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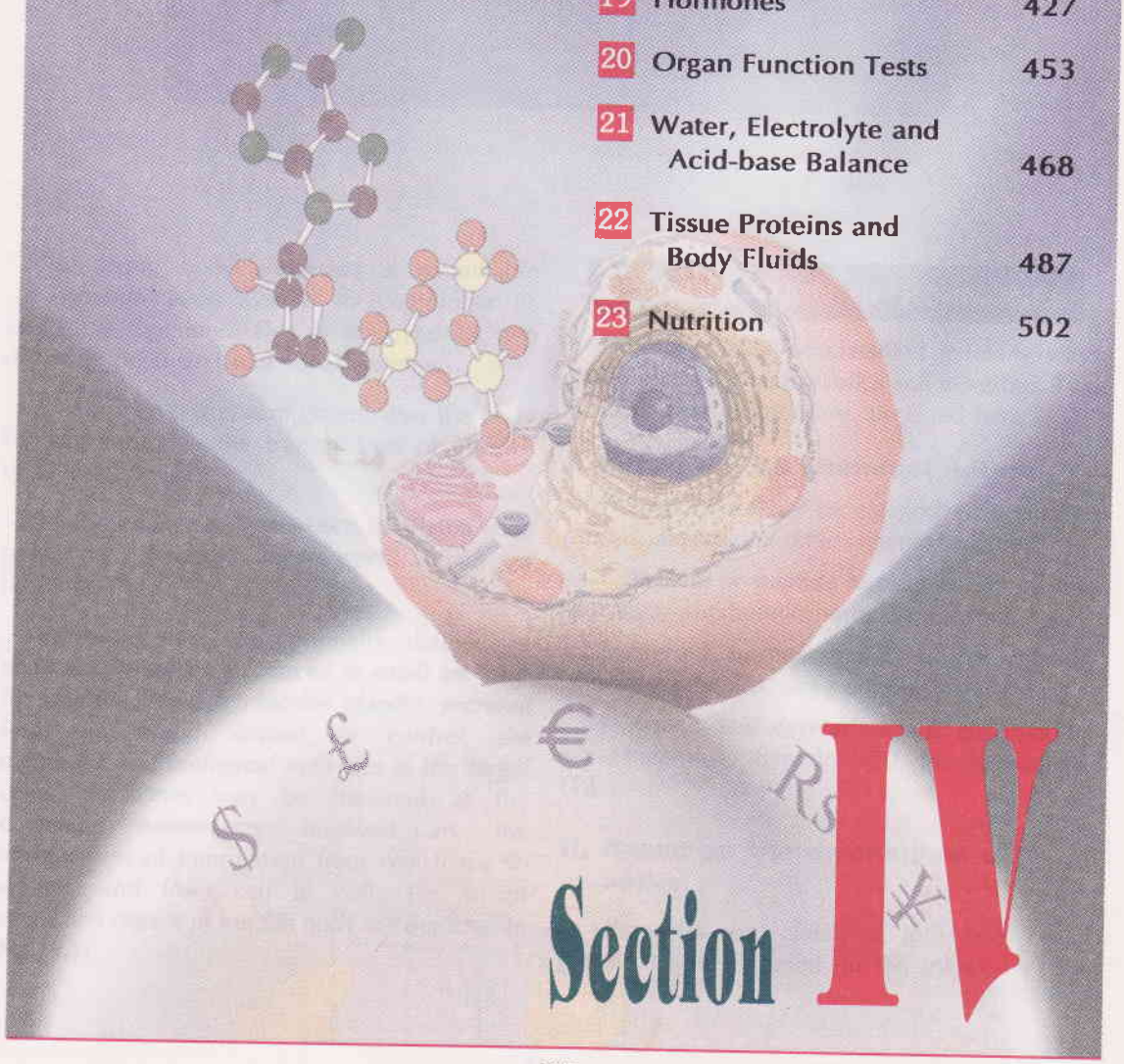
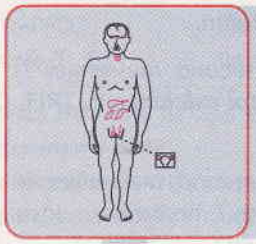


Section IV

19 Hormones



The hormones speak :

"We are the chemical messengers of the body!
Diversified in our structure and function;
Act either directly or through messengers;
'Growth, health and welfare' is our motto."

The living body possesses a remarkable communication system to coordinate its biological functions. This is achieved by two distinctly organized functional systems.

1. The nervous system coordinates the body functions through the transmission of electro-chemical impulses.

2. The ***endocrine system*** acts through a wide range of ***chemical messengers*** known as hormones.

Hormones are conventionally defined as ***organic substances, produced in small amounts by specific tissues (endocrine glands), secreted into the blood stream to control the metabolic and biological activities in the target cells***. Hormones may be regarded as the ***chemical messengers*** involved in the transmission of information from one tissue to another and from cell to cell. The major endocrine organs in human body are depicted in ***Fig.19.1***).

CLASSIFICATION OF HORMONES

Hormones may be classified in many ways based on their characteristics and functions. Two types of classification are discussed here

I. Based on the chemical nature

The hormones can be categorized into three groups considering their chemical nature.

1. **Protein** or **peptide hormones** e.g. insulin, glucagon, antidiuretic hormone, oxytocin.

2. **Steroid hormones** e.g. glucocorticoids, mineralocorticoids, sex hormones.

3. **Amino acid derivatives** e.g. epinephrine, norepinephrine, thyroxine (T_4), triiodothyronine (T_3).

II. Based on the mechanism of action

Hormones are classified into two broad groups (I and II) based on the location of the

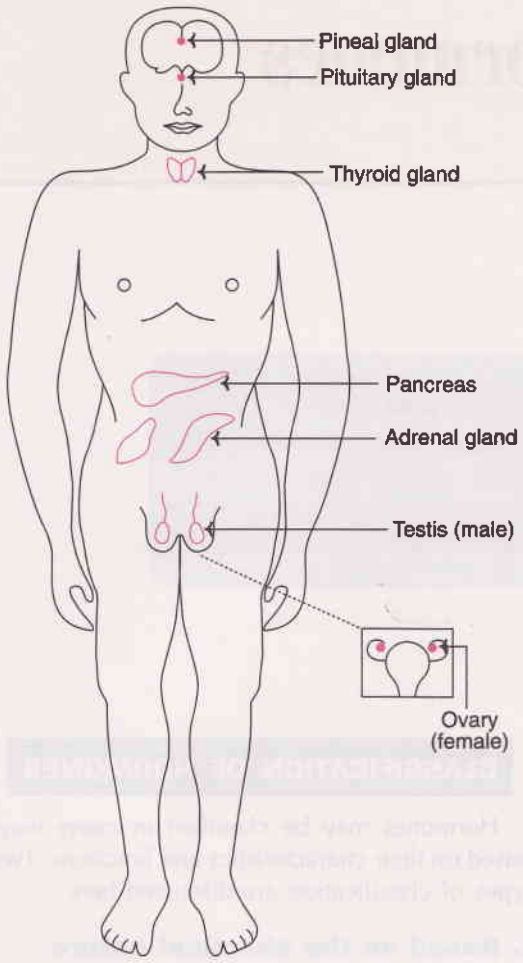


Fig. 19.1 : Diagrammatic representation of major endocrine glands.

receptors to which they bind and the signals used to mediate their action.

1. **Group I hormones :** These hormones ***bind to intracellular receptors*** to form receptor-hormone complexes (the intracellular messengers) through which their biochemical functions are mediated. Group I hormones are lipophilic in nature and are mostly derivatives of cholesterol (exception—T_3 and T_4). e.g. estrogens, androgens, glucocorticoids, calcitriol.

2. **Group II hormones :** These hormones bind to cell surface (plasma membrane) receptors and stimulate the release of certain molecules, namely the ***second messengers*** which, in turn, perform the biochemical functions. Thus, hormones themselves are the first messengers.

Group II hormones are subdivided into three categories based on the chemical nature of the second messengers.

(a) The second messenger is cAMP e.g. ACTH, FSH, LH, PTH, glucagon, calcitonin.

(b) The second messenger is ***phosphatidyl-inositol/calcium*** e.g. TRH, GnRH, gastrin, CCK.

(c) The second messenger is ***unknown*** e.g. growth hormone, insulin, oxytocin, prolactin.

The principal human hormones, their classification based on the mechanism of action, and major functions are given in ***Table 19.1***.

Mechanism of action of group I hormones

These hormones are lipophilic in nature and can easily pass across the plasma membrane. They act through the ***intracellular receptors*** located either in the cytosol or the nucleus. The hormone-receptor complex binds to specific regions on the DNA called hormone responsive element (HRE) and causes increased expression of specific genes (***Fig.19.2***). It is believed that the interaction of hormone receptor complex with HRE promotes initiation and, to a lesser extent, elongation and termination of RNA synthesis (transcription). The ultimate outcome is the production of specific proteins (translation) in response to hormonal action.

Mechanism of action of group II hormones

These hormones are considered as the first messengers. They exert their action through mediatory molecules, collectively called ***second messengers***.

Table 19.1 Principal human hormones—classification (by mechanism of action), origin and major functions

Hormone(s)	*Origin*	*Major Function(s)*
Group I. HORMONES THAT BIND TO INTRACELLULAR RECEPTORS		
Estrogens	Ovaries and adrenal cortex	Female sexual characteristics, menstrual cycle.
Progestins	Ovaries and placenta	Involved in menstrual cycle and maintenance of pregnancy.
Androgens	Testes and adrenal cortex	Male sexual characteristics, spermatogenesis.
Glucocorticoids	Adrenal cortex	Affect metabolisms, suppress immune system.
Mineralocorticoids	Adrenal cortex	Maintenance of salt and water balance.
Calcitriol (1, 25–DHCC)	Kidney (final form)	Promotes absorption of Ca^{2+} from intestine, kidney and bone.
Thyroid hormones (T_3, T_4)	Thyroid	Promote general metabolic rate.
Group II. HORMONES THAT BIND TO CELL SURFACE RECEPTORS		
A. The second messenger is cAMP		
Adrenocorticotropic hormone (ACTH)	Anterior pituitary	Stimulates the release of adrenocorticosteroids.
Follicle stimulating hormone (FSH)	Anterior pituitary	In females, stimulates ovulation and estrogen synthesis. In males, promotes spermatogenesis.
Luteinizing hormone (LH)	Anterior pituitary	Stimulates synthesis of estrogens and progesterone and causes ovulation. Promotes androgen synthesis by testes.
Chorionic gonadotropin (hCG)	Anterior pituitary	Stimulates progesterone release from placenta.
Thyroid stimulating hormone (TSH)	Anterior pituitary	Promotes the release of thyroid hormones (T_3, T_4).
β-Endorphins and enkephalins	Anterior pituitary	Natural endogenous analgesics (pain relievers).
Antidiuretic hormone (ADH)	Posterior pituitary (stored)	Promotes water reabsorption by kidneys.
Glucagon	Pancreas	Increases blood glucose level, stimulates glycogenolysis and lipolysis.
Parathyroid hormone (PTH)	Parathyroid	Increases serum calcium, promotes Ca^{2+} release from bone.
Calcitonin	Thyroid	Lowers serum calcium. Decreases Ca^{2+} uptake by bone and kidney.
Epinephrine	Adrenal medulla	Increases heart rate and blood pressure. Promotes glycogenolysis in liver and muscle and lipolysis in adipose tissue.
Norepinephrine	Adrenal medulla	Stimulates lipolysis in adipose tissue.
B. The second messenger is phosphatidyl inositol/calcium		
Thyrotropin-releasing hormone (TRH)	Hypothalamus	Promotes TSH release.
Gonadotropin-releasing hormone (GnRH)	Hypothalamus	Stimulates release of FSH and LH.
Gastrin	Stomach	Stimulates gastric HCl and pepsinogen secretion.
Cholecystokinin (CCK)	Intestine	Stimulates contraction of gall bladder and secretion of pancreatic enzymes.
C. The second messenger is unknown/unsettled		
Growth hormone (GH)	Anterior pituitary	Promotes growth of the body (bones and organs).
Prolactin (PRL)	Anterior pituitary	Growth of mammary glands and lactation.
Oxytocin	Posterior pituitary (stored)	Stimulates uterine contraction and milk ejection.
Insulin	Pancreas	Lowers blood glucose (hypoglycemic effect), promotes protein synthesis and lipogenesis.
Somatomedins (insulin-like growth factors, IGF-I, IGF-II)	Liver	Growth related functions of GH are mediated. Stimulates growth of cartilage.

cAMP—THE SECOND MESSENGER

Cyclic AMP (cAMP, cyclic adenosine 3′,5′-monophosphate) is a ubiquitous nucleotide. It consists of adenine, ribose and a phosphate (linked by 3′,5′ linkage). cAMP acts as a second messenger for a majority of ***polypeptide hormones.***

The membrane-bound enzyme adenylate cyclase converts ATP to cyclic AMP. cAMP is hydrolysed by phosphodiesterase to 5′-AMP (***Fig.19.3***).

Adenylate cyclase system

A series of events occur at the membrane level that influence the activity of adenylate cyclase leading to the synthesis of cAMP. This process is mediated by ***G-proteins***, so designated due to their ability to bind to ***g***uanine nucleotides.

Action of cAMP—a general view

Once produced, cAMP performs its role as a second messenger in eliciting biochemical responses (***Fig.19.4***). cAMP activates protein kinase **A** (A stands for c**A**MP). This enzyme is a heterotetramer consisting of 2 regulatory subunits (R) and 2 catalytic subunits (C).

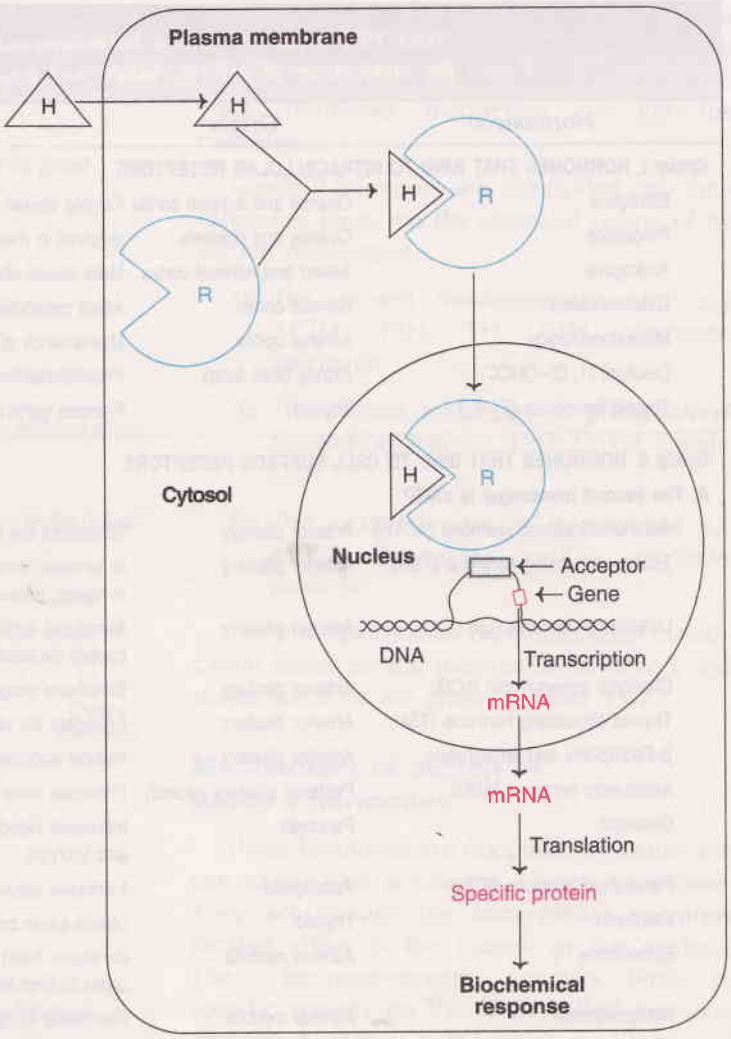


Fig. 19.2 : Mechanism of action of steroid hormones (H–Hormone; R–Receptor; HR–Hormone-receptor complex).

cAMP binds to inactive protein kinase and causes the dissociation of R and C subunits.

$$4cAMP + R_2C_2 \longrightarrow R_2(4\ cAMP) + 2C$$

(inactive) (inactive) (active)

The active subunit (C) catalyses phosphorylation of proteins (transfer of phosphate group to serine and threonine residues). It is the phosphoprotein that ultimately causes the biochemical response.

It should, however, be remembered that cAMP does not act on all protein kinases. For instance, on protein kinase C (the second messenger is diacylglycerol).

Dephosphorylation of proteins : A group of enzymes called protein phosphatases hydrolyse and remove the phosphate group added to proteins.

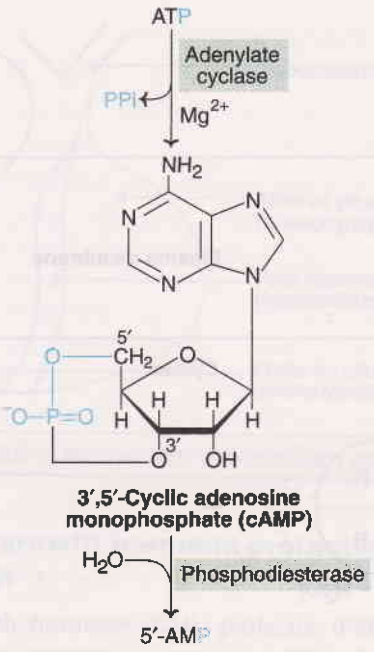


Fig. 19.3 : Synthesis and degradation of cAMP.

Degradation of cAMP : cAMP undergoes rapid hydrolysis, catalysed by the enzyme phosphodiesterase to 5′ AMP which is inactive. Hence, the effect of cAMP will be shortlived if the hormone stimulating adenylate cyclase is removed. Caffeine and theophylline (methylxanthine derivatives) can inhibit phosphodiesterases and increase the intracellular levels of cAMP.

HYPOTHALAMIC AND PITUITARY HORMONES

The pituitary gland or hypophysis (weighing about 1 g) is located below the hypothalamus of the brain. It consists of two distinct parts—the anterior pituitary (adenohypophysis) and the posterior pitutitary (neurohypophysis) connected by pars intermedia (***Fig.19.5***). The latter is almost absent in humans, although found in lower organisms.

Hypothalamus is a specialized center in the brain that functions as a ***master coordinator of hormonal action***. In response to the stimuli of central nervous system, hypothalamus liberates certain releasing factors or hormones. These factors stimulate or inhibit the release of corresponding tropic hormones from the anterior pituitary. Tropic hormones stimulate the target endocrine tissues to secrete the hormones they synthesize. The relationship between hypothalamus and pituitary with endocrine glands is illustrated in ***Fig.19.6***. ***In general, the hormonal system is under feedback control***. For instance, adrenocorticotropic hormone (ACTH) inhibits the release of corticotropin releasing hormone (CRH).

HYPOTHALAMIC HORMONES

Hypothalamus produces at least six releasing factors or hormones.

1. **Thyrotropin-releasing hormone (TRH) :** It is a tripeptide consisting of glutamate derivative (pyroglutamate), histidine and proline. TRH stimulates anterior pituitary to release thyroid-stimulating hormone (TSH or thyrotropin) which, in turn, stimulates the release of thyroid hormones (T_3 and T_4).

2. **Corticotropin-releasing hormone (CRH) :** It stimulates anterior pituitary to release adrenocorticotropic hormone (ACTH) which in turn, acts on adrenal cortex to liberate adrenocorticosteroids. CRH contains 41 amino acids.

3. **Gonadotropin-releasing hormone (GnRH) :** It is a decapeptide. GnRH stimulates anterior pituitary to release ***gonadotropins***, namely luteinizing hormone (LH) and follicle stimulating hormone (FSH).

4. **Growth hormone-releasing hormone (GRH)** with 44 amino acids stimulates the release of growth hormone (GH or somatotropin) which promotes growth.

5. **Growth hormone release-inhibiting hormone (GRIH) :** It contains 14 amino acids and is also known as ***somatostatin***. GRIH inhibits the release of growth hormone from the anterior pituitary.

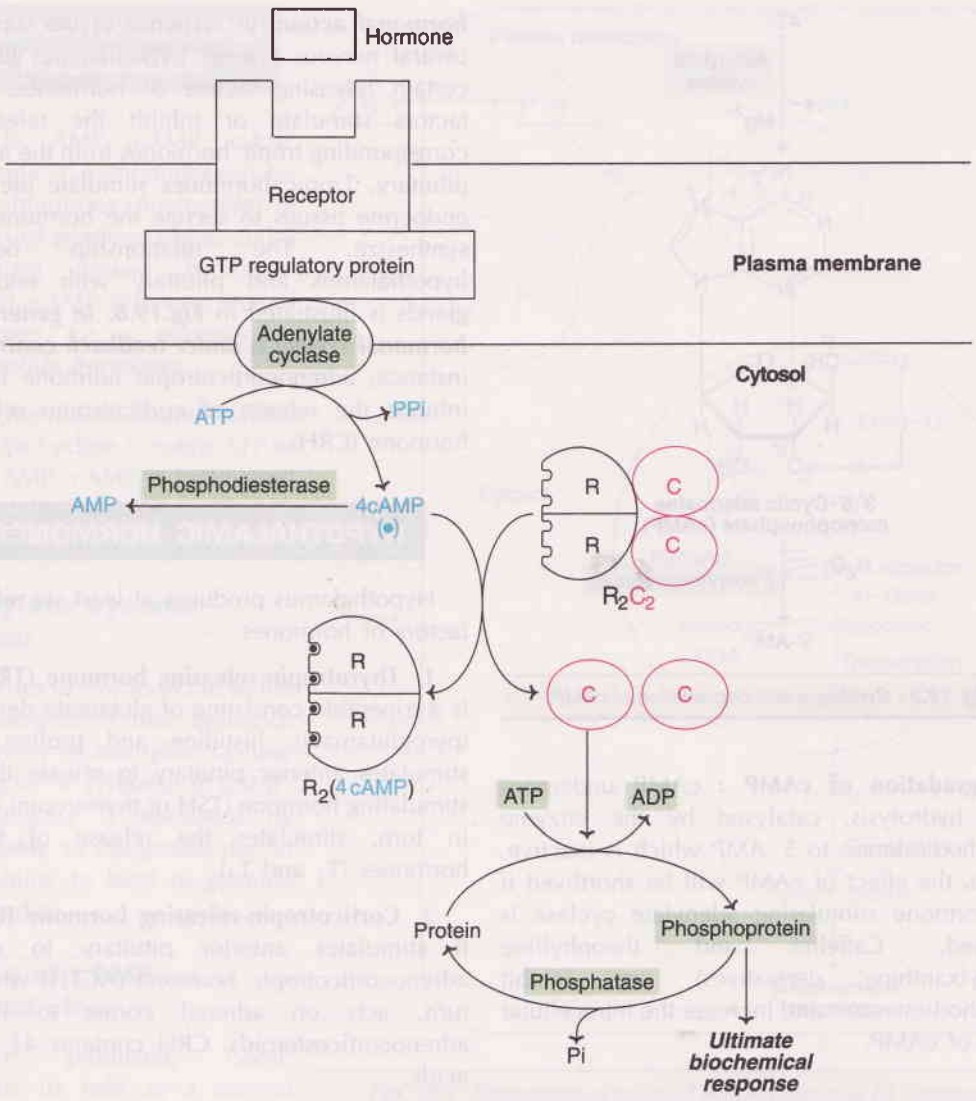


Fig. 19.4 : *Overview of synthesis and action of cAMP (R_2C_2–cAMP dependent protein kinase A; R_2–Regulatory subunits; C_2–Catalytic subunits; C–Active catalytic unit of R_2C_2).*

6. **Prolactin release-inhibiting hormone (PRIH) :** It is believed to be a dopamine and/or a small peptide that inhibits the release of prolactin (PRL) from anterior pituitary.

ANTERIOR PITUITARY HORMONES

Anterior pituitary or adenohypophysis is truly the ***master endocrine organ.*** It produces several hormones that influence—either directly or indirectly—a variety of biochemical processes in the body. The hormones of adenohypophysis are broadly classified into three categories.

I. The growth hormone-prolactin group.

II. The glycoprotein hormones.

III. The pro-opiomelanocortin peptide family.

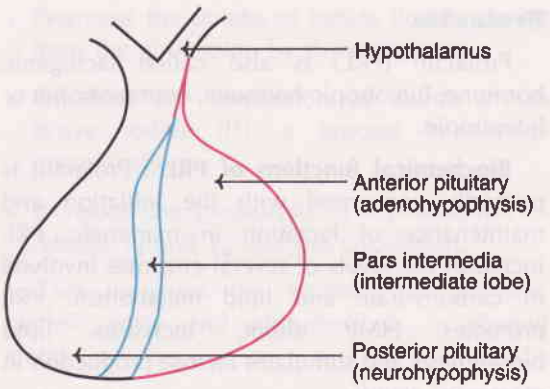


Fig. 19.5 : A diagrammatic view of pituitary gland.

I. The growth hormone-prolactin group

Growth hormone (GH), prolactin (PRL) and chorionic somatomammotropin (CS; placental lactogen) are protein hormones with many striking similarities in their structure.

Growth hormone (GH)

The growth hormone (or somatotropin) is produced by somatotropes, a special group of acidophilic cells of anterior pituitary.

Regulation of GH release : Two hypothalamic factors play a prominent role in the release of growth hormones. These are the growth hormone-releasing hormone (GRH) that stimulates and the growth hormone release-inhibiting hormone (GRIH, somatostatin) that inhibits. This, in turn, is regulated by a feedback mechanism.

Growth hormone production is influenced by many factors such as sleep, stress (pain, cold, surgery), exercise, food intake etc. It is observed that the largest increase in the production of GH occurs after the onset of sleep. This supports the ***adage "If you don't sleep, you won't grow."***

Biochemical functions of GH : Growth hormone promotes growth, and also influences the normal metabolisms (protein, carbohydrate, lipid and mineral) in the body.

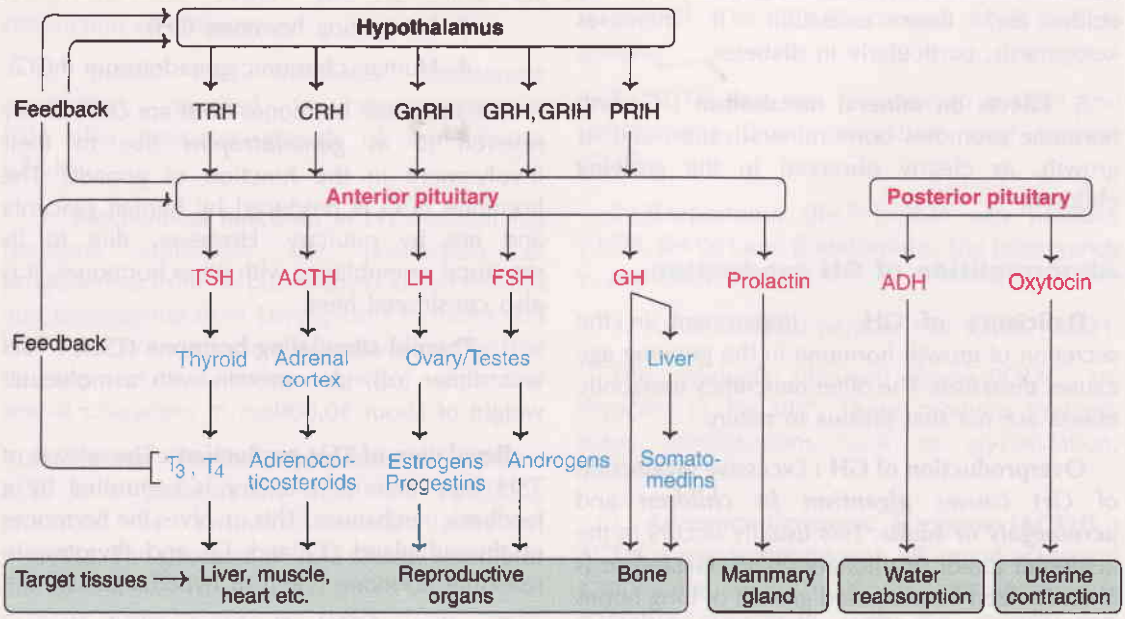


Fig. 19.6 : Hormonal heirarchy relationships between hypothalamus and pituitary with other endocrine glands [TRH–Thyrotropin releasing hormone; CRH–Corticotropin releasing hormone; GnRH–Gonadotropin releasing hormone; GRH–Growth hormone releasing hormone; GRIH–Growth hormone release inhibiting hormone; TSH–Thyroid stimulating hormone; ACTH–Adrenocorticotropic hormone; LH–Luteinizing hormone; FSH–Follicle stimulating hormone; GH–Growth hormone; ADH–Antidiuretic hormone; T_3–Triiodothyronine; T_4–Tetraiodothyronine (thyroxine)].

1. **Effects on growth :** As is obvious from the name, GH is essential for the growth. The growth-related effects of GH are mediated through insulin like growth factor I (IGF-I) which is also known as ***somatomedin C*** (formerly sulfation factor), produced by liver.

2. **Effects on protein metabolism :** Growth hormone has an anabolic effect on protein metabolism. It promotes the uptake of amino acids into the tissues and increases the protein synthesis. The overall effect of GH is a positive nitrogen balance that leads to increase in body weight.

3. **Effects on carbohydrate metabolism :** Growth hormone is antagonistic to insulin and causes hyperglycemia. GH increases gluconeogenesis, decreases glucose utilization, impairs glycolysis and reduces the tissue uptake of glucose.

4. **Effects on lipid metabolism :** Growth hormone promotes lipolysis in the adipose tissue and increases the circulatory levels of free fatty acids and their oxidation. It increases ketogenesis, particularly in diabetes.

5. **Effects on mineral metabolism :** Growth hormone promotes bone mineralization and its growth, as clearly observed in the growing children.

Abnormalities of GH production

Deficiency of GH : Impairment in the secretion of growth hormone in the growing age causes ***dwarfism***. The other deficiency metabolic effects are not that serious in nature.

Overproduction of GH : Excessive production of GH causes ***gigantism in children*** and ***acromegaly in adults***. This usually occurs in the acidophil tumor of pituitary gland. Gigantism is characterized by increased growth of long bones and this is observed before the epiphyseal plates close. Acromegaly occurs after epiphyseal closure and is characterized by increase in the size of hands, facial changes (enlarged nose, protruding jaw), excessive hair, thickening of skin etc.

Prolactin

Prolactin (PRL) is also called lactogenic hormone, luteotropic hormone, mammotropin or luteotropin.

Biochemical functions of PRL : Prolactin is primarily concerned with the initiation and maintenance of lactation in mammals. PRL increases the levels of several enzymes involved in carbohydrate and lipid metabolism. PRL promotes HMP shunt, increases lipid biosynthesis and stimulates lactose production in mammary glands.

Prolactin promotes the growth of corpus luteum (hence also known as luteotropic hormone) and stimulates the production of progesterone.

II. The glycoprotein hormones

The following four hormones are glycoprotein in nature and possess certain structural similarities, despite their functional diversity.

1. Thyroid stimulating hormone (TSH)
2. Follicle stimulating hormone (FSH)
3. Luteinizing hormone (LH)
4. Human chorionic gonadotropin (hCG).

The last three hormones (***2-4***) are collectively referred to as ***gonadotropins*** due to their involvement in the function of gonads. The hormone hCG is produced by human placenta and not by pituitary. However, due to its structural resemblance with other hormones, it is also considered here.

1. **Thyroid stimulating hormone (TSH) :** TSH is a dimer ($\alpha\beta$) glycoprotein with a molecular weight of about 30,000.

Regulation of TSH production : The release of TSH from anterior pituitary is controlled by a feedback mechanism. This involves the hormones of thyroid gland (T_3 and T_4) and thyrotropin-releasing hormone (TRH) of hypothalamus.

Functions of TSH : The biochemical effects of TSH on thyroid gland are briefly discussed here. TSH binds with plasma membrane receptors and stimulates adenylate cyclase with a consequent increase in cAMP level. TSH, through the mediation of cAMP, exerts the following effects.

- Promotes the uptake of iodide (iodide pump) from the circulation by thyroid gland.
- Enhances the conversion of iodide (I^-) to active iodide (I^+), a process known as organification.
- Increases the proteolysis of thyroglobulin to release T_3 and T_4 into the circulation.

TSH increases the synthesis of proteins, nucleic acids and phospholipids in thyroid gland.

Gonadotropins : The follicle-stimulating hormone (***FSH***), luteinizing hormone (***LH***) and human chorionic gonadotropin (***hCG***) are commonly known as gonadotropins. All three are glycoproteins.

The release of FSH and LH from the anterior pituitary is controlled by gonadotropin-releasing hormone (GnRH) of hypothalamus.

2. **Biochemical functions of FSH :** In females, FSH stimulates follicular growth, increases the weight of the ovaries and enhances the production of estrogens.

In males, FSH stimulates testosterone production, required for spermatogenesis. FSH also promotes growth of seminiferous tubules.

3. **Biochemical functions of LH :** Luteinizing hormone stimulates the production of progesterone from corpus luteum cells in females and testosterone from Leydig cells in males. LH and FSH are collectively responsible for the development and maintenance of secondary sexual characters in males.

4. **Human chorionic gonadotropin (hCG) :** hCG is a glycoprotein (mol. wt. 100,000), produced by syncytiotrophoblast cells of placenta. The structure of hCG closely resembles that of LH.

The levels of hCG in plasma and urine increase almost immediately after the implantation of fertilized ovum. The detection of ***hCG in urine*** is conveniently used for the ***early detection*** (within a week after missing the menstrual cycle) ***of pregnancy***.

III. The pro-opiomelanocortin (POMC) peptide family

This family consists of the hormones—adrenocorticotropic hormone (***ACTH***), lipotropin (***LPH***) and melanocyte stimulating hormone (***MSH***) and several (about 24) neuromodulators such as endorphins and enkephalins.

The synthesis of POMC family. is very interesting. All the members of POMC are produced from a single gene of the anterior and intermediate lobes of pituitary. It is fascinating that a single polypeptide—pro-opiomelanocortin—is the precursor (approximately 285 amino acids) that contains multiple hormones. The name ***pro-opiomelano-cortin*** is derived since it is a ***pro***hormone to ***opio***ids, ***melano***cyte-stimulating hormone and ***corti***cotropi***n***.

Products of POMC : The pituitary multihormone precursor is synthesized as pre-proopiomelanocortin from which POMC is formed. The POMC consists of 3 peptide groups.

1. ACTH that can give rise to α-MSH and corticotropin like intermediate lobe peptide (CLIP).

2. β-Lipotropin (β-LPH) that can produce γ-LPH, β-MSH and β-endorphin. The latter yields γ- and α-endorphins.

3. An N-terminal peptide that forms γ-MSH.

The products obtained from POMC are depicted in ***Fig.20.7***. These products undergo many modifications such as glycosylation, acetylation etc.

1. **Adrenocorticotropic hormone (ACTH) :** ACTH is a polypeptide with 39 amino acids and a molecular weight of 4,500. This hormone is primarily concerned with the growth and functions of adrenal cortex.

Regulation of ACTH production : The release of ACTH from the anterior pituitary is under the regulation of hypothalamic hormone, namely corticotropin releasing hormone (CRH).

Biochemical functions of ACTH

- ACTH promotes the conversion of cholesterol to pregnenolone in the adrenal cortex.
- It enhances RNA and protein synthesis and thus promotes adrenocortical growth.
- ACTH increases lipolysis by activating lipase of adipose tissue.

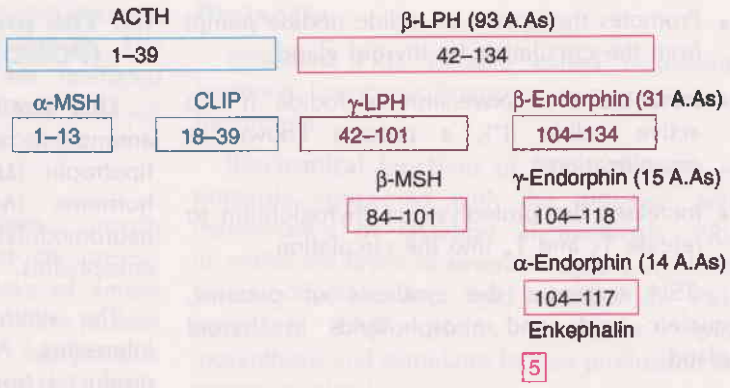


***Fig. 19.7** : The members of the pro-opiomelanocortin (POMC) family derived from POMC cleavage. (Numbers in blocks represent amino acids in sequence; In the brackets are the number of amino acids–AAs; (ACTH–Adrenocorticotropic hormone; LPH–Lipotropin; MSH–Melanocyte-stimulating hormone; CLIP–Corticotropin like intermediate lobe peptide).*

Overproduction of ACTH : Cushing's syndrome is caused by an excessive production of ACTH which may be due to a tumor. This syndrome is characterized by hyper-pigmentation and increased production of adrenocorticosteroids. The associated symptoms include negative nitrogen balance, impaired glucose tolerance, hypertension, edema, muscle atrophy etc.

2. **β-Lipotropin (β-LPH) :** β-LPH is derived from POMC and contains 93 carboxy terminal amino acids. This polypeptide consists of γ-LPH and β-endorphin from which β-MSH and γ-endorphin are, respectively, formed. γ-Endorphin can be converted to α-endorphin and then to enkephalins (***Fig.19.7***). β-LPH is found only in the pituitary and not in other tissues since it is rapidly degraded.

The biochemical functions of β-LPH, as such, are limited. It promotes lipolysis and increases the mobilization of fatty acids. The most important function of β-LPH is its precursor role for the formation of β-endorphin and enkephalins.

Endorphins and enkephalins : These are the ***natural analgesics*** that control pain and emotions. They were discovered after an unexpected finding of opiate receptors in the human brain.

Synthesis : Endorphins and enkephalins are produced from β-endorphin which, in turn, is derived from POMC (***Fig.19.7***). β-Lipotropin has 31 amino acids while its modified products α and γ-endorphins have 15 and 14 amino acids, respectively. Methionine enkephalin (Tyr–Gly–Gly–Phe–***Met***) and leucine enkephalin (Tyr-Gly-Gly-Phe-***Leu***) are the two important pentapeptide derivatives of β-endorphin.

Biochemical actions : Endorphins and enkephalins are peptide neurotransmitters that produce opiate-like effects on the central nervous system, hence they are also known as ***opioid-peptides***. They bind to the same receptors as the morphine opiates and are believed to ***control*** the ***endogenous pain perception***. Endorphins and enkephalins are more potent (20-30 times) than morphine in their function as analgesics.

It is ***believed that the pain relief*** through ***acupuncture and placebos is mediated through opioid peptides***.

3. **Melanocyte-stimulating hormone (MSH) :** Three types of MSH (α, β and γ) are present in the precursor POMC molecule. In humans, γ MSH is important while in some animals α and β are functional. The activity of γ-MSH is contained in the molecule γ-LPH or its precursor β-LPH (***Fig.19.7***).

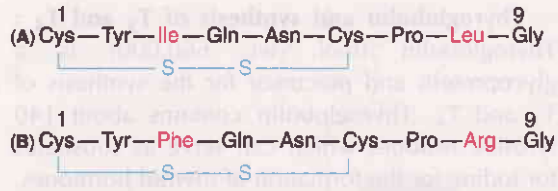


Fig. 19.8 : Structures of (A) Human oxytocin and (B) Human antidiuretic hormone (ADH).

The functions of MSH has been clearly established in some animals. MSH promotes the synthesis of skin pigment melanin (melanogenesis) and disperses melanin granules that ultimately leads to darkening of the skin. In humans, MSH does not appear to play any role in melanin synthesis.

POSTERIOR PITUITARY HORMONES

Two hormones namely ***oxytocin*** and ***antidiuretic hormone*** (ADH, vasopressin) are produced by the posterior pituitary gland (neurohypophysis). Both of them are nonapeptides (9 amino acids). Their structures are depicted in ***Fig.19.8.***

Oxytocin

The release of oxytocin from posterior pituitary gland is caused by the neural impulses of nipple stimulation. The other stimuli responsible for oxytocin release include vaginal and uterine distention.

Biochemical functions

1. **Effect on uterus :** Oxytocin causes the contraction of pregnant uterus (smooth muscles) and induces labor.

2. **Effect on milk ejection :** In mammals, oxytocin causes contraction of myoepithelial cells (look like smooth muscle cells) of breast. This stimulates the squeezing effect, causing milk ejection from the breast.

3. Oxytocin synthesized in the ovary appears to ***inhibit the synthesis of steroids.***

Antidiuretic hormone (ADH)

The release of ADH (also called ***vasopressin***) is mostly controlled by osmoreceptors (of hypothalamus) and baroreceptors (of heart). Any increase in the osmolarity of plasma stimulates ADH secretion.

Biochemical functions : ADH is primarily concerned with the regulation of water balance in the body. It stimulates kidneys to retain water and, thus, increases the blood pressure.

In the absence of ADH, the urine output would be around 20 l/day. ADH acts on the distal convoluted tubules of kidneys and causes water reabsorption with a result that the urine output is around 0.5-1.5 l/day.

Mechanism of action : ADH stimulates adenylate cyclase causing production of cAMP. Water reabsorption is promoted by cAMP. Inhibitors of adenylate cyclase (e.g. calcium) inhibit the activity of ADH. This supports the view that ADH action is mostly mediated through cAMP.

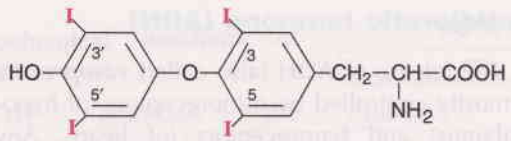
Diabetes insipidus : This disorder is characterized by the excretion of large volumes of dilute urine ***(polyuria)***. It may be due to insufficient levels of ADH or a defect in the receptors of target cells.

THYROID HORMONES

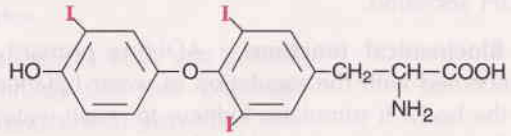
Thyroid gland (weighs about 30 g in adults) is located on either side of the trachea below the larynx. It produces two principal hormones (***Fig.19.9***)—***thyroxine*** (T_4; 3,5,3′,5′-tetraiodothyronine) and ***3,5,3′-triiodothyronine*** (T_3)—which regulate the metabolic rate of the body. Thyroid gland also secretes calcitonin, a hormone concerned with calcium homeostasis (discussed under calcium metabolism, ***Chapter 18***).

Biosynthesis of thyroid hormones

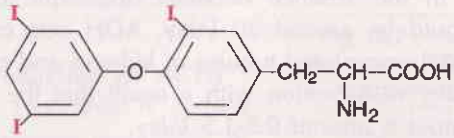
Iodine is essential for the synthesis of thyroid hormones. More than half of the body's total iodine content is found in the thyroid gland.



3, 5, 3′, 5′-Tetraiodothyronine (thyroxine, T_4)



3,5,3′-Triiodothyronine (T_3)

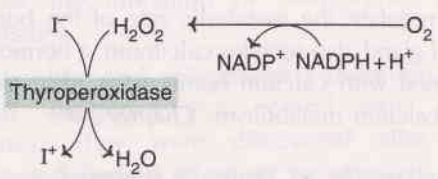


3, 3′, 5′-Triiodothyronine (reverse T_3, rT_3)

Fig. 19.9 : Structures of thyroid hormones (Refer Fig. 15.21 for their biosynthesis).

Uptake of iodide : The uptake of iodide by the thyroid gland occurs against a concentration gradient (about 20 : 1). It is an energy requiring process and is linked to the ATPase dependent Na^+-K^+ pump. Iodide uptake is primarily controlled by TSH. Antithyroid agents such as thiocyanate and perchlorate inhibit iodide transport.

Formation of active iodine : The conversion of iodide (I^-) to active iodine (I^+) is an essential step for its incorporation into thyroid hormones. Thyroid is the only tissue that can oxidize I^- to a higher valence state I^+. This reaction requires H_2O_2 and is catalysed by the enzyme thyroperoxidase (mol. wt. 60,000). An NADPH dependent system supplies H_2O_2.



TSH promotes the oxidation of iodide to active iodine while the antithyroid drugs (thiourea, thiouracil, methinazole) inhibit.

Thyroglobulin and synthesis of T_3 and T_4 : Thyroglobulin (mol. wt. 660,000) is a glycoprotein and precursor for the synthesis of T_3 and T_4. Thyroglobulin contains about 140 tyrosine residues which can serve as substrates for iodine for the formation of thyroid hormones.

Tyrosine (of thyroglobulin) is first iodinated at position 3 to form monoiodotyrosine (MIT) and then at position 5 to form diiodotyrosine (DIT). Two molecules of DIT couple to form thyroxine (T_4). One molecule of MIT, when coupled with one molecule of DIT, triiodothyronine (T_3) is produced. The mechanism of coupling is not well understood. The details of synthesis of T_3 and T_4 are given under tyrosine metabolism (***Chapter 15***). A diagrammatic representation is depicted in ***Fig.19.10***.

As the process of iodination is completed, each molecule of thyroglobulin contains about 6-8 molecules of thyroxine (T_4). The ratio of T_3 to T_4 in thyroglobulin is usually around 1 : 10.

Storage and release of thyroid hormones

Thyroglobulin containing T_4 and T_3 can be stored for several months in the thyroid gland. It is estimated that the stored thyroid hormones can meet the body requirement for 1-3 months.

Thyroglobulin is digested by lysosomal proteolytic enzymes in the thyroid gland. The free hormones thyroxine (90%) and triiodothyronine (10%) are released into the blood, a process stimulated by TSH. MIT and DIT produced in the thyroid gland undergo deiodination by the enzyme deiodinase and the iodine thus liberated can be reutilized.

Transport of T_4 and T_3

Two specific binding proteins—thyroxine binding globulin (TBG) and thyroxine binding prealbumin (TBPA)—are responsible for the transport of thyroid hormones. Both T_4 and T_3 are more predominantly bound to TBG. A small fraction of free hormones are biologically active. T_4 has a half-life of 4-7 days while T_3 has about one day.

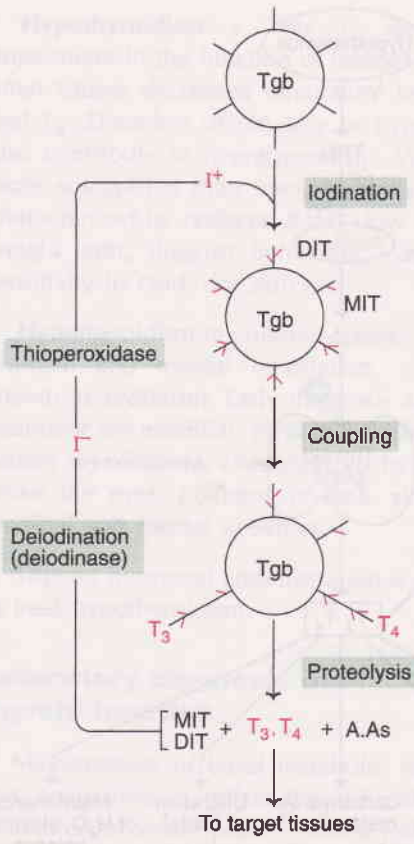


***Fig. 19.10** : Biosynthesis of thyroid hormones—diagrammatic representation [**Note : Refer Fig. 15.21** for synthesis with structures; Tgb–Thyroglobulin; I^+–Active iodine; T_3–Triiodothyronine; T_4–Thyroxine; MIT–Monoiodotyrosine; DIT–Diiodotyrosine; A. As–Amino acids].*

Biochemical functions of thyroid hormones

Triiodothyronine (***T_3***) is about four times ***more active*** in its biological functions ***than*** thyroxine (***T_4***). The following are the biochemical functions attributed to thyroid hormones (T_3 and T_4).

1. **Influence on the metabolic rate :** Thyroid hormones ***stimulate the metabolic activities*** and increases the oxygen consumption in most of the tissues of the body (exception—brain, lungs, testes and retina).

Na^+-K^+ ATP pump : This is an energy dependent process which consumes a major share of cellular ATP. Na^+-K^+ ATPase activity is directly correlated to thyroid hormones and this, in turn, with ATP utilization. Obesity in some individuals is attributed to a decreased energy utilization and heat production due to diminished Na^+-K^+ ATPase activity.

2. **Effect on protein synthesis :** Thyroid hormones act like steroid hormones in promoting protein synthesis by acting at the transcriptional level (activate DNA to produce RNA). Thyroid hormones, thus, function as anabolic hormones and cause positive nitrogen balance and promote growth and development.

3. **Influence on carbohydrate metabolism :** Thyroid hormones promote intestinal absorption of glucose and its utilization. These hormones increase gluconeogenesis and glycogenolysis, with an overall effect of enhancing blood glucose level (hyperglycemia).

4. **Effect on lipid metabolism :** Lipid turnover and utilization are stimulated by thyroid hormones. Hypothyroidism is associated with elevated plasma cholesterol levels which can be reversed by thyroid hormone administration.

Regulation of T_3 and T_4 synthesis

The synthesis of thyroid hormones is controlled by feedback regulation (***Fig. 19.11***). T_3 appears to be more actively involved than T_4 in the regulation process. The production of thyroid stimulating hormone (TSH) by pituitary, and thyrotropin releasing hormone (TRH) by hypothalamus are inhibited by T_3 and, to a lesser degree, by T_4. The increased synthesis of TSH and TRH occurs in response to decreased circulatory levels of T_3 and T_4. As already discussed, the body has sufficient stores of hormones to last for several weeks. Hence it takes some months to observe thyroid functional deficiency.

Metabolic fate of T_3 and T_4

Thyroid hormones undergo deiodination in the peripheral tissues. The iodine liberated may be reutilized by the thyroid. T_3 and T_4 may get conjugated with glucuronic acid or sulfate in the liver and excreted through bile. Thyroid hormones are also subjected to deamination to

produce tetraiodothyroacetic acid (from T_4) and triiodothyroacetic acid (from T_3) which may then undergo conjugation and excretion.

Abnormalities of thyroid function

Among the endocrine glands, ***thyroid is the most susceptible for hypo- or hyperfunction.***

Three abnormalities associated with thyroid functions are known.

Goiter : Any abnormal increase in the size of the thyroid gland is known as goiter. Enlargement of thyroid gland is mostly to compensate the decreased synthesis of thyroid hormones and is associated with ***elevated TSH.*** Goiter is primarily due to a failure in the autoregulation of T_3 and T_4 synthesis. This may be caused by deficiency or excess of iodide.

Goitrogenic substances (goitrogens) : These are the substances that interfere with the production of thyroid hormones. These include thiocyanates, nitrates and perchlorates and the drugs such as thiourea, thiouracil, thiocarbamide etc. Certain plant foods—cabbage, cauliflower and turnip—contain goitrogenic factors (mostly thiocyanates).

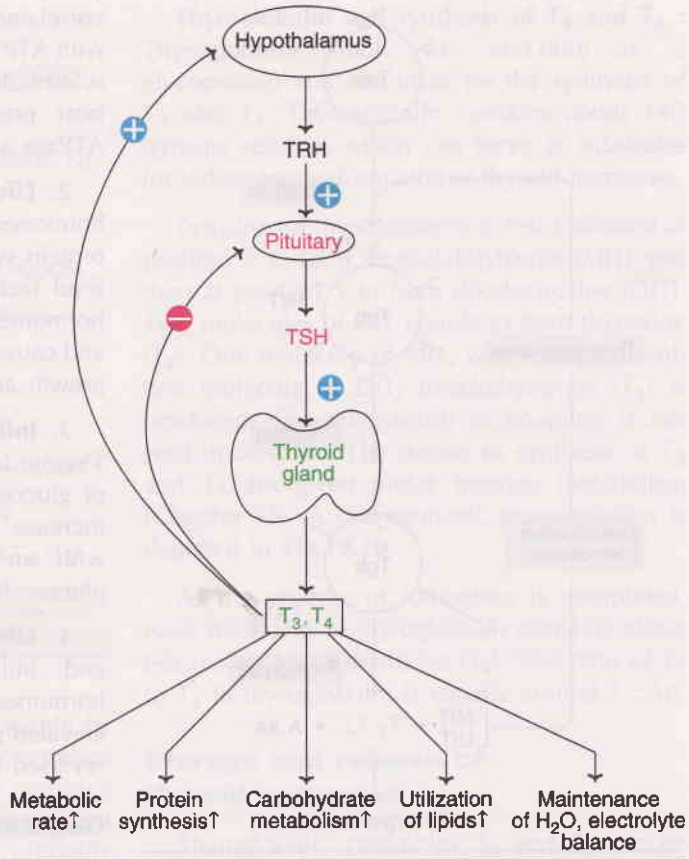


Fig. 19.11 : *Regulation of synthesis and functions of thyroid hormones—an overview (TRH–Thyrotropin-stimulating hormone; TSH–Thyroid stimulating hormone; T_3–Triiodothyronine; T_4–Thyroxine; ⊕–Promoting effect; ⊖–Inhibitory effect).*

Simple endemic goiter : This is due to iodine deficiency in the diet. It is mostly found in the geographical regions away from sea coast where the water and soil are low in iodine content. Consumption of iodized salt is advocated to overcome the problem of endemic goiter. In certain cases, administration of thyroid hormone is also employed.

Hyperthyroidism : This is also known as ***thyrotoxicosis*** and is associated with overproduction of thyroid hormones. Hyperthyroidism is characterized by increased metabolic rate (***higher BMR***) nervousness, irritability, anxiety, rapid heart rate, loss of weight despite increased appetite, weakness, diarrhea, sweating, sensitivity to heat and often protrusion of eyeballs (exopthalmos).

Hyperthyroidism is caused by ***Grave's disease*** (particularly in the developed countries) or due to increased intake of thyroid hormones. Grave's disease is due to elevated ***thyroid stimulating IgG*** also known as long acting thyroid stimulator (LATS) which activates TSH and, thereby, increases thyroid hormonal production.

Thyrotoxicosis is diagnosed by scanning and/or estimation of T_3, T_4 (both elevated) and TSH (decreased) in plasma. The treatment includes administration of antithyroid drugs. In severe cases, thyroid gland is surgically removed.

Hypothyroidism : This is due to an impairment in the function of thyroid gland that often causes decreased circulatory levels of T_3 and T_4. Disorders of pituitary or hypothalamus also contribute to hypothyroidism. Women are more susceptible than men. Hypothyroidism is characterized by ***reduced BMR***, slow heart rate, weight gain, sluggish behaviour, constipation, sensitivity to cold, dry skin etc.

Hypothyroidism in children is associated with physical and mental retardation, collectively known as ***cretinism***. Early diagnosis and proper treatment are essential. Hypothyroidism in adult causes ***myxoedema,*** characterized by bagginess under the eyes, puffiness of face, slowness in physical and mental activities.

Thyroid hormonal administration is employed to treat hypothyroidism.

Laboratory diagnosis of thyroid function

Measurement of basal metabolic rate (BMR) was once used to reflect thyroid activity. The estimation of serum protein bound iodine (PBI), representing the circulating thyroid hormones, was employed for a long time to assess thyroid function. The normal serum PBI concentration is 3-8 µg/100 ml.

Hypothyroidism is associated with decreased PBI and hyperthyroidism with increased PBI.

In recent years, more sensitive and reliable tests have been developed to assess thyroid activity. The concentration of free T_3 and T_4, and TSH are measured (by RIA or ELISA) and their serum normal concentrations are

Free triiodothyronine (T_3)	— 80–220 ng/dl
Free thyroxine (T_4)	— 0.8–2.4 ng/dl
Total thyroxine (T_4)	— 5–12 µg/dl
Thyroid stimulating hormone (TSH)	— <10 µU/ml

Radioactive iodine uptake (RAIU) and scanning of thyroid gland are also used for diagnosis.

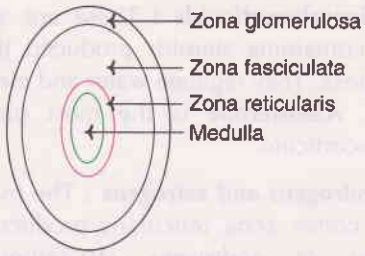


Fig. 19.12 : Adrenal gland with zones (3) and medulla.

Thyroid activity and serum cholesterol

Serum cholesterol level is ***increased in hypothyroidism*** and decreased in hyperthyroidism. Unfortunately, cholesterol estimation will be of no value in the assessment of thyroid function. This is due to the fact that serum cholesterol level is elevated in many other disorders (diabetes, obstructive jaundice, nephrotic syndrome etc.). However, cholesterol estimation may be utilized for monitoring thyroid therapy.

HORMONES OF ADRENAL CORTEX

The adrenal glands are two small organs (each weighing about 10 g), located above the kidneys. Each adrenal consists of two distinct tissues—an outer cortex (with 3 zones) and inner medulla (***Fig.19.12***).

As many as 50 steroid hormones (namely adrenocorticosteroids), produced by adrenal cortex, have been identified. However, only a few of them possess biological activity.

Adrenocorticosteroids are classified into three groups according to their dominant biological action. However, there is some overlap in their functions.

1. **Glucocorticoids** : These are 21-carbon steroids, produced mostly by zona fasciculata. They affect glucose (hence the name), amino acid and fat metabolism in a manner that is opposite to the action of insulin. ***Cortisol*** (also known as hydrocortisone) is the most important glucocorticoid in humans. Corticosterone is predominantly found in rats.

2. **Mineralocorticoids :** These are also 21-carbon containing steroids produced by zona glomerulosa. They regulate water and electrolyte balance. ***Aldosterone*** is the most prominent mineralocorticoid.

3. **Androgens and estrogens :** The innermost adrenal cortex zona reticularis produces small quantities of androgens (19-carbon) and estrogens (18-carbon). These hormones affecting sexual development and functions are mostly produced by gonads. Dehydroepiandrosterone—a precursor for androgens—is synthesized in adrenal cortex.

Synthesis of adrenocorticosteroids

Cholesterol undergoes cleavage with an elimination of a 6-carbon fragment to form pregnenolone. ***Pregnenolone is the common precursor for the synthesis of all steroid hormones.***

Conversion of cholesterol to pregnenolone is catalysed by cytochrome P_{450} side chain cleavage enzyme. This reaction is promoted by ACTH. The enzymes—hydroxylases, dehydrogenases/isomerases and lyases associated with mitochondria or endoplasmic reticulum—are responsible for the synthesis of steroid hormones. The metabolic pathway for the formation of major adrenocorticosteroids is given in ***Fig.19.13.***

Biochemical functions of adrenocorticosteroids

1. **Glucocorticoid hormones :** The important glucocorticoids are—***cortisol***, ***cortisone*** and ***corticosterone***. They bring about several biochemical functions in the body.

(a) **Effects on carbohydrate metabolism :** Glucocorticoids promote the synthesis of glucose (gluconeogenesis). This is brought about by increasing the substrates (particularly amino acids) and enhancing the synthesis of phosphoenolpyruvate carboxykinase, the rate limiting enzyme in gluconeogenesis.

The overall influence of glucocorticoids on carbohydrate metabolism is to increase blood glucose concentration. The biological actions of glucocorticoids generally oppose that of insulin.

(b) **Effects on lipid metabolism :** Glucocorticoids increase the circulating free fatty acids. This is caused by two mechanisms.

(i) Increased breakdown of storage triacylglycerol (lipolysis) in adipose tissue.

(ii) Reduced utilization of plasma free fatty acids for the synthesis of triacylglycerols.

(c) **Effects on protein and nucleic acid metabolism :** Glucocortiocoids exhibit both catabolic and anabolic effects on protein and nucleic acid metabolism. They promote transcription (RNA synthesis) and protein biosynthesis in liver. These anabolic effects of glucocorticoids are caused by the stimulation of specific genes.

Glucocorticoids (particularly at high concentration) cause catabolic effects in extrahepatic tissues (e.g. muscle, adipose tissue, bone etc.). This results in enhanced degradation of proteins.

(d) **Effects on water and electrolyte metabolism :** The influence of glucocorticoids on water metabolism is mediated through antidiuretic hormone (ADH). Deficiency of glucocorticoids causes increased production of ADH. ADH decreases glomerular filtration rate causing water retention in the body.

(e) **Effects on the immune system :** Glucocorticoids (particularly cortisol), in high doses, suppress the host immune response. The steroid hormones act at different levels—damaging lymphocytes, impairment of antibody synthesis, suppression of inflammatory response etc.

(f) **Other physiological effects of glucocorticoids :** Glucocorticoids are involved in several physiological functions.

(i) Stimulate the fight and flight response (to face sudden emergencies) of catecholamines.

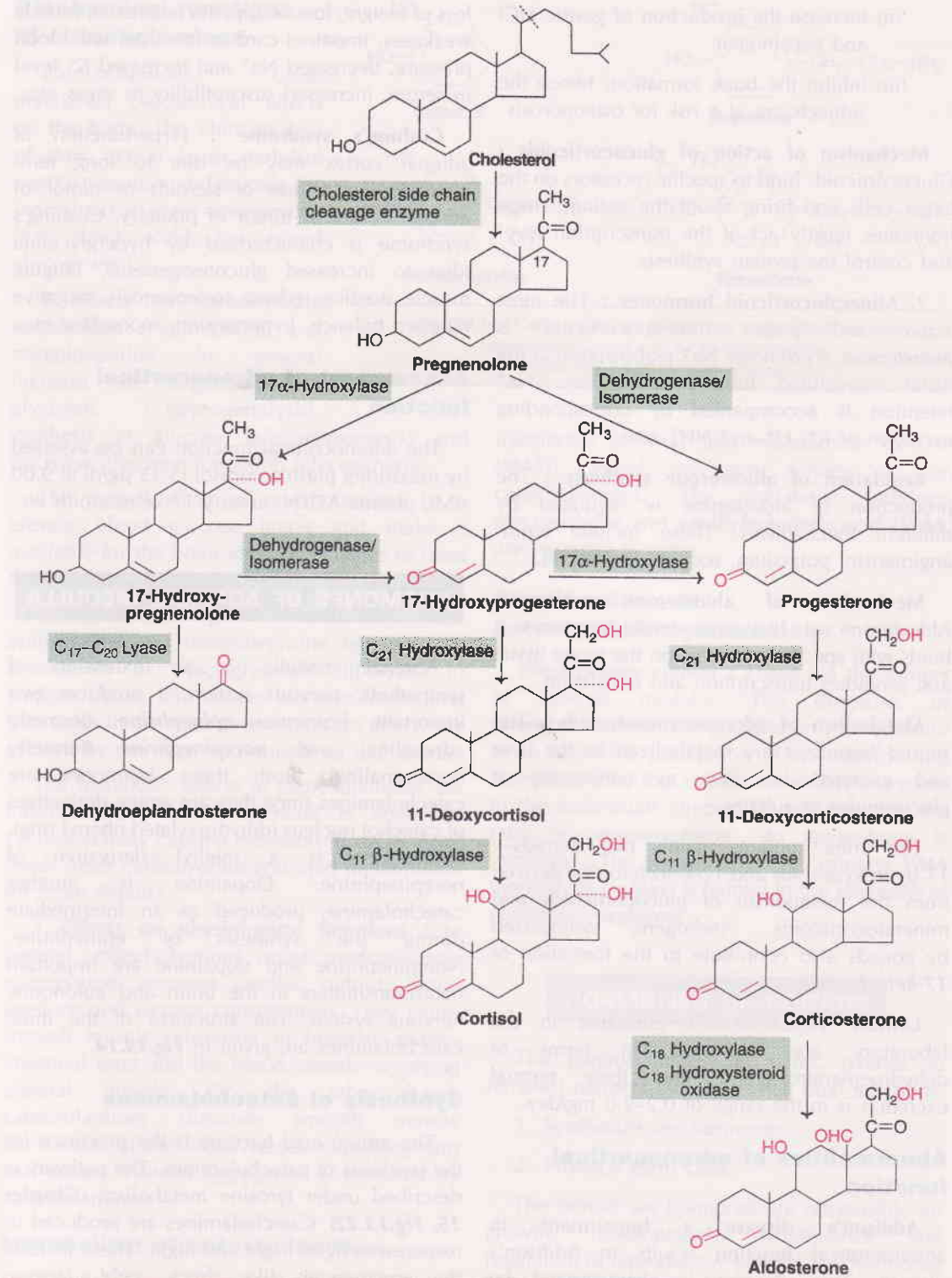


Fig. 19.13 : Biosynthesis of major adrenocorticosteroids.

(ii) Increase the production of gastric HCl and pepsinogen.

(iii) Inhibit the bone formation, hence the subjects are at a risk for osteoporosis.

Mechanism of action of glucocorticoids : Glucocorticoids bind to specific receptors on the target cells and bring about the action. These hormones mostly act at the transcription level and control the protein synthesis.

2. **Mineralocorticoid hormones :** The most active and potent mineralocorticoid is ***aldosterone***. It promotes Na^+ reabsorption at the distal convoluted tubules of kidney. Na^+ retention is accompanied by corresponding excretion of K^+, H^+ and NH_4^+ ions.

Regulation of aldosterone synthesis : The production of aldosterone is regulated by different mechanisms. These include renin-angiotensin, potassium, sodium and ACTH.

Mechanism of aldosterone action : Aldosterone acts like other steroid hormones. It binds with specific receptors on the target tissue and promotes transcription and translation.

Metabolism of adrenocorticosteroids : The steroid hormones are metabolized in the liver and excreted in urine as conjugates of glucuronides or sulfates.

The urine contains mainly two steroids—17-hydroxysteroids and 17-ketosteroids—derived from the metabolism of glucocorticoids and mineralocorticoids. Androgens synthesized by gonads also contribute to the formation of ***17-ketosteroids***.

Urinary 17-ketosteroids estimated in the laboratory are expressed in terms of dehydroepiandrosterone and their normal excretion is in the range of 0.2–2.0 mg/day.

Abnormalities of adrenocortical function

Addison's disease : Impairment in adrenocortical function results in Addison's disease. This disorder is characterized by decreased blood glucose level (hypoglycemia), loss of weight, loss of appetite (anorexia), muscle weakness, impaired cardiac function, low blood pressure, decreased Na^+ and increased K^+ level in serum, increased susceptibility to stress etc.

Cushing's syndrome : Hyperfunction of adrenal cortex may be due to long term pharmacological use of steroids or tumor of adrenal cortex or tumor of pituitary. Cushing's syndrome is characterized by hyperglycemia (due to increased gluconeogenesis), fatigue, muscle wasting, edema, osteoporosis, negative nitrogen balance, hypertension, moon-face etc.

Assessment of adrenocortical function

The adrenocortical function can be assessed by measuring plasma cortisol (5-15 µg/dl at 9.00 AM), plasma ACTH, urinary 17-ketosteroids etc.

HORMONES OF ADRENAL MEDULLA

Adrenal medulla is an extension of sympathetic nervous system. It produces two important hormones—***epinephrine*** (formerly adrenaline) and ***norepinephrine*** (formerly noradrenaline). Both these hormones are catecholamines since they are amine derivatives of catechol nucleus (dihydroxylated phenyl ring). Epinephrine is a methyl derivative of norepinephrine. Dopamine is another catecholamine, produced as an intermediate during the synthesis of epinephrine. Norepinephrine and dopamine are important neurotransmitters in the brain and autonomic nervous system. The structures of the three catecholamines are given in ***Fig.19.14.***

Synthesis of catecholamines

The amino acid tyrosine is the precursor for the synthesis of catecholamines. The pathway is described under tyrosine metabolism (***Chapter 15, Fig.15.22***). Catecholamines are produced in response to fight, fright and flight. These include the emergencies like shock, cold, fatigue, emotional conditions like anger etc.

Biochemical functions of catecholamines

Catecholamines cause diversified biochemical effects on the body. The ultimate goal of their action is to mobilize energy resources and prepare the individuals ***to meet emergencies*** (e.g. shock, cold, low blood glucose etc.).

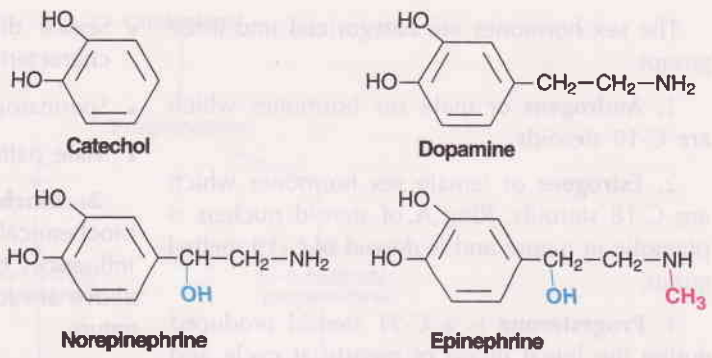


Fig. 19.14 : *Catecholamines (dopamine, norepinephrine and epinephrine) produced by adrenal medulla (Refer **Fig. 15.22** for biosynthesis).*

1. **Effects on carbohydrate metabolism :** Epinephrine and norepinephrine in general increase the degradation of glycogen (glycogenolysis), synthesis of glucose (gluconeogenesis) and decrease glycogen formation (glycogenesis).

The overall effect of catecholamines is to elevate blood glucose levels and make it available for the brain and other tissues to meet the emergencies.

2. **Effects on lipid metabolism :** Both epinephrine and norepinephrine enhance the breakdown of triacylglycerols (lipolysis) in adipose tissue. This causes increase in the free fatty acids in the circulation which are effectively utilized by the heart and muscle as fuel source.

The metabolic effects of catecholamines are mostly related to the increase in adenylate cyclase activity causing elevation in cyclic AMP levels (refer carbohydrate and lipid metabolisms for more details).

3. **Effects on physiological functions :** In general, catecholamines (most predominantly epinephrine) increase cardiac output, blood pressure and oxygen consumption. They cause smooth muscle relaxation in bronchi, gastro-intestinal tract and the blood vessels supplying skeletal muscle. On the other hand, catecholamines stimulate smooth muscle contraction of the blood vessels supplying skin and kidney. Platelet aggregation is inhibited by catecholamines.

Metabolism of catecholamines

Catecholamines are rapidly inactivated and metabolized. The enzymes—catechol-O methyl-transferase (COMT) and monoamine oxidase (MAO), found in many tissues act on catecholamines. The metabolic products metanephrine and ***vanillylmandelic acid (VMA)*** are excreted in urine.

Abnormalities of catecholamine production

Pheochromocytomas : These are the tumors of adrenal medulla. The diagnosis of pheochromocytoma is possible only when there is an excessive production of epinephrine and norepinephrine that causes severe hypertension. In the individuals affected by this disorder, the ratio of norepinephrine to epinephrine is increased. The measurement of urinary ***VMA*** (normal <8 mg/day) is helpful in the ***diagnosis*** of pheochromocytomas.

HORMONES OF GONADS

The gonads (testes in males, ovaries in females) perform closely related dual functions.

1. Synthesize sex hormones;
2. Produce germ cells.

The steroid sex hormones are responsible for growth, development, maintenance and regulation of reproductive system. Sex hormones are essentially required for the development of germ cells.

The sex hormones are categorized into three groups

1. **Androgens** or male sex hormones which are C-19 steroids.

2. **Estrogens** or female sex hormones which are C-18 steroids. Ring A of steroid nucleus is phenolic in nature and is devoid of C-19 methyl group.

3. **Progesterone** is a C-21 steroid produced during the luteal phase of menstrual cycle and also during pregnancy.

ANDROGENS

The male sex hormones or androgens are produced by the Leydig cells of the testes and to a minor extent by the adrenal glands in both the sexes. Ovaries also produce small amounts of androgens.

Biosynthesis of androgens

Cholesterol is the precursor for the synthesis of androgens. It is first converted to pregnenolone which then forms androstenedione by two pathways—either through progesterone or through 17-hydroxypregnenolone (***Fig.19.15***). Testosterone is produced from androstenedione. The production of androgens is under the control of LH and FSH.

Active form of androgen : The primary product of testes is testosterone. However, the active hormone in many tissues is not testosterone but its metabolite ***dihydrotestosterone*** (DHT). Testosterone, on reduction by the enzyme 5 α-reductase, forms DHT. This conversion mostly occurs in the peripheral tissues. Some workers consider testosterone as a prohormone and dihydrotestosterone, the more potent form as the hormone.

Physiological and biochemical functions of androgens

1. **Sex-related physiological functions :** The androgens, primarily DHT and testosterone, influence :

- Growth, development and maintenance of male reproductive organs.
- Sexual differentiation and secondary sexual characteristics.
- Spermatogenesis.
- Male pattern of aggressive behavior.

2. **Biochemical functions :** Many specific biochemical effects of androgens that ultimately influence the physiological functions stated above are identified. Androgens are anabolic in nature.

- **Effects on protein metabolism :** Androgens promote RNA synthesis (transcription) and protein synthesis (translation). Androgens cause positive nitrogen balance and increase the muscle mass.
- **Effects on carbohydrate and fat metabolisms :** Androgens increase glycolysis fatty acid synthesis and citric acid cycle.
- **Effects on mineral metabolism :** Androgens promote mineral deposition and bone growth before the closure of epiphyseal cartilage.

ESTROGENS

Estrogens are predominantly ovarian hormones, systhesized by the follicles and corpus luteum of ovary. These hormones are responsible for maintenance of menstrual cycle and reproductive process in women.

Synthesis of estrogens

Estrogen synthesis occurs from the precursor cholesterol (***Fig.19.15***). Estrogens are produced by aromatization (formation of aromatic ring) of androgens. The ovary produces estradiol (E_2) and estrone (E_1) while the placenta synthesizes these two steroid hormones and estriol (E_3). The synthesis of estrogens is under the control of LH and FSH.

Physiological and biochemical functions of estrogens

1. **Sex-related physiological functions :** The estrogens are primarily concerned with

- Growth, development and maintenance of female reproductive organs.

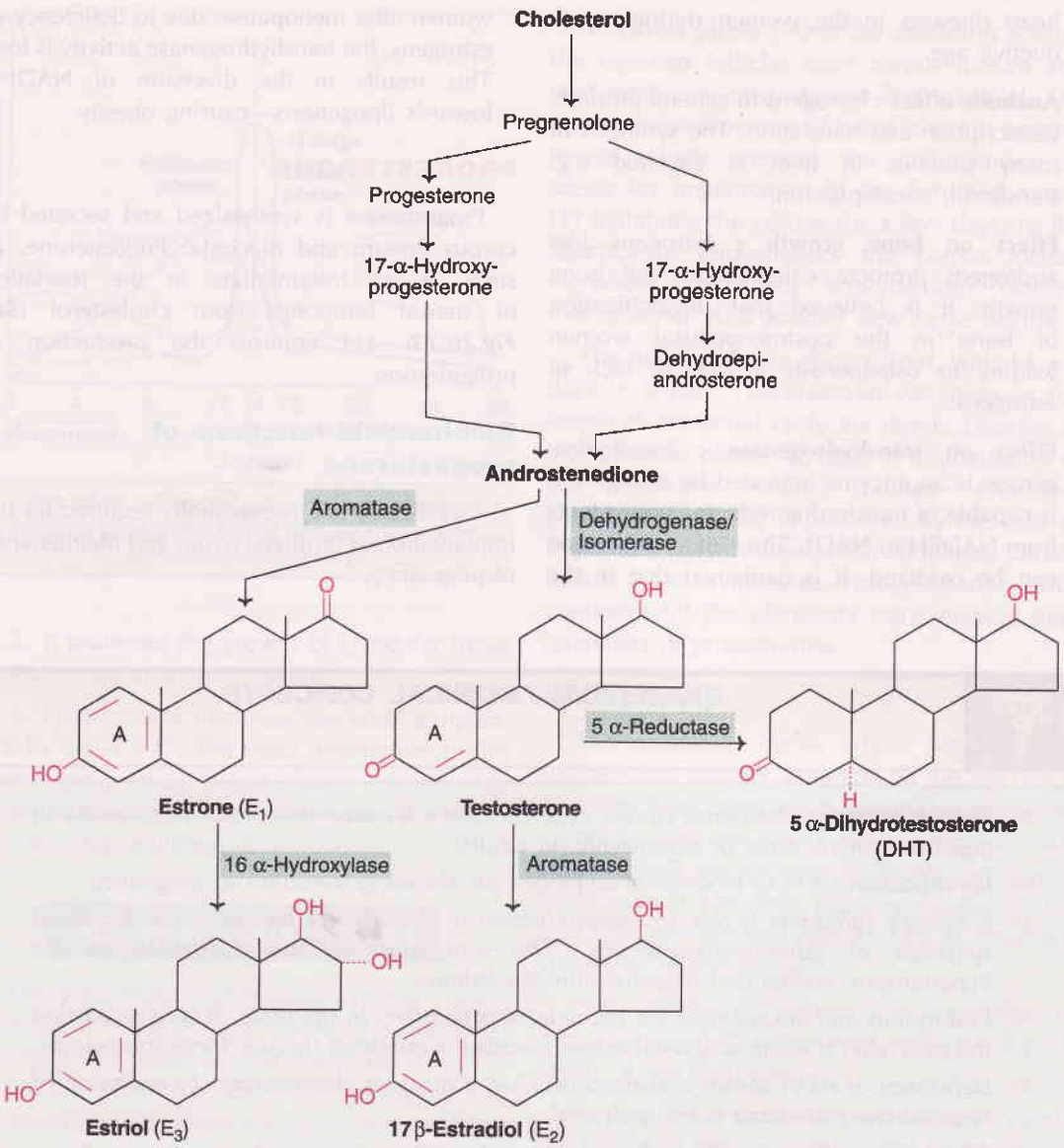


Fig. 19.15 : *Biosynthesis of steroid sex hormones from cholesterol*
(Note : *Male and female sex hormones are given together).*

- Maintenance of menstrual cycles.
- Development of female sexual characteristics.

2. **Biochemical functions :** Estrogens are involved in many metabolic functions.

- **Lipogenic effect :** Estrogens increase lipogenesis in adipose tissue and, for this reason, women have relatively more fat (about 5%) than men.
- **Hypocholesterolemic effect :** Estrogens lower the plasma total cholesterol. The LDL fraction of lipoproteins is decreased while the HDL fraction is increased. This explains the low incidence of atherosclerosis and coronary

heart diseases in the women during reproductive age.

- **Anabolic effect :** Estrogens in general promote transcription and translation. The synthesis of many proteins in liver is elevated e.g. transferrin, ceruloplasmin.
- **Effect on bone growth :** Estrogens like androgens promote calcification and bone growth. It is believed that decalcification of bone in the postmenopausal women leading to osteoporosis is due to lack of estrogens.
- **Effect on transhydrogenase :** Transhydrogenase is an enzyme activated by estrogen. It is capable of transferring reducing equivalents from NADPH to NAD^+. The NADH so formed can be oxidized. It is explained that in the women after menopause, due to deficiency of estrogens, the transhydrogenase activity is low. This results in the diversion of NADPH towards lipogenesis—causing obesity.

PROGESTERONE

Progesterone is synthesized and secreted by corpus luteum and placenta. Progesterone, as such, is an intermediate in the formation of steroid hormones from cholesterol (***See Fig.20.13***). LH controls the production of progesterone.

Biochemical functions of progesterone

1. Progesterone is essentially required for the implantation of fertilized ovum and maintenance of pregnancy.

BIOMEDICAL / CLINICAL CONCEPTS

- *Growth hormone deficiency causes dwarfism while its excessive production results in gigantism (in children) or acromegaly (in adults).*
- *Identification of hCG in urine is employed for the early detection of pregnancy.*
- *Cushing's syndrome is due to overproduction of ACTH that results in the increased synthesis of adrenocorticosteroids. The symptoms of this syndrome include hypertension, edema and negative nitrogen balance.*
- *Endorphins and enkephalins are the natural pain-killers in the brain. It is believed that the pain relief through acupuncture and placebos is mediated through these compounds.*
- *Deficiency of ADH causes diabetes insipidus, a disorder characterized by excretion of large volumes of dilute urine (polyuria).*
- *Thyroid hormones directly influence $Na^+ - K^+$ ATP pump which consumes a major share of cellular ATP. Obesity in some individuals is attributed to decreased energy utilization (heat production) due to diminished $Na^+ - K^+$ ATPase activity.*
- *Catecholamines are produced in response to fight, fright and flight. The ultimate goal of catecholamine function is to mobilize energy resources and prepare the individual to meet emergencies such as shock, cold, fatigue, anger etc.*
- *Pheochromocytomas are the tumors of adrenal medulla, characterized by excessive production of epinephrine and norepinephrine, associated with severe hypertension.*
- *Sex hormones are primarily responsible for growth, development, maintenance and regulation of reproductive system.*
- *The low incidence of atherosclerosis and coronary heart disease in the women during reproductive age is due to estrogens.*

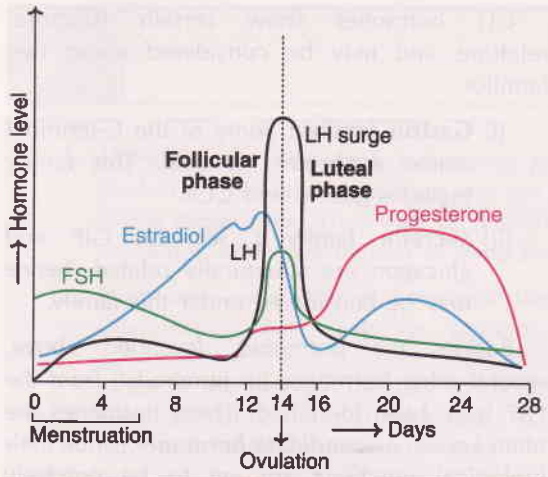


Fig. 19.16 : Hormonal pattern in women during mestrual cycle (FSH–Follicle stimulating hormone; LH–Luteinizing hormone).

2. It promotes the growth of glandular tissue in uterus and mammary gland.

3. Progesterone increases the body temperature by 0.5–1.5 F°. The exact mechanism of this thermogenic effect is not clearly known. The measurement of temperature was used as an indicator for ovulation.

THE MENSTRUAL CYCLE

The occurrence of menstrual cycle is a good example of coordination among the hormonal functions. In humans, the menstrual cycle is under the control of FSH, LH, estrogens and progesterone. The cycle normally varies between 25 and 35 days in length, with a mean of 28 days. The menstrual cycle can be divided into two phases—follicular phase and luteal phase (***Fig.19.16***).

1. **Follicular phase :** Follicular stimulating hormone (FSH) causes the development and maturation of ovarian follicles. As the follicle enlarges, estradiol progessively rises and reaches its peak value 24 hours before LH and FSH attain their respective maximum levels. LH surge or peak initiates ovulation-release of ovum from the ruptured follicles. The levels of progesterone are low during follicular phase

2. **Luteal phase :** After the ovulation occurs, the ruptured follicles form corpus luteum and start producing progesterone and estradiol. The predominant hormone of luteal phase is progesterone which prepares the endometrium of uterus for implantation of the fertilized ovum. LH maintains the corpus for a few days. In the absence of implantation, the corpus luteum regresses and sheds endometrium causing menstruation. And another new cycle begins.

The luteal phase is always fixed, with 14 ± 2 days in length. The observed variations in the length of menstrual cycle are due to changes in the follicular phase. In case of implantation of the fertilized ovum, human chorionic gonadotropin (hCG) is produced by the cells of implanted early embryo. hCG stimulates corpus luteum to synthesize progesterone. This continues till the plancenta starts making high quantities of progesterone.

Menopause

The menstrual cycles which begin in the women after puberty, continue till the age of 45-50 years. The cycles cease around this age which coincides with the loss of ovarian function. The progesterone and estrogen levels are very low in these women. However, the concentration of LH and FSH are elevated due to lack of feedback inhibition by estrogens.

Post-menopausal women are susceptible to two complications associated with insufficient levels of sex hormones.

1. **Atrophy of secondary sex tissues :** Mainly the epithelial tissue of vagina and lower urinary tract.

2. **Osteoporosis :** Decreased density of bones and increased susceptibility to fractures.

GASTROINTESTINAL (OR GUT) HORMONES

The digestion and absorption of nutrients (***Chapter 8***) is a complicated process which is regulated by the autonomic nervous system. This occurs in association with peptide hormones of gastrointestinal tract (GIT).

The specialized cells lining the GIT are responsible for the production of GIT hormones. Hence GIT may be considered as the largest mass of cells that secrete hormones. A large number of GIT hormones have been identified. However, only four GIT hormones have been well characterised.

1. **Gastrin :** This hormone contains 17 amino acids and is produced by gastric mucosa. It stimulates the secretion of gastric HCl and pepsinogen (proenzyme of pepsin). The release of gastrin is stimulated by vagus nerve of stomach and partially digested proteins. HCl and certain other hormones inhibit gastrin release.

2. **Secretin :** It is a 27-amino acid containing polypeptide and resembles glucagon in many ways. Secretin is synthesized by the mucosa of the upper small intestine. It is released in response to the presence of HCl in chyme in the duodenum which is passed on from the stomach. Secretin stimulates pancreatic cells to produce bicarbonate (HCO_3^-) in order to neutralize HCl.

3. **Cholecystokinin (CCK) :** It contains 33 amino acids and is produced by the upper part of small intestine. The secretion of CCK is stimulated by the products of protein and lipid digestion, namely peptides, amino acids, mono- or diacylglycerols, fatty acids and glycerol.

Cholecystokinin stimulates the contraction of gall bladder and increases the flow of bile into duodenum. It also promotes the secretion of *digestive enzymes and* HCO_3^- from pancreas.

4. **Gastric inhibitory peptide (GIP) :** It contains 43 amino acids and is produced by duodenal mucosa. The release of GIP is stimulated by the presence of glucose in the gut. The most important function of GIP is to stimulate the release of insulin from pancreas. This is evident from the fact that the plasma insulin level is elevated much before the increase in blood glucose. GIP also inhibits gastric HCl secretion, gastric motility and its emptying.

GIT hormones show certain structural relations and may be considered under two families.

(i) **Gastrin family :** Some of the C-terminal amino acids are identical. This family includes gastrin and CCK.

(ii) **Secretin family :** Secretin, GIP and glucagon are structurally related, hence may be considered under this family.

Besides the hormones described above, several other hormones (in hundreds!) from the GIT have been identified. These hormones are often known as ***candidate hormones,*** since their biological functions are yet to be precisely identified. The candidate hormones include ***vasoactive intestinal peptide*** (VIP), ***motilin, enteroglucagon, substance P, neurotensin, somatostatin*** and ***enkephalins***.

Mechanism of action of GIT hormones

Many of the GIT hormones have receptor sites specific for their action. At least two distinct mechanisms have been identified through which these hormones act.

1. Production of cAMP through the activation of adenylate cyclase e.g. secretin, VIP etc.

2. Stimulation of intracellular Ca^{2+} usually mediated through the metabolism of phosphatidylinositol e.g. gastrin , CCK.

Both these mechanisms ultimately influence the enzyme secretions/other biological effects.

Other hormones

Besides the hormones discussed above, there are a few other important hormones which are not referred to in this chapter. ***Insulin and glucagon*** are described under diabetes mellitus (***Chapter 36***) while ***parathyroid hormone*** and ***calcitonin*** are discussed under calcium metabolism (***Chapter 18***) These hormones are not given here to avoid repetition.

SUMMARY

1. *Hormones are the organic substances, produced in minute quantities by specific tissues (endocrine glands) and secreted into the blood stream to control the biological activities in the target cells. They may be regarded as the chemical massengers involved in the regulation and coordination of body functions.*
2. *Hormones are classified based on their chemical nature or mechanism of action. Chemically, they may be proteins or peptides (insulin, oxytocin), steroids (glucocorticoids, sex hormones) and amino acid derivatives (epinephrine, thyroxine). By virtue of the function, group I hormones bind to the intracellular receptors (estrogens, calcitriol), while group II hormones (ACTH, LH) bind to the cell surface receptors and act through the second messengers.*
3. *Cyclic AMP (cAMP) is an intracellular second messenger for a majority of polypeptide hormones. Membrane bound adenylate cyclase enzyme, through the mediation of G proteins, is responsible for the synthesis of cAMP. cAMP acts through protein kinases that phosphorylate specific proteins which, in turn, cause the ultimate biochemical response. Phosphatidylinositol/calcium system also functions as a second messenger for certain hormones (TRH, gastrin).*
4. *Hypothalamus is the master coordinator of hormonal action as it liberates certain releasing factors or hormones (TRH, CRH, GRH, GRIH) that stimulate or inhibit the corresponding trophic hormones from the anterior pituitary.*
5. *Anterior pituitary gland is the master endocrine organ that produces several hormones which influence either directly or indirectly (through the mediation of other endocrine organs) a variety of biochemical processes in the body. For instance, growth hormone is directly involved in growth promoting process while TSH, FSH and ACTH, respectively influence thyroid gland, gonads and adrenal cortex to synthesize hormones.*
6. *Thyroid gland produces two principal hormones—thyroxine (T_4) and triiodothyronine (T_3)—which are primarily concerned with the regulation of the metabolic activity of the body. Goiter is a disorder caused by enlargement of thyroid gland and is mainly due to iodine deficiency in the diet.*
7. *Adrenal cortex synthesizes glucocorticoids (e.g. cortisol) that influence glucose, amino acid and fat metabolism, and mineralocorticoids (e.g. aldosterone) that regulate water and electrolyte balance. Androgens and estrogens (sex hormones) in small quantities are also synthesized by the adrenal cortex.*
8. *Adrenal medulla produces two important hormones—epinephrine and norepinephrine (catecholamines). They influence diversified biochemical functions with an ultimate goal to mobilize energy resources and prepare the individual to meet emergencies (shock, anger, fatigue etc.)*
9. *The steroid sex hormones, primarily androgens in males and estrogens in females, are respectively synthesized by the testes and ovaries. These hormones are responsible for growth, development, maintenance and regulation of reproductive system in either sex.*
10. *Several gastrointestinal hormones (e.g. gastrin, secretin) have been identified that are closely involved in the regulation of digestion and absorption of foodstuffs.*

SELF-ASSESSMENT EXERCISES

I. Essay questions

1. Describe the role of second messengers in hormonal action.
2. Write an account of the anterior pituitary hormones.
3. Discuss in detail the synthesis and biochemical functions of thyroid hormones.
4. Describe the hormones of adrenal cortex with special reference to glucocorticoids.
5. Write briefly on the synthesis and biochemical functions of sex hormones.

II. Short notes

(a) 'G'-Proteins, (b) Inositol triphosphate, (c) Hypothalamic hormones, (d) ACTH, (e) Goiter, (f) Epinephrine, (g) Cortisol, (h) Gastrin, (i) ADH, (j) Aldosterone.

III. Fill in the blanks

1. The enzyme that catalyses the formation of cAMP from ATP is _____.
2. The inorganic ion that can act as a second messenger for certain hormones is _____.
3. The endocrine organ responsible for the synthesis of trophic hormones is _____.
4. The compounds that produce opiate-like effects on the central nervous system are _____.
5. The enzyme that converts iodide (I^-) to active iodine (I^+) _____.
6. The most predominant mineralocorticoid synthesized by adrenal cortex _____.
7. The major urinary excretory product of catecholamines _____.
8. The male sex hormone, testosterone, is converted to a more active form, namely _____.
9. The precursor for the synthesis of steroid hormones _____.
10. The gastrointestinal hormone that increases the flow of bile from the gall bladder _____.

IV. Multiple choice questions

11. Impairment in the synthesis of dopamine by the brain is a major causative factor for the disorder
    (a) Parkinson's disease (b) Addison's disease (c) Cushing's syndrome (d) Goiter.
12. One of the following hormones is an amino acid derivative
    (a) Epinephrine (b) Norepinephrine (c) Thyroxine (d) All of them.
13. The most active mineralocorticoid hormone is
    (a) Cortisol (b) Aldosterone (c) 11-Deoxycorticosterone (d) Corticosterone.
14. Name the hormone, predominantly produced in response to fight, fright and flight
    (a) Thyroxine (b) Aldosterone (c) Epinephrine (d) ADH.
15. The hormone essentially required for the implantation of fertilized ovum and maintenance of pregnancy
    (a) Progesterone (b) Estrogen (c) Cortisol (d) Prolactin.

20 Organ Function Tests

The liver speaks :

"Master organ I am, for the body's metabolism !
Damage to my cells causes malfunction;
Raising serum bilirubin and certain enzymes markedly,
Tested in lab for my functional measurement."

Each organ of the body has to perform its biochemical functions to keep the body, as a whole, in a healthy state. This is possible only when the cells of the organ are intact in structure and function. Any abnormality in the tissue, caused by exogenous or endogenous factors, will seriously impair the organ function which, in turn, influences the health of the organism.

Based on the functional capabilities of the organs, ***specific biochemical investigations*** have been developed ***in the laboratory***, to assess their function. In this chapter, the biochemical investigations to assess the functioning of liver, kidney, stomach and pancreas are discussed. The tests to evaluate the function of endocrine organs are discussed elsewhere (***Chapter 19***).

LIVER FUNCTION TESTS

Liver performs several diversified functions. It is the central organ of body's metabolism.

Functions of liver

1. **Metabolic functions :** Liver actively participates in carbohydrate, lipid, protein, mineral and vitamin metabolisms.

2. **Excretory functions :** Bile pigments, bile salts and cholesterol are excreted in the bile into intestine.

3. **Protective functions and detoxification :** Kupffer cells of liver perform phagocytosis to eliminate foreign compounds. Ammonia is detoxified to urea. Liver is responsible for the metabolism of xenobiotics (detoxification).

4. **Hematological functions :** Liver participates in the formation of blood (particularly in the embryo), synthesis of plasma proteins (including blood clotting factors) and destruction of erythrocytes.

5. **Storage functions :** Glycogen, vitamins A, D and B_{12} and trace element iron are stored in liver.

The above list is an oversimplification and inconclusive with regard to the role of liver in the body.

Tests to assess liver function

The liver function tests (LFT) are the biochemical investigations to assess the capacity of the liver to carry out any of the functions it performs. LFT will help to detect the abnormalities and the extent of liver damage.

Two important facts should be borne in mind while carrying out LFT.

1. Liver is a large-size factory of safety. Therefore, it can perform many of its functions almost normally, despite the damage.

2. Selection of the right test is important in LFT. This is due to the fact that since liver participates in several functions, the function that is measured in LFT may not be the one that is adversely affected.

The major liver function tests may be classified as follows

1. Tests based on ***excretory function***—Measurement of bile pigments, bile salts, bromosulphthalein.

2. Tests based on ***serum enzymes*** derived from liver—Determination of transaminases, alkaline phosphatase, 5'-nucleotidase, γ-glutamyl-transpeptidase.

3. Tests based on ***metabolic capacity***—Galactose tolerance, antipyrine clearance.

4. Tests based on ***synthetic functions***—Prothrombin time, serum albumin.

5. Tests based on ***detoxification***—Hippuric acid synthesis.

This above list, although inconclusive, contains the most important biochemical investigations to assess LFT. Among these, the commonly used tests are described in the following pages.

Markers of liver function

The important liver functions and the common plasma/serum markers for the impaired functions are listed in ***Table 20.1***. The most important markers namely, bilirubin, enzymes, albumin, prothrombin time and drug metabolism with special reference to jaundice and other liver diseases are described.

Table 21.1 A list of liver (hepatic) functions and the common markers in plasma for the impaired function

Hepatic function	*Common plasma/serum marker(s) for impaired function*
Heme catabolism	↑Bilirubin
Enzymes	↑Alanine transaminase ↑Aspartate transaminase ↑γ-Glutamyltranspeptidase
Protein synthesis	↓Albumin ↑Prothrombin time
Protein catabolism	↑Urea ↑Ammonia
Lipid metabolism	↑Cholesterol ↑Triglycerides
Drug metabolism	↑Half-lives of drugs
Bile acid metabolism	↑Bile acids

BILIRUBIN

Bilirubin is a bile pigment, and is the excretory end product of heme degradation. It is conjugated in the liver to form bilirubin diglucuronide, and excreted in bile. The details of bilirubin metabolism are discussed elsewhere (***Chapter 10***).

Serum bilirubin

The normal concentration of serum bilirubin is in the range of ***0.2-1.0 mg/dl***. Of this, the conjugated bilirubin (diglucuronide 75%; monoglucuronide 25%) is about 0.2-0.4 mg/dl, while the unconjugated bilirubin is 0.2-0.6 mg/dl.

Icterus index

This is a simple test to ***measure*** the ***yellow colour of serum due to bilirubin***. This test is rather crude and almost outdated. However, it is often useful for a rapid assessment of neonatal jaundice.

van den Bergh reaction

This is a specific reaction to identify the increase in serum bilirubin (above the reference level). Normal serum gives a negative van den Bergh reaction.

Mechanism of the reaction : van den Bergh reagent is a mixture of equal volumes of sulfanilic acid (in dilute HCl) and sodium nitrite. The principle of the reaction is that diazotised sulfanilic acid (in the above mixture) reacts with bilirubin to form a purple coloured azobilirubin.

Direct and indirect reactions : Bilirubin as such is insoluble in water while the conjugated bilirubin is soluble. van den Bergh reagent reacts with ***conjugated bilirubin*** and gives a purple colour immediately (normally within 30 seconds). This is referred to as a ***direct positive*** van den Bergh reaction. Addition of methanol (or alcohol) dissolves the ***unconjugated bilirubin*** which then gives the van den Bergh reaction (normally within 30 minutes) positive and this is referred to as ***indirect positive***. If the serum contains both unconjugated and conjugated bilirubin in high concentration, the purple colour is produced immediately (direct positive) which is further intensified by the addition of alcohol (indirect positive). This type of reaction is known as ***biphasic***.

van den Bergh reaction and jaundice : This reaction is highly useful in understanding the nature of jaundice. This is due to the fact that the type of jaundice is characterized by increased serum concentration of unconjugated bilirubin (hemolytic), conjugated bilirubin (obstructive) or both of them (hepatic). Therefore, the response of van den Bergh reaction can differentiate the jaundice as follows

Indirect positive	—	Hemolytic jaundice
Direct positive	—	Obstructive jaundice
Biphasic	—	Hepatic jaundice.

Bilirubin in urine

The conjugated bilirubin, being water soluble, is excreted in urine. This is in contrast to unconjugated bilirubin which is not excreted. Bilirubin in urine can be detected by Fouchet's test or Gmelin's test.

Bromosulphthalein (BSP) test

Bromosulphthalein is a ***dye*** used ***to assess*** the ***excretory function of liver***. It is a non-toxic compound and almost exclusively excreted by the liver (through bile). BSP is administered intravenously (5 mg/kg body weight) and its serum concentration is measured at 45 min and at 2 hrs. In normal individuals, less than 5% of the dye is retained at the end of 45 min. Any impairment in liver function causes an ***increased retention of the dye***. This test is quite sensitive to assess liver abnormality with particular reference to excretory function.

SERUM ENZYMES DERIVED FROM LIVER

Liver cells contain several enzymes which may be released into the circulation in liver damage. Measurement of selected enzymes in serum is often used to assess the liver function. It must, however, be noted that there is no single enzyme that is absolutely specific to liver alone. Despite this fact, serum enzymes provide valuable information for LFT. Some of these enzymes are discussed hereunder.

Transaminases or aminotransferases

The activities of two enzymes—namely serum glutamate pyruvate transaminase (SGPT; recently called as ***alanine transaminase***—ALT) and serum glutamate oxaloacetate transaminase (SGOT; recently known as ***aspartate transaminase***—AST) —are widely used to assess the liver function. ALT is a cytoplasmic enzyme while AST is found in both cytoplasm and mitochondria. The activity of these enzymes is low in normal serum (ALT 5-40 IU/l; AST 5-45 IU/l). Serum ***ALT*** and AST are increased in liver damage. However, alanine transaminase is ***more sensitive*** and reliable for the assessment of LFT.

Estimation of serum transaminases cannot identify the causes (etiology) of hepatic damage. Further, they do not have much prognostic value.

Alkaline phosphatase

Alkaline phosphatase (ALP) is mainly derived from bone and liver (the cells lining the bile canaliculi). A rise in serum ALP (normal 3-13 KA units/dl), usually associated with elevated serum bilirubin is an indicator of biliary obstruction (***obstructive/posthepatic jaundice***). ALP is also elevated in cirrhosis of liver and hepatic tumors.

Liver is not the sole source of alkaline phosphatase. Therefore, its measurement has to be carefully viewed (along with others) before arriving at any conclusion. The liver and bone isoenzymes of ALP can be separated by electrophoresis.

γ-Glutamyl transpeptidase

This is a microsomal enzyme widely distributed in body tissues, including liver. Measurement of γ-glutamyl transpeptidase (GGT) activity provides a sensitive index to asses liver abnormality. The activity of this enzyme almost parallels that of transaminases in hepatic damage. Serum GGT is highly elevated (normal 5-40 IU/l) in ***biliary obstruction*** and ***alcoholism***. Further, several drugs (e.g. phenytoin) induce (liver synthesis) and increase this enzyme in circulation.

5′-Nucleotidase

The serum activity of 5′-nucleotidase (normal 2-15 U/l) is elevated in hepatobiliary disease and this parallels ALP. The advantage with 5′-nucleotidase is that it is not altered in bone diseae (as is the case with ALP).

Other enzymes

Serum isocitrate dehydrogenase and isoenzymes of lactate dehydrogenase (LDH_4 and LDH_5) are also useful in LFT.

Enzyme combinations

Very often, a combination of serum enzyme estimations (instead of a single one) is used for a better understanding of liver functions. For instance, a large increase in transaminases (particularly ALT) relative to a small increase in alkaline phosphatase indicates hepatocellular damage. On the other hand, a small increase in transaminases and a large increase of alkaline phosphatase shows biliary obstruction.

JAUNDICE

Jaundice (*French* : jaune—yellow) is characterized by yellow ***coloration of sclera (of eyes)*** and skin. This is due to the elevated ***serum bilirubin*** level, usually ***beyond 2 mg/dl*** (normal < 1 mg/dl).

The metabolism of heme to produce bilirubin and its conjugated derivatives and the types of jaundice have already been described. The reader must refer this (***Chapter 10***) now. The biochemical changes and the related parameters for the differential diagnosis of the three types of jaundice (hemolytic, obstructive and hepatic) are given in ***Table 20.2***.

In the ***Fig.20.1***, the normal and abnormal bilirubin metabolism (along with the associated

Table 20.2 Biochemical changes for the differential diagnosis of three types of jaundice

Parameter	*Hemolytic jaundice (preheptic jaundice)*	*Obstructive jaundice (posthepatic jaundice)*	*Hepatic jaundice (Intrahepatic jaundice)*
Serum bilirubin	Unconjugated bilirubin ↑	Conjugated bilirubin ↑	Both ↑
van den Bergh reaction	Indirect positive	Direct positive	Biphasic
Serum enzymes	ALT, AST and ALP →	ALP ↑↑, ALT and AST marginal ↑	ALT and AST ↑↑, ALP marginal ↑
Bilirubin in urine	Not excreted	Excreted	Excreted
Urobilinogen in urine	Excretion ↑	→ or ↓	→ or ↓

ALT : Alanine transaminase; AST : Aspartate transaminase; ALP : Alkaline phosphatase; ↑ : Increase; ↓ : Decrease; → : Normal.

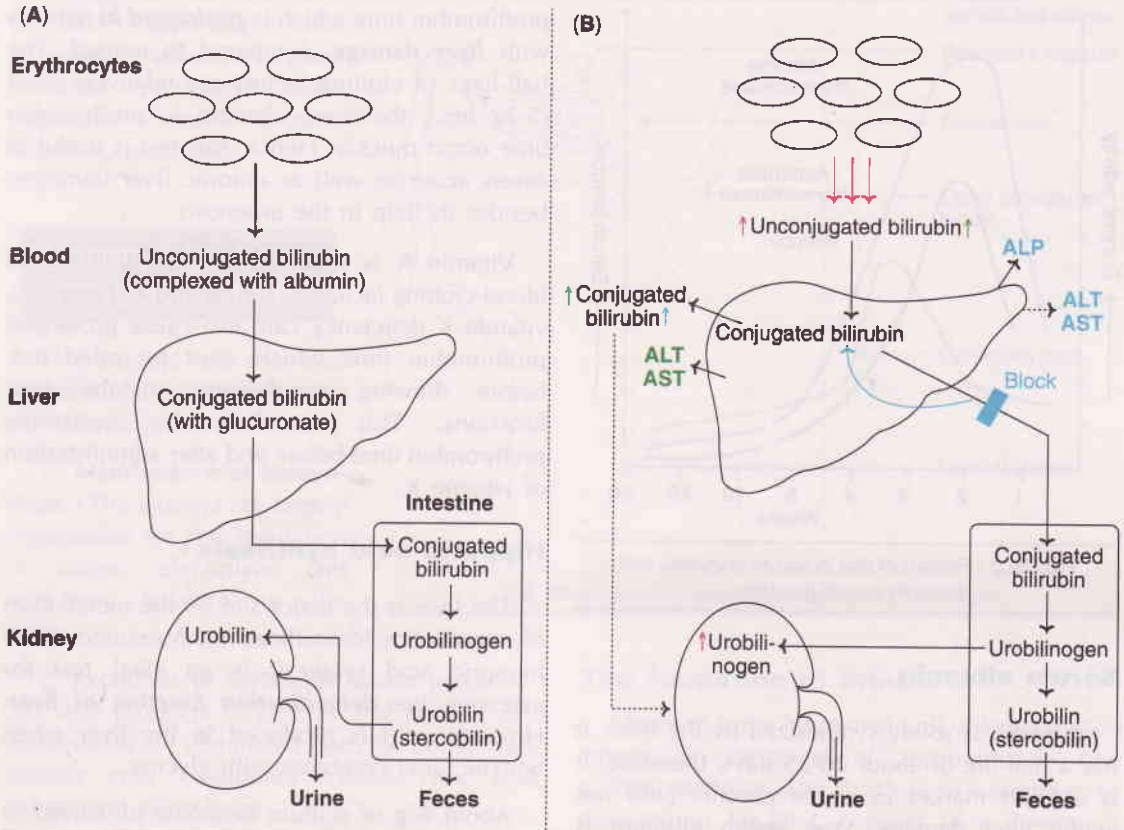


*Fig. 20.1 : Normal and abnormal bilirubin metabolism (A) Normal bilirubin metabolism (B) Alterations in bilirubin metabolism along with enzymes in three types of jaundice (**Note :** Colours indicate major changes; Red–changes in hemolytic jaundice; Green–changes in hepatic jaundice; Blue–changes in obstructive jaundice; Dotted lines indicate minor pathways; ALT–Alanine transaminase; AST–Aspartate transaminase; ALP–Alkaline phosphatase).*

enzyme changes) are depicted. The major changes in the 3 types of jaundice are listed below

Hemolytic jaundice : Elevated serum unconjugated bilirubin, and increased urinary excretion of urobilinogen.

Obstructive jaundice : Elevated serum conjugated bilirubin and increased activities of alkaline phosphatase (ALP), alanine transaminase (ALT) and aspartate transaminase (AST).

Hepatic jaundice : Elevated serum unconjugated and conjugated bilirubin, and increased activities of ALT and AST.

The pattern of rise in the serum alanine transaminase, aspartate transaminase and bilirubin in acute viral hepatitis is depicted in ***Fig.21.2***. It may be noted that the transaminase activities (more predominantly ALT) are elevated much before the bilirubin starts increasing.

Galactose tolerance

Galactose is a monosaccharide, almost exclusively metabolized by the liver. The liver function can be assessed by measuring the utilization of galactose. This is referred to galactose tolerance test. The subject is given intravenous administration of galactose (about 300 mg/kg body weight). Blood is drawn at 10 minute intervals for the next 2 hours and galactose estimated. In the normal individuals, the ***half-life of galactose*** is about 10-15 minutes. This is markedly ***elevated*** in hepatocellular ***damage*** (infective hepatitis, cirrhosis).

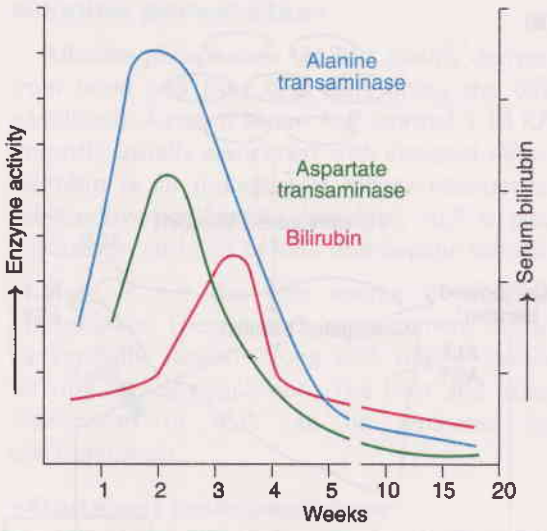


Fig. 20.2 : Pattern of rise in serum enzymes and bilirubin in viral hepatitis.

Serum albumin

Albumin is solely synthesized by the liver. It has a half-life of about 20-25 days, therefore, it is a good marker to assess chronic (and not acute) liver damage. Low serum albumin is commonly observed in patients with severe liver damage. It must, however, be noted that the serum albumin concentration is also ***decreased*** due to other factors such as ***malnutrition.***

Functional impairment of liver is frequently associated with increased synthesis of globulins. Cirrhosis of the liver causes a ***reversal*** of albumin/globulin ratio (***A/G ratio***). Serum electrophoresis of proteins reveals increased albumin and decreased γ-globulin concentration. This, however, may not have much diagnostic importance since several diseases are associated with altered electrophoretic pattern of serum proteins.

Prothrombin time

The liver synthesizes all the factors concerned with blood clotting. A decrease in the concentration of plasma clotting factors is found in the impairment of liver function. This can be assessed in the laboratory by measuring prothrombin time which is ***prolonged in*** patients with ***liver damage***, compared to normal. The half-lives of clotting factors are relatively short (5-72 hrs.), therefore, changes in prothrombin time occur quickly. Hence, this test is useful to assess acute as well as chronic liver damages; besides its help in the prognosis.

Vitamin K is required for the synthesis of blood clotting factors II, VII, IX and X. Therefore, vitamin K deficiency can also cause prolonged prothrombin time which must be ruled out, before drawing conclusions on the liver functions. This is done by measuring prothrombin time before and after administration of vitamin K.

Hippuric acid synthesis

The liver is the major site for the metabolism of xenobiotics (detoxification). Measurement of hippuric acid synthesis is an ideal ***test for*** assessing the ***detoxification function of liver***. Hippuric acid is produced in the liver when benzoic acid combines with glycine.

About 6 g of sodium benzoate (dissolved in about 250 ml water), is orally given to the subject, after a light breakfast (usually 2 hrs later) and after emptying the bladder. Urine collections are made for the next 4 hours and the amount of hippuric acid excreted is estimated. Theoretically, 6 g of sodium benzoate should yield 7.5 g of hippuric acid. In the healthy persons, about 60% of sodium benzoate (equivalent to 4.5 g hippuric acid) is excreted in urine. A ***reduction*** in hippuric acid excretion (particularly < 3 g) ***indicates hepatic damage***.

Choice of liver functions tests

The choice of biochemical tests to measure liver functions mostly depends on the purpose of the investigation. The clinical history of the subject is often a guiding factor in this regard. A single test in isolation may have a little diagnostic value.

Frequently, a ***combination of laboratory investigations are employed in LFT***. These include serum bilirubin (conjugated and

unconjugated), alanine transaminase, aspartate transaminase, alkaline phosphatase, γ-glutamyl transpeptidase and proteins (albumin, globulins).

KIDNEY (RENAL) FUNCTION TESTS

The kidneys are the vital organs of the body, performing the following major functions.

1. **Maintenance of homeostasis :** The kidneys are largely responsible for the regulation of water, electrolyte and acid-base balance in the body.

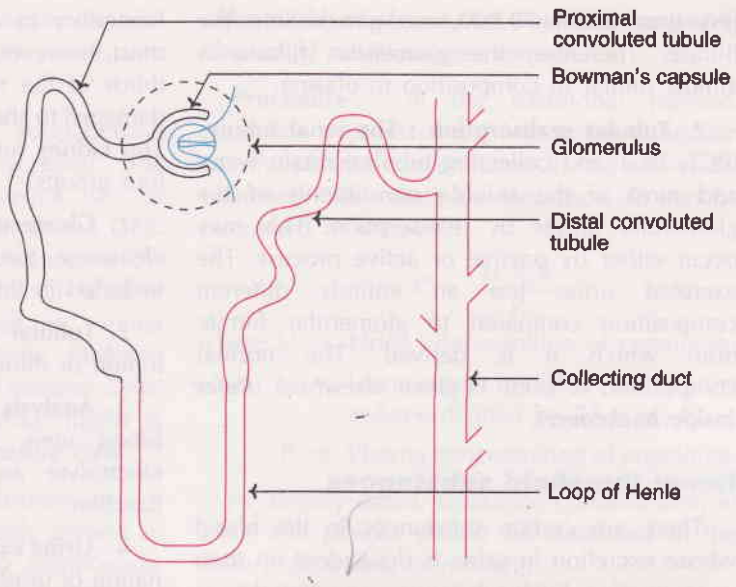


Fig. 20.3 : Diagrammatic representation of a nephron.

2. **Excretion of metabolic waste products :** The end products of protein and nucleic acid metabolism are eliminated from the body. These include urea, creatinine, creatine, uric acid, sulfate and phosphate.

3. **Retention of substances vital to body :** The kidneys reabsorb and retain several substances of biochemical importance in the body e.g. glucose, amino acids etc.

4. **Hormonal functions :** The kidneys also function as endocrine organs by producing hormones.

- **Erythropoietin,** a peptide hormone, stimulates hemoglobin synthesis and formation of erythrocytes.
- 1,25-Dihydroxycholecalciferol ***(calcitriol)*** – the biochemically active form of vitamin D – is finally produced in the kidney. It regulates calcium absorption from the gut.
- **Renin,** a proteolytic enzyme liberated by kidney, stimulates the formation of angiotensin II which, in turn, leads to aldosterone production. Angiotensin II and aldosterone are the hormones involved in the regulation of electrolyte balance.

The formation of urine

Nephron is the functional unit of kidney. Each kidney is composed of approximately one million nephrons. The structure of a nephron, as depicted in ***Fig.20.3***, consists of a Bowman's capsule (with blood capillaries), proximal convoluted tubule (PCT), loop of Henle, distal convoluted tubule (DCT) and collecting tubule.

The blood supply to kidneys is relatively large. About 1200 ml of blood (650 ml plasma) passes through the kidneys, every minute. From this, about ***120-125 ml is filtered per minute by the kidneys*** and this is referred to as ***glomerular filtration rate*** (GFR). With a normal GFR (120-125 ml/min), the glomerular filtrate formed in an adult is about 175-180 litres per day, out of which only 1.5 litres is excreted as urine. Thus, more than 99% of the glomerular filtrate is reabsorbed by the kidneys.

The process of urine formation basically involves two steps—glomerular filtration and tubular reabsorption.

1. **Glomerular filtration :** This is a passive process that results in the formation of ultrafiltrate of blood. All the (unbound) constituents of plasma, with a molecular weight

less than about 70,000, are passed into the filtrate. Therefore, the glomerular filtrate is almost similar in composition to plasma.

2. **Tubular reabsorption :** The renal tubules (PCT, DCT and collecting tubules) retain water and most of the soluble constituents of the glomerular filtrate by reabsorption. This may occur either by passive or active process. The excreted urine has an entirely different composition compared to glomerular filtrate from which it is derived. The normal composition of urine is given elsewhere (***Refer inside backcover***).

Renal threshold substances

There are certain substances in the blood whose excretion in urine is dependent on their concentration. Such substances are referred to as renal threshold substances. At the normal concentration in the blood, they are completely reabsorbed by the kidneys, with a result that their excretion in urine is almost negligible.

The renal threshold of ***a substance*** is defined as its ***concentration in blood (or plasma) beyond which*** it is ***excreted into urine***. The renal threshold for glucose is 180 mg/dl; for ketone bodies 3 mg/dl; for calcium 10 mg/dl and for bicarbonate 30 mEq/l. While calculating the renal threshold of a particular compound, it is assumed that both the kidneys are optimally functioning, without any abnormality. But this is not always true—in which case the renal threshold is altered. For instance, ***renal glycosuria*** is associated with reduced threshold for glucose due to its diminished tubular reabsorption.

The term ***tubular maximum*** (***Tm***) is used to indicate the maximum capacity of the kidneys to absorb a particular substance. For instance, tubular maximum for glucose (TmG) is 350 mg/min.

Tests to assess renal function

In view of the important and sensitive functions the kidney performs (described already), it is essential that the abnormalities (renal damages), if any, must be detected at the earliest. Several tests are employed in the laboratory to assess kidney (renal) function. It must, however, be remembered that about two-thirds of the renal tissue must be functionally damaged to show any abnormality by these tests. The kidney function tests may be divided into four groups.

1. **Glomerular function tests :** All the clearance tests (inulin, creatinine, urea) are included in this group.

2. **Tubular function tests :** Urine concentration or dilution test, urine acidfication test.

3. **Analysis of blood/serum :** Estimation of blood urea, serum creatinine, protein and electrolyte are often useful to assess renal function.

4. **Urine examination :** Simple routine examination of urine for volume, pH, specific gravity, osmolality and presence of certain abnormal constituents (proteins, blood, ketone bodies, glucose etc.) also helps, of course to a limited degree, to assess kidney functioning.

Some of the important renal function tests are discussed in the following pages.

CLEARANCE TESTS

The clearance tests, measuring the glomerular filtration rate (GFR) are the most useful in assessing the renal function. The excretion of a substance can be expressed quantitatively by using the concept of clearance.

Clearance, in general, is defined as the ***volume of plasma that would be completely cleared of a substance per minute***. In other words, ***clearance of a substance refers to the milliliters of plasma which contains the amount of that substance excreted by kidney per minute.*** Clearance (C), expressed as ml/minute, can be calculated by using the formula

$$C = \frac{U \times V}{P}$$

where U = Concentration of the substance in urine.

V = Volume of urine in ml excreted per minute.

P = Concentration of the substance in plasma.

Care should be taken to express the concentrations of plasma and urine in the same units (mmol/l or mg/dl).

The clearance of a given substance is determined by its mode of excretion. The maximum rate at which the plasma can be cleared of any substance is equal to the GFR. This can be easily calculated by measuring the clearance of a plasma compound which is freely filtered by the glomerulus and is neither absorbed nor secreted in the tubule. ***Inulin*** (a plant carbohydrate, composed of fructose units) and ^{51}Cr-EDTA satisfy this criteria. Inulin is intravenously administered ***to measure GFR***.

In practice, however, measurement of clearance for the substances already present in the blood is preferred. The two compounds, namely ***creatinine*** and ***urea***, are commonly employed for this purpose. Creatinine clearance (~145 ml/min) is marginally higher than the GFR as it is secreted by the tubules. On the other hand, urea clearance (~75 ml/min) is less than the GFR, since it is partially reabsorbed by the tubules.

Diodrast (diiodopyridone acetic acid) is used as a contrast medium to take urinary tract X-rays. Diodrast and ***para amino hippuric acid (PAH)*** are peculiar substances as they are entirely excreted by a single passage of blood through the kidneys. It is partly filtered by the glomerulus and mostly excreted by the tubules. PAH has a clearance of about 700 ml/min (or 1,200 ml, if expressed as blood). Thus clearance of PAH represents the ***renal plasma flow***.

Creatinine clearance test

Creatinine is an excretory product derived from creatine phosphate (largely present in muscle). The excretion of creatinine is rather constant and is not influenced by body metabolism or dietary factors. As already stated, creatinine is filtered by the glomeruli and only marginally secreted by the tubules. The value of creatinine clearance is close to GFR, hence its measurement is a sensitive and good approach to assess the renal glomerular function. Creatinine clearance may be defined as ***the volume (ml) of plasma that would be completely cleared of creatinine per minute***.

Procedure : In the traditional method, creatinine content of a 24 hr urine collection and the plasma concentration in this period are estimated. The creatinine clearance (C) can be calculated as follows :

$$C = \frac{U \times V}{P}$$

where U = Urine concentration of creatinine

V = Urine output in ml/min (24 hr urine volume divided by 24 × 60)

P = Plasma concentration of creatinine.

As already stated, creatinine concentration in urine and plasma should be expressed in the same units (mg/dl or mmol/l).

Modified procedure : Instead of a 24 hr urine collection, the procedure is modified to collect urine for 1 hr, after giving water. The volume of urine is recorded. Creatinine contents in plasma and urine are estimated. The creatinine clearance can be calculated by using the formula referred above.

Reference values : The normal range of creatinine clearance is around ***120-145 ml/min***. These values are slightly lower in women. In recent years, creatinine clearance is expressed in terms of body surface area.

Diagnostic importance : A decrease in creatinine clearance value (< 75% normal) serves as sensitive indicator of a decreased GFR, due to renal damage. This test is useful for an early detection of impairment in kidney function, often before the clinical manifestations are seen.

Urea clearance test

Urea is the end product of protein metabolism. After being filtered by the glomeruli, it is partially reabsorbed by the renal tubules. Hence, urea clearance is less than the GFR and, further, it is influenced by the protein content of the diet. For these reasons, ***urea clearance is not as sensitive as creatinine clearance*** for assessing renal function. Despite this fact, several laboratories traditionally use this test.

Urea clearance is defined as the volume (ml) of plasma that would be completely cleared of urea per minute. It is calculated by the formula

$$C_m = \frac{U \times V}{P}$$

where C_m = Maximum urea clearance
U = Urea concentration in urine (mg/ml)
V = Urine excreted per minute in ml
P = Urea concentration in plasma (mg/ml).

The above calculation is applicable if the ***output of urine is more than 2 ml per minute.*** This is referred to as ***maximum urea clearance*** and the normal value is around 75 ml/min.

Standard urea clearance : It is observed that the urea clearance drastically changes when the volume of urine is less than 2 ml/min. This is known as ***standard urea clearance (C_s)*** and the normal value is around 54 ml/min. It is calculated by a modified formula

$$C_s = \frac{U \times \sqrt{V}}{P}$$

Diagnostic importance : A urea clearance value below 75% of the normal is viewed seriously, since it is an indicator of renal damage. Blood urea level as such is found to increase only when the clearance falls below 50% normal. As already stated, creatinine clearance is a better indicator of renal function.

Urine concentration test

This is a test to assess the renal tubular function. It is a simple test and involves the accurate measurement of specific gravity which depends on the concentration of solutes in urine. A specific gravity of 1.020 in the early morning urine sample is considered to be normal.

Several measures are employed to concentrate urine and measure the specific gravity. These include overnight water deprivation and administration of antidiuretic hormone. If the specific gravity of urine is above 1.020 for at least one of the samples collected, the tubular function is considered to be normal.

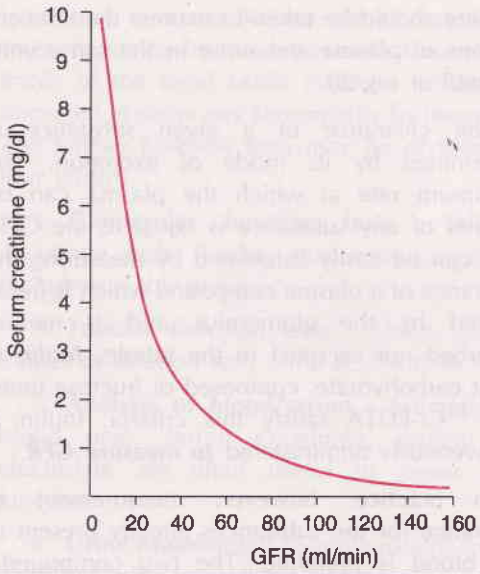


Fig. 20.4 : The relationship between glomerular filtration rate (GFR) and serum creatinine concentration.

Osmolality and specific gravity : The osmolality of urine is variable. In normal individuals, it may range from 500-1,200 milliosmoles/kg. The plasma osmolality is around 300 milliosmoles/kg. The normal ratio of the osmolality between urine and plasma is around 2-4. It is found that the urine (without any protein or high molecular weight substance) with an osmolality of 800 mosm/kg has a specific gravity of 1.020. Therefore, measurement of urine osmolality will also help to assess tubular function.

Analysis of blood (or serum)

Estimation of serum creatinine and blood urea are often used to assess the overall kidney function, although these tests are less sensitive than the clearance tests. ***Serum creatinine*** is a ***better*** indicator than urea in this regard. The diagnostic importance of urea and creatinine estimations are discussed elsewhere (***Refer Chapter 15***).

The relationship between GFR and serum creatinine levels is depicted in ***Fig.20.4***. It is observed that the GFR must fall to about 50% of its normal value before a significant increase in serum creatinine occurs. Therefore, a normal

serum creatinine level does not necessarily mean that all is well with the kidney. It is estimated that a loss of 50% of the functions of nephrons leads to (approximate) doubling of serum creatinine concentration.

Urine examination

The routine urine examination is undoubtedly a guiding factor for renal function. The volume of urine excreted, its pH, specific gravity, osmolality, the concentration of abnormal constituents (such as proteins, ketone bodies, glucose and blood) may help to have some preliminary knowledge of kidney function. More information on urine laboratory tests is given in the ***appendix***.

Choice of renal function tests

In general, the assessment of kidney function starts with the routine urine examination, followed by serum creatinine and/or blood urea estimations and, finally, the specific tests to measure the tubular and glomerular functions (clearance tests).

GASTRIC FUNCTION TESTS

The stomach is a major organ of digestion and performs the following functions

1. Stomach is a reservoir of ingested foodstuffs.

2. It has a great churning ability which promotes digestion.

3. Stomach elaborates HCl and proteases (pepsin) which are responsible for the initiation of digestive process.

4. The products obtained in the stomach (peptides, amino acids) stimulate the release of pancreatic juice and bile.

Secretion of gastric HCl

The parietal (oxyntic) cells of gastric glands produce HCl. The pH in the gastric lumen is as low as 0.8 (against the blood pH 7.4). Therefore, the protons are transported against the concentration gradient by an active process.

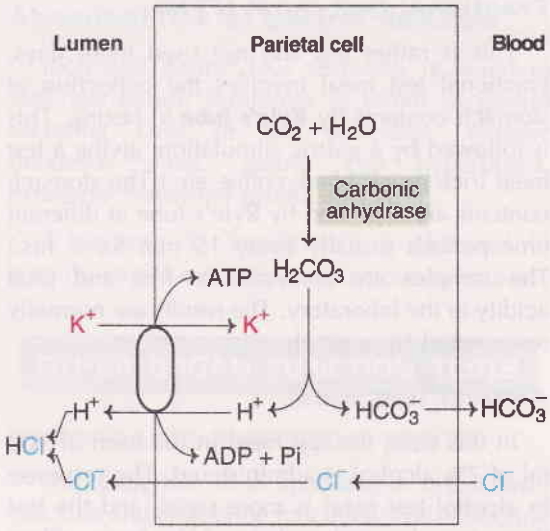


Fig. 20.5 : Mechanism of HCl secretion (O—represents K^+ activated ATPase).

A unique enzyme—namely K^+ activated ATPase—present in the parietal cells is connected with the mechanism of HCl secretion (***Fig.20.5***). The process involves an exchange of H^+ ions (of the parietal cells) for K^+ ions (of the lumen). This is coupled with the consumption of energy, supplied by ATP. The H^+ are continuously generated in the parietal cells by the dissociation of carbonic acid which, in turn, is produced from CO_2. The bicarbonate ions (HCO_3^-), liberated from the carbonic acid (H_2CO_3) dissociation, enter the blood in exchange for Cl^- ions. The latter diffuse into the gastric lumen to form HCl. Gastrin—a peptide hormone of gastrointestinal tract—stimulates HCl secretion.

Following a meal, there is a slight ***elevation in the plasma bicarbonate concentration*** which is linked to the gastric HCl secretion. This is referred to as ***alkaline tide***.

TESTS TO ASSESS GASTRIC FUNCTION

There are several tests for gastric function evaluation, some of the important ones are briefly discussed.

Fractional test meal (FTM)

This is rather old and not used these days. Fractional test meal involves the collection of stomach contents by ***Ryle's tube*** in fasting. This is followed by a gastric stimulation, giving a test meal (rice gruel, black coffee etc.) The stomach contents are aspirated by Ryle's tube at different time periods (usually every 15 min for 2 hrs.) The samples are analysed for free and total acidity in the laboratory. The results are normally represented by a graph.

Alcohol test meal

In this case, the test meal in the form of 100 ml of 7% alcohol is administered. The response to alcohol test meal is more rapid, and the test time can be reduced to $1^1/_2$ hour. Clear specimens can be collected by this test, and the free acidity levels are relatively higher compared to FTM.

Pentagastrin stimulation test

Pentagastrin is a synthetic peptide which stimulates the gastric secretion in a manner similar to the natural gastrin. The test procedure adapted is as follows

The stomach contents are aspirated by Ryle's tube in a fasting condition. This is referred to as residual juice. The gastric juice elaborated for the next one hour is collected and pooled which represents the basal secretion. Pentagastrin (5 mg/kg body weight) is now given to stimulate gastric secretion. The gastric juice is collected at 15 minute intervals for one hour. This represents the maximum secretion.

Each sample of the gastric secretion collected is measured for acidity by titrating the samples with N/10 NaOH to pH 7.4. The end point may be detected by an indicator (phenol red) or a pH meter.

Basal acid output (BAO) refers to the acid output (millimol per hour) under the basal conditions i.e. basal secretion.

Maximal acid output (MAO) represents the acid output (millimol per hour) after the gastric stimulation by pentagastrin i.e. maximum secretion.

In normal individuals, the BAO is 4-10 mmol/hr while the MAO is 20-50 mmol/hr.

Augmented histamine test meal

Histamine is a powerful stimulant of gastric secretion. The basal gastric secretion is collected for one hour. Histamine (0.04 mg/kg body weight) is administered subcutaneously and the

BIOMEDICAL / CLINICAL CONCEPTS

- *The impairment in the functions of any organ in the body will adversely influence the health of the organism. Organ function tests are the laboratory tools to biochemically evaluate the working of a given organ.*
- *Acute viral hepatitis is associated with elevated alanine transaminase (predominantly), aspartate transaminase and bilirubin.*
- *Increase in serum γglutamyl transpeptidase is observed in biliary obstruction and alcoholism.*
- *A combination of laboratory investigations—instead of a single one—are commonly employed in assessing organ function. Kidney function can be accurately assessed by clearance tests, measuring glomerular filtration rate. A reduction in clearance reflects renal damage.*
- *Zollinger-Ellison syndrome, a tumor of gastrin secreting cells of the pancreas, is associated with increased gastric HCl production.*

gastric contents are aspirated for the next one hour (at 15 minute intervals). The acid content is measured in all these samples.

Insulin test meal

This is also known as ***Hollander's test.*** It is mainly done to assess the completeness of vagotomy (vagal resection). Insulin (0.1 unit/kg body weight) is administered intravenously which causes hypoglycemia (blood glucose about 40 mg/dl), usually within 30 minutes, in normal persons.

If the vagotomy operation is successful, *insulin administration does not cause any* increase in the acid output, compared to the basal level. This test has to be carefully perfomed, since hypoglycemia is dangerous.

Tubeless gastric analysis

In the traditional methods of gastric analysis, a tube is invariably passed into the stomach to collect the gastric juice. This causes inconvenience to the subject. Recently, some tests involving tubeless gastric analysis have been developed. Such tests, however, are mostly useful for preliminary screening.

The principle of tubeless gastric analysis involves administration of a cation exchange resin that gets quantitatively exchanged with the H^+ ions of the gastric juice. The resin is then excreted into urine which can be estimated for an indirect measure of gastric acidity (concentration of H^+ ions).

Diagnex blue containing ***azure-A-resin*** is employed in the tubeless gastric analysis.

Abnormalities of gastric function

Increased gastric HCl secretion is found in Zollinger-Ellison syndrome (a tumor of gastrin secreting cells of the pancreas), chronic duodenal ulcer, gastric cell hyperplasia, excessive histamine production etc.

A decrease in gastric HCl is observed in gastritis, gastric carcinoma, pernicious anemia etc.

OTHER ORGAN FUNCTION TESTS

PANCREATIC FUNCTION TESTS

The pancreas is a specialized organ with exocrine and endocrine functions. The endocrine functions are discussed under the topic diabetes mellitus (***Chapter 36***).

The exocrine functions involve the synthesis of pancreatic juice containing several enzymes (for the digestion of foodstuffs) and bicarbonate. The major enzymes of pancreatic juice are trypsin, chymotrypsin, elastase, carboxy-peptidase, amylase and lipase.

Pancreatic enzymes in serum : *Serum amylase* and ***lipase measurements*** are commonly employed to assess the pancreatic function. Both these enzyme activities are elevated in acute pancreatitis, obstruction in the intestine and/or pancreatic duct.

THYROID FUNCTION TESTS

Thyroid gland produces two principal hormones—thyroxine (T_4) and triiodothyronine which regulate the metabolic rate of the body. The laboratory tests employed for the diagnosis of thyroid function are described in the ***Chapter 19*** on hormones.

SUMMARY

1. *Specific laboratory biochemical investigations are employed to assess the functioning of the organs such as liver, kidney, stomach and pancreas.*
2. *The liver function can be evaluated by the tests based on its excretory function (serum bilirubin), serum enzymes (transaminases), metabolic capability (galactose tolerance test) and synthetic functions (prothrombin time).*
3. *Serum bilirubin (normal < 1mg/dl) is derived from heme degradation. It is mostly (75%) found in the conjugated form. van den Bergh reaction is a specific test to identify the increased serum bilirubin. Conjugated bilirubin gives a direct positive test while the unconjugated bilirubin gives an indirect positive test.*
4. *The serum enzymes—namely alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) and γ glutamyltranspeptidase (GGT)—are frequently used for LFT. Increase in the activities of these enzymes indicates an impairment in liver function.*
5. *Jaundice is due to elevated serum bilirubin level (>2 mg/dl). The three types of jaundice (hemolytic, obstructive and hepatic) can be differentially diagnosed by biochemical tests. Thus, unconjugated bilirubin (indirect positive) is increased in hemolytic jaundice, conjugated bilirubin (direct positive) in obstructive jaundice and both of them (biphasic) are increased in hepatic jaundice.*
6. *Impaired galactose tolerance test, diminished serum albumin concentration and prolonged prothrombin time are also associated with liver malfunction.*
7. *The renal (kidney) function is usually assessed by evaluating either the glomerular (clearance tests) or tubular function (urine concentration test). This is often guided by blood analysis (for urea, creatinine) and/or urine examination.*
8. *The clearance is defined as the volume of the plasma that would be completely cleared of a substance per minute. Inulin clearance represents glomerular filtration rate (GFR). Creatinine clearance and urea clearance tests are often used to assess renal function. A decrease in their clearance is an indication of renal damage.*
9. *Impairment in renal function is often associated with elevated concentration of blood urea, serum creatinine, decrease in osmolality and specific gravity of urine (by urine concentration test).*
10. *The tests to evaluate gastric function include fractional test meal, pentagastrin stimulation test, augmented histamine test and tubeless gastric analysis. Gastric HCl secretion is elevated in chronic duodenal ulcer and gastric hyperplasia. Gastritis and pernicious anemia are associated with decreased gastric HCl. Pancreatic function is assessed by serum amylase and lipase. Both of them are elevated in acute pancreatitis.*

SELF-ASSESSMENT EXERCISES

I. Essay questions

1. Write briefly on the different laboratory investigations employed to assess liver function.
2. Discuss the biochemical parameters for the differential diagnosis of jaundice.
3. Give an account of the serum enzymes derived from liver and their importance in LFT.
4. Describe the renal function tests.
5. Discuss the different laboratory investigations to evaluate gastric function.

II. Short notes

(a) Serum bilirubin, (b) van den Bergh reaction, (c) Galactose tolerance test, (d) Prothrombin time as LFT, (e) Renal threshold substances, (f) Glomerular filtration rate, (g) Creatinine clearance, (h) Standard urea clearance, (i) Urine concentration test, (j) Gastric function tests.

III. Fill in the blanks

1. Bilirubin is the excretory end product of _____.
2. The laboratory reaction most commonly employed to detect the elevation of serum bilirubin is _____.
3. The serum enzyme most predominantly elevated in viral hepatitis is _____.
4. Obstructive jaundice is characterized by an increase in the serum enzyme _____.
5. The excretory function of liver can be evaluated by using a dye _____.
6. The renal threshold for glucose is _____.
7. The exogenous substance used to measure glomerular filtration rate (GFR) is _____.
8. Standard urea clearance is calculated when the volume of urine output is less than _____.
9. Name the stomach tube used to aspirate gastric juice _____.
10. Name the synthetic peptide used to stimulate gastric secretion for evaluation of gastric function _____.

IV. Multiple choice questions

11. In hemolytic jaundice, van den Bergh reaction is

    (a) Indirect positive (b) Direct positive (c) Biphasis (d) None of these.

12. The serum enzyme elevated in alcoholic cirrhosis of liver is

    (a) Alanine transaminase (b) Aspartate transaminase (c) Alcohol dehydrogenase (d) γ-Glutamyl transpeptidase.

13. Bilirubin is not excreted in urine in

    (a) Obstructive jaundice (b) Hepatic jaundice (c) Hemolytic jaundice (d) All three.

14. Urea clearance is less than GFR because it is

    (a) Partially secreted by the renal tubules (b) Partially reabsorbed by the tubules (c) Only filtered by glomeruli (d) None of these.

15. The serum enzyme used to evaluate pancreatic function is

    (a) Alkaline phosphatase (b) Amylase (c) Aspartate transaminase (d) Lactate dehydrogenase.

21 Water, Electrolyte and Acid-base Balance

$$pH = pK_a + \log \frac{[HCO_3^-]}{[H_2CO_3]}$$

The acid-base homeostasis speaks :

"We maintain the blood pH at 7.4!
Regulated by buffers, lungs and kidneys;
Increased hydrogen ion causes acidosis;
Decreased hydrogen ion leads to alkalosis."

The organism possesses tremendous capacity to survive against odds and maintain homeostasis. This is particularly true with regard to water, electrolyte and acid-base status of the body. These three are interrelated, hence they are considered together for the discussion in this chapter. Kidney actively participates in the regulation of water, electrolyte and acid-base balance. The general functions of kidney have already been described (***Chapter 20***).

WATER BALANCE

Water is the solvent of life. Undoubtedly, water is more important than any other single compound to life. It is involved in several body functions.

Functions of water

1. Water provides the aqueous medium to the organism which is essential for the various biochemical reactions to occur.

2. Water directly participates as a reactant in several metabolic reactions.

3. It serves as a vehicle for transport of solutes.

4. Water is closely associated with the regulation of body temperature.

Distribution of water

Water is the major body constituent. An adult human contains about 60% water (men 55-70%, women 45-60%). The women and obese individuals have relatively less water which is due to the higher content of stored fat in an anhydrous form.

A 70 kg normal man contains about 42 litres of water. This is distributed in intracellular (inside the cells 28l) and extracellular (outside the cells 14l) compartments, respectively known as ***intracellular fluid*** (***ICF***) and ***extracellular fluid*** (***ECF***). The ECF is further divided into interstitial fluid (10.5l) and plasma (3.5l). The distribution of water in man is given in ***Table 21.1***.

Table 21.1 Distribution of water in an adult man, weighing 70 kg.

Compartment	% Body weight	Volume (l)
Total	60	42
Intracellular fluid (ICF)	40	28
Extracellular fluid (ECF)	20	14
Interstitial fluid	15	10.5
Plasma	5	3.5

WATER TURNOVER AND BALANCE

The body possesses tremendous capacity to regulate its water content. In a healthy individual, this is achieved by balancing the daily water intake and water output.

Water intake

Water is supplied to the body by ***exogenous*** and ***endogenous*** sources.

Exogenous water : Ingested water and beverages, water content of solid foods-constitute the exogenous source of water. Water intake is highly variable which may range from 0.5-5 litres. It largely depends on the social habits and climate. In general, people living in hot climate drink more water. Ingestion of water is mainly controlled by a ***thirst centre*** located in the ***hypothalamus***. Increase in the osmolality of plasma causes increased water intake by stimulating thirst centre.

Endogenous water : The ***metabolic water*** produced within the body is the endogenous water. This water (300-350 ml/day) is derived from the oxidation of foodstuffs. It is estimated that 1 g each of carbohydrate, protein and fat, respectively, yield 0.6 ml, 0.4 ml and 1.1 ml of water. On an average, about 125 ml of water is generated for 1,000 Cal consumed by the body.

Water output

Water losses from the body are variable. There are four distinct routes for the elimination of water from the body—urine, skin, lungs and feces.

Urine : This is the ***major route*** for water loss from the body. In a healthy individual, the urine output is about 1-2 l/day. Water loss through kidneys although highly variable, is well regulated to meet the body demands—to get rid of water or to retain. It should, however, be remembered that man cannot completely shut down urine production, despite there being no water intake. This is due to the fact that some amount of water (about 500 ml/day) is essential as the medium to eliminate the waste products from the body.

Hormonal regulation of urine production : It is indeed surprising to know that about 180 litres of water is filtered by the glomeruli into the renal tubules everyday. However, most of this is reabsorbed and only 1-2 litres is excreted as urine. Water excretion by the kidney is tightly controlled by ***vasopressin*** also known as ***antidiuretic hormone (ADH)*** of the posterior pituitary gland. The secretion of ADH is regulated by the osmotic pressure of plasma. An increase in osmolality promotes ADH secretion that leads to an increased water reabsorption from the renal tubules (less urine output). On the other hand, a decrease in osmolality suppresses ADH secretion that results in reduced water reabsorption from the renal tubules (more urine output). Plasma osmolality is largely dependent on the sodium concentration, hence sodium indirectly controls the amount of water in the body.

Diabetes insipidus is a disorder characterized by the ***deficiency of ADH*** which results in an ***increased loss of water*** from the body.

Skin : Loss of water (450 ml/day) occurs through the body surface by perspiration. This is an unregulated process by the body which mostly depends on the atmospheric temperature and humidity. The loss is more in hot climate. Fever causes increased water loss through the skin. It is estimated that for every 1°C rise in body temperature, about 15% *increase is observed in the loss of water (through* skin).

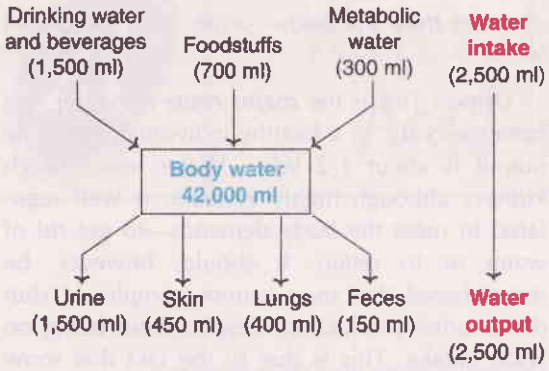


Fig. 21.1 : Water balance in the body, represented by daily intake and output (values are variable).

Lungs : During respiration, some amount of water (about 400 ml/day) is lost through the expired air. The latter is saturated with water and expelled from the body. In hot climates and/or when the person is suffering from fever, the water loss through lungs is increased.

The loss of water by perspiration (via skin) and respiration (via lungs) is collectively referred to as ***insensible water loss.***

Feces : Most of the water entering the gastrointestinal tract is reabsorbed by the intestine. About 150 ml/day is lost through feces in a healthy individual. Fecal loss of water is tremendously increased in diarrhea.

A summary of the water intake and output in the body is depicted in ***Fig.21.1***. It may be noted that water balance of the body is regulated predominantly by controlling the urine output. This happens after an obligatory water loss via skin, lungs and feces.

The abnormalities associated with water balance—dehydration and overhydration—will be described, following a discussion on electrolyte balance.

ELECTROLYTE BALANCE

Electrolytes are the compounds which readily dissociate in solution and ***exist as ions*** i.e. positively and negatively charged particles. For instance, NaCl does not exist as such, but it exists as cation (Na^+) and anion (Cl^-). The concentration of electrolytes are ***expressed as milliequivalents (mEq/l)*** rather than milligrams. A gram equivalent weight of a compound is defined as its weight in grams that can combine or displace 1 g of hydrogen. One gram equivalent weight is equivalent to 1,000 milliequivalents.

The following formula is employed to convert the concentration mg/l to mEq/l.

$$mEq/l = \frac{\text{mg per litre} \times \text{Valency}}{\text{Atomic weight}}$$

Electrolyte composition of body fluids

Electrolytes are well distributed in the body fluids in order to maintain the osmotic equilibrium and water balance. A comparison of electrolytes present in extracellular (plasma) and intracellular (muscle) fluids is given in ***Table 21.2***. The total concentration of cations and anions in each body compartment (ECF or ICF) is equal to maintain electrical neutrality.

There is a marked difference in the concentration of electrolytes (cations and anions) between the extracellular and intracellular fluids. Na^+ is the principal ***extracellular cation*** while K^+ is the ***intracellular cation***. This difference in the concentration is essential for the cell survival which is maintained by ***$Na^+ - K^+$ pump*** (for details, ***Refer Chapter 33***). As regards anions, Cl^- and HCO_3^- predominantly occur in extracellular fluids, while HPO_4^-, proteins and organic acids are found in the intracellular fluids.

Osmolarity and osmolality of body fluids

There are two ways of expressing the concentration of molecules with regard to the osmotic pressure.

1. **Osmolarity :** The number of moles (or millimoles) per liter of solution.

2. **Osmolality :** The number of moles (or millimoles) per kg of solvent.

Table 21.2 Composition of electrolytes in the body fluids (expressed as mEq/l)

Extracellular fluid (plasma)				*Intracellular fluid (muscle)*			
Cations		Anions		Cations		Anions	
Na^+	142	Cl^-	103	K^+	150	HPO_4^{2-}	140
K^+	5	HCO_3^-	27	Na^+	10	HCO_3^-	10
Ca^{2+}	5	HPO_4^{2-}	2	Mg^{2+}	40	Cl^-	2
Mg^{2+}	3	SO_4^{2-}	1	Ca^{2+}	2	SO_4^{2-}	5
		Proteins	16			Proteins	40
		Organic acids	6			Organic acids	5
	155		155		202		202

If the solvent is pure water, there is almost no difference between osmolarity and osmolality. However, for biological fluids (containing molecules such as proteins), the osmolality is more commonly used. This is about 6% greater than osmolarity.

Osmolality of plasma

Osmolality is a measure of the solute particles present in the fluid medium. The osmolality of plasma is in the range of 285-295 milliosmoles/kg (***Table 21.3***). Sodium and its associated anions make the largest contribution (~90%) to plasma osmolality. Osmolality is generally measured by osmometer.

For practical purposes, ***plasma osmolality*** can be computed from the concentrations (mmol/l) of Na^+, K^+, urea and glucose as follows

$$2(Na^+) + 2(K^+) + \text{Urea} + \text{Glucose}$$

The factor 2 is used for Na^+ and K^+ ions to account for the associated anion concentration (assuming complete ionization of the molecules). Since plasma Na^+ is the most predominant contributor to osmolality, the above calculation is further simplified as follows

$$\underset{\text{(mmol/kg)}}{\text{Plasma osmolality}} = 2 \times \underset{\text{(mmol/l)}}{\text{Plasma } Na^+}$$

The above calculation holds good only if plasma concentration of glucose and urea are in the normal range. This calculation, however, will not be valid in severe hyperproteinemia and lipemia.

Osmolality of ECF and ICF

Movement of water across the biological membranes is dependent on the osmotic pressure differences between the intracellular fluid (ICF) and extracellular fluid (ECF). In a healthy state, the osmotic pressure of ECF, mainly due to Na^+ ions, is equal to the osmotic pressure of ICF which is predominantly due to

Table 21.3 Distribution of constituents in plasma osmolality

Constituent (solute)	*Osmolality (mosm/kg)*
Sodium	135
Associated anions	135
Potassium	3.5
Associated anions	3.5
Calcium	1.5
Associated anions	1.5
Magnesium	1.0
Associated anions	1.0
Urea	5.0
Glucose	5.0
Protein	1.0
Total	293

K^+ ions. As such, there is no net passage of water molecules in or out of the cells, due to this osmotic equilibrium.

Regulation of electrolyte balance

Electrolyte and water balance are regulated together and the kidneys play a predominant role in this regard. The regulation is mostly achieved through the hormones aldosterone, ADH and renin-angiotensin.

Aldosterone : It is a ***mineralocorticoid*** produced by adrenal cortex. Aldosterone increases Na^+ reabsorption by the renal tubules at the expense of K^+ and H^+ ions. The net effect is the retention of Na^+ in the body.

Antidiuretic hormone (ADH) : An increase in the plasma osmolality (mostly due to Na^+) stimulates hypothalamus to release ADH. ADH effectively ***increases water reabsorption*** by renal tubules.

Renin-angiotensin : The secretion of aldosterone is controlled by renin-angiotensin system. Decrease in the blood pressure (due to a fall in ECF volume) is sensed by juxtaglomerular apparatus of the nephron which secrete renin. Renin acts on angiotensinogen to produce angiotensin I. The latter is then converted to angiotensin II which stimulates the release of aldosterone.

The relation between renin, angiotensin and aldosterone in the regulation of Na^+ balance is depicted in ***Fig.21.2***. Aldosterone and ADH coordinate with each other to maintain the normal fluid and electrolyte balance.

Atrial natriuretic peptide : This is a polypeptide hormone secreted by the right atrium of the heart. Atrial natriuretic peptide increases the urinary Na^+ excretion. The significance of this hormone, however, is not clear.

Na^+ concentration and ECF

It is important to realise that Na^+ and its anions (mainly Cl^-) are confined to the extracellular fluid. And the retention of water in the ECF is directly related to the osmotic effect of these ions (Na^+ and Cl^-). Therefore, the amount of Na^+ in the ECF ultimately determines its volume.

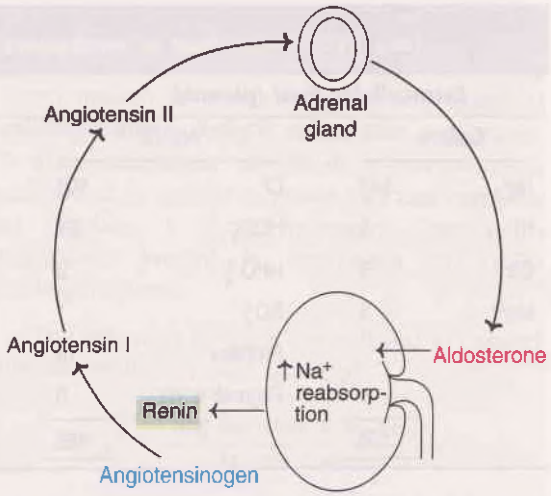


Fig. 21.2 : Hormonal regulation of Na^+ balance by the kidney.

Dietary intake and electrolyte balance

Generally, the consumption of a well-balanced diet supplies the body requirement of electrolytes. Humans do not possess the ability to distinguish between the salt hunger and water hunger. Thirst, however, may regulate electrolyte intake also. In hot climates, the loss of electrolyte is usually higher. Sometimes it may be necessary to supplement drinking water with electrolytes.

Dehydration

Dehydration is a condition characterized by ***water depletion in the body***. It may be due to insufficient intake or excessive water loss or both. Dehydration is generally classified into two types.

1. Due to loss of water alone.
2. Due to deprivation of water and electrolytes.

Causes of dehydration : Dehydration may occur as a result of diarrhea, vomiting, excessive sweating, fluid loss in burns, adrenocortical

dysfunction, kidney diseases (e.g. renal insufficiency), deficiency of ADH (diabetes insipidus) etc.

Characteristic features of dehydration : There are three degrees of dehydration—mild, moderate and severe.

The salient features of dehydration are given hereunder

1. The volume of the ***extracellular fluid*** (e.g. plasma) is ***decreased*** with a concomitant rise in electrolyte concentration and osmotic pressure.

2. Water is drawn from the intracellular fluid that results in shrunken cells and disturbed metabolism e.g. increased protein breakdown.

3. ADH secretion is increased. This causes increased water retention in the body and consequently urine volume is very low.

4. ***Plasma protein and blood urea*** concentrations are ***increased.***

5. Water depletion is often accompanied by a loss of electrolytes from the body (Na^+, K^+ etc.).

6. The principal clinical symptoms of severe dehydration include increased pulse rate, low blood pressure, sunken eyeballs, decreased skin turgor, lethargy, confusion and coma.

Treatment : The treatment of choice for dehydration is ***intake of plenty of water.*** In the subjects who cannot take orally, water should be administered intravenously in an isotonic solution (usually 5% glucose). If the dehydration is accompanied by loss of electrolytes, the same should be administered by oral or intravenous routes. This has to be done by carefully monitoring the water and electrolyte status of the body.

Osmotic imbalance and dehydration in cholera

Cholera is transmitted through water and foods, contaminated by the bacterium ***Vibrio cholerae.*** This bacterium produces a toxin which stimulates the intestinal cells to secrete various ions (Cl^-, Na^+, K^+, HCO_3^- etc.) into the intestinal lumen. These ions collectively raise the osmotic pressure and suck the water into lumen. This results in diarrhea with a heavy loss of water (5–10 liters/day). If not treated in time, the victims of cholera will die due to dehydration and loss of dissolved salts. Thus, cholera and other forms of severe diarrhea are the major killers of young children in many developing countries.

Oral rehydration therapy (ORT) is commonly used to treat cholera and other diarrheal diseases.

Overhydration

Overhydration or water intoxication is caused by excessive ***retention of water in the body***. This may occur due to excessive intake of large volumes of salt free fluids, renal failure, overproduction of ADH etc. Overhydration is observed after major trauma or operation, lung infections etc.

Water intoxication is associated with dilution of ECF and ICF with a decrease in osmolality. The clinical symptoms include headache, lethargy and convulsions. The treatment advocated is stoppage of water intake and administration of hypertonic saline.

Water tank model

The distribution of body water (in the ECF and ICF), dehydration and overhydration can be better understood by a water tank model (***Fig.21.3***). The tank has an inlet and outlet, respectively, representing the water intake (mostly oral) and water output (mainly urine) by the body.

Dehydration is caused when the water output exceeds the intake. On the other hand, overhydration is due to more water intake and less output.

Metabolism of electrolytes

The body distribution, dietary intake, intestinal absorption and biochemical functions of individual electrolytes are discussed under the section mineral metabolism (***Chapter 18***). The

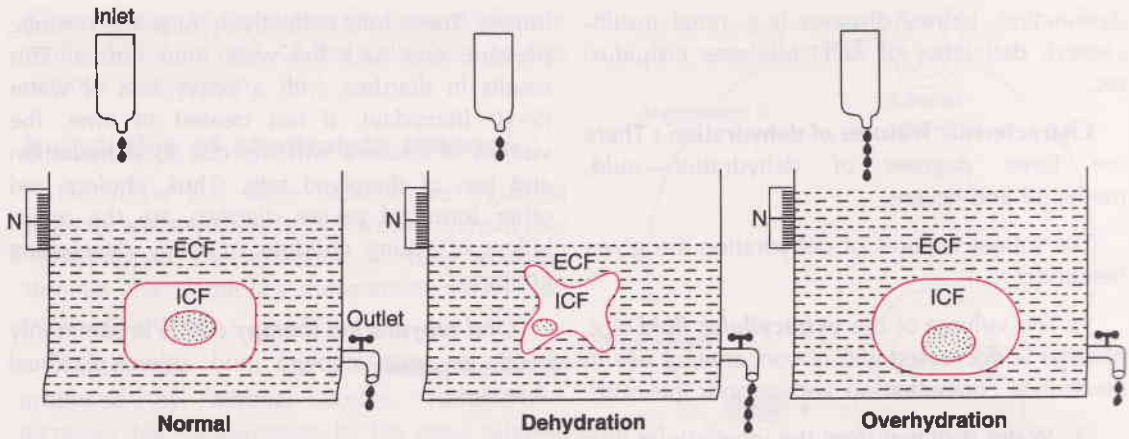


Fig. 21.3 : Water tank model representing body fluid compartments (N–Normal level; ECF–Extracellular fluid; ICF–Intracellular fluid).

electrolyte disorders, particularly hypernatremia and hyponatremia (of sodium); hyperkalemia and hypokalemia (of potassium) must also be referred.

ACID-BASE BALANCE

The ***normal pH*** of the ***blood*** is maintained in the narrow range of ***7.35-7.45***, i.e. slightly alkaline. The pH of intracellular fluid is rather variable. Thus, for erythrocytes the pH is 7.2, while for skeletal muscle, it may be as low as 6.0.

Maintenance of blood pH is an important homeostatic mechanism of the body. In normal circumstances, the regulation is so effective that the blood pH varies very little. Changes in blood pH will alter the intracellular pH which, in turn, influence the metabolism e.g. distortion in protein structure, enzyme activity etc. It is estimated that the blood pH compatible to life is 6.8-7.8. (For a good understanding of acid-base balance, adequate knowledge on acids, bases, pH and buffers is essential. The reader, therefore, must first refer ***Chapter 40*** for this purpose. These basic aspects are not discussed here to avoid repetition).

Production of acids by the body

The metabolism of the body is accompanied by an overall production of acids. These include the volatile acids like carbonic acid (most predominent, about 20,000 mEq/day) or non-volatile acids (about 80 mEq/day) such as lactic acid, sulfuric acid, phosphoric acid etc. Carbonic acid is formed from the metabolic product CO_2; lactic acid is produced in anaerobic metabolism; sulfuric acid is generated from proteins (sulfur containing amino acids); phosphoric acid is derived from organic phosphates (e.g. phospholipids). All these acids add up H^+ ions to the blood. A ***diet rich in animal proteins results in more acid production*** by the body that ultimately leads to the excretion of ***urine*** which is profoundly ***acidic***.

Production of bases by the body

The formation of basic compounds in the body, in the normal circumstances, is negligible. Some amount of bicarbonate is generated from the organic acids such as lactate and citrate. The ammonia produced in the amino acid metabolism is converted to urea, hence its contribution as a base in the body is insignificant. A vegetarian diet has a tendency for a net production of bases. This is due to the fact that vegetarian diet produces salts of organic acids such as sodium lactate which can utilize H^+ ions produced in the body. For this reason, a ***vegetarian diet has an alkalizing effect on the body.*** This is reflected by the excretion of neutral or slightly alkaline urine by these subjects.

MAINTENANCE OF BLOOD pH

The body has developed three lines of defense to regulate the body's acid-base balance and maintain the blood pH (around 7.4).

I. Blood buffers

II. Respiratory mechanism

III. Renal mechanism.

I. Blood buffers

A buffer may be defined as a solution of a weak acid (HA) and its salt (BA) with a strong base. The buffer resists the change in pH by the addition of acid or alkali and the buffering capacity is dependent on the absolute concentration of salt and acid. It should be borne in mind that the buffer cannot remove H^+ ions from the body. It temporarily acts as a shock absorbant to reduce the free H^+ ions. The H^+ ions have to be ultimately eliminated by the renal mechanism (described later).

The blood contains 3 buffer systems.

1. Bicarbonate buffer
2. Phosphate buffer
3. Protein buffer.

1. **Bicarbonate buffer system :** Sodium bicarbonate and carbonic acid ($NaHCO_3 - H_2CO_3$) is the most ***predominant buffer*** system of the extracellular fluid, particularly the plasma. Carbonic acid dissociates into hydrogen and bicarbonate ions.

$$H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

By the law of mass action, at equilibrium

$$K_a = \frac{[H^+][HCO_3^-]}{[H_2CO_3]} \quad \ldots\ldots (1)$$

(K_a = Dissociation constant of H_2CO_3).

The equation may be rewritten as follows

$$[H^+] = K_a \frac{[H_2CO_3]}{[HCO_3^-]}. \quad \ldots\ldots (2)$$

We know that $pH = \log \frac{1}{[H^+]}$.

By taking the reciprocals and logarithms (for logs, multiplication becomes addition).

$$\log \frac{1}{[H^+]} = \log \frac{1}{K_a} + \log \frac{[HCO_3^-]}{[H_2CO_3]} \quad \ldots\ldots (3)$$

$$\log \frac{1}{K_a} = pK_a$$

The equation 3 may now be written as

$$pH = pK_a + \log \frac{[HCO_3^-]}{[H_2CO_3]}. \quad \ldots\ldots (4)$$

The above equation is valid for any buffer pair. The general equation referred to as ***Henderson-Hasselbalch equation*** for any buffer is written as

$$pH = pK_a + \log \frac{[Base]}{[Acid]}. \quad \ldots\ldots (5)$$

It is evident from this equation that the pH is dependent on ratio of the concentration of the base to acid (HCO_3^- and H_2CO_3 in equation 4).

Blood pH and the ratio of HCO_3^- to H_2CO_3 : The plasma bicarbonate (HCO_3^-) concentration is around 24 mmol/l (range 22-26 mmol/l). Carbonic acid is a solution of CO_2 in water. Its concentration is given by the product of p^{CO2} (arterial partial pressure of CO_2 = 40 mm Hg) and the solubility constant of CO_2 (0.03).

Thus $H_2CO_3 = 40 \times 0.03 = 1.2$ mmol/l.

The Henderson-Hasselbalch equation for bicarbonate buffer is

$$pH = pK_a + \log \frac{[HCO_3^-]}{[H_2CO_3]}.$$

Substituting the values (blood pH = 7.4; pK_a for H_2CO_3 = 6.1; HCO_3^- = 24 mmol/l; $H_2CO_3^-$ = 1.2 mmol/l), in the above equation

$$7.4 = 6.1 + \log \frac{24}{1.2}$$

$$= 6.1 + \log 20$$

$$= 6.1 + 1.3$$

$$= 7.4$$

It is evident that at a blood pH 7.4, the ***ratio of bicarbonate to carbonic acid is 20 : 1***. Thus, the bicarbonate concentration is much higher (20 times) than carbonic acid in the blood. This is referred to as ***alkali reserve*** and is responsible for the effective buffering of H^+ ions, generated in the body. In normal circumstances, the concentration of bicarbonate and carbonic acid determines the pH of blood. Further, the bicarbonate buffer system serves as an index to understand the disturbances in the acid-base balance of the body.

2. **Phosphate buffer system :** Sodium dihydrogen phosphate and disodium hydrogen phosphate ($NaH_2PO_4 - Na_2HPO_4$) constitute the phosphate buffer. It is mostly an intracellular buffer and is of less importance in plasma due to its low concentration. With a pK of 6.8 (close to blood pH 7.4), the phosphate buffer would have been more effective, had it been present in high concentration. It is estimated that the ratio of base to acid for phosphate buffer is 4 compared to 20 for bicarbonate buffer.

3. **Protein buffer system :** The plasma proteins and hemoglobin together constitute the protein buffer system of the blood. The buffering capacity of proteins is dependent on the pK of ionizable groups of amino acids. The imidazole group of histidine (pK = 6.7) is the most effective contributor of protein buffers. The plasma proteins account for about 2% of the total buffering capacity of the plasma.

Hemoglobin of RBC is also an important buffer. It mainly buffers the fixed acids, besides being involved in the transport of gases (O_2 and CO_2). More details on hemoglobin are given under respiratory mechanism for regulation of pH.

II. Respiratory mechanism for pH regulation

Respiratory system provides a rapid mechanism for the maintenance of acid-base balance. This is achieved by regulating the concentration of carbonic acid (H_2CO_3) in the blood i.e. the denominator in the bicarbonate buffer system. The details of CO_2 transport and the role of hemoglobin in this process are described elsewhere (***Chapter 10, Refer Fig.10.6***).

The large volumes of CO_2 produced by the cellular metabolic activity endanger the acid-base equilibrium of the body. But in normal circumstances, all of this CO_2 is eliminated from the body in the expired air via the lungs, as summarized below.

$$H_2CO_3 \xleftrightarrow{\text{Carbonic anhydrase}} CO_2 + H_2O$$

The rate of respiration (or the rate of removal of CO_2) is controlled by a respiratory centre, located in the medulla of the brain. This centre is highly sensitive to changes in the pH of blood. Any decrease in blood pH causes hyperventilation to blow off CO_2, thereby reducing the H_2CO_3 concentration. Simultaneously, the H^+ ions are eliminated as H_2O.

Respiratory control of blood pH is rapid but only a short term regulatory process, since hyperventilation cannot proceed for long.

Hemoglobin as a buffer : Hemoglobin of erythrocytes is also important in the respiratory regulation of pH. At the tissue level, hemoglobin binds to H^+ ions and helps to transport CO_2 as HCO_3^- with a minimum change in pH (referred to as ***isohydric transport***). In the lungs, as hemoglobin combines with O_2, H^+ ions are removed which combine with HCO_3^- to form H_2CO_3. The latter dissociates to release CO_2 to be exhaled (***Refer Fig.10.6***).

Generation of HCO_3^- by RBC : Due to lack of aerobic metabolic pathways, RBC produce very little CO_2. The plasma CO_2 diffuses into the RBC along the concentration gradient where it combines with water to form H_2CO_3. This reaction is catalysed by ***carbonic anhydrase*** (also called carbonate dehydratase). In the RBC, H_2CO_3 dissociates to produce H^+ and HCO_3^-. The H^+ ions are trapped and buffered by hemoglobin. As the concentration of HCO_3^- increases in the RBC, it diffuses into plasma along with the concentration gradient, in exchange for Cl^- ions, to maintain electrical neutrality. This phenomenon, referred to as ***chloride shift***, helps to generate HCO_3^- (***Fig.21.4***).

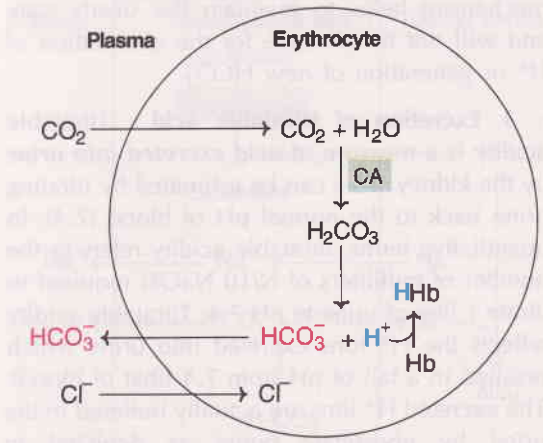


Fig. 21.4 : Generation of bicarbonate by the erythrocyte (CA–Carbonic anhydrase; Hb–Hemoglobin).

III. Renal mechanism for pH regulation

The role of kidneys in the maintenance of acid-base balance of the body (blood pH) is highly significant. The ***renal mechanism*** tries to provide a ***permanent solution to the acid-base disturbances***. This is in contrast to the temporary buffering system and a short term respiratory mechanism, described above.

The kidneys regulate the blood pH by maintaining the alkali reserve, besides excreting or reabsorbing the acidic or basic substances, as the situation demands.

Urine pH normally lower than blood pH : The pH of urine is normally acidic (***~6.0***). This clearly indicates that the kidneys have contributed to the acidification of urine, when it is formed from the blood plasma (pH 7.4). In other words, the H^+ ions generated in the body in the normal circumstances, are eliminated by acidified urine. Hence the pH of urine is normally acidic (~6.0), while that of blood is alkaline (7.4). Urine pH, however, is variable and may range between 4.5-9.5, depending on the concentration of H^+ ions.

Carbonic anhydrase and renal regulation of pH : The enzyme carbonic anhydrase (inhibited by ***acetazolamide***) is of central importance in the renal regulation of pH which occurs by the following mechanisms.

1. Excretion of H^+ ions
2. Reabsorption of bicarbonate
3. Excretion of titratable acid
4. Excretion of ammonium ions.

1. **Excretion of H^+ ions :** Kidney is the only route through which the H^+ can be eliminated from the body. H^+ excretion occurs in the proximal convoluted tubules (renal tubular cells) *and is coupled with the regeneration of* HCO_3^-. The process depicted in ***Fig.21.5***, occurs as follows.

Carbonic anhydrase catalyses the production of carbonic acid (H_2CO_3) from CO_2 and H_2O in the renal tubular cell. H_2CO_3 then dissociates to H^+ and HCO_3^-. The H^+ ions are secreted into the tubular lumen in exchange for Na^+. The Na^+ in association with HCO_3^- is reabsorbed into the blood. This is an effective mechanism to eliminate acids (H^+) from the body with a simultaneous generation of HCO_3^-. The latter adds up to the alkali reserve of the body. The H^+ combines with a non-carbonate base and is excreted in urine.

2. **Reabsorption of bicarbonate :** This mechanism is primarily responsible to conserve the blood HCO_3^-, with a simultaneous excretion of H^+ ions. The normal urine is almost free from HCO_3^-. This is explained as follows (***Fig.21.6***).

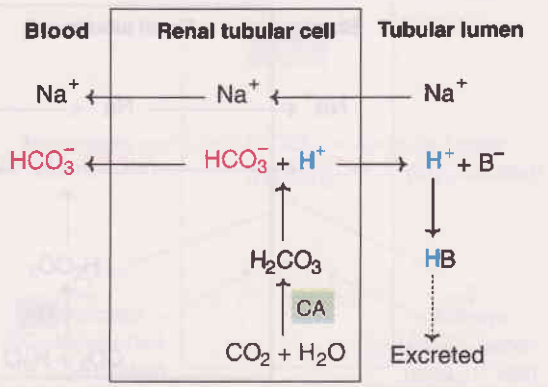


Fig. 21.5 : Renal regulation of blood pH–Excretion of H^+ ions (CA–Carbonic anhydrase).

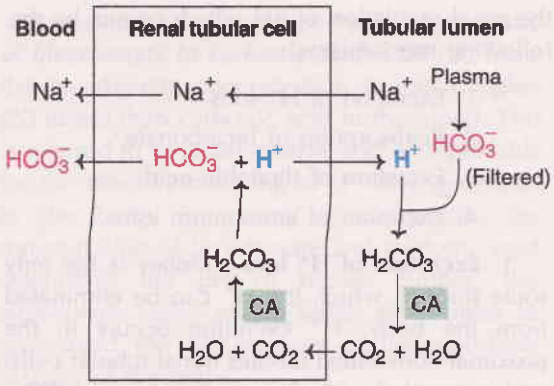


Fig. 21.6 : Renal regulation of blood pH–Reabsorption of bicarbonate (CA–Carbonic anhydrase).

Bicarbonate freely diffuses from the plasma into the tubular lumen. Here HCO_3^- combines with H^+, secreted by tubular cells, to form H_2CO_3. H_2CO_3 is then cleaved by carbonic anhydrase (of tubular cell membrane) to form CO_2 and H_2O. As the CO_2 concentration builds up in the lumen, it diffuses into the tubular cells along the concentration gradient. In the tubular cell, CO_2 again combines with H_2O to form H_2CO_3 which then dissociates into H^+ and HCO_3^-. The H^+ is secreted into the lumen in exchange for Na^+. The HCO_3^- is reabsorbed into plasma in association with Na^+. Reabsorption of HCO_3^- is a cyclic process with the net excretion of H^+ or generation of new HCO_3^-. This is because the H^+ is derived from water. This mechanism helps to maintain the steady state and will not be effective for the elimination of H^+ or generation of new HCO_3^-.

3. **Excretion of titratable acid :** Titratable acidity is a measure of ***acid excreted into urine*** by the kidney. This can be estimated by titrating *urine back to the normal pH of blood (7.4).* In quantitative terms, titratable acidity refers to the number of milliliters of N/10 NaOH required to titrate 1 liter of urine to pH 7.4. Titratable acidity reflects the H^+ ions excreted into urine which resulted in a fall of pH from 7.4 (that of blood). The excreted H^+ ions are actually buffered in the urine by phosphate buffer as depicted in ***Fig.21.7***, and briefly described hereunder.

As already discussed, H^+ ion is secreted into the tubular lumen in exchange for Na^+ ion. This Na^+ is obtained from the base, disodium hydrogen phosphate (Na_2HPO_4). The latter in turn combines with H^+ to produce the acid, sodium dihydrogen phosphate (NaH_2PO_4), in which form the major quantity of titratable acid in urine is present. As the tubular fluid moves down the renal tubules, more and more H^+ ions are added, resulting in the acidification of urine. This causes a fall in the pH of urine to as low as 4.5. Any further fall in the pH will cause depletion of Na^+ ions.

4. **Excretion of ammonium ions :** This is another mechanism to buffer H^+ ions secreted into the tubular fluid. The H^+ ion combines with

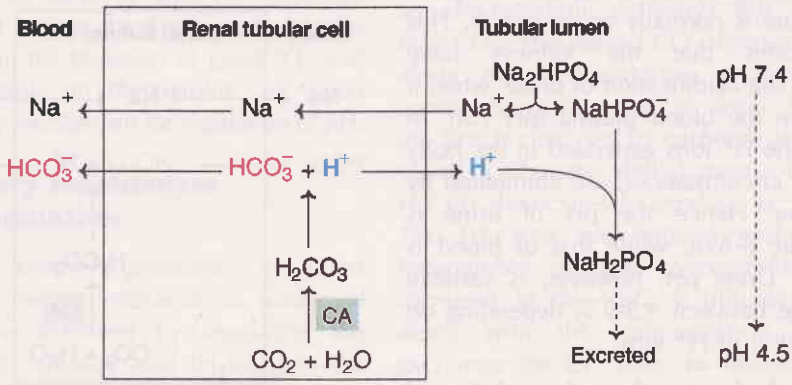


Fig. 21.7 : Renal regulation of blood pH–Excretion of titratable acid by phosphate buffer mechanism (CA–Carbonic anhydrase).

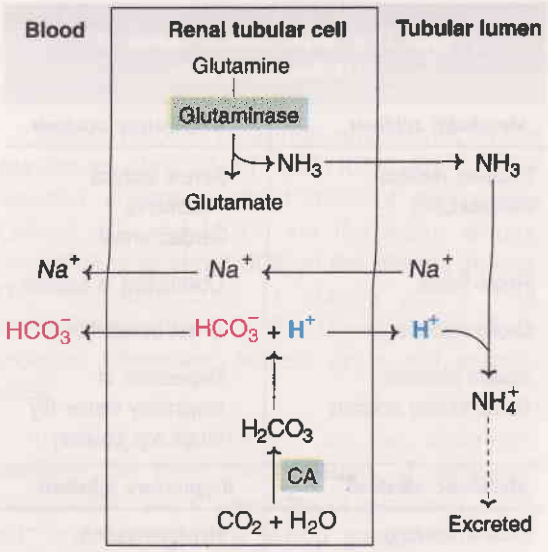


Fig. 21.8 : Renal regulation of blood pH–Excretion of ammonium ions (CA–Carbonic anhydrase).

NH_3 to form ammonium ion (NH_4^+). The renal tubular cells deamidate glutamine to glutamate and NH_3. This reaction is catalysed by the enzyme glutaminase. The NH_3, liberated in this reaction, diffuses into the tubular lumen where it combines with H^+ to form NH_4^+ (***Fig.21.8***). Ammonium ions cannot diffuse back into tubular cells and, therefore, are excreted into urine.

NH_4^+ is a major urine acid. It is estimated that about half to two-thirds of body acid load is eliminated in the form of NH_4^+ ions. For this reason, renal regulation via NH_4^+ excretion is very effective to eliminate large quantities of acids produced in the body. This mechanism becomes predominant particularly in acidosis.

Carbon dioxide—the central molecule of pH regulation

As is observed from the foregoing discussion, CO_2 is of central importance in the acid-base balance of the body. It has the ability to combine with H_2O to from H_2CO_3 which can dissociate to HCO_3^- and H^+. A summary of the interaction between the lungs, erythrocytes and kidneys in handling CO_2 to maintain pH of the blood is depicted in ***Fig.21.9***. The CO_2 generated by aerobic metabolism may be exhaled via lungs, or converted to HCO_3^- by erythrocytes and kidneys to add up to the alkali reserve of the body.

Buffers of intracellular fluids

The regulation of pH within the cells is as important as that discussed above for the extracellular fluid. The H^+ ions generated in the cells are exchanged for Na^+ and K^+ ions. This is particularly observed in skeletal muscle which reduces the potential danger of H^+ accumulation in the cells.

DISORDERS OF ACID-BASE BALANCE

The body has developed an efficient system for the maintenance of acid-base equilibrium with a result that the pH of blood is almost constant (7.4). The ***blood pH compatible to life is 6.8-7.8***, beyond which life cannot exist.

For a better understanding of the disorders of acid-base balance, the Henderson-Hasselbalch equation must be frequently consulted.

$$pH = pK_a + \log \frac{[HCO_3^-]}{[H_2CO_3]}$$

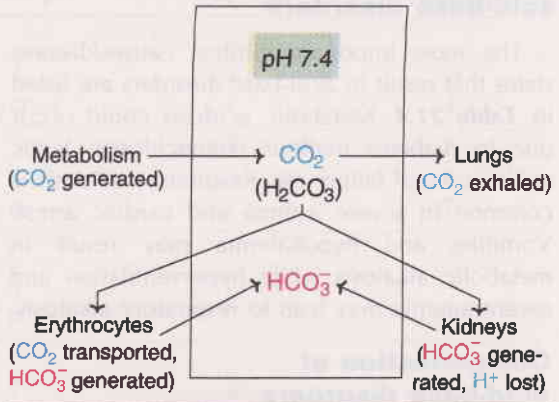


Fig. 21.9 : Carbon dioxide—the central molecule of blood pH regulation.

It is evident from the above equation that the blood pH (H^+ ion concentration) is dependent on the relative concentration (ratio) of bicarbonate (HCO_3^-) and carbonic acid (H_2CO_3).

The acid-base disorders are mainly classified as

1. **Acidosis—a decline in blood pH**
   (a) *Metabolic acidosis*—due to a decrease in bicarbonate.
   (b) *Respiratory acidosis*—due to an increase in carbonic acid.
2. **Alkalosis—a rise in blood pH**
   (a) *Metabolic alkalosis*—due to an increase in bicarbonate.
   (b) *Respiratory alkalosis*—due to a decrease in carbonic acid.

The four acid-base disorders referred above are primarily due to alterations in either bicarbonate or carbonic acid. It may be observed that the metabolic acid-base balance disorders are caused by a direct alteration in bicarbonate concentration while the respiratory disturbances are due to a change in carbonic acid level (i.e. CO_2). This type of classification is more theoretical. In the actual clinical situations, mixed type of disorders are common.

The terms ***acidemia*** and ***alkalemia,*** respectively, refer to an increase or a decrease in $[H^+]$ ion concentration in blood. They are, however, not commonly used.

Clinical causes of acid-base disorders

The most important clinical causes/disease states that result in acid-base disorders are listed in ***Table 21.4***. Metabolic acidosis could occur due to diabetes mellitus (ketoacidosis), lactic acidosis, renal failure etc. Respiratory acidosis is common in severe asthma and cardiac arrest. Vomiting and hypokalemia may result in metabolic alkalosis while hyperventilation and severe anemia may lead to respiratory alkalosis.

Compensation of acid-base disorders

To counter the acid-base disturbances, the body gears up its homeostatic mechanism and makes every attempt to restore the pH to normal level (7.4). This is referred to as compensation which may be partial or full. Sometimes the acid-base disorders may remain uncompensated.

Table 21.4 Major clinical causes of acid-base disorders

Metabolic acidosis	*Respiratory acidosis*
Diabetes mellitus (ketoacidosis)	Severe asthma Pneumonia Cardiac arrest
Renal failure	Obstruction in airways
Lactic acidosis	Chest deformities
Severe diarrhea Renal tubular acidosis	Depression of respiratory center (by drugs e.g. opiates)
Metabolic alkalosis	***Respiratory alkalosis***
Severe vomiting	Hyperventilation
Hypokalemia	Anemia High altitude
Intravenous administration of bicarbonate	Salicylate poisoning

The principal acid-base disturbances, along with the blood concentration of HCO_3^- and H_2CO_3, in acute and compensated states are given in the ***Table. 21.5***.

For the acute metabolic disorders (due to changes in HCO_3^-), respiratory compensation sets in and regulates the H_2CO_3 (i.e. CO_2) by hyper- or hypoventilation. As regards acute respiratory disorders (due to changes in H_2CO_3), the renal compensation occurs to maintain the HCO_3^- level, by increasing or decreasing its excretion.

In the ***Table 21.6***, a summary of the acid-base disorders with primary changes and compensatory mechanisms is given.

Anion gap

For a better understanding of acid-base disorders, adequate knowledge of anion gap is essential. The total concentration of cations and

anions (expressed as mEq/l) is equal in the body fluids. This is required to maintain electrical neutrality.

The commonly measured electrolytes in the plasma are Na^+, K^+, Cl^- and HCO_3^-. Na^+ and K^+ together constitute about 95% of the plasma cations. Cl^- and HCO_3^- are the major anions, contributing to about 80% of the plasma anions. The remaining 20% of plasma anions (not normally measured in the laboratory) include proteins, phosphate, sulfate, urate and organic acids.

Anion gap is defined as the difference between the total concentration of measured cations (Na^+ and K^+) and that of measured anion (Cl^- and HCO_3^-). The ***anion gap (A^-)*** in fact represents the ***unmeasured anions in the plasma*** which may be calculated as follows, by substituting the normal concentration of electrolytes (mEq/l).

$$Na^+ + K^+ = Cl^- + HCO_3^- + A^-$$

$$136 + 4 = 100 + 25 + A^-$$

$$A^- = 15 \text{ mEq/l}$$

The anion gap in a healthy individual is around 15 mEq/l (range 8-18 mEq/l). Acid-base disorders are often associated with alterations in the anion gap.

Metabolic acidosis

The primary defect in metabolic acidosis is a ***reduction in bicarbonate concentration*** which leads to a fall in blood pH. The bicarbonate concentration may be decreased due to its utilization in buffering H^+ ions, loss in urine or gastrointestinal tract or failure to be regenerated.

The most important cause of metabolic acidosis is due to an excessive production of organic acids which combine with $NaHCO_3^-$ and deplete the alkali reserve.

$$NaHCO_3^- + \text{Organic acids} \longrightarrow \text{Na salts of organic acids} + CO_2$$

Metabolic acidosis is commonly seen in ***severe uncontrolled diabetes mellitus*** which is associated with excessive production of acetoacetic acid and β-hydroxybutyric acid (both are organic acids).

Anion gap and metabolic acidosis : Increased production and accumulation of organic acids causes an ***elevation*** in the anion gap. This type of picture is seen in metabolic acidosis associated with diabetes (***ketoacidosis***).

Table 21.5 Acid-base disorders along with the concentrations of bicarbonate (HCO_3^-) and carbonic acid (H_2CO_3) in plasma

Disorder	*Blood pH*	*$[HCO_3^-]$*	*$[H_2CO_3]$*
Metabolic acidosis			
Acute	↓	↓	→
Compensated (by ↑ ventilation)	↘ or →	↓	↓
Respiratory acidosis			
Acute	↓	→	↑
Compensated (HCO_3^- retained by kidney)	↘ or →	↑	↑
Metabolic alkalosis			
Acute	↑	↑	→
Compensated (by ↓ ventilation)	↗ or →	↑	↑
Respiratory alkalosis			
Acute	↑	→	↓
Compensated (↑HCO_3^- excretion by kidney)	↗ or →	↓	↓

↑ : Increased; ↓ : Decreased; → : Normal; ↘: Marginally decreased; ↗ : Marginally increased.

Table 21.6 Acid-base disorders with primary changes and compensatory mechanisms

Disorder	*Primary change*	*Compensatory mechanism*	*Timescale for compensation*
Metabolic acidosis	Decreased plasma bicarbonate	Hyperventilation (decrease in pCO_2)	Minutes to hours
Metabolic alkalosis	Increased plasma bicarbonate	Hypoventilation (increase in pCO_2)	Minutes to hours
Respiratory acidosis	Increased pCO_2	Elevation in plasma bicarbonate; increase in renal reabsorption of bicarbonate	Days
Respiratory alkalosis	Decreased pCO_2	Reduction in plasma bicarbonate; decrease in renal reabsorption of bicarbonate	Days

Compensation of metabolic acidosis : The acute metabolic acidosis is usually compensated ***by hyperventilation of lungs***. This leads to an increased elimination of CO_2 from the body (hence $H_2CO_3\downarrow$). but respiratory compensation is only short-lived. Renal compensation sets in within 3-4 days and the H^+ ions are excreted as NH_4^+ ions.

Respiratory acidosis

The primary defect in respiratory acidosis is due to a ***retention of CO_2*** ($H_2CO_3\uparrow$). There may be several causes for respiratory acidosis which include depression of the respiratory centre (overdose of drugs), pulmonary disorders (bronchopneumonia) and breathing air with high content of CO_2.

The renal mechanism comes for the rescue to compensate respiratory acidosis. More HCO_3^- is generated and retained by the kidneys which adds up to the alkali reserve of the body. The excretion of ***titratable acidity*** and NH_4^+ is ***elevated in urine***.

Metabolic alkalosis

The primary abnormality in metabolic alkalosis is an ***increase in HCO_3^- concentration***. This may occur due to excessive vomiting (resulting in loss of H^+) or an excessive intake of sodium bicarbonate for therapeutic purposes (e.g. control of gastric acidity). Cushing's syndrome (hypersecretion of aldosterone) causes increased retention of Na^+ and loss of K^+ from the body. Metabolic alkalosis is commonly associated with low K^+ concentration (hypokalemia). In severe K^+ deficiency, H^+ ions are retained inside the cells to replace missing K^+ ions. In the renal tubular cells, H^+ ions are exchanged (instead of K^+) with the reabsorbed Na^+. Paradoxically, the patient excretes acid urine despite alkalosis.

The respiratory mechanism initiates the ***compensation by hypoventilation*** to retain CO_2 (hence $H_2CO_3\uparrow$). This is slowly taken over by renal mechanism which excretes more HCO_3^- and retains H^+.

Respiratory alkalosis

The primary abnormality in respiratory alkalosis is a ***decrease in H_2CO_3 concentration***. This may occur due to prolonged hyperventilation resulting in increased exhalation of CO_2 by the lungs. Hyperventilation is observed in conditions such as hysteria, hypoxia, raised intracranial pressure, excessive artificial ventilation and the action of certain drugs (salicylate) that stimulate respiratory centre.

The renal mechanism tries to compensate by increasing the urinary excretion of HCO_3^-.

Mixed acid-base disorders

Sometimes, the patient may have two or more acid-base disturbances occurring simultaneously. In such instances, both HCO_3^- and H_2CO_3 are altered. In general, if the biochemical data (of blood gas analysis) cannot be explained by a specific acid-base disorder, it is assumed that a mixed disturbance is occurring. Many a times, ***compensatory mechanisms may lead to mixed acid-base disorders***.

Acid-base disorders and plasma potassium

Plasma potassium concentration (normal 3.5-5.0 mEq/l) is very important as it affects the contractility of the heart. Hyperkalemia (high plasma K^+) or hypokalemia (low plasma K^+) can be life-threatening. The relevance of potassium balance in certain acid-base disorders is discussed briefly.

Potassium and diabetic ketoacidosis : The hormone insulin increases K^+ uptake by cells (particularly from skeletal muscle). The patient of severe uncontrolled diabetes (i.e. with metabolic acidosis) is usually with hypokalemia. When such a patient is given insulin, it stimulates K^+ entry into cells. The result is that plasma K^+ level is further depleted. Hypokalemia affects heart functioning, and is life threatening.

Potassium and alkalosis : Low plasma concentration of K^+ (hypokalemia) leads to an increased excretion of hydrogen ions, and thus may cause metabolic alkalosis. Conversely, metabolic alkalosis is associated with increased renal excretion of K^+.

BIOMEDICAL / CLINICAL CONCEPTS

- *Existence of life is unimaginable in the absence of water.*
- *Kidneys play a predominant role in the regulation of water, electrolyte and acid-base balance.*
- *Electrolyte and water balance regulation occurs through the involvement of hormones—aldosterone, ADH and renin-angiotensin.*
- *Severe dehydration is characterized by low blood pressure, sunken eyeballs, lethargy, confusion and coma.*
- *Sodium is the principal extracellular cation while K^+ is intracellular. The maintenance of the differential concentration of these electrolytes is essential for the survival of life which is brought about by Na^+-K^+ pump.*
- *The body metabolism is accompanied by the production of acids such as carbonic acid, sulfuric acid, phosphoric acid etc.*
- *Vegetarian diet has an alkalizing effect on the body. This is attributed to the formation of organic acids such as sodium lactate which can deplete H^+ ions by combining with them.*
- *The blood pH is maintained by blood buffers, respiratory and renal mechanisms.*
- *Carbon dioxide is the central molecule of acid-base regulation.*
- *Disturbances in acid-base regulation result in acidosis (decreased blood pH) or alkalosis (raised blood pH).*
- *Uncontrolled diabetes mellitus is associated with metabolic acidosis, commonly referred to as ketoacidosis (due to the overproduction of ketone bodies).*

In view of the importance discussed above, the measurement of plasma K^+ concentration assumes significance in the acid-base disorders. In cases of these disorders associated with hypokalemia, potassium supplementation (with carefull monitoring of plasma K^+) needs to be considered.

BLOOD GAS MEASUREMENT

The measurement of blood gas is an important investigation in the laboratory service. In certain conditions associated with respiratory failure and/or acid-base disorders, blood gas (CO_2 and O_2) measurement assumes significance. Based on the results obtained and the severity of the condition, oxygen treatment or artificial ventilation is carried out.

For blood gas analysis, a sample of arterial blood collected from (most commonly) radial artery in the forearm, or (less commonly) from the femoral artery in the leg is used. The biochemical profile measured include pO_2, pCO_2, and pH (H^+ ion concentration). The concentration of bicarbonate is calculated by using Henderson-Hasselbalch equation. In fact, the blood gas analysers employed in the hospitals are designed to perform the various calculations automatically and give the final results. The reference ranges of blood gas analysis are given in ***Table 21.7.***

Table 21.7 Reference ranges of arterial blood gas analysis

Parameter	*Concentration/value*
$[H^+]$	35–43 mmol/l
pH	7.35–7.45
pCO_2	4.5–6.0 kPa
pO_2	10.5–13.5 kPa
Bicarbonate*	24–30 mmol/l

**Bicarbonate concentration is calculated from pH and pCO_2 values.*

SUMMARY

1. *Water is the solvent of life and constitutes about 60% of the total body weight, distributed in intracellular and extracellular fluids. The daily water intake (by drinking, from foodstuffs and metabolic water) and output (loss via urine, skin, lungs and feces) maintain the body balance of water.*
2. *Electrolytes are distributed in the intracellular and extracellular fluids to maintain the osmotic equilibrium and water balance, Na^+ is the principal extracellular cation while K^+ is the intracellular cation. As regards anions, Cl^- and HCO_3^- predominantly occur in the extracellular fluids while HPO_4^{2-}, proteins and organic acids are present in the intracellular fluids.*
3. *The osmolality of plasma is about 285 milliosmoles/kg, which is predominantly contributed by Na^+ and its associated anions. Thus, for practical purposes, plasma osmolality can be calculated from Na^+ concentration ($2 \times Na^+$ in mmol/l).*
4. *Water and electrolyte balance are usually regulated together and this is under the control of hormones—aldosterone, antidiuretic hormone and renin.*
5. *Dehydration of the body may be due to insufficient water intake or its excessive loss or both. Depletion of water in the ICF causes disturbance in metabolism. The manifestations of severe dehydration include increased pulse rate, low blood pressure, sunken eyeballs, decreased skin turgor, lethargy and coma.*
6. *The normal pH of blood is maintained in the narrow range of 7.35–7.45. The metabolism of the body is accompanied by an overall production of acids. The body has developed three lines of defense (blood buffers, respiratory and renal mechanisms) to regulate the acid-base balance and maintain the blood pH.*
7. *Among the blood buffers, bicarbonate buffer (with a ratio of HCO_3^- to H_2CO_3 as 20 : 1) is the most important in regulating blood pH. Phosphate and protein buffer systems also contribute in this regard. The respiratory system regulates the concentration of carbonic acid by controlling the elimination of CO_2 via lungs.*
8. *The renal (kidney) mechanism regulates blood pH by excreting H^+ and NH_4^+ ions besides the reabsorption of HCO_3^-. The pH of urine is normally acidic which indicates that the kidneys have contributed to the acidification of urine.*
9. *The acid-base disorders are classified as acidosis (metabolic or respiratory) and alkalosis (metabolic or respiratory), respectively, due to a rise or fall in blood pH. The metabolic disturbances are associated with alterations in HCO_3^- concentration while the respiratory disorders are due to changes in H_2CO_3 (i.e. CO_2).*
10. *Blood gas measurement includes the parameters pO_2, pCO_2, pH and bicarbonate, and it is very important to evaluate and treat acid-base disorders.*

SELF-ASSESSMENT EXERCISES

I. Essay questions

1. Describe the role of kidney in the regulation of blood pH.
2. Give an account of the water distribution and its balance in the body.
3. Campare the composition of electrolytes in the extracellular and intracellular fluids. Discuss the regulation of electrolyte balance.
4. Describe the role of blood buffers in the acid-base balance.
5. Classify acid-base disorders and discuss them with compensatory mechanisms.

II. Short notes

(a) Dehydration, (b) Vasopressin and water balance, (c) Osmolality of plasma, (d) Acids produced in the body, (e) Henderson-Hasselbalch equation, (f) Bicarbonate buffer, (g) Excretion of H^+ by kidney, (h) Titratable acidity, (i) Metabolic acidosis, (j) Anion gap.

III. Fill in the blanks

1. The hormone controlling water excretion via kidneys is _____.
2. The principal cation of extracellular fluid is _____.
3. The normal osmolality of plasma is _____.
4. Na^+ reabsorption by renal tubules is increased by the hormone _____.
5. The most predominant volatile acid generated in the body is _____.
6. The most important buffer system regulating blood pH is _____.
7. At a normal blood pH 7.4, the ratio of bicarbonate to carbonic acid is _____.
8. The body acid load is predominantly eliminated in the form of _____.
9. The primary defect in metabolic acidosis is a reduction in the plasma concentration of _____.
10. The respiratory alkalosis is primarily associated with a decrease in the plasma concentration of _____.

IV. Multiple choice questions

11. The metabolic (endogenous) water is derived by the oxidation of

    (a) Carbohydrate (b) Protein (c) Fats d) All of them.

12. The most predominant anion in the extracellular fluids

    (a) Cl^- (b) HCO_3^- (c) HPO_4^{2-} (d) Protein.

13. The only route through which H^+ ions are eliminated from the body

    (a) Lungs (b) Stomach (c) Kidneys (d) None of them.

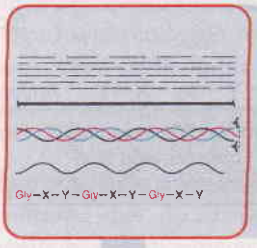
14. Name the amino acid from which ammonia is derived in the renal tubular cells which is finally excreted as NH_4^+

    (a) Asparagine (b) Glutamine (c) Glutamate (d) Aspartate.

15. The anion gap refers to the unmeasured plasma anion concentration (in the laboratory) and is represented by

    (a) Proteins and organic acids (b) Phosphate and sulfate (c) Urate (d) All of them.

22 Tissue Proteins and Body Fluids



The protein, collagen, speaks :

"I am the most abundant protein in mammals;
Triple helical in structure, with distinct types;
Predominantly composed of glycine and proline;
I give strength, support and shape to tissues."

The body possesses a vast number of proteins designed with specific structures to perform specialized functions. A selected few of the most important proteins that are intimately connected with the tissue structure and functions are briefly described in this chapter. In addition, the body fluids are also discussed.

CONNECTIVE TISSUE PROTEINS

The connective tissue or ***extracellular matrix (ECM)*** refers to the complex material surrounding the mammalian cells in tissues. The major protein components of ECM include collagen, elastin, fibrillin, fibronectin, laminin and proteoglycans. Besides these proteins, the structural proteins namely keratins are also described.

COLLAGEN

Collagen is the most abundant protein in mammals, comprising approximately one-third of the total body protein. Collagen is the predominant component of the connective tissue, although its distribution varies in different tissues. For instance, collagen forms 90% of the organic matrix of bones, 85% of tendons, 70% of skin, and 4% of liver.

Functions of collagen

1. Being a major component of the connective tissue, collagen ***gives strength, support*** and ***shape to the tissues.*** The tensile strength of collagen fiber is impressive. To break a collagen fiber of 1 mm in diameter, a load of 10–40 kg is needed! However, in diseased states with altered collagen structure, the tensile strength is reduced.

2. Collagen contributes to proper alignment of cells, which in turn helps in cell proliferation, and their differentiation to different tissues and organs.

3. Collagen (that is exposed in blood vessels) contributes to thrombus formation.

Types of collagen

Collagen is not a single homogeneous protein, but a group of structurally related and genetically distinct proteins. In humans, at least 19 different types of collagens, composed of 30 distinct polypeptide chains (encoded by separate genes), have been identified. The types of collagen are numbered (by Roman numerals) as I, II...XIX. The different types of collagen are suited to perform specialized functions in tissues. For instance, collagens type I and type II are respectively found in skin and bone.

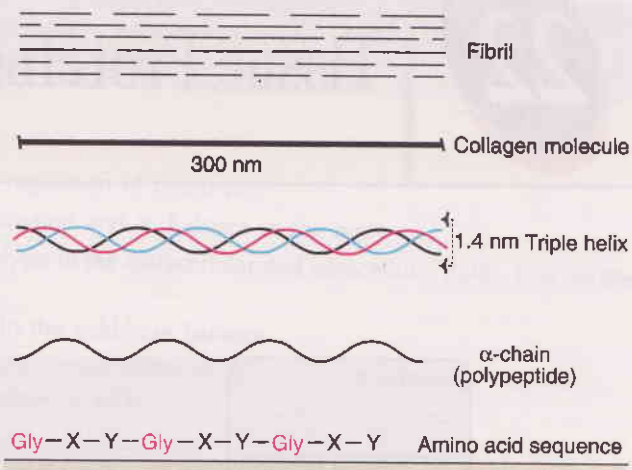


Fig. 22.1 : A diagrammatic representation of the structure of collagen and fibril
(X and Y represent amino acids other than glycine)

Structure of collagen

In principle, all types of collagen are ***triple helical structures***. The triple helix may occur throughout the molecule, or only a part of the molecule.

Type I mature collagen, containing about 1000 amino acids (for each polypeptide chain) possesses triple helical structure throughout the molecule. It is composed of three similar polypeptide chains twisted around each other to form a rod like molecule of 1.4 nm diameter, and about 300 nm length (***Fig.22.1***). The amino acid composition of collagen is unique. Approximately $^1/_3$rd of the amino acids are contributed by glycine i.e. every third amino acid in collagen is glycine. Hence, the repetitive amino acid sequence of collagen is represented by $(Gly\text{-}X\text{-}Y)_n$, where X and Y represent other amino acids. Thus, collagen may be regarded as a polymer of glycine-led tripeptide. Among the other amino acids, ***proline*** and ***hydroxyproline*** are present in large quantities (about 100 residues each). These two amino acids ***confer rigidity*** to the collagen molecule.

The triple helical structure of collagen is stabilized by an extensive network of hydrogen bonds, covalent cross-links, electrostatic and hydrophobic interactions, and van der Waals forces.

The triple helical molecules of collagen assemble and form elongated fibrils, and then rod like fibers in the tissues. The fibril formation occurs by a quarter staggered alignment i.e. each triple helix of collagen is displaced longitudinally from its neighbour by about one-quarter of its length (***Fig.22.1***).

The strength of the collagen fibers is contributed by the covalent cross links formed between lysine and hydroxylysine residues. The degree of collagen cross-linking increases with age. Thus, in older people, the collagen containing tissues (e.g. skin, blood vessels) become less elastic and more stiff, contributing to health complications.

Biosynthesis of collagen

Collagen synthesis occurs in fibroblasts, and the cells related to them e.g. osteoblasts in bones, chondroblasts in cartilage, odontoblasts in teeth.

Collagen is synthesized on the ribosomes in a precursor form namely ***preprocollagen***. This contains a signal peptide which directs the protein to reach the endoplasmic reticulum (ER). In the ER, the signal peptide is cleaved to form ***procollagen***. The latter undergoes extensive post-translational modifications (hydroxylation and glycosylation) and disulfide bonds formation. The procollagen so formed is secreted into the extracellular medium, and subjected to the action of aminoproteinase and carboxy-proteinase to remove the terminal amino acids.

This is followed by a spontaneous assembly of the polypeptide chains (with about 1000 amino acids in each) to form triple helical structures of collagen.

Abnormalities associated with collagen

The biosynthesis of collagen is a complex process, involving at least 30 genes (in humans), and about 8 post-translational modifications. Expectedly, many inherited diseases due to gene mutations, linked with collagen formation have been identified. A few of them are listed below.

- **Ehlers-Danlos syndrome**–a group of inherited disorders characterized by hyperextensibility of skin, and abnormal tissue fragility.
- **Alport syndrome**–due to a defect in the formation of type IV collagen fibres found in the basement membrane of renal glomeruli. The patients exhibit hematuria and renal diseases.
- **Osteogenesis imperfecta**–characterized by abnormal fragility of bones due to decreased formation of collagen.
- **Epidermolysis bullosa**–due to alteration in the structure of type VII collagen. The victims exhibit skin breaks and blisters formation even for a minor trauma.

Scurvy : This is a disease due to the ***deficiency of vitamin C*** (ascorbic acid). Although not a genetic disease, scurvy is related to the improper formation of collagen, hence referred here (vitamin C is needed for the post-translational modifications of collagen). Scurvy is characterized by bleeding of gums, poor wound healing and subcutaneous hemorrhages.

Lathyrism : It is a ***disease of bone deformities*** caused by the consumption of Kesari dal (*Lathyrus sativa*) in some parts of India. The toxic compound namely β-oxalyl aminoalanine (BOAA), found in kesari dal, interferes with the cross-linking of lysine amino acids in collagen. BOAA is found to inhibit enzyme lysyl oxidase.

ELASTIN

Elastin is another important (besides collagens) connective tissue protein. It is mainly responsible for the extensibility and elasticity of tissues. Elastin is found in large quantities in lungs, arterial blood vessels, elastic ligments etc.

Elastin is synthesized as tropoelastin which undergoes post-translational modifications (formation of hydroxyproline, and no hydroxylysine). Compared to collagen, elastin structure is simple—no triple helix, no repeat sequence of $(Gly\text{-}X\text{-}Y)_n$.

Abnormalities associated with elastin

- **Williams syndrome** is a genetic disease due to impairment in elastin synthesis. The connective tissue and central nervous system are affected.
- Decreased synthesis of elastin is found in aging of skin and pulmonary emphysema.

FIBRILLIN

Fibrillin is a structural component of myofibrils found in various tissues.

Marfan syndrome is a genetic disorder due to a mutation in the gene for fibrillin. It is characterized by hyperextensibility of joints and skeletal system. Consequently, the patients of Marfan syndrome are tall, and have long digits. These patients may also have cardiovascular complications. Some researchers believe that Abraham Lincoln was a victim of Marfan syndrome.

FIBRONECTIN

Fibronectin, a glycoprotein, is closely involved in the interaction of cells with extracellular matrix. It actively participates in cell adhesion and cell migration. In general, tumor cells are deficient in fibronectin which results in the lack of adhesion among the tumor cells that may often lead to metastasis.

LAMININ

The basal lamina of glomerular membrane (of renal cells) contains laminin. In fact, laminin is one of the first extracellular proteins synthesized during embryogenesis. It is actively involved in

neuronal growth and nerve regeneration. In the patients of Alzheimer's disease, high concentrations of laminin are found.

KERATINS

Keratins are structural ***proteins*** found ***in hair***, skin, nails, horns etc. The 3 polypeptides of keratin form α-helical structure and are held together by disulfide bonds. The toughness and strength of keratin are directly related to the number of disulfide bonds. Thus, the harder keratin possesses more disulfide bonds. The mechanical strength of the hair is attributed to disulfide bonds.

Hair waving (curling)

When the hair is exposed to moist heat, the α-helices of α-keratin can be stretched. This results in the formation of β-conformation from α-helices. On cooling, the hair structure is reverted back to α-conformation. This property of ***α- and β-conformations of keratin*** is exploited in hair waving or curling.

The hair to be curled is first bent to appropriate shape. By applying a reducing agent, the disulfide bonds (of cystine) are converted to sulfhydyl groups (cysteine). This results in the uncoiling of α-helical structure. After some time, th reducing agent is removed, and an oxidizing agent is added. This allows the formation of some new disulfide bonds between cysteine residues (***Fig.22.2***). The hair is now washed and cooled. The desired curls are formed on the hair due to new disulfide bonds and altered α-helical structure of keratin. It may however, be noted that a permanent curling of hair is not possible. The new hair that grows will be the native original hair only (without curls).

PROTEOGLYCANS

Proteoglycans are ***conjugated proteins containing glycosaminoglycans*** (GAGs). Several proteoglycans with variations in core proteins and GAGs are known e.g. syndecan, betaglycan, aggrecan, fibromodulin. For more information on the structure and functions of proteoglycans ***Refer Chapter 2***. GAGs, the components of proteoglycans, are affected in a group of genetic disorders namely mucopolysaccharidoses (***Chapter 13***).

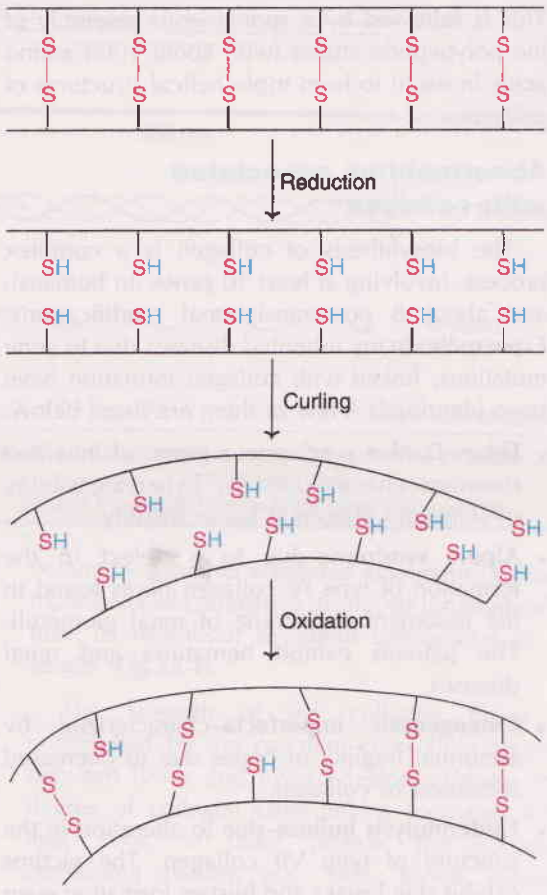


Fig. 22.2 : A diagrammatic representation of hair waving with suitable alterations in keratin structure (— S S corresponds to disulfide bonds of cystine; — SH indicates sulfhydryl groups of cysteine)

CONTRACTILE PROTEINS

The proteins that are involved in the ***movement of body organs*** (e.g. muscle, heart, lung) are regarded as contractile proteins. It is worthwhile to understand the basic structure of muscle before learning the contractile proteins.

STRUCTURE OF MUSCLE

Muscle is the ***single largest tissue*** of the human body. Muscle constitutes about 20% of body mass at birth, 40% in young adults and

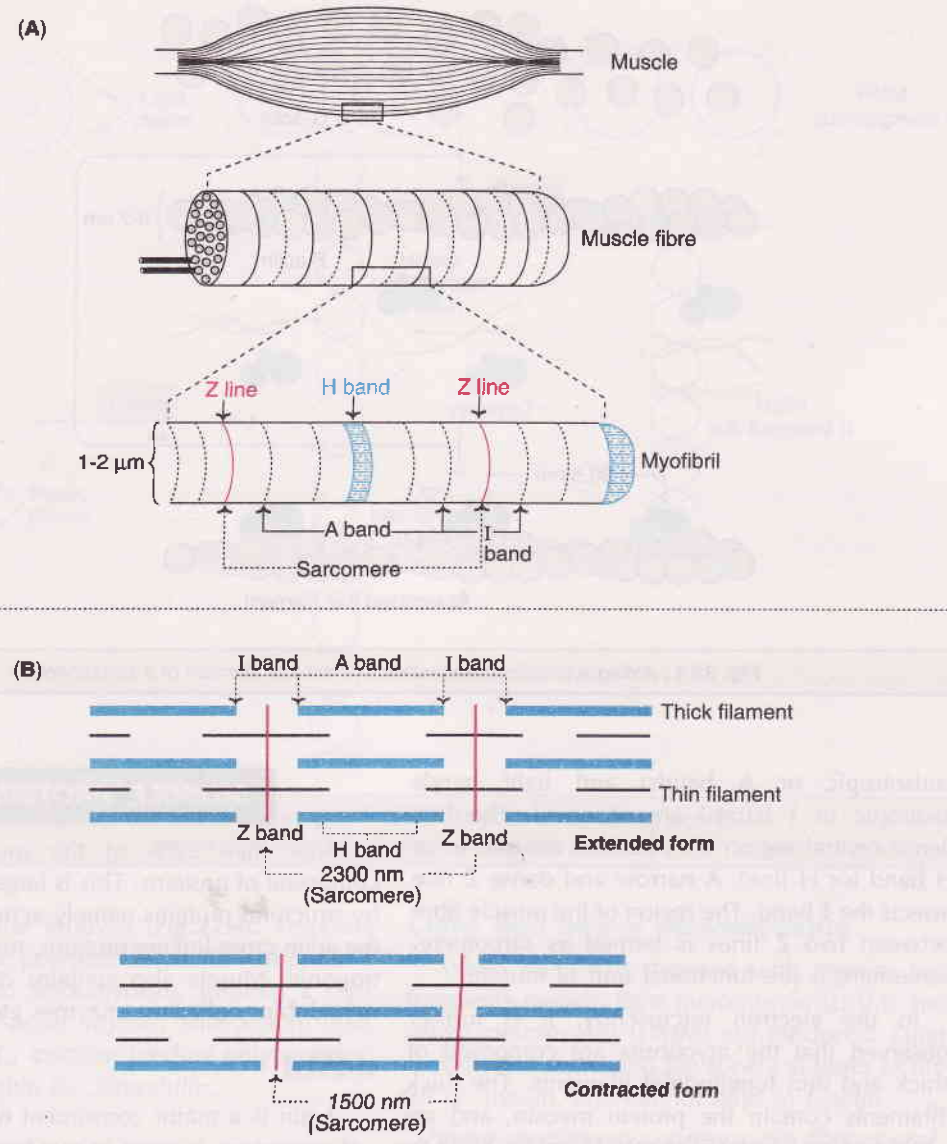


*Fig. 22.3 : (A) Structure of myofibril of a straited muscle (B) Arrangement of filaments of myofibril in extended and contracted form. (**Note :** The length of sarcomere is reduced from 2300 nm to 1500 nm during contraction)*

30% in aged adults. Three types of muscles are found in vertebrates-skeletal, cardiac and *smooth. The skeletal and cardiac muscles are* ***striated*** while the smooth muscles are ***non-striated***.

The structure of striated muscle is represented in ***Fig.22.3***. It is composed of bundles of multinucleated muscle fibre cells. Each cell is surrounded by an electrically excitable plasma membrane, the sarcolemma. The muscle fibre cells are long which may extend the entire length *of the muscle. The intracellular fluid of fibre cells* is the sarcoplasm (i.e. cytoplasm) into which the ***myofibrils*** are embedded. The sarcoplasm is rich in glycogen, ATP, creatine phosphate, and the enzymes of glycolysis.

When the myofibril is examined under electron microscope, alternating dark bands

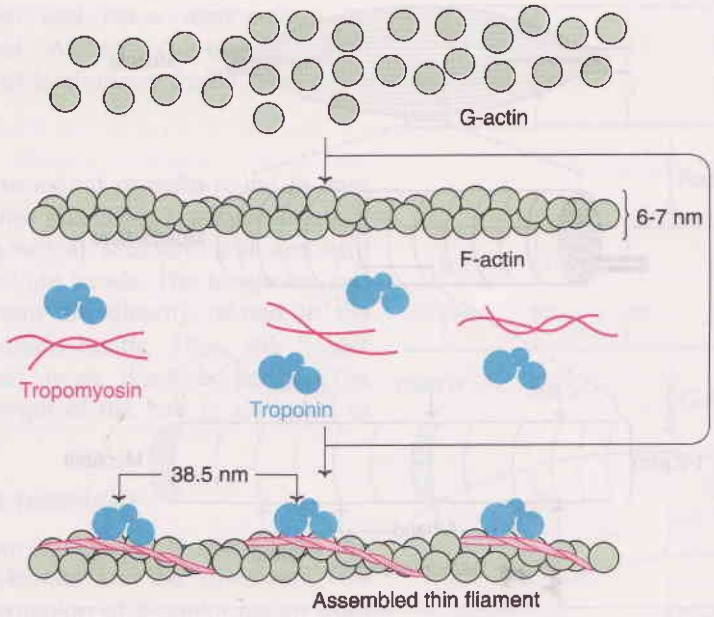


Fig. 22.4 : A diagrammatic representation of the thin filament of a sarcomere.

(anisotropic or A bands) and light bands (isotropic or I bands) are observed. The less dense central region of A band is referred to as H band (or H line). A narrow and dense Z line bisects the I band. The region of the muscle fibre between two Z lines is termed as sarcomere. Sarcomere is the functional unit of muscle.

In the electron microscopy, it is further observed that the myofibrils are composed of thick and thin longitudinal filaments. The thick filaments contain the protein myosin, and are confined to A band. The thin filaments lie in the I band, and can extend into A band (but not into H line). These thin filaments contain the proteins actin, tropomyosin and troponin.

During the course of ***muscle contraction***, the thick and thin filaments slide over each other (***sliding filament model*** of muscle contraction). Consequently, the H bands and I bands shorten. However, there is no change in the length of thick and thin filaments. The length of sarcomere which is around 2300 nm in an extended form of myofibril is reduced to 1500 nm in a contracted form (***Fig.22.3B***).

MUSCLE PROTEINS

More than 20% of the muscle mass is composed of proteins. This is largely contributed by structural proteins namely actin, myosin, and the actin cross-linking proteins, tropomyosin and troponin. Muscle also contains other proteins – myoglobin, collagen, enzymes etc.

ACTIN

Actin is a major constituent of thin filaments of sarcomere. It exists in two forms – monomeric ***G-actin*** (i.e. ***g***lobular actin) and polymeric ***F-actin*** (i.e. ***f***ilament actin). G-actin constitutes about 25% of the muscle proteins by weight. In the presence of Mg^{2+} ions, G-actin polymerizes (non-covalently) to form an insoluble double helical F-actin with a thickness of 6-7 nm (***Fig.22.4***).

Tropomyosin and troponin : These two are cross-linking proteins found in association with actin. Although, minor in terms of mass, they are important in terms of their function. Tropomyosin, composed of two chains, attaches

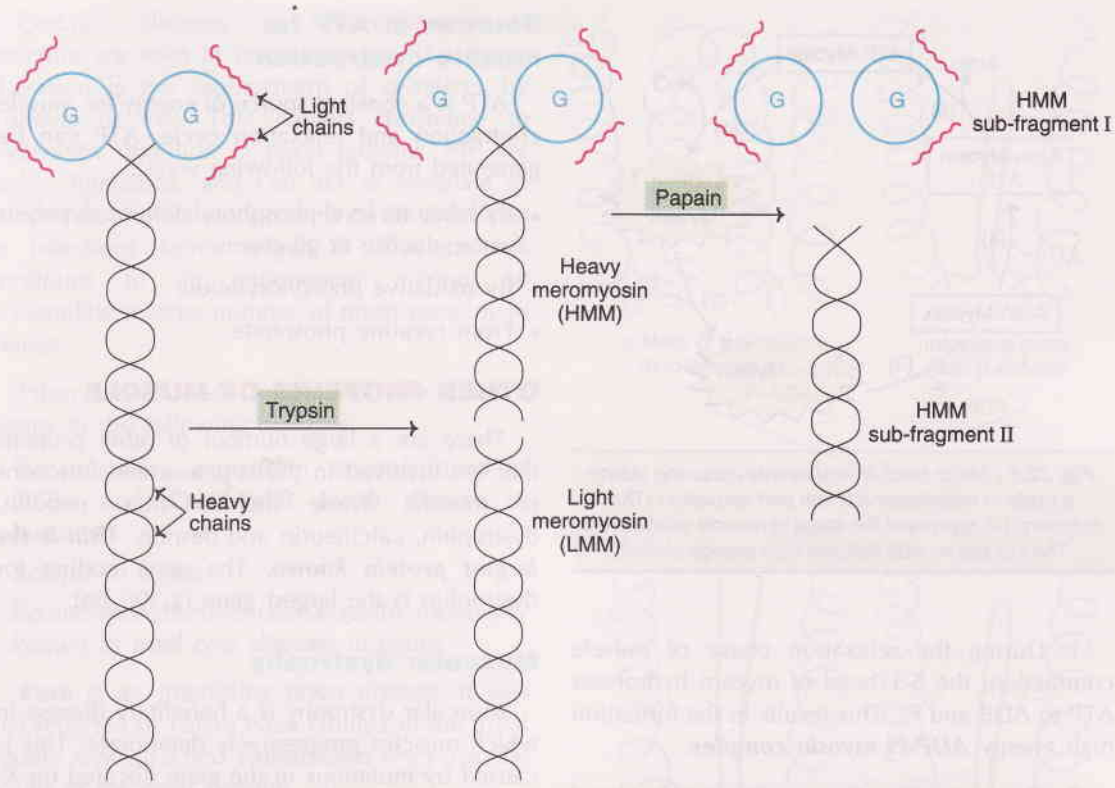


Fig. 22.5 : A diagrammatic representation of myosin along with its digested products by trypsin and papain (G-Globular head).

to F-actin in the grooves (***Fig.22.4***). Troponin consists of three polypeptide chains – troponin T (TpT binding to tropomyosin), tropinin I (TpI that inhibits F-actin myosin interaction) and troponin C (TpC, calcium binding polypeptide). TpC is comparable to calmodulin.

MYOSINS

Myosins are actually a family of proteins with about 15 members. The myosin that is predominantly present in muscle is myosin II.

In terms of quantity, myosin constitutes approximately 55% of muscle protein, and is found in thick filaments. Myosin is composed of six polypeptide chains (hexamer). It contains one pair of heavy (H) chains, and two pairs of light (L) chains.

Limited digestion of myosin with trypsin and papain has helped to understand its structure and function (***Fig.22.5***).

Light and heavy meromyosins

When myosin is digested with trypsin, two fragments namely light meromyosin (LMM) and heavy meromyosin (HMM) are produced. Light meromyosin represents the α-helical fibres of the tail of myosin, and cannot bind to F-actin.

Heavy meromyosin contains the fibrous and globular portions of myosin. HMM inhibits ATPase activity and binds to F-actin.

On digestion by papain, heavy meromyosin yields two sub-fragments S-1 and S-2 (HMM S-1, HMM S-2). HMM S-2 fragment is fiber-like, does not bind to F-actin and has no ATPase activity. On the other hand, HMM S-1 is globule-like, binds to L-chains, and possesses ATPase activity.

MUSCLE CONTRACTION

An outline of the reactions involving muscle contraction is depicted in ***Fig.22.6***, and briefly described in the next page.

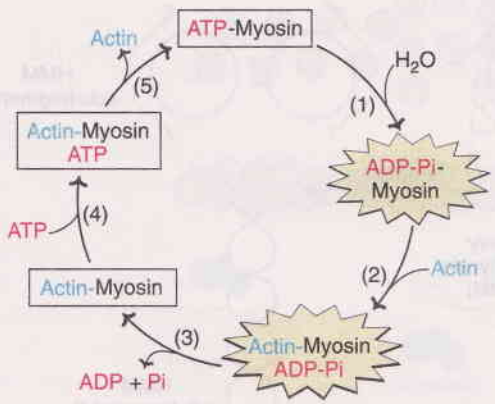


Fig. 22.6 : Major biochemical events occurring during a cycle of muscle contraction and relaxation (The numbers 1-5 represent the steps in muscle contraction; The zig zag rounds indicate high energy states).

1. During the relaxation phase of muscle contraction, the S-1 head of myosin hydrolyses ATP to ADP and Pi. This results in the formation high energy ***ADP-Pi myosin complex***.

2. On contraction, the muscle gets stimulated (through the participation of actin, Ca^{2+}, troponin, tropomyosin etc.) to finally form actin-myosin-ADP-Pi complex.

3. The next step is the ***power stroke*** which drives movement of actin filaments over myosin filaments. This is followed by the release of ADP and Pi, and a conformation change in myosin. The actin-myosin complex is in a low energy state.

4. A fresh molecule of ATP now binds to form actin-myosin ATP complex.

5. Actin is released, as myosin-ATP has low affinity for actin. This step is crucial for relaxation which is dependent on the binding of ATP to actin-myosin complex.

A fresh cycle of muscle contraction and relaxation now commences with the hydrolysis of ATP and the formation of ADP-Pi-myosin complex. It has to be noted that it is ultimately the ATP that is the immediate source of energy for muscle contraction.

Sources of ATP for muscle contraction

ATP is a constant source of energy for muscle contraction and relaxation cycle. ATP can be generated from the following ways.

- By substrate level phosphorylation of glycolysis using glucose or glycogen.
- By oxidative phosphorylation.
- From creatine phosphate.

OTHER PROTEINS OF MUSCLE

There are a large number of other proteins that are involved in the structure and functions of muscle. These include titin, nebulin, dystrophin, calcineurin and desmin. ***Titin is the largest protein known***. The gene coding for dystrophin is the largest gene (2,300 bp).

Muscular dystrophy

Muscular dystrophy is a hereditary disease in which muscles progressively deteriorate. This is caused by mutations in the gene (located on X-chromosome) coding for the protein dystrophin.

PROTEIN MISFOLDING AND DISEASES

The process of protein folding is complex and has been briefly described in ***Chapter 25***. Sometimes, improperly folded proteins may be formed (either spontaneous or by gene mutations). Such misfolded proteins usually get degraded within the cell. However, as the individuals age, the misfolded proteins accumulate and cause a number of diseases. Prion diseases and amyloidosis, two groups of diseases due to protein misfolding are briefly discussed.

Prion diseases

The term prion represents ***pro***teinous ***in***fectious agents. ***Prion proteins*** (***PrP***) are the altered forms of normal proteins. However, no differences in the primary structure (i.e. amino acid sequence) and post-translational modifications are observed.

Certain changes in three-dimensional structure are seen in prion proteins. The major alteration is the replacement of α-helices by β-sheets in PrP. This confers resistance to proteolytic digestion of prion proteins. PrP are highly infectious, and can act as template to convert non-infectious proteins (with α-helices) to infectious forms (***Fig.22.7***). This process continues in an exponential manner to accumulate a large number of prion proteins in tissues.

Prion proteins are implicated as causative agents in the following diseases.

- Transmissible spongiform encephalopathies (TSEs) and ***Creutzfeldt Jacob disease*** in humans.
- ***Scrapie*** in sheep
- Bovine spongiform encephalopathy (popularly known as ***mad cow disease***) in cattle.

Kuru *is an interesting prion disease.* It was first reported in Papau New Guinea in the tribal people who practice ***cannibalism*** (they eat the brains of the dead people).

As of now, there is no treatment for prion diseases. Transmissible spongiform encephalopathies are invariably fatal in humans.

Amyloidosis

The term amyloids is used to refer to the ***altered proteins*** (with ***β-sheets***) that ***accumulate in the body***, particularly in the nervous system. Amyloids are formed by protein misfolding or due to gene mutations. They are not infectious agents as prion proteins. However, as the age advances, amyloids accumulate, and they have been implicated in many degenerating diseases. A total of at least 15 different proteins are involved in amyloidosis.

Alzheimer's disease is a ***neurodegerative disorder***, affecting about 5-10% of the people above 60 years of age. It is characterized by memory loss, confusion, hallucinations, personality changes with abnormal behaviour. As the disease progresses, the patient may enter a vegetative state, and may die after 10 years after the onset of the disease manifestations. The accumulation of amyloids (in the form of amyloid plaque) has been clearly demonstrated in the patients of Alzheimer's disease.

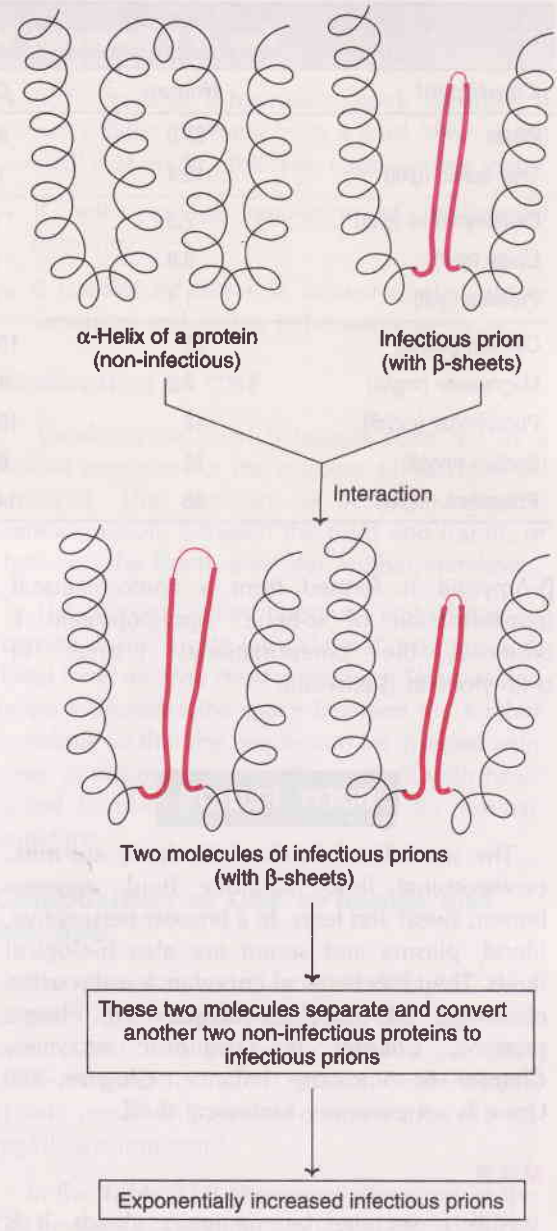


Fig. 22.7 : *A model for the formation of infectious prions (Red thick lines represent β-sheets in protein).*

A specific protein, namely β-amyloid which is prone for self aggregation is believed to be the causative agent of Alzheimer's disease,

Table 22.1 Composition of milk in different species

Constituent	Human	Cow	Buffalo	Goat
Water	87.6	87.2	83.5	87.0
Total solids (g/dl)	12.4	12.8	16.5	13.0
Carbohydrates (g/dl)	7.5	4.4	5.4	4.6
Lipids (g/dl)	3.8	3.8	6.5	3.5
Proteins (g/dl)	1.1	3.3	4.3	3.7
Calcium (mg/dl)	35	150	160	175
Magnesium (mg/dl)	2.2	13	10	8
Phosphorus (mg/dl)	16	100	100	70
Sodium (mg/dl)	15	60	60	50
Potassium (mg/dl)	55	140	130	85

β-Amyloid is formed from a conformational transformation of α-helix. Apolipoprotein E promotes the conformational change of α-amyloid to β-amyloid.

BODY FLUIDS

The specialized fluids of the body are milk, cerebrospinal fluid, amniotic fluid, aqueous humor, sweat and tears. In a broader perspective, blood, plasma and serum are also biological fluids. Their biochemical importance is discussed elsewhere (Hemoglobin, ***Chapter 10***; Plasma proteins, ***Chapter 9***; Diagnostic enzymes, ***Chapter 6***; Acid-base balance, ***Chapter 21***). Urine is an excretory biological fluid.

MILK

Milk is secreted by mammary glands. It is almost a ***complete natural food***. Milk is the only food for the offsprings of mammals on their birth.

COMPOSITION OF MILK

The major constituents of milk in different species—human, cow, buffalo and goat are given in ***Table 22.1***. Water is the major constituent, with a concentration in the range of 83–87%, depending on the species. The remaining 13–17% is made up of solids–carbohydrates, lipids, proteins, minerals and vitamins.

Carbohydrates in milk

Milk contains the disaccharide lactose which imparts sweetness. Human milk has a higher concentration of lactose (7.5%) compared to milk of other species. Thus, human milk is sweet enough for the babies to relish. Milk sugar (lactose) serves two major functions.

1. It provides galactose, a structural unit for the growing infant.
2. In the intestine, it gets metabolized to lactic acid which eliminates harmful bacteria.

Lipids in milk

The lipids in the milk are dispersed as small globules. Milk fat is mainly composed of triacylglycerols. Mono- and diacylglycerols are also present in trace quantities. The fatty acids found in milk (i.e. in TG) are mostly medium or short chain, and saturated e.g. palmitic acid, myristic acid, stearic acid, lauric acid and butyric acid. Oleic acid, an unsaturated fatty acid, is also present.

Proteins in milk

The major milk proteins are casein (about 80%) and lactalbumin. Small concentrations of

enzymes (proteases, lipase, xanthine oxidase, lysozyme) and immunoglobulins are also found.

Milk casein (a phosphoprotein) is almost a complete protein (next to egg albumin), containing all the essential amino acids. It is present in milk in the form of aggregates called micelles. The ***white colour of milk*** is due to the dispersion of ***calcium caseinate micelles***.

Whey proteins : If milk is acidified, casein gets precipitated at isoelectric point (pH 4.7). The supernatant fluid contains whey proteins (20% of milk proteins). These include lactalbumin, lactoglobulin and various enzymes.

Minerals in milk

Milk is rich in calcium, magnesium, phosphorus, sodium, potassium and chlorine. However, milk is a ***poor source of iron*** and ***copper***.

Vitamins in milk

Both fat soluble and water soluble vitamins are found in good concentration in milk. However milk is ***deficient in vitamin C***.

Calorific value of milk

Due to variability in the nutrient composition (carbohydrates, fats and proteins), the calorific value of milk from different species varies. Thus, human milk can provide about 70 Cal/100 ml, while for buffalo milk, it is around 95 Cal/100 ml.

CEREBROSPINAL FLUID (CSF)

Cerebrospinal fluid is a clear, colourless liquid formed within the cavities (ventricles) of brain and around the spinal cord. CSF originates in the choroid plexus (as an ultrafiltrate of plasma) and returns to blood through arachnoid villi. About 500 ml of CSF is formed everyday. However, at any given time, there is about 120–150 ml CSF in the system. Further, CSF is completely replaced about three times a day.

Functions of CSF

As the brain has no lymphatic system, CSF drains into the ventricular system and moves into spaces surrounding the brain and spinal cord. The major functions of CSF are listed.

- CSF serves as a hydraulic shock absorber. It can diffuse the force from a hard blow to the skull that might otherwise cause severe injury.
- It helps in the regulation of intracranial pressure.
- It is believed that CSF influences the hunger sensation and eating behaviours.

Collection of CSF

Cerebrospinal fluid is usually collected by a spinal puncture for the purpose of biochemical analysis. The puncture is performed in the lumbar region, between the third and fourth, or between the fourth and fifth lumbar vertebrae.

The sterile lumbar puncture (spinal tap) is carried out in a side lying (lateral) position with head fixed into the chest and knees. This position helps to increase the space between the lumbar vertebrae so that the needle can be inserted with ease. A sitting position of the patient with head flexed to chest can also be used for lumbar puncture.

Composition of CSF in health and disease

The normal composition of cerebrospinal fluid is given in the ***Table 22.2***. From the diagnostic point of view, the total cell count of lymphocytes (Reference : 0-5 $\times$ 10^6/1), protein concentration (15–45 mg/dl) and glucose concentration (45–85 mg/dl) are important.

In the ***Table 22.3***, the major alterations in the CSF in the disease states are given. The total ***cell count*** and ***protein content*** are ***increased*** while ***glucose*** concentration is ***reduced in tuberculosis meningitis***. In case of brain tumors, there is no change in total cell count while the protein concentration may be marginally increased.

The colour and appearance of CSF is sometimes a guiding factor in the disease diagnosis. For instance, CSF is opalescent and slightly yellow coloured in tuberculosis meningitis.

Table 22.2 Normal composition of cerebrospinal fluid

Parameter	*Description/concentration*
Volume	90–150 ml
Appearance	Clear and colourless
Specific gravity	1.006–1.008
Osmolality	280–290 mOsm/kg
Total cell count (lymphocytes)	0–5 × 10^6/l
pH	7.3–7.4
Protein	15–45 mg/dl
A/G ratio (albumin/globulin)	8 : 1
Glucose	45–85 mg/dl
Chloride	118–130 mEq/l
Calcium	2.1–2.7 mEq/l
Sodium	145–155 mEq/l
Potassium	2.0–3.5 mEq/l

AMNIOTIC FLUID

Amniotic fluid is a liquid produced by the membranes and the fetus. It surrounds the fetus throughout pregnancy. The volume of amniotic fluid increases with the gestational age. Thus, the volume increases from 30 ml (at 2 weeks of gestation) to 350 ml (at 20 weeks), and thereafter to 500–1000 ml. Amniotic fluid is almost clear with some desquamated fetal cells and a little lipid.

Functions of amniotic fluid

- It provides physical protection to the fetus.
- Amniotic fluid is a medium for the exchange of various chemicals.

Diagnostic importance of amniotic fluid

The term ***amniocentesis*** is used for the process by which amniotic fluid is collected for analysis. The diagnostic importance of amniotic fluid is given below.

Assessment of fetal maturity : Fetal maturity can be assessed by cytological staining of fat cells, and estimation of creatinine concentration (> 1.6 mg/dl indicates fetal maturity).

Lung maturity : The fetal lung maturity is evaluated by measuring ***lecithin–sphingomyelin (L/S) ratio***. A L/S ratio of 2 : 1 or more indicates lung maturity. If L/S ratio is less than 1.2 : 1, it is better to delay the induced delivery until the lung has become more mature.

Diagnosis of congenital disorders : Amniotic fluid analysis is useful for the prenatal diagnosis of congenital disorders. Some of the important ones are listed.

- Chromosomal disorders such as Down's syndorme.
- Metabolic disorders e.g. cystic fibrosis.
- Sex-linked disorders e.g. hemophilia.
- Enzyme defects e.g. Tay-Sachs disease.

Table 22.3 Changes in cerebrospinal fluid in the disease states

Disease	*Colour and appearance*	*Total cell count*	*Protein*	*Glucose*
Normal	Clear and colourless	0–5 × 10^6/l	15–45 mg/dl	45–85 mg/dl
Tuberculosis meningitis	Opalescent and slightly yellow	Increased	Increased	Relatively low
Bacterial meningitis	Opalescent and turbid	Markedly increased	Markedly increased	Markedly decreased
Brain tumour	Clear and colourless	No change	Increased	Low
Subarachnoid hemorrhage	Slightly blood colour	RBC and WBC present	Increased	Almost normal

Assessment of hemolytic diseases : Estimation of bilirubin in amniotic fluid is useful to evaluate the severity of hemolytic diseases.

Measurement of α-fetoprotein : Increased levels of α-fetoprotein (normal 15–40 µg/ml during gestation; at 40 weeks < 1.0 µg/ml) are associated with neural tube defects, fetal distress, Turner syndrome. Elevated α-fetoprotein may also indicate a possible death of the fetus.

AQUEOUS HUMOR

Aqueous humor is the fluid that fills the anterior chamber of the eye. This fluid is responsible for maintaining the intraocular tension. Aqueous humor, secreted by the ciliary body, enters the anterior chamber. Blockade in the flow of aqueous humor causes ***glaucoma*** due to increased intraocular pressure.

BIOMEDICAL / CLINICAL CONCEPTS

- *Improper formation of collagen is associated with certain genetic diseases e.g. Ehlers-Danlos syndrome (abnormal tissue fragility), osteogenesis imperfecta (abnormal fragility of bones).*
- *Defective formation of collagen is observed in scurvy, caused by vitamin C deficiency. This results in bleeding of gums and poor wound healing.*
- *Hair waving (curling) through artificial means is possible with suitable alterations in the structure of keratins.*
- *Muscular dystrophy occurs due a mutation in the gene coding for the protein dystrophin.*
- *Protein misfolding results in prion diseases (e.g. mad cow disease) and amyloidosis (Alzheimer's disease).*
- *Biochemical analysis of cerebrospinal fluid is useful for the diagnosis of certain diseases – tuberculosis meningitis (increased protein and decreased glucose concentrations).*
- *Amniotic fluid is analysed to assess fetal maturity (creatinine concentration > 1.6 mg/dl), lung maturity (lecithin–sphingomyelin ratio > 2 : 1) and for the prenatal diagnosis of congenital disorders (e.g. hemophilia, Down's syndrome).*

SUMMARY

1. *The major proteins of connective tissue are collagen, elastin, fibrillin, laminin and proteoglycans. Among these, collagen is the most abundant, constituting one-third of the total body proteins.*

2. *Type I mature collagen is a triple helical structure i.e. contains three polypeptide chains each with about 1000 amino acids. The repetitive amino acid sequence of collagen is $(Gly\text{-}X\text{-}Y)_n$. Glycine constitutes about 1/3 rd of the amino acids while X and Y represent other amino acids.*

3. *Keratins are structural proteins found in hair, skin, nails and horns. The strength of the keratins is directly related to the number of disulfide bonds.*

4. *Muscle is the single largest tissue of the human body (30–40% of body weight). It is composed of fibre cells into which myofibrils are embedded. Each myofibril contains alternating A and I bands. Sarcomere is the functional unit of muscle.*

5. *Actin, myosin, tropomyosin and troponin are the major contractile proteins found in muscles. The muscle contraction and relaxation occur due to the active involvement of these proteins. ATP is the immediate source of energy for muscle contraction.*

6. *Proper folding of proteins is essential for their structure. Misfolding of proteins results in certain diseases e.g. mad cow disease, Alzheimer's disease.*

7. *The specialized fluids of the body include milk, cerebrospinal fluid, amniotic fluid, aqueous humor, sweat and tears.*

8. *Milk is almost a complete food with various nutrients—carbohydrates, lipids, proteins, vitamins and minerals. However, milk is deficient in vitamin C, iron and copper.*

9. *Cerebrospinal fluid is an ultrafiltrate of plasma. In the disease, tuberculosis meningitis, the total cell count and protein concentration are increased, while glucose concentration is decreased in CSF.*

10. *Amniotic fluid is a liquid produced by the fetus. Its biochemical analysis is important for the diagnostic purpose – assessment of fetal maturity, diagnosis of congenital diseases.*

SELF-ASSESSMENT EXERCISES

I. Essay questions

1. Give an account of the structure and functions of collagen. Add a note on the abnormalities associated with collagen.
2. Describe the muscle proteins, and muscle contraction.
3. Discuss the protein misfolding and various diseases related to it.
4. Give an account of the composition of milk.
5. Describe the functions and composition of cerebrospinal fluid. Add a note on the alterations in CSF in diseased states.

II. Short notes

(a) Biosynthesis of collagen, (b) Collagen and scurvy, (c) Elastin, (d) Light and heavy meromyosins, (e) Prion diseases, (f) Amyloidosis, (g) Hair waving, (h) Vitamins and minerals in milk, (i) Collection of CSF, (j) Amniotic fluid.

III. Fill in the blanks

1. The most abundant protein in mammals _____.
2. The amino acid that contributes to one-third of the total number of amino acids in collagen _____.
3. The toxic compound that interferes with the cross-linking of lysine in collagen, causing lathyrism _____.
4. Marfan syndrome is a genetic disorder due to a mutation of the gene coding for _____.
5. Name the carbohydrates associated with the structure of proteoglycans _____.
6. The region of the muscle fibre between two Z lines is termed as _____.
7. Name the major protein found in the structure of thin filaments of sarcomere _____.
8. The white colour of milk is due to the dispersion of _____.
9. Name the vitamin deficient in milk _____.
10. The fetal lung maturity is evaluated by measuring _____ ratio.

IV. Multiple choice questions

11. The number of polypeptide chains present in collagen
    (a) 1 (b) 2 (c) 3 d) 4.
12. The functional unit of muscle
    (a) Fibre cell (b) Myofibril (c) H band d) Sarcomere.
13. The immediate source of energy for muscle contraction
    (a) ATP (b) Creatine phosphate (c) GTP d) Phosphoenol pyruvate.
14. One of the following minerals is lacking in milk
    (a) Calcium (b) Sodium (c) Iron d) Potassium.
15. One of the following biochemical parameters is increased in tuberculosis meningitis
    (a) Glucose (b) Protein (c) Sodium d) Chloride.

23 Nutrition

The nutrition speaks :

"Some eat to live,
And some live to eat!
My function is
To cater for all."

Whether a man eats for living or lives for eating, food is his prime concern. ***Nutrition*** may be defined as the ***utilization of food by living organisms.*** Biochemists have largely contributed to the science of nutrition.

Nutrition significantly promotes man's development, his health and welfare. The subject nutrition, perhaps, is the most controversial. This is due to the fact that nutrition is concerned with food, and everyone feels competent enough to talk like an expert on nutrition. Further, high public awareness and the controversial reports by scientists also contribute to the controversy.

Methodology in nutrition : Most of the existing knowledge on nutrition is originally derived from animal experimentation. This is despite the fact that there may exist several differences in the biochemical composition between man and animals! For instance, some animals can synthesize ascorbic acid while man cannot do so.

Study of human nutrition : The study of nutrition may be logically divided into three areas—***ideal nutrition***, ***undernutrition*** and ***overnutrition***. Ideal nutrition is the concern of everyone. Undernutrition is the prime concern of developing countries while overnutrition is a serious concern of developed countries.

A sound knowledge of chemistry and metabolism of foodstuffs (carbohydrates, lipids, proteins, vitamins and minerals) is an essential prerequisite for a better understanding of nutrition. The reader must, therefore, first refer these chapters. The principles of nutrition with special reference to energy demands, carbohydrates, fats, proteins, recommended dietary/daily allowances (RDA), balanced diet and nutritional disorders are discussed in the following pages.

NUTRITION AND ENERGY SUPPLY

Food is the fuel source of the body. The ingested food undergoes metabolism to liberate energy required for the vital activities of the body.

Table 23.1 Calorific values of foodstuffs

Foodstuff	Energy value (Cal/g) In bomb calorimeter	In the body
Carbohydrate	4.1	4
Fat	9.4	9
Protein	5.4	4
Alcohol	7.1	7

Energy content of foods

The calorific value (energy content) of a food is calculated from the heat released by the total combustion of food in a calorimeter.

Unit of heat : ***Calorie*** is the unit of heat. One calorie represents the amount of ***heat required to rise the temperature of one gram of water by 1°C*** (i.e. from 15° to 16°C). A calorie is too small a unit. Therefore, it is more conveniently expressed as kilocalories (1,000 times calorie) which is represented by kcal or simply ***Cal*** (with capital '***C***').

The ***joule*** is also a unit of energy used in some countries. The relationship between calories and joules (J) is

$$1 \text{ Cal } (1 \text{ kcal}) = 4.128 \text{ KJ}$$

The joule is less commonly used by nutritionists.

Calorie value of foods : The energy values of the three principal foodstuffs—carbohydrate, fat and protein—measured in a bomb calorimeter and in the body are given in the ***Table 23.1***. The carbohydrates and fats are completely oxidized (to CO_2 and H_2O) in the body; hence their fuel values, measured in the bomb calorimeter or in the body, are almost the same. Proteins, however, are not completely burnt in the body as they are converted to products such as urea, creatinine and ammonia, and excreted. Due to this reason, calorific value of protein in the body is less than that obtained in a bomb calorimeter. The energy values of ***carbohydrates***, ***fats*** and ***proteins*** (when utilized in the body) respectively, are ***4***, ***9*** and ***4 Cal/g***.

Alcohol is a recent addition to the calorie (7 Cal/g) contribution, as it is a significant dietary component for some people.

It must be noted that the nutrients, namely vitamins and minerals, have no calorific value, although they are involved in several important body functions, including the generation of energy from carbohydrates, fats and proteins.

Respiratory quotient of foodstuffs

The respiratory quotient (***R. Q.***) is the ***ratio of*** the volume of CO_2 produced to the volume of O_2 utilized in the oxidation of foodstuffs.

Carbohydrates : The carbohydrates are completely oxidized and their R. Q. is close to 1, as represented below for glucose.

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O$$

$$\text{R. Q. for carbohydrate} = \frac{CO_2}{O_2} = \frac{6}{6} = 1.$$

Fats : Fats have relatively lower R.Q. since *they have a low oxygen content. For this reason,* fats require more O_2 for oxidation. The R.Q. for the oxidation of the fat, tristearin is given below.

$$2\,C_{57}H_{110}O_6 + 16\,3O_2 \longrightarrow 114\,CO_2 + 110\,H_2O$$

$$\text{R. Q. for fat} = \frac{CO_2}{O_2} = \frac{114}{163} = 0.7.$$

Proteins : *The chemical nature of proteins is highly variable, and this cannot be represented* by any specific formula. By indirect measurements, the R.Q. of protein is found to be around 0.8.

Mixed diet : The R. Q. of the diet consumed is dependent of the relative composition of carbohydrates, fats and proteins. For a normally ingested diet, it is around 0.8.

UTILIZATION OF ENERGY IN MAN

Man consumes energy to meet the fuel demands of the three ongoing processess in the body.

1. Basal metabolic rate
2. Specific dynamic action
3. Physical activity.

Besides the above three, additional energy supply is needed during growth, pregnancy and lactation.

BASAL METABOLIC RATE

Basal metabolism or basal metabolic rate (BMR) is defined as the ***minimum amount of energy required by the body*** to maintain life ***at complete physical and mental rest*** in the post-absorptive state (i.e. 12 hours after the last meal). It may be noted that ***resting metabolic rate (RMR)*** is in recent use for BMR.

Under the basal conditions, although the body appears to be at total rest, several functions within the body continuously occur. These include working of heart and other organs, conduction of nerve impulse, reabsorption by renal tubules, gastrointestinal motility and ion transport across membranes (Na^+-K^+ pump consumes about 50% of basal energy).

Measurement of BMR

Preparation of the subject : For the measurement of BMR the subject should be awake, at complete physical and mental rest, in a post-absorptive state and in a comfortable surrounding (at 25°C).

Measurement : The BMR is determined either by the ***apparatus of Benedict and Roth*** (closed circuit device) or by the Douglas bag method (open circuit device). The former is more frequently used.

By Benedict-Roth method, the volume of O_2 consumed (recorded on a graph paper) by the subject for a period of 2-6 minutes under basal conditions is determined. Let this be A liters for 6 minutes. The standard calorific value for one liter O_2 consumed is 4.825 Cal.

Heat produced in 6 min = $4.825 \times A$

Heat produced in one hour = $4.825A \times 10$

Units of BMR : BMR is expressed as Calories per square meter of body surface area per hour i.e. Cal/sq.m/hr.

For the calculation of body surface area, the simple formula devised by Du Bois and Du Bois is used.

$$A = H^{0.725} \times W^{0.425} \times 71.84$$

where A = Surface area in cm^2

H = Height in cm

W = Weight in kg.

To convert the surface area into square meters (m^2), divide the above value (cm^2) by 10,000. Nomograms of body surface area (directly in m^2) from heights and weights are readily available in literature.

Normal values of BMR : ***For an adult man 35–38 Cal/sq. m/hr; for an adult woman 32-35 Cal/sq.m/hr.*** A BMR value between –15% and +20% is considered as normal.

Some authors continue to represent BMR as Cal/day. ***For an adult man BMR is around 1,600 Cal/day, while for an adult woman around 1,400 Cal/day.*** This is particularly important for easily calculating energy requirements per day.

Factors affecting BMR

1. **Surface area :** The BMR is directly proportional to the surface area. Surface area is related to weight and height.

2. **Sex :** Men have marginally higher (about 5%) BMR than women. This is due to the higher proportion of lean muscle mass in men.

3. **Age :** In infants and growing children, with lean muscle mass, the BMR is higher. In adults, BMR decreases at the rate of about 2% per decade of life.

4. **Physical activity :** BMR is increased in persons (notably athletes) with regular exercise. This is mostly due to increase in body surface area.

5. **Hormones :** Thyroid hormones (T_3 and T_4) have a stimulatory effect on the metabolism of the body and, therefore, BMR. Thus, BMR is ***raised in hyperthyroidism*** and ***reduced in hypothyroidism***. In fact, the measurement of BMR was earlier used to assess thyroid function.

The other hormones such as epinephrine, cortisol, growth hormone and sex hormones increase BMR.

6. **Environment :** In cold climates, the BMR is higher compared to warm climates.

7. **Starvation :** During the periods of starvation, the energy intake has an inverse relation with BMR, a decrease up to 50% has been reported. This may be an adaptation by the body.

8. **Fever :** Fever causes an increase in BMR. An elevation by more than 10% in BMR is observed for every 1°C rise in body temperature.

9. **Disease states :** BMR is elevated in various infections, leukemias, polycythemia, cardiac failure, hypertension etc. In Addison's disease (adrenal insufficiency), BMR is marginally lowered.

10. **Racial variations :** The BMR of Eskimos is much higher. The BMR of Oriental women living in USA is about 10% less than the average BMR of American women.

Significance of BMR

BMR is important to calculate the ***calorie requirement*** of an individual and ***planning of diets***. Determination of BMR is useful for the assessment of thyroid function. In hypothyroidism, BMR is lowered (by about −40%) while in hyperthyroidism it is elevated (by about + 70%). Starvation and certain disease conditions also influence BMR (described above).

SPECIFIC DYNAMIC ACTION

The phenomenon of the ***extra heat production by the body***, over and above the calculated caloric value, when a given ***food is metabolized*** by the body, is known as specific dynamic action (SDA). It is also known as ***calorigenic action*** or ***thermogenic action*** or thermic action (effect) of food.

SDA for different foods : For a food containing 25 g of protein, the heat production from the caloric value is 100 Cal (25 × 4 Cal). However, when 25 g protein is utilized by the body, 130 Cal of heat is liberated. The extra 30 Cal is the SDA of protein. Likewise, consumption of 100 Cal of fat results in 113 Cal and 100 Cal of carbohydrate in 105 Cal, when metabolized in the body. SDA for protein, fat and carbohydrate are 30%, 13% and 5%, respectively. Thus, ***proteins*** possess the ***highest SDA*** while ***carbohydrates*** have the ***lowest***.

SDA for mixed diet : For a mixed diet, the SDA is not an additive value of different foods but it is much less. The presence of fats and carbohydrates reduces the SDA of proteins. Fats are most efficient in reducing SDA of foodstuffs. For a regularly consumed mixed diet, the SDA is ***around 10%***.

Significance of SDA : For the utilization of foods by the body, certain amount of energy is consumed from the body stores. This is actually an expenditure by the body for the utilization of foodstuffs. It is the highest for proteins (30%) and lowest for carbohydrates (5%) and for a mixed diet around 10%. It is, therefore, essential that an additional 10% calories should be added to the total energy needs (of the body) towards SDA. And the diet should be planned, accordingly. (SDA is quite comparable to the handling charges levied by a bank for an outstation cheque).

The higher SDA for protein indicates that it is not a good source of energy. Fat is the best source of energy due to its lowering effect on SDA. However, excessive utilization of fat leads to ketosis.

Mechanism of SDA : The exact cause of SDA is not known. It is generally believed that SDA of foods is due to the energy required for digestion, absorption, transport, metabolism and storage of foods in the body.

Intravenous administration of amino acids or the oral ingestion of proteins gives the same SDA. This shows that the SDA of proteins is not due to their digestion and absorption. Hepatectomy abolishes SDA, thereby indicating that SDA is closely connected with the metabolic functions of liver. The SDA of proteins is primarily to meet the energy requirements for

Table 23.2 Type of physical activity and energy expenditure (over and above BMR, about 65 Cal/hr).

Physical activity	*Energy requirement (Cal/hr)*
Sitting (quietly)	25
Standing (quietly)	30
Writing/eating/reading	30
Car driving	60
Typing	75
Household work (dish washing)	80
Walking (slow)	130
Sexual intercourse	140
Cycling (slow)	150
Running (moderate)	500
Swimming	600
Walking upstairs	800

deamination, synthesis of urea, biosynthesis of proteins, synthesis of triacylglycerol (from carbon skeleton of amino acids). It has been demonstrated that certain amino acids (phenylalanine, glycine and alanine) increase the SDA. It is a common experience that consumption of a protein rich diet makes us feel warm and comfortable in cold weather. This is due to the high SDA of proteins.

The SDA of carbohydrates is attributed to the energy expenditure for the conversion of glucose to glycogen.

As regards fat, the SDA may be due to its storage, mobilization and oxidation.

PHYSICAL ACTIVITY OF THE BODY

The physical activity of the individual is highly variable. The amount of energy needed for this depends mainly on the duration and intensity of muscular activity. The expenditure of energy for the various physical activities has been calculated (***Table 23.2***).

For the sake of convenience, the individuals are grouped into four categories with regard to their physical activity and the requirement of energy.

Light work — 30–40% of BMR
(teachers, office workers, doctors)

Moderate work — 40–50% of BMR
(housewives, students)

Heavy work — 50–60% of BMR
(agricultural labourers, miners)

Very heavy work — 60–100% of BMR
(construction workers, rickshaw pullers)

Energy requirements of man

As already stated, the three factors—basal metabolic rate, specific dynamic action and physical activity—determine the energy needed by the body. In an individual with light work, ***about 60% of the calories are spent towards BMR, about 30% for physical activity and about 10% to take care of the SDA.***

The daily requirement of energy is rather variable which depends on the BMR (in turn depends on age, sex, body size etc.) and physical activity. As per some rough calculation, caloric requirements of adults per day (Cal/day) are in the following ranges.

Light work — 2,200–2,500
Moderate work — 2,500–2,900
Heavy work — 2,900–3,500
Very heavy work — 3,500–4,000

NUTRITIONAL IMPORTANCE OF CARBOHYDRATES

Dietary carbohydrates are the ***chief source of energy***. They contribute to 60-70% of total caloric requirement of the body. Incidentally, carbohydrate rich foods cost less.

Carbohydrates are the most abundant dietary constituents, despite the fact that they are ***not essential nutrients to the body***. From the nutritional point of view, carbohydrates are grouped into 2 categories.

1. Carbohydrates ***utilized*** by the body—starch, glycogen, sucrose, lactose, glucose, fructose etc.

2. Carbohydrates ***not utilized*** (not digested) by the body—cellulose, hemicellulose, pectin, gums etc.

Among the carbohydrates utilized by the body, starch is the most abundant. The consumption of starch has distinct advantages due to its bland taste, satiety value and slow digestion and absorption. Sucrose (the table sugar), due to its sweetness, can be consumed to a limited extent. ***Excessive intake of sucrose*** causes dental caries, and an increase in plasma lipid levels is associated with many health complications.

Functions of carbohydrates

1. **Major sources of energy :** Carbohydrates are the principal source of energy, supplying 60–80% of the caloric requirements of the body.

2. **Protein sparing action :** Proteins perform a specialized function of body building and growth. The wasteful expenditure of proteins to meet the energy needs of the body should be curtailed. Carbohydrates come to the rescue and spare the proteins from being misused for caloric purpose.

3. **Absolute requirement by brain :** The brain and other parts of central nervous system are dependent on glucose for energy. Prolonged hypoglycemia may lead to irreversible brain damage.

4. **Required for the oxidation of fat :** Acetyl CoA is the product formed in fatty acid oxidation. For its further oxidation via citric acid cycle, acetyl CoA combines with oxaloacetate, the latter is predominantly derived from carbohydrates. It may therefore be stated '***Fat burns in a fuel of carbohydrate***'. Excess utilization of fats coupled with deficiency of carbohydrates leads to ketosis.

5. **Energy supply for muscle work :** The muscle glycogen is broken down to lactic acid (glycolysis) to provide energy for muscle contraction.

6. **Synthesis of pentoses :** Pentoses (e.g. ribose) are the constituents of several compounds in the body e.g. nucleic acids (DNA, RNA), coenzymes (NAD^+, FAD). These pentoses are produced in carbohydrate metabolism.

7. **Synthesis of non-essential amino acids :** The intermediates of carbohydrate metabolism, mainly the keto acids (e.g. pyruvic acid), serve as precursors for the synthesis of non-essential amino acids.

8. **Synthesis of fat :** Excess consumption of carbohydrates leads to the formation of fat which is stored in the adipose tissue.

9. **Importance of non-digestible carbohydrates :** These are the carbohydrates not utilized by the body. Yet, they are important since they improve bowel motility, prevent constipation, lower cholesterol absorption and improve glucose tolerance (details discussed later).

Glycemic index

There are variations in the increase and fall of blood glucose levels after the ingestion of different carbohydrate containing foods. These quantitative differences are assayed by glycemic index which measures the ***time course of post-prandial glucose concentrations*** from a graph. Glycemic index may be defined as the area under the blood glucose curve after the ingestion of a food compared with the area under the blood glucose curve after taking the same amount of carbohydrate as glucose. It is expressed as percentage.

$$\text{Glycemic index} = \frac{\text{Area under the blood glucose curve after ingestion of test meal}}{\text{Area under the curve after ingestion of glucose}} \times 100$$

A graphic representation of high and low glycemic indices is depicted in ***Fig.23.1***.

The glycemic index of a complex carbohydrate (i.e. starch) is lower than a refined carbohydrate (i.e. glucose). This is explained on the basis of slow digestion and absorption of

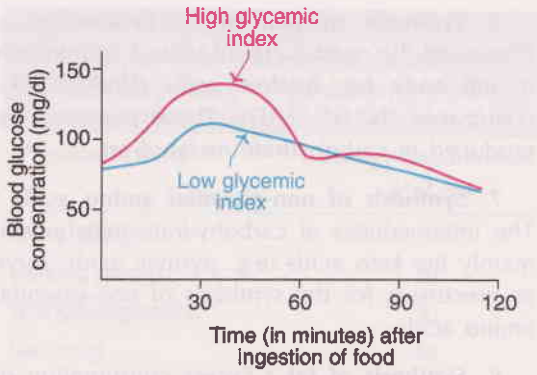


Fig. 23.1 : The glycemic index curve after the ingestion of two different foods.

complex carbohydrates. Further, the glycemic index of carbohydrate is usually lower when it is combined with protein, fat or fiber. The glycemic index of some selected foods is given in ***Table 23.3.***

The food item like ice cream has relatively lower glycemic index. This may be explained on the basis of high fat content which lowers the glucose absorption.

The nutritional importance of glycemic index is controversial. This is due to the fact that the foods with low glycemic index need not be good for health. However, low glycemic index foods usually have higher satiety value (creating a sense of stomachfulness), and thus may be helpful in limiting the caloric intake. Nutritionists are of the opinion that foods with high fiber content and low glycemic index (e.g. whole grains, fruits, vegetables) should be preferred for consumption.

Sources of carbohydrates

Carbohydrates are abundant in several naturally occurring foods. These include table sugar (99%), cereals (60–80%), pulses (50–60%), roots and tubers (20–40%) and bread (50–60%).

Requirement of carbohydrates

In a well balanced diet, at least 40% of the caloric needs of the body should be met from carbohydrates.

FIBER IN NUTRITION

The ***complex carbohydrates*** that are ***not digested*** by the human enzymes are collectively referred to as dietary fiber. These include ***cellulose***, hemicellulose, pectin, lignin, gums and mucilage. It may, however, be noted that some of the fibers are digestible by the enzymes of intestinal bacteria (e.g. pectins, gums). For a long time, fiber was regarded as nutritional waste. And now nutritionists attach a lot of importance to the role of fiber in human health. The most important beneficial and the adverse effects of dietary fiber are briefly described.

Beneficial effects of fiber

1. **Prevents constipation :** Fiber helps to maintain the normal motility of gastrointestinal tract (GIT) and prevents constipation.

2. **Eliminates bacterial toxins :** Fiber adsorbs large quantities of water and also the toxic compounds produced by intestinal bacteria that lead to increased fecal mass and its easier expulsion.

3. **Decreases GIT cancers :** The lower incidence of cancers of gastrointestinal tract (e.g. colon and rectum) in vegetarians compared to non-vegetarians is attributed to dietary fiber.

Table 23.3 Glycemic index of some selected foods

Food item	*Glycemic index*
Glucose	100
Carrots	90–95
Honey	80–90
Bread, rice	70–80
Banana, potato	60–70
Sweet potato	50–60
Oranges, apples	40–45
Ice cream, milk	35–40
Fructose	20–25
Soy beans	15–20

4. **Improves glucose tolerance :** Fiber improves glucose tolerance by the body. This is mainly done by a diminished rate of glucose absorption from the intestine.

5. **Reduces plasma cholesterol level :** Fiber decreases the absorption of dietary cholesterol from the intestine. Further, fiber binds with the bile salts and reduces their enterohepatic circulation. This causes increased degradation of cholesterol to bile salts and its disposal from the body.

6. **Satiety value :** Dietary fiber significantly adds to the weight of the foodstuff ingested and gives a sensation of stomachfullness. Therefore, satiety is achieved without the consumption of excess calories.

Adverse affects of fiber

Some of the food fads went to the extent of ingesting huge quantities of rice bran to achieve all the benefits of fiber. This led to several complications. In general, the harmful effects are mostly observed in people consuming ***large quantities*** of dietary fiber.

1. Digestion and ***absorption of protein*** are adversely ***affected.***

2. The intestinal absorption of certain minerals (e.g. Ca, P, Mg) is decreased.

3. Intestinal bacteria ferment some fibers, causing ***flatulence*** and often discomfort.

Sources of dietary fiber

Fruits, ***leafy vegetables***, vegetables, whole wheat legumes, rice bran etc. are rich sources of fiber. The ideal way to increase fiber intake is to reduce intake of refined carbohydrates, besides eating vegetables, fresh fruits and whole grains. In general, vegetarians consume more fiber than non-vegetarians. An average daily intake of about 30 g fiber is recommended.

NUTRITIONAL IMPORTANCE OF LIPIDS

Triacylglycerols (fats and oils) are the concentrated dietary source of fuel, contributing 15-50% of the body energy requirements. Phospholipids and cholesterol (from animal sources) are also important in nutrition. The nutritional and biochemical functions of fat, phospholipids and cholesterol have already been discussed in detail and the reader must invariably refer them now (***Chapters 3*** and ***14***).

Major nutritional functions of lipids

Dietary lipids have two major nutritive functions.

1. Supply triacylglycerols that normally constitute about 90% of dietary lipids which is a concentrated source of fuel to the body.

2. Provide essential fatty acids and fat soluble vitamins (A, D, E and K).

ESSENTIAL FATTY ACIDS

The unsaturated fatty acids which the body cannot synthesize and, therefore, must be ***consumed in the diet*** are referred to as essential fatty acids (EFA).

The fatty acids—***linoleic*** and ***linolenic acid***—cannot be synthesized by humans. In a strict sense, only these two are essential fatty acids. Arachidonic acid can be synthesized from linoleic acid in some animal species, including man. However, the conversion efficiency of linoleic acid to arachidonic acid is not clearly known in man. And for this reason, some nutritionists recommend that it is better to include ***some amount of arachidonic acid*** also in the diet.

Functions of EFA

1. Essential fatty acids are the structural components of biological membranes.

2. Participate in the transport and utilization of cholesterol.

3. Prevent fat accumulation in the liver.

4. Required for the synthesis of prostaglandins.

5. Maintain proper growth and reproduction of the organisms.

Deficiency of EFA

Essential fatty acid deficiency is associated with several complications. These include impairment in growth and reproduction, increased BMR and high turnover of phospholipids. The EFA deficiency in humans is characterized by a scaly dermatitis on the posterior and lateral parts of limbs and buttocks. This condition is referred to as ***phrynoderma*** or ***toad skin***. Poor wound healing and hair loss is also observed in EFA deficiency.

EFA content of foods

The essential fatty acids, more frequently called polyunsaturated fatty acids (PUFA), are predominantly present in ***vegetable oils*** and ***fish oils***. The rich vegetable sources include sunflower oil, cotton seed oil, corn oil, soyabean oil etc.

The fat of animal origin (exception—fish), contain less PUFA e.g. butter, fat of meat, pork and chicken.

Dietary intake of EFA

Nutritionists recommend that at least 30% of the dietary fat should contain PUFA. Very high intake of PUFA (i.e. totally replacing saturated fatty acids) may not be advisable. This is due to the fact that excess PUFA, unless accompanied by antioxidants (vitamin E, carotenes), is believed to be injurious to the cells due to the overproduction of free radicals.

CHOLESTEROL IN NUTRITION

It is proved beyond doubt that the elevated serum cholesterol (> 200 mg/dl) increases the risk of atherosclerosis and coronary heart diseases (For details, ***Refer Chapter 14***). But the role of dietary cholesterol in this regard is still controversial. Cholesterol synthesis continuously occurs in the body which is under a feedback regulation. Some nutritionists believe that dietary cholesterol may not have much influence on the body levels while others recommend to avoid the consumption of cholesterol rich foods (e.g. egg yolk) for a better health.

It is an accepted fact that reduction in serum cholesterol level lowers the risk of heart diseases.

REQUIREMENT OF DIETARY FAT

Consumption of dietary fats and oils is considered in terms of their contribution towards the energy needs of the body. There is a wide variation in fat intake. It is much higher (up to 50% of daily calories) in affluent societies compared to the poorer sections of the people (about 15% of calories). The recommended fat intake is around 20–30% of the daily calorie requirement, containing about 50% of PUFA.

NUTRITIONAL IMPORTANCE OF PROTEINS

Proteins have been traditionally regarded as '***body-building foods***'. However, 10-15% of the total body energy is derived from proteins. As far as possible, carbohydrates spare proteins and make the latter available for body-building process. The functions carried out by proteins in a living cell are innumerable, a few of them are listed hereunder.

Functions of proteins

1. Proteins are the fundamental basis of cell structure and its function.

2. All the enzymes, several hormones, immunoglobulins, transport carriers etc., are proteins.

3. Proteins are involved in the maintenance of osmotic pressure, clotting of blood, muscle contraction etc.

4. During starvation, proteins (amino acids) serve as the major suppliers of energy. It may be noted that the structural proteins themselves serve as 'storage proteins' to meet the emergency energy needs of the body. This is in contrast to lipids and carbohydrates which have the respective storage forms triacylglycerols (in adipose tissue) and glycogen (in liver and muscle).

Essential amino acids

The nutritional importance of proteins is based on the content of essential amino acids. The details of the essential amino acids are described under chemistry of proteins (***Chapter 4***). There are ten essential amino acids—arginine, valine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, tryptophan and threonine (code to recall—AV HILL MP TT). Of these two—namely arginine and histidine—are semi-essential. The requirement of 8 essential amino acids per kg body weight per day is given in ***Table 23.4***. Cysteine and tyrosine can, respectively, spare the requirement of methionine and phenylalanine.

NITROGEN BALANCE

Dietary protein is almost an exclusive source of nitrogen to the body. Therefore, the term nitrogen balance truly represents the protein (16% of which is nitrogen) utilization and its loss from the body.

Nitrogen balance is determined by comparing the intake of nitrogen (chiefly by proteins) and the excretion of nitrogen (mostly undigested protein in feces; urea and ammonia in urine). A normal healthy adult is in a nitrogen equilibrium since the daily dietary intake (I) is equal to the loss through urine (U), feces (F) and sweat (S).

$$I = U + F + S$$

Table 23.4 Requirements of essential amino acids

Amino acid	*Requirement (mg/kg body weight/day)*
Valine	14
Isoleucine	12
Leucine	16
Lysine	12
Methionine*	10
Phenylalanine*	16
Tryptophan	3
Threonine	8

** Cysteine and tyrosine can, respectively, spare (partly) the requirement of methionine and phenylalanine.*

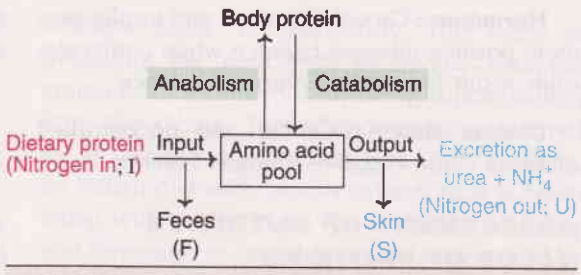


Fig. 23.2 : Overview of nitrogen balance (At equilibrium N input = N output; For positive N balance, N input > N output; for negative N balance N input < N output).

Thus, an individual is said to be in a nitrogen balance if the intake and output of nitrogen are the same (***Fig. 23.2***). There are two other situations—a positive and a negative nitrogen balance.

Positive nitrogen balance : This is a state in which the ***nitrogen intake is higher than the output.*** Some amount of nitrogen is retained in the body causing a net increase in the body protein. Positive nitrogen balance is observed in growing children, pregnant women or during recovery after serious illness.

Negative nitrogen balance : This is a situation in which the ***nitrogen output is higher than the input.*** The result is that some amount of nitrogen is lost from the body depleting the body protein. Prolonged negative nitrogen balance may even lead to death. This is sometimes observed in children suffering from kwashiorkor or marasmus.

Negative nitrogen balance may occur due to inadequate dietary intake of protein (deficiency of even a single essential amino acid) or destruction of tissues or serious illness. In all these cases, the body adapts itself and increases the breakdown of tissue proteins causing loss of nitrogen from the body.

Other factors influencing nitrogen balance

Besides the major factors discussed above (growth, pregnancy, protein deficiency, injury, illness) several other factors influence nitrogen balance.

Hormones : Growth hormone and insulin promote positive nitrogen balance while corticosteroids result in negative nitrogen balance.

Disease states : Cancer and uncontrolled diabetes cause negative nitrogen balance.

ASSESSMENT OF NUTRITIVE VALUE OF PROTEINS

Knowledge on the quantity of dietary protein alone is not sufficient to evaluate the nutritional importance of proteins. This is in contrast to dietary carbohydrates and lipids. The quality of the proteins which depends on the composition of essential amino acids is more important. Several laboratory methods are in use to assess the nutritive value of proteins. Of these, four methods—protein efficiency ratio, biological value, net protein utilization and chemical score—are discussed briefly.

Protein efficiency ratio (PER)

This test consists of feeding weaning (21 day old) albino rats with a 10% test protein diet and recording the gain in body weight for a period of 4 weeks. PER is represented by gain in the weight of rats per gram protein ingested.

$$\text{PER} = \frac{\text{Gain in body weight (g)}}{\text{Protein ingested (g)}}$$

The PER for egg protein is 4.5; for milk protein 3.0; for rice protein 2.2.

Biological value (BV)

The biological value of protein is defined as the ***percentage of absorbed nitrogen retained by the body.***

$$\text{BV} = \frac{\text{Nitrogen retained}}{\text{Nitrogen absorbed}} \times 100$$

For the measurement of BV, the experimental animals, namely weaning albino rats are chosen. They are first fed with a protein-free diet for 10 days. Then they are kept on a 10% protein diet to be tested for BV. Urine and feces are collected for both the periods i.e. protein-free diet and protein diet. Nitrogen is estimated in the diet, feces and urine samples. Biological value can be calculated by the following formula

$$\text{BV} = \frac{(\text{N absorbed} - \text{N lost in metabolism})}{\text{N absorbed}} \times 100$$

$$\text{BV} = \frac{[I_n - (F_n - F_c)] - (U_n - U_c)}{I_n - (F_n - F_c)} \times 100$$

where I_n = Nitrogen ingested

F_n = Nitrogen in feces (on protein diet)

F_c = Nitrogen in feces (on protein-free diet)

U_n = Nitrogen in urine (on protein diet)

U_c = Nitrogen in urine (on protein-free diet)

For the calculation of BV of proteins, experiments can be done even in human subjects. The BV for different protein sources is given in ***Table 23.5***.

The biological value provides a reasonably good index for the nutritive value of proteins. But unfortunately this method has an inherent drawback. It cannot take into account the nitrogen that might be lost during the digestion process. For instance, if the ingested nitrogen is 100 mg, absorbed is 10 mg and retained is 8 mg, the BV 8/10 × 100 = 80. This figure is erroneous, since the major part of the protein (90 mg) did not enter the body at all for utilization.

Net protein utilization (NPU)

NPU is a better nutritional index than biological value, since it takes into account the digestibility factor. The experimental procedure for NPU is similar to that of BV. Net protein utilization can be calculated as

$$\text{NPU} = \frac{\text{Nitrogen retained}}{\text{Nitrogen ingested}} \times 100$$

Chemical score

This is ***based on the chemical analysis of the protein for*** the composition of ***essential amino acids*** which is then compared with a reference protein (usually egg protein). The chemical score is defined as the ratio between the quantitity of the most limiting essential amino acid in the test

protein to the quantity of the same amino acid in the egg protein, expressed as percentage.

$$\text{Chemical score} = \frac{\text{mg of the limiting amino acid /g test protein}}{\text{mg of the same amino acid /g egg protein}} \times 100$$

The chemical score of egg protein, for any one of the essential amino acids, is taken as 100 and the rest of the proteins are compared.

In the ***Table 23.5***, the four methods employed (PER, BV, NPU and chemical score) for the assessment of nutritive value of proteins are compared with regard to the different sources of dietary proteins. Although there are certain variations, anyone of these methods provides sufficient information on the nutritive value of proteins.

Mutual supplementation of proteins.

As is observed from the ***Table 23.5***, the animal proteins are superior in their nutritive value compared to the proteins of vegetable origin. Further, some of the essential amino acids are limiting in certain foods. For instance, ***rice*** and ***wheat*** proteins are ***limiting in lysine*** and ***threonine*** while the protein of Bengal gram is limited in sulfur-containing amino acids (methionine and cystine).

It is fortunate that humans (worldover) have the habit of consuming a mixed diet, with different foods, simultaneously. This helps to overcome the deficiency of certain essential amino acids in one food by being supplemented from the others. This phenomenon is referred to as ***mutual supplementation***. For instance, an Indian diet with cereals (wheat, rice) is taken along with pulses (dal). The limitation of lysine and threonine in cereal proteins is overcome by their supplementation from dal proteins. Simultaneously, the limitation of sulfur-containing amino acids in dal is also compensated by the cereals which are rich in them.

The nutritive value of protein of a particular food can be enhanced by appropriate combination with other foods. Due to the consumption of mixed diets, ***dietary deficiency of essential amino acids is most uncommon***. Further, the principle of mixed diet takes care to supply adequate quantities of essential amino acids to the people subsisting on pure vegetarian diets. It has to be remembered that the effect of mutual supplementation in proteins is best observed with the same meal (or at least on the same day).

Requirement of proteins

The requirement of protein is dependent on its nutritive value, caloric intake and physiological states (growth, pregnancy,

Table 23.5 Nutritive value of food proteins, assessed by PER, BV, NPU and chemical score

Source of protein	*PER*	*BV*	*NPU*	*Chemical score*	*Limiting amino acid(s)*
Egg	4.5	94	90	100	Nil
Milk	3.0	84	75	65	S-Containing amino acids
Fish	3.0	85	70	60	Tryptophan
Meat	2.7	75	76	70	S-Containing amino acids
Rice	2.2	68	60	60	Lysine, threonine
Wheat	1.5	58	47	42	Lysine, threonine
Bengal gram	1.7	58	47	45	S-Containing amino acids
Red gram	1.5	57	46	45	S-Containing amino acids
Groundnut	1.7	55	45	44	Lysine, threonine, S-amino acids
Soyabean	2.1	65	55	55	S-Containing amino acids

PER–Protein efficiency ratio; BV–Biological value; NPU–Net protein utilization; S–Sulfur.

lactation) of the individual. For an ***adult, 0.8-1.0 g protein/kg body weight/day*** is adequate. The requirement should be nearly double for growing children, pregnant and lactating women.

Dietary sources of proteins

The protein content of foods is variable, cereals have 6-12%; pulses 18-22%; meat 18-25%, egg 10-14%; milk 3-4% and leafy vegetables 1-2%. In general, the ***animal proteins*** are ***superior*** than vegetable proteins as the dietary source.

NUTRITIONAL IMPORTANCE OF VITAMINS AND MINERALS

The nutritional aspects including metabolism, biochemical functions, dietary sources, requirements and associated disorders for vitamins (***Chapter 7***) and for minerals (***Chapter 18***) have already been discussed in much detail.

RECOMMENDED DIETARY ALLOWANCES (RDA)

The recommended dietary/daily allowances (RDA) represents the ***quantities of the nutrients to be provided in the diet daily*** for maintaining good health and physical efficiency of the body. It must be remembered that RDA is not the minimum amount to just meet the body needs, but allowance is given for a safe margin.

Factors affecting RDA

1. **Sex :** The RDA for ***men*** is about ***20% higher*** than that for ***women***. Iron is an exception as the requirement is greater in menstruating women. Additional requirements (20-30% above normal) are needed for pregnant and lactating women.

2. **Age :** In general, the nutrient requirement is much ***higher in the growing age***. For instance, the protein requirement for a growing child is about 2 g/kg body wt/day compared to 1 g/kg body wt/day for adults.

RDA an for adult man

The details of RDA for each of the nutrients in relation to age, sex and physiological status is described in the respective chapters. For a quick recapitulation, the RDA of macronutrients (carbohydrate, fat and protein) and selected micronutrients (vitamins and minerals) for an adult man weighing 70 kg are given in ***Table 23.6***.

BALANCED DIET

After discussing the nutritional aspects of dietary ingredients and their RDA, it is worthwhile to formulate a diet for man. A

Table 23.6 Recommended dietary allowance (RDA) of important nutrients for an adult man, weighing 70 kg.

Nutrient(s)	*RDA*
Carbohydrates	400 g
Fats	70 g
Proteins	56 g*
Essential fatty acids	4 g
Vitamin A	1,000 µg **
Vitamin D	5 µg***
Vitamin E	10 µg
Vitamin K	70 µg
Ascorbic acid	60 mg
Thiamine	1.5 mg
Riboflavin	2 mg
Niacin	20 mg
Pyridoxine	2 mg
Folic acid	150 µg
Cobalamin	2 µg
Calcium	800 mg
Phosphorus	800 mg
Iron	10 mg

** 0.8 g/kg body weight/day; ** Retinol equivalents; *** As cholecalciferol*

balanced diet or ***prudent diet*** is defined as the diet which contains different types of foods, possessing the nutrients—carbohydrates, fats, proteins, vitamins and minerals—in a proportion to meet the requirements of the body. A balanced diet invariably supplies a little more of each nutrient than the minimum requirement to withstand the short duration of leanness and keep the body in a state of good health.

The basic composition of balanced diet is highly variable, as it differs from country to country, depending on the availability of foods. Social and cultural habits, besides the economic status, age, sex and physical activity of the individual largely influence the intake of diet.

The Nutrition Expert Group, constituted by the Indian Council of Medical Research has recommended the composition of balanced diets for Indians. This is done taking into account the commonly available foods in India. The composition of balanced diet (vegetarian and non-vegetarian), for an adult man and an adult woman are, respectively, shown is ***Table 23.7*** and ***Table 23.8***.

The Indian balanced diet is composed of cereals (rice, wheat, jowar), pulses, vegetables, roots and tubers, fruits, milk and milk products, fats and oils, sugar and groundnuts. Meat, fish and eggs are present in the non-vegetarian diets. In case of vegetarians, an additional intake of milk and pulses is recommended. The nutritional composition of the most commonly consumed Indian foods given in the Appendix VII. The nutritional aspects of milk are given in the ***Chapter 22***.

Balanced diet in developed countries

Some people in developed countries (e.g. U.S.A) consume excessive quantities of certain nutrients. It is recommended that such people have to reduce the intake of total calories, total fat, saturated fatty acids, cholesterol, refined sugars and salt. The U.S. Government recommends a daily intake of less than 30% fat against the present 40–50% towards calories.

NUTRITIONAL DISORDERS

While the people of developing countries suffer from undernutrition, overnutrition is the major concern of the developed countries. Some of the important nutritional diseases are discussed hereunder.

Table 23.7 Balanced diet for an adult man*

	Sedentary work		*Moderate work*		*Heavy work*	
	Vegetarian (g)	*Non-vegetarian (g)*	*Vegetarian (g)*	*Non-vegetarian (g)*	*Vegetarian (g)*	*Non-vegetarian (g)*
Cereals	400	400	475	475	650	650
Pulses	70	55	80	65	80	65
Green leafy vegetables	100	100	125	125	125	125
Other vegetables	75	75	75	75	100	100
Roots and tubers	75	75	100	100	100	100
Fruits	30	30	30	30	30	30
Milk	200	100	200	100	200	100
Fats and oils	35	40	40	40	50	50
Meat and fish	...	30	...	30	...	30
Eggs	...	30	...	30	...	30
Sugar and jaggery	30	30	40	40	55	55
Groundnuts	...	...	...	...	50	50

**Formulations based on the recommended dietary (daily) allowances (RDA) of the Indian Council of Medical Research (1989)*

Protein-energy malnutrition

Protein-energy malnutrition (PEM)—sometimes called ***protein-calorie malnutrition (PCM)***—is the most common nutritional disorder of the developing countries. PEM is widely prevalent in the infants and pre-school children. ***Kwashiorkor*** and ***marasmus*** are the two extreme forms of protein-energy malnutrition.

Kwashiorkor

The term kwashiorkor was introduced by Cicely Williams (1933) to a nutritional disease affecting the people of Gold Coast (modern Ghane) in Africa. Kwashiorkor literally means ***sickness of the deposed child*** i.e. a disease the child gets when the next baby is born.

Occurrence and causes : Kwashiorkor is predominantly found in children between 1-5 years of age. This is primarily due to insufficient intake of proteins, as the diet of a weaning child mainly consists of carbohydrates.

Clinical symptoms : The major clinical manifestations of kwashiorkor include stunted growth, edema (particularly on legs and hands), diarrhea, discoloration of hair and skin, anemia, apathy and moonface.

Biochemical manifestations : Kwashiorkor is associated with a ***decreased plasma albumin*** concentration (< 2 g/dl against normal 3–4.5 g/dl), fatty liver, deficiency of K^+ due to diarrhea. Edema occurs due to lack of adequate plasma proteins to maintain water distribution between blood and tissues. Disturbances in the metabolism of carbohydrate, protein and fat are also observed. Several vitamin deficiencies occur. Plasma retinol binding protein ***(RBP) is reduced***. The immunological response of the child to infection is very low.

Treatment : Ingestion of ***protein-rich foods*** or the dietary combinations to provide about 3–4 g of protein/kg body weight/day will control kwashiorkor. The treatment can be monitored by measuring plasma albumin concentration, disappearance of edema and gain in body weight.

Table 23.8 Balanced diet for an adult woman*

	Sedentary work		*Moderate work*		*Heavy work*		*Additional allowances during*	
	Vegetarian (g)	*Non-vegetarian* (g)	*Vegetarian* (g)	*Non-vegetarian* (g)	*Vegetarian* (g)	*Non-vegetarian* (g)	*Pregnancy* (g)	*Lactation* (g)
Cereals	300	300	350	350	475	475	50	100
Pulses	60	45	70	55	70	55	...	10
Green vegetables	125	125	125	125	125	125	25	25
Other vegetables	75	75	75	75	100	100	...	...
Roots and tubers	50	50	75	75	100	100	...	...
Fruits	30	30	30	30	30	30	...	...
Milk	200	100	200	100	200	100	125	125
Fats and oils	30	35	35	40	40	45	...	15
Sugar and jaggery	30	30	30	30	40	40	10	20
Meat and fish	...	30	...	30	...	30	...	...
Eggs	...	30	...	30	...	30	...	...
Groundnuts	...	...	...	...	40	40	...	...

*Formulations based on the recommended dietary allowances (RDA) of the Indian council of Medical Research (1989)

Table 23.9 Comparison between kwashiorkor and marasmus

Clinical/biochemical parameter	*Kwashiorkor*	*Marasmus*
Age of onset	Pre-school children (1-5 yr)	Weaned infants (< 1 yr)
Main nutritional cause	Low protein intake	Low calorie intake
Body weight	60–80% of normal	Less than 60% of normal
Growth	Mild retardation	Severe retardation
Oedema	Present	Absent
Facial appearance	Moon face	Like old man's face
Abdomen	Protruding	Shrunken
Skin	Dermatitis	Dry and atrophic
Muscles	Undergo wasting	Weak and atrophic
Subcutaneous fat	Present	Absent
Vitamin deficiencies	Present	Present
Serum albumin	0.5–2 g/dl	2–3 g/dl
Serum cortisol	Normal or decreased	Increased
Fasting blood glucose	Decreased	Decreased
Serum K^+	Decreased	Normal

Marasmus

Marasmus literally means 'to waste'. It mainly occurs in children under one year age. Marasmus is predominantly due to the ***deficiency of calories.*** This is usually observed in children given watery gruels (of cereals) to supplement the mother's breast milk.

The symptoms of marasmus include growth retardation, muscle wasting (emaciation), anemia and weakness. A marasmic child does not show edema or decreased concentration of plasma albumin. This is a major difference to distinguish marasmus from kwashiorkor.

In the ***Table 23.9***, a comparison between kwashiorkor and marasmus is given.

Signs comparable to marasmus in advanced cancer and AIDS

The patients of certain chronic diseases like cancer and AIDS are fequently undernourished, resulting in a codition called ***cachexia***. This is mainly due to the loss of body proteins as a result of ***hypermetabolism***, particularly increased basal metabolic rate. Further, increased oxidation of metabolic fuels leading to thermogenesis is also observed in cancer and AIDS.

Nutritional anemias

Anemia is characterized by lower concentration of hemoglobin (reference 14–16 g/dl) with a reduced ability to transport oxygen. Nutritional anemias are classified based on the size of erythrocytes.

- **Microcytic anemia**—most common, with reduced RBC size. Occurs due to the deficiency of iron, copper and pyridoxine.
- **Macrocytic anemia**—RBC are large and immature. Mostly due to the deficiency of folic acid and vitamin B_{12}.

- **Normocytic anemia**—Size of the RBC is normal, but their quantity in blood is low. Mostly found in protein-energy malnutrition.

OTHER NUTRITIONAL DISORDERS

There are several other nutritional disorders which have been discussed elesewhere. These include obesity, body mass index and atherosclerosis (***Chapter 14***); vitamin deficiency disorders—xerophthalmia, rickets, beri-beri, pellagra, scurvy and pernicious anemia (***Chapter 7***); goiter and other disorders of minerals (***Chapter 18***). The biochemical ramifications of starvation are discussed along with the integration of metabolism (***Chapter 16***).

NUTRITIONAL STATUS AND CLINICAL PRACTICE

The nutritional status of an individual is important in clinical practice. The dietary requirements of nutrients are variable, and are mostly related to age and sex, and physiological status. Some examples of listed

- Infants and young children have increased needs of protein, iron and calcium.
- During teenage, high calcium and magnesium are recommended.
- In pregnancy, and lactation, the requirements of iron, calcium, magnesium, folic acid and vitamin B_6 and B_{12} are increased.
- Elderly people have to take more of vitamins B_6 and B_{12}, folic acid and vitamin D, and minerals chromium, zinc etc.

BIOMEDICAL / CLINICAL CONCEPTS

- *Most of the information on human nutrition is based on the research carried out in experimental animals.*
- *The body at total rest (physical and mental) requires energy to meet the basal requirements such as working of heart, conduction of nerve impulse, membrane transport etc.*
- *Carbohydrates are the most abundant dietary constituents despite the fact that they are not essential nutrients to the body.*
- *Adequate intake of dietary fiber prevents constipation, eliminates bacterial toxins, reduces GIT cancers, improves glucose tolerance and reduces plasma cholesterol.*
- *In general, vegetable oils are good sources for essential fatty acids while animal proteins are superior for the supply of essential amino acids.*
- *The biological value (BV) of protein represents the percentage of absorbed nitrogen retained in the body. The BV for egg protein is 94 while that for rice is 68.*
- *The recommended dietary allowance (RDA) of nutrients depends on the sex and age, besides pregnancy and lactation in the women.*
- *The habit of consuming mixed diet by man is largely responsible to enhance the nutritive value of foods, besides preventing several nutritional deficiencies (e.g. amino acids).*
- *Kwashiorkor and marasmus, the two extreme forms of protein-energy malnutrition in infants and pre-school children, are highly prevalent in developing countries.*

In general, illness and metabolic stress increase the nutritional demands. For instances, liver and kidney diseases reduce the formation of active vitamin D (calcitriol), and storage and utilization of vitamins—folic acid, vitamin B_{12} and vitamin D.

Drug and nutrient interactions

Many drugs are known to lead to potential nutrient deficiencies (***Table 23.10***). For instance, oral contraceptives may result in vitamin B_6, B_{12} and folic acid deficiencies.

Table 23.10 Drug and nutrient interactions

Drug	*Risk of nutrient deficiencies*
Oral contraceptives	Vitamin B_6, vitamin B_{12}, folic acid
Diuretics	Potassium, zinc
Anticonvulsants	Folic acid, vitamin D, vitamin K
Isoniazid	Vitamin B_6
Corticosteroids	Vitamin D, calcium, potassium, zinc
Alcohol	Thiamine, vitamin B_6, folic acid

SUMMARY

1. *The calorific values of carbohydrates, fats and proteins respectively are 4, 9 and 4 Cal/g. These three nutrients (macronutrients) supply energy to the body to meet the requirements of basal metabolic rate, specific dynamic action and physical activity.*
2. *Basal metabolic rate (BMR) represents the minimum amount of energy required by the body to maintain life at complete physical and mental rest, in the post-absorptive state. The normal BMR for an adult man is 35-38 Cal/m^2 body surface/hr.*
3. *Specific dynamic action (SDA) is the extra heat produced by the body over and above the calculated calorific value of foodstuff. It is higher for proteins (30%), lower for carbohydrates (5%), and for a mixed diet, it is around 10%.*
4. *Carbohydrates are the major source of body fuel supplying about 40-70% of body calories. The non-digested carbohydrates (cellulose, pectin) are referred to as fiber. Adequate intake of fiber prevents constipation, improves glucose tolerance and reduces plasma cholesterol.*
5. *Lipids are the concentrated source of energy. They also provide essential fatty acids (linoleic and linolenic acids) and fat-soluble vitamins (A, D, E and K).*
6. *Proteins are the body building foods that supply essential amino acids, besides meeting the body energy requirement partly (10-15%).*
7. *Several methods are employed to assess the nutritive value of proteins. These include protein efficiency ratio, biological value, net protein utilization and chemical score.*
8. *The recommended dietary allowance (RDA) represents the quantities of nutrients to be provided daily in the diet for maintaining good health and physical efficiency. The RDA for protein is 1g/kg body weight/day.*
9. *A balanced diet is the diet which contains different types of foods with the nutrients, namely carbohydrates, fats, proteins, vitamins and minerals, in a proportion to meet the body requirements.*
10. *Protein-energy malnutrition (PEM) is the most common nutritional disorder in the developing countries. Kwashiorkor is primarily due to inadequate protein intake while marasmus is mainly caused by calorie deficiency.*

SELF-ASSESSMENT EXERCISES

I. Essay questions

1. Define BMR. Discuss the factors affecting BMR.
2. Describe the different methods employed for the nutritional evaluation of proteins.
3. Define a balanced diet. Formulate a diet for a medical student.
4. Discuss the protein-energy malnutrition with special reference to kwashiorkor.
5. Give an account of the recommended dietary allowance (RDA) for macro- and micronutrients.

II. Short notes

(a) Essential amino acids, (b) Mutual supplementation of proteins, (c) Caloric value of foods, (d) Specific dynamic action, (e) Energy requirements of man, (f) Fiber in nutrition, (g) Kwashiorkor, (h) Limiting amino acids, (i) Nitrogen balance, (j) Biological value of proteins.

III. Fill in the blanks

1. One calorie of energy is equivalent to _____ Joules (KJ).
2. The endocrine organ most predominantly associated with BMR is _____.
3. The non-digestible carbohydrates are collectively known as _____.
4. The major source of energy to the body is supplied by _____.
5. The nutritional assessment method used to know the most limiting essential amino acid in relation to a standard protein is _____.
6. The daily normal requirement of protein in an adult is _____.
7. The percentage of absorbed nitrogen retained in the body represents _____.
8. The proteins of Bengal gram are limiting in the amino acids _____.
9. The nutrient required in greater amounts in menstruating women compared to men is _____.
10. The biochemical parameter often used as an index for monitoring the recovery from kwashiorkor is _____.

IV. Multiple choice questions

11. The specific dynamic action (SDA) is the greatest for the following foodstuff

    (a) Protein (b) Carbohydrate (c) Fat (d) Vitamins.

12. The reference protein for the calculation of chemical score

    (a) Meat protein (b) Fish protein (c) Milk protein (d) Egg protein.

13. The essential amino acid limiting in rice

    (a) Methionine (b) Tryptophan (c) Lysine (d) Histidine.

14. A continuous supply of energy to the body is necessary to meet the requirements of

    (a) Basal metabolic rate (b) Specific dynamic action (c) Physical activity (d) All of them.

15. One of the following is the most important essential fatty acid in the diet

    (a) Linoleic acid (b) Arachidonic acid (c) Oleic acid (d) Palmitic acid.