an ultrasound machine. The CRL of a 1-month-old embryo is about 5 mm, and that of a 2-month-old embryo is about 30 mm. At full term the CRL is about 300 mm. However, because of variations in the degree of curvature of fetuses, CRL is not a very accurate index of fetal age. Various other measurements are also used. For example (with the use of ultrasound), we can measure the dimensions of some parts of the fetus (e.g. head, foot length).

Carnegie Embryonic Staging System

This system divides the development of an embryo into various stages. It is a numeric system for characterizing developmental stages. This system is used internationally by embryologists and research workers. The stages range from 1 to 23 and cover 1–56 days of embryonic development.

FURTHER GROWTH OF THE FETUS

At the beginning of the fetal period (9th week to 3rd month), the embryo has developed into a recognizable human being and the primordia of all organ system have formed.

During the fetal period, development is mainly directed toward the rapid growth in body size and toward differentiation of tissues, organs and organ systems.

During this period, the growth of the head is slow as compared to that of the rest of body. At the beginning of 3rd month, the head is half the CRL, while at birth it is about one-fourth of CRL. Fetal weight gain is very rapid in the last month of pregnancy.

A brief summary of growth in the embryonic and fetal periods is given in Tables 21.1 and 21.2.

TABLE 21.1: Developmental events during embryonic period (Figs 21.2 to 21.5)

Age in weeks	*Changes in external form*
3rd week (Figs 21.1 and 21.2)	• Germ disc can be identified
4th week	• At the beginning of the 4th week, folding of embryonic disc • Formation of the foregut, the midgut and the hindgut • Pharyngeal arches start appearing at the end of the 4th week • Heart produces a large prominence on the ventral aspect of the embryo and starts functioning by the end of the 4th week • Forebrain is the most cranial and most prominent structure of the embryo • Upper limbs appear as paddle-shaped buds • Otic pits and the lens placodes become visible
5th week	• Further development of the head and of the face occurs rapidly • Mesonephric kidney starts forming
6th week	• Upper limbs show further differentiation so that the elbow and digits can be recognized • Lower limb buds and the external ear start forming • Further development is seen in the eyes, eyelids and ear
7th week	• Hand shows formation of digits • Ossification of bones starts in the upper limb
8th week	• Limbs and digits are fully formed • Movements start taking place in the limbs • Bone formation begins in the lower limbs • The neck appears between the head and the thorax • The external genitalia may start showing sex differences • The tail disappears

TABLE 21.2: Developmental events during fetal period (Fig. 21.6)

Age	*Developmental features*
3rd month (9–12 weeks)	• Eyes and ears are now in their definitive positions • Process of ossification is seen in all long bones • Intestinal loops that had herniated out of the abdominal cavity now return into it • Male and female external genitalia can be visualized using ultrasound imaging • Urine formation begins
4th month (13–16 weeks)	• Proportion of the size of the head relative to the rest of the body is less as compared to that in the 3rd fetal month • Length of the fetus increases rapidly • Lower limbs reach their final relative length as compared to the rest of the body • Movements in the limbs are not very strong but can be seen on ultrasound examination
5th month (17–20 weeks)	• Length of fetus increases. Increase in weight is however slow • Mother can now feel the movements of the fetus (quickening) • Hair on the head, and eyebrows, can be seen • Skin becomes covered with sebaceous gland secretion (vernix caseosa)
6th month (21–24 weeks)	• Skin is wrinkled due to absence of subcutaneous tissue • Lung alveoli begin to secrete surfactant, which helps to maintain the patency of the alveoli of the lung. This is a sign of the maturity of the respiratory system • Rapid eye movements begin • Fetus now starts gaining body weight rapidly
7th month (25–29 weeks)	• Blood formation now begins to shift from spleen to bone marrow • Central nervous system is now mature enough to be able to control respiration, if the fetus is born at this stage • Respiratory system is mature enough to perform gaseous exchanges between pulmonary vessels and lung alveoli • A fetus born prematurely in the 7th month can survive. Such fetuses are said to be viable
8th month (30–34 weeks)	• Skin is smooth due to deposition of subcutaneous fat. It is pink due to increase in blood supply • Body weight increases rapidly • The pupillary light reflex can be elicited
9th month (35–38 weeks)	• Head circumference is almost the same as that of the abdomen in the terminal weeks of pregnancy • At the end of the fetal period, the skull has the largest circumference of all parts of body • The testes usually lie in the scrotum • The length of the foot is slightly more than the length of the femur

Fig. 21.2: Tubal gestation showing gestational sac with embryonic disc—3 weeks of age

Fig. 21.3: Opened gestational sac with embryonic disc

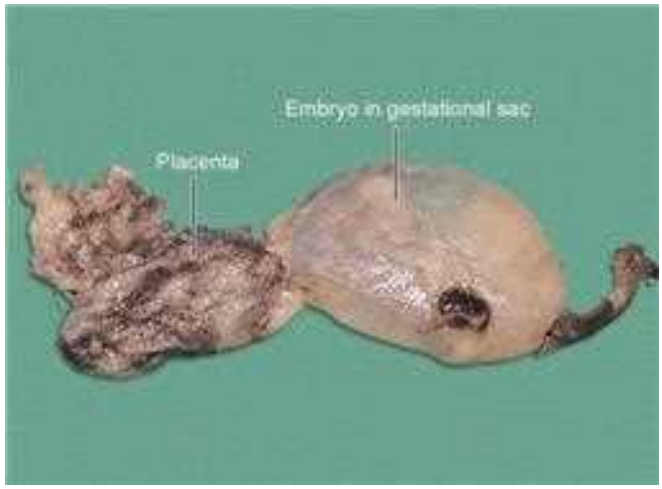


Fig. 21.4: Six weeks of embryo in gestational sac and placenta

Fig. 21.5: Six weeks of embryo after opening gestational sac

DETERMINING THE AGE OF A LIVING FETUS

The ability to find out the age of a living fetus is very important clinically. The age of such a fetus can be determined by making measurements using ultrasound examination.

1. Gestational sac can be identified as an echo-free space containing the amniotic fluid, embryo and extraembryonic structures (umbilical cord, placenta) (Figs 21.7 and 21.8).
2. Between 7 weeks and 14 weeks, the CRL can be measured.
3. Estimation of fetal age in the second and third trimesters of pregnancy is based on the measurements of various body parts. These are:
   - Biparietal diameter
   - Circumference of the head (Fig. 21.9)
   - Circumference of the abdomen (Fig. 21.10)
   - Length of femur
   - Foot length.

Estimation of fetal age facilitates management of pregnancy. The various congenital anomalies that can be identified include neural tube defects like anencephaly (Fig. 21.11) (Fig. 10.22), encephalocele (Fig. 21.12), spina bifida, abdominal wall defects like omphalocele (Fig. 21.13), facial defects like microcephaly (Fig. 21.14), cleft lip and palate (Fig. 9.14), for identifying growth proportion (Fig. 21.15), growth of limbs (Fig. 21.16), etc.

CONTROL OF FETAL GROWTH

Intrauterine growth of the fetus is influenced by maternal factors, placental factors and fetal factors.

Maternal Factors

Adequate availability of nutrition in maternal blood and its transfer across the placenta are essential for normal growth of

Fig. 21.6: Changes in external appearance and body proportions at different gestational ages

Fig. 21.7: Six months embryo in gestational sac with placenta

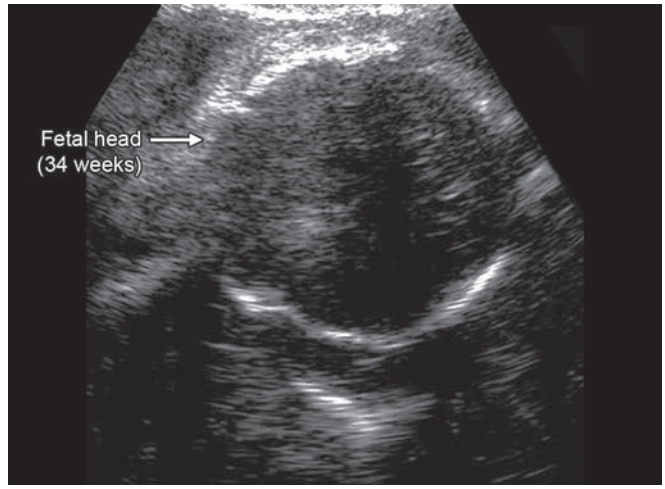


Fig. 21.9: Ultrasound image of fetal head (34 weeks)
Image Courtesy: Dr K Bhanu

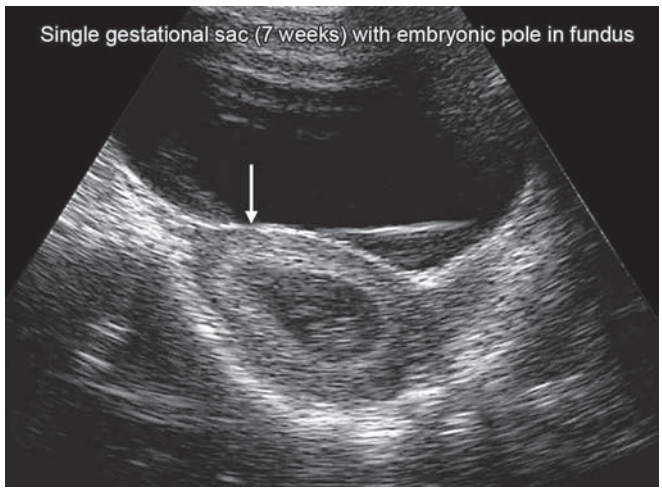


Fig. 21.8: Transabdominal ultrasound showing single gestational sac of 7 weeks in fundus with embryonic pole
Image Courtesy: Dr K Bhanu

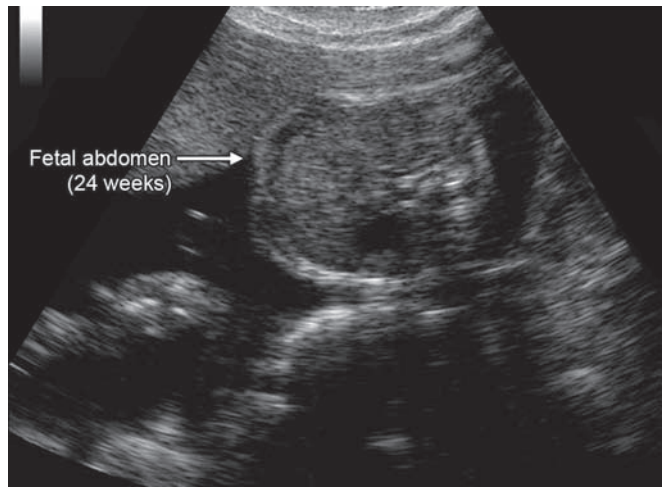


Fig. 21.10: Ultrasound image of fetal abdomen (24 weeks)
Image Courtesy: Dr K Bhanu

Fig. 21.11: Anencephalic fetus with macrostomia and externally placed parotid gland

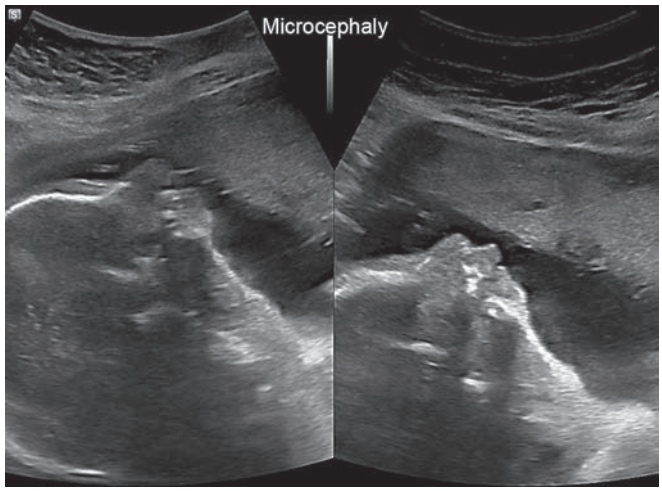


Fig. 21.14: Ultrasound image of a case of microcephaly
Image Courtesy: Dr Ganesh Kumar and Dr Sasikala

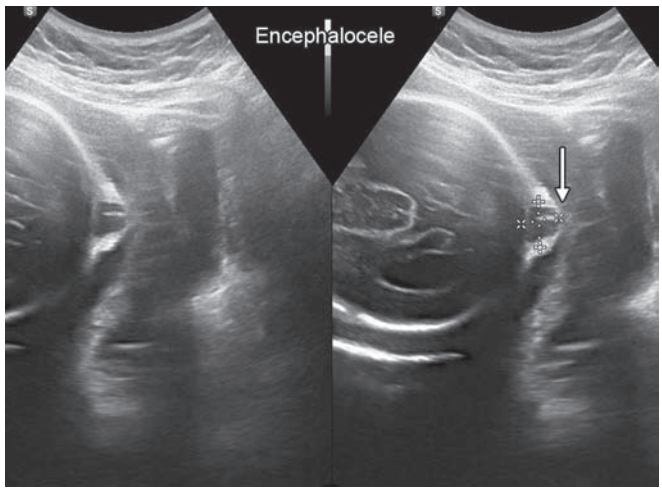


Fig. 21.12: Ultrasound image of a case of encephalocele
Image Courtesy: Dr Ganesh Kumar and Dr Sasikala

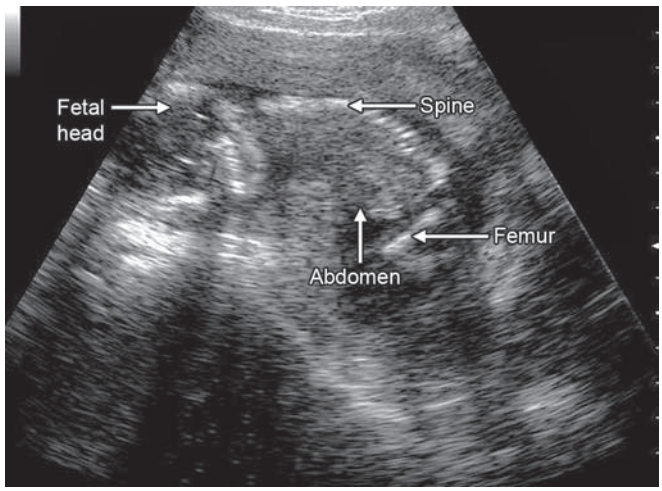


Fig. 21.15: Ultrasound image of a fetus of 16 weeks of gestational age showing fetal head, abdomen and femur
Image Courtesy: Dr K Bhanu

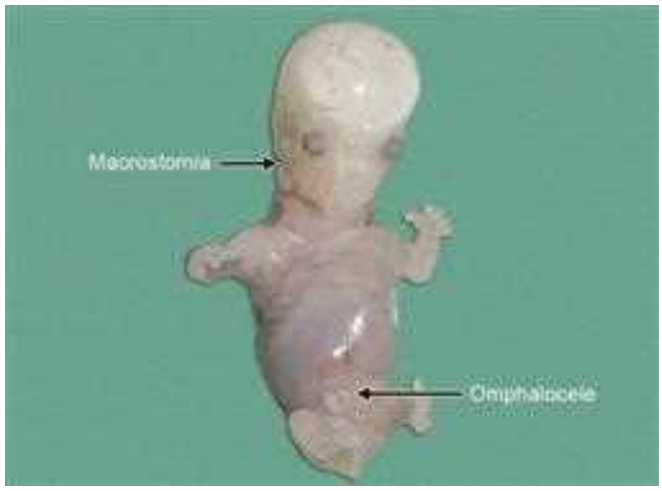


Fig. 21.13: A 10-week fetus with omphalocele and unilateral macrostomia

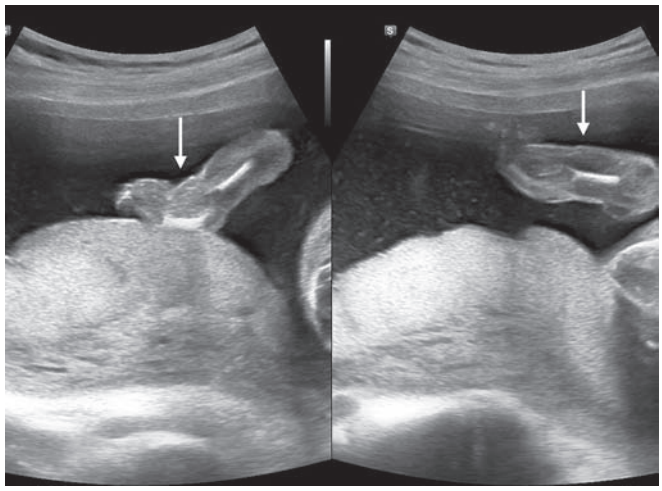


Fig. 21.16: Ultrasound image for estimating growth of limb
Image Courtesy: Dr Ganesh Kumar and Dr Sasikala

the fetus. Malnutrition in the mother affects fetal growth and can possibly cause fetal malformations. As a rule, maternal hormones do not pass through the placenta and hence they cannot affect fetal growth. However, they can influence the fetus indirectly by controlling maternal metabolic processes.

Placental Factors

- Hormones secreted by the placenta can influence the fetus indirectly by influencing maternal metabolism. For example, somatomammotropin secreted by the placenta has an anti-insulin effect leading to increased plasma levels of glucose and amino acids in maternal blood. The availability of these to the fetus is, thereby, increased.
- Placental hormones also have a direct influence on fetal growth. Somatomammotropin increases fetal growth. Human chorionic gonadotropin (hCG) stimulates growth of the fetal testis.

Fetal Factors

- Fetal growth is influenced by genetic factors. However, genetic factors that determine the height of the individual operate mainly in postnatal life (through the action of the growth hormone and of the thyroid hormone).
- Fetal endocrine glands start functioning near the middle of intrauterine life. The effects of hormones produced by them may be different from those seen in postnatal life, the modifications being necessary for requirements of the fetus.
  - For example, the fetal adrenal gland starts producing cortisol in the 9th week. In an adult, cortisol has a catabolic effect. To prevent this, cortisol secreted by the fetus is converted to cortisone (which does not have this effect).
  - Growth hormone (produced by the hypophysis cerebri) and thyroid hormones have very little effect on fetal growth. Infants in whom these hormones are deficient do not show growth retardation.

However, sex hormones produced by developing gonads greatly influence differentiation of genital organs in both sexes.

Fetal Growth Retardation

When the growth of a fetus is less than that seen in 90% of fetuses (i.e. it is below the 90th percentile), the phenomenon is described as *intrauterine growth retardation (IUGR)*. Such infants are also described as *small for gestational age*. Such fetuses have an increased risk of congenital malformations, Apart from genetic factors like chromosomal abnormalities, growth retardation can also be caused by infections, poor nutrition, cigarette smoking, alcohol and use of harmful drugs by the mother.

CAUSATION OF CONGENITAL ANOMALIES (TERATOGENESIS)

- One of the main objectives of the study of embryology, by medical students, is to understand the causation of congenital anomalies.
- If a growing embryo is exposed to certain agents (chemical or physical), abnormalities in development can result. Such agents are called *teratogens*. The study of congenital malformations constitutes the science of *teratology*.
- The development of the embryo is dependent primarily on genetic influences. However, environmental conditions can also exert an important effect. It, therefore, follows that congenital anomalies may occur either as a result of genetic or environmental defects, or by a combination of both.
- Embryos with major abnormalities are aborted early in pregnancy, and this may occur even before the mother is aware of the pregnancy. According to some estimates, the total number of abnormal embryos may be as high as 50% and their spontaneous abortion may be nature's way of reducing the birth of malformed babies.
- In spite of this fact, 2–3% of infants born alive, show one or more congenital malformations. Some anomalies are not obvious at birth but are discovered later. The total incidence of malformations may, therefore, be as high as 5% of live births.

These figures are cited to highlight the great importance of the need to understand the causation of congenital abnormalities. The list of known teratogens keeps increasing. It has also been observed that some particular organs are most sensitive to teratogens when they are passing through critical phases in their development. This period of greatest susceptibility to teratogens differs from organ to organ.

Mode of action of teratogens—some general principles may be stated as follows:

1. *Stage of action of embryonic development*: The susceptibility to a teratogen, and the degree of damage it causes, depends upon the stage of embryonic development at which the embryo or fetus is exposed to the teratogen. When a teratogen acts before differentiation of germ layers, the effects are drastic and often lead to death of the embryo.
2. *Period of organogenesis and critical period of development*: The organ systems of the fetus are established between 3 weeks and 8 weeks of pregnancy, and this is referred to as the embryonic period or *period of organogenesis*. Most anomalies are produced during this period. Unfortunately this is an early stage of pregnancy and the mother may not even be aware of her pregnancy. Therefore, she may not take the necessary precautions. She may keep on consuming harmful products like drugs, alcohol or cigarettes. During the

fetal period that follows, teratogenic influences become much less severe. The type of malformation produced depends on the exact timing of the teratogenic influence. Each organ seems to have a critical period during which it is most sensitive to teratogens.

3. *Genetic and metabolic influences*: The susceptibility to a teratogen is influenced by genetic factors. A fetus of one genotype can be much more susceptible to the same teratogen than a fetus of another genotype. Teratogenic agents act by influencing metabolic processes.
4. *Dose and duration of exposure*: The dose and duration of exposure to teratogen is also important. High concentration and long period of exposure to a teratogen is relatively more harmful.

About 80% of all congenital malformations are produced by a combination of genetic and environmental factors. Of the remaining 20%, about half are caused exclusively by genetic or chromosomal factors and the remaining half exclusively by environmental factors.

Hereditary Causes

- Anomalies may be caused by defects in a specific chromosome or in a specific gene. Chromosomal defects owe their effects to the absence of certain genes, or presence of extraneous ones on them. Hence, all hereditary defects are ultimately caused by failure of the cells to synthesize the right proteins (especially enzymes) at the right time.
- In producing an anomaly, the genetic defect may directly affect the organ, or may have an indirect effect. For example, a genetic defect that leads to agenesis of the testis may indirectly influence the developing external genitalia by interfering with the production of hormones necessary for their development. Similarly, an anomaly of a blood vessel may interfere with the blood supply of an organ and hence adversely affect its development.

Environmental Causes (Teratogens)

Infections

Syphilis, chickenpox, human immunodeficiency virus (HIV), measles and toxoplasmosis. There is a well-known correlation between a disease known as *German measles* and congenital anomalies. When the mother suffers from this disease in the early months of pregnancy, the offspring often has cataract (opaque lens of eye), anomalies of the heart, or deafness.

Malnutrition

Deficiencies of *vitamins, minerals* (like calcium or phosphorus), certain trace elements, and of some amino acids have been shown to cause anomalies. It is believed that *iodine deficiency* causes *endemic cretinism*. However, the extent to which nutritional deficiencies are responsible for anomalies in humans is controversial.

Antigenic Reactions

Hemolytic disease of the newborn.

Drugs and Chemicals

Thalidomide, aminopterin (a folic acid antagonist); *diphenylhydantoin* and *trimethadione* (used for epilepsy*); phenothiazine, lithium, meprobamate, chlordiazepoxide* and *diazepam* (which are used as tranquilizers). *Alcohol* in fetal blood produces the *fetal alcohol syndrome*.

Hormones

Administration of synthetic *estrogens* or *progestins* can cause malformations of external genitalia. Fetuses exposed to diethylstilbestrol (a synthetic estrogen) in intrauterine life, show increased incidence of carcinoma of the vagina and cervix in later life. Maternal diabetes can also cause congenital malformations.

Physical Factors

- Abnormal intrauterine environment due to an abnormal site of implantation, due to the presence of twins, because of an abnormal position of the fetus within the uterus, because of too much amniotic fluid (*hydramnios*) or because of too little fluid (*oligoamnios*).
- Insufficient or excessive availability of oxygen. Too much oxygen leads to a condition called *retrolental fibroplasia*.
- *Hyperthermia* or increased body temperature is teratogenic. Increase in temperature may be due to fever secondary to infection, or due to bathing with hot water for long duration. Hyperthermia leads to mental retardation, cleft lip and cleft palate, limb deficiency, spina bifida and anencephaly.

PRENATAL DIAGNOSIS OF FETAL DISEASES AND MALFORMATIONS

A physician can now detect congenital malformation or disease of the fetus even during early pregnancy. Early detection of severe abnormality helps the patient to decide the desirability of early termination of pregnancy. Many procedures are now available by which we can assess the fetal status within the womb of mother. These procedures are described briefly below.

Ultrasonography

Ultrasonography is widely used to assess the condition of the developing fetus in utero because the investigation is cheap, readily available, and has no harmful effects. It can be done either by transvaginal or transabdominal method. By this procedure, one can determine:

- Fetal age and age-related growth of the fetus
- Presence or absence of congenital anomalies
- Position of fetus and placenta
- Whether there are multiple gestations
- Amount of amniotic fluid.

Alpha-fetoprotein Assay

Alpha-fetoprotein is normally produced in the liver and in the gut of a fetus in the second trimester of pregnancy. It reaches amniotic fluid and maternal serum through the placenta. Its concentration increases when the fetus is suffering from congenital malformations like omphalocele, bladder exstrophy and intestinal atresia. However, its concentration decreases in a few chromosomal anomalies like Down syndrome and trisomy 18.

Amniocentesis

In this procedure, a needle is passed through the abdominal wall of the mother and made to enter the amniotic cavity of the fetus. About 20 mL of amniotic fluid is withdrawn. This procedure is usually performed between the 15 weeks and 18 weeks of gestation. Amniotic fluid obtained is used for chemical analysis (alpha-fetoprotein and acetylcholinesterase). Amniotic fluid also contains epithelial cells from the skin of the fetus. These can be used for karyotyping. These epithelial cells can also be used for determining the sex of the fetus by the simple procedure of detecting sex chromatin. Such tests are however illegal in India as they often lead to female feticide.

Chorionic Villus Sampling

In this procedure, a sample of chorionic villus tissue is collected by entering the uterus either by an abdominal route or by a cervical route. The procedure is performed between 10 weeks to 12 weeks of gestation. The biopsies are used for detecting chromosomal abnormalities, inborn errors of metabolism or X-linked disorders.

Fetoscopy

The fetal body may be directly observed for congenital anomalies by using a fiberoptic lighting instrument. This procedure is usually performed during 17–20 weeks of gestation.

Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) of a fetus can be performed to get further information about conditions that have been detected in ultrasonographic images especially for fetal brain and spine for making a decision about the therapies. MRI is safe and provides high soft tissue contrast and resolution.

Percutaneous Umbilical Cord Blood Sampling

In this procedure, fetal blood is drawn from the umbilical vein for diagnosis of many genetic conditions. The same procedure is also used for blood transfusion into the fetus or for injection of drugs.

FETAL THERAPIES

Fetal therapies are therapeutic for treating a fetal condition (medical line of management) or correcting an anomaly (surgical line of management). In selected cases, treatment of a third trimester fetus is possible with limited success. Such treatment has been used for *fetal anemias, hemolytic disease, fetal cardiac arrhythmia, thyroid dysfunction* and *surgical corrections.*

Fetal Transfusion

Fetal transfusion is given directly into the umbilical vein by the percutaneous umbilical cord blood sampling (PUBS) procedure.

Fetal Surgery

Surgical correction of some birth defects like congenital diaphragmatic hernias, urinary tract obstruction, etc. in the fetus has become possible. In this procedure, the uterus is opened by cesarean section and the fetus is operated upon directly. After repair of the defect, the fetus is placed back into the uterus. Most of the surgeries in utero are performed after 28th weeks of pregnancy.

REVIEW QUESTIONS

1. What are the stages of gestational period?
2. Write a note on prenatal diagnosis of fetal anomalies.
3. Name the environmental teratogens.

Chapter 22

Embryology Ready Reckoner

DEVELOPMENTAL ANATOMY AT A GLANCE

Adrenal Gland

- Superficial cortex—coelomic epithelium (mesoderm) that lies in the angle between the developing gonad and the attachment of the mesentery.
- Deeper medulla—neural crest cells (ectoderm).

Arch of Aorta

- Ventral aortic sac
- Left horn of ventral aortic sac
- Left fourth arch artery.

Arteries of Gut

- Foregut—celiac artery
- Midgut—superior mesenteric artery
- Hindgut—inferior mesenteric artery.

Azygos and Hemiazygos Veins

- *Azygos vein* is formed from:
  - The vein of the right azygos line; and
  - The most cranial part of the right posterior cardinal vein through which it opens into the superior vena cava (formed from the right common cardinal).
- *Hemiazygos* and the *accessory hemiazygos veins*:
  - Vertical parts of these veins represented by left azygos line.
  - Horizontal parts are formed by postaortic anastomoses between the azygos lines of the two sides.

Cecum and Appendix

- Cecal bud arising from postarterial segment of midgut loop.
- Proximal part of bud grows rapidly to form cecum. Distal part remains narrow and forms appendix.

Chorionic Villi

- Primary villi—outer syncytiotrophoblast + inner cytotrophoblast.
- Secondary villi—syncytiotrophoblast +cytotrophoblast + primary mesoderm.
- Tertiary villi—Syncytiotrophoblast + cytotrophoblast + primary mesoderm + fetal blood vessel.
- Intervillous space filled with maternal blood.

Celiac Artery

- Fusion of ventral splanchnic arteries of foregut.

Coronary Sinus

- Medial part from left horn of the sinus venosus.
- Lateral part from proximal part of left common cardinal vein.

Duodenum

- First part and the upper half of second part from foregut.
- Rest of the duodenum from midgut.
- Proximal part of the duodenum is supplied by branches of celiac artery, and the distal part by branches of superior mesenteric artery.

Diaphragm

- Septum transversum forms central tendon.
- Dorsal mesentery of esophagus.
- Body wall mesoderm forms peripheral rim.
- Pleuroperitoneal membrane closes the communication between pleural and peritoneal cavities.

Ear

- External ear: Ectoderm of first branchial cleft.
- Middle ear: Endoderm of first pharyngeal pouch.
- Internal ear: Ectodermal thickening the otic placode.

Ear Ossicles

- Malleus, incus—1st pharyngeal arch mesoderm.
- Stapes—2nd pharyngeal arch mesoderm.

Extrahepatic Biliary Apparatus

- Gallbladder and cystic duct from pars cystica of hepatic bud.
- Common bile duct from narrow part of hepatic bud between pars cystica and duodenal part of foregut.
- Common hepatic duct from undivided part of pars hepatica distal to the origin of pars cystica.
- Right and left hepatic ducts from right and left branches of pars hepatica.

Eye—Neuroectodermal Derivatives

- Retina
- Muscles—sphincter pupillae, dilator pupillae, ciliaris.
- Optic nerve.

Face

- *Contribution of five processes and their differentiation*:
  - Unpaired—frontonasal
  - Paired—maxillary and mandibular processes.
- *Components of each process*:
  - Core of mesenchyme
  - Covering surface ectoderm.
- Derivatives from each process are:
  - Frontonasal process
    - Forehead
    - Nasal septum
    - Philtrum of upper lip
    - Premaxilla bearing four incisor teeth.
  - Maxillary process
    - Whole upper lip except philtrum
    - Most of palate except the part formed by premaxilla.
  - Mandibular process
    - Whole lower lip.

Facial Anomalies

- Unilateral cleft lip (upper lip)—failure of fusion of globular process with maxillary process.
- Bilateral cleft lip—failure of fusion of maxillary process and globular swellings bilaterally.
- Mandibular and lower lip cleft—nonfusion of mandibular process.
- Unilateral cleft lip with cleft palate—nonfusion of palatine process with primitive palate and nasal septum.
- Facial cleft—nonfusion of maxillary process with lateral nasal process.

Fertilization—Results

- Completion of second meiotic division in ovum
- Restoration of diploid chromosomes
- Genetic sex of embryo decided
- Axis of embryo defined
- Initiation of cleavage.

First Pharyngeal Arch Derivatives

- Skeletal—malleus, incus, maxilla, mandible, zygomatic bone, sphenomandibular ligament.
- Muscular—muscles of mastication, tensor tympani, tensor veli palatini, mylohyoid, anterior belly of digastric.
- Nerve—mandibular nerve.
- Artery—part of maxillary artery.

Fetal Circulation (Fig. 22.1)

Factors necessary for fetal circulation in addition to placenta:

- Left umbilical vein
- Ductus venosus
- Valve of inferior vena cava (IVC)
- Foramen ovale
- Ductus arteriosus
- Umbilical arteries—both.

Fourth Pharyngeal Arch Derivatives

- Skeletal—lamina of thyroid cartilage.
- Muscle—cricothyroid muscle.
- Nerve—superior laryngeal nerve.
- Artery—right subclavian artery, and left side part of arch of aorta.

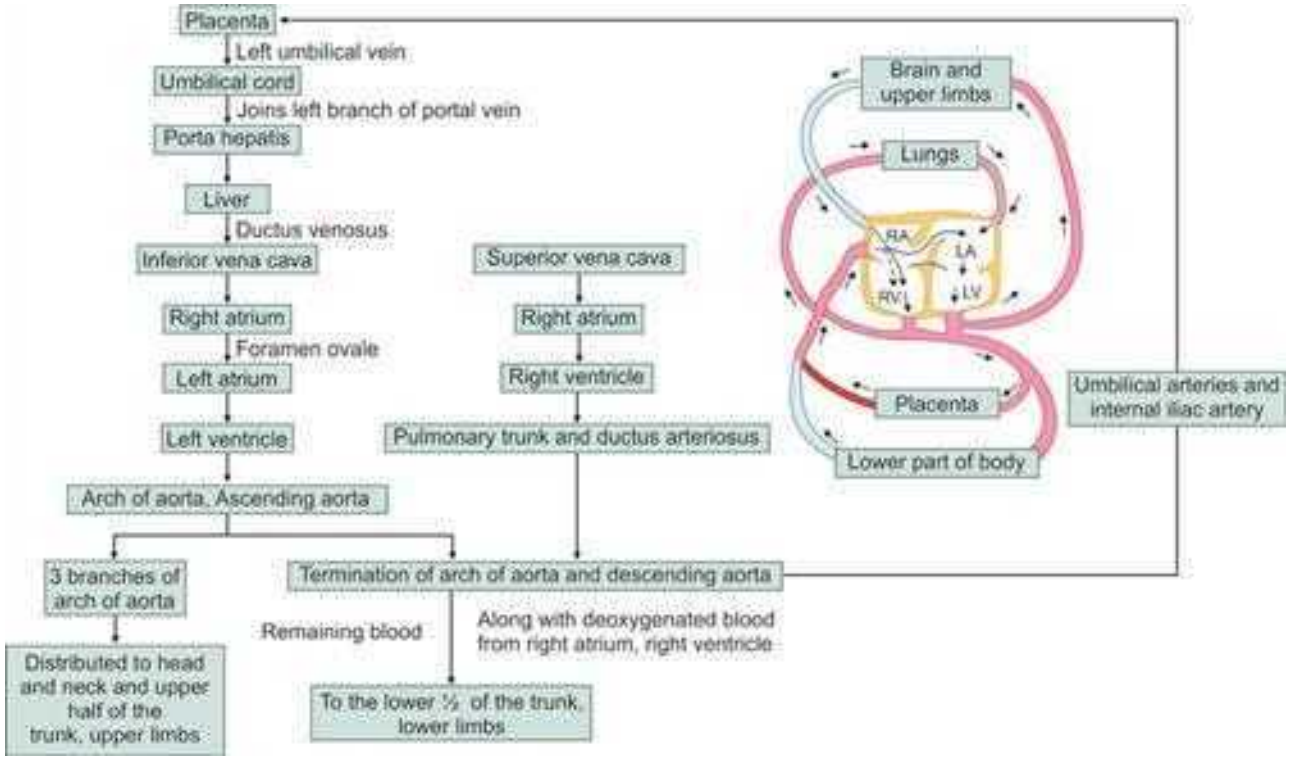


Fig. 22.1: Fetal circulation

Glands Derived from Various Germ Layers

- Ectoderm—e.g. sweat gland, mammary gland, adenohypophysis, parotid gland, lacrimal gland, and sebaceous gland. Neurohypophysis and pineal from modified surface ectoderm (neuroectoderm).
- Endoderm—e.g. pancreas, liver, submandibular, and sublingual salivary glands.
- Mesoderm—e.g. adrenal cortex.

Gut Derivatives Supplied by Arteries from Two Different Sources

- Duodenum—branch of celiac and superior mesenteric arteries.
- Transverse colon—superior and mesenteric artery branches.
- Anal canal—branches from inferior mesenteric and internal iliac arteries.
- Pancreas—branches from celiac and superior mesenteric arteries.

Implantation—Abnormal Sites

- Lower uterine segment (placenta previa)
- Tubal
- Ovarian
- Abdominal implantation.

Heart: Divisions of Endothelial Heart Tube and Their Derivatives

- Truncus arteriosus—pulmonary trunk and ascending aorta.
- Bulbus cordis—outflow part of right and left ventricle.
- Primitive ventricle—inflow part of right and left ventricle.
- Primitive atrium—rough part of right and left atrium.
- Right horn of sinus venosus—smooth part of right atrium.
- Left horn of sinus venosus—coronary sinus.

Inferior Vena Cava: From below Upward

- Right posterior cardinal vein (between its junction with the supracardinal, and the anastomosis between the two posterior cardinals)
- Right supracardinal vein (between its junction with the posterior cardinal, and the supracardinal-subcardinal anastomosis)
- Right suprasubcardinal anastomosis
- Right subcardinal vein

- Subcardinal-hepatocardiac anastomosis (vessel communicating right subcardinal with common hepatic vein)
- Right hepatocardiac channel (common hepatic vein).

Interatrial Septum

- Septum primum
- Septum intermedium
- Septum secundum.

Interventricular Septum

- Ventricular septum—muscular part from the floor of the bulboventricular cavity.
- Proximal bulbar septum—by fusion of right and left bulbar ridges from conus arteriosus.
- Septum intermedium—membranous part—from atrioventricular cushions.

Kidney

- Collecting part—collecting tubules, minor and major calyces and the pelvis are derived from the dilated upper end of the ureteric diverticulum.
- Secreting part—Bowman's capsule, proximal convoluted tubule, loop of Henle, distal convoluted tubule of nephrons derived from the metanephric blastema.

Left Atrium

- Left half of the primitive atrium
- Dilated terminal parts of the pulmonary veins
- Left half of atrioventricular canal.

Liver

- It develops as endodermal diverticulum from the ventral wall of junction of the foregut and midgut that grows ventrally and cranially into the septum transversum.
- It gives off two solid buds which give rise to the right and left lobes of the liver.
- Connective tissue part from the mesoderm of septum transversum.
- Parenchyma is derived from the endoderm.
- Sinusoids from absorption and breakdown of vitelline and umbilical veins.

Lower Limb Arteries

- Axis artery is derived from dorsal root of umbilical artery.
- Remnants of axis artery of lower limb are inferior gluteal arteries, arteria comitans nervi ischiadici, anastomosis of profunda femoris.
- Femoral artery develops from capillary plexus in the ventral aspect of thigh communicates with external iliac and axis arteries.
- Popliteal artery sprouts from axis artery at the distal border of popliteus.

Metanephric Blastema

- Bowman's capsule
- Proximal convoluted tubules
- Loops of Henle
- Distal convoluted tubules.

Midgut Rotation

- Total rotation about 270°
- 90° rotation occurs in the physiological umbilical hernia.
- 180° rotation occurs within the abdominal cavity.

Pancreas

- Dorsal and ventral pancreatic buds at the junction of foregut and midgut.
- Upper part of head, neck, body, and tail of pancreas from dorsal pancreatic bud.
- Lower part of head and uncinate process from ventral pancreatic bud.
- During gut rotation ventral bud fuses with dorsal bud.

Parathyroid Glands

- Upper pair from the 4th pharyngeal pouch.
- Lower pair from the 3rd pharyngeal pouch.

Pharyngeal Arch Artery Derivatives

- 1st—part of maxillary artery.
- 2nd—part of stapedial artery.
- 3rd—common carotid, part of internal carotid on both sides.
- 4th—right side part of subclavian artery, left side part of arch of aorta.
- 5th—disappear.
- 6th—right side of pulmonary trunk, left side left pulmonary trunk and ductus arteriosus.

Pharyngeal Pouches—Derivatives

- Endocrine glands:
  - Thyroid
  - Parathyroids.
- Lymphoid organs:
  - Palatine tonsil
  - Thymus.

- Others:
  - Auditory tube
  - Tympanic cavity
  - Mastoid antrum, air cells
  - Tympanic membrane.

Pharyngeal Pouches Derivatives

- 1st pouch—tubotympanic recess, mucous lining of middle ear cavity, Eustachian tube, inner layer of tympanic membrane.
- 2nd pouch—tonsil, remnant of 2nd pouch is intratonsillar cleft.
- 3rd pouch—inferior parathyroid, thymus.
- 4th pouch—superior parathyroid, ultimobranchial body.

Pituitary Gland

- Adenohypophysis—ectodermal diverticulum from the roof of the stomodeum—Rathke's pouch.
- Neurohypophysis—neuroectodermal downgrowth from the floor of the diencephalon.

Portal Vein

- Dorsal anastomotic channel.
- Part of right vitelline vein between cephalic ventral and dorsal anastomosis.
- Right branch from intrahepatic part of right vitelline vein.
- Left branch from cephalic ventral anastomosis and intrahepatic part of vitelline vein.

Prostate

- Number of epithelial outgrowths from the proximal part of the urethra, which invade the surrounding dense mesenchyme forms the glandular component.
- The mesoderm differentiates into the muscular and the connective tissue part of the gland.
- Inner glandular zone is mesodermal.
- Outer glandular zone is endodermal.

Rectum and Anal Canal

- The endodermal cloaca is shut off from the ectodermal cloaca by means of the cloacal membrane.
- As a result of the development of the urorectal septum, the endodermal cloaca is divided into an anterior part which develops into the vesicourethral part and the urogenital sinus, and a dorsal segment called the primitive rectum. The rectum develops from the primitive rectum.
- Anal canal is both ectodermal and endodermal in development. The part of anal canal above the white line of Hilton (upper 2/3rds) is derived from endoderm of primitive rectum. The part caudal to white line (lower 1/3rd) is derived from ectoderm of proctodeum.

Right Atrium

- Right half of the primitive atrium.
- Sinus venosus.
- Right half of the atrioventricular canal.

Second Pharyngeal Arch Derivatives

- Skeletal—stapes, lesser cornu and upper part of body of hyoid bone, stylohyoid ligament.
- Muscular—muscles of facial expression including scalp and auricular muscles.
- Nerve—facial nerve.
- Artery—part of stapedial artery.

Second Week of Gestation—Week of 2's

- Inner cell mass differentiates into two germ layers
  1. Hypoblast
  2. Epiblast.
- Appearance of two cavities
  1. Amniotic
  2. Yolk sac—primary and secondary.
- Two layers of trophoblast
  1. Cytotrophoblast
  2. Syncytiotrophoblast.
- Formation extraembryonic components
  1. Extraembryonic mesoderm (EEM)
  2. Extraembryonic coelom.
- Division of EEM into two layers
  1. Somatopleuric
  2. Splanchnopleuric.
- Two cavity membranes
  1. Amniogenic membrane
  2. Heuser's membrane.
- Two fetal membranes
  1. Amnion
  2. Chorion.
- Two ends of embryo—axis differentiation by prochordal plate
  1. Cephalic
  2. Caudal axis.

Septum Transversum

- It is the unsplit part of intraembryonic mesoderm at the cranial end of pear-shaped embryonic disc.

- Before the formation of head fold, it is cranial to developing pericardial cavity and heart.
- With the formation of head fold, it lies caudal to pericardial cavity and heart. It is between pericardial cavity and yolk sac cavity.
- It contributes for the formation of ventral mesogastrium (lesser omentum, falciform ligament, diaphragm and connective tissue capsule of liver).

Seventh Cervical Intersegmental Artery—Contributions

- Main stem—subclavian artery.
- Dorsal division—stem of the vertebral artery.
- Lateral division—axillary and brachial arteries.
- Ventral division—stem of the internal thoracic (mammary) artery.

Sixth Pharyngeal Arch Derivatives

- Skeletal—laryngeal cartilages.
- Muscles—intrinsic muscles of larynx.
- Nerve—recurrent laryngeal nerve.
- Arteries—right side right pulmonary trunk, left side proximal part develops into left pulmonary trunk, distal part into ductus arteriosus.

Smooth Muscles Derived from Ectoderm

- Sphincter pupillae
- Dilator pupillae
- Myoepithelial cells of sweat gland.

Spermiogenesis

- Transformation of spermatids to spermatozoa
- Golgi apparatus forms acrosomal cap
- Nucleus forms the head
- Controls form axial filaments of body and tail
- Mitochondria forms sheath
- Cytoplasm extruded out as residual bodies.

Spleen

- It develops from mesoderm in the dorsal mesogastrium as small spleniculi. These later fuse to form single mass of spleen. Presence of splenic notches along the upper border of adult spleen indicates persistence of fetal lobulation.
- The splenic mass projects into the left layer of dorsal mesogastrium.
- Capsule, septa, connective tissue from mesoderm.

Superior Vena Cava

- Right duct of Cuvier
- Terminal portion of right anterior cardinal vein caudal to transverse anastomosis in the cervical region.

Subclavian Artery

Right:
- Proximal part—from the right fourth arch artery.
- Remaining part—from the seventh cervical intersegmental artery.

Left:
Entirely from the seventh cervical intersegmental artery.

Testis

- Seminiferous tubule, rete testis, interstitial cells, fibrous septa and coverings develop from medulla of genital ridge.
- Efferent ductules from proximal 12–15 mesonephric tubules.
- Canal of epididymis and vas deferens from mesonephric duct.
- Appendix of testis is a paramesonephric duct remnant.
- Appendix of epididymis is a mesonephric duct remnant.

Third Pharyngeal Arch Derivatives

- Skeletal—lower half of body of hyoid bone and its greater cornu.
- Muscle—stylopharyngeus muscle.
- Nerve—glossopharyngeal nerve.
- Artery—part of common carotid artery and internal carotid artery.

Thoracic Duct

- Caudal part of right lymphatic duct.
- Cross anastomosis between right and left lymphatic ducts.
- Cephalic part of left lymphatic duct.

Thyroid Gland

- Isthmus and pyramidal lobe and lateral lobes are derived from the proliferation of endodermal cells at the lower end of the median thyroid diverticulum which arises from the floor of the pharynx between the tuberculum impar and hypobranchial eminence.
- Lateral thyroid from ultimobranchial body.

Tongue

- Anterior two-thirds forms lingual swellings, tuberculum impar (1st arch)—supplied by mandibular branch of trigeminal nerve (general sensation), chorda tympani branch of facial (taste sensation).
- Posterior one-third forms cranial part of hypobranchial eminence (3rd, 4th arches)—glossopharyngeal (both general and special), branch of vagus (general sensation).
- Muscles develop from occipital myotomes supplied by hypoglossal nerve.

Tympanic Membrane

- Outer cuticular layer—ectodermal.
- Middle fibrous layer—mesodermal.
- Inner mucous layer—endodermal.

Upper Limb Arteries

- Axis artery of the upper limb—lateral branch of 7th intersegmental artery.
- Axis artery forms the axillary, brachial and anterior interosseous artery.
- Median artery develops from anterior interosseous artery.
- Radial and ulnar arteries develop from axis artery at the elbow region.
- Ulnar artery communicates with deep palmar plexus.

Ureteric Bud Derivatives

- Collecting tubules and ducts
- Minor and major calyces
- Pelvis of kidney
- Ureter.

Urethra in Males

- Prostatic urethra up to the openings of the ejaculatory ducts caudal part of the vesicourethral canal (endoderm). Posterior wall of this part by absorbed mesonephric ducts (mesoderm).
- Rest of the prostatic urethra, membranous urethra from the pelvic part of the definitive urogenital sinus.
- Penile urethra from phallic part of the definitive urogenital sinus.
- Urethra in glans from ectoderm.

Urethra in Females

- It is homologous with that part of the male prostatic urethra which is proximal to the opening of the prostatic utricle.
- It is entirely formed from the vesicourethral portion of the endodermal cloaca, and the caudal ends of the mesonephric ducts.
- A small contribution from pelvic part of the urogenital sinus.

Urinary Bladder

- Cranial dilated part of vesicourethral canal (endoderm) and proximal portion of allantois. The allantois gets obliterated.
- Trigone of the bladder from the incorporated (absorbed) caudal ends of the mesonephric ducts.
- The epithelium of the urinary bladder is endodermal.
- The muscular and serous walls of the organ are derived from splanchnopleuric mesoderm.

Uterine Anomalies

- Didelphys—complete failure of fusion of paramesonephric ducts results in double uterus, double cervix, double vagina.
- Bicornis bicollis—double uterus, double cervix, single vagina.
- Septate uterus—septum in the uterine cavity.
- Subseptate—incomplete septum in the uterus.
- Arcuate uterus—fundus is concave.
- Unicornuate uterus—unilateral suppression of paramesonephric duct.

Vertebral Artery

- First part—from its origin to the point of entry into the foramen transversarium of the sixth cervical vertebra—dorsal division of the seventh cervical intersegmental artery.
- The vertical part (second part), lying in the foramina transversaria, postcostal anastomoses between the first to sixth cervical intersegmental arteries.
- The horizontal (third) part, running transversely on the arch of the atlas—spinal branch of the first cervical intersegmental artery.

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