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FACULTY OF SCIENCE AND TECHNOLOGY

DEPARTMENT OF BIOMEDICAL LABORATORY SCIENCES

CLINICAL CHEMISTRY III (CCH 3512)

MODULE HANDOUT 2018/2019

Year III, Semester I

Lecturer: Mr NZEYIMANA GODEFROID

COURSE CONTENTS OUTLINE

OVERVIEW OF AND THE ENDOCRINE SYSTEM

- hypothalamus
- pituitary gland
- Peripheral glands:
- Thyroid hormone
- parathyroid hormones
- Adrenal gland hormones
- Ovary hormones
- Testacles hormones
- Growth hormone
- Prolactin
- Vasopressin
- Reproductive endocrinology; aspects of pregnancy& Infertility and testing
- Physiological processes and functions of various hormones

- Liver function, Renal, Gastric, pancreatic and intestinal function tests mediated by hormones,
- Tumor markers
- Vitamins
- Definition
- Sources of vitamins
- Fat and water soluble vitamins
- Vitamins A, B, C, D,E ,K, Folic Acid

- Functions , clinical significance of vitamins & disorders due to lack of vitamins
- Laboratory analysis of various vitamins
- Apply quality assurance and quality control of biochemical tests

CHAPTER I

OVERVIEW OF THE ENDOCRINE SYSTEM

1.Introduction on human body glands

The human body presents two types of glands which are: Exocrine glands and endocrine glands.

- Exocrine glands are glands that produce and secrete substances Into an epithelial surface by way of a duct.
- Examples of exocrine glands include sweat, salivary, mammary, sebaceous, and mucous.
- Exocrine glands are one of two types of glands in the human body, the other being endocrine glands, which secrete their products directly into the bloodstream.
- The liver and pancreas are both exocrine and endocrine glands; they are exocrine glands because they secrete products—bile and pancreatic juice—into the gastrointestinal tract through a series of ducts, and endocrine because they secrete other substances directly into the bloodstream.
- Endocrine and nervous systems are the two major regulatory systems of the body.
- The endocrine system consists of glands present in different parts of the body.
- They are called endocrine glands, because they release their secretions directly into the blood stream without the help of ducts (ductless glands).
- Endocrine glands are present scattered throughout the body. They are connected to each other and other organs of the body through vascular system.
- The chemicals secreted by the endocrine glands are called **hormones**

HORMONE

Definition

- A hormone is a chemical substance secreted by the endocrine glands directly into the extracellular fluid mainly blood .It regulates the function of a distant organ (target organ).They alter the activity of the cell quantitatively ,which influence :metabolism, growth , reproduction and adaptation to the environment..

TYPES OF HORMONE

- **Classical hormones:** the hormones are liberated from the endocrine gland and carried by the blood or lymph. They reach the target cell and produce a desired effect. Example includes thyroid hormones and hormones of pancreas.
- **Neurohormones:** these are secreted from the exon terminals and released into the circulation .Examples are hypothalamic releasing hormones.
- **Paracrine hormones:** these are the hormones which have effect on the adjacent cells. For example Alfa and *B*eta cells of islets of Langerhans exert paracrine effect on each other.
- **Autocrine hormones:** the cells regulate its own function.

CHEMICAL CLASSES OF HORMONES

Chemically hormones are of three types

- Proteins or peptides
- Lipid derived hormones (Steroid hormones)
- Amines

Peptide Hormones

- Peptide hormones consist of chains of amino acids linked by peptide bonds.
- They are water soluble hormones and are carried in unbound form in the plasma.
- All peptide hormones are hydrophilic and are therefore unable to cross the plasma membrane alone.
- They are synthesized by rough endoplasmic reticulum.

Proteins or peptide hormones are:

- Anterior pituitary hormones
- Posterior pituitary hormones
- Insulin, glucagon, and parathormone .
- Some peptide hormones contain carbohydrate side chains and are termed glyco-proteins, such as the follicle-stimulating hormone.

Lipid-Derived Hormones

- Lipid and phospholipid-derived hormones are produced from lipids such as linoleic acid and arachnidonic acid.

- Steroid hormones, which form the majority of lipid hormones, are derived from cholesterol; for example, testosterone and cortisol, estrogen, progesterone, aldosterone.
- Eicosanoids are also lipid hormones that are derived from fatty acids in the plasma membrane. Example is prostaglandins. Unlike other hormones, eicosanoids are not stored in the cell, they are synthesized as required.
- Both steroids and eicosanoids are lipophilic and can cross the plasma membrane.
- They are formed from smooth endoplasmic reticulum.

Amines-derived hormones:

- There are derivatives of amino acids (tyrosine and tryptophan). Examples are catecholamine and thyroxin.
- They are synthesized in the cell cytoplasm**.**

CONTROL OF HORMONE SECRETION

- The following points highlight the three ways to control secretions of hormones.
- The three ways are:
- 1. Neural Control
- 2. Endocrine Control
- 3. Feedback Control.

NEURAL CONTROL

- Some endocrine secretions are solely controlled by nerve impulses.
- Secretion of adrenal medullary hormones ,secretion of neuro-hypophysial hormones and various releasing hormones of hypothalamus are under this category.
- For instance, in mammals, the act of suckling of baby stimulates tactile receptors in the nipple of mother and this impulse stimulates hypothalamic cells through sensory nerve and spinal cord. Latter, hypothalamic neuro-secretion stimulates neurohypophysis for secretion of oxytocin. Oxytocin helps in secretion of milk.

ENDOCRINE CONTROL

- Some endocrine secretions are controlled by other endocrine glands.
- For instance, different releasing hormones of hypothalamus control the secretion of anterior pituitary hormones.
- TSH-RH of hypothalamus controls the secretion TSH from anterior pituitary.
- Similarly, TSH and ACTH secretion of anterior pituitary stimulate thyroid gland and adrenal cortex respectively for secretion of thyroid hormone and adrenal cortical hormone.

FEEDBACK CONTROL

- The process of inhibiting or stimulating the first step by the final step in a hormonal reaction pathway, is called feedback regulation. The secretion of a hormone may be stimulated or inhibited by the feedback effect of some other hormone or metabolite

This feedback control can be divided into two ways:

- Negative feedback mechanism:
- Positive feedback mechanism:

Negative feedback mechanism

- In this type, rising concentration of a hormone inhibits the release of second hormone from other gland, called negative feedback control.
- Eg:High secretion of Cortisol of the adrenal cortex inhibits the secretion anterior pituitary corticotrophin.

Positive feedback mechanism

- In this mechanism, rising concentration of a hormone acts on another gland to release second hormone, which further stimulates the first hormone, called positive feedback mechanism.

- Example: At pre ovulatory phase, a gonadal hormone estrogen increases the release of pituitary LH, which in turn stimulates ovarian estrogen production. Thus estrogen and LH levels go on increasing continuously.

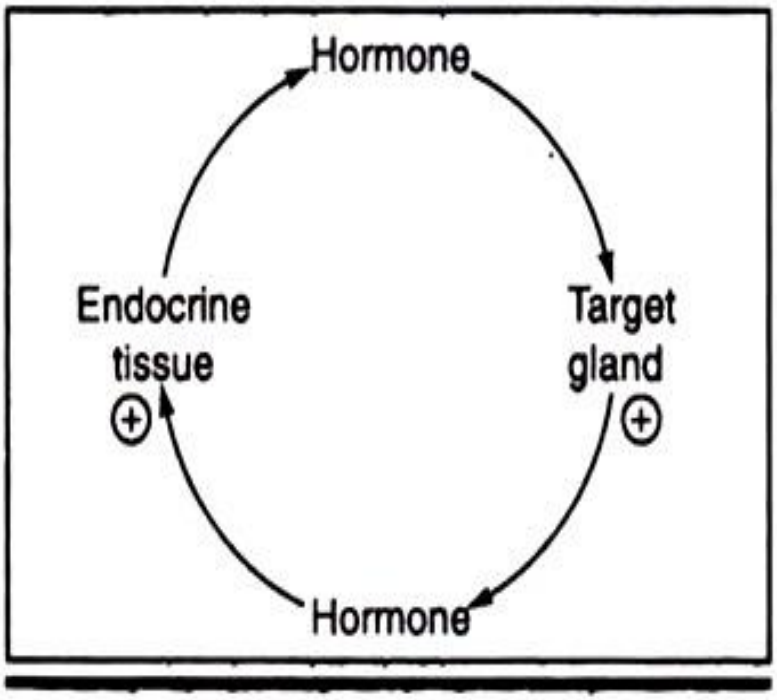


Fig. 3.3: Positive feedback mechanism

MECHANISM OF ACTION OF HORMONE

- Hormone regulates functioning of its target tissues by activating the receptors. Hormone binds to the receptor to form a hormone-receptor complex. This complex alters the structure and function of receptor then Altered receptor produces desired response by
- Change in membrane permeability of the cell: Hormones like epinephrine and norepinephrine secreted by the adrenal medulla act by this mechanism. They cause opening and

closing of ion channels, there by bringing about a change in the membrane permeability.

- Activation of an intracellular enzyme: Binding of hormone to its receptor activates an enzyme inside the cell membrane.
- For example, activation of adenylcyclase .This in turn catalyzes the formation of CAMP.It is called second messenger since it does not directly cause the effect.
- Activation of genes: Some hormones bind to the protein receptors inside the cell. The hormone-receptor complex binds to the proteins of DNA in the nucleus. This initiates transcription of genes to form mRNA.

HORMONE RECEPTORS

Overview

- Only cells with receptors for a specific hormone respond; those cells lacking the specific receptors are unaffected.
- Hormone receptors found on the target cell membrane are termed external receptors, and those within the cytoplasm and nucleus are termed internal receptors.
- Hormone-sensitive cells respond to high concentrations of certain hormones by reducing the number of receptors on the cell surface.
- For example, elevated insulin concentration causes a loss or inactivation of insulin receptors in liver cells.
- Cell membrane receptors

Proteins, peptide, and catecholamine hormones have specific membrane receptors. Three general categories of cell-surface

receptors include:, G-protein, ion-channel and enzyme-linked protein receptors.

- **Cytoplasmic receptor**

Receptor for steroid hormones is usually found almost entirely in the cell cytoplasm.

- **Nucleus of the cell**

Receptor for the thyroid hormones is present in the nucleus.

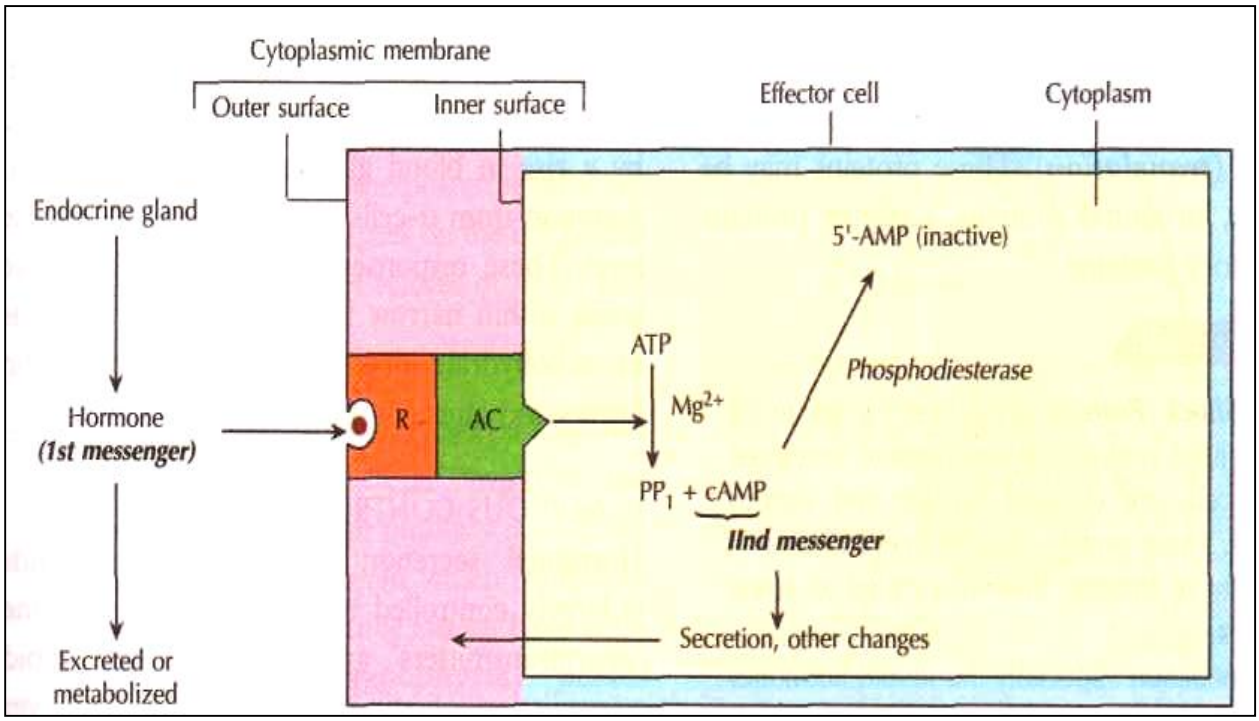
G-protein Or G protein–coupled receptors

- G protein–coupled receptors (GPCRs), also known as seven-transmembrane domain receptors, 7TM receptors, heptahelical receptors, serpentine receptor, and G protein–linked receptors (GPLR).
- It constitutes a large protein family of receptors, the G-protein-coupled receptor (GPCR) Family, that sense molecules outside the cell and activate inside signal transduction pathways and, ultimately, cellular responses.
- G protein–coupled receptors are found only in eukaryotes, including yeast and animals.
- The ligands that bind and activate these receptors include light-sensitive compounds like hormones and neurotransmitters, and vary in size from small molecules peptides to large proteins.
- G protein–coupled receptors are involved in many diseases, and are also the target of approximately 40% of all modern medicinal drugs.

There are two principal signal transduction pathways involving the G protein–coupled receptors:

1.The cAMP signal pathway

cAMP: cyclic Adenosine monophosphate



2.The phosphatidylinositol signal pathway

- In the phosphatidylinositol signal pathway,the extracellular signal molecule binds with the G protein receptor on the cell surface and activates phospholipase c,so that they will be formation of second messenger(Inositol triphosphate-IP3 and diacylglycerol) which begins when there is binding of an extracellular regulatory molecule to a membrane receptor.

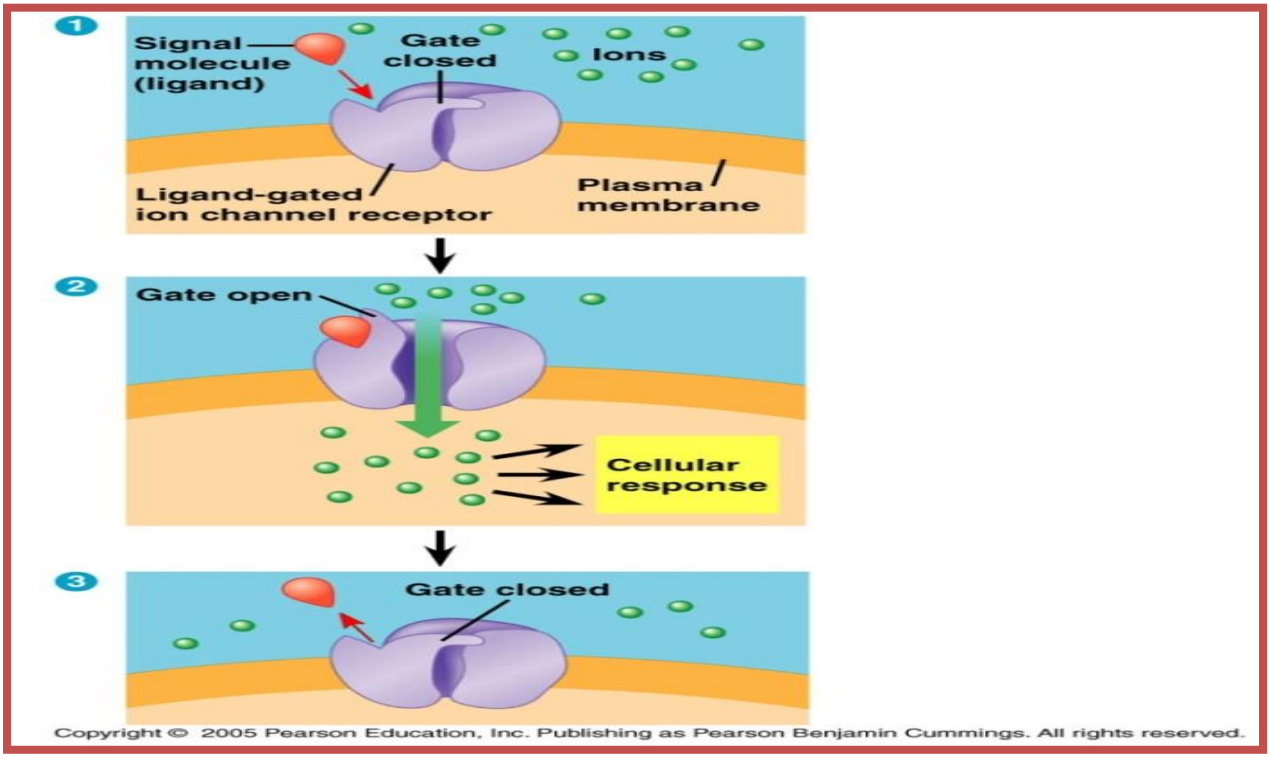
Enzyme-linked receptor

- An enzyme-linked receptor, also known as a catalytic receptor, is a Transmembrane, where the binding of an extracellular ligand causes enzymatic activity on the intracellular side.
- Hence a catalytic receptor is an integral membrane protein possessing both enzymatic catalytic and receptor functions.
- They have two important domains, an extra-cellular ligand binding domain and an intracellular domain, which has a Catalytic function.
- The signaling molecule binds to the receptor on the outside of the cell and causes a conformational change on the catalytic function located on the receptor.
- Examples of the enzymatic activity include**:**

➢ **Receptor tyrosine kinase**

➢ **Guanylate cyclase**

Ion channel linked receptors



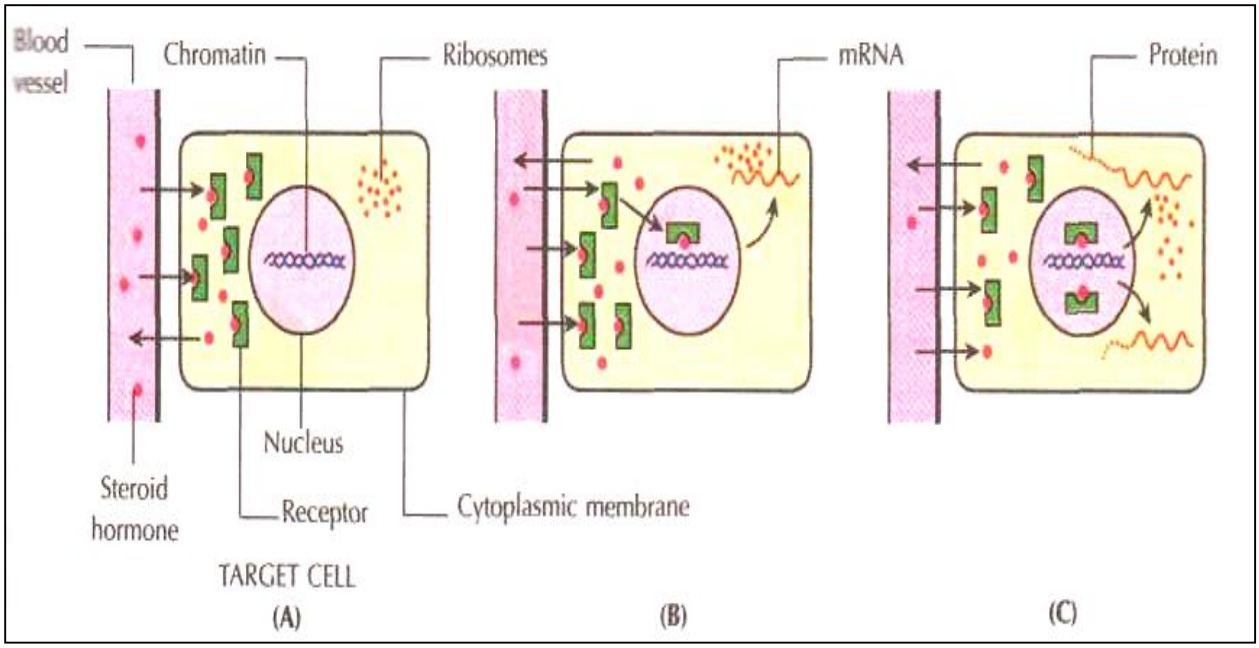
INTRACELLULAR RECEPTORS

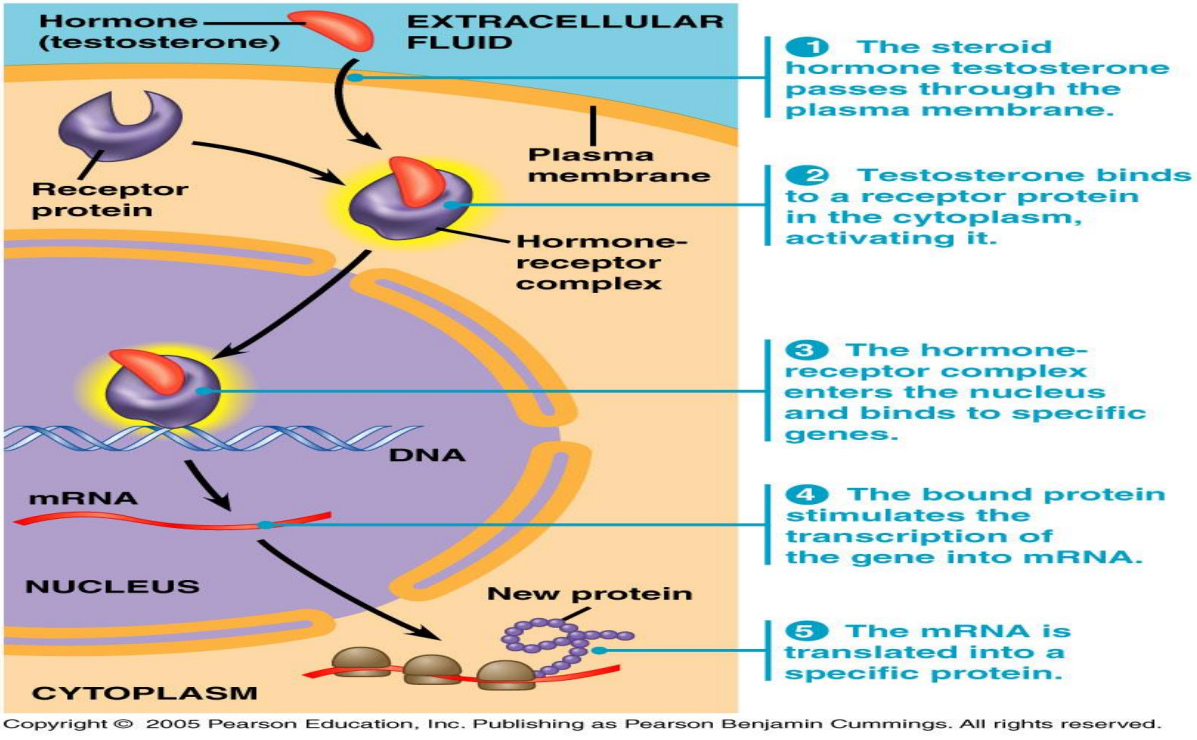
Transcription and Translation Effect

- Lipid soluble hormones, such as Steroid and thyroid hormones can easily enter into the target cells and exert their effect by combining to a specific cytoplasmic receptor protein in a target cell

i.e. cell that responds to the hormone

- Each receptor molecule binds two molecules of hormone, forming a complex that enters the nucleus and becomes attached to the chromatin, the genetic material.
- The complex reacts with DNA, stimulates the transcription {i.e. formation of mRNA) of a particular gene, and specific messenger RNA (mRNA) synthesis increases.
- The specific mRNA enters the cytoplasm, where it directs the ribosomes to synthesize specific proteins (translation).
- These proteins may be enzymes, structural proteins, receptor proteins .

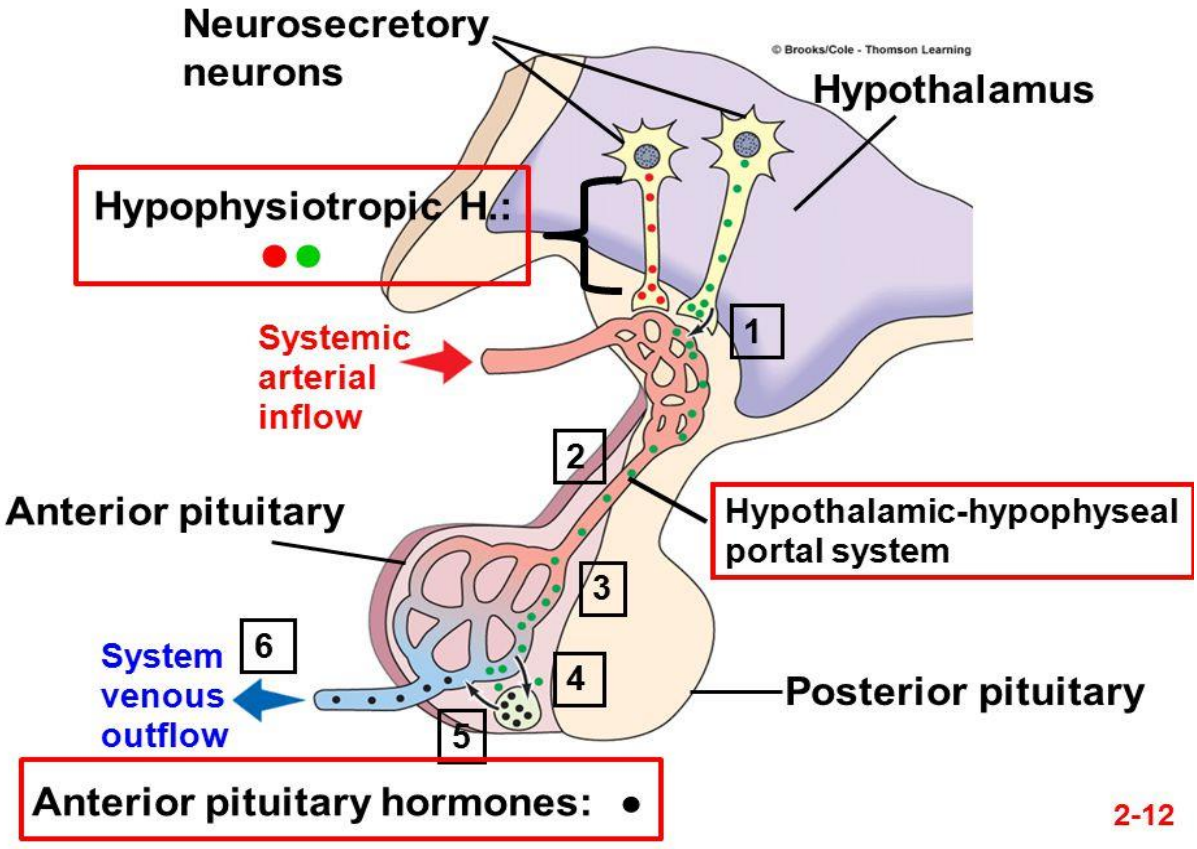




HORMONE TRANSPORT

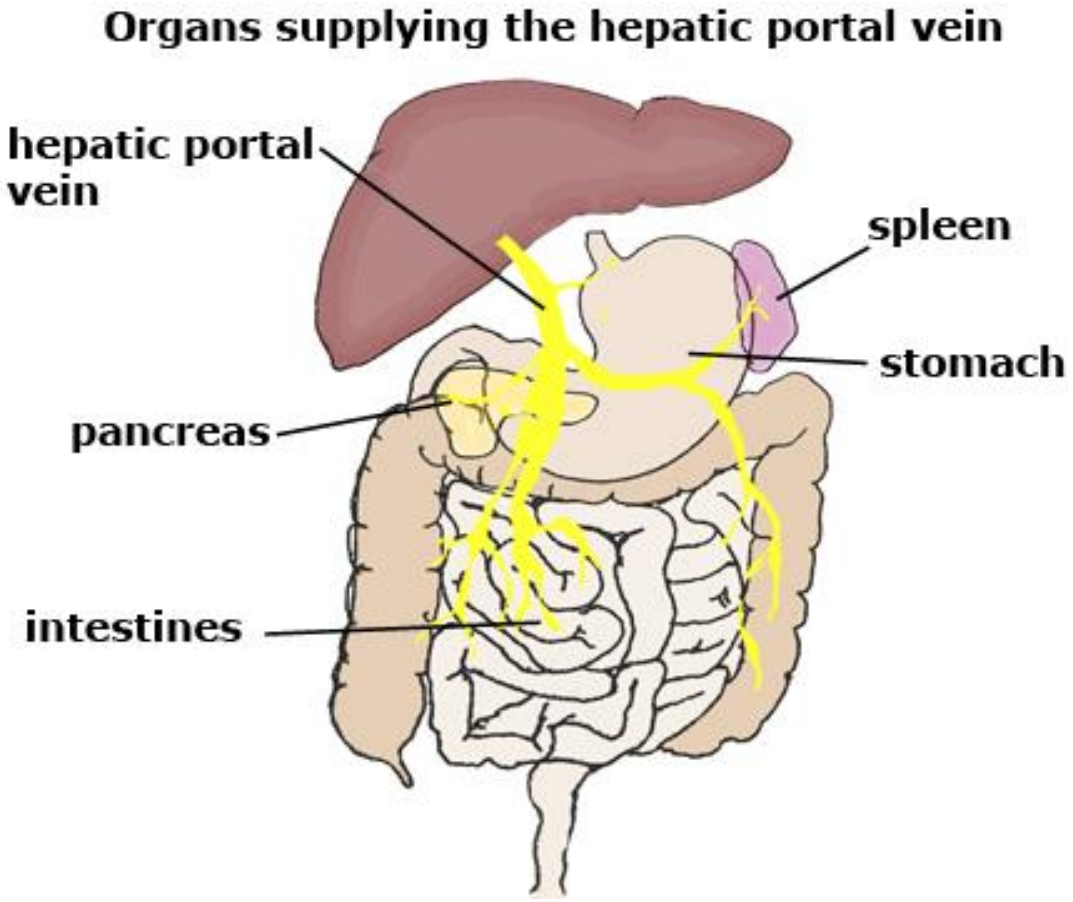
- Most hormones are secreted into the general circulation to exert their effects on appropriate distant target tissues.

- Hormones after secretion enter the bloodstream and circulate in the plasma .They are carried either in free form or bound to the plasma proteins .Usually the steroid hormones circulate in the free form .
- Two portal circulations in which hormones which are transported ,are present in the human body.
- 1. First system, the hypothalamic-hypophyseal portal circulation, collects blood from capillaries originating in the hypothalamus and, through a plexus of veins surrounding the pituitary stalk, directs the blood into the anterior pituitary gland, . This allows the neurohormones secreted by the neuroendocrine cells of the hypothalamus to be transported directly to the cells of the anterior pituitary.

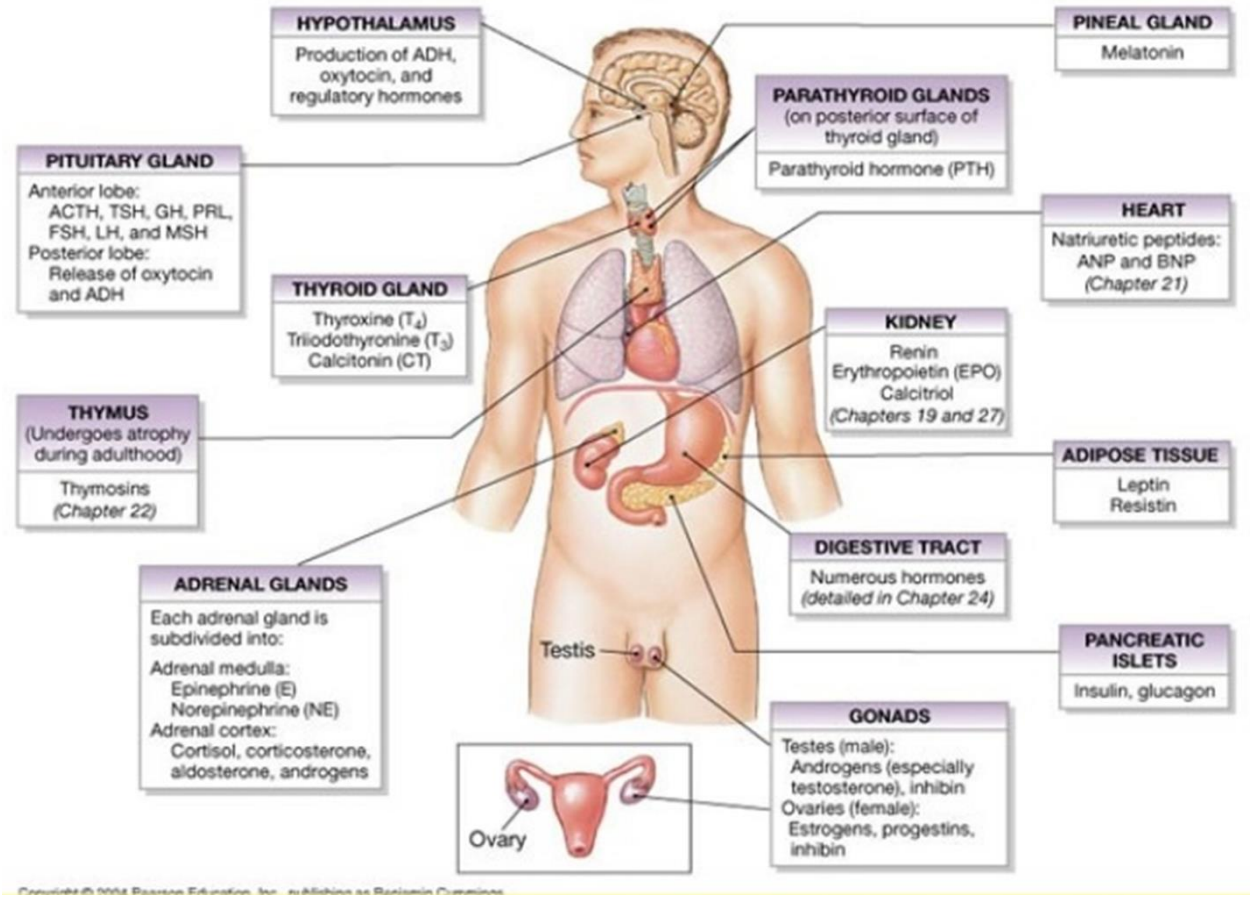


- **2.** In the second system, the hepatic portal circulation, capillaries originating in the gastrointestinal tract and the

spleen merge to form the portal vein, which enters the liver and divides to form portal capillaries. This allows hormones from the islets of Langerhans of the pancreas, such as insulin and glucagon, as well as certain nutrients absorbed from the intestine, to be transported into the liver before being distributed through the general circulation.

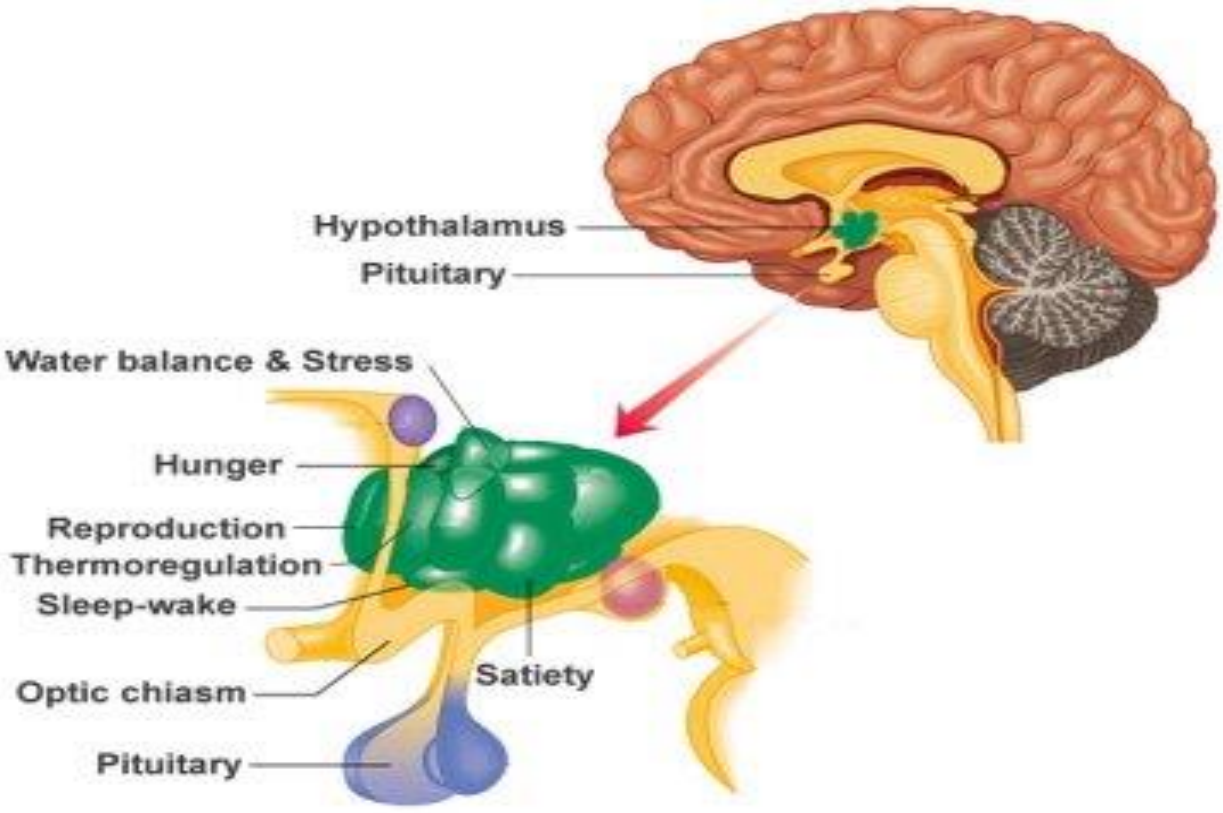


I.2.MAJOR ENDOCRINE GLANDS



HYPOTHALAMUS

- **STRUCTURE AND PHYSIOLOGY**



- The hypothalamus is a portion of the brain that contains a number of small nuclei with a variety of functions.

- One of the most important functions of the hypothalamus is to link the nervous system to the endocrine system via the pituitary gland (hypophysis).
- The hypothalamus is responsible for certain metabolic processes and other activities of the autonomic nervous system.
- It synthesizes and secretes certain neurohormones, called releasing hormones or hypothalamic hormones, and these in turn stimulate or inhibit the secretion of pituitary hormones.
- The hypothalamus controls body temperature, hunger, important aspects of parenting and attachment behaviors, thirst, fatigue, sleep.

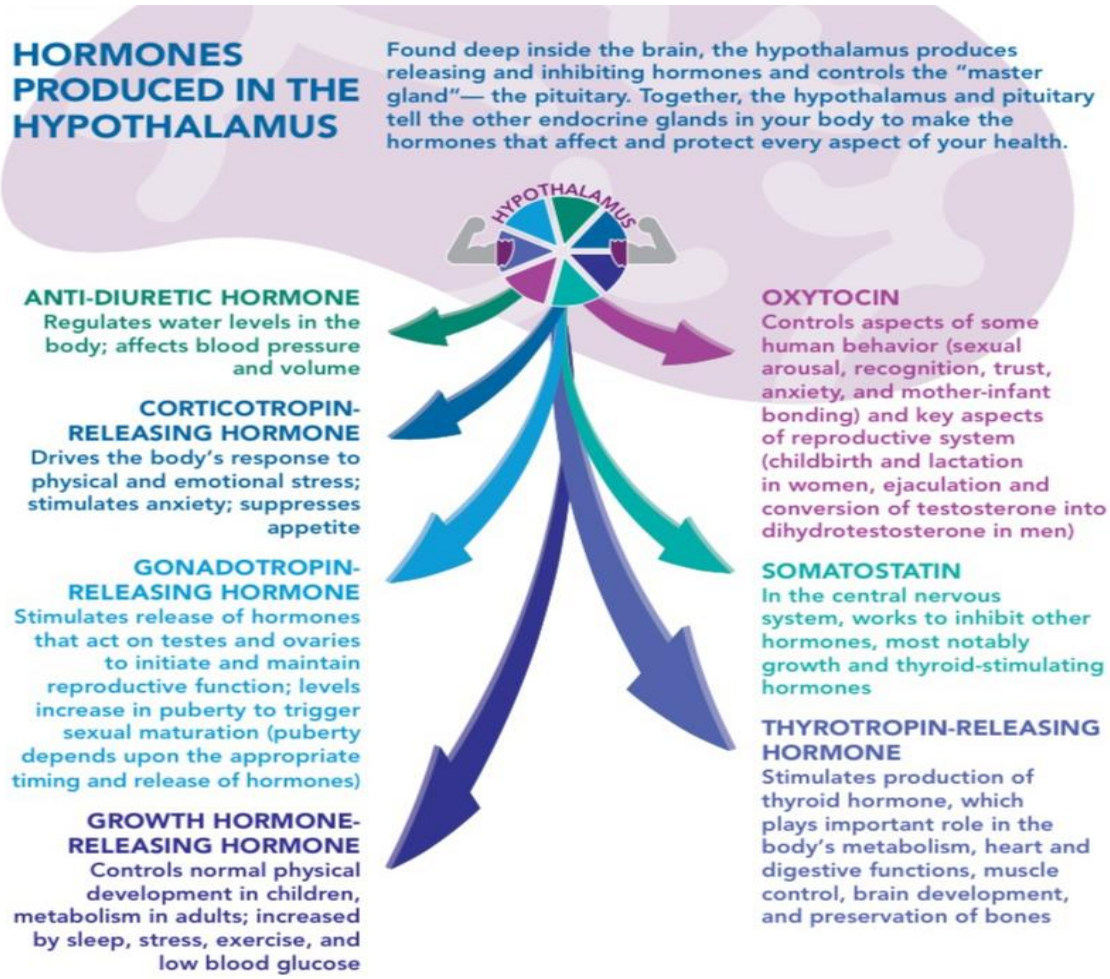
SECRETION

- The hypothalamus secretes hypothalamus-releasing and inhibiting factors which control the secretions of the pituitary gland
- Hormones released by hypothalamus are conducted through the hypothalamohypophysial portal system. These are extensive capillary networks connecting the hypothalamus and anterior pituitary.
- The hypothalamic hormones are secreted by the specialized cells in it.
- Hormones secreted from these nerve terminals are immediately absorbed into the hypothalamohypophysial portal system and are carried to the sinuses of the anterior pituitary gland.
- The anterior pituitary in turn secretes various hormones that act on other endocrine glands.These endocrine glands produce hormones which act on the target cells.

- The hormones of the posterior pituitary are ADH and oxytocin.
- They are synthesized in the hypothalamus ,stored and released from the posterior pituitary.

Important hypothalamic hormones are as under:

- TRH (Thyrotropin-releasing hormone): it causes release of thyroid –stimulating hormone.
- CRH (Corticotropin-releasing hormone): it causes release of ACTH.
- GHIH (Growth hormone inhibitory hormone- somatostatin): It inhibits the release of growth hormone.
- GnRH(Gonadotropin-releasing hormone):It causes release of two gonadotropic hormones, FSH and LH.
- PIH (Prolactin –inhibiting hormone): it causes inhibition of prolactin secretion.



MECHANISM OF ACTION

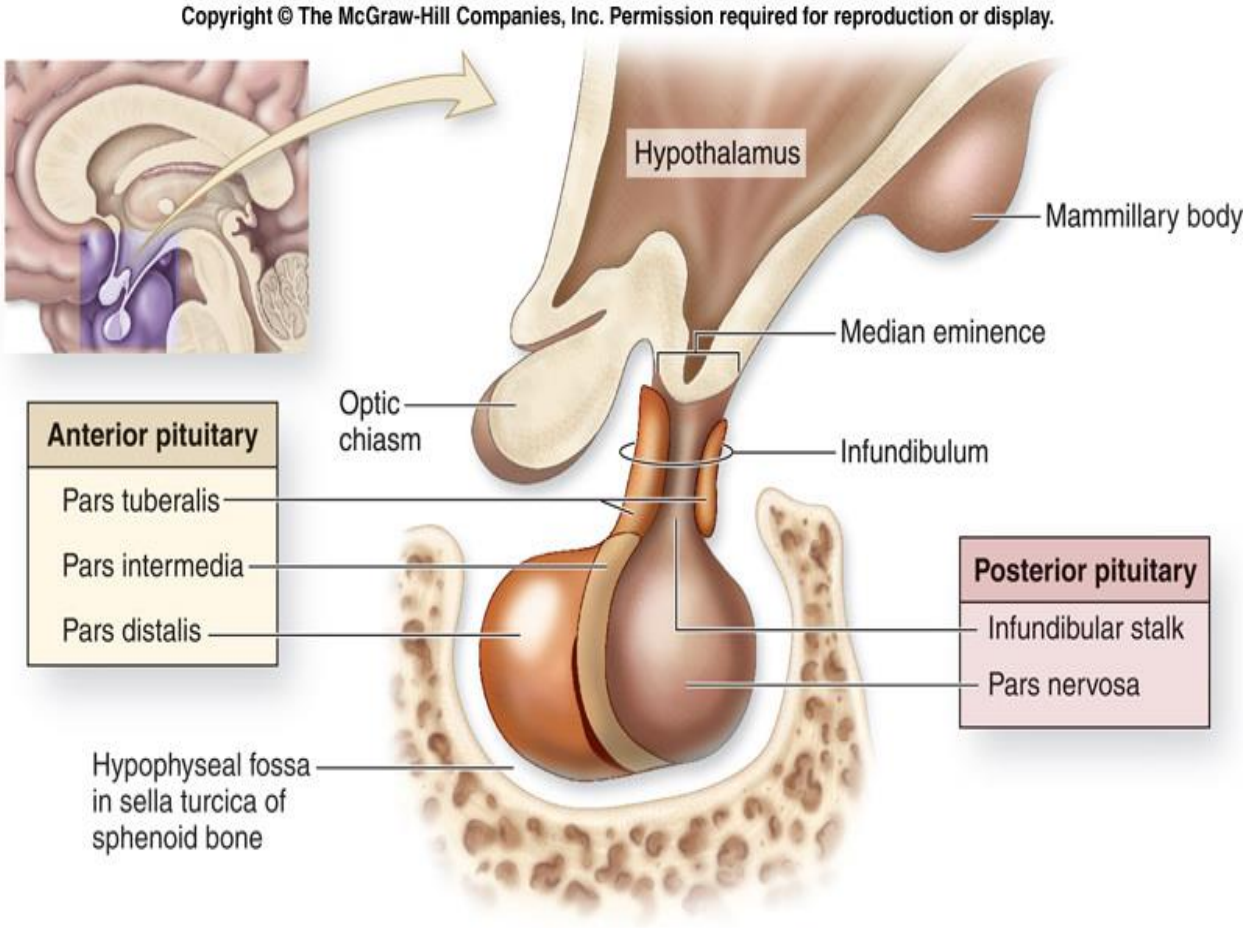
- Hypothalamic-releasing hormones act on the anterior pituitary by binding to specific membrane receptors on the cells. Most of the hormones act by stimulating cAMP.

PITUITARY GLAND

Structure and physiology

- Pituitary gland or hypophysis is a small endocrine gland with a diameter of 1 cm and weight of 0.5 to 1 g. It is situated in a depression called 'sella turcica', present in the sphenoid bone

at the base of skull. It is connected with the hypothalamus by the pituitary stalk or hypophyseal stalk.



DIVISIONS OF PITUITARY GLAND

Pituitary gland is divided into two divisions:

- Anterior pituitary or adenohypophysis
- Posterior pituitary or neurohypophysis
- Both the divisions are situated close to each other. Still both are entirely different in their development, structure and function.

- Between the two divisions, there is a small and relatively a vascular structure called pars intermedia. Actually, it forms a part of anterior pituitary.

REGULATION OF SECRETION

- Hypothalamo-hypophyseal Relationship
- The relationship between hypothalamus and pituitary gland is called hypothalamo-hypophyseal relationship.
- Hormones secreted by hypothalamus are transported to anterior pituitary and posterior pituitary,But the mode of transport of these hormones is different.
- Hormones from hypothalamus are transported to anterior pituitary through hypothalamo-hypophysial portal blood vessels.
- But, the hormones from hypothalamus to posterior pituitary are transported by nerve fibers of hypothalamo-hypophyseal tract.
- Anterior pituitary is also known as the master gland because it regulates many other endocrine glands through its hormones.

ANTERIOR PITUITARY OR ADENOHYPOPHYSIS

Anterior pituitary consists of three parts

- Pars distalis
- Pars tuberalis

- Pars intermedia.

REGULATION OF ANTERIOR PITUITARY SECRETION

- Hypothalamus controls anterior pituitary by secreting the releasing and inhibitory hormones (factors), which are called neurohormones.

HORMONES SECRETED BY ANTERIOR PITUITARY

Six hormones are secreted by the anterior pituitary:

- Growth hormone (GH) or somatotropic hormone (STH)
- Thyroid-stimulating hormone (TSH) or thyrotropic hormone
- Adrenocorticotropic hormone (ACTH)
- Follicle-stimulating hormone (FSH)
- Luteinizing hormone (LH) in females or interstitial- cell-stimulating hormone (ICSH) in males
- Prolactin.
- Recently, the hormone -lipotropin is found to be secreted by anterior pituitary. It stimulates melanocytes to produce melanin, and can also be cleaved into smaller peptides. In humans, γ-lipotropin and β-endorphin, are all possible fragments of β-lipotropin.β-Lipotropin also performs lipid-mobilizing functions such as lipolysis .

TROPIC HORMONES

- A tropic hormone is a hormone that stimulates an endocrine gland to grow and secrete it's hormones.(One hormone causes another hormone to do something). In humans, tropic

hormones are secreted by the adenohypophysis(anterior pituitary gland)

- First five hormones of anterior pituitary stimulate the other endocrine glands.
- Growth hormone also stimulates the secretory activity of liver and other tissues. Therefore, these five hormones are called tropic hormones. Prolactin is concerned with milk production.

Gonadotropic Hormones

- Follicle-stimulating hormone and the luteinizing hormone are together called gonadotropic hormones or gonadotropins because of their action on gonads.

GROWTH HORMONE

Source of Secretion

- Growth hormone is secreted by somatotropes which are the cells of anterior pituitary.

Chemistry and Blood Level

- GH is protein in nature, having a single-chain polypeptide with 191 amino acids. Its molecular weight is 21,500.
- Basal level of GH concentration in blood of normal adult is up to 300 g/dL and in children, it is up to 500 g/dL.

Transport

- Growth hormone is transported in blood by GH-binding proteins (GHBPs)

Half-life and Metabolism

- Half-life of circulating growth hormone is about 20 minutes. It is degraded in liver and kidney.
- GH is responsible for the general growth of the body.

Actions of Growth Hormone

- GH is responsible for the growth of almost all tissues of the body, which are capable of growing.
- It increases the size and number of cells by mitotic division.
- GH also causes specific differentiation of certain types of cells like bone cells and muscle cells.
- GH also acts on the metabolism of all the three major types of food stuffs in the body: proteins, lipids and carbohydrates.

Action of GH On metabolism

- GH increases the synthesis of proteins, mobilization of lipids and conservation of carbohydrates.

On protein metabolism

GH accelerates the synthesis of proteins by:

- Increasing amino acid transport through cell membrane
- Increasing ribonucleic acid (RNA) translation
- Increasing transcription of DNA to RNA

- Decreasing catabolism of protein
- Promoting anabolism of proteins indirectly

On fat metabolism

- GH mobilizes fats from adipose tissue. So, the concentration of fatty acids increases in the body fuids.
- These fatty acids are used for the production of energy by the cells.
- During the utilization of fatty acids for energy production, lot of acetoacetic acid is produced by liver and is released into the body fuids, leading to ketosis.
- Sometimes, excess mobilization of fat from the adipose tissue causes accumulation of fat in liver, resulting in fatty liver.

On carbohydrate metabolism

- Major action of GH on carbohydrates is the conservation of glucose.

Effects of GH on carbohydrate metabolism:

- Decrease in the peripheral utilization of glucose for the production of energy
- Increase in the deposition of glycogen in the cells
- Decrease in the uptake of glucose by the cells

On bones

- In embryonic stage, GH is responsible for the differentiation and development of bone cells.
- In later stages, GH increases the growth of the skeleton.
- It increases both the length as well as the thickness of the bones

Mode of Action of GH – Somatomedin

- GH acts on bones, growth and protein metabolism through somatomedin secreted by liver.
- GH stimulates the liver to secrete somatomedin.
- Sometimes, in spite of normal secretion of GH, growth is arrested (dwarfism) due to the absence or deficiency of somatomedin.

Somatomedin

- Somatomedin is defined as a substance through which growth hormone acts.
- It is a polypeptide with the molecular weight of about 7,500.

Types of somatomedin

Somatomedins are of two types:

- Insulin-like growth factor-I (IGF-I), which is also called somatomedin C
- Insulin-like growth factor-II.
- Somatomedin C (IGF-I) acts on the bones and protein metabolism.

- Insulin-like growth factor-II plays an important role in the growth of fetus.

Duration of action of GH and somatomedin C

- GH is transported in blood by loose binding with plasma protein.
- So, at the site of action, it is released from plasma protein rapidly.
- Its action also lasts only for a short duration of 20 minutes.
- But, the somatomedin C binds with plasma proteins very strongly.
- Because of this, the molecules of somatomedin C are released slowly from the plasma proteins.
- Thus, it can act continuously for a longer duration.

The action of somatomedin C lasts for about 20 hours

<u>Mode of action of somatomedin C</u>

- Somatomedin C acts through the second messenger called cyclic AMP.

Growth hormone receptor

- GH receptor is called growth hormone secretagogue (GHS) receptor.
- It is a transmembrane receptor, belonging to cytokine receptor family.
- GH binds with the receptor situated mainly in liver cells and forms the hormone- receptor complex.

- Hormone-receptor complex induces various intracellular enzyme pathways, resulting in somatomedin secretion.
- Somatomedin in turn, executes the actions of growth hormone.

Regulation of GH Secretion

Growth hormone secretion is altered by various factors.

GH secretion is stimulated by:

- Hypoglycemia
- Fasting
- Starvation
- Exercise
- Stress and trauma

GH secretion is inhibited by:

- Hyperglycemia
- Increase in free fatty acids in blood

OTHER HORMONES OF ANTERIOR PITUITARY

Thyroid-stimulating Hormone (TSH)

TSH is necessary for the growth and secretory activity of the thyroid gland. It has many actions on the thyroid gland. Controls the rate of secretion of T3 and T4 hormones.

- **Adrenocorticotropic Hormone (ACTH)**

ACTH is necessary for the structural integrity and the secretory activity of adrenal cortex. Actually ACTH controls secretion of adrenocortical hormones, which in return control carbohydrate ,protein and fat metabolism

- **Follicle-stimulating Hormone (FSH)**

Follicle-stimulating hormone (FSH) is a gonadotropin, a glycoprotein polypeptide hormone. FSH is synthesized and secreted by the gonadotropic cells of the anterior pituitary gland, and regulates the development, growth, pubertal maturation, and reproductive processes of the body. FSH and luteinizing hormone (LH) work together in the reproductive system.The half-life of FSH is about 3 to 4 hours.

Actions of FSH

- In males, FSH acts along with testosterone and accelerates the process of spermiogenesis(the final stage of spermatogenesis).
- In females FSH,Causes the development of graafian follicle from primordial follicle
- Stimulates the theca cells of graafian follicle and causes secretion of estrogen.

Luteinizing Hormone (LH)

- Luteinizing hormone also known as lutropin and sometimes lutrophin is a hormone produced by gonadotropic cells in the anterior pituitary gland.

Actions of LH

- In males, LH is known as interstitial cell-stimulating hormone (ICSH) because it stimulates the interstitial cells of Leydig in testes. This hormone is essential for the secretion of testosterone from Leydig cells.

In females, LH:

- Causes maturation of vesicular follicle into graafian follicle along with follicle-stimulating hormone
- Induces synthesis of androgens from theca cells of growing follicle
- Is responsible for ovulation

Prolactin

- Prolactin is a single chain polypeptide with 199 amino acids. Its half-life is about 20 minutes.
- Prolactin is necessary for the final preparation of mammary glands for the production and secretion of milk.

POSTERIOR PITUITARY GLAND

- Hormones of posterior pituitary are secreted in the hypothalamus and stored in the posterior pituitary.

Hormones of posterior pituitary are

- antidiuretic hormone(vasopressin)
- Oxytocin

ANTIDIURETIC HORMONE(VASOPRESSIN)

- An antidiuretic hormone controls the amount of water in the body ,by regulating the urine output

Source of Secretion

- Antidiuretic hormone (ADH) is secreted mainly by supraoptic nucleus of hypothalamus. It is also secreted by paraventricular

nucleus in small quantity. From here, this hormone is transported to posterior pituitary through the nerve fibers of hypothalamo-hypophyseal tract.

Chemistry and Half-life

- Antidiuretic hormone is a polypeptide containing 9 amino acids. Its half-life is 18 to 20 minutes.
- **Actions**

Antidiuretic hormone has two actions:

- Retention of water
- Vasopressor action.

Retention of water

- Major function of ADH is retention of water by acting on kidneys. It increases the facultative reabsorption of water from distal convoluted tubule and collecting duct in the kidneys.

Vasopressor action

- In large amount, ADH shows vasoconstrictor action. Particularly, causes constriction of the arteries in all parts of the body. Due to vasoconstriction, the blood pressure increases. ADH acts on blood vessels through receptor **.**
- However, the amount of ADH required to cause the vasopressor effect is greater than the amount required to cause the antidiuretic effect.

Regulation of Secretion

ADH secretion depends upon the volume of body fuid and the osmolarity of the body fuids. Potent stimulants for ADH secretion are:

- Decrease in the extracellular fuid (ECF) volume
- Increase in osmolar concentration in the ECF.

OXYTOCIN

Source of Secretion

- Oxytocin is secreted mainly by paraventricular nucleus of hypothalamus.
- It is also secreted by supraoptic nucleus in small quantity and it is transported from hypothalamus to posterior pituitary through the nerve fibers of hypothalamo-hypophyseal tract.
- In the posterior pituitary, the oxytocin is stored in the nerve endings of hypothalamo-hypophyseal tract.
- When suitable stimuli reach the posterior pituitary from hypothalamus, oxytocin is released into the blood.

Chemistry and Half-life

- Oxytocin is secreted in both males and females
- Oxytocin is a polypeptide having 9 amino acids. It has a half-life of about 6 minutes.

Actions in Females

- In females, oxytocin acts on mammary glands and uterus
- Oxytocin causes ejection of milk from the mammary glands.
- Ducts of the mammary glands are lined by myoepithelial cells.
- Oxytocin causes contraction of the myoepithelial cells and flow of milk from alveoli of mammary glands to the exterior through duct system and nipple.

- The process by which the milk is ejected from alveoli of mammary glands is called milk ejection reflex or milk let-down reflex.
- It is one of the neuroendocrine reflexes.
- On uterus ,oxytocin causes contraction of smooth muscles of the pregnant uterus and relaxation of cervix
- In non pregnant uterus ,oxytocin facilitates sperm transport.

Action in Males

- In males, the release of oxytocin increases during ejaculation.

Mode of Action of Oxytocin

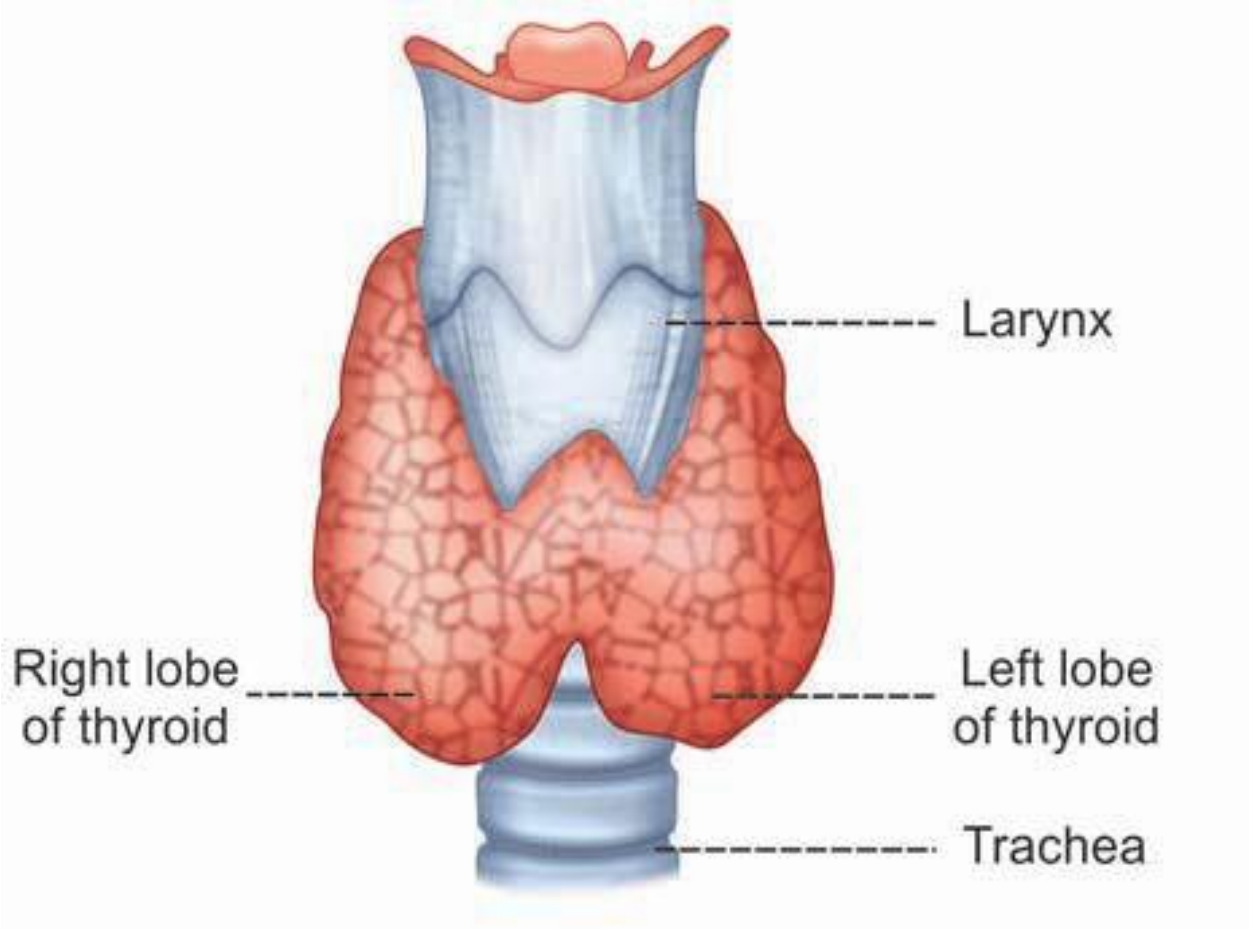
- Oxytocin acts on mammary glands and uterus by activating G-protein coupled oxytocin receptor.

THYROID HORMONES

STRUCTURE AND PHYSIOLOGY OF THYROID GLAND

- Thyroid is an endocrine gland situated at the root of the neck on either side of the trachea.
- It has two lobes, which are connected in the middle by an isthmus.
- It weighs about 20 to 40 g in adults.
- Thyroid is larger in females than in males.

- The structure and the function of the thyroid gland change in different stages of the sexual cycle in females.
- Its function increases slightly during pregnancy and lactation and decreases during menopause.
- The functional unit of the thyroid gland is the follicle, a roughly spherical group of cells arranged around a protein-rich storage material called colloid.



HORMONES OF THYROID GLAND

- Thyroid gland secretes four hormones:
- 1. Thyroxine (T4): It constitutes about 90% of the thyroid hormone.

- 2. Tri-iodothyronine or(T3): it constitutes about 9% of the thyroid hormone.
- 3. Reverse T3(RT3): It constitutes only 1%of the thyroid hormone.
- 4. Calcitonin : it is secreted from the Para follicular cells

SYNTHESIS AND RELEASE OF THYROID HORMONES

- Thyroid hormones are synthesized in adults as long as the dietary iodine .
- This supply prevents goiter formation.
- The daily ingestion of iodide is 400-500 μg daily in many areas and the same amount is excreted in the urine .

MECHANISM OF ACTION OF THYROID HORMONES

- Thyroid hormone binds to specific high-affinity receptors in the target cell nucleus.
- Thyroid hormone-receptor complex then binds to DNA & increase the expression of specific genes.
- The resultant mRNAs then trigger production of various enzymes that alter cell function.
- The transcription of the growth hormone gene is also enhanced, hence more Growth hormone produced as well.

FUNCTIONS OF THYROID HORMONES

Thyroid hormones have two major effects on the body:

- I. To increase basal metabolic rate

- II. To stimulate growth in children.

1. ACTION ON BASAL METABOLIC RATE (BMR)

- Thyroxine increases the metabolic activities in most of the body tissues, except brain, retina, spleen, testes and lungs. It increases BMR by increasing the oxygen consumption of the tissues. The action that increases the BMR is called calorigenic action.
- In hyperthyroidism, BMR increases by about 60% to 100% above the normal level and in hypothyroidism it falls by 20% to 40% below the normal level.

2. ACTION ON PROTEIN METABOLISM

- Thyroid hormone increases the synthesis of proteins in the cells. The protein synthesis is accelerated by the following ways:
- By Increasing the Translation of RNA
- Thyroid hormone increases the translation of RNA in the cells. Because of this, the ribosomes are activated and more proteins are synthesized.
- By Increasing the Transcription of DNA to RNA
- Thyroid hormone also stimulates the transcription of DNA to RNA. This in turn accelerates the synthesis of proteins in the cells .
- By Increasing the Activity of Mitochondria
- In addition to acting at nucleus, thyroid hormone acts at mitochondrial level also. It increases the number and the activity of mitochondria in most of the cells of the body. Thyroid hormone accelerates the synthesis of RNA and other substances from mitochondria, by activating series of enzymes.

In turn, the mitochondria increase the production of ATP, which is utilized for the energy required for cellular activities.

- By Increasing the Activity of Cellular Enzymes
- Thyroid hormones also increase the activity of at least 100 or more intracellular enzymes such as alpha- glycerophosphate dehydrogenase and oxidative enzymes. These enzymes accelerate the metabolism of proteins and the carbohydrates.
- Though thyroxine increases synthesis of protein, it also causes catabolism of proteins.

3. ACTION ON CARBOHYDRATE METABOLISM

- Thyroxine stimulates almost all processes involved in the metabolism of carbohydrate.
- Increases the absorption of glucose from GI tract.
- Enhances the glucose uptake by the cells, by accelerating the transport of glucose through the cell membrane.
- Increases the breakdown of glycogen into glucose.
- Accelerates gluconeogenesis.

ACTION ON FAT METABOLISM

- Thyroxine decreases the fat storage by mobilizing it from adipose tissues and fat depots. The mobilized fat is converted into free fatty acid and transported by blood. Thus, thyroxine increases the free fatty acid level in blood.

ACTION ON PLASMA AND LIVER FATS

- Even though there is an increase in the blood level of free fatty acids, thyroxine specifically decreases the cholesterol, phospholipids and triglyceride levels in plasma. So, in hyposecretion of thyroxine, the cholesterol level in plasma increases, resulting in atherosclerosis.
- Thyroxine also increases deposition of fats in the liver, leading to fatty liver.
- Thyroxine decreases plasma cholesterol level by increasing its excretion from liver cells into bile. Cholesterol enters the intestine through bile and then it is excreted through the feces.

ACTION ON VITAMIN METABOLISM

- Thyroxine increases the formation of many enzymes.Since vitamins form essential parts of the enzymes, it is believed that the vitamins may be utilized during the formation of the enzymes.

ACTION ON BODY TEMPERATURE

- Thyroid hormone increases the heat production in the body, by accelerating various cellular metabolic processes and increasing BMR.
- It is called thyroid hormone- induced thermogenesis. During hypersecretion of thyroxine, the body temperature increases greatly, resulting in excess sweating.

ACTION ON GROWTH

- Thyroid hormones have general and specific effects on growth.
- Increase in thyroxine secretion accelerates the growth of the body, especially in growing children.
- Lack of thyroxine arrests the growth.
- At the same time, thyroxine causes early closure of epiphysis. So, the height of the individual may be slightly less in hypothyroidism.
- Thyroxine is more important to promote growth and development of brain during fetal life and first few years of postnatal life.
- Deficiency of thyroid hormones during this period leads to mental retardation.

ACTION ON BODY WEIGHT

- Thyroxine is essential for maintaining the body weight. Increase in thyroxine secretion decreases the body weight and

fat storage. Decrease in thyroxine secretion increases the body weight because of fat deposition.

ACTION ON BLOOD

- Thyroxine accelerates erythropoietic activity and increases blood volume. It is one of the important general factors necessary for erythropoiesis. Polycythemia (an abnormally increased concentration of hemoglobin in the blood, either through reduction of plasma volume or increase in red blood cell)is common in hyperthyroidism.

ACTION ON CARDIOVASCULAR SYSTEM

- Thyroxine increases the overall activity of cardiovascular system.

I.On Heart Rate

- Thyroxine acts directly on heart and increases the heart rate. It is an important clinical investigation for diagnosis of hypothyroidism and hyperthyroidism.

On the Force of Contraction of the Heart

- Due to its effect on enzymatic activity, thyroxine generally increases the force of contraction of the heart. But in hyperthyroidism or in thyrotoxicosis, the heart may become weak due to excess activity and protein catabolism. So, the patient may die of cardiac decompensation.
- Cardiac decompensation refers to failure of the heart to maintain adequate circulation associated with

dyspnea(shortness of breath), venous engorgement (veins overfilled with blood) and edema.

iii .On Blood Vessels

- Thyroxine causes vasodilatation by increasing the metabolic activities. During increased metabolic activities, a large quantity of metabolites is produced. These metabolites cause vasodilatation.

iv. On Arterial Blood Pressure

- Because of increase in rate and force of contraction of the heart, increase in blood volume and blood flow by the infuence of thyroxine, cardiac output increases. This in turn, increases the blood pressure

ACTION ON RESPIRATION

- Thyroxine increases the rate and force of respiration indirectly. The increased metabolic rate (caused by thyroxine) increases the demand for oxygen and formation of excess carbon dioxide. These two factors stimulate the respiratory centers to increase the rate and force of respiration.

- **ACTION ON GASTROINTESTINAL TRACT**

  Generally, thyroxine increases the appetite and food intake. It also increases the secretions and movements of GI tract. So, hypersecretion of thyroxine causes diarrhea and the lack of thyroxine causes constipation.

ACTION ON CENTRAL NERVOUS SYSTEM

- Thyroxine is very essential for the development and maintenance of normal functioning of central nervous system (CNS).

i.On Development of Central Nervous System

Thyroxine is very important to promote growth and development of the brain during fetal life and during the first few years of postnatal life. Thyroid deficiency in infants results in abnormal development of synapses, defective myelination and mental retardation

- **ii.On the Normal Function of Central Nervous System**

Thyroxine is a stimulating factor for the central nervous system, particularly the brain. So, the normal functioning of the brain needs the presence of thyroxine. Thyroxine also increases the blood flow to brain.

- **ACTION ON SKELETAL MUSCLE**

Thyroxine is essential for the normal activity of skeletal muscles. Slight increase in thyroxine level makes the muscles to work with more vigour.

- **ACTION ON SLEEP**

  Normal thyroxine level is necessary to maintain normal sleep pattern.

- **ACTION ON SEXUAL FUNCTION**

  Normal thyroxine level is essential for normal sexual function.

- **ACTION ON OTHER ENDOCRINE GLANDS**

  Because of its metabolic effects, thyroxine increases the demand for secretion by other endocrine glands.

<u>PARATHYROID GLAND</u>

- Human beings have four parathyroid glands, which are situated on the posterior surface of upper and lower poles of thyroid gland .Parathyroid glands are very small in size, measuring about 6 mm long, 3 mm wide and 2 mm thick, with dark brown color.

  Fig: Parathyroid glands on the posterior surface of thyroid gland

- Each parathyroid gland is made up of chief cells and oxyphil cells. Chief cells secrete parathormone. Oxyphil cells are the degenerated chief cells and their function is unknown. However, these cells may secrete parathormone during pathological condition called parathyroid adenoma. The number of oxyphil cells increases after puberty.
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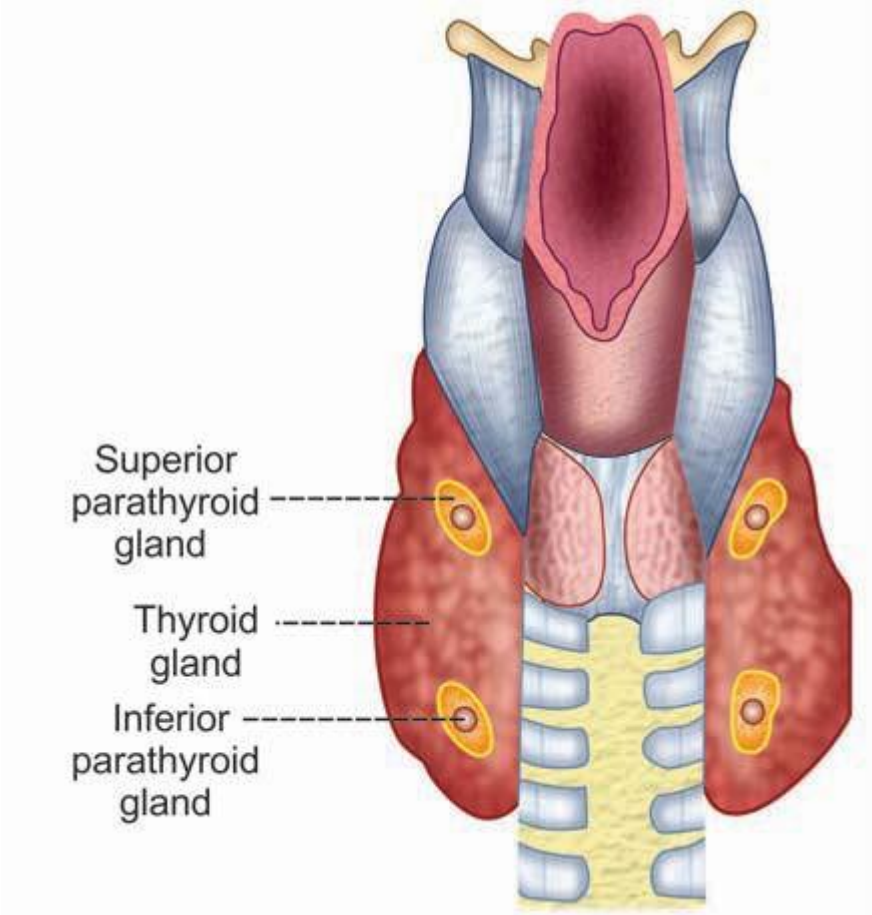

Histology

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PARATHORMONE

- Parathormone secreted by parathyroid gland is essential for the maintenance of blood calcium level within a very narrow critical level. Maintenance of blood calcium level is necessary

because calcium is an important inorganic ion for many physiological functions

- Source of Secretion : Parathormone (PTH) is secreted by the chief cells of the parathyroid glands.
- Chemistry: Parathormone is protein in nature, having 84 amino acids. Its molecular weight is 9,500.
- **Half-life and Plasma Level**

  Parathormone has a half-life of 10 minutes. Normal plasma level of PTH is about 1.5 to 5.5 ng/dL.

- **Synthesis**

  Parathormone is synthesized from the precursor called prepro-PTH containing 115 amino acids. First, the prepro-PTH enters the endoplasmic reticulum of chief cells of parathyroid glands. There it is converted into a prohormone called pro-PTH, which contains 96 amino acids. Pro-PTH enters the Golgi apparatus, where it is converted into PTH.

- **Metabolism**

  Sixty to seventy percent of PTH is degraded by Kupffer cells of liver, by means of proteolysis. Degradation of about 20% to 30% PTH occurs in kidneys and to a lesser extent in other organs.

ACTIONS OF PARATHORMONE

- PTH plays an important role in maintaining blood calcium level. It also controls blood phosphate level.

ACTIONS OF PARATHORMONE ON BLOOD CALCIUM LEVEL

- Primary action of PTH is to maintain the blood calcium level within the critical range of 9 to 11 mg/dL. The blood calcium level has to be maintained critically because, it is very important for many of the activities in the body.

  PTH maintains blood calcium level by acting on:

- 1. Bones
- 2. Kidney
- **3. Gastrointestinal tract.**

1.On Bone

- Parathormone enhances the resorption of calcium from the bones (osteoclastic activity) by acting on osteoblasts and osteoclasts of the bone.
- PTH increases calcium resorption from bone by stimulating the proliferation of osteoclasts also.
- Osteoblasts and osteoclasts are types of cells the human body uses to repair broken bones.

  Osteoclasts break down old bone tissue allowing osteoblasts to replace it with new material. Together, these cells facilitate bone healing and bone growth.

2.On Kidney

- PTH increases the reabsorption of calcium from the renal tubules along with magnesium ions and hydrogen ions. It increases calcium reabsorption mainly from distal convoluted tubule and proximal part of collecting duct.
- PTH also increases the formation of 1,25-dihydroxycholecalciferol (activated form of vitamin D) from 25-hydroxycholecalciferol in kidneys

3. On Gastrointestinal Tract

- PTH increases the absorption of calcium ions from the GI tract indirectly. It increases the formation of 1,25-dihydroxycholecalciferol in the kidneys. This vitamin, in turn increases the absorption of calcium from GI tract.
- Thus, the activated vitamin D is very essential for the absorption of calcium from the GI tract. And PTH is essential for the formation of activated vitamin D.

Role of PTH in the activation of vitamin D

Vitamin D is very essential for calcium absorption from the GI tract. But vitamin D itself is not an active substance. Instead, vitamin D has to be converted into 1, 25-dihydroxycholecalciferol in the liver and kidney in the presence of PTH. The 1,25-dihydroxycholecalciferol is the active product.

MODE OF ACTION OF PARATHORMONE

Parathormone Receptors

- Parathormone receptors (PTH receptors) are of three types, PTHR1, PTHR2 and PTHR3, which are G protein- coupled receptors. PTHR1 is physiologically more important than the other two types. PTHR1 mediates the actions of PTH and PTH-related protein Role of PTHR2 and PTHR3 is not known clearly.
- On the target cells, PTH binds with PTHR1 which is coupled to G protein and forms hormone-receptor complex. Hormone-

receptor complex causes formation of cAMP, which acts as a second messenger for the hormone

PANCREATIC HORMONES

- Endocrine function of pancreas is performed by the islets of Langerhans. Human pancreas contains about 1 to 2 million islets.
- Islets of Langerhans consist of four types of cells:
- 1. A cells or α-cells, which secrete glucagon
- 2. B cells or –β cells, which secrete insulin
- 3. D cells or δ-cells, which secrete somatostatin
- 4. F cells or PP cells, which secrete pancreatic polypeptide.

INSULIN

Synthesis

- Synthesis of insulin occurs in the rough endoplasmic reticulum of β-cells in islets of Langerhans. It is synthesized as preproinsulin that gives rise to proinsulin. Proinsulin is converted into insulin and C peptide through a series of peptic cleavages. C peptide is a connecting peptide that connects α and β chains. At the time of secretion, C peptide is detached.

Source of Secretion

- Insulin is secreted by B cells or the β-cells in the islets of Langerhans of pancreas.

Chemistry and Half-Life

- Insulin is a polypeptide with 51 amino acids and a molecular weight of 5,808. It has two amino acid chains called α and β chains, which are linked by disulfide bridges. The α-chain of insulin contains 21 amino acids and β-chain contains 30 amino acids. The biological half-life of insulin is 5 minutes.

Metabolism

- Binding of insulin to insulin receptor is essential for its removal from circulation and degradation. Insulin is degraded in liver and kidney by a cellular enzyme called insulin protease or insulin-degrading enzyme

Actions of insulin

- Insulin is the important hormone that is concerned with the regulation of carbohydrate metabolism and blood glucose level. It is also concerned with the metabolism of proteins and fats.

On Carbohydrate Metabolism

- Insulin is the only antidiabetic hormone secreted in the body.

i.e. It is the only hormone in the body that reduces blood glucose level. Insulin reduces the blood glucose level by its following actions on carbohydrate metabolism:

i. Increases transport and uptake of glucose by the cells

ii. Promotes peripheral utilization of glucose

iii. Promotes storage of glucose – glycogenesis

iv. Inhibits glycogenolysis

v. Inhibits gluconeogenesis

On Protein Metabolism

- Insulin facilitates the synthesis and storage of proteins and inhibits the cellular utilization of proteins by the following actions:
- i. Facilitating the transport of amino acids into the cell from blood, by increasing the permeability of cell membrane for amino acids
- ii. Accelerating protein synthesis by influencing the transcription of DNA and by increasing the translation of mRNA
- iii. Preventing protein catabolism by decreasing the activity of cellular enzymes which act on proteins
- iv. Preventing conversion of proteins into glucose.
- Thus, insulin is responsible for the conservation and storage of proteins in the body.

On Fat Metabolism

Insulin stimulates the synthesis of fat. It also increases the storage of fat in the adipose tissue.

- **Actions of insulin on fat metabolism are:**

i.Synthesis of fatty acids and triglycerides

Insulin promotes the transport of excess glucose into cells, particularly the liver cells. This glucose is utilized for the synthesis of fatty acids and triglycerides. Insulin promotes the synthesis of lipids by activating the enzymes which convert:

a.Glucose into fatty acids

b. Fatty acids into triglycerides.

ii. Transport of fatty acids into adipose tissue.

- Insulin facilitates the transport of fatty acids into the adipose tissue.

iii. Storage of fat

- Insulin promotes the storage of fat in adipose tissue by inhibiting the enzymes which degrade the triglycerides.
-

On growth

- Along with growth hormone, insulin promotes growth of body by its anabolic action on proteins. It enhances the transport of amino acids into the cell and synthesis of proteins in the cells.

i.e. it causes conservation of proteins by increasing the glucose utilization by the tissues.

MODE OF ACTION OF INSULIN

- On the target cells, insulin binds with the receptor protein and forms the insulin-receptor complex. This complex executes the action by activating the intracellular enzyme system.

Insulin Receptor

- Insulin receptor is a glycoprotein with a molecular weight of 340,000. It is present in almost all the cells of the body.

REGULATION OF INSULIN SECRETION

- Insulin secretion is mainly regulated by blood glucose level. In addition, other factors like amino acids, lipid derivatives, gastrointestinal and endocrine hormones
- And autonomic nerve fibers also stimulate insulin secretion

1.Role of Blood Glucose Level

- When blood glucose level is normal (80 to 100 mg/dL), the rate of insulin secretion is low (up to 10 μU/minute). When blood glucose level increases between 100 and 120 mg/dL, the rate of insulin secretion rises rapidly to 100 μU/minute. When blood glucose level rises above 200 mg/dL, the rate of insulin secretion also rises very rapidly up to 400 μU/minute.

2. Role of Proteins

- Excess amino acids in blood also stimulate insulin secretion. Potent amino acids are arginine and lysin. Without any increase in blood glucose level, the amino acids alone can cause a slight increase in insulin secretion. However, amino acids potentiate the action of glucose on insulin secretion so that, in the presence of amino acids, elevated blood glucose level increases insulin secretion to a great extent

3. Role of Lipid Derivatives

- The α-ketoacids such as acetoacetate also increase insulin secretion.

4. Role of Gastrointestinal Hormones

- Insulin secretion is increased by some of the gastrointestinal hormones such as gastrin, secretin, .

5. Role of Endocrine Hormones

- Diabetogenic hormones like glucagon, growth hormone and cortisol also stimulate insulin secretion, indirectly.

GLUCAGON

Source of Secretion

- Glucagon is secreted from A cells or α-cells in the islets of Langerhans of pancreas. It is also secreted from A cells of stomach and L cells of intestine.
- **Chemistry and half-life:** Glucagon is a polypeptide with a molecular weight of 3,485. It contains 29 amino acids. Half-life of glucagon is 3 to 6 minutes.
- **Synthesis:** Glucagon is synthesized from the preprohormone precursor called preproglucagon in the α-cells of islets. Preproglucagon is converted into proglucagon, which gives rise to glucagon.
- **Metabolism:** About 30% of glucagon is degraded in liver and 20% in kidney. The cleaved glucagon fragments are excreted through urine. 50% of the circulating glucagon is degraded in blood itself by enzymes such as serine and cysteine proteases.

Actions of glucagon

Actions of glucagon are antagonistic to those of insulin

1. On Carbohydrate Metabolism

- Glucagon increases the blood glucose level by:

i. Increasing glycogenolysis in liver and releasing glucose from the liver cells into the blood.

ii. Increasing of gluconeogenesis

REGULATION OF GLUCAGON SECRETION

- Secretion of glucagon is controlled mainly by glucose and amino acid levels in the blood.

1.Role of Blood Glucose Level

- Important factor that regulates the secretion of glucagon is the decrease in blood glucose level. When blood glucose level decreases below 80 mg/dL of blood, α-cells of islets of Langerhans are stimulated and more glucagon is released. Glucagon, in turn increases the blood glucose level. On the other hand, when blood glucose level increases, α-cells are inhibited and the secretion of glucagon decreases.

2.Role of Amino Acid Level in Blood

Increase in amino acid level in blood stimulates the secretion of glucagon. Glucagon, in turn converts the amino acids into glucose.

3.Role of Other Factors

Factors which increase glucagon secretion:

- **i.** Exercise
- ii. Stress

Factors which inhibit glucagon secretion:

- **i.** Somatostatin
- ii. Insulin

SOMATOSTATIN

- Somatostatin, also known as growth hormone–inhibiting hormone (GHIH) or by several other names, is a peptide hormone that regulates the endocrine system and affects neurotransmission and cell proliferation via interaction with G protein-coupled somatostatin receptors and inhibition of the release of numerous secondary hormones. Somatostatin inhibits insulin and glucagon secretion.

SOURCE OF SECRETION

Somatostatin is secreted from:

- 1. Hypothalamus
- 2. D cells (δ-cells) in islets of Langerhans of pancreas
- 3. D cells in stomach and upper part of small intestine.

CHEMISTRY AND HALF-LIFE:

- Somatostatin is a polypeptide. It is synthesized in two forms, namely somatostatin-14 (with 14 amino acids) and somatostatin-28 (with 28 amino acids). Both the forms have similar actions. Half-life of somatostatin is 2 to 4 minutes.

-

SYNTHESIS

- Somatostatin is synthesized from the precursor prosomatostatin. Prosomatostatin is converted mostly into somatostatin-14 in the D cells of islets in pancreas. However, in the intestine, large amount of somatostatin- 28 is produced from prosomatostatin.

METABOLISM

Somatostatin is degraded in liver and kidney

ACTIONS OF SOMATOSTATIN

1. Somatostatin acts within islets of Langerhans and, inhibits β and α cells, i.e. it inhibits the secretion of both glucagon and insulin

2. It decreases the motility of stomach, duodenum and gallbladder

3. It reduces the secretion of gastrointestinal hormones ,gastrin, CCK (Cholecystokinin), GIP (Gastric inhibitory polypeptide) and VIP(Vasoactive intestinal peptide)

4. Hypothalamic somatostatin inhibits the secretion of GH and TSH from anterior pituitary. That is why, it is also called growth hormone-inhibitory hormone (GHIH).

REGULATION OF SECRETION OF SOMATOSTATIN

Pancreatic Somatostatin

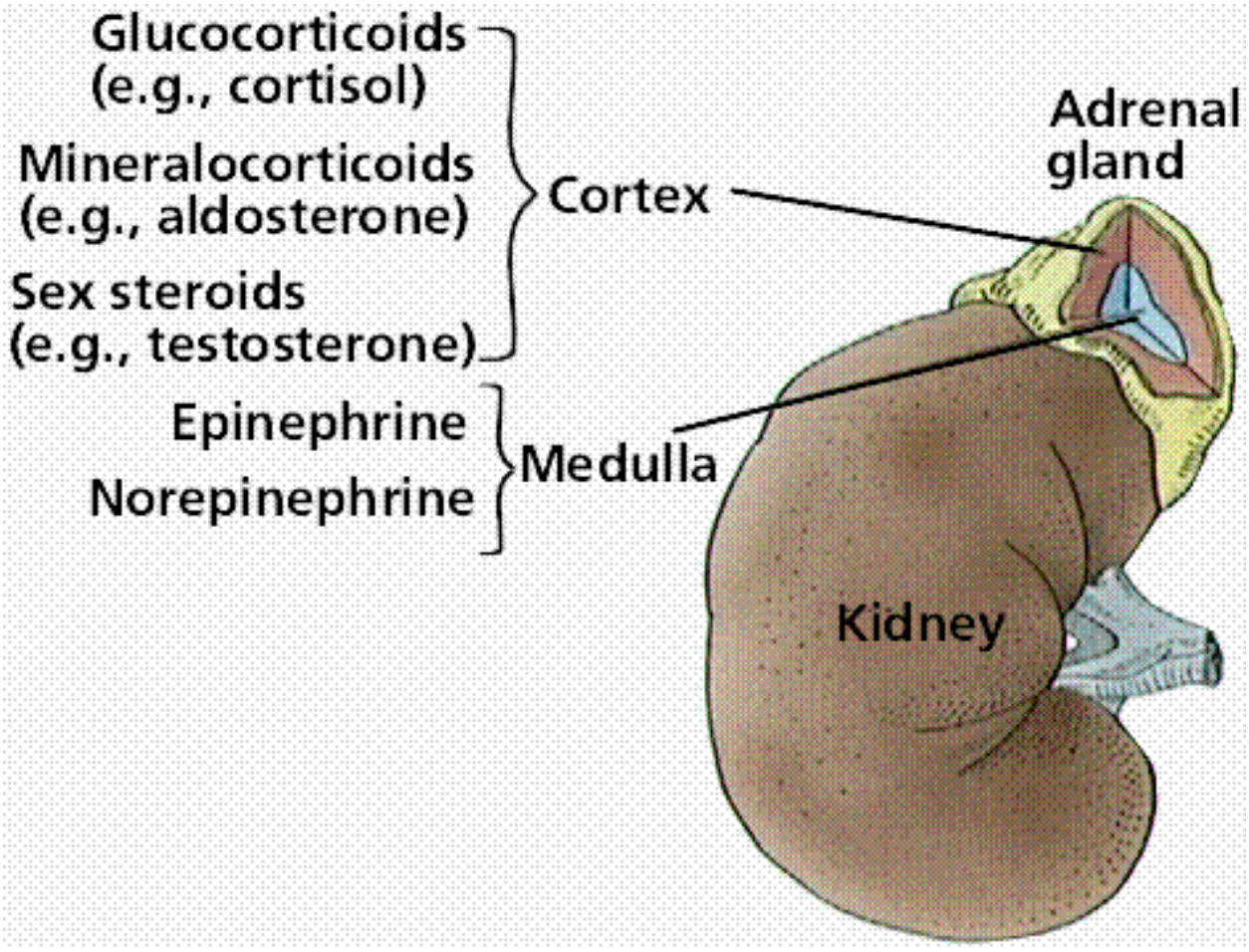
- Secretion of pancreatic somatostatin is stimulated by glucose, amino acids and CCK (Cholecystokinin). The tumor of D cells of islets of Langerhans causes hypersecretion of somatostatin. It leads to hyperglycemia and other symptoms of diabetes mellitus.

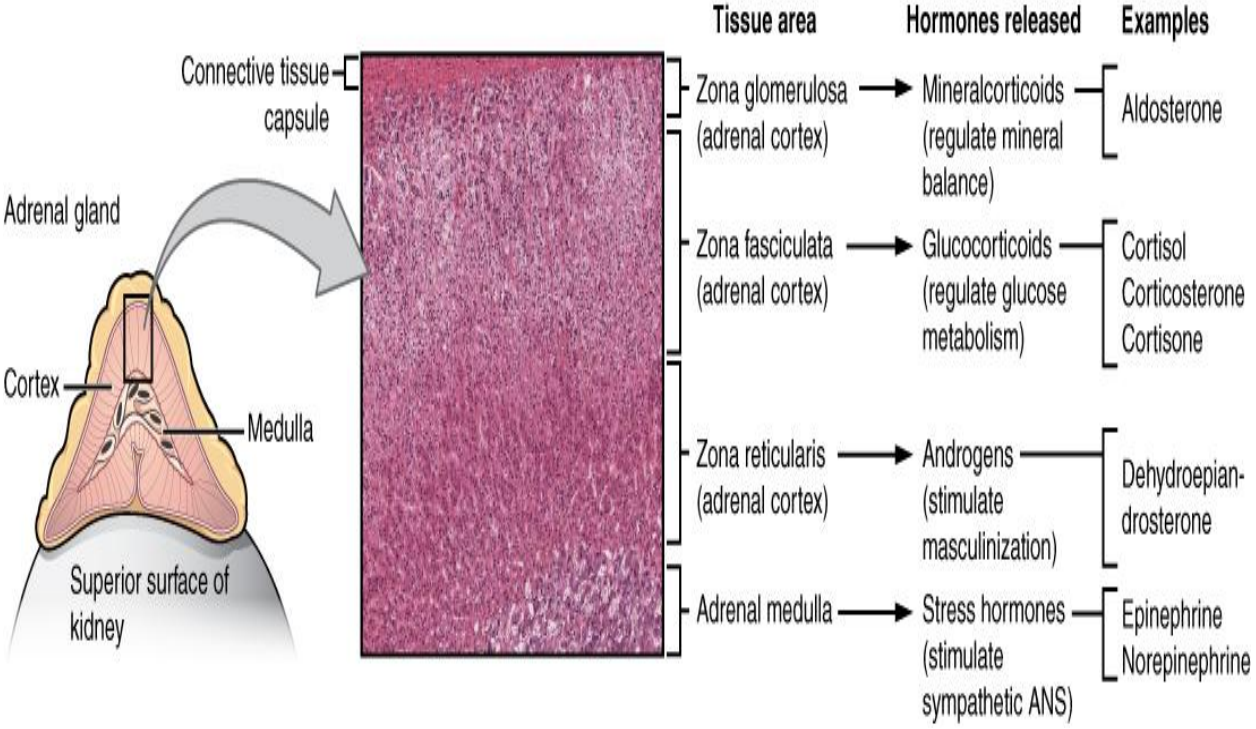
Gastrointestinal Tract Somatostatin

- Secretion of somatostatin in GI tract is increased by the presence of chyme-containing glucose and proteins in stomach and small intestine.

ADRENAL GLANDS

- Adrenal glands are called the 'life-saving glands' or 'essential endocrine glands'. It is because the absence of adrenocortical hormones causes death within 3 to 15 days and absence of adrenomedullary hormones, drastically decreases the resistance to mental and physical stress.


Glucocorticoids
(e.g., cortisol)
Mineralocorticoids
(e.g., aldosterone)
Sex steroids
(e.g., testosterone)
Cortex
Epinephrine
Norepinephrine
Medulla
Adrenal gland
Kidney


Connective tissue capsule
Adrenal gland
Cortex
Medulla
Superior surface of kidney
Tissue area
Hormones released
Examples
Zona glomerulosa (adrenal cortex)
Mineralcorticoids (regulate mineral balance)
Aldosterone
Zona fasciculata (adrenal cortex)
Glucocorticoids (regulate glucose metabolism)
Cortisol
Corticosterone
Cortisone
Zona reticularis (adrenal cortex)
Androgens (stimulate masculinization)
Dehydroepian-drosterone
Adrenal medulla
Stress hormones (stimulate sympathetic ANS)
Epinephrine
Norepinephrine

FUNCTIONAL ANATOMY OF ADRENAL GLANDS

- There are two adrenal glands. Each gland is situated on the upper pole of each kidney.

  The adrenal glands (also known as suprarenal glands) are endocrine glands that produce a variety of hormones including adrenaline and the steroids aldosterone and cortisol. Each gland weighs about 4 g.

PARTS OF ADRENAL GLAND

Adrenal gland is made of two distinct parts:

1. Adrenal cortex: Outer portion, constituting 80% of the gland
2. Adrenal medulla: Central portion, constituting 20% of the gland.

HORMONES OF ADRENAL CORTEX

Adrenocortical hormones are steroids in nature, hence the name 'corticosteroids'. Based on their functions, corticosteroids are classified into three groups:

1. Mineralocorticoids
2. Glucocorticoids
3. Sex hormones.

SYNTHESIS AND TRANSPORT OF ADRENOCORTICAL HORMONES

SYNTHESIS

- All adrenocortical hormones are steroid in nature and are synthesized mainly from cholesterol that is absorbed directly from the circulating blood. Small quantity of cholesterol is also synthesized within the cortical cells from acetylcoenzyme A (acetyl-CoA).

TRANSPORT

Mineralocorticoids

- Mineralocorticoids are transported in blood by binding with plasma proteins, especially globulins. The binding is loose and 50% of these hormones are present in free form.

Glucocorticoids

- Glucocorticoids are transported by a special plasma protein known as glucocorticoids-binding globulin. Ninety four percent of glucocorticoids are transported by this protein, where as about 6% of them are found free in plasma. Albumin plays a very little role in glucocorticoid transport.

Sex hormones

- Adrenal sex hormones are transported by another special plasma protein known as sex hormone-binding globulin.

MINERALOCORTICOIDS

- Ninety percent of mineralocorticoid activity is provided by aldosterone.

Life-saving Hormone

- Aldosterone is very essential for life and it maintains the osmolarity and volume of ECF. It is usually called life-saving

hormone because, its absence causes death within 3 days to 2 weeks.

Aldosterone has three important functions.

- It increases:

1.Reabsorption of sodium from renal tubules

2.Excretion of potassium through renal tubules

3.Secretion of hydrogen ion into renal tubules

Actions of aldosterone :

1.on sodium ions

- Aldosterone acts on the distal convoluted tubule and the collecting duct and increases the reabsorption of sodium. During hypersecretion of aldosterone, the loss of sodium through urine is only few milligram per day. But during hyposecretion of aldosterone, the loss of sodium through urine increases (hypernatriuria) up to about 20 g/day. It proves the importance of aldosterone in regulation of sodium ion concentration and osmolality in the body.

- **2. On Potassium Ions**

- Aldosterone increases the potassium excretion through the renal tubules. When aldosterone is deficient, the potassium ion concentration in ECF increases leading to hyperkalemia. Hyperkalemia results in serious cardiac toxicity, with weak contractions of heart and development of arrhythmia. In very severe conditions, it may cause cardiac death. When aldosterone secretion increases, it leads to hypokalemia and muscular weakness**.**

3. On Hydrogen Ion Concentration

- While increasing the sodium reabsorption from renal tubules, aldosterone causes tubular secretion of hydrogen ions. To some extent, secretion of hydrogen ions is in exchange for sodium ions. It obviously reduces the hydrogen ion concentration in the ECF. In normal conditions, aldosterone is essential to maintain acid- base balance in the body. In hypersecretion, it causes alkalosis and in hyposecretion, it causes acidosis

GLUCOCORTICOIDS

- Glucocorticoids act mainly on glucose metabolism.
- Glucocorticoids are:
- 1. Cortisol
- 2. Corticosterone
- 3. Cortisone.

FUNCTIONS OF GLUCOCORTICOIDS

- Cortisol or hydrocortisone is more potent and it has 95% of glucocorticoid activity.
- Corticosterone is less potent showing only 4% of glucocorticoid activity.
- Cortisone with 1% activity is secreted in minute quantity.

Life-protecting Hormone

- Like aldosterone, cortisol is also essential for life but in a different way. Aldosterone is a life-saving hormone, whereas cortisol is a life-protecting hormone because, it helps to withstand the stress and trauma in life

- Glucocorticoids have metabolic effects on carbohydrates, proteins, fats and water. These hormones also show mild mineralocorticoid effect. Removal of adrenal glands in human beings and animals causes disturbances of metabolism. Exposure to even mild harmful stress after adrenalectomy, leads to collapse and death.

On Carbohydrate Metabolism

- Glucocorticoids increase the blood glucose level by two ways:
- i. By promoting gluconeogenesis in liver from amino acids: Glucocorticoids enhance the breakdown of proteins in extrahepatic cells, particularly the muscle. It is followed by release of amino acids into circulation. From blood, amino acids enter the liver and get converted into glucose (gluconeogenesis)
- ii. By inhibiting the uptake and utilization of glucose by peripheral cells: This action is called anti- insulin action of glucocorticoids.
- Hypersecretion of glucocorticoids increases the blood glucose level, resulting in hyperglycemia, glucosuria and adrenal diabetes. Hyposecretion of these hormones causes hypoglycemia and fasting during adrenal insufficiency will be fatal. It decreases blood glucose level to a great extent, resulting in death.

On Muscles

- Glucocorticoids increase the catabolism of proteins in muscle. So, hypersecretion causes muscular weakness due to loss of protein.

On Blood Cells

- Glucocorticoids decrease the number of circulating eosinophils by increasing the destruction of eosinophils in reticuloendothelial cells. These hormones also decrease the number of basophils and lymphocytes and increase the number of circulating neutrophils, RBCs and platelets.

On Resistance to Stress

- Exposure to any type of stress, either physical or mental, increases the secretion of adrenocorticotropic hormone (ACTH), which in turn increases glucocorticoid secretion. The increase in glucocorticoid level is very essential for survival during stress conditions, as it offers high resistance to the body against stress.

MODE OF ACTION

- Glucocorticoids bind with receptors to form hormone-receptor complex, which activates DNA to form mRNA. mRNA causes activation of enzymes, which alter the cell function.

REGULATION OF SECRETION

- Anterior pituitary regulates glucocorticoid secretion by secreting adrenocorticotropic hormone (ACTH). ACTH secretion is regulated by hypothalamus through corticotropin-releasing factor (CRF).

ADRENAL SEX HORMONES

- Adrenal sex hormones are secreted mainly by zona reticularis. Zona fasciculata secretes small quantities of sex hormones. Adrenal cortex secretes mainly the male sex hormones, which are called androgens. But small quantity of estrogen and progesterone are also secreted by adrenal cortex.

Androgens secreted by adrenal cortex:

- **1.** Dehydroepiandrosterone
- 2. Androstenedione
- 3. Testosterone
- Dehydroepiandrosterone is the most active adrenal androgen. Androgens, in general, are responsible for masculine features of the body. But in normal conditions, the adrenal androgens have insignificant physiological effects, because of the low amount of secretion both in males and females.

HORMONES OF ADRENAL MEDULLA

- Adrenal medullary hormones are the amines derived from catechol and so these hormones are called catecholamines.

  Catecholamines secreted by adrenal medulla

  1. Adrenaline or epinephrine
  2. Noradrenaline or norepinephrine
  3. Dopamine.

ACTIONS OF ADRENALINE AND NORADRENALINE

- Adrenaline and noradrenaline stimulate the nervous system. Adrenaline has significant effects on metabolic functions and both adrenaline and noradrenaline have significant effects on cardiovascular system.

OVARIAN HORMONES

- The ovaries maintain the health of the female reproductive system.
- They secrete two main hormones—estrogen and progesterone.

Hormones of the Ovaries

- Ovaries produce and release two groups of sex hormones—progesterone and estrogen
- There are actually three major estrogens, known as

  1.Estradiol

  2.Estrone

  3. Estriol
- These substances work together to promote the healthy development of female sex characteristics during puberty and to ensure fertility.
- Estrogen (estradiol, specifically) is instrumental in breast development, fat distribution in the hips, legs, and breasts, and the development of reproductive organs.
- To a lesser extent, the ovaries release the hormone relaxin prior to give birth. Another minor hormone is inhibin, which is important for signaling to the pituitary to inhibit follicle-stimulating hormone secretion

PROGESTERONE

- Progesterone is one of the hormones in our bodies that stimulates and regulates various functions.
- Progesterone plays a role in maintaining pregnancy.
- The hormone is produced in the ovaries, the placenta (when a woman gets pregnant) and the adrenal glands.
- It helps prepare your body for conception and pregnancy and regulates the monthly menstrual cycle. It also plays a role in sexual desire

TESTICULAR HORMONES

- The testes secrete testosterone, which is necessary for proper physical development in boys.
- In adult hood, testosterone maintains libido(sexual desire), muscle strength, and bone density.
- Testosterone is necessary for proper physical development in boys.
- It is the primary androgen, which is the term for any substance that stimulates and/or maintains masculine development.
- During puberty, testosterone is involved in many of the processes that transition a boy to manhood, including:

➢ Healthy development of male sex organs

➢ Growth of facial and body hair

➢ Lowering of the voice

➢ Increase in height

- Increase in muscle mass
- Growth of the Adam's apple
- The importance of testosterone is not limited to puberty. Throughout adulthood, the hormone is integral in a variety of functions, such as:
- Maintaining libido
- Sperm production
- Maintaining muscle strength and mass
- Promoting healthy bone density

Testosterone Production

The hypothalamus and pituitary gland control how much testosterone the testes produce and secrete.

- **The hypothalamus sends a signal to the pituitary gland to release gonadotrophic substances (follicle stimulating hormone and luteinizing hormone).**
- **Luteinizing hormone (LH) stimulates testosterone production. If too much testosterone is produced, the hypothalamus alerts the pituitary gland to make less LH, which tells the testes to decrease testosterone levels.**

I.3PATHOPHYSIOLOGY

Pathophysiology of hypothalamus

- Disorder presenting in the hypothalamus, may be caused by damage resulting from malnutrition, including anorexia ,eating disorders, genetic disorders, radiation, surgery, head trauma, lesion, tumour or other physical injury to the hypothalamus.

  •The hypothalamus is the control center for several endocrine functions.

- Numerous dysfunctions manifest as a result of hypothalamic disease.

- Damage to the hypothalamus may cause disruptions in body temperature regulation, growth, weight, sodium and water balance, milk production, emotions, and sleep cycles. Hypopituitarism, neurogenic diabetes insipidus, hypothyroidism…..

HORMONE	TOO HIGH	TOO LOW
ANTI-DIURETIC HORMONE	Water retention, diluted blood, seizure	Dehydration, blood pressure drop
CORTICOTROPIN-RELEASING HORMONE	Diabetes, high blood pressure, osteoporosis, abdominal obesity, acne, dysfunctional menstrual cycle, infertility, muscle loss and weakness (i.e. Cushing's syndrome)	Weight loss, low blood pressure, gastrointestinal distress, anorexia nervosa, increased skin pigmentation in areas not exposed to sun (e.g. hand creases, gentials)
GONADOTROPIN-RELEASING HORMONE	Disrupted connection between the hypothalamus, pituitary gland, and gonads (i.e. menopause, removal of the testes or ovaries)	Poor bone health, no puberty, infertility (i.e. Kallmann syndrome)
GROWTH HORMONE-RELEASING HORMONE	Abnormal enlargement of hands, feet, and skull which alter facial features (i.e. acromegaly), diabetes, menstrual disorders	In children—delayed physical growth, delayed puberty In adults—decreased muscle mass and increased body fat
OXYTOCIN	Beyond the brain, linked to enlarged prostate resulting in urination difficulty	Linked to breastfeeding difficulty in women, and autism/poor social functioning in developing children
SOMATOSTATIN (aka GROWTH HORMONE-INHIBITING HORMONE)	Beyond the brain, diabetes, gallstones, intolerance to fat in the diet, and diarrhea	Variety of physiological problems, including uncontrolled growth hormone secretion
THYROTROPIN-RELEASING HORMONE	Weight loss, weak muscles, excessive sweating, excessive menstrual flow (i.e. hyperthyroidism)	Fatigue, depression, weight gain, feeling cold, constipation, dry skin and hair, hair loss, heart problems, dyslipidemia, irregular menstrual cycles (i.e. hypothyroidism)

PATHOPHYSIOLOGY OF PITUITARY GLAND

HYPERACTIVITY OF ANTERIOR PITUITARY

1. Gigantism

Gigantism is the pituitary disorder characterized by excess growth of the body. The subjects look like the giants with average height of about 7-8 feet.

Cause

- Gigantism is due to hypersecretion of GH in childhood or in the pre-adult life before the fusion of epiphysis of bone . It occurs due to pituitary tumors.

Signs and symptoms

- The general over growth of the person leads to the development of a huge stature with a height of more than 7 or 8 feet. The limbs are disproportionately long
- The giants are hyperglycemic and they develop glycosuria and pituitary diabetes.

2. Acromegaly

- It is the disorder characterized by the enlargement, thickening and broadening of bones, particularly in the extremities of the body.

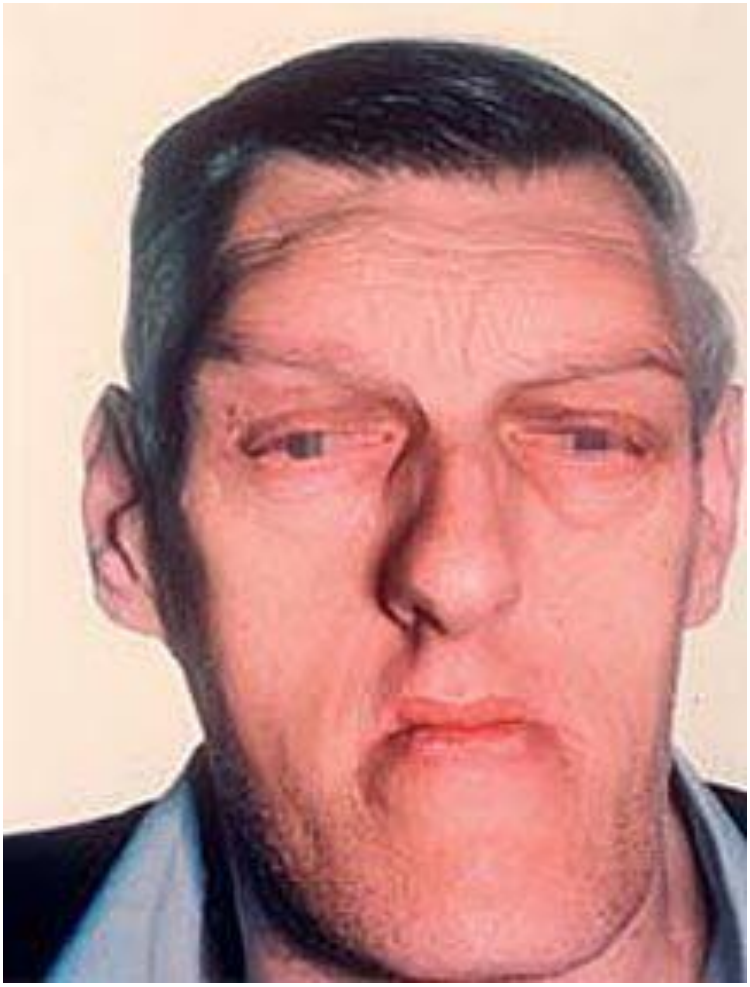
Cause

- Acromegaly is due to hypersecretion of GH in adults after the fusion of epiphysis with shaft of the bone. Hypersecretion of GH is due to adenomatous tumor of anterior pituitary involving the acidophil cells.

Signs and symptoms

- The striking facial features are protrusion of supraorbital ridges, broadening of nose, thickening of lips, thickening and wrinkles formation on forehead, and protrusion of lower jaw

(prognathism). The face with these features is called acromegalic or guerrilla face.



- Enlargement of hands and feet with bowing of spine (kyphosis)
- The scalp is thickened and thrown into folds or wrinkles like bulldog scalp. There is general overgrowth of body hair
- The visceral organs such as lungs, heart, liver and spleen are enlarged
- Thyroid gland, parathyroid glands and the adrenal glands show hyperactivity
- Hyperglycemia and glucosuria occur resulting in diabetes mellitus


MEDMTube
ACROMEGALY
Symptoms & Treatments

- Hypertension
- Headache
- Visual disturbances

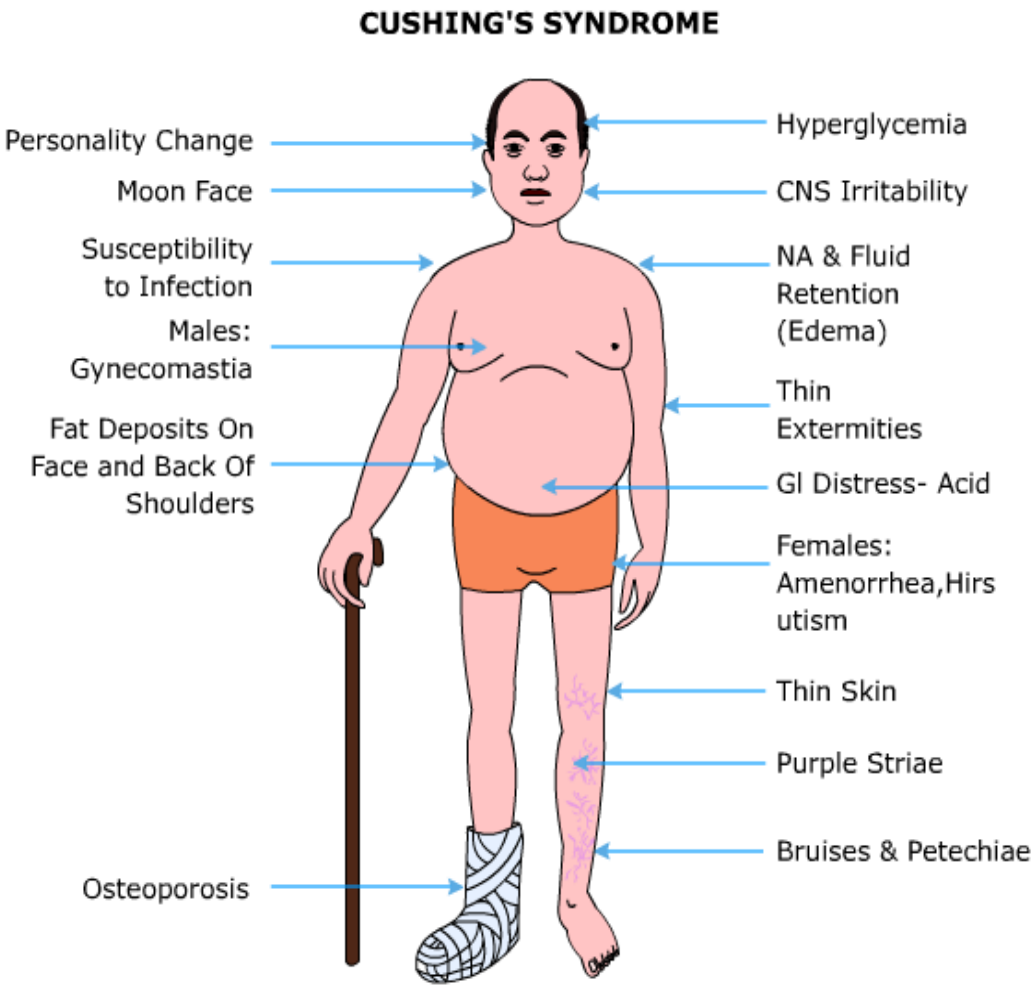
3. Acromegalic Gigantism

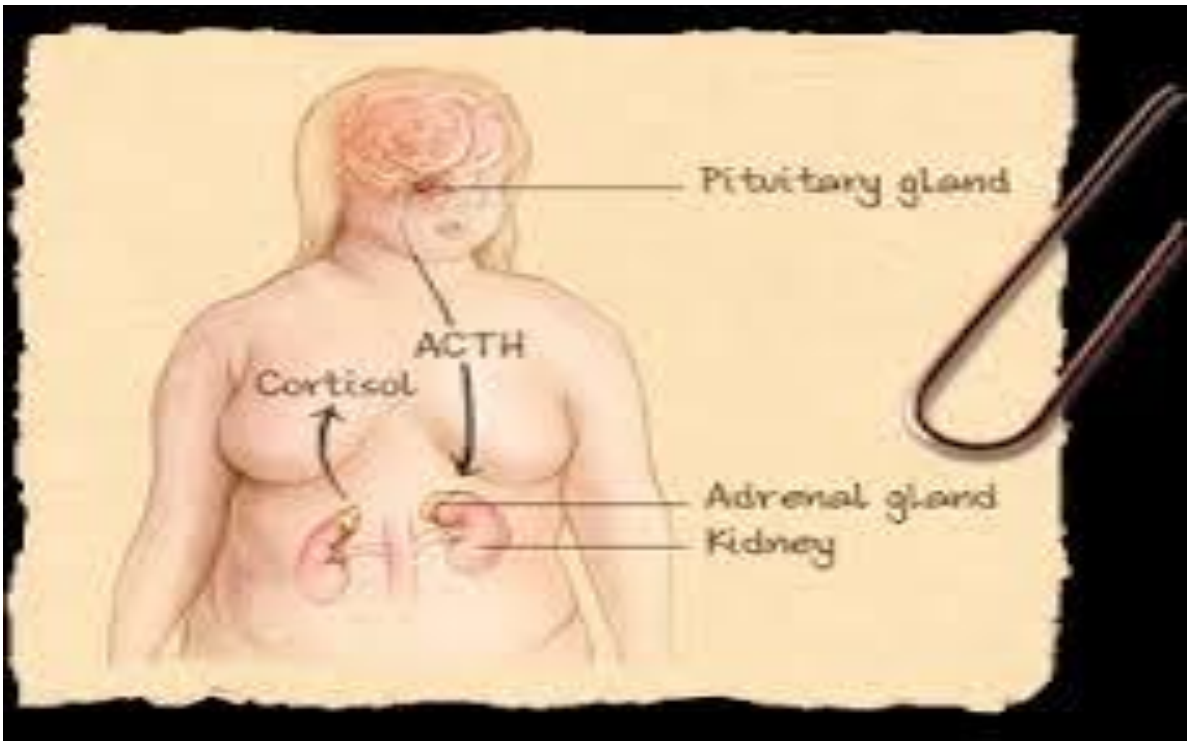
- It is a rare disorder with symptoms of both gigantism and acromegaly.
- Hypersecretion of GH in children, before the fusion of epiphysis with shaft of the bones causes gigantism.

- And, if hypersecretion of the GH is continued even after the fusion of epiphysis, the symptoms of acromegaly also appear.

4. Cushing's Disease

- It is also a rare disease characterized by obesity.





HYPOACTIVITY OF ANTERIOR PITUITARY

- **1. Dwarfism**

  It is a pituitary disorder in children characterized by the stunted growth.

  Causes

- Reduction in the GH secretion in infancy or early childhood causes dwarfism. It occurs because of the following reasons:
- Deficiency of GHRH from hypothalamus.
- Deficiency of somatomedin-C
- Atrophy or degeneration of acidophilic cells in the anterior pituitary.
- Tumor of chromophobes: It is a nonfunctioning tumor, which compresses and destroys the normal GH secreting cells.

- **<u>Pan hypopituitarism</u>**: In this condition, there is reduction in the secretion of all the hormones of anterior pituitary gland. This type of dwarfism is associated with other symptoms due to the deficiency of other anterior pituitary hormones

<u>Signs and symptoms</u>

- The primary symptom of hypopituitarism in children is the stunted skeletal growth. The maximum height of anterior pituitary dwarf at the adult age is only about 3 feet
- But the proportions of different parts of the body are almost normal. Only, the head becomes slightly larger in relation to the body .
- Pituitary dwarfs do not show any deformity and their mental activity is normal with no mental retardation
- Reproductive function is not affected, if there is only GH deficiency. However, in pan hypopituitarism, the dwarfs do not obtain puberty due to deficiency of gonadotropic hormones.

Laron dwarfism

- Laron dwarfism is a genetic disorder that occurs due to the presence of abnormal GH secretagogue receptors.

Psychogenic dwarfism

- Dwarfism occurs if the child is exposed to extreme emotional deprivation or stress. The short stature is because of deficiency of GH. This type of dwarfism is called psychogenic dwarfism, psychosocial dwarfism or stress dwarfism.

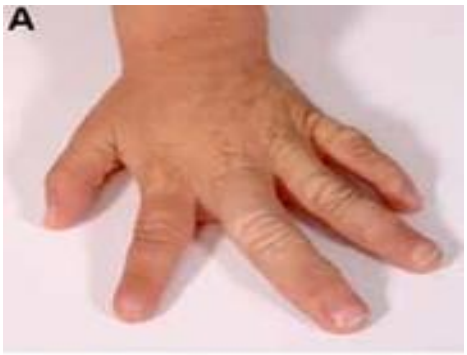
2. Acromicria

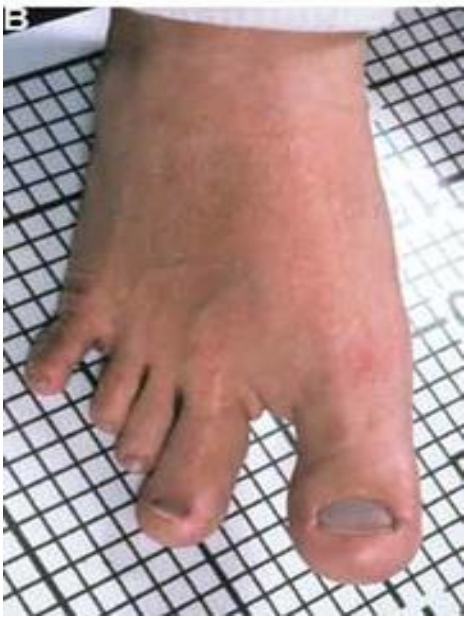
- It is a rare disease in adults characterized by the atrophy of the extremities of the body.

Causes

- Deficiency of GH in adults causes acromicria. The secretion of GH decreases in the following conditions
- Deficiency of GH releasing hormone from hypothalamus
- Atrophy or degeneration of acidophilic cells in the anterior pituitary
- Tumor of chromophobes: It is a nonfunctioning tumor, which compresses and destroys the normal cells secreting the GH
- Panhypopituitarism: In this condition, there is reduction in the secretion of all the hormones of anterior pituitary gland.

Acromicria is associated with other symptoms due to the deficiency of other anterior pituitary hormones.





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Signs and symptoms

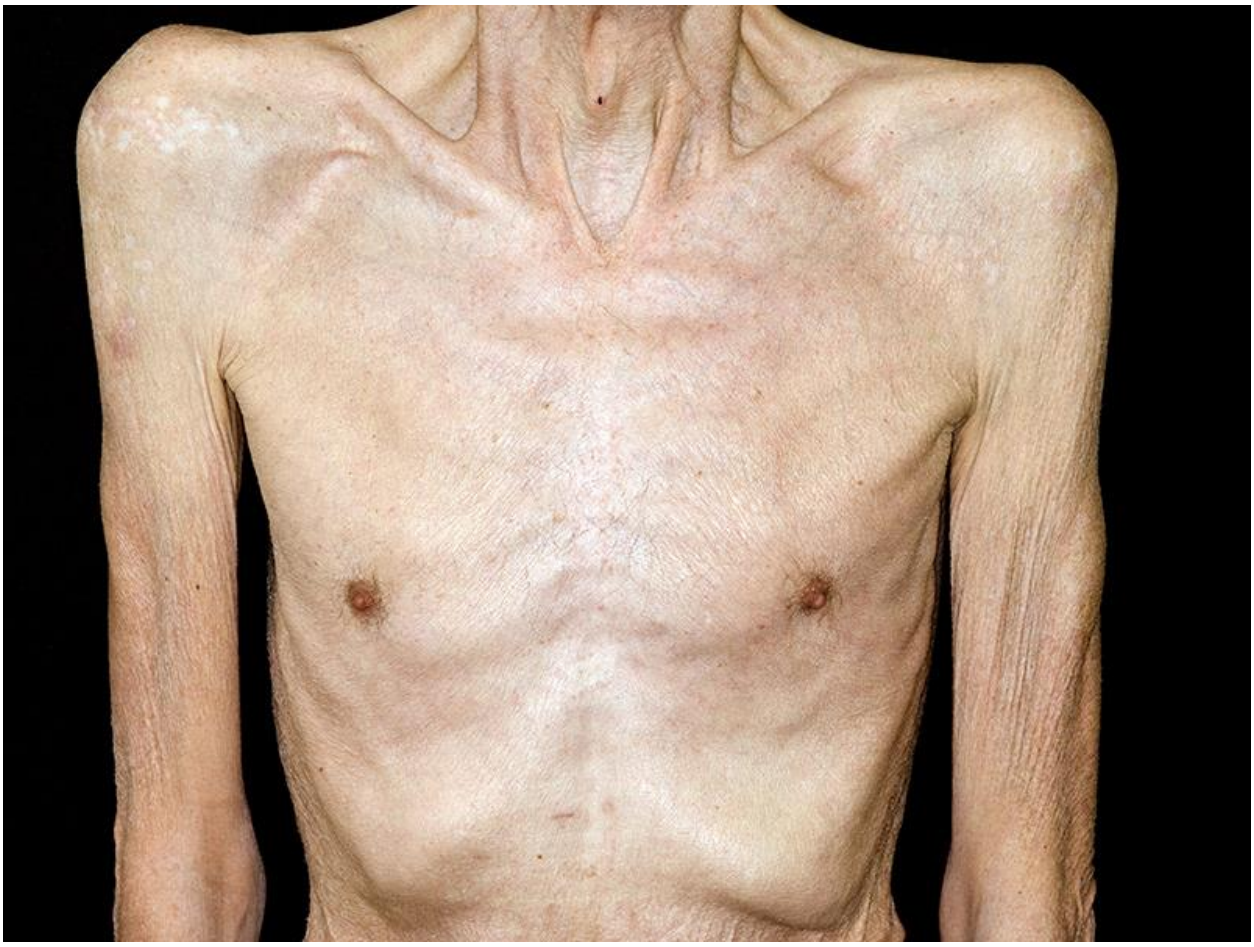
- Atrophy and thinning of extremities of the body, (hands and feet) are the major symptoms in acromicria
- Acromicria is mostly associated with hypothyroidism and hyposecretion of adrenocortical hormones
- The person becomes lethargic and obese
- There is loss of sexual functions.

Simmond's Disease

- It is a rare pituitary disease. It is also called pituitary cachexia.

Causes

- It occurs mostly in pan hypopituitarism, i.e. hyposecretion of all the anterior pituitary hormones due to the atrophy or degeneration of anterior pituitary.



Symptoms

- A major feature of Simmond's disease is the rapidly developing senile decay. Thus, a 30 years old person looks like a 60 years old person.

- There is loss of hair over the body and loss of teeth.
- The skin on face becomes dry and wrinkled. So, there is shrunken appearance of facial features. It is the most common feature of this disease.

ADH

- In the absence of ADH, the distal convoluted tubule and collecting duct are totally impermeable to water.
- So, reabsorption of water does not occur in the renal tubules and dilute urine is excreted. This leads to loss of large amount of water through urine. This condition is called diabetes insipidus and the excretion of large amount of water is called diuresis.

PATHOPHYSIOLOGY/ DISORDERS OF THYROID GLAND

HYPERTHYROIDISM

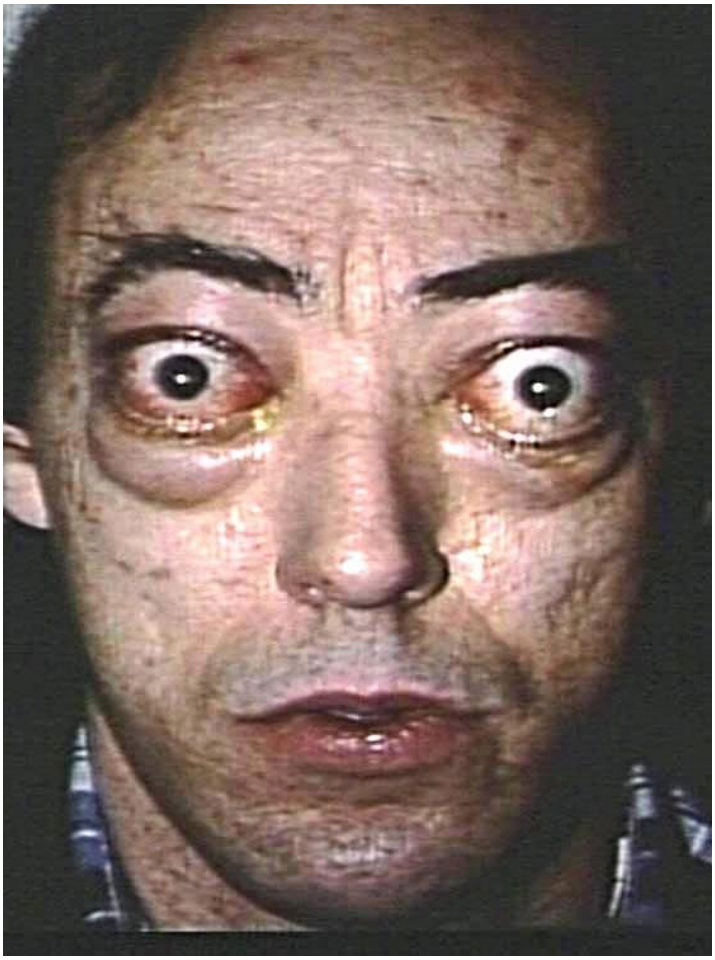
- Increased secretion of thyroid hormones is called hyperthyroidism.

Causes of Hyperthyroidism

- Hyperthyroidism is caused by:
- 1. Graves' disease
- 2. Thyroid adenoma.

1. Graves' disease

- Graves' disease is an autoimmune disease and it is the most common cause of hyperthyroidism.
- Normally, TSH combines with surface receptors of thyroid cells and causes the synthesis and secretion of thyroid hormones.
- In Graves' disease, the B lymphocytes (plasma cells) produce autoimmune antibodies called thyroid-stimulating autoantibodies (TSAbs).
- These antibodies act like TSH by binding with membrane receptors of TSH and activating cAMP system of the thyroid follicular cells. This results in hypersecretion of thyroid hormones.
- Antibodies act for a long time even up to 12 hours in contrast to that of TSH, which lasts only for an hour .
- The high concentration of thyroid hormones caused by the antibodies suppresses the TSH production also.
- So, the concentration of TSH is low or almost zero in plasma of most of the hyperthyroid patients.



2. Thyroid adenoma

- Sometimes, a localized tumor develops in the thyroid tissue.
- It is known as thyroid adenoma and it secretes large quantities of thyroid hormones.
- It is not associated with autoimmunity.

- As far as this adenoma remains active, the other parts of thyroid gland cannot secrete the hormone.
- This is because; the hormone secreted from adenoma depresses the production of TSH.
-

Signs and Symptoms of Hyperthyroidism

- 1.Intolerance to heat as the body produces lot of heat due to increased basal metabolic rate caused by excess of thyroxine
- 2. Increased sweating due to vasodilatation
- 3. Decreased body weight due to fat mobilization
- 4. Diarrhea due to increased motility of GI tract
- 5. Muscular weakness because of excess protein catabolism
- 6. Nervousness, extreme fatigue, inability to sleep
- Extreme anxiety or worry. All these symptoms are due to the excess stimulation of neurons in the central nervous system

7. Toxic goiter:multinodular goiter associated with hyperthyroidism

8.Polycythemia

9.Cardiac failure

HYPOTHYROIDISM

- Decreased secretion of thyroid hormones is called hypothyroidism. Hypothyroidism leads to myxedema in adults and cretinism in children.

Myxedema

- Myxedema is the hypothyroidism in adults, characterized by generalized edematous appearance.

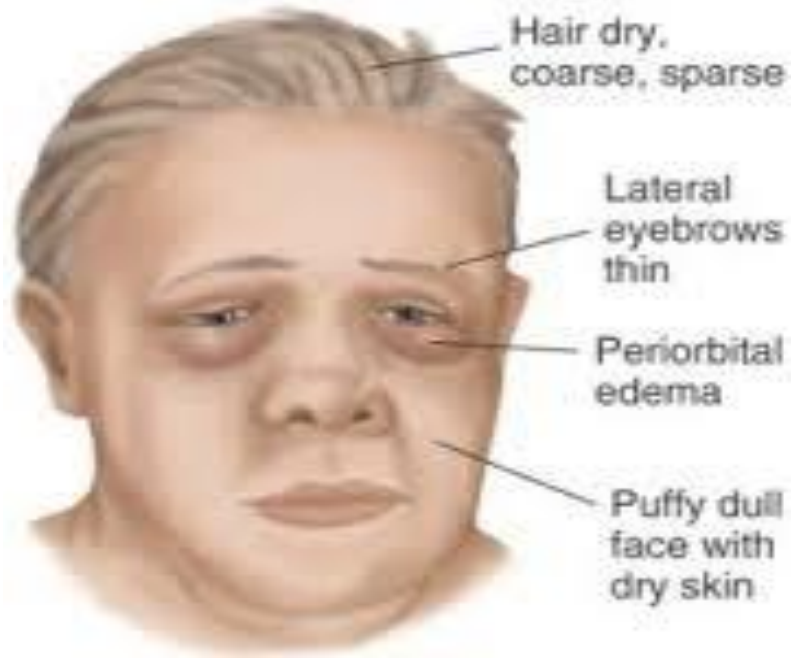
Causes for myxedema

- Myxedema occurs due to diseases of thyroid gland, genetic disorder or iodine deficiency.
- In addition, it is also caused by deficiency of thyroid-stimulating hormone or thyrotropin-releasing hormone.
- Common cause of myxedema is the autoimmune disease called Hashimoto's thyroiditis, which is common in late middle-aged women ,In most of the patients, it starts with glandular infammation called thyroiditis caused by autoimmune antibodies.
- Later it leads to destruction of the glands.

Signs and symptoms of myxedema

- Typical feature of this disorder is an edematous appearance throughout the body. It is associated with the following symptoms:
- 1. Swelling of the face
- 2. Bagginess under the eyes
- 3. Non-pitting type of edema, i.e. when pressed, it does not make pits and the edema is hard.
- It is because of accumulation of proteins with hyaluronic acid and chondroitin sulfate, which form a hard tissue with increased accumulation of fluid

- 4.Atherosclerosis: It is the hardening of the walls of arteries because of accumulation of fat deposits and other substances. In myxedema, it occurs because of increased plasma level of cholesterol which leads to deposition of cholesterol on the walls of the arteries.
- Atherosclerosis produces arteriosclerosis, which refers to thickening and stiffening of arterial wall.
- Arteriosclerosis causes hypertension.
- Other general features of hypothyroidism in adults are:
- 1.Anemia
- 2.Fatigue
- 3.Extreme somnolence with sleeping up to 14 to 16 hours per day
- 4.Decreased cardiovascular functions such as reduction in rate and force of contraction of the heart, cardiac output and blood volume
- 5.Increase in body weight
- 6.Constipation
- 7. Depressed hair growth
- 8. Frog-like husky voice
- 9. Cold intolerance.



Cretinism

- **Cretinism** is the hypothyroidism in children, characterized by stunted growth.

Causes for cretinism

- Cretinism occurs due to congenital absence of thyroid gland, genetic disorder or lack of iodine in the diet.

Features of cretinism

- A newborn baby with thyroid deficiency may appear normal at the time of birth because thyroxine might have been supplied from mother. But a few weeks after birth, the baby starts developing the signs like sluggish movements and croaking sound while crying. Unless treated immediately, the baby will be mentally retarded permanently.
- Skeletal growth is more affected than the soft tissues. So, there is stunted growth with bloated body. The tongue becomes so big that it hangs down with dripping of saliva. The big tongue obstructs swallowing and breathing. The tongue produces characteristic guttural breathing that may sometimes choke the baby.

Cretin Vs dwarf

- A cretin is different from pituitary dwarf. In cretinism, there is mental retardation and the different parts of the body are disproportionate. Whereas, in dwarfism, the development of nervous system is normal and the parts of the body are proportionate .The reproductive function is affected in cretinism but it may be normal in dwarfism.

GOITER

- Goiter means enlargement of the thyroid gland. It occurs both in hypothyroidism and hyperthyroidism.
- Goiter in Hyperthyroidism – Toxic Goiter
- Toxic goiter is the enlargement of thyroid gland with increased secretion of thyroid hormones, caused by thyroid tumor.
- Goiter in Hypothyroidism – Non-toxic Goiter
- Non-toxic goiter is the enlargement of thyroid gland without increase in hormone secretion. It is also called hypothyroid goiter.

Based on the cause, the non-toxic hypothyroid goiter is classified into two types.

1. **Endemic colloid goiter**
2. **Idiopathic non-toxic goiter**

1.Endemic colloid goiter

- Endemic colloid goiter is the non-toxic goiter caused by iodine deficiency. It is also called iodine deficiency goiter. Iodine deficiency occurs when intake is less than 50 μg/day. Because of lack of iodine, there is no formation of hormones. By feedback mechanism, hypothalamus and anterior pituitary are stimulated.

- It increases the secretion of TRH and TSH. The TSH then causes the thyroid cells to secrete tremendous amounts of thyroglobulin into the follicle. As there are no hormones to be cleaved, the thyroglobulin remains as it is and gets accumulated in the follicles of the gland. This increases the size of gland.

- In certain areas of the world, especially in the Swiss Alps, Andes, Great Lakes region of United States and in India, particularly in Kashmir Valley, the soil does not contain enough iodine. Therefore, the foodstuffs also do not contain iodine. The endemic colloid goiter was very common in these parts of the world before the introduction of iodized salts.

- Idiopathic non-toxic goiter is the goiter due to unknown cause. Enlargement of thyroid gland occurs even with- out iodine deficiency. The exact cause is not known.
- It is suggested that it may be due to thyroiditis and deficiency of enzymes such as peroxidase, iodinase and deiodi- nase, which are required for thyroid hormone synthesis. Some foodstuffs contain goiterogenic substances (goitrogens).

2.Idiopathic non-toxic goiter

- These substances contain antithyroid substances like propylthiouracil. Goitrogens suppress the synthesis of thyroid hormones.
- Therefore, TSH secretion increases, resulting in enlargement of the gland. Such goitrogens are found in vegetables like turnips and cabbages. Soybean also contains some amount of goitrogens.

- The goitrogens become active only during low iodine intake.

TREATMENT FOR THYROID DISORDERS

TREATMENT FOR HYPERTHYROIDISM

1.By using Antithyroid Substances

- Antithyroid substances are the drugs which suppress the secretion of thyroid hormones. Hyperthyroidism in early stage can be treated by antithyroid substances.

Three well-known antithyroid substances are:

i. Thiocyanate
ii. Thiourylenes
iii.High concentration of inorganic iodides.

i.Thiocyanate

- Thiocyanate prevents synthesis of thyroxine by inhibiting iodide trapping.

ii.Thiourylenes

- Thiourylenes are the thiourea-related substances such as propylthiouracil and methimazole, which prevent the formation of thyroid hormone from iodides and tyrosine

2. By Surgical Removal

- In advanced cases of hyperthyroidism, treatment by using antithyroid substances is not possible. So, thyroid gland of these patients must be removed. Surgical removal of thyroid gland is called thyroidectomy

TREATMENT FOR HYPOTHYROIDISM

- The only treatment for hypothyroidism is the administration of thyroid extract or ingestion of pure thyroxine in the form of tablets, orally.

PARATHYROID

DISORDERS OF PARATHYROID GLANDS

- Disorders of parathyroid glands are of two types:
  I. Hypoparathyroidism
  II. Hyperparathyroidism.

HYPOPARATHYROIDISM – HYPOCALCEMIA

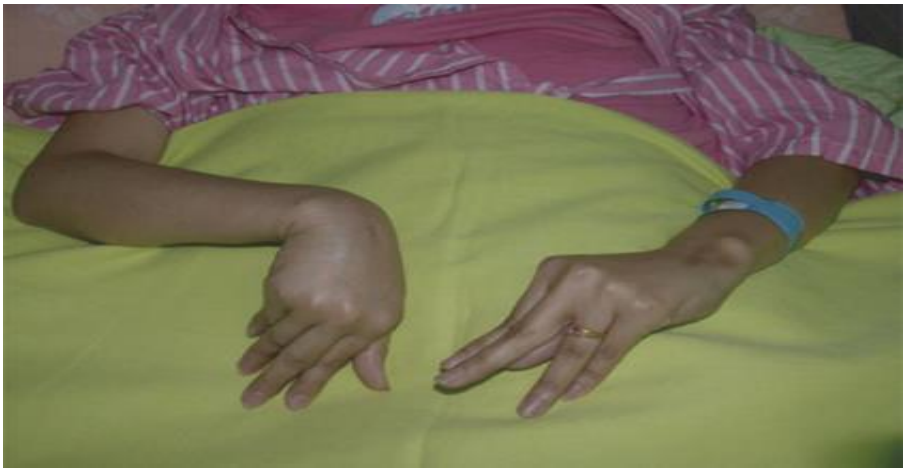
- Hyposecretion of PTH is called hypoparathyroidism
-
- **Causes for Hypoparathyroidism**

  1.Surgical removal of parathyroid glands (parathyroidectomy)
  2. Removal of parathyroid glands during surgical removal of thyroid gland (thyroidectomy)
  3. Autoimmune disease
  4. Deficiency of receptors for PTH in the target cells. In this, the PTH secretion is normal or increased but the hormone cannot act on the target cells. This condition is called pseudo hypoparathyroidism.
- Hypoparathyroidism leads to hypocalcemia, by decreasing the resorption of calcium from bones. Hypocalcemia causes neuromuscular hyperexcitability, resulting in hypocalcemic tetany. Normally, tetany occurs when plasma calcium level falls below 6 mg/dL from its normal value of 9.4 mg/dL.

- **Hypocalcemic Tetany**

- Tetany is an abnormal condition characterized by violent and painful muscular spasm (spasm = involuntary muscular

contraction), particularly in feet and hand. It is because of hyperexcitability of nerves and skeletal muscles due to calcium deficiency.



HYPERPARATHYROIDISM – HYPERCALCEMIA

- Hypersecretion of PTH is called hyperparathyroidism.
- It results in hypercalcemia. Hyperparathyroidism is of three types:

1. Primary hyperparathyroidism

- Primary hyperparathyroidism is due to the development of tumor in one or more parathyroid glands. Sometimes, tumor may develop in all the four glands.

2. Secondary hyperparathyroidism

- Secondary hyperparathyroidism is due to the physiological compensatory hypertrophy of parathyroid glands, in response to hypocalcemia which occurs due to other pathological conditions such as:

i.Chronic renal failure
ii. Vitamin D deficiency
iii. Rickets.

3. Tertiary hyperparathyroidism

-
- Tertiary hyperparathyroidism is due to hyperplasia (abnormal increase in the number of cells) of all the parathyroid glands that develops due to chronic secondary hyperparathyroidism

Hypercalcemia

- Hypercalcemia is the increase in plasma calcium level. It occurs in hyperparathyroidism because of increased resorption of calcium from bones.

Signs and symptoms of hypercalcemia

- Depression of the nervous system
- Lack of appetite
- Constipation.
- Depressive effects of hypercalcemia are noticed when the blood calcium level increases to 12 mg/dL. The condition becomes severe with 15 mg/dL and it becomes lethal when blood calcium level reaches 17 mg/dL.

PATHOPHYSIOLOGY OF PANCREATIC HORMONES HYPOACTIVITY – DIABETES MELLITUS

- Diabetes mellitus is a metabolic disorder characterized by high blood glucose level, associated with other manifestations.
- 'Diabetes' means 'polyuria' and 'mellitus' means 'honeyed or sweet'.
- The name 'diabetes mellitus' was coined by Thomas Willis, who discovered sweetness of urine from diabetics in 1675.
- In most of the cases, diabetes mellitus develops due to deficiency of insulin.

Classification of Diabetes Mellitus

- There are several forms of diabetes mellitus, which occur due to different causes.
- Diabetes may be primary or secondary. Primary diabetes is unrelated to another disease.
- Secondary diabetes occurs due to damage or disease of pancreas by another disease or factor.
- Type I diabetes mellitus is due to deficiency of insulin because of destruction of β cells in islets of Langerhans.

Type I Diabetes Mellitus

- This type of diabetes mellitus may occur at any age of life. But, it usually occurs before 40 years of age and the persons

affected by this require insulin injection. So it is also called **insulin-dependent diabetes mellitus** (IDDM).

- When it develops at infancy or childhood, it is called **juvenile diabetes**.
- Type I diabetes mellitus develop rapidly and progresses at a rapid phase.
- It is not associated with obesity, but may be associated with acidosis or ketosis.

Causes of type I diabetes mellitus

- 1. Degeneration of β-cells in the islets of Langerhans of pancreas
- 2. Destruction of β-cells by viral infection
- 3. Congenital disorder of β-cells
- 4. Destruction of β-cells during autoimmune diseases.
- It is due to the development of antibodies against β-cells.

- Diabetes mellitus that occurs before 25 years. It is due to hereditary defects in insulin secretion.

Type II Diabetes Mellitus

- Type II diabetes mellitus is due to insulin resistance (failure of insulin receptors to give response to insulin). So, the body is unable to use insulin. About 90% of diabetic patients have type II diabetes mellitus. It usually occurs after 40 years.
- Only some forms of Type II diabetes require insulin. In most cases, it can be controlled by oral hypoglycemic drugs. So it is also called **non insulin- dependent diabetes mellitus** (NIDDM).

- Type II diabetes mellitus may or may not be associated with ketosis, but often it is associated with obesity.

Causes for type II diabetes mellitus

- In this type of diabetes, the structure and function of β-cells and blood level of insulin are normal. But insulin receptors may be less, absent or abnormal, resulting in insulin resistance. Common causes of insulin resistance are:
  1. Genetic disorders (significant factors causing type II diabetes mellitus)
  2. Lifestyle changes such as bad eating habits and physical inactivity, leading to obesity
  3. Stress.

Other forms of type II diabetes mellitus

1.Gestational diabetes: It occurs during pregnancy. It is due to many factors such as hormones secreted during pregnancy, obesity and lifestyle before and during pregnancy. Usually, diabetes disappears after delivery of the child. However, the woman has high risk of development of type II diabetes later.
2.Pre-diabetes: It is the stage between normal condition and diabetes. The person does not show overt (observable) symptoms of diabetes but there is an increase in blood glucose level. Though pre-diabetes is reversible, the affected persons are at a high risk of developing type II diabetes mellitus.

Secondary Diabetes Mellitus

Secondary diabetes mellitus is rare and only about 2% of diabetic patients have secondary diabetes. It may be temporary or may become permanent due to the underlying cause.

Causes of secondary diabetes mellitus

1. Endocrine disorders such as gigantism, acromegaly and Cushing's syndrome.
Hyperglycemia in these conditions causes excess stimulation of β-cells. Constant and excess stimulation, in turn causes burning out and degeneration of β-cells. The β-cell exhaustion leads to permanent diabetes mellitus.
2.Damage of pancreas due to disorders such as chronic pancreatitis, cystic fibrosis and hemochromatosis (high iron content in body causing damage of organs)
3.Pancreatectomy (surgical removal)
4.Liver diseases such as hepatitis C and fatty liver
5.Autoimmune diseases such as celiac disease
6. Excessive intake of alcohol and opiates

Signs and Symptoms of Diabetes Mellitus

Various manifestations of diabetes mellitus develop because of three major setbacks of insulin deficiency.
1. Increased blood glucose level (300 to 400 mg/dl) due to reduced utilization by tissue
2. Mobilization of fats from adipose tissue for energy purpose, leading to elevated fatty acid content in blood. This causes deposition of fat on the wall of arteries and development of atherosclerosis
3. Depletion of proteins from the tissues.

Following are the signs and symptoms of diabetes mellitus:

1. ***Glucosuria***: Glucosuria is the loss of glucose in urine. Normally, glucose does not appear in urine. When glucose level rises above 180 mg/dL in blood, glucose appears in urine. It is the renal threshold level for glucose.

2. ***Osmotic diuresis***: Osmotic diuresis is the diuresis caused by osmotic effects. Excess glucose in the renal tubules develops osmotic effect. Osmotic effect decreases the re-absorption of water from renal tubules, resulting in diuresis. It leads to polyuria and polydipsia.

3. ***Polyuria:*** Excess urine formation with increase in the frequency of voiding urine is called polyuria. It is due to the osmotic diuresis caused by increase in blood glucose level.

4. ***Polydipsia:*** Increase in water intake is called polydipsia. Excess loss of water decreases the water content and increases the salt content in the body. This stimulates the thirst center in hypothalamus. Thirst center, in turn increases the intake of water.

5. Polyphagia: Polyphagia means the intake of excess food. It is very common in diabetes mellitus.

6. Asthenia: Loss of strength is called asthenia. Body becomes very weak because of this. Asthenia occurs due to protein depletion, which is caused by lack of insulin. Lack of insulin causes decrease in protein synthesis and increase in protein breakdown, resulting in protein depletion. Protein depletion

also occurs due to the utilization of proteins for energy in the absence of glucose utilization.

7. ***Acidosis:*** During insulin deficiency, glucose cannot be utilized by the peripheral tissues for energy. So, a large amount of fat is broken down to release energy. It causes the formation of excess ketoacids, leading to acidosis.
One more reason for acidosis is that the ketoacids are excreted in combination with sodium ions through urine (ketonuria).

8. **Acetone breathing:** In cases of severe ketoacidosis, acetone is expired in the expiratory air, giving the characteristic acetone or fruity breath odor. It is a life-threatening condition of severe diabetes.

9. Kussmaul breathing: Kussmaul breathing is the increase in rate and depth of respiration caused by severe acidosis.

10. Circulatory shock: Osmotic diuresis leads to dehydration, which causes circulatory shock. It occurs only in severe diabetes.

11. Coma : Due to Kussmaul breathing, large amount of carbon dioxide is lost during expiration. It leads to drastic causing severe acidosis and coma. It occurs in severe cases of diabetes mellitus. Increase in the blood glucose level develops hyperosmolarity of plasma which also leads to coma. It is called hyperosmolar coma.

Complications of Diabetes Mellitus

- Prolonged hyperglycemia in diabetes mellitus causes dysfunction and injury of many tissues, resulting in some complications. Development of these complications is directly proportional to the degree and duration of hyperglycemia. However, the patients with well- controlled diabetes can postpone the onset or reduce the rate of progression of these complications.
- Initially, the untreated chronic hyperglycemia affects the blood vessels, resulting in vascular complications like atherosclerosis.

Vascular complications are responsible for the development of most of the complications of diabetes such as:

Cardiovascular complications like:

- i. Hypertension
- ii. Myocardial infarction: type of the blockage of the blood flow to the heart muscle

Diagnostic Tests for Diabetes Mellitus

Diagnosis of diabetes mellitus includes the determination of:

- 1. Fasting blood glucose
- 2. Postprandial blood glucose
- 3. Glucose tolerance test (GTT)
- 4. Glycosylated (glycated) hemoglobin.

- Determination of glycosylated hemoglobin is commonly done to monitor the glycemic control of the persons already diagnosed with diabetes mellitus.

DYSORDERS OF ADRENAL CORTEX

HYPERACTIVITY OF ADRENAL CORTEX

- Hypersecretion of adrenocortical hormones leads to the following conditions:
- 1. Cushing syndrome
- 2. Hyperaldosteronism
- 3. Adrenogenital syndrome.

HYPERALDOSTERONISM

Increased secretion of aldosterone is called hyperaldosteronism.

Causes and Types

Depending upon the causes, hyperaldosteronism is classified into two types:

- i. Primary hyperaldosteronism
  ii. Secondary hyperaldosteronism.

Primary Hyperaldosteronism

Primary hyperaldosteronism is otherwise known as Conn syndrome. It develops due to tumor in zona glomerulosa of adrenal cortex

Secondary Hyperaldosteronism

Secondary hyperaldosteronism occurs due to extra causes such as:
i.Congestive cardiac failure
ii.Nephrosis
iii. Toxaemia of pregnancy
iv. Cirrhosis of liver.

Signs and Symptoms

i.Increase in ECF volume and blood volume

ii.Hypertension due to increase in ECF volume and blood volume

iii. Severe depletion of potassium, which causes renal damage. The kidneys fail to produce concentrated urine. It leads to polyuria and polydipsia

iv. Muscular weakness due to potassium depletion

v.Metabolic alkalosis due to secretion of large amount of hydrogen ions into the renal tubules. Metabolic alkalosis reduces blood calcium level causing tetany.

ADRENOGENITAL SYNDROME

- Under normal conditions, adrenal cortex secretes small quantities of androgens which do not have any significance effect on sex organs or sexual function. However, secretion of abnormal quantities of adrenal androgens develops adrenogenital syndrome. Testosterone is responsible for the androgenic activity in adrenogenital syndrome.

Causes

- Adrenogenital syndrome is due to the tumor of zona reticularis in adrenal cortex.

Symptoms

- Adrenogenital syndrome is characterized by the tendency for the development of secondary sexual character of opposite sex.

Symptoms in females

- Increased secretion of androgens causes development of male secondary sexual characters. The condition is called adrenal virilism.
- Symptoms are:

- i.Masculinization due to increased muscular growth
- ii. Deepening of voice
- iii. Amenorrhea
- iv. Enlargement of clitoris
- v.Male type of hair growth.

Symptoms in males

Sometimes, the tumor of estrogen secreting cells produces more than normal quantity of estrogens in males.

- It produces some symptoms such as:

- i. Feminization
- ii. Gynecomastia (enlargement of breast)
- iii. Atrophy of testis
- iv. Loss of interest in women.

HYPOACTIVITY OF ADRENAL CORTEX

Hyposecretion of adrenocortical hormones leads to the following conditions:

- 1. Addison disease or chronic adrenal insufficiency
- 2. Congenital adrenal hyperplasia

ADDISON DISEASE OR CHRONIC ADRENAL INSUFFICIENCY

- Addison disease is the failure of adrenal cortex to secrete corticosteroids.

Types of Addison Disease

- i. Primary Addison disease due to adrenal cause

- ii. Secondary Addison disease due to failure of anterior pituitary to secrete ACTH
- iii. Tertiary Addison disease due failure of hypothalamus to secrete corticotropin-releasing factor (CRF).

Causes for Primary Addison Disease

- i. Atrophy of adrenal cortex due to autoimmune diseases
- ii. Destruction of the gland because of tuberculosis
- iii. Destruction of hormone-secreting cells in adrenal cortex by malignant tissues
- iv. Congenital failure to secrete cortisol
- v. Adrenalectomy and failure to take hormone therapy.

<u>Ovarian gland dysfunction</u>

Diseases and Disorders of the Ovaries

- **Osteoporosis:** Osteoporosis is commonly associated with menopause,..
- Menopause is marked by the rapid loss of estrogen.
- The role estrogen play in bone loss can best be described in terms of a battle between *osteoclasts* (bone absorbing cells) and *osteoblasts* (bone producing cells).
- Estrogen is on the side of the osteoblasts, but as the estrogens diminish, the osteoblasts are discouraged from producing more bone.
- As such, the osteoclasts win by absorbing more bone than is being produced by the osteoblasts.

- Estrogen replacement therapy during menopause protects bone mass and helps protect against the risk of osteoporotic fractures.
- **Ovarian Cancer:** Ovarian cancer is an extremely serious, but rare, disease. Its symptoms usually don't become apparent until the cancer has progressed into the later stages.
- **Ovarian Cysts:** Ovarian cysts are fluid-filled sacs that affect women of all ages, though mostly women of child-bearing age. Cysts are very common—and they can range in size from a pea to a grapefruit. The majority of cysts are harmless, though larger cysts (those larger than 5 cm in diameter) may need to be surgically removed because large cysts can twist the ovary and disrupt its blood supply.
- Below are some common symptoms of pathological cysts:
  - The most telltale symptom is pain and discomfort in the abdomen, vagina, low back.
  - Breast tenderness
  - Bloating
  - Increased hair growth on your face, back, and chest
  - Pain before or after your menstrual cycle and irregular periods
  - Infertility
  - Weight gain
  - Fatigue

DISORDERS OF THE TESTES: HYPOGONADISM

Hypogonadism is a testicular disorder associated with low testosterone.
Having testosterone levels that are too low causes a variety of problems, including:

- Decreased sex drive

- Diminished muscle mass
- Low sperm count (reduced fertility)
- Loss of body hair
- There are two types of hypogonadism—primary and secondary. Primary refers to a defect with the testicles, and secondary involves a problem in the pituitary gland that indirectly affects testosterone production.

CHAPTER II

TUMOR MARKERS

Definition

- A Tumor marker is a substance found in increased amounts in the blood,other body fluids and tissues that may suggests the presence of cancer.
- Many cancers are associated with the abnormal production of some molecules which can be measured in plasma. These molecules are known as tumor markers.

A good tumor maker should have those properties:

- **1.** A tumor marker should be present in or produced by tumor itself.
- **2.** A tumor marker should not be present in healthy tissues.
- **3.** Plasma level of a tumor marker should be at a minimum level in healthy subjects and in benign conditions.
- **4.** A tumor marker should be specific for a tissue, it should have different immunological properties when it is synthesized in other tissues.

- **5.** Plasma level of the tumor marker should be in proportion to the both size of tumor and activity of tumor.
- **6.** Half life of a tumor should not be very long
- **7.** A tumor marker should be present in plasma at a detectable level, eventhough tumor size is very small

Tumor markers can be classified as respect with the type of the molecule:

- **1.** Enzymes or isoenzymes (ALP, PAP)
- **2.** Hormones (calcitonin)
- **3.** Oncofetal antigens (AFP, CEA)
- **4.** Carbonhydrate epitopes recognised by
- monoclonal antibodies (CA 15-3,CA 19-9,
- CA125)
- **5.** Receptors (Estrogen, progesterone)
- 6. Genetic changes (mutations in some oncogenes and tumor suppressor genes. Some mutations in BRCA1 and 2 have been linked to hereditary breast and over cancer)

Potential uses of tumor markers

- Screening in general population
- Differential diagnosis of symptomatic patients
- Clinical staging of cancer
- Estimating tumor volume
- As a prognostic indicator for disease progression
- Evaluating the success of treatment
- Detecting the recurrence of cancer
- Monitoring reponse to therapy
- Radioimmunolocalization of tumor masses
- In order to use a tumor marker for screening in the presence of cancer in asymptomatic individuals in general population, the

marker should be produced by tumor cells and not be present in healthy people.

- However, **most tumor markers are present in normal, benign and cancer tissues and are not specific enough to be used for screening cancer**.
- Few markers are specific for a single individual tumor, most are found with different tumors of the same tissue type.
- They are present in higher quantities in blood from cancer patients than in blood from both healthy subjects and patients with benign diseases.
- Some tumor markers have a plasma level in proportion to the size of tumor while some tumor markers have a plasma level in proportion to the activity of tumor.
- The clinical staging of cancer is aided by quantitation of the marker.
- Serum level of marker reflects tumor burden.
- The level of the marker at the time of diagnosis may be used as a prognostic indicator for disease progression and patient survival. After successful initial treatment, such as surgery, the marker value should decrease. The rate of the decrease can be predicted.
- The magnitude of marker reduction may reflect the degree of success of the treatment.
- In the case of recurrence of cancer, marker increases again.
- Most tumor marker values correlate with the effectiveness of treatment.

ENZYMES

Alkaline Phosphatase (ALP)

- Increased alkaline phosphatase activities are seen in primary or secondary liver cancer. Its level may be helpful in evaluating metastatic cancer with bone or liver involvement. Placental ALP, regan isoenzyme, elevates in a variety of malignancies, including ovarian, lung, gastrointestinal cancers and Hodgkin's disease.

Prostatic acid phosphatase (PAP)

- It is used for staging prostate cancer and for monitoring therapy. Increased PAP activity may be seen in osteogenic sarcoma, multiple myeloma and bone metastasis of other cancers and in some benign conditions such as osteoporosis and hyperparathyroidism.

Prostate Specific Antigen (PSA)

- The clinical use of PAP has been replaced by PSA. PSA is much more specific for screening or for detection early cancer. It is found in mainly prostatic tissue.
- PSA exists in two major forms in blood circulation. The majority of PSA is complexed with some proteins. A minor component of PSA is free.
- PSA testing itself is not effective in detecting early prostate cancer. Urinary bladder catheterization and digital rectal examination may lead an increased PSA level in serum.
- The ratio between free and total PSA is an reliable marker for differentiation of prostatic cancer from benign prostatic hyperplasia.
- The use of PSA should be together with digital rectal examination and followed by transrectal ultrasonography for an accurate diagnosis of cancer.
- Serum level of PSA was found to be correlated with clinical stage, grade and metastasis

- The greatest clinical use of PSA is in the monitoring of treatment.
- This treatment includes radical prostatectomy, radiation therapy and antiandrogen therapy.
- The PSA level should fall below the detection limit.
- This may require 2-3 weeks. If it is still at a high level after 2-3 weeks, it must me assumed that residual tumor is present.
- Androgen deprivation therapy may have direct effect on the PSA level . **This subject must be considered always.**

HORMONES

Calcitonin

- Calcitonin is a hormone which decreases blood calcium concentration.
- Its elevated level is usually associated with medullary thyroid cancer.
- Calcitonin levels appear to correlate with tumor volume and metastasis.
- Calcitonin is also useful for monitoring treatment and detecting the recurrence of cancer.
- However calcitonin levels are also at a high levels in some patients with cancer of lung, breast, kidney, liver and in non malignant conditions such as pulmonary diseases, pancreatitis, Paget's disease, hyperparathyroidism, myeloproliferative disordes and pregnancy.

Human Chorionic Gonadotropin (hCG)

- It is a glycooprotein appears in pregnancy. Its high levels is a useful marker for tumors of placenta and some tumors of testes.

- hCG is also at a high level in patients with primary testes insufficiency.
- hCG does not cross the blood-brain barier. Higher levels in BOS may indicate metastase to brain.

ONCOFETAL ANTIGENS

- Most reliable markers in this group are α-fetoprotein and carcinoembryonic antigen (CEA)

α-Fetoprotein (AFP)

- α-fetoprotein is a marker for hepatocellular and germ cell carcinoma.
- It is also increased in pregnancy and chronic liver diseases.
- AFP is useful for screening (AFP levels greater than 1000 μg/L are indicative for cancer except pregnancy), determining prognosis and monitoring therapy of liver cancers.
- AFP is also a prognostic indicator of survival.
- Serum AFP levels is less than 10 μg/L in healthy adults. Elevated AFP levels are associated with shorter survival time.
- AFP and hCG combined are useful in classifying and staging germ cell tumors.One or both markers are increased in those tumors.

Carcinoembryonic antigen (CEA)

- It is a cell-surface protein and a well defined tumor marker.

- CEA is a marker for colorectal, gastrointestinal, lung and breast carcinoma.
- CEA levels are also elevated in smokers and some patients having benign conditions such as cirrhosis, rectal polyps, ulcerative colitis and benign breast disease.
- CEA testing should not be used for screening. Some tumors don't produce CEA. It is useful for staging and monitoring therapy.

CARBOHYDRATE MARKERS

- These markers either are antigens on the tumor cell surface or are secreted by tumor cells.
- They are high-molecular weight mucins . Monoclonal antibodies have been developed against these antigens.
- Most reliable markers in this group are CA 15-3, CA 125 and CA19-9.

CA 15-3

- CA 15-3 is a marker for breast carcinoma. Elevated CA 15-3 levels are also found in patients with pancreatic, lung, ovarian, colorectal and liver cancer and in some benign breast and liver diseases.
- It is not useful for diagnosis. It is most useful for monitoring therapy.

CA 125

- Although CA 125 is a marker for ovarian and endometrial carcinomas, it is not specific. CA 125 elevates in pancreatic, lung, breast, colorectal and gastrointestinal cancer, and in benign conditions such as cirrhosis, hepatitis, endometriosis, pericarditis and early pregnancy.

- It is useful in detecting residual disease in cancer patients following initial therapy.
- A preoperative CA 125 level of less than 65 kU/L is associated with a greater 5 years survival rate than is a level greater 65 kU/L.
- It is also useful in differentiating benign from malignant disease in patients with ovarian masses.
- In the detection of recurrence, use of CA 125 level as an indicator is about 75 % accurate.

CA 19-9

- CA 19-9 is a marker for both colorectal and pancreatic carcinoma.However elevated levels were seen in patients with hepatobiliary, gastric, hepatocellular and breast cancer and in benign conditions such as pancreatitis and benign gastrointestinal diseases.
- CA 19-9 levels correlate with pancreatic cancer staging.
- It is useful in monitoring pancreatic and colorectal cancer.
- Elevated levels of CA 19-9 can indicate recurrence before detected by radiography or clinical findings in pancreatic and colorectal cancer.

PROTEIN MARKERS

- Most reliable markers in this group are β_2-microglobulin, ferritin, thyroglobulin and immunoglobulin.

β_2-microglobulin

- β_2-microglobulin is a marker for multiple myeloma, Hodgkin lymphoma. It also increases in chronic inflammation and viral hepatitis.

Ferritin

- Ferritin is a marker for Hodgkin lymphoma, leukemia, liver, lung and breast cancer.

Thyroglobulin

- It is a useful marker for detection of differentiated thyroid cancer.

Immunoglobulin: Monoclonal immunoglobulin has been used as marker for multiple myeloma for more than 100 years.

- Monoclonal paraproteins appear as sharp bands in the globulin area of the serum protein .
- Bence-Jones protein is a free monoclonal immunoglobulin light chain in the urine and it is a reliable marker for multiple myeloma.

RECEPTOR MARKERS

- Estrogen and progesterone receptors are used in breast cancer as indicators for hormonal therapy.
- Patients with positive estrogen and progesterone receptors tend to respond to hormonal treatment.
- Those with negative receptors will be treated by other therapies.
- Hormone receptors also serve as a prognostic factors in breast cancer. Patients with positive receptor levels tend to survive longer.
- Cytoplasmic estrogen receptors are now routinely measured in samples of breast tissue after surgical removal of a tumor Of patients with breast cancer, 60 % have tumors with estrogen receptor.

- Approximately two thirds of patients with estrogen receptor (+) tumors respond to the hormonal therapy. 5% of patients with estrogen receptor (-) tumors respond to the hormonal therapy.
- Progesterone receptor testing is a useful adjunt to the estrogen receptor testing. Because progesterone receptor synthesis appears to be dependent on estrogen action.
- Measurement of progesterone receptors provides a confirmation that all the steps of estrogen action are intact. Indeed breast cancer patients with both progesterone and estrogen receptor (+) tumors have a higher response rate to hormonal therapy.

C-erbB2 (HER-2 Neu)

- It is receptor for epidermal growth factor (EGF) but it doesn't contain EGF binding domain. It serves as a co-receptor in EGF action
- In the case of increased expression of C-erbB2 leads the auto-activation and increased signal transduction
- Increased expression of C-erbB2 was determined in some cancers. It was suggested as an important factor for carcinogenesis and metastasis

GENETIC CHANGES

Four classes of genes are implicated in development of cancer:

- 1) **protooncogenes** which are responsible for normal cell growth and differentiation
- 2) **tumor suppressor genes** which are involved in recognition and repair of damaged DNA.
- 3)**apoptosis-related genes** are responsible for regulation of apoptosis

- 4)**DNA repair genes**

- Alterations on these genes may lead tumor development.

Susceptible protooncogenes:

- K-ras, N-ras mutations are found to be correlated acute myeloid leukemia, neuroblastoma

Susceptible DNA repair genes:

- BRCA1 and BRCA2 are specific genes in inherited predisposition for developing breast and over cancer, and mutations on these genes are newly measured in some laboratories.
- Mismatch-repair genes are mutated in some colon cancers

Susceptible tumor suppressor genes:

- Retinoblastoma gene
- P53 gene
- P21 gene
- Those genetic markers are very new and not routinely measured in laboratories.

Chromosomal translocation

- c-myc gene has been found to be translocated from 8chromosome to 14chromosome and than become activated in Burkitt's lymphoma.
- myc gene encodes a DNA-binding protein which stimulates cell dividing.

- In chronic myeloid leukemia, there is a translocation between chromosomes 9 and 22.

CHAPTER III

HORMONE METABOLISM IN REPRODUCTIVE FUNCTION

INTRODUCTION

Gonadotropin-releasing hormone (GnRH), a decapeptide synthesized and released from the hypothalamus, stimulates the production and release of luteinizing hormone (LH) and follicle stimulating hormone (FSH) by the anterior pituitary.

In the adult female, LH is responsible for promoting ovulation through its menstrual midcycle surge, for transforming the ruptured follicle into the corpus luteum, and for stimulating production of estradiol and progesterone from the corpus luteum.

In the adult male, LH stimulates the leydig cells of the testes to synthesize and secrete testosterone. Like LH, FSH is also released in a pulsatile fashion in response to GnRH.

In the adult female, FSH promotes the growth and development of the ovarian follicles and stimulates estradiol production and secretion by these maturing follicles.

In the adult male, FSH promotes spermatogenesis.

Under the influence of FSH testosterone is coverted to estradiol by the aromatase enzyme.

Estradiol causes the granulose cells to produce more FSH receptors, making them more sensitive to FSH,

which leads to the synthesis of more estradiol which leads to the growth and thickening of the endometrial layer.

The rising levels of estradiol that occurs in the early to mid–follicular phase inhibits the production of pituitary FSH through a negative feedback mechanism.

CORPUS LUTEUM

The corpus luteum, which means yellow body in Latin, is what is left of the follicle after a woman ovulates.

During the follicular phase of a woman's cycle, several follicles develop under the influence of FSH (follicle stimulating hormone).

Each follicle contains an egg.

In a typical cycle only one egg will become mature enough for ovulation.

When a woman ovulates the egg will burst from the follicle.

Then what is left of the follicle will become the corpus luteum.

The luteal phase, named after the corpus luteum, is the second half of a woman's menstrual cycle.

The luteal phase begins after ovulation and continues until menstruation occurs.

The corpus luteum produces progesterone.

Progesterone makes the lining of the uterus thick for implantation and is necessary to sustain a healthy pregnancy.

The corpus luteum produces progesterone until the placenta begins to take over progesterone production around ten weeks gestation.

After a woman ovulates, the corpus luteum only lasts for about 12-14 days unless it begins receiving HCG (human chorionic gonadotropin) from a developing embryo.

If the egg is not fertilized, the corpus luteum dies and progesterone production stops.

When progesterone levels drop, the uterus lining stops thickening and is consequently shed during menstruation.

If the egg is fertilized, the corpus luteum will begin receiving HCG from the embryo.

HCG tells the corpus luteum to keep producing progesterone.

The corpus luteum lasts for about ten weeks after ovulation.

After ten weeks the placenta takes over progesterone production through the end of pregnancy.

If a woman is pregnant and has low progesterone levels this may result in miscarriage.

The CNS and sex steroid hormones control GnRH release; testosterone in males and the estrogens in females provide negative feedback control while estrogen regulates via positive feedback just prior to ovulation in menstruating females.

Regulation of FSH and LH release in the adult female is provided through negative feedback from estradiol produced by the developing follicles and the corpus luteum.

CONTROL OF SECRETION

Also, the secretion of LH is controlled by positive feedback from the rapidly rising levels of estrogen just prior to ovulation.

In males, testosterone from the testicular Leydig cells controls FSH and LH release through negative feedback.

In males, testosterone from the testicular Leydig cells controls FSH and LH release through negative feedback.

The peptide inhibin produced by the seminiferous tubules and the ovaries regulates FSH secretion also.

Male androgenic steroids include testosterone, dihydrotestosterone (DHT),

androstenediol, androstenedione, Dehydroepiandrosterone (DHEA) and Dehydroepiandrosterone sulphate (DHEAS).

DHEA) and DHEAS are the most abundant adrenal androgens.

The most important androgen is testosterone

Responsible for musculine differentiation of fetal genital tract and development and maintenance of the male sec. sex characteristics and spermatogenesis.

Estradiol is the most potent natural estrogen that is produced mainly by the testes in males and ovaries in non pregnant females.

Responsible for dev.of female sec. sex characteristics.

In the adult female, it also causes the proliferation of endometrium, ↑es the thickness of the vaginal epithelium, ↑es vascularity of cervix,

↑es cervical mucous elasticity to allow sperm penetration and dilates the cervix.

Like testosterone, it circulates mainly bound to (SHBG).

REPRODUCTIVE FUNCTION

Reproductive hormones are employed to differentially diagnose a myriad of reproductive endocrinology disorders.

For example testosterone is used to evaluate hirsutism and virilisation in females and primary hypogonadism in males.

Estradiol measurements are used clinically for the differential diagnosis of amenorrhea, for the evaluation of precocious puberty in girls, for the assessment of follicular maturation in ovulation, as a prognostic indicator of assisted reproductive treatment protocols, and the evaluation of gynecomastia in males.

Progesterone is used clinically for ovulation detection and confirmation, the detection of luteal-phase defects, the verification of ovulation induction, monitoring progesterone replacement therapy, and the evaluation of patients at risk for early-gestation abortion.

Normal Adult Female Reproductive Endocrinology

The adult female menstrual cycle is divided into three phases- follicular, ovulatory, and luteal.

The cycle, beginning with the first day of uterine bleeding, generally lasts 28 days.

The follicullar phase begins with the first day of menstrual bleeding and ends just prior to the ovulatory surge.

During the follicular stage, FSH stimulates the maturation of an ovarian follicle.

The follicle secretes increasing amounts of estrogen as it grows in size.

The estrogen, in turn, stimulates the proliferation of the uterine endometrium.

LH levels begin to rise slowly in the early follicular phase, usually 1-2 days after the rise in FSH.

LH is responsible for stimulating the synthesis of androstenedione and testosterone, which are subsequently converted to estrogens.

Overall, sex steroid production is low and constant during the first half of the follicular phase.

Seven to eight days before the LH surge ,the developing follicle is producing ever increasing amounts of estradiol.

Maximum estradiol levels are achieved 1 day prior to the LH surge.

In the later part of of the follicular phase , FSH secretion from the pituitary is selectively inhibited because of the high estradiol levels and the release of inhibin from the developing follicle.

The ovulatory phase encompasses 1 day on either side of the LH surge.

Estradiol positive feedback causes the preovulatory pulsatile surge of LH, which causes the mature follicle to release its ovum.

The ovum deficient follicle, now known as corpus luteum, decreases its estradiol production early in the luteal phase, causing a simultaneous small increase in FSH.

At this point, the corpus luteum begins to release progesterone and prostagladins, signaling the beginning of the luteal phase.

The progesterone produced by the corpus luteum to maintain the uterine lining reaches a maximum concentration 6-8 days after the LH surge.

In the middle of the luteal phase, estradiol levels increase while LH and FSH decrease until just prior to menstruation.

In the absence of fertilization, the corpus luteum degenerates and estradiol and progesterone levels decline and eventually result in endometrial bleeding.

Pregnacy

During pregnacy, cells in the placenta produce HCG.

This hormone has several functions, inlcuding maintenace of the corpus leuteum in early preg, stimulation of fetal gonadal development, promotion of steroidogenesis in the fetal placental unit and stimulation of fetal testicular secretion of testosterone.

Menopause:

IT is the cessation of the cyclic ovarian that usually occurs in the the fifth decade of life.

Signs and symptoms of menopause result from the waning of ovarian follicular activity and decreased estradiol production, and menstrual cycle becomes irregular.

FSH levels are increased, while LH is normal and estradiol and progesterone are decreased when compared to normal ovulatory cycles.

With true menopause, both FSH and LH are greatly increased while estrogen levels are markedly reduced.

Female Reproductive Endocrinology Disorders

Female reproductive disorders consist of either hypofunction or hyperfunction conditions.

Increased levels of LH and FSH suggest gonadal dysfunction that result in an absence of negative feedback to the pituitary.

Decreased levels of LH and FSH are due to either hypothalamic or pituitary disease.

> Normal menstrual periods in an adult female imply normal levels of LH, FSH and estradiol and thus no reproductive endocrinology disorder.

Female Hypogonadism

In primary female hypogonadism,(hypergonadotropic hypogonadism) the defect is due to ovarian hypofunction.

Causes include Turner's syndrome ($45X_0$), menopause, testicular feminization and polycystic ovary disease.

The level of the defect for secondary hypogonadism(hypogonadotropic hypogonadism) is at the level of either the pituitary or the hypothalamus.

Causes of female secondary hypogonadism include hypopituitarism, hypothalamic disorders, pregnancy, hypothyroidism, and central

disorders (anorexia nervosa, stress, intense physical training, weight lose and malnutrition.)

Female Hypergonadism

Estrogen secreting tumors are the primary cause of female primary **hypergonadism**(hypogonadotropic hypergonadism).

The major causes of female secondary hypergonadism(hypergonaditropic hypergonadism) are precocious puberty and the premature maturation of the CNS.

Male Reproductive Endocrinology Disorders

In males, most clinical dysfunction is due to hypofunction.

Male Hypogonadism: The presentation of male hypogonadism depends on the age of onset and the degree of testosterone deficiency.

Variations in presentation include ambiguous genetalia, delayed puberty, postpubertal gonadal failure, gynecomastia, and infertility.

In males primary hypogonadism, the disorder is due to gonadal failure.

Causes include Klinefelter's syndrome (47XXY), (males have extra X chrom)

male climacteric (Leydig cell failure in older men), castration and incomplete androgen sensitivity.

In secondary hypogonadism(hypogonadotropic hypogonadism) the abnormality is at the level of the pituitary or the hypothalamus.

PROLACTIN HORMONE

Is a polypeptide that is under inhibitory control by hypothalamic dopamine.

Prolactin exhibits a diurnal variation with highest levels occuring during sleep.

Has no known function in males.

In females it is responsible for the initiation and maintenance of lactation.

Prolactin acts directly without the production of a target gland hormone.

Thus it is a non-tropic and self regulating hormone with no feedback mechanism.

Prolactin inhibits the pulsatile secretion of GnRH and the positive feedback of estradiol on LH release.

Increased prolactin levels result in hypogonadism due to the hormone's ability to inhibit GnHR pulsatile secretion.

A pituitary adenoma is the predominant cause of hyperprolactnemia.

Females with a prolactinoma present with amenorrhea, galactorrhea,

and infertility while adult males exhibit impotency, decreased libido, and infertility.

Clinically, serum prolactin is monitored to assess infertility and to diagnose and monitor prolactinomas.

LABORATORY ASSESSMENT OF REPRODUCTIVE FUNCTION.

- Testing for all the reproductive hormones is done using immunoassays, RIA, IRMA, ELISA and chemiluminescence.

CHAPTER IV

LIVER,RENAL,GASTROINTESTINAL AND PANCREATIC FUNCTION MEDIATED BY HORMONES

ENDOCRINE HORMONES SECRETED BY THE LIVER

- The liver holds sway as the largest internal organ in the human body. However, its importance and significant is not just due to its size. There are several roles that the liver performs without which the body will be unable to function efficiently.

Some of the functions of the liver include helping the body digest fats, storage of nutrient reserves, filtering poisons and wastes from the blood, synthesis of a variety of proteins, and regulating the levels of many chemicals found in the bloodstream. Another important function of the liver is revealed by an overview of endocrine hormones secreted by the liver. In other words, the liver also serves as an endocrine organ.

HORMONES SECRETED BY THE LIVER

The liver is responsible for the secretion of the following hormones:

- Angiotensinogen
- Thrombopoietin
- Insulin-like Growth factor 1 (IGF-1)

1.ANGIOTENSINOGEN

Angiotensin is a peptide hormone that causes vasoconstriction and a subsequent increase in blood pressure. It is part of the renin-angiotensin system, which is a major target for drugs that raises blood pressure. Angiotensin also stimulates the release of aldosterone, another hormone, from the adrenal cortex.

2. Thrombopoietin

Also known as TPO, **thrombopoietin** is a glycoprotein hormone synthesized majorly by the liver and also to some levels by the kidneys. It is made up of about 332 amino acids and the major function is the stimulation of bone marrow precursor cells to become megakaryocytes.

Megakaryocytes in turn fragment to form platelets (megakaryocytopoiesis), which are responsible for blood clotting. The parenchymal cells and sinusoidal endothelial cells of the liver are responsible for its production.

3.INSULIN-LIKE GROWTH FACTOR 1 (IGF-1)

IGF-1 is similar in molecular structure to insulin. It is made up of 70 amino acids and plays an important role in the regulation of cell growth and development and also has some insulin-like effects. A deficiency of this hormone results in Laron's

dwarfism. Production of IGF-1 in the liver is stimulated by growth hormone.

KIDNEY OR RENAL HORMONES

The kidney plays an essential role in the maintenance of life in higher organisms, not only through regulating the blood pressure and body fluid homeostasis and clearing the wastes, but also by acting as a major endocrine organ.

Hormones secreted by kidney or renal and their function:

- Renin
- Erythropoietin
- Calcitriol

1. **Renin's** primary function is therefore to eventually cause an increase in blood pressure, leading to restoration of perfusion pressure in the kidneys. Renin is secreted from juxtaglomerular kidney cells.

2. **Erythropoietin** (EPO) is a hormone produced by the **kidney** that promotes the formation of red blood cells by the bone marrow. The kidney cells that make erythropoietin are sensitive to low oxygen levels in the blood that travels through the kidney.

Calcitriol is a steroid hormone that has long been known for its important role in regulating body levels of calcium and phosphorus, and in mineralization of bone

In addition, the kidney serves as an important endocrine

target organ for a number of hormones, thereby

controlling the extracellular fluid volume, electrolyte

balance, acid-base balance, and blood pressure.

GASTROINTESTINAL HORMONES

Hormones secreted by gastrointestinal :

-Gastrin

-Cholecystokinin

Gastrin and cholecystokinin (CCK) are structurally and functionally related peptide hormones that serve as regulators of various digestive processes and feeding behaviors.

Gastrin and CCK(are important hormonal regulators that are known to induce gastric secretion, stimulate pancreatic secretion, increase blood circulation and water secretion in the stomach and intestine, and stimulate smooth muscle contraction. Originally found in the gut, these hormones have since been shown to be present in various parts of the nervous system.

PANCREAS HORMONES

The pancreas is a glandular organ in the upper abdomen, but really it serves as two glands in one: a digestive exocrine gland and a hormone-producing endocrine gland. Functioning as an exocrine gland, the pancreas excretes enzymes to break down the proteins, lipids, carbohydrates, and nucleic acids in food. Functioning as an endocrine gland, the pancreas secretes the hormones insulin and glucagon to control blood sugar levels through out the day.

PANCREAS HORMONES

- Pancreatic polypeptide
- Gastrin
- Cholecystokinin
- Glucagon
- Somatostatin
- Secretin

Their function:

- **Glucagon/GIP(Gastric inhibitory polypeptide)/secretin/VIP hormones(Vasoactive intestinal peptide)** are a family of evolutionarily related peptide hormones that regulate activity of G-protein coupled receptors from secretin receptor family.
- **GIP(Gastric inhibitory polypeptide):** is an inhibiting hormone of the secretin family of hormones.
- **Glucagon** causes the liver to convert stored glycogen into glucose, which is released into the bloodstream. ... Thus, glucagon and insulin are part of a feedback system that keeps blood glucose levels stable
- **Vasoactive intestinal** polypeptide (VIP) is a potent vasodilator, regulates smooth muscle activity, epithelial cell secretion, and blood flow in the gastrointestinal tract .
- **secretin** is released into the bloodstream and stimulates the acinar cells of the pancreas to secrete water and bicarbonate into the pancreatic ducts that drain into the duodenum.
- **Cholecystokinin** stimulates the gallbladder to contract and release stored bile into the intestine.

Somatostatin

- **The Somatostatin** is a hormone which inhibits the release of the pituitary somatotropin (growth hormone), and inhibits the release of glucagon and insulin from the pancreas of fasted animals.

CHAPTER V

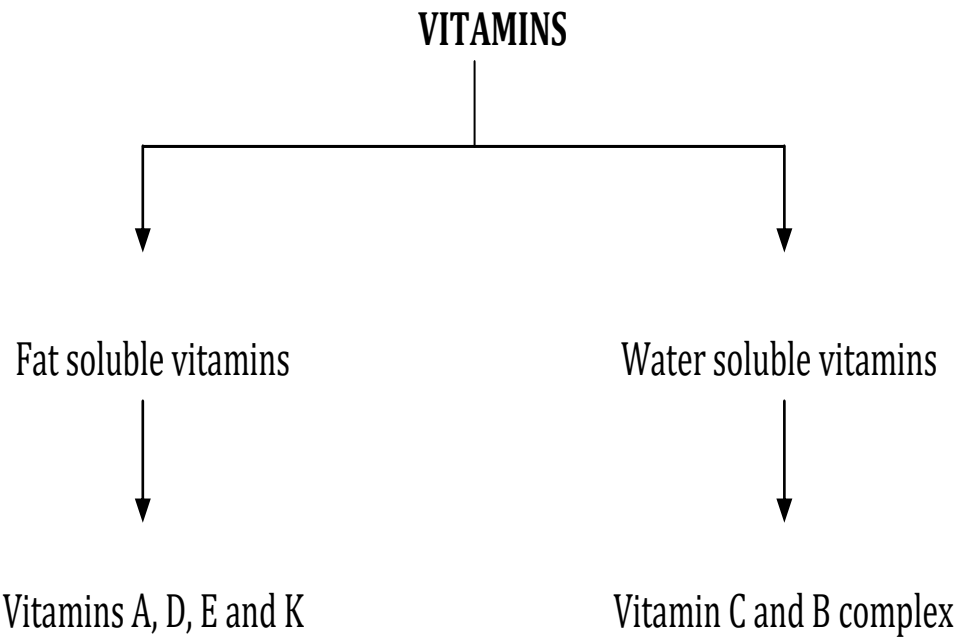
VITAMINS

Definition

- Vitamins are organic substances occurring in many foods in small amounts and they are required for normal metabolic functions of the body.

CLASSIFICATION:

- Vitamins are classified into 2 groups based on their solubility.



1. Fat soluble vitamins

- These vitamins are soluble in fat and fat solvents but insoluble in water.
- E.g: Vitamin A, D, E and K.

2. Water soluble vitamins

Example;

Vitamin C (Ascorbic acid)

Viatmin B1 (Thiamine)

Vitamin B2 (Riboflavin)

Vitamin B3 (Niacin)

Vitamin B5 (Pantothenic acid)

Vitamin B6 (Pyridoxin)

Vitamin B7 (Biotin)

Vitamin B9 (Folic acid)

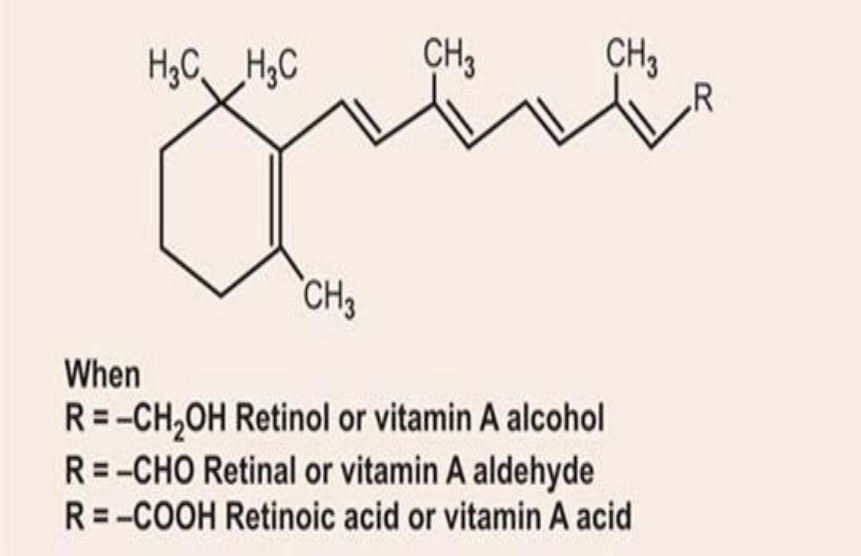
Vitamin B12 (Cyanocobalamine)

FAT SOLUBLE VITAMINS
VITAMIN A (RETINOL)

OTHER NAMES

- Retinol (alcohol form)
- Retinal (aldehyde form)
- Retinoic acid (acid form)
- Provitamin A

STRUCTURE:



FUNCTIONS:

- Retinol is necessary for growth and reproduction.
- Vitamin A plays an important role in vision in dim light.
- It participates in protein synthesis.
- It is also essential for normal bone formation.
- It is involved in the synthesis of mucopolysaccharides and glycoproteins.

- Beta – carotene acts as an antioxidant against disease like cancer, cataract etc.

SOURCES:

Plant sources:

Carrot, mango, orange, papaya, green leafy vegetables, spinach, pumpkin, jack fruit, drumstick leaves, tomato, apricot, peaches, plums, cherries, mangoes, sweet potatoes, corn and papayas.

- **Animal sources:**

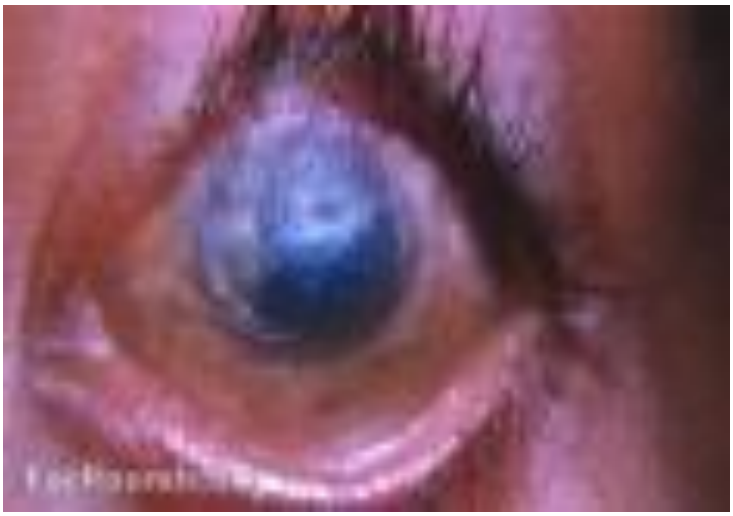
Sheep liver, butter, ghee, egg, milk, curd, cheese

REQUIREMENTS

Group	Retinol (µg)	Beta-carotene (µg)
Infants	350	1400
Childrens 1 – 3 years6 7 – 9 years	 400 600	 1600
Boys and Girls 10 – 18 years	 600	2400
Man	600	2400
Woman	600	2400
Pregnant woman	600	2400
Lactating woman	900	3800

DEFICIENCY:

- 1. **Night blindness (nyctalopia)**: in case of Vit A deficiency The person cannot see in dim light.
- 2. **Xerophthalmia**: The conjunctiva becomes dry, thickened, wrinkled and pigmented. The cornea becomes dry, dull (Xerosis cornea).



- 3. **Keratomalacia**: **Cornea:** White opaque spots called ***Bitot's spots*** appear in the conjunctiva on either side in each eye. Corneal epithelium becomes Keratinised, opaque and may become softened and ulcerated

- Defective teeth formation.

- Growth retardation.
- Decreased protein synthesis
- **Respiratory tract:** Keratinisation occurring in the mucous membrane of respiratory tract leads to increased susceptibility to infection and lowered resistance to disease.
- 8. **Urinary tract:** Keratinisation of UT leads to **calculi formation.**

EXCESS (HYPERVITAMINOSIS A)

- Intake of large amount of vitamin A for long period is called hypervitaminosis A.

The symptoms are:

- Nausea and vomiting
- Head ache
- Drowsiness
- coarsening and falling of hair,
- skeletal decalcification
- Roughening of skin,
- Irritability
- Enlargement of liver in children.

VITAMIN D

OTHER NAMES

- Calciferol
- Sunshine vitamin

STRUCTURE

- Vitamin D includes a number of compounds that are chemically related to sterols, occurring chiefly in animals.

FUNCTIONS:

- Vitamin D helps in the absorption of calcium and phosphorus.
- It is required for calcification of bones.
- It helps to maintain the calcium and phosphorus levels in the body.
- It increases the citrate level of blood, bone, kidney, and heart tissues and also the excretion of citric acid.
- **Action of calcitriol on the kidney:** Calcitriol (active form of vit D)is also involved in minimizing the excretion of calcium and phosphate through the kidney, by decreasing their excretion and enhancing reabsorption.

SOURCES:

- Liver, fatty fish, cod liver oil, egg, butter, ghee, cheese, milk and milk products.
- Spinach, cabbage, exposure to sunlight.

REQUIREMENTS

The daily requirements are

- Infants and preschool children – 10µg/day
- Children and adults - 5µg/day
- Nursing mothers - 10µg/day

DEFICIENCY:

RICKETS:

- Deficiency of vitamin D leads to rickets in children. There is weakness and abnormalities in bone formation.

The symptoms are:

- Head is enlarged and flattened
- Deformities of ribs
- Enlargement of ends of long bones
- Bowing of legs
- Muscle weakness
- Flat feet.

2. OSTEOMALACIA

Osteomalacia is a condition in adults mainly women who are not exposed to sunlight where the quality of bone is reduced.

The symptoms are:

- Softening of bones
- Deformities of ribs, pelvis, legs
- Muscle weakness
- Frequent bone fractures

3. OSTEOPOROSIS

It is the disease of bone occurs in old people

4. RENAL OSTEODYSTROPHY

- When renal parenchyma is lost or diseased quite significantly, it is unable to form calcitriol and calcium absorption is impaired. Hypocalcemia leads to increase in PTH which acts on bone to increase Ca++. Consequently there is excessive bone turnover and structural changes. This condition is known as renal osteodystrophy.

EXCESS:

Intake of excess amount of vitamin D leads to hypervitaminosis.

The symptoms are

- Nausea and vomiting
- Irritability
- Constipation
- Hypercalcemia
- Urinary lithiasis
- Polyuria
- Polydipsia
- Dehydration

VITAMIN E

OTHER NAMES

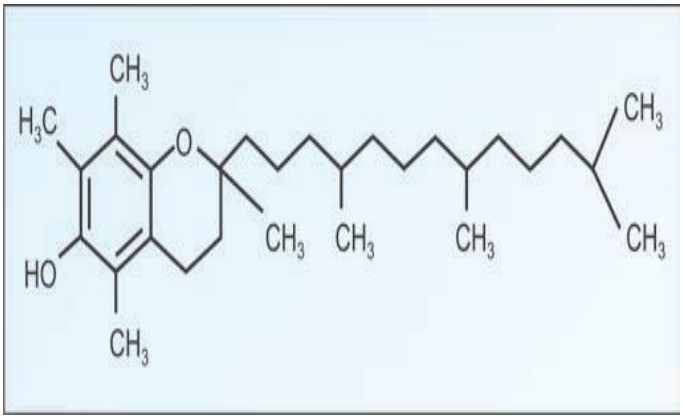
- Antisterility vitamin
- Tocopherol

STRUCTURE

- Derivatives of 6-hydroxy chroman
- Possess antisterility property

Four tocopherols:

- **α-tocopherol:** 5, 7, 8 trimethyl tocol
- **β-tocopherol:** 5, 8 dimethyl tocol
- **γ-tocopherol:** 7, 8 dimethyl tocol
- **δ-tocopherol:** 8 methyl tocol



FUNCTIONS

- It acts as an antioxidant in the body.
- It is essential for maintenance of nervous tissues.
- Vitamin E is called **anti-ageing vitamin**.
- It is essential for normal reproduction
- It is required for proper storage of creatine in skeletal muscle.
- Vitamin E is needed for optimal absorption of amino acids from the intestine.
- It is involved in proper synthesis of nucleic acids.

- Vitamin E protects liver from being damaged by toxic compounds such as carbon tetrachloride.

It works in association with vitamins A , C and beta-carotene, to delay the onset of cataract

SOURCES

- Vegetable oils, Sunflower seeds, Butter, Fruits, Cereal grains, Eggs, Vegetables, Milk, Sesame oil, Mustard oil, Almonds

REQUIREMENTS

❖

Group	Vitamin E (mg/day)
Infants (0 – 1 year)	3 – 4
Children (2 – 10 years)	5 – 7
Adolescents (11 – 18 years)	8 – 10
Adults	10
Pregnancy	12
Lactation	13

DEFICIENCY

- Vitamin E deficiency leads to uncoordinated movement, weakness and sensory disturbances.
- It causes hemolytic anemia in low birth weight infants

- Reproductive failure
- Defective functioning of the retina leads to permanent blindness
- Severe liver disease
- Muscular dystrophy
- Hemolytic anemia

EXCESS

Increased intake of vitamin E leads to **hypervitaminosis E**.

It leads to

- Liver and kidney failure
- Growth retardation
- Increased prothrombin time
- Bone calcification

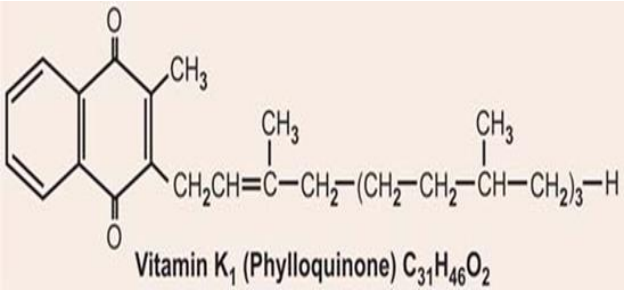
VITAMIN K

OTHER NAMES

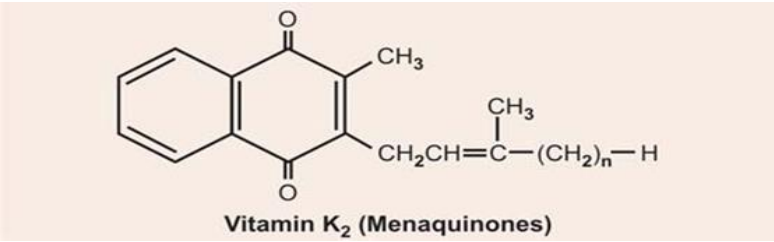
- Anti-haemorrhagic factor
- Koagulation vitamin

STRUCTURE

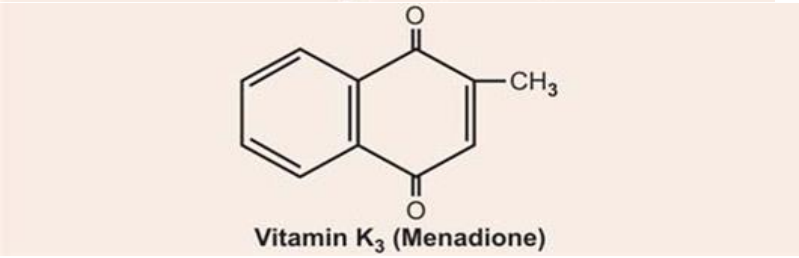
- Vitamin K is derivatives of 2-methyl-1-4-napthoquinone.
- It differs from each other in the composition of their side chain present at carbon 3 of the naphthoquinone ring
- Vitamin K1 = 2-methyl-3-phytyl-1-4-napthoquinone from alfalfa leaves and Phytylchain attached in position 3 of the menadione nucleus
- Vitamin K2 = 2-methyl-3-diffarnesyl-1-4-napthoquinone from putrified fish meal and difarnesyl chain attached in position 3 of the menadione nucleus
- Vitamin K3 = 2-methyl-1-4-napthoquinone and it is a Simplest and most potent synthetic Vit. K



Vitamin K_1 (Phylloquinone) $C_{31}H_{46}O_2$



Vitamin K_2 (Menaquinones)



Vitamin K_3 (Menadione)

FUNCTIONS

- It is essential for blood coagulation
- It helps in activation of calcium binding protein
- It acts as a cofactor for carboxylation reaction.
- Vitamin K is a necessary cofactor in oxidative phosphorylation being associated with mitochondrial lipids.

SOURCES

- Green leafy vegetables, fruits, Alfalfa, Spinach, Cabbage, Tomato, Cauliflower, Soyabean, Carrots, Potatoes, seeds, cereals, legumes, milk, fish, dairy, meat products, Milk, Fish, eggs .

REQUIREMENTS

❖

Group	Vitamin K µg/day
Infants	
0 – 1 year	12 - 20
Childrens	
1 – 3 years	15 – 30
4 – 6 years	20 – 40
7 – 10 years	30 - 60
Adolescents	
11 – 18 years	50 - 100
Adults	70 – 140
Pregnant woman	200
Lactating woman	150

DEFICIENCY

- Deficiency of vitamin K leads to delayed blood clotting in infants (Haemorrhagic disease)
- It occurs in patients given antibiotic which reduce the intestinal bacterial flora.
- Malabsorption and biliary tract obstruction.

EXCESS

- Vitamin K does not produce any toxic effects.

- Vitamin K analogues .e.g: menadione produce toxic effects in infants like hemolytic anaemia and jaundice due to increased breakdown of RBC .

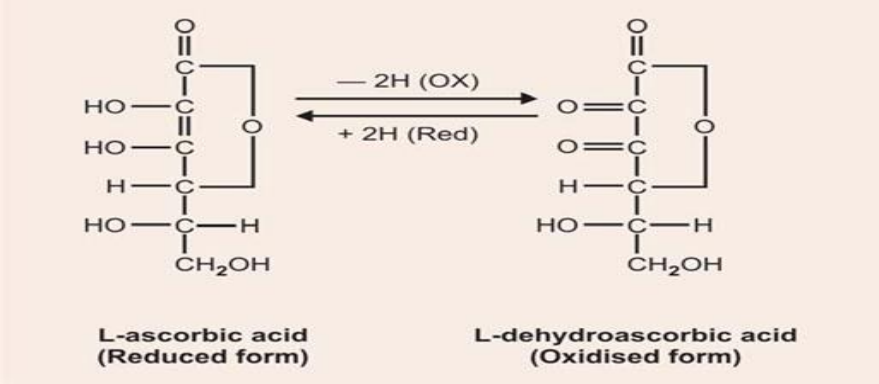
WATER SOLUBLE VITAMINS
VITAMIN C (ASCORBIC ACID)

OTHER NAMES:

- **Anti-scorbutic vitamin**

STRUCTURE

- Ascorbic acid has a structure similar to that of L-glucose and it is a derivative of glucose.



FUNCTIONS

- It is necessary for hydroxylation of proline and hydroxyproline which is essential for formation of collagen.
- It helps in the synthesis of non-essential aminoacids, nor-epinephrine (neurotransmitter) and cartinine.
- It is required for absorption of iron.
- It is required for normal wound healing.

- It is essential for drug detoxification.
- Vitamin C is an excellent antioxidant.
- It regulates oxidation – reduction reactions, acting as hydrogen transporting agent (co enzyme)
- It is involved in the tyrosine and tryptophan metabolism
- It is necessary for the normal growth, maturation and reproduction of cells.
- It is also necessary for the maturation of RBC.
- It also builds up body resistance against infections by inactivating bacterial toxins.
- Ascorbic acid is necessary for the formation of tissue "ferritin".

SOURCES

- Amla is the richest source of vitamin C.
- Guava, orange, lime, tomatoes, apple, grapes, banana, jack fruit, guava
- Drumstick leaves, agathi, bitter gourd, cabbage
- Cereals, pulses, meat, liver, milk are poor sources.

REQUIREMENTS

Group	Vitamin C mg/day
Infants	
0 – 1 year	25
Childrens	40
Adolescents	
Boys	40
Girls	40
Man	40
Woman	40
Pregnant woman	40
Lactating woman	80

DEFICIENCY

- Deficiency of vitamin C leads to a disease called Scurvy in both infants and adults.
- 1. Infantile scurvy:
  Loss of appetite
- Pain in the limbs
- Bleeding gums
- Delayed wound healing
- Defective bone growth
- Loss of weight
- Irritability
- Hemorrhage under the skin

2. Adult scurvy

- Fever
- Delayed wound healing
- Weakness
- Shortness of breath
- Pain in bones, joints and muscles
- Anaemia
- Spongy and bleeding gums
- Skin becomes rough and dry

EXCESS

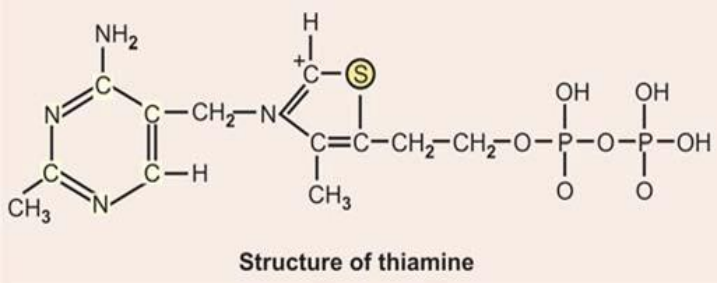
- Unpleasant diarrhea
- Gastrointestinal disturbances
- Excess vitamin C interferes with anticoagulant therapy.

VITAMIN B COMPLEX
VITAMIN B1 (THIAMINE)

OTHER NAMES:

- Anti-beriberi vitamin
- Anti-neuritic vitamin

- It contains pyrimidine ring and thiazole ring held by a methylene bridge.
- It contains sulphur (sulphur containing vitamin)



Structure of thiamine

FUNCTIONS

- It is involved in transmission of nerve impulses across the cells.
- It is involved in the conversion of tryptophan to niacin.
- Essential for growth.
- Thiamine is converted to thiamine pyrophosphate (TPP), which is an important coenzyme in carbohydrate metabolism.
- It required for pyruvate dehydrogenase(contributes to transforming pyruvate into acety-coA), α-ketoacid dehydrogenase (decarboxylase).

SOURCES

- Good sources - Yeast, whole wheat, millets, hand pounded rice, parboiled rice.
- Other sources - Gingelly seeds, groundnut, soyabean, cashewnut, organ meats, milk, pork, liver and eggs.

REQUIREMENTS

recommended dietary allowances for vitamin B1

Group	Vitamin B1 mg/day
Infants 0 – 1 year	 50 – 55mg/kg
Childrens (1 – 9 years)	0.6 – 1.2
Boys (10 – 18 years)	1.1 – 1.3
Girls (10 – 18 years)	1.0
Man Sedentary Moderate Heavy work	 1.2 1.4 1.6
Woman Sedentary Moderate Heavy work	 0.9 1.1 1.2
Pregnant woman	+0.3
Lactating woman	+0.2 – +0.3

DEFICIENCY:

Deficiency of thiamine leads to beri-beri.

1. Dry beri-beri

- Loss of appetite
- Tingling, numbness
- Burning sensation in hands and feet
- Muscles becomes weak
- Mental depression and confusions.

2.Wet beri-beri:

- Oedema (accumulation of fluids) in legs, face.
- Enlargement of heart
- Breathlessness

3. Infantile beri-beri:

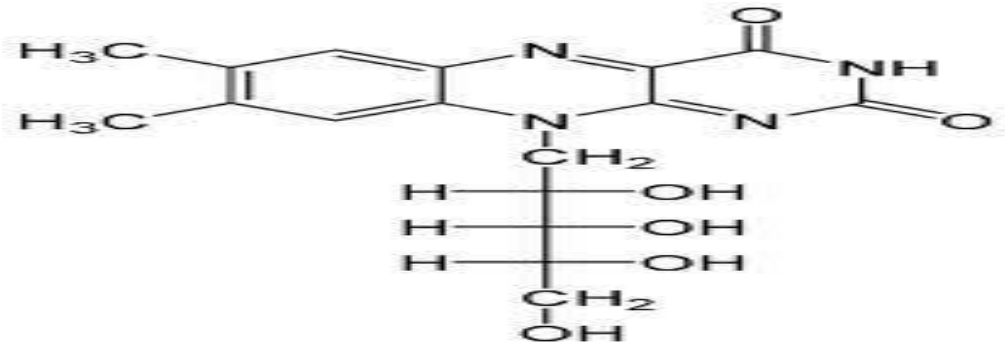
- Restlessness
- Sleeplessness
- Breathlessness
- Constipation
- Enlargement of heart.
- Impairment in nerve impulse transmission.
- Peripheral polyneuritis which include the symptoms like tingling sensation on the skin, numbness, and weakness of the limbs and paraplegia

VITAMIN B2 (RIBOFLAVIN)

- Vitamin B2 is the yellow enzyme, which is heat stable**.**

STRUCTURE

- The ribose and a heterocyclic substance – isoalloxazine
- First carbon of ribose – attached to 9th position of isoalloxazine – three rings – benzene, azine, pyrimidine



FUNCTIONS

- The coenzyme form of riboflavin (FMN and FAD) is involved in oxidation- reduction and dehydrogenation reactions.
- It is involved in the conversion of Vitamin B6 and folate to active form.
- It is essential for the formation of RBC
- It is required for the synthesis of glycogen.
- The coenzymes, FAD and FMN are associated with certain enzymes involved in carbohydrate, lipid, protein and purine metabolisms, besides the electron transport chain.

SOURCES

- Rich sources - Liver, meat, fish, egg, dried yeast, milk and milk products.
- Other sources - Whole grains, legumes and green leafy vegetables.

REQUIREMENTS

Group	Vitamin B2 (mg/day)
Infants 0 – 1 year	 60 – 65mg/day
Childrens (1 – 9 years)	0.7 – 1.2
Boys (10 – 18 years)	1.3 – 1.6
Girls (10 – 18 years)	1.2
Man Sedentary Moderate Heavy work	 1.4 1.6 1.9
Woman Sedentary Moderate Heavy work	 1.1 1.5 1.2
Pregnant woman	+0.2
Lactating woman	+0.3

DEFICIENCY:

It is characterized by:

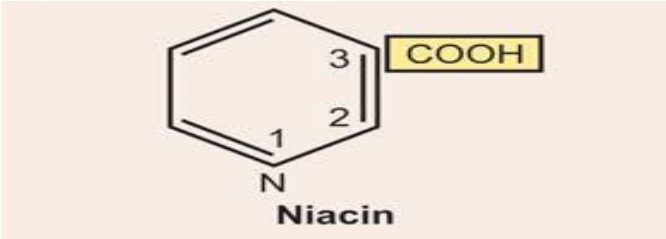
- Burning of the mouth and tongue
- Angular stomatitis - lesions at the angle of the mouth
- Glossitis – inflammation of the tongue
- Skin becomes dry
- Burning sensation of the eyes
- Anaemia
- Photophobia of eyes.
- Ulceration of cornea and some times cataract formation

VITAMIN B3 (NIACIN)

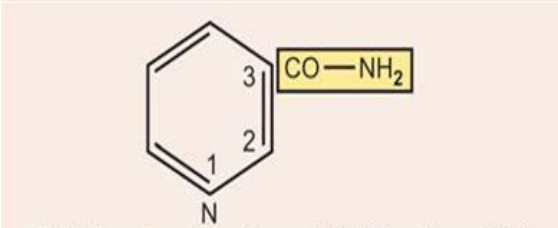
OTHER NAMES:

- **Nicotinamide**

STRUCTURE

- 

Niacin



Pyridine-3-carboxylic amide (Nicotinamide)

FUNCTIONS

- It is essential for tissue metabolism
- Coenzyme form of Niacin (NAD^+ AND $NADP^+$) is involved in oxidation-reduction reaction.
- NAD^+ is involved in catabolic reactions and $NADP^+$ is involved in anabolic reactions.
- Niacin inhibits lipolysis in the adipose tissue and decrease the circulatory free fatty acids.
- Niacin is used in the treatment of hyperlipoproteinemia type II b (elevation of LDL and VLDL).

SOURCES

Liver, yeast, meat, fish, milk, rice polishing, peanut, legumes, cereals, whole grains.

- Tryptophan in diet is converted to niacin.

i.e: 60mg of tryptophan forms 1mg of niacin.

REQUIREMENTS

Group	Vitamin B3 (mg/day)
Infants 0 – 1 year	650 – 710mg/kg
Childrens (1 – 9 years)	8 - 13
Boys (10 – 18 years)	15 – 17
Girls (10 – 18 years)	13 - 14
Man	16 - 21
Woman	12 - 16
Pregnant woman	+2
Lactating woman	+4

DEFICIENCY

Deficiency of niacin leads to **pellagra**. It is characterized by **3D's (dermatitis, diarrhea and dementia).**

1. Dermatitis – inflammation of skin especially neck, arms, feet and knees.

2. Diarrhea – Form of loose stools with blood. Prolonged diarrhea leads to weight loss

3. Dementia – it is associated with nervous tissue. There is irritability, depression, poor concentration, loss of memory and insomnia (sleeplessness).

4. If not treated leads to death

EXCESS

- Liver damage
- Increased levels of glucose and uric acid in the circulation.

VITAMIN B5 (PANTOTHENIC ACID)

OTHER NAMES:

- Anti-dermatitis factor
- Filtrate factor.

STRUCTURE

- Pantothenic acid consists of β-alanine in peptide linkage with a dihydroxy dimethyl butyric acid ('Pantoic' acid).

FUNCTIONS

- It is a constituent of coenzyme A, which is involved in metabolism.
- Coenzyme A serves as an activated acetyl or acyl group.
- Succinyl CoA is also involved in many reactions,

including the synthesis of porphyrins of heme.

- It is a component of fatty acid synthase.
- It is essential for the synthesis of vitamin B12, hemoglobin and cytochromes.

REQUIREMENT

Group	Vitamin B5 (mg/day)
Infants	1.5 – 2.5
Children	3 - 4
Adolescents	4 – 5
Adults	4 – 7
Pregnancy and Lactation	4 - 8

SOURCES:

- The rich sources are liver, meat, egg, milk, yeast, kidney and fresh vegetables.

DEFICIENCY

- Growth failure
- Dermatitis
- Diarrhea
- Greying of hair
- Sleeplessness
- Burning feet syndrome (Pain & numbness in the toes

- Hemorrhage
- Fatty liver
- Reproductive failure
- Anaemia
- Decreased steroid synthesis

EXCESS

- Diarrhea
- Nausea
- Heart burn.

VITAMIN B6 (PYRIDOXINE)

OTHER NAMES:

- Vitamin B6 occurs in 3 forms – pyridoxine, pyridoxal and pyridoxamine.

STRUCTURE

- **Pyridoxol (Pyridoxine):** also called as ***Adermin*** is chemically 2-methyl-3OH-4, 5-di (hydroxymethyl) pyridine.
- **Pyridoxal (Aldehyde form)**
- **Pyridoxamine (Amine form)**

FUNCTIONS

- Coenzyme form of vitamin B6 as pyridoxal phosphate is required for many reactions like:

a) Transamination or aminotransfer: is chemical reaction that transfers an amino group to a ketoacid to form new amino acids.

b) Deamination:is the process by which a a are broken down if there is an excess protein intake(the amino group is removed from a a and converted to ammonia).

c) Decarboxylation

- It is involved in the conversion of tryptophan to niacin.
- It is involved in the synthesis of mRNA to hemoglobin
- It is involved in the conversion of linoleic acid to arachidonic acid.
- It is essential for the release of glycogen from liver and muscle.

REQUIREMENT

Group	**Vitamin B6 (mg/day)**
Infants 0 – 1 year	0.1 – 0.4
Childrens (1 – 9 years)	0.9 – 1.6
Boys and Girls (10 – 12 years)	1.6
Boys and Girls (13 – 18 years)	2.0
Man	2.0
Woman	2.0
Pregnant woman	2.5
Lactating woman	2.5

SOURCES

- **Rich sources** - Meat, egg yolk, fish, milk, pulses, wheat, corn, cabbage, roots .

Cooking and processing of food leads to loss of vitamin B6.

DEFICIENCY

Vitamin B6 deficiency leads to abnormalities in protein metabolism, which is characterized by,

- Poor growth
- Anaemia
- Decreased antibody formation

Hypochromic microcytic anaemia due to reduction in heme production

The symptoms are:

- Weakness
- Nervousness
- Irritability
- Depression
- Mental confusion
- Sleep disturbances
- Difficulty in walking.

EXCESS

- Nerve damage

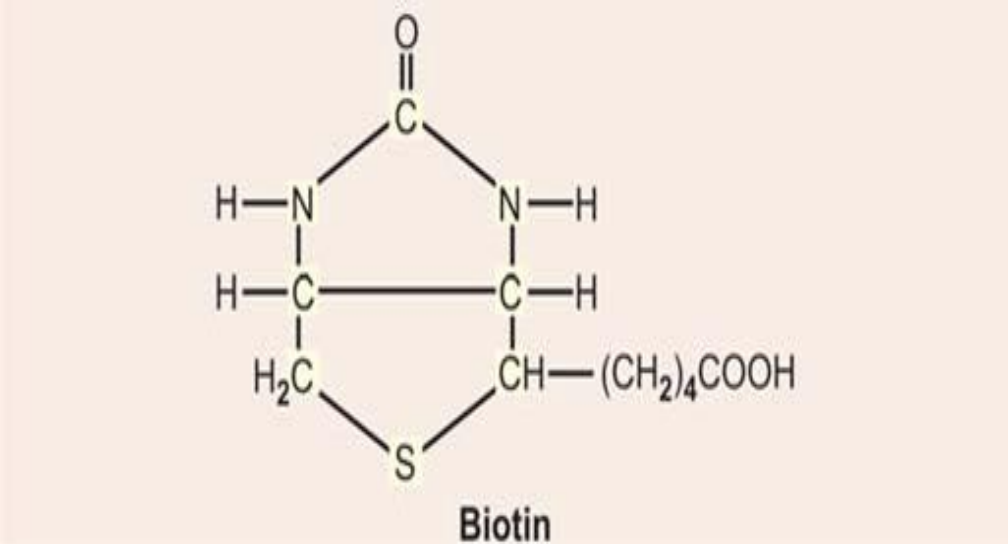
VITAMIN B7 (BIOTIN)

OTHER NAMES:

- Anti-egg white injury factor
- Vitamin H

STRUCTURE

- Biotin is a heterocyclic monocarboxylic acid, it is a ***sulphur-containing*** water-soluble B-vitamin. The structure is formed by fusion of imidazole and thiophene rings with a valeric acid side chain.



Biotin

FUNCTIONS

- It serves as a carrier of CO2 in carboxylation reactions like:

a) **Acetyl CoA carboxylase:is** a biotin dependent enzyme that catalyse the irreversible carboxylation of acetyl coA to produce malonyl coA.

b) **Pyruvate carboxylase**: is an enzyme that catalyzes the physiologically irreversible carboxylation of pyruvate to form oxaloacetate

c) **Propionyl CoA carboxylase:is** a biotin dependent enzyme that catalyses the carboxylation of propionyl coA to form methylmalony coA.

SOURCES

- Good sources are liver, soyabeans, egg yolk, rice polishing, yeast
- Cereals, legumes, meat, fish, milk, oil seeds, nuts
- Vegetables and fruits are **poor sources**.

REQUIREMENTS

❖

Group	Vitamin B7 (µg/day)
Infants	35 – 50
Children	65 - 120
Adolescents	150 – 200
Adults	150 – 200
Pregnancy and Lactation	250

DEFICIENCY

- Depression
- Muscle pain
- Anaemia
- Nausea
- Loss of appetite
- Dermatitis
- Alopecia.

VITAMIN B9 (FOLIC ACID)

Folic acid was first extracted from dark green vegetables.

FUNCTIONS

It is required for normal growth and division of cells.

Coenzyme of folic acid (Tetrahydrofolate)is required for many reactions:

i) Synthesis of purine

ii) Thymine- essential compound of DNA

iii) Formation of heme group of hemoglobin

iv) Conversion of phenyl alanine to tyrosine

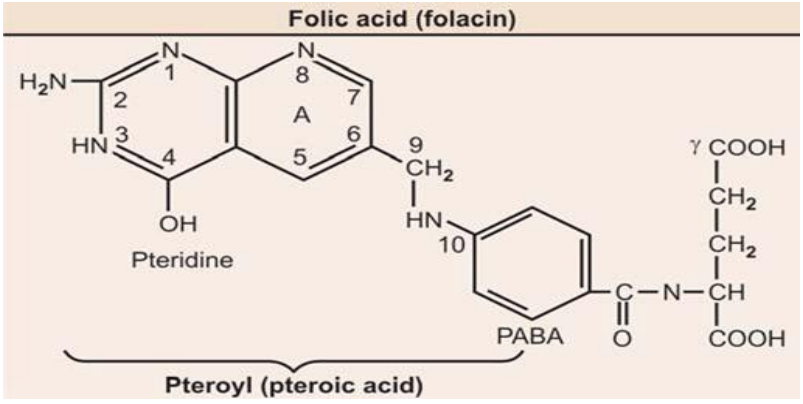
v) It helps in the development of RBC

STRUCTURE

Folic acid consists of three components

- Pteridine ring,
- p-amino benzoic acid (PABA) and
- Glutamic acid

Folic acid mostly has one glutamic acid residue and is known as pteroyl-glutamic acid (PGA).



SOURCES

- **Rich sources** - Green leafy vegetables, whole grains, cereals, liver, kidney, yeast, rice polishing and eggs.
- **Poor source** - Milk

REQUIREMENTS

❖

Group	Vitamin B9 (µg/day)
Infants	25
Children (1 – 9 years)	30 - 60
Boys and Girls (10 – 18 years)	70 - 100
Man and Woman	100
Pregnancy	400
Lactation	150

DEFICIENCY

- Megaloblastic anemia – reduced oxygen carrying capacity
- Folate deficiency during pregnancy causes neural defects in the fetus.
- Folate deficiency impairs the ability of the immune system to fight infection.
- Folic acid is associated with the metabolism of histidine.

EXCESS

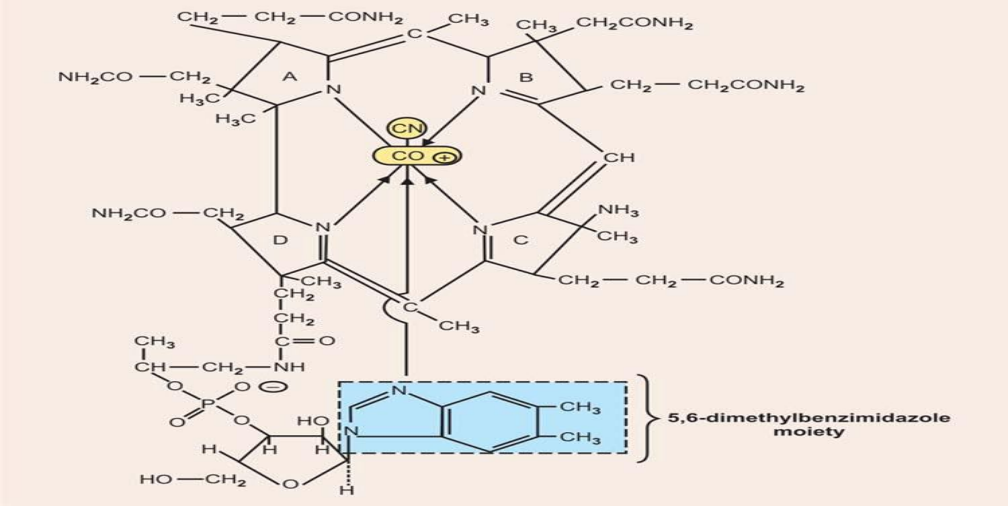
- Excess of folic acid lead to kidney injury.

VITAMIN B12 (CYANOCOBALAMINE)

- **OTHER NAMES:**
- Anti-pernicious anaemia vitamin

STRUCTURE:

- It is a unique vitamin, synthesized by only microorganisms and not by animals and plants.
- Vitamin B12 is the only vitamin with a complex structure. The empirical formula of vitamin B12 (cyanocobalamin) is $C_{63}H_{90}N_{14}O_{14}PCo$.



FUNCTIONS

- It is necessary for normal growth and maintenance of healthy nervous tissue.

- It is essential for normal blood formation
- It is involved in DNA synthesis
- It facilitates the formation of folate coenzymes which is required for nucleic acid synthesis.
- It is required for the synthesis of myelin sheath that surrounds the nerve fiber.
- It is required for the synthesis of Methionine from homocysteine.
- Isomerization of methyl malonyl CoA to succinyl CoA

SOURCES

Good sources - Sheep liver, kidney, mutton, egg, milk, curd, shrimp, fish, pork.

- It is also synthesized by bacteria in colon.

REQUIREMENTS

❖

Group	Vitamin B12 (mg/day)
Infants	0.2
Children (Boys and Girls) (1 – 18 years)	0.2 – 1.0
Man	1.0
Woman	1.0
Pregnancy	1.0
Lactation	1.5

- Deficiency of vitamin B12 leads to pernicious anaemia
- Pernicious anaemia is characterized by very large, immature RBC with normal amounts of hemoglobin.

The causes of pernicious anaemia

- Inadequate ingestion of vitamin B12
- Inadequate absorption and utilization
- Increased requirements during infancy and pregnancy
- It is associated with neuronal degeneration and demyelination of nervous system. The symptoms include paresthesia (numbness and tingling) of fingers and toes. In advanced stages, confusion, loss of memory and even psychosis may be observed

EXCESS:

- Excess of vitamin B12 does not have any toxic effects.

CHAPTER VI

ENZYME -LINKED IMMUNOSORBENT ASSAY (ELISA)

- The enzyme linked immunosorbent assay (ELISA), uses an enzyme labeled to measure the formation of antigen-antibody complex.
- The Immunoassay Uses Known Antigens (Ag's) or Antibodies (Ab's) to measure specific analytes
- The enzyme label used in this assay is conjugated to a ligand, which can be an antigen or an antibody specific for the antibody or antigen of interest respectively.
- Some enzymes used in ELISA include alkaline phosphatase, horseradish peroxidase, glucose-6-dehydrogenase or Beta-galactosidase. Depending on the type of enzyme substrate utilized, the end product may be detected by spectrophotometric methods.

REAGENTS WHICH ARE USED IN ELISA

- **Antibodies**: monoclonal or polyclonal, soluble or mobilized into solid support and can be used as unlabeled or enzyme-conjugated.
- **Antigens:** used as labeled or enzyme conjugated and immobilized or soluble, depending on the assay protocol.
- **Standards and control**
- **Enzyme-conjugates**: are either antigens or antibodies.
- **Buffers/diluents**: used to maintain reaction PH and ion concentration or for samples dilutions .
- **Substrates substance**: the reaction of enzyme with substrate produces a color change.
- **Stopping reagent**: 1-N sulfuric acid is used to inhibit the enzyme activity and stabilize the final colored reaction product

COMPETETIVE AND NON COMPETITIVE ASSAY

- Most ELISAs are solid phase assays in which an antigen or antibody is adsorbed onto a solid support.
- Both competitive and noncompetitive techniques can be used for detecting either antigens or antibodies in test samples depending on assays protocol.

A. COMPETITIVE-BINDING ASSAY

Principle: The unlabeled ligand (patient sample) competes with enzyme-conjugated ligand for a limited number of immobilized antibody/antigen-binding sites in wells. After a brief incubation, separation of bound and free enzyme-

conjugated ligand follows, substrate is added and enzyme present in the bound fraction converts substrate to a colored product. The amount of product formed is inversely related to the concentration of the unlabeled ligand in the test sample.

- **Procedure:**

- The test sample (unlabeled ligand) and enzyme-conjugated ligand are mixed in wells or solid support containing a limited amount of antigens or antibodies immobilized.

- The mixture is incubated to allow antigen-antibody complex to be formed.

- The beads of solid support are washed with buffered water to remove unbound antigens/antibodies.

- The substrate having a chromogenic substance is added, which reacts with the enzyme-conjugate during an incubation period, with development of a color.

- Then, the stop solution is added, to stop the enzyme reaction. After, the end product can be detected using a spectrophotometer. The amount of color that develops is inversely proportional to the amount of ligand in the sample.

B.NONCOMPETITIVE ASSAY

- **Principle:** Sample solutions are incubated with the antibody-coated beads so that all of the antigen present in the test sample will be bound by the immobilized antibody.

After removal of unbound material, the amount of antigen bound to the antibody is quantitated by adding an enzyme-conjugated antibody that recognizes a second antigenic determinant on the same antigen. Following a second incubation and separation step, the enzyme activity remaining associated with the sandwich is determined by the addition of the substrate. product formation is directly related to the amount of antigen in the standards, controls and test sample.

Procedure:

Add the patient's serum which may contain the specific antigen or antibody to the test well containing the fixed antigen or antibody depending on assay protocol.

Incubate allowing Ag/Ab reaction to occur. During incubation , antigens/antibodies in wells will attach to unknown in sample

- To remove antigen or antibodies, which did not bind the fixed antibodies or antigens, buffered wash solution is added. The free unattached patient antigens or antibodies become suspended in the wash solution while the attached antigens or antibodies remain bound to the fixed antigens or antibodies on the sides of the well.
- An Enzyme-labeled antibody/antigen is then added to the Test well and the system is incubated to allow Enzyme-labeled antibody/antigen to bind to the fixed antigen or antibody.
- Enzyme-labeled antigens or antibodies which have not attached must be washed from the well with buffered wash solution. This leaves patient's specific antigen or antibody sandwiched between fixed antibodies or antigens and the Enzyme-labeled antibodies or antigens respectively.

After the Test sandwich is formed, it is still not visible**.** Substrate is added to the well. Any Enzyme-labeled

antigen/antibody in the test system will react with the enzyme substrate during incubation. The action of the Enzyme on the substrate causes a color change in proportion to the Enzyme-linked antibodies/antigens bound to the patient's antigens/antibodies respectively

- Then, the stopping solution is added to stop the enzyme reaction. The amount of color that develops is proportional to the amount of antibody in the specimen.

Immunoassay Sensitivity and detectors

- ELISA assay is able to measure the value of analyte <1ug/dl.
- Colorimetric, chemiluminescent or fluorescent systems may provide qualitative or quantitative results,The signal levels are measured in a spectrophotometer, luminometer or fluorometer.

LABORATORY APPLICATIONS

- ELISA solid phase assays have become the standard method of screening for viral antigens or antibodies produced by host in response to viral infections . viruses like hepatitis, HIV,cytomegalovirus(CMV) and Epstein-Barr virus(EBV).
- ELISA can be used to detect hormones like follicle-stimulating hormone ,HCG, luteinizing hormone, prolactin, and thyroid-stimulating hormone.
- ELISA is also used to detect enzymes like creatine kinase isoenzyme(CK-MB), and prostatic acid phosphatase.
  ELISA is used to measure tumor markers like carcinoembryonic antigen, alpha-fetoprotein

ADVANTAGES AND DISADVANTAGES OF ELISA ASSAY

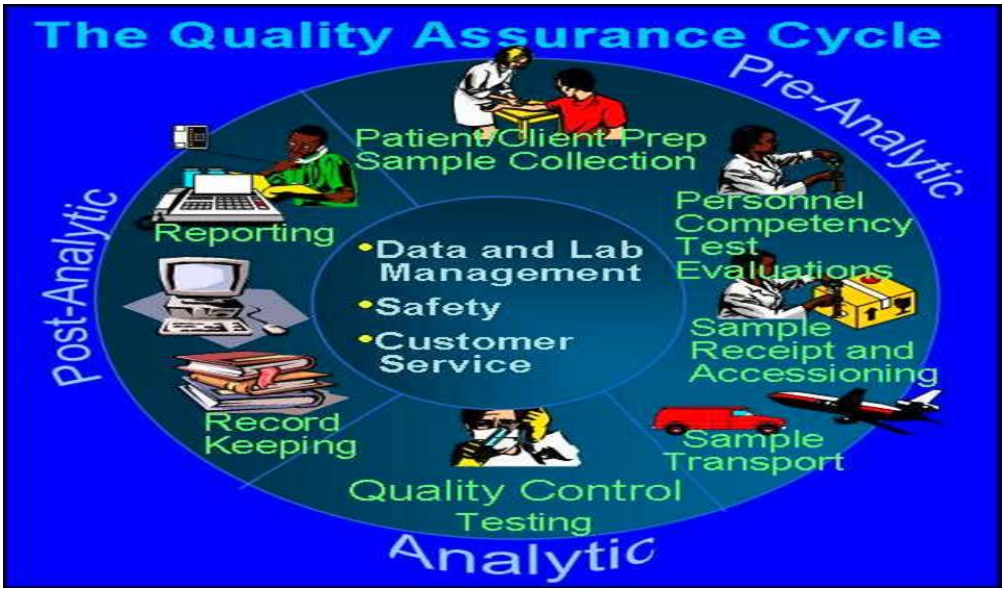
ADVANTAGES	DISADVANTAGES
➢ Utilizes small amounts of patient sample. ➢ Uses small volumes of reagents ➢ Can be designed to detect patient specific antibody or patient antigen. ➢ The competitive and noncompetitive methods are highly sensitive, detecting pictogram quantities of hormones and other substances. ➢ Enzyme –conjugated antigens and antibodies can be stored under sterile conditions and used for years without any appreciable loss of their enzymatic and immunologic activities.	➢ Monoclonal antibodies more difficult to find. ➢ ELISAs are the assays that req multiple incubation and separ steps and take longer to perfo than either RIA(radioimmunoassay) or IRMA(immunoradiometric as increasing the chance of error

CHAPTER VII

QUALITY ASSURANCE

INTRODUCTION

- QA is defined as the overall program that ensures that the final results reported by the laboratory are correct.
- Planned and systematic activities assure quality and reliability of the entire testing process from test order to test interpretation.
- QA includes pre-analytical, analytical, and post-analytical quality assurance monitors



Purpose of Quality Assurance

- Helps physicians, patients and clients
- Creates good reputation
- Motivates staff
- Is cost-effective(productive)
- Prevents complaints
- Builds trust

Key Components

- Internal quality assessment (IQA)
- External quality assessment (EQA)

- Standardization of processes and procedures (pre-analytic, analytic and post-analytic phases)
- Management and 0rganization

- **<u>Internal Quality Assessment</u>**

- Internal quality assessment can be maintained by the following ways,
- i) Use of standardized glassware, reagents and equipment
- ii) Employment of conscientious and well trained staff
- iii) Maintenance of
- Proper analytical skill from start to finish
- Required quality reagents
- Desired performance of the instruments
- iv) Selection of accurate and precise methods
-
- There are 2 phases in internal quality control
-
- a) Preventive phase
- b) Retrospective method

a) Preventive phase

In this phase, preventive precautions are taken at the following stages of specimen analysis:

- Collection of specimen
- Separation of serum
- Specimen analysis
- Photometric analysis and
- Calculations of test values etc.

b) Retrospective method

This phase includes the comparison between
i) Optimal condition variance (OCV)
ii) Routine condition variance (RCV)

- OCV refers to the test results obtained under optimum conditions i.e by using freshly prepared reagents and standardization A grade glass wares.
- RCV refers to the results obtained by using routine requirements (by using routine stored reagents and glassware in regular use).

- The difference between OCV and RCV should not be more than 3%.

In brief internal lab assessment include:

- Monitoring lab procedures
- Track lab processes
- Instrument calibration
- Equipment maintenance
- Result reporting and archiving
- Specimen collection and handling

EXTERNAL QUALITY ASSESSMENT

The term external quality assessment, is used to describe a method that allows for comparison of a laboratory‘s testing to

a source outside the laboratory. This comparison can be made to the performance of a group of laboratories or to the performance of a reference laboratory

Following are the various ways to observe external quality control

- i) Use of a recognized sample
- ii) Checking of the similarity of the reported values by sending the specimen to a recognized laboratory.
- iii) Analysis of some specimen brought from recognized laboratories and comparative studies of the analyzed specimen.

STANDARDIZATION OF PROCESSES AND PROCEDURES

- Reproducible lab results
- Uniform activities
- Standard operating procedures (SOP)

Taking care during the three stages of analysis

- Pre-analytical (Pre examination)
- Analytical (Examination)
- Post-analytical (Post examination)

WHY DO ERROR OCCURS?

Some causes include:

- Unclear Individual responsibilities
- No written procedures
- To do not follow the Written procedures .
- Training is not done or not completed
- Checks not done for transcription errors
- Test kits not stored properly
- Equipment not properly maintained
- Errors can occur throughout the testing process

PRE ANALYTIC ERRORS

- Errors at any stage of the collection, testing and reporting process can potentially lead to a serious patient misdiagnosis.
- Errors during the collection process are not inevitable but can be prevented with a diligent application of quality control, continuing education and effective collection systems.
- 32 - 75% of all test errors occur in the pre-analytical phase

The Pre-Analytical process

- 1. Patient Identification
- 2. Sampling Technique (example phlebotomy)
- 3. Test Collection Procedures
- 4. Specimen Transport
- 5. Specimen Processing

Collection of sample:

- Locate Patient
- Prepare Patient
- Draw Sample
- Label
- Dispose of supplies

PATIENT IDENTIFICATION

- When identifying the patient, you have to provide their full name, address, identification number and/or date of birth.

- Hospital No in ,patients should be wearing an identification band with the above information, which the phlebotomist should confirm before the venipuncture.
- It is important to identify a patient accurately so that blood is collected from the correct person.
- Drowning blood from the wrong person or labeling the correct patient's sample with a different patient's label can certainly contribute to laboratory error. (Mislabeling)

Factor which can affect lab results

- Diet & Nutrition status
- Genetic variation
- Obesity
- Exercise
- Posture
- Hemolysis, lipidemia & icterus
- Special habits
- Drugs
- Diagnostic and therapeutic procedures(endoscopy)

PHLEBOTOMY

Phlebotomy is a highly complex skill requiring expert knowledge, and critical judgment

- Venipuncture is a frequent medical procedure.
- Phlebotomy errors may cause harm to patients or result in needle stick injury to the phlebotomist

Posture

- The patient should be comfortably seated or supine for 20 minutes before sampling. Not standing
- The patient arm should be extended in a straight line from the shoulder to the wrist.

Collection site.

- The median cubital vein is the preferred site.
  Veins on the hand or at ankle may be used

PHLEBOTOMY TECHNIQUES ERRORS

Tourniquet Application

- Tourniquet tied too close to the venipuncture site can cause hematoma
- Veins may not become prominent if tourniquet is tied too high (more than 3 to 4 inches above venipuncture site)
- Tourniquet left on longer than one minute can result in hemo concentration , affecting some test results
- Tourniquet should be released as soon as needle is in the lumen of the vein and blood flow established

- **Additive** : ex: EDTA, citrate, lithium heparin , oxalate, fluoride
  • Collected insufficient Blood to the amount of additive in tube.
- **Hemolysis**
  - Traumatic venipuncture

- Blood collected from area with hematoma
- Vigorous shaking of tubes after collection

- **Proper Tube Mixing**: All tubes with additives need to be inverted to mix the additive evenly with the blood. Improper mixing of the tube after venipuncture could contribute to sample clotting.

SPECIMEN TRANSPORT

- **Temperature**
- Specimens must be transported at the appropriate temperature for the required test
    - Avoid temperature extremes if transported via vehicle from other collection site
- **Light**
    - Some samples need to be protected from light, for example sample for bilirubin.

- **Blood Specimen Transport**

Transport of blood specimens in the proper manner after collection ensures the quality of the sample

- **Timing**

- Some specimens must be transported immediately after collection, for example Arterial Blood Gases.
- Specimens for serum or plasma chemistry testing should be centrifuged and separated within two hours

ANALYTICAL FACTORS THAT CAN AFFECT LABORATORY TESTS :

- Instrument calibration and maintenance
- Standards and procedure control
- Test procedure logistics (reagents, pipetting, timing etc).

Preventing and Detecting Errors – During Testing

- Perform and review Quality Control (QC)
- Follow safety precautions
- Conduct test according to written procedures
- Correctly interpret test results

POST-ANALYTICAL FACTORS

- Transcription error in reporting
- Report sent to the wrong location
- Information system not maintained
- Actually ,in reporting and charting of the results, including transcription and clerical errors.
- Use of computers as well as proper patient identifiers, laboratory request form, labels and specimen containers can greatly aid in minimizing these errors.

Preventing and Detecting Errors – After testing

- Re-check patient/client identifier
- Write legibly
- Clean up and dispose of contaminated waste
- Package EQA specimens for re-testing, if needed
  EQA = external quality assurance

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