4/*ENDOCRINES*

Introduction. The glands of the body may be divided into those with an internal secretion (endocrine glands) and those with an external secretion (exocrine glands). Examples of exocrine glands are the sweat, lacrimal and mammary glands which pass their secretion along ducts to the external surface of the body, and the glands of the mouth, stomach and intestines whose secretions are passed along ducts into the alimentary tract. On the other hand, the endocrine (ductless) glands do not possess any ducts or openings to the exterior. In the unicellular animal, all physiological functions for life processes are served by the one single cell. But with the onset of evolution and formation of the multicellular forms, special systems have been evolved for performing different activities. With the evolution of different systems, necessity arose for correlating their functions; otherwise, the organism cannot live as a harmonious unit. Two systems are empowered with this act of co-ordination, they are the nervous and endocrine systems.

The endocrine (Greek, *endon*=within; *crinein*=to set apart) system consists of a number of ductless glands (Fig. 4.1) which manufacture certain

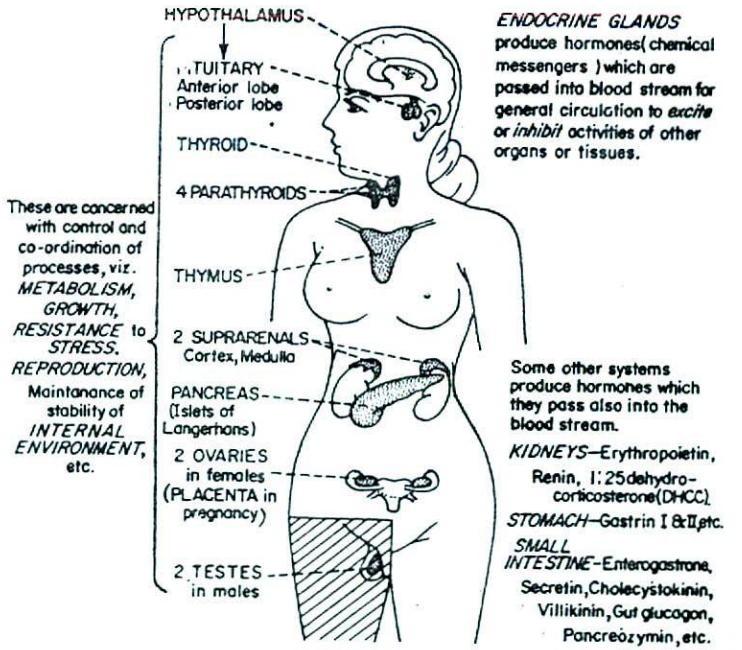


Fig. 4.1. Diagrammatic representation of appropriate anatomical positions of some endocrine glands. Needless to say, although each endocrine gland has specific functions, but all are interdependent, underactivity or overactivity of one tends to affect the whole system.

chemical substances that take an essential part in the life processes of the body. In 1902, Starling and Bayliss while working on the control of digestive secretions found that an extract from the intestinal mucosa contained a substance which, when injected intravenously, brought about a secretion of the pancreatic juice. They went on to show that this substance which they termed **secretin** was released into the blood stream when food entered the small intestine. Following discussions with colleagues at Cambridge in 1905, the word *hormone* (from the Greed, *hormaō*, to excite or arouse or set in motion) was coined for such substances which circulate in the blood and bring about an effect on a distant organ. The word has tended more recently to be restricted to the substances produced by the endocrine glands, although the digestive tract humoral agents (gastrin, secretin, pancreozymin, etc.) are strictly speaking, hormones. However these hormones guide and control the growth, multiplication, differentiation and metabolic activities of various tissues and systems and thereby bring about a correct physiological balance between them. They also regulate the compositon of the various body fluids surrounding the tissue cells and thus establish the normal physiological relation between the cells and the surrounding fluid (internal environment or *milieu interne*).

In this way, throughout the whole life—from the early stage of foetal development right up to the moment of death—the endocrine glands exert a profund influence on various bodily activities including the mental and behavioural patterns.

It has, however, been noted that although endocrines are ideal co-ordinating system of sustained nature, they are not efficient enough where quickness, exact localisation, etc., are required. For this reason, another system of regulators has evolved. This was the nervous system. Functions which are slow and sustained, are carried on by the endocrines. But those that require quickness, accuracy and higher co-ordination have been passed on to the nervous system. However, it has lately been observed that the nervous and hormonal systems are interrelated playing a major role in homeostasis. They are interdependent and their function in homeostasis is integrated since there exists a reciprocal relationship between them. The two systems correlate at the hypothalamic level. This highest centre of the autonomic nervous system also elaborates several hormones that control major endocrine activities of the body through the pituitary, and thus represent a single **neuroendocrine system.**

Definition. Starling and Bayliss have defined hormone as a chemical agent which is released from one group of cells and travel via the blood stream to affect one or more different groups of cells. But Huxley (1935) has defined hormones as **information-transferring molecules,** the essential function of which is to transfer information from one set of cells to another, for the good of the cell population as a whole.

The organ, influenced by a particular hormone is called a **target organ.** When the target organ is an endocrine organ itself, the hormone is named a **trophic** (*trophe*=to nourish) **hormone.** All trophic hormones are secreted by the anterior pituitary alone.

Glands generally possess ducts. But these glands have got no duct. They secrete internally and pour their secretions in the blood stream (or lymphatics). Hence, they are called *ductless* glands.

Some glands are composite in nature, having both internal and external secretions, viz., pancreas, testes, and ovaries. A gland may be temporary, lasting only for a certain period and then dying away, e.g., thymus. Some gland are periodic and recurrent, e.g., placenta and corpus luteum. These are provisional structures, formed to carry out functions on special occasions.

GENERAL CONSIDERATIONS OF HORMONES

The hormones act as bodily catalysts resembling enzymes in certain aspects and are not used during their catalytic actions. The hormones differ from enzymes in the following ways: (a) they are not always protein in structure, the known hormones include protein with mol. wt. of 30,000 or less; (b) the hormones are secreted into the blood stream prior to use, because, circulating levels can indicate the activity of endocrine gland and the exposure of target organs; (c) the hormones are produced in an organ other than that in which they finally effect the action.

1. **Action in low concentration.** Hormones act in very low concentrations, like the vitamins. Frequently, intravenous injection of a hormone in microgram quantities produces an appropriate measurable change in the variable, controlled by the hormone.

2. **Storage, destruction and excretion.** Hormones are not ordinarily stored, except in the gland of origin. They do not have any cumulative action, because they are destroyed and excreted as soon as their functions are over. Some hormones work quickly and are destroyed quickly, e.g., epinephrine (adrenaline); others perform their work slowly and are also disposed of slowly, e.g., thyroxine.

3. **Mode of action.** The hormones influence the activity or extent of certain processes in the body, but do not appear to initiate such processes. In many instances the quantitative changes effected by a hormone cover so wide a range, raising the level of activity of a process from almost zero to +4, that what actually is a quantitative change appears to be qualitative at first sight. It is believed by some that the action of hormones is morphologic. As soon as a hormone reaches its target cell, it can affect the intracellular metabolism to modify the cell function. Peptide and protein hormones somewhat being large molecules will not enter cells rapidly unless specialised transport system is present. For example, insulin may affect cell metabolism by binding to receptors on the surface rather than by entering the cell itself. When the hormone is restricted to surface binding, there are two mechanisms of action—(i) a hormonally induced change in membrane permeability for ions or substrates and (ii) production of a *second messenger* within the cell that will transmit the signals of the hormone (Fig. 4.2).

Ions, glucose and amino cacids are commonly used for hormonal *control of permeability*. Increased entry of glucose into cells through a special transport system is the major path when insulin controls glucose utilisation by muscle. Growth hormone, glucagon, glucocorticoids, testosterone, oestrogen and vasopressin can change membrane permeability whether changes in membrane permeability are primary or secondary

effects of these hormones are debatable. A number of hormones can regulate intracellular metabolism via a second-messenger, *cyclic AMP.*

(a) CYCLIC AMP AND HORMONE ACTION. Cyclic AMP (adenosine 3',5'-monophosphate) is a nucleotide. Robinson, Butcher and Sutherland (1971) have described cyclic AMP as a director of foreign affairs. In this system (Fig. 4.2), the hormone can bind to a receptor on the cell surface that is a part and parcel of adenyl cyclase involved in the formation of cyclic AMP. As a substrate ATP is used by adenyl cyclase which produces cyclic AMP and inorganic pyrophosphate. Changes

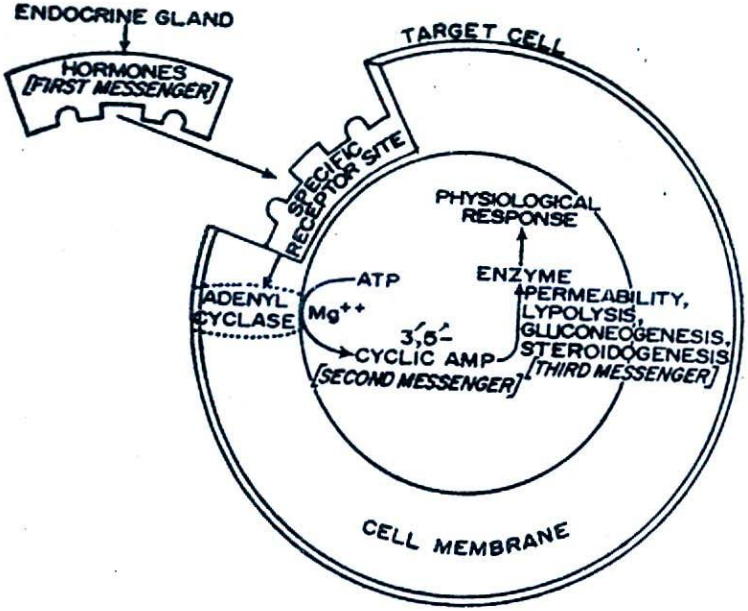


Fig. 4.2. Diagram showing the role of cyclic AMP as a second messenger for hormone action.

in the activity of adenyl cyclase commonly control the intracellular levels of cyclic AMP. But cyclic levels are effected with the destruction of messenger by phosphodiesterase which is inhibited by methyl xanthines. For example caffeine results in much higher cyclic levels in response to activation of adenyl cyclase. Effects of the second messenger are: (a) *there are changes in membrane permeability to amino acids, water and ions;* (b) *the rate of enzymatic reactions can be modified;* (c) *there is release of stored hormones from endocrine glands and* (d) *induction of enzyme* formation. A cyclic AMP-dependent protein kinase may be involved in a number of these effects. Cyclic AMP activates protein kinase which catalyses phosphorylation of either enzymes or controls transcription of DNA by histones. Effects of regulation by cyclic AMP are as follows:

*Process affected or Enzymes	Changes in rate or activity	Tissue/Cell
Growth hormone (GH) release	**Increased**	**Anterior pituitary**
Leutinising hormone (LH) release	,,	,,
Thyrotrophin (TSH) release	,,	,,
Adrenocorticotrophic hormone (ACTH) release	,,	,,
Insulin release	,,	**Pancreas**
Thyroid hormone release	,,	**Thyroid glands**
Calcitonin release	,,	**Parafollicular cell**
Melanocyte dispersion	,,	**Skin**
HCl secretion	,,	**Gastric mucosa**
RNA synthesis	,,	**Bacteria**
DNA synthesis	,,	**Thymocytes**
Amylase release	,,	**Parotid glands**
Amino acid uptake	,,	**Liver and Uterus**
Renin production	,,	**Kidneys**
Calcium resorption	,,	**Bone**
Contractility	,,	**Cardiac muscle**
Water permeability	,,	**Epithelial tissue**
Ion permeability	,,	**Several tissues**
Lipolysis	,,	**Adipose tissues**
Steroidogenesis	,,	**Several tissues**
Kitogenesis	,,	**Liver**
Tyrosine transaminase induction	,,	,,
Serine dehydratase induction	,,	,,
PEP carboxykinase induction	,,	,,
Phosphorylase	,,	**Several tissues**
Protein kinase	,,	,,
Glyconeogenesis	,,	**Liver**
Glycogen synthetase	**Decreased**	**Several tissues**
Overall protein synthesis	,,	,,
Tension	,,	**Smooth muscle**
Amino acid uptake	,,	**Adipose tissue**
Histamine release	,,	**Leucocytes**
Aggregation	,,	**Platelets**
Cell growth	,,	**Tumour cells**
Discharge frequency	,,	**Cerebellar Purkinje cell**
Membrane potential	**Hyperpolarised**	**Smooth muscle**

(b) SYNTHESIS OF ENZYME AT THE NUCLEAR LEVEL. It is enumerated that certain hormones, particularly the steroid hormones are able to penetrate the cell membrane due to their smaller size and lipid permeability. As a result, these compounds can modify intracellular metabolism directly. For example, oestrogen can penetrate the cell membrane and binds to a receptor protein in the cytoplasm. Then it is transported

*From Sutherland.

in the nucleus where it is transferred to a nuclear receptor protein. Steroid hormone appears to modify cell metabolism by inducing new enzyme formation. Such type of control is exerted by regulating the transcription of portions of DNA resulting in the formation of messenger RNA. mRNA leaves the nucleus and is translated by the protein synthesis pathway in the cytoplasm. Effects of steroid hormone commonly require 60 minutes or so to be exerted and may be blocked by inhibition of RNA and protein synthesis. Steroid hormones also appear to exert *permisive effects* on the action of other hormones. The effect of rapidly acting hormones, viz., glucagon depends upon the presence of normal levels of steroid hormones in order to exert their effects.

(c) STIMULATION OF ENZYME SYNTHESIS AT THE RIBOSOMAL LEVEL. It is possible that hormones may alter the rate of synthesis of new enzymes by either activating or inhibiting particular genes directly. Either messenger, transfer or ribosomal RNA might be altered. Actinomycin, puromycin, etc., block such synthesis of RNA. This gene-activating action of some hormones may function both in vertebrates and invertebrates. According to Korner (1965), ribosomes from growth hormone-treated animals have got a modified capacity to synthesise protein in the presence of normal mRNA.

(d) ACTIVATION OF ENZYMES. Certain hormones may act through direct activation of intracellular enzyme systems similar to certain vitamins acting as coenzymes. Minute quantities of certain hormones either activate or inhibit some enzymes both *in vitro* and *in vivo*.

(e) ALTERATION OF MEMBRANE PERMEABILITY. Certain hormones have capacity to alter the permeability of cell membrane so that movement of substances into the cell is affected. Thus insulin increases the transfer of glucose into the cells like muscle and fat. Similarly somatotrophin helps in the entry of amino acids intracellularly.

4. Rate-limiting action. Hormones govern the rates of certain critical or rate-limiting reactions or processes. For example, thyroxine increases oxygen utilisation and O_2 utilisation does not drop to zero after thyroidectomy. Similarly, some glucose utilisation occurs even in the absence of insulin.

5. Dual control. There are many instances—such as, regulation of blood sugar, growth, sex, etc., where more than one hormone take part—some helping the process, others inhibiting it—and thus exerting a dual control.

6. Multiple secretion. A gland may secrete many hormones serving altogether different functions, e.g., pituitary, adrenal cortex, etc.

7. Chemical nature. The majority of hormones are peptides, protein, glycoproteins or amino acid derivatives, steroids. Normally, they are transported in combination with certain fractions of plasma protein. This is advantageous, in that (a) it increases the solubility of the highly insoluble hormone. (b) It provides a reserve of hormone so that ready supply is available. (c) It may slow the rate of hormone destruction, and (d) it determines the rapidity and the onset and duration times of hormonal action.

8. Interrelations of endocrines. None of the endocrine glands is completely independent. They are closely interrelated and interdependent. The interrelationship may be synergistic or complementary, permis-

sive and inhibitory. They live like a harmonious family, guiding and controlling one another, being complementary to some and antagonistic to others. Consequently, hypoactivity or hyperactivity of a gland will lead to corresponding changes in the other related endocrines. For instance, hyperpituitarism (anterior) may lead to hyperthyroidism. A reciprocal relation is often observed, e.g., if a gland A stimulates another gland B, then B depresses A, directly or indirectly through other glands. For instance, anterior pituitary stimulates thyroid, gonads and adrenal cortex; while the secretion of any one of three glands depresses the particular trophic hormone of anterior pituitary. As a corollary to this, one may say that a hormone often regulates its own secretion indirectly through other glands. This is known as the **feedback** or **push-pull** mechanism. This may be a fundamental rule of endocrine regulation.

9. Control of endocrine system. The principal function of endocrine system is to maintain the meliem interieur (milieu interne) constant.

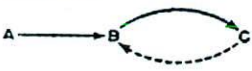


Fig. 4.3. Negative feedback showing when variable *A* increases, variable *B* increases leading to an increase in variable *C*. But an increase in variable *B* leads to a decrease in variable *A*.

For the maintenance of such constancy many homeostatic mechanism come into play. Many of such homeostatic processes are of negative feedback type and a few are of possitive feedback type. Fig. 4.3 demonstrates that A stimulates B, and B stimulates the formation of C. But increased formation of C inhibits B. This is the negative feedback. Fig. 4.4 demonstrates the possitive feedback. Here A stimulates B, and B stimulates the formation of C and again C stimulates B.

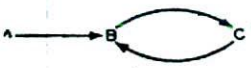


Fig. 4.4. Diagram shows positive feedback.

The simplest form of endocrine control that operates in case of secretions of insulin, glucagon, parathyroid, thyrocalcitonin (calcitonin)



Fig. 4.5. Endocrine control showing action of hormones on specific cell promoting thereby a change in the extracellular fluid which regulates the output of hormones by the end organ.

and also of aldosterone (partly) has been shown in Fig. 4.5. In this system endocrine glands secrete hormones which stimulate the target cells. The target cells, on the other hand, release substances that by negative feedback mechanism decreases the rate of release of hormones. In this system, neural or pituitary control seems to be absent. A and A_1 represent other factors operating independently of feedback loop and influencing respectively the activity of endorine gland and target cell.

A more complex system of control operates in the regulation of secretion of aldosterone from the adrenal cortex (Fig. 4.6). Renin plays an important role in such regulation. Renin is a protein-spliting enzyme produced by the juxtaglomerular cells which surround the afferent arteriole just before it reaches the glomerular capillary tuft. Renin converts circulat-

ing renin substrate to the decapeptide, angiotensin I. A converting enzyme is produced in lungs and blood by the removal of two C-terminal amino acids of angiotensin I to form biologically active octapeptide, angiotensin II. Angiotensin II has an immediate effect on the peripheral arterioles to cause vasoconstriction and increased systemic blood pressure, and also stimulates secretion of aldosterone by the adrenal cortex and the thirst centre. The renin-angiotensin system is discussed in detail elsewhere.

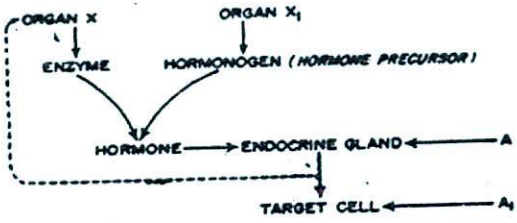


Fig. 4.6. Diagrammatic representation of endocrine control by release of hormonogen (a hormone precursor) from two organs—x and x_1. A and A_1 represent the same function as in Fig. 4.5. Dotted line indicates inhibition.

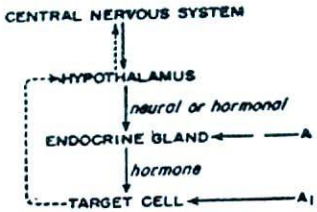


Fig. 4.7. Diagram showing the endocrine control achieved by the hypothalamus. A and A_1 indicate the same function as in Fig. 4.5. Dotted line represents inhibition.

In next order of complexity, certain endocrine glands (EG) are under the direct control of hypothalamus. This type of control system has been described in Fig. 4.7. Adrenaline of adrenal medulla, growth hormone of anterior pituitary and vasopressin of posterior pituitary fall within this control system. Hypothalamus controls the endocrine activity either by neural mechanism or by hormonal mechanism. But the activity of the hypothalamus is not controlled by the hormone of the gland itself but indirectly by the change in the plasma constituent occurs due to hormone.

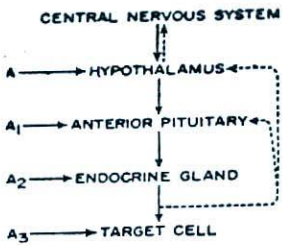


Fig. 4.8. Diagram represents the complex feedback systems that operate in controlling the endocrine function through hypothalamus → anterior pituitary → endocrine gland → target cell. In this system, feedback control is achieved at the level of the hypothalamus and anterior pituitary. A, A_1, A_2, A_3, operate independently and separately for controlling the function of the hypothalamus, anterior pituitary, endocrine gland and target cell respectively.

The highest complexity in the regulation of endocrine function is observed in case of hormones secreted from the target glands of the anterior pituitary. The general principle of controlling mechanism has been presented in Fig. 4.8. In such system anterior pituitary function is controlled by the hypothalamus through secretion of specific-releasing factors (hormonal). The anterior pituitary hormones are specific for specific target endocrine glands.

The normal control of endocrine mechanism is achieved through the negative feedback mechanism at the level of hypothalamus by the product of the final target endocrine glands. TSH and ACTH fall within this category. Sometimes, feedback control is exerted at the level of anterior pituitary by the hormones of the target endocrine gland.

10. **Interrelations with vitamins.** Vitamins are directly or indirectly concerned with hormone functions. For instance, vitamin D (calciferol) and parathyroid, vitamin E (tocopherol) and gonads, ascorbic acid (vitamin C) and adrenal cortex, gonads and so on.

11. **Dysfunction of an endocrine gland**

(i) *Nature of dysfunction.* A gland may be upset in two ways—producing either *too little* or *too much* of its normal secretion, but as a rule *does* not produce any abnormal compound. (Although theoretically probable.)

(ii) *Effects of dysfunction.* The effects produced by the hypoactivity or the hyperactivity of a particular gland should not be explained by the fault of that gland alone but also (a) by the changes produced in other related glands (central action) and (b) by the disturbance of the peripheral actions of the other related hormones. As for instance, diabetes mellitus should not be regarded as due to the lack of insulin alone but due to the unopposed activity of the antagonistic group, e.g., anterior pituitary, etc.

(iii) *Causes of dysfunction.* The cause of a glandular disturbance may not always lie in the gland itself, but somewhere outside the gland. The causes may be summarised as follows: (a) The gland itself may be diseased. (b) There may be lack of the raw material with which the hormone is manufactured, e.g., iodine deficiency in Goitre. (c) The primary seat of the disease may be in some other gland which normally controls its action, e.g., hyperthyroidism may be secondary to hyperactivity of the anterior pituitary. (d) Overwork of the gland, e.g., excessive carbohydrate feeding is supposed to be a cause of diabetes mellitus. (e) Disturbed nervous system—stress, strain, faulty—adaptation, etc., may lead to endocrine troubles (vide Adrenal Cortex). (f) Some of the endocrine disorders are genetic, due to inborn defects. Examples are hereditary diabetes, some types of hypothyroidism, adrenal cortical disturbances, etc.

12. **Therapeutic administration of hormones.** In selecting a particular hormone for therapeutic purposes, the nature of endocrine deficiency, whether acute or chronic, is to be looked into. Accordingly, a quick acting or slow and prolonged acting preparation is administered. Some hormones are effective by mouth while others are not. The principle is that *if a hormone be broken down in the digestive tract or inactivated by liver while being absorbed through the portal vein—it cannot be effective by oral route.* Thyroxine and some sex hormones are effective by mouth, while protein hormones like insulin, pituitary hormones, etc., are not. Hence, they are administered by other routes.

In some cases the chemical structure of natural hormone molecule has been altered in the laboratory to make it suitable for therapeutic purposes.

The following is a comprehensive list of endocrine glands in the human body:

I. **Glands with definite endocrine functions:**

1. **Pituitary**—anterior lobe and posterior lobe.
2. **Thyroid.**
3. **Parathyroid.**
4. **Adrenals**—cortex and medulla.
5. **Ovary.**
6. **Testes.**
7. **Islets of Langerhans** (endocrine pancreas).
8. **Placenta**—during pregnancy.

II. **Glands with probable endocrine functions:**
1. **Thymus.** 2. **Pineal body.**

In addition to above, there are certain other substances which should be regarded as hormones. For instance, (1) *Gastrin* I & II and the *haemopoietic factor*—secreted by stomach. (2) *Secretin, enterogastrone, cholecystokinin-pancreozymin, villikinin,* etc., —produced by small intestine.

Methods of investigation

The following methods may be adopted to study the functions of an endocrine gland:

I. **Histological:**

1. *Histometric method.* Measurement of the size of the cell as in the case of hyperfunction or hypofunction of the thyroid.

2. *Histochemical method.* Nature of cells, their straining reactions and their changes in hypofunction and hyperfunction of the gland.

II. **Clinical:** Effects of (1) *hypofunction* and (2) *hyperfunction* of the gland due to disease or hereditary abnormality.

III. **Experimental:**

1. (i) *Extirpation or surgical removal of the gland.* Different degree of hypofunction of the gland may be produced by its removal to varying degree. Abnormality due to removal of a gland might also be due to unopposed release of hormone from another gland. Sometimes clinical condition due to genetic endocrine abnormality is noted. As for example, when the anterior lobe of the pituitary is removed the following important abnormalities appear : (a) there is arrested growth; (b) atrophy of the gland and indirectly of the accessory sex organs appear; (c) there is atrophy of the adrenal cortex and thyroid; (d) involution of the mammary gland and suppression of milk secretion occur; (e) there is a tendency to hypoglycaemia and glycogen reduction in muscle and liver; (f) diminished resistance to infection and shock and depression of spontaneous activity may appear; (g) there is lowered metabolic rate. Since this gland secretes hormones which control other endocrine glands, effects of hypophysectomy are widespread. (ii) *Chemical ablation:* (a) destruction of thyroid gland by ionising radiations of administered ^{131}I, (b) damage to the β-cells of the islets of Langerhans by alloxan, (c) hypofunction of adrenal cortex produced by amphenone, are a few examples. (d) Recently improved technique immunochemistry in producing antibodies (antihormones) by heterologous hormones also produce hypofunction of the endocrine organs.

2. *Administration* (oral or injection) of a suitable extract or *implantation* of the gland—(a) should rectify the effects of clinical hypofunction or experimental extirpation, (b) should produce effects similar to clinical hyperfunction if given to a normal animal. As for classical example, when the pancreas of dogs is removed, the glucose content of blood begins to rise within a very short time, urine gives a positive test for sugar, excretion of nitrogen is elevated and the animal is died within 2 to 3 weeks.

IV. **Chemical:**

1. *Isolation of the active principle from* the gland and also from blood. Sometimes the urine provides a good source. A convenient chemical method for the estimation of hormones, either qualitative or quantitative,

is not available in most cases. Methods of chromatographic separation have facilitated such estimations.

2. *Artificial synthesis* of the active principle.

V. **Radio-isotopic methods:** Radio-isotopic tracer method has provided a valuable tool in the study of metabolism of the hormones, both synthesis and degradation. Application of 'autoradiographic' (radioautographic) method is an additional implement in such study.

VI. **In vitro studies:** In vitro techniques, which are supposed to provide identical results as in vivo ones are also often employed. They may be of the following types: (1) perfusion of whole organs, (2) biochemical study with very thin slices of the living organ suspending in appropriate physiologic solutions, and (3) studying enzymatic transformations with tissue minces and homogenates.

Essential Properties of a Hormone

1. **Easy solubility.** Since the hormones are carried in the blood stream, they should be soluble in water or should remain in such a form in which they can be easily carried.

2. **Low molecular weight.** Since they readily pass out of the capillaries, hormones should have small molecular weight.

3. **Easily diffusible.** This is true almost in all cases excepting insulin, thyroglobulin, etc., having comparatively bigger molecules.

4. **No cumulative action.** It must be readily destroyed or inactivated or excreted. This is necessary in order to prevent cumulative or continued action. As soon as their functions are finished they should be disposed of.

Major endocrine glands and hormones in controlling body functions. Release of hormones into the blood stream and spread of

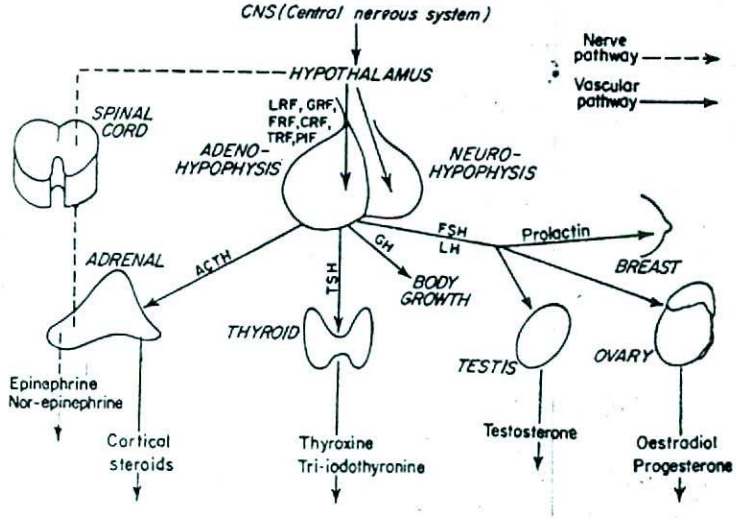


Fig. 4.9. Diagram shows the some relationship between the endocrine and nervous system and also shows that neurosecretory structures (i.e., hypothalamus, neurohypophysis and adrenal medulla) are controlled by direct nerve supply and other glands' activity is regulated by hormonal or humoral agents carried in the blood.

depolarisation through nerves transmit signals which allow an animal to respond to changes in its environment. Either a nerve impulse or a change in the concentration of some substance into the blood which perfuses the gland may trigger such release (Fig. 4.9). Such a substance called hormone is produced by a gland or specialised neurosecretory cell, is often stored in this cell, and is released into the blood stream in response to some specific stimulus. As soon as the hormone reaches its target cells, it modifies the function of the cell to remove the stimulus that primarily caused the release of the hormone. This *negative feedback* mechanism operates to maintain homeostatis within the organism. Signals that are transmitted through nerves possess the advantage of localisation and speed to single cells within a large group of similar cells.

1. **Hypophysis.** ACTH (adrenocorticotrophic hormone), GH (growth hormone), FSH (follicle-stimulating hormone), MSH (melanocyte-stimulating hormone, TSH (thyrotrophin), LH (leutinising hormone), prolactin, vasopressin and oxytocin.
2. **Thyroid.** Thyroxine, tri-iodothyronine, calcitonin.
3. **Parathyroid.** Parathyroid hormone.
4. **Adrenal gland.** Glucocorticoids, mineralocorticoids, epinephrine, norepirephrine,
5. **Ovary.** Oestrogen, progesterone.
6. **Testis.** Testosterone.
7. **Islets of Langerhans** (pancreatic islets). Insulin, glucagon.
8. **Skin** Vitamin C.
9. **Hypothalamus:** TRF (thyrotrophin-releasing factor), CRF (corticotrophin-releasing factor), GRF (growth hormone-releasing factor), FHRF (follicle-stimulating hormone-releasing factor), LHRF (leutinising hormone-releasing factor), PIF (prolactin-inhibiting factor) GHIR (growth hormone-inhibiting factor), MIF (melanocyte-inhibiting factor).

An outline of synthesis and release of and assay of hormones. Endocrine glands commonly possess some anatomical characteristics. These typical glands, except for the hypothalamus and neurohypophysis which are a special class of neurosecretory organs, are composed of masses of secretory cells which are arranged in clumps and columns. As for example, the thyroid gland is arranged in thyroid acini or alveoli with their secretory products being stored in the thyroid lumen. However, generally the hormones or their parent substances are stored in the cells as granules or dropletes. Though they are glandular in nature, there is no presence of ducts. Since the blood exercises as a *duct* and takes away secretory products, they are highly vascularised.

Many of the hormones are peptides or proteins in nature. The synthesis of protein hormones takes place on ribosomes attached to the endoplasmic reticulum. These hormones are packaged into zymogen or storage granules in the Golgi complex. The formation of this type of granules differ from cell to cell and serves as a *means of identifying the hormone which is in the granule.*

The synthesis of adrenal and gonadal steroid hormones takes place in a complicated series of reactions. Steroid hormones which are synthesised from cholesterol occur into both the cytoplasm and mitochondria of the cells. These cells are commonly rich in mitochondria and adaptation

of mitochondrial structure to facilitate steroid synthesis are present in some cases. In these glands, cholesterol is stored into lipid droplets as cholesterol esters. During the stimulation of the gland, the hormone is produced and is synthesised either from the stored precursor or from cholesterol present in the plasma.

The process of release of hormone is called **emiocytosis** or reverse pinocytosis. The membrane of storage zymogen granules fuses with the plasmalemma of the cell and the product is released into the interstitial space.

When the hormone is present in extremely low concentration, it has effects. For this reason, assay methods are sufficiently sensitive and specific. Initially **bioassays** measure all hormones, but they are often insensitive and non-specific. Bioassays are dependent on the effect of a hormone either on a living animal or on a tissue preparation. Once a hormonal activity has been identified in an extract of a gland, the substance responsible for the activity could be purified and the chemical structure determined. Then **chemical assays** were divised. By these methods thyroid and steroid (adrenal and gonadal) hormones are satisfactorilly estimated. But the estimation of peptide hormone in the concentration in plasma in the range of millimicrogram per ml and other protein hormones in mg per ml, found difficult to purify and quantitate by usual fractionation, then **radioimmunoassay** was devised (1964). By the use of this method a pure hormone can be prepared by the gland which synthesises the compound. At present radioimmunoassays have been established for growth hormone, insulin, glucagon, follicle-stimulating hormone, leutinising hormone, thyrotrophin, adrenocorticotrophic hormone, vasorpressin, parathyroid hormone, calcitonin, α- and β-melanocyte-stimulating hormones. When a hormone is injected in an appropriate species of animal, antibodies against the hormone are formed. If repeated injections of hormone are given, the serum of the animal comprises a high titer of antibody against the purified hormone. The immunoreactive hormone in plasma and other body fluids determines the measurement of the serum. Radioactive hormone can be prepared by reaction of the thyroid residues of the protein with ^{131}I. Radioimmunoassay of non-protein hormones is based on the presence of specific-binding protein for thyroid, adrenal and gonadal (steroid) hormones in plasma. Normal plasma contains a specifically bound carrier protein.

HYPOPHYSIS (PITUITARY GLAND)

In 16th century Vesalius named this gland the pituitary (L. *pituita* = phlegm or mucus) filtered and collected wastes from the brain and these lubricated the nasal and pharyngeal passages. When the true function of the gland was established the term hypophysis (*hypo* + Gr. *phyein* = to grow) was substituted. But the gland is still often called the pituitary.

Anatomy. The pituitary gland is a reddish-grey, small oval-shaped structure, situated at the base of the brain, in the sella turcica of the sphenoid bone. It is attached to the floor of the third ventricle by a stalk. Average weight in adults 0·5 to 0·6 g. The weight varies with age, sex and physiologic state. It is somewhat larger in the females, weighing

from 0·6 to 0·7 g. It increases in size through the fourth decade of life, then slowly decreases. It also enlarges during pregnancy. Average dimensions are 10 mm (anteroposteriorly) × 6 mm (dorsoventrally) × 13 mm (laterally). Anatomically, it consists of two parts: the **anterior lobe** and **posterior lobe.** But microscopically, it consists of six parts: (1) **Pars distalis** or **Pars anterior,** (2) **Pars tuberalis,** (3) **Pars intermedia,** (4) **Pars nervosa (Lobus nervosus)** or **Pars posterior (Processus infundibuli),** (5) **Median eminence of tuber cinereum,** and (6) **Infundibulum (Pituitary stalk).** The pars intermedia is separated from the pars distalis or pars anterior by the *interglandular cleft.* The first three parts are collectively known as the **glandular division** or **lobus glandularis** (adenohypophysis); the last three as the **neural division** (neurohypophysis) (Fig. 4.10).

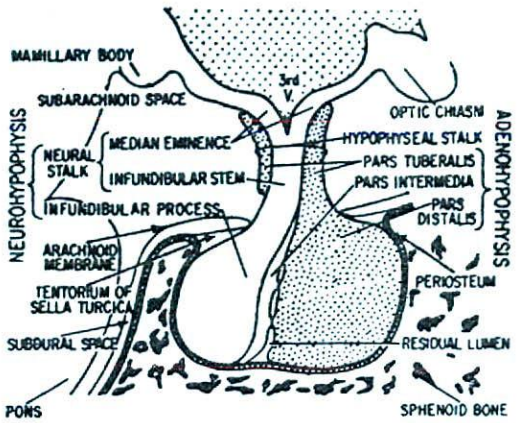


Fig. 4.10. Diagram showing the component structures and systems of nomenclature for the hypophysis and its anatomical position.

Development. The pituitary body develops from two outgrowths. A *glandular* diverticulum—**Rathke's pouch**—grows upwards from the primitive buccal cavity (ectoderm) and meets with a similar *neural* downgrowth from the floor of the third ventricle. The stalk of Rathke's pouch disappears. But that of the neural downgrowth persists and forms the pituitary stalk **(infundibulum).** From the anterior wall of Rathke's

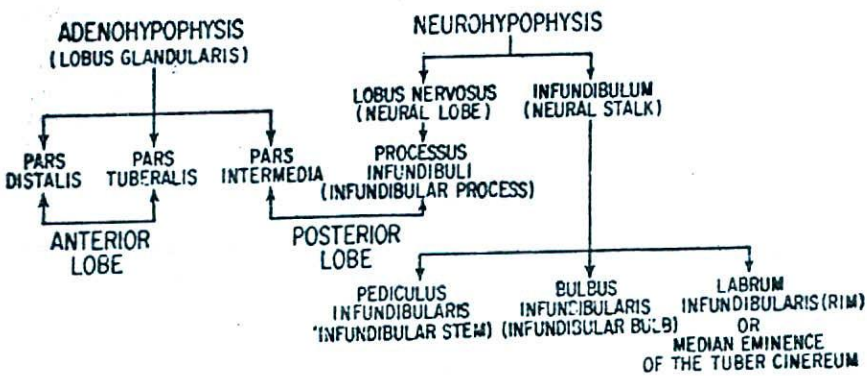


Fig. 4.11. Schematic reproduction of systems of anatomical nomenclature for the hypophysis.

pouch develops the pars distalis or pars anterior; from the top arises the pars tuberalis; from the posterior wall develops the pars intermedia. The pars intermedia invests the pars posterior or pars nervosa to some extent. The original cavity of Rathke's pouch forms the interglandular cleft. The original lumen of the nervous downgrowth completely disappears in man but persists in cat. Thus the so-called **anterior lobe** consists of pars distalis (pars anterior) and pars tuberalis, whereas the **posterior lobe** consists of pars intermedia and the processus infundibuli. Sometimes the term 'posterior lobe' is loosely used as a synonym for the 'pars nervosa'.

Divisions of the pituitary gland as recommended by the International Commission on Anatomical Nomenclature have been presented in Fig. 4.11.

Throughout the book this nomenclature will be followed.

The pituitary gland secretes several hormones which have been presented in pages 4-16, 4-17.

STRUCTURE AND FUNCTIONS OF ADENOHYPOPHYSIS

Adenohypophysis includes **pars distalis, pars tuberalis** and **pars intermedia.**

Histology of pars distalis (Fig. 4.12). **Pars distalis or Pars anterior** forms about 75% of the hypophysis and consists of columns or masses of epithelioid cells with numerous blood sinuses.

Schonemann in 1892 first recorded the diversity of cell types in the anterior pituitary gland.

The earlier classification of the cells of pars distalis into **acidophils, basophils** and **chromophobes** are inadequate. The present idea is that the different pituitary hormones are liberated by separate cell types. So possibly six kinds of cells secrete six separate hormones. Studies employing histochemical, immunofluorescent and electron microscopic techniques have made such classifications possible.

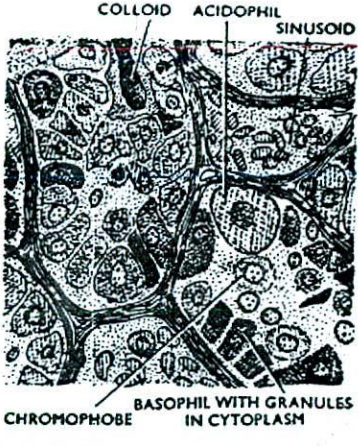


Fig. 4.12. Diagrammatic representation of histological structure of pars distalis only.

(a) **Chromophobes** [C (chief) cells or reserve cells, 50%] are so named because these sells are not stained with either basic dyes or acid dyes and lack typical secretory granules. Chromophobe cells in general have got less cytoplasm than chromophils. The earlier hypothesis is that the chromophobes are non-functional and have recently been doubted. Small secretory granules have been identified inside these cells and it is postulated that they may be either resting reserve ones or cells depleted of secretions or contain precursors not yet differentiated. Tumours in man producing excess corticotropin and prolactin cause alterations in these cell types. Romeis classified the chromophobes as gamma cells.

The major hormones secreted by the pituitary gland

HORMONES	ORIGIN	CELLULAR SOURCE	CHEMISTRY	CONTROL	TARGET CELLS OR ORGANS	PRINCIPAL ACTIONS
Growth (GH) or STH	Pars distalis	Acidophils	Protein	Blood sugar, Hypothalamus	Somatic cells	Controls growth of bone and muscle; anabolic effect on nitrogen metabolism; carbohydrate and fat metabolism; elevates glycogen stores of skeletal and cardiac muscles.
Thyroid-stimulating (TSH) or Thyrotrophin	Pars distalis	Basophils	Glycoprotein	TRF, Thyroxine	Thyroid gland	Controls rate of iodine uptake by thyroid tissue and influences synthesis of the thyroid hormones.
Adrenocorticotrophin (ACTH)	Pars distalis	Basophils	Polypeptide	CRF, Cortisone	Adrenal cortex	Stimulates secretion of adrenal cortical steroids by adrenal cortex, certain extracortical actions.
Luteinising (LH) or Interstitial cell-stimulating (ICSH)	Pars distalis	Basophils	Glycoprotein	LRF, Oestrogen and Progesterone	Corpus luteum or interstitial cells of testes	*Testis:* Stimulates interstitial cells of Leydig, thus promoting the production of androgen. *Ovary:* Controls formation of corpora lutea, secretion of progesterone; possibly acts in conjugation with FSH.
Follicle-stimulating (FSH)	Pars distalis	Basophils	Glycoprotein	FSH-RF and Oestrogen	Graafian follicles	*Testis:* Has possible action on seminiferous tubules to promote spermatogenesis. *Ovary:* Controls growth of ovarian follicles; function with ICSH to cause oestrogen secretion and ovulation.

The major hormones secreted by the pituitary gland

HORMONES	ORIGIN	CELLULAR SOURCE	CHEMISTRY	CONTROL	TARGET CELLS OR ORGANS	PRINCIPAL ACTIONS
Prolactin or Luteotrophic (LTH)	Pars distalis	Acidophils	Protein	PIF	Mammary gland	Controls proliferation of mammary gland and initiation of milk secretion; prolongs functional life of the corpus luteum, the secretion of progesterone.
Melanocyte-stimulating (MSH) or Intermedin	Pars intermedia	Basophils and Acidophils	Polypeptide	MIF	Melanoblast cells	Controls dispersion of pigment granules in melanophores; darkening of the skin.
Vasopressin (antidiuretic hormone, ADH)	Neurohypophysis	Hypothalamic supra-optical and Paraventricular nuclei	Polypeptide	CNS	Kidneys, Blood vessels, etc.	Elevates blood pressure through action on arterioles; promotes resorption of water by kidney tubules.
Oxytocin	Neurohypophysis	Hypothalamic paraventricular nuclei	Polypeptide	CNS	Uterus, Mammary glands, etc.	Affects postpartum mammary glands, causing ejection of milk; promotes contraction of uterine muscle; probable action in parturition and in sperm transport in female tract.

(b) **Chromophil cells** are so named as they have got affinity toward dyes. Early staining methods show only two types of chromophils: (i) acidophil (α-cell or eosinophil or oxyphil, 35%) and (ii) basophil (β-cell, 15%). *These proportions vary depending upon physiological state, age and sex.* The cytoplasm of acidophil is stained with acid dye and that of basophil, with basic dye. But studies with most modern histochemical, immunofluorescent and also with electron microscopic techniques, in recent years have permitted the demonstration of different pituitary cell types in each of acidophil and basophil cells as specific hormone is secreted by a distinct cell type.

(i) **Acidophil cells.** Recent studies claim that acidophil cell secretes both growth hormone and lactogenic hormone. For the secretion of these two hormones there are separate cell types—somatotrophic and lactotrophic, and these have been recognised by electron microscopic studies. Differential centrifugation of homogenates of the anterior lobe, the rapid sedimenting granules, which are mainly acidophilic, have been shown to contain somatotrophin and prolactin.

Somatotrophic cells, orangeophils or **α-acidophils.** These cells are oval or rounded and the cytoplasm is packed with dense, round granules—350-400 nm in size. These cell types are stained with orange G of an Azan stain. Tumours of acidophil cells are associated with acromegaly and gigantism.

Lactotrophic cells, carminophils or **ϵ-acidophils.** These types of acidophil cells are preferably stained with azocarmine, erythropsin or acid fuchsin. Granules of this type of cells are fewer and larger, ranging from 600-900 nm in size. Number of these cells varies in relation to the reproductive cycle—increasing significantly during pregnancy and lactation. These cells are believed to secrete prolactin. These are often ovoid or ellipsoid.

(ii) **Basophil or mucoid cells.** There are different kinds of basophil cells secreting different types of trophic hormones like ACTH, TSH, FSH and LH. ACTH is protein whereas the last three hormones are glycoprotein (mucoid cells). Differential staining methods have been able to stain the different basophilic cell types.

Thyrotrophic cells. The cell that makes TSH has been termed as *thyrotroph* and is a large polygonal one with small nuclei, taking PA-Schiff (periodic acid-Schiff) stain combined with aldehyde thionine. These thyrotrophic cells reveal blue-purple granules with above stain. Ablation of thyroid causes hypertrophy of these cells which are then called commonly as "thyroidectomy cells".

Gonadotrophic or castration or signet-ring cells. The gonadotrophic cells are also positive with PA-Schiff stain. Cells that secrete FSH (folliculotrophs) are larger rounded cells whose secretory granules are spherical between 150 and 300 nm in diameter. Granules of these cells become red with the combined PA-Schiff and aldehyde thionine stain.

The cells that secrete LH or ICSH (luteotrophs or interstitiotrophs) are comparatively small and with scant cytoplasm. The cells are rounded or polygonal and found in proximity of the sinusoidal capillaries. These cells also contain spherical secretory granules having diameter 100-300 nm. Granules of these cells become coloured purple with combined PA-Schiff and aldehyde thionine stain.

Corticotrophic cells. Immunofluorescent studies with antibodies that react with corticotrophin have definitely localised the hormone within basophilic cells, but some cells also may fluorescence with antibodies against thyrotrophin. Basophil-cell tumours are associated with Cushing's syndrome. It has been suggested that certain granule-containing chromophobe may be concerned with the production of ACTH. Considering the similarities in the amino acid sequences in the structure of melanocyte-stimulating hormone (MSH) and ACTH, it is reported that these two hormones are secreted from the same cells. In lower vertibrates where the pars intermedia is distinctly present, MSH is secreted from the specific cell type of this lobe. But in man there is no distinct pars intermedia and existence of separate cell type for MSH is doubtful. Pathophysiological observations in man suggest that ACTH and MSH may have common cell type.

Blood supply. The anterior lobe of pituitary gland gets blood supply from several **superior hypophyseal arteries** originating from the internal carotid artery and circle of Willis. The superior hypophyseal arteries supply the anterior lobe through two sets of blood vessels (Fig. 4.13). One set of blood vessels supplies the lobe directly and forms a sinusoid. Another set of blood vessels reaches the capillary plexus of the median eminence and the infundibular stem. This capillary plexus is a spiral structure called gomitoli around the hypophyseal stalk. This capillary plexus is drained by a long portal vein which ultimately ends in the sinusoids of anterior lobe. This second set of blood supply is the **hypothalamico - hypophyseal portal system** and is important for controlling the anterior pituitary secretory function through hypothalamic releasing factors (RF). Vascular connections of the anterior lobe with the hypothalamic nuclei are thus made through this vessel. **Inferior hypophyseal arteries** give off several endarteries. Venous drainage from the anterior lobe is made through the cavernous sinus.

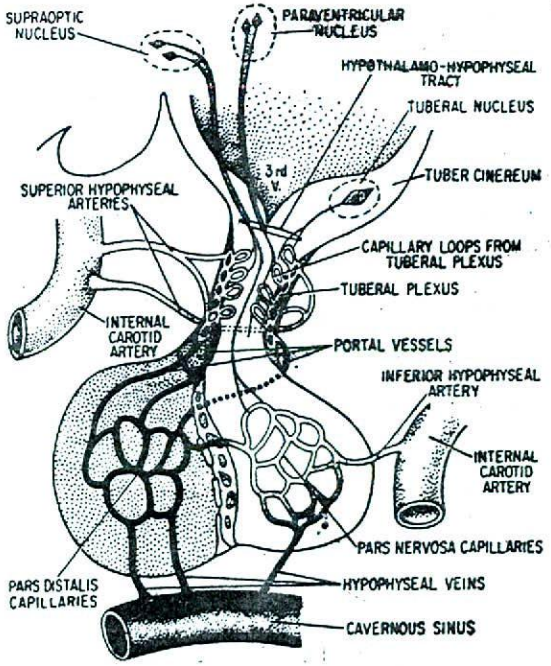


Fig. 4.13. Diagram showing the hypophyseal blood supply.

Nerve supply. There is definite evidence of a nervous control of

the anterior lobe, but only a few fibres either from the hypothalamo hypophyseal tract of nerve fibres or from the carotid plexus of the cervica sympathetic or from the greater superfical petrosal nerves have bee traced to it. They are probably vasomotor nerves.

FUNCTIONS OF THE PARS DISTALIS OF PITUITARY GLAND

Anterior lobe is the master gland of the endocrine system because it produces protein trophic hormones which affect the other ductless glands. This lobe secretes several hormones of which six have been isolated ir almost pure forms. The following hormones are believed to be secreted

(I) growth hormone or somatotrophic hormone (GH or STH) (II) thyrotrophic hormone or thyroid-stimulating hormone (TSH or thyrotrophin, (III) adrenocorticotrophic hormone (ACTH or adrenotrophic hormone or adrenocorticotrophin, (IV) gonadotrophic hormones or gonadotrophins: (a) follicle-stimulating hormone (FSH), (b) luteinising hormone (LH) or interstitial cell-stimulating hormone (ICSH), (c) lactogenic hormone or prolactin or mammotrophic hormone (MH) or luteotrophic hormone (LTH) or luteotrophin, which is also **gonadotrophic hormone** in some species.

There is also an another hormone named **exophthalmos-producing substance (EPS)** in thyrotoxicosis. Recent evidences indicate that another hormone β-lipotrophin (β-LPH) is also released.

A brief description of the hormones is given below:

I. **Growth hormone or Somatotrophic hormone (GH or STH)** —secreted by acidophil cells.

Chemistry—isolated as a crystalline protein with molecular weight of about 21,000-48,000*. That of the human preparation is in the region of 21,500. Human growth hormone has a single straigth chain polypeptide structure containing two intramolecular disulphide bridges (Fig. 4.14). These disulphide bridges are not essential for biological activities. The human growth hormone is composed of 188 amino acids** without carbohydrate substituents. It is not effective by oral administration as it is inactivated by pepsin and trypsin.

It has been noted that human growth hormone (HGH) exerts lactogenic effect along with the growth stimulating one. This can be correlated with the fact that human placental lactogen (HPL)and somatotrophin are chemically and immunologically similar.

Assay. (1) The method consists in measurement of the increase in width of the proximal epiphyseal cartilage of the tibia of the hypophysectomised female rats following injection of the GH. (2) The other method involves measurement of the increase in body weight of normal, plateaued, or hypophysectomised female rats following injection of the hormonal preparation containing GH (STH).

Functional evidences

A. **Experimental.** 1. Extirpation in the young retards skeletal growth and produces dwarfs. If the anterior pituitary be then grafted or the extract injected, growth is restored.

*Bovine GH weighs about 46,000 (400 amino acids).

**Recently it has been proposed that human growth hormone (HGH) may contain 190 amino acids (Niall).

2. If the extract be injected in normal young animals, growth is stimulated and giant animals are formed.

3. Somatostain (GH-release inhibiting hormone, GR-IH) derived from extracts of ovine hypothalamus, has been found to inhibit the normal GH response to insulin hypoglycaemia in man.

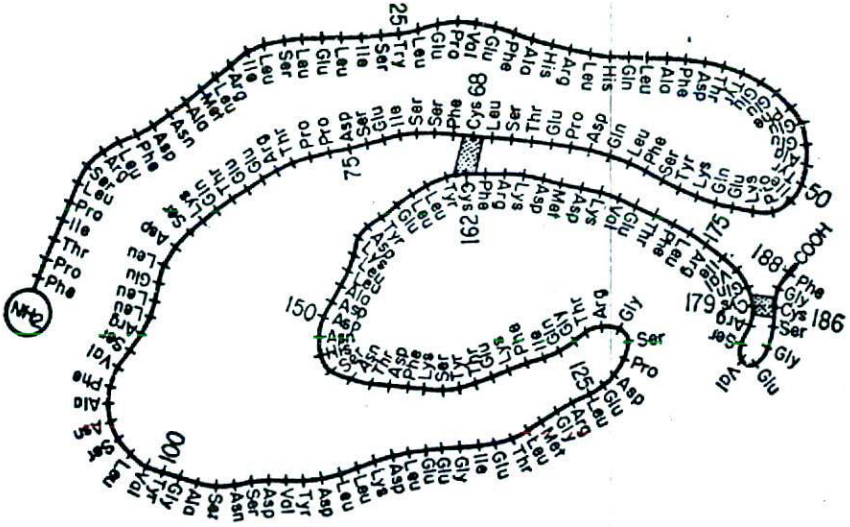


Fig. 4.14. Schematic representation of amino acid sequence of HGH.

B. **Clinical.** 1. Hypopituitarism (atrophy or ill-development of acidophil cells) in infants and children (e.g., before union of epiphyseal cartilage) retards skeletal growth and produces dwarfs **(dwarfism).**

2. Hyperpituitarism (acidophil tumour) in the young increases skeletal growth producing unusually tall stature (2 to 2·50 metres). This condition is known as **gigantism.**

3. Hyperpituitarism in the adults (e.g., after union of epiphysis) increases the thickness of lower jaw, hands and feet, producing a *gorilla-like* appearance called—**acromegaly** (reversion to *gorilla type*).

Actions of growth hormone (GH or STH)

1. **Skeletal growth.** *Stimulates the multiplication of the epiphyseal cartilage and thus increases the length of the cartilage bones.* In hypophysectomised animals a membrane appears at the epiphyseal line which inhibits the growth of the cartilage cells. On injection of growth hormone, the membrane disappears and bone growth is resumed again. In adult animals with closed epiphysis growth hormone causes thickening of bone. Membrane bones are believed not to be affected.

2. **Regulates general body growth.** After administration of this hormone there is an increased body growth due to its direct effect in the tissues. (a) *Muscles*—Stimulates the growth of muscles. In gigantism and acromegaly there is an increased growth of muscles. (b) *Viscera*—In

hypophysectomised animal the liver cells are reduced in size. RNA and protein content of the cytoplasm and nuclei are much reduced and kidneys remain small with impaired functions. After administration of growth hormone all these defective growths are found to be rectified. (c) It stimulates the growth of the thymus. (d) It increases the secretion of milk during lactation. (e) Nervous system is not affected by STH. Growth of brain does not stop in young hypophysectomised animal and exogenous STH does not increase growth of this again.

3. **Metabolism.** As STH has metabolic effect over and above growth, the secretion of the hormone does not stop at adulthood, but continues throughout the life. (a) **On protein metabolism**—STH increases nucleic acid and protein synthesis, decreases nitrogen excretion in the urine and the nitrogen thus retained helps in the synthesis of tissue protein. STH is a protein-anaebolic hormone and prevents the catabolism of amino acids. In nephrectomised animals conversion of amino acids to urea is diminished after administration of STH. When the supply of protein becomes limited, STH helps in retaining N_2. It diminishes the amino acid content of the plasma by transferring it into the tissue and helps in the growth of the tissue. STH increases the transport of amino acids across the cell membrane and thus helps in the synthesis of proteins. GH increases the rate of incorporation of α-amino-isobutyric acid. [Similar action of insulin on glucose transport, vide Action of Insulin.] There is also increased serum alkaline phosphatase which helps in the protein synthesis and ossification of bones. On liver protein synthesis, GH increases the synthesis by modifying the activity of ribosomes in the translation of mRNA and also produces marked increase in liver RNA synthesis and content. Eventually GH stimulates liver cell division and increases overall hepatic size. These effects of GH are actually brought about through the mediation of insulin. (b) **On fat metabolism**—Growth hormone has got an important effect on fat metabolism. Administration of the hormone causes mobilisation of peripheral fat depot to the liver. The mobilised fat is transported in the plasma as non-esterified fatty acids (NEFA). In human subjects plasma level of NEFA increases 2-3 folds after administration of 1-2 mg of primate GH. It is of opinion that alteration of fat metabolism is the primary effect of the GH. Increased oxidation of fat decreases the catabolism of amino acids causing utilisation of amino acids in the synthesis of protein. (c) **On carbohydrate metabolism**—The primary effect of STH on carbohydrate metabolism is to stimulate its storage. Administration of growth hormone in animal or in man produces hyperglycaemia and glycosuria. The high blood glucose level leads to overproduction of insulin by β-cells and finally to its exhaustion and atrophy. So the growth hormone is diabetogenic specially in man. The hormone, however, increases in the glycogen content of cardiac muscle. If the pituitary gland is removed in pancreatomised animal, hyperglycaemia, glycosuria and ketone bodies disappear and the animals live for months. This experiment was first done by Houssay and such animal is known as Houssay animal. Administration of ACTH produces similar effects as induced by growth hormone. Both STH and ACTH increase gluconeogenesis and diminish the rate of oxidation of glucose. STH and ACTH exert antagonistic actions to those of insulin.

4. **Ion or mineral metabolism.** GH (STH) increases intestinal absorption of calcium as well as its excretion. In addition to calcium, sodium, potassium, magnesium, phosphate and chloride are also retained.

5. STH stimulates proliferation of thymic lymphocytes both *in vivo* and *in vitro*.

6. The growth hormone also has lactogenic effects.

Regulation of growth hormone secretion

(a) *Hypothalamus.* The secretion of growth hormone by pituitary acidophil cells is regulated by the hypothalamic hormone, the GHRF (growth hormone-releasing factor). This specific hypothalamic hormone has been isolated and identified as a small acidic peptide of 11 amino acid residues. If the extract of the median eminence is administered into an immature young rat, then growth is increased. Lesions in this area arrest growth. It is believed that this releasing factor, GHRF or SRF (somatotrophin-releasing factor), is secreted from the median eminence.

GHRF levels in the hypothalamus are reduced by starvation or corticosteroids and increased by thyroxine. Drug-induced stimulation or inhibition of growth hormone release is also mediated through the hypothalamic releasing factor. Growth hormone secretion is enhanced by α-adrenergic agents, but whether at pituitary or hypothalamic level is unknown as yet.

(b) *Other factors*

Other than the hypothalamus, certain factors may affect the GH secretion. These are discussed below:

Stimuli which increase GH secretion are: hypoglycaemia, 2-deoxy-glucose, exercise, fasting, protein meal, infusion of amino acid specially arginine, glucagon, pyrogen, lysine vasopressin, physiological REM sleep.

(i) **Hypoglycaemia.** Insulin-induced hypoglycaemia is considered to be potent stimulus for GH secretion. To have an effective stimulus, blood sugar must fall to 50% or less of the basal level. This effect is not due to direct action of insulin but that of hypoglycaemia.

(ii) **Fasting.** Fasting causes a rise in plasma growth hormone in man, rat and rabbit. In severe malnutrition plasma GH level is sometimes increased. ACTH and catecholamines may stimulate HGH secretion and lipid mobilisation during fasting.

(iii) **Exercise.** Plasma level of HGH is increased even after light exercise. The resultant rise in plasma FFA provides source of energy-sparing glucose.

(iv) **Amino acid infusion.** Amino acid administration causes an elevation of HGH. Rise in concentration of precursor makes condition favourable for protein synthesis.

(v) **Stress.** Different stressful stimuli affect the secretion of GH. Surgical stress, emotional stress, bacterial endotoxins stimulate stress. Insulin hypoglycaemia stimulates secretion of human growth hormone (HGH) possibly through stress mechanism. Darkness inhibits growth. Crowding stimulates growth slowly. Exposure to high or low temperature influences growth. Noise sometimes inhibits growth. Gentling or handling stimulates growth.

(vi) **Sleep.** Deep sleep stimulates growth. The young allowed to sleep always stimulate growth.

(vii) **Hormones.** (a) Thyroid hormone stimulates growth through the secretion of growth hormone. (b) Small or large doses of androgens stimulate growth, but on prolonged treatment stops growth by closing the epiphysis. Relation of this hormore with the release of growth hormone has not yet been established. ACTH infusion can stimulate HGH release during starvation.

Following factors may inhibit growth.

(a) *Hormonal Factors.* Oestrogen and other ovarian hormones have got influence on growth as because females are shorter than the males. It is suggested that ovarian hormones retard growth through inhibition of GH secretion.

(b) *Adrenal Corticoids.* Corticoids interfere with the growth process. Administration of corticoids causes dwarfism. The corticoids inhibit the stimulating effect of GH on the epiphysis.

Mechanism of action

It is recently advocated that the action of STH is mediated through cyclic AMP as increase in the mitotic activity of thymus has been noted both after STH and direct application of the nucleotide.

Growth without growth hormone

In the foetal and neonatal stages, growth is mostly independent of growth hormone secretion. Plasma 'sulphation factor' which has an effect on cartilage metabolism, is growth hormone dependent. This plasma factor stimulates sulpher-35 incorporation into cartilage and is present in high concentration in acromegaly and in low concentration in hypopituitarism. Besides this, peptides with lipotrophic activity have been prepared from GH by other investigators. These derivatives are possibly released from the pituitary with varying quantities of the parent hormone, or they may be formed in peripheral tissues. It is suggested that renal uptake and metabolism of GH are possibly an essential step in biological activation of the pituitary hormones and most probably by the formation of secondary peptides with growth and metabolic activity.

II. **Thyrotrophic Hormone or Thyrotrophin or Thyroid-stimulating Hormone (TSH)**—secreted by the basophil cells.

Chemistry. It is a glycoprotein with molecular weight of about 25,000. It is not effective by oral administration as it is inactivated by proteolytic enzymes. Bovine TSH consists of an α-chain of 96 amino acid residues and a β-chain with 113, but the detailed composition of human TSH is not yet known.

Action—controls the growth and activity of the thyroid gland. Primary physiologic action of TSH is probably to stimulate the release of thyroid hormone from the intrafollicular thyroglobulin. TSH is necessary for coupling of di-iodotyrosine to form thyroxine (T_4). Recent evidence has implicated cyclic AMP as an intracellular mediator of TSH action. TSH increases adenyl cyclase activity in thyroid homogenates and also cyclic AMP accumulation in thyroid slices. TSH accelerates lipolysis by isolated rat adipose tissue *in vitro*. TSH may exert a direct effect upon

extra-ocular retrobulbar structures (lipid, muscle, connective tissue), with consequent protrusion of the eyeball (exophthalmos).

Evidences

1. After hypophysectomy following changes take place: (i) Thyroid takes up less inorganic iodide from the plasma. Formation of monoiodotyrosine, di-iodotyrosine, tri-iodothyronine and thyroxine is reduced and as a result there is less discharge of thyroxine into the blood. (ii) Diminution of size, weight and vascularisation of the gland. (iii) Acini contain larger amount of colloid and the epithelial cells of the acini are reduced in height. There are (iv) reduced BMR and (v) reduced heart rate.

2. After injection of TSH in hypophysectomised animal the normal activity of the thyroid gland returns. In excessive doses it causes hyperplasia of the thyroid, increased vascularity of the gland and also causes hypertrophy of thyroid. The epithelial cells of the acini are increased with changes in the polarity of the mitochondria and Golgi apparatus within them. Due to these changes signs of hyperthyroidism appear.

After injection of TSH the following morphological and metabolic phenomena of increased thyroid action are observed: (a) resorption of follicular colloid, (b) increase in thyroid acinar cell height, (c) increase of Golgi apparatus, proteolytic activity, oxidase granules and O_2 consumption of thyroid cells, (d) increase of thyroid RNA and protein, (e) increase in uptake of radio-active iodine by the thyroid, (f) increase' in the iodine content of the gland as well as in the protein-bound iodine of plasma.

3. In hypopituitarism, development of thyroid is retarded and a condition of thyroid hormone deficiency occurs.

4. In hyperpituitarism, thyroid often becomes hyperactive.

5. It has been suggested that the action of TSH on thyroid cell is mediated through cyclic AMP. It is also enumerated that TSH may act at the nuclear level and ultimately influence protein synthesis.

It may be possible that anterior pituitary produces more than one type of thyrotrophin. According to Dobyns and his co-workers pituitary extracts from patients suffering from Graves' disease contain a substance specially capable of producing exophthalmos. This **exophthalmos-producing substance (EPS)** may not be a separate anterior pituitary hormone but an abnormal variety of TSH. Thyroid hormone which depresses the release of TSH also cures exophthalmos. In man TSH in the blood is increased in myxoedema, cretinism, etc., but no exophthalmos occurs.

Assay

1. Determination of the number of colloid droplets in the cells of guinea-pig thyroid following injection of preparation containing TSH.

2. Uptake of ^{131}I by the thyroid of hypophysectomised rat.

3. Measurement of the thyroid acinar cell height, and extention of the hind limb of the statis (starved, non-metamorphosing tadpole) following injection of preparation containing TSH.

4. Iodine depletion in the thyroid of one-day-old chick.

Control of thyrotrophic hormone or thyrotrophin or TSH secretion. Thyrotrophin secretion is under the control of (1) hypothalamus, and (2) thyroxine level in the blood.

1. **Hypothalamus.** Stimulation of the hypothalamus by fine electrodes causes release of a tripeptide humoral factor known as *thyrotrophin-*

releasing factor (TRF) which circulates through the blood stream (hypophyseal portal system) and helps in the liberation of thyrotrophin from the anterior pituitary.

Control of TSH output is higher when the anterior pituitary is in its normal position. There are at least two factors: (i) it is inhibited by TH acting directly on the pituitary cells, and (ii) the basal levels of TH and TSH are determined by negative feedback mechanism. Any rise in TH depresses TSH output which in turn leads to a fall in TH output. Any fall in TH has the reverse effect. Minute injection of TH into the anterior pituitary or thyroid tissue transplanted there, causes a fall in TSH output. Thus the negative feedback relationship tends to maintain plasma levels of TH and TSH constant (Fig. 4.15).

Control of TRF output depends upon: (a) It is linked with control of appetite, factors which stimulate the one also stimulating the other. (b) Cooling of the anterior hypothalamus, by implanting tubes through which cold water can be passed or into which ether can be placed or evaporated, stimulates the release of TSH and activation of the thyroid. On the other hand, lesions in the median eminence prevents the release of TSH in response to cooling. (c) Minute implants of the thyroid hormone in the hypothalamus diminish TSH output due to a diminution of TRF release or to diffusion of TH into the pars distalis where it could inhibit TSH directly (Fig 4.15).

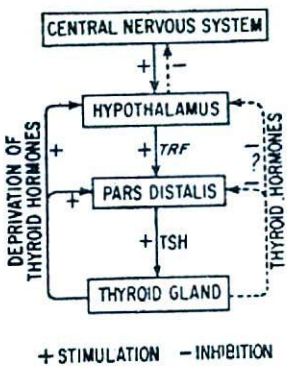


Fig. 4.15. Diagram showing the relationship between hypothalamus, pars distalis and thyroid gland.

2. **Thyroxine level in the blood.** The rate of the secretion of thyrotrophin is controlled by the thyroxine content of the blood. High thyroxine content in the blood inhibits and low thyroxine content in the blood stimulates secretion. Inhibition of thyrotrophin secretion by thyroxine might possibly be due to its effects both on anterior pituitary and hypothalamus. If the low thyroxine content persists for a prolonged period there will be continuous secretion of a large amount of thyrotrophin which causes thyroid hyperplasia.

3. **Hypothalamic-filter hypothesis.** Considering the capability of local changes in concentration of thyroid hormones in stimulation or inhibiting the secretion of TSH, a hypothesis has been put forward by Euler, Brown-Grant, Purves and Averill. According to this hypothesis, hypothalamus acts as filter, resulting reduced circulation of thyroid hormones through the anterior pituitary. They have postulated that local variation in extraction rate as a result of changes in hypothalamus may provide a stimulus in the form of local variation in blood hormone. In certain species thyroxine and tri-iodothyronine are concentrated in the median eminence and posterior pituitary. This increased concentration of thyroid hormones may suggest a removal of thyroid hormones from the blood during its passage through the primary plexus of the portal system.

III. **Adrenocorticotrophic hormone (ACTH*) or Adrenotrophic hormone or Adrenocorticotrophin**—secreted by basophil cells under the control of CRF from the hypothalamus, and is subject to indirect (and possibly direct) regative feedback mechanisms involving cortisol.

Chemistry. Polypeptide in nature consisting of 39 amino acid residues. Molecular weight about 4,500. The amino acids are numbered from the end with the free NH_2 group (the N-terminal end). The first 24 are common to ACTH from man and other mammals and biological activity is provided by the first 20. The arrangement of amino acids 25 to 33 varies in different species and that found in man is shown. It has been isolated in α- and β-forms. Both these forms contain a large number of

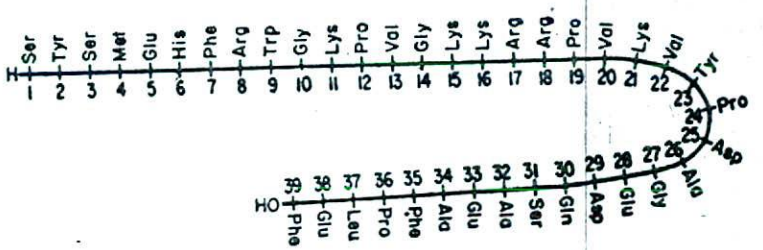


Fig. 4.16. Schematic representation of amino acid sequence of humen ACTH.

amino acids and have got straight chain linkage. Unlike other pituitary hormones, can withstand heating to 100°C.

Action. *ACTH controls the growth of adrenal cortex and the synthesis of cortisol, and is therefore essential to life.* The action of ACTH on adrenal cortex is mediated through cyclic AMP. ACTH increases adenyl cyclase activity in rat adrenal homogenates. ACTH in normal subjects produces the same effects as *cortisone* (*vide* Adrenals).

Evidences. 1. Hypophysectomy leads to atrophy of adrenal cortex specially, the zona fasciculata and zona reticularis. Zona glomerulosa in rats, dogs, etc., undergoes slight change after hypophysectomy and there are also no marked changes in electrolyte metabolism. From these findings it has been suggested that zona glomerulosa secretes alsosterone which is only under minor control of the pituitary gland. Injection of ACTH into these hypophysectomised animals produces: (i) Increased size of the adrenal gland, (ii) Increased release of the adrenal steroid hormones, (iii) Diminution of the lipid, cholesterol, and ascorbic acid content of the adrenal cortex.

2. If one adrenal gland be removed, the other undergoes compensatory hypertrophy. But if anterior pituitary be removed before, such hypertrophy fails to occur.

3. In hyperpituitarism, adrenal cortex hypertrophies. Similarly, in hypopituitarism it degenerates or fails to develop.

4. Tumours of the basophil cells (Cushing's disease) causes enlargement (even tumours) of adrenal cortex.

The influence of anterior pituitary on metabolism specially on carbohydrate metabolism, is partially due to the effects of this hormone on

*ACTH is used in the treatment of rheumatoid arthritis.

secretion of glucocorticoids by the adrenal cortex. Hypophysectomy reduces and the injection of large doses of ACTH increases the secretion of glucocorticoids. It also increases total protein synthesis as indicated by increased adrenal RNA and adrenal tissue protein. It affects steroid hormone synthesis in the adrenals at an early stage in the conversion of cholesterol to pregnenolone, the primary precursor of steroid.

Administration of ACTH to normal human beings produces the following effects: (1) increased excretion of nitrogen, potassium and phosphorus, (2) retention of sodium chloride and secondary retention of water, (3) elevation of fasting blood sugar and a diabetic glucose tolerance curve, (4) increase in circulating free fatty acids, (5) increased excretion of uric acid, (6) decline in circulating eosinophils and lymphocytes, and increase in neutrophils. (7) It has a slight melanocyte-stimulating effect.

ACTH exerts some effects which are not mediated through adrenal cortex. One of such effects is an increase in the concentration of NEFA in the adipose tissue. The other effect is the stimulation of melanin synthesis. Due to structural similarities ACTH exhibits these effects similar to MSH and β-LPH.

Assay

1. Repair test consists in determination of the re-appearence of lipid substance in the regressed adrenal cortices of hypophysectomised rats following injection of preparation containing ACTH.
2. Maintenance test consists in determination of the quantity of ACTH-containing substance required to maintain the adrenal gland's weights of rats injected immediately after hypophysectomy.
3. Measurement of decrease in adrenal ascorbic acid of hypophysectomised rats one hour, after intravenous injection of ACTH-containing material, is another method. This is a highly specific and sensitive assay method.

Control of ACTH secretion

ACTH secretion is under the control of (1) hypothalamus, (2) nervous structures other than the hypothalamus, (3) steroid feedback mechanism, (4) short feedback mechanism, and (5) stress control mechanism.

1. **Hypothalamus.** The role of hypothalamus in the secretion of ACTH from the anterior pituitary can be studied under the following conditions: (a) **Hypothalamic stimulation**—Electrical stimulation of various hypothalamic regions causes increased discharge of ACTH. Sometimes mere presence of electrodes in the median eminence causes discharge of ACTH. Now it is well established that hypothalamic nuclei control the secretion of ACTH through their releasing factor CRF. (b) **Hypothalamic lesions**—Lesions at certain regions of the hypothalamus cause decreased secretion of ACTH. Weight of the pituitary is decreased after lesions of median eminence. (c) **Transplantation of the anterior pituitary**—If the anterior pituitary is removed from its normal position and is detached from its normal neurovascular link and then transplanted at a remote side as for instance at the anterior chamber of the eye or under the kidney capsule, then weight of the adrenal gland is decreased. But if such pituitary is transplanted just beneath the median

eminence of the hypophysectomised animals then mostly the normal functions of the adrenal gland are retained. This latter effect is due to regeneration of blood vessels creating vascular link in between the hypothalamus and anterior pituitary since misplacement of the transplant a few mm, breaks up the link and the adrenal gland's weight is decreased.

2. **Nervous structures other than the hypothalamus.** Practically hypothalamus plays as sort of funnel through which all kinds of information collected from the pituitary, are transmitted to the anterior pituitary. The informations that are received by the hypothalamus from different parts of the body are the following: (a) **Peripheral nerves and spinal cord**—It has been observed by Gordon (1950) and Egdahl (1959) that peripheral nerves and spinal cord facilitate the release of ACTH. Role of the spinal cord in the release of ACTH depends upon the nature of stimulation. (b) **Midbrain**—Midbrain has got both activating and inhibiting (dualistic) effects on the secretion of ACTH from the anterior pituitary. Main functions of the midbrain are that of inhibition of ACTH release. Transection or lesion at midbrain level causes increased secretion of ACTH. This inhibition possibly has got some role on the control of circadian rhythm. Stimulations of reticular component of the midbrain, tegmentum and of the peripheral aqueductal region activate ACTH secretion. (c) **Limbic system**—Different limbic system structures have been observed to participate in the control of secretion of ACTH. Amygdaloid nuclei have got stimulating effects on the secretion of ACTH but forebrain and rhinencephalic structures have got inhibiting roles on the secretion of ACTH. Posterior orbital surface also stimulates the secretion of ACTH. Extrahypothalamic role on the secretion of ACTH has been summarised in Fig. 4.17.

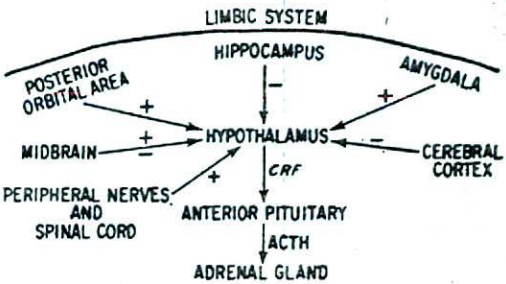


Fig. 4.17. Diagram representing the relationship between extrahypothalamic nervous structures, hypothalamus, anterior pituitary and adrenal gland.

3. **Steroid feedback mechanism.** Blood level of steroid hormones plays an essential role in modifying the activity of adrenal-pituitary axis. The existence of steroid-ACTH feedback mechanism has been evidenced by the following classical observations: (a) compensatory adrenal hypertrophy and hyperfunction in uuilateral adrenalectomy, (b) prevention of compensatory hypertrophy in the adrenal by adequate doses of cortical hormones, and (c) atrophy of the adrenal glands by increased doses of cortical hormones—corticosterone, cortisol, etc.

Steroid feedback mechanism may operate through the following ways: (a) direct actions on the adrenal cortex, (b) modifications of the responsiveness of the adrenal cortex to ACTH, (c) modifying the synthesis and release of ACTH, taking place in the pituitary, (d) acting directly

on the hypothalamus or on the other areas of the brain that control the activity of the hypothalamic pituitary unit, and (e) modification of the responsiveness of the pituitary to its physiologic activators like vasopressin, CRF, etc. Steroid feedback mechanism has been summarised in Fig. 4.18.

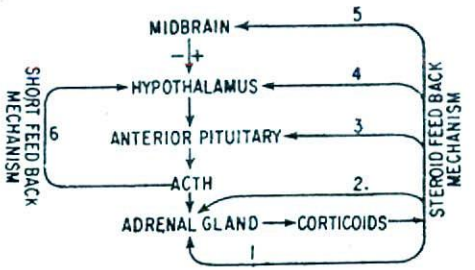


Fig. 4.18. Schematic representation of steroid and 'short' feedback systems controlling ACTH secretion and midbrain modulating system. 1,2,3,4 & 5 depict repectively the direct action on adrenals, modification of responsiveness of adrenal cortex to ACTH, action on anterior pituitary, action on hypothalamic receptors, & action on midbrain receptors. 6 denotes the short feedback mechanism in which ACTH reaching hypothalamic receptors through general circulation and pituitary portal vessels carrying blood to the brain controls the secretion of hypothalamic releasing factors—CRF, vasopressin etc.

4. **Short feedback or servo mechanism.** Not only the steroid hormones control the secretion of ACTH but also the blood level of ACTH controls the endogenous secretion of ACTH. This is the short, direct or autofeedback mechanism. Increased blood level of ACTH inhibits the endogenous secretion of ACTH (Fig. 4.18).

5. **Role of stress in the control of secretion of ACTH.** Different stressful stimuli have got some modifying effects on the secretion of ACTH. Pituitary-adrenal axis is activated by stress. It is suggested that adrenal gland may be activated independently or indirectly through ACTH during stress. There is no such clear evidence that the same nervous pathways that control ACTH secretion through blood corticosteroid feedback mechanism are also involved in the activation of the pituitary during stress. Non-specific stresses, such as cold, pyrogens, epinephrine, oestrogen, trauma or psychic reactions may also stimulate the hypothalamus resulting in increased ACTH production and hence protective compensation against stress.

In summary, the hypothalamus is primarily concerned with the synthesis and release of ACTH from the anterior pituitary as because the hypothalamus is the area where the CRF is synthesised and stored and released when necessary for activating the anterior pituitary. Besides this, the hypothalamus is the area where the feedback receptors, sensitive to corticoids and to ACTH are predominantly situated.

IV. **Gonadotrophic hormones (GTH) or Gonadotrophins.** The basophil cells secrete gonadotrophins which control the growth and activity of the gonad and indirectly all the other processes connected with it (i.e., growth of sex organs, secondary or accessorty sex characters and in females, the phases of menstrual cycle, pregnancy, lactation, etc.). The anterior pituitary controls the gonads is proved by the following facts:

Evidences

1. Hypophysectomy, in the young, retards growth and activity of the gonads.

2. If the anterior pituitary is grafted in the subarachnoid space below the hypothalamus of young hypophysectomised animals their normal sexual growth takes place, because normal maturation of Graafian follicles and ovulation with the formation of corpus luteum along with cyclic changes in oestrus are observed in females.

3. Hypopituitarism in children (Dwarfism) retards sexual development and in adults leads to sexual degeneration (impotency, amenorrhoea, etc.).

There are two gonadotrophins: (a) **follicle-stimulating hormone (FSH),** and (b) **luteinising hormone (LH)** or **interstitial cell-stimulating hormone (ICSH).**

Chemistry. The two gonadotrophic hormones are glycoprotein in nature. The amino acid sequences of either FSH or LH have not been completely determined in man but both have a mol. wt. of about 30,000. The LH is made up of two subunits of about 15,000 each, the fact about FSH is undiscovered.

Assay of FSH

Hypophysectomised animal is to be employed to avoid influence of LH. The following procedure may be used: (1) Production of increase of ovarian weight or follicular development in hypophysectomised female rats. (2) Production of increase in weight of testes without stimulation of secondary sex organs in hypophysectomised male rats. (3) Binding of ^{131}I labelled FSH to a proper antiserum is used as a sensitive radioimmunoassay method.

Assay of LH

(1) Ascorbic acid depletion from the luteinised ovary of pseudopregnant rat. (2) Measurement of the degree of hyperaemia in immature rat ovary by using ^{131}I labelled serum albumin. (3) Increase in weight of seminal vesicles or prostate of immature or hypophysectomised rat. (4) The change in colour (melanisation) of the feathers of castrated or non-breeding weaver finch.

FUNCTIONS OF GONADOTROPHINS

Functions of FSH

1. **In females.** (a) Increases the number and size (maturation) of Graafian follicles and prepares them for ovulation. After the menopause the production rate of FSH is increased approximately 15-fold.

2. **In males.** Stimulates spermatogenesis. In old age in the male (as testicular function wanes) the production of FSH increases.

[Due to its action on both male and female gametes this hormone is also called **gametokinetic factor.**]

Functions of LH

1. **In females.** (a) Complete development of the ovarin follicles to secretory stage and secretion of oestrogen. (b) Rupture of the follicles and ovulation, **due to combined action of FSH and LH.** (c) Causes appearance, growth and persistence of corpus luteum. In the ovary LH can stimulate the non-germinal elements to produce androgens, testosterone, etc., giving rise to hirsutism. After menopause a 5-fold increase of LH.

The actual site of LH action on progesterone synthesis in corpora lutea is not known.

It activates phosphorylase. It is suggested that LH may act to increase cyclic AMP production. It has been shown that cyclic AMP levels are elevated after addition of LH to incubating corpora lutea. Added cyclic AMP duplicates the effect of LH both at site of acetate incorporation into cholesterol and at site of cholesterol incorporation into progesterone (Fig. 4.19.). Protein synthesis may be involved in the actions of LH and in turn of its mediator cyclic AMP, on steroidogenesis.

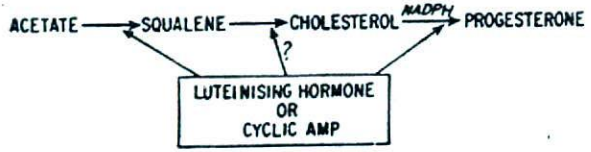


Fig. 4.19. Scheme showing the effect of LH or cyclic AMP on progesterone synthesis in corpora lutea.

2. **In males.** ICSH (the same hormone) stimulates the development and functional activity of Leydig (interstitial) cells, and consequently, the production of testicular androgen. Its administration, therefore, produces effects in the organism (except on the testis) similar to those which follow administration of testosterone.

Control of gonadotrophin secretion

Gonadotrophin secretion is under the control of (1) **hypothalamus,** and (2) **sex hormones.**

1. **Hypothalamus.** Hypothalamic nuclei are known to secrete specific releasing factors for the release of specific gonadotrophic hormones. *Luteinising hormone-releasing factor* (*LRF*) for LH (ICSH) and *Follicle-stimulating hormone-releasing factor* (*FSH-RF*) for FSH are secreted from hypothalamus when they are necessary. The following facts prove that the hypothalamus controls gonadotrophin secretion.

(a) In the rabbits, ovulation takes place normally 12-18 hours after mating, in presence of an intact anterior pituitary.

(b) By electrical stimulation of the hypothalamus, ovulation can be induced. This is probably due to release of some chemical substances(LRF) from hypothalamus, which circulate in the blood stream and stimulate the anterior pituitary to secrete gonadotrophin—LH.

(c) In the female ferrets, exposure to light under certain conditions, stimulates ovulation, provided anterior pituitary and optic nerves are intact. The retinal impulse caused by the falling of light reaches the brain and stimulates the hypothalamus to release LRF which stimulates the anterior pituitary to secrete gonadotrophin—LH. In pigeons by similar visual impulses ovulation has also been demonstrated.

(d) Stimulation of the hypothalamus in the rabbit causes ovulation, but direct stimulation of the anterior pituitary is ineffective. This shows that LRF from hypothalamus is required for the secretion of LH from the anterior pituitary.

(e) Secretion of FSH is also under the influence of the hypothalamus. Lesion of the hypothalamus depresses the release of FSH. (Hypothalamic lesions, e.g., pinealoma in boys, can cause sexual precocity and early

true puberty, i.e., those 'boys' are fertile.) [Relation to the menstrual cycle is not considered here, but cyclical change in the blood levels of the gonadotrophic hormones is responsible for the regulation of menstruation.]

It has been found that the reticular system and amygdaloid nuclei help in the release of LH.

Recently Schally *et al.* (1971) have claimed that one polypeptide secreted from the hypothalamus seems to control the secretion of both the FSH and LH from the anterior pituitary of several species. They have isolated a polypeptide from porcine hypothalamus which stimulates the release of both LH and FSH. This polypeptide has been synthesised by them and structurally identified as (pyro) Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2. Both the synthetic and naturally occurring polypeptides have got the property of releasing both LH and FSH.

2. **Sex hormones.** Injections of oestrogens or androgens for long periods, cause degeneration of the anterior pituitary. Secretion of gonadotrophin is inhibited causing atrophy of gonads.

This shows that concentration of sex hormones in blood regulates secretion of gonadotrophins. A high concentration inhibits, whereas a low concentration stimulates secretion.

After injection of oestrogens, secretion of FSH is diminished and secretions of LH and LTH are increased. This shows that the concentration of the ovarian hormones regulates the secretion of FSH, LH and LTH. At the onset of menstruation the concentration of oestrogen, and progesterone in the blood falls. FSH is secreted and thus helps in maturation of Graafian follicles and secretion of oestrogen. Secretion of oestrogen rises and it inhibits the secretion of FSH and stimulates the secretion of LH and LTH. LH stimulates ovulation, formation of corpus luteum, secretion of progesterone, and oestrogen from corpus luteum. Progesterone inhibits the secretion of LH.

(c) **Lactogenic hormone or Prolactin or Luteotrophic hormone (LTH) or Mammotrophic hormone (MH) or Luteotrophin.** It is secreted during pregnancy and lactation in women by acidophil 'pregnancy' cells. It is a peptide hormone, isolated in pure form and contains tyrosine, tryptophan, cystine, methionine, arginine and sulphur. Molecular weight is about 25,000. Its structure is not fully known, but it is likely to contain between 180 and 190 amino acids and to have several disulphide bridges. Human prolactin resembles ovine prolactin and not HGH in its immunological properties.

Action of prolactin

1. Prolactin (LTH) is responsible for lactation in the postpartum women, the breast having been prepared by oestrogen and progesterone. It helps in initiating (lactogenesis) rather than maintaining milk secretion. Growth or somatotrophic and thyroid hormones help in the maintenance of the secretion of milk (galactopoiesis). The level of prolactin increases during the night. Oestradiol stimulates prolactin release whereas L-dopa inhibits it by promoting the discharge of PIF.

2. It stimulates slightly the proliferation of the glandular elements of the mammary glands during pregnancy and thus helps to complete the development of breasts.

3. It helps in maintenance of secretory activity of corpus luteum and secretion of the hormone, progesterone, due to combined action of LH and prolactin.

In the hypophysectomised-oöphorectomised-adrenalectomised rat, GH, glucocorticoids, oestrogen and progesterone in conjugation with prolactin stimulate the development of mammary glands. Prolactin in conjugation with progesterone stimulates alveolar development of the gland. It is probably concerned with the suppression of ovulation during lactation.

If glucocorticoids and insulin as well as prolactin are present during the period of cell division, casein production is observed. Prolactin, insulin and glucocorticoids in combination will induce the synthesis of lactose synthetase system.

Prolactin produces growth of oesophagus with histological evidence of epithelial proliferation and desquamation. It has metabolic action similar to HGH and HPL (human placental lactogen).

Evidences. 1. Hypophysectomy (or ischaemic necrosis of pituitary) in lactating animals prevents the onset of lactation (e.g., Simmonds' disease). If in this condition the anterior lobe extract be injected lactation starts.

2. Injection of prolactin stimulates milk secretion in the fully developed mammary glands.

3. Synthetic ergot alkaloids, which diminish prolactin secretion, probably inhibit the release of preformed hormone granules from the lactotrophs.

This shows that the anterior pituitary produces a hormone (LTH) which controls the activity of mammary glands.

The physiological role of prolactin in the male is not known.

Assay

The method, usually employed, depends upon the increase in weight of Corp sac of the pigeon after injection of prolactin. An international unit is defined as the specific activity contained in 0·1 mg of (international) standard preparation.

Control of prolactin secretion

1. **Hormones.** The **sex hormones** and the **placental gonadotrophins** inhibit prolactin secretion.

Evidences. (a) Although breasts are fully developed, yet lactation does not occur during pregnancy. It starts after parturition and expulsion of placenta. (b) Lactation is inhibited by injections of oestrogen and progesterone. Low oestrogen content in blood activates the action of prolactin. During pregnancy this action of oestrogen is inhibited by progesterone. (c) It is inhibited by the injections of placental gonadotrophins. Excessive secretion of prolactin under pathological conditions may lead to abnormal lactation (galactorrhoea) in either sex. The Forbes-Albright syndrome is persistent laclation in patients with pituitary tumours, but without acromegaly. The tumours would be classified as chromophobe adenomas.

These observations suggest that in the intact animals, prolactin secretion is inhibited by higher concentration of sex hormones and placental hormone in the blood during pregnancy. After expulsion of placenta these factors are absent, hence lactation starts.

2. **Nervous system.** Suckling of the baby generates afferent impulses which reflexly stimulate prolactin secretion through hypothalamus (Fig. 4.20). It is believed that suckling inhibits the median eminence to secrete prolactin-inhibiting factor (PIF) and thus prolactin secretion from the anterior pituitary is increased. Only minutes quantities are produced by lactotrophs and released into blood stream. It may regulate its own secretion by a short negative feedback control system. PIF inhibits the secretion of prolactin (LTH) in the mammal, but in birds this factor has got opposite effects.

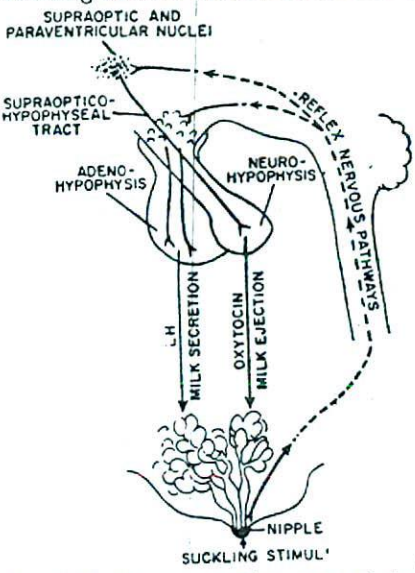


Fig. 4.20. Diagram showing some of the neural and hormonal components involved in controlling lactation (after *Harris*).

Evidence. Although nerves play an important role, yet they are not essential. Because, denervation of the mammary glands does not stop lactation. Even a grafted mammary gland secretes.

Ejection of milk. Suckling of the baby not only stimulates prolactin secretion but also stimulates secretion of oxytocin. The afferent impulses after stimulation of the nipple by suckling of the baby reach the supraoptic nuclei of the hypothalamus. From the hypothalamus through the nervous tract the posterior pituitary is stimulated. Oxytocin is released. It circulates in the blood stream, reaches the mammary glands and produces contraction of the myoepithelial cells and helps in the ejection of milk (Fig. 4.20). According to some, oxytocin may have certain effects on the secretion of LTH and STH.

Prolactin stimulates the proliferation of the Crop glands of the male and female pigeons and doves and stimulates the secretion of Crop milk.

Lipotrophin, Lipolytic hormone (LPH), Fat-mobilising agent

Lipotrophin (β-LPH) has been isolated as an adenohypophyseal hormone having chemical and biological characteristics similar to ACTH or MSH, but certain differences exist.

All the three hormones have seven amino acids common to their chemical structures and they all stimulate chromatophores. ACTH and β-LPH cause lypolysis of rabbit fat pad and β-LPH is named for such lypolytic activity.

INTERRELATIONS OF PARS DISTALIS WITH GONADS, THYROID GLANDS AND ADRENAL CORTEX

Pars distalis, by releasing different trophic hormones, controls the activity of gonads, thyroid and adrenal cortex. The postulated existance

of diabetogenic, ketogenic, pancreatrophic hormones has not been established, except β-LPH. Thus two gonadotrophins, FSH and LH or ICSH control growth, development and activity of male and female gonads. Thyrotrophic hormone (TSH) and adrenocorticotrophic hormone

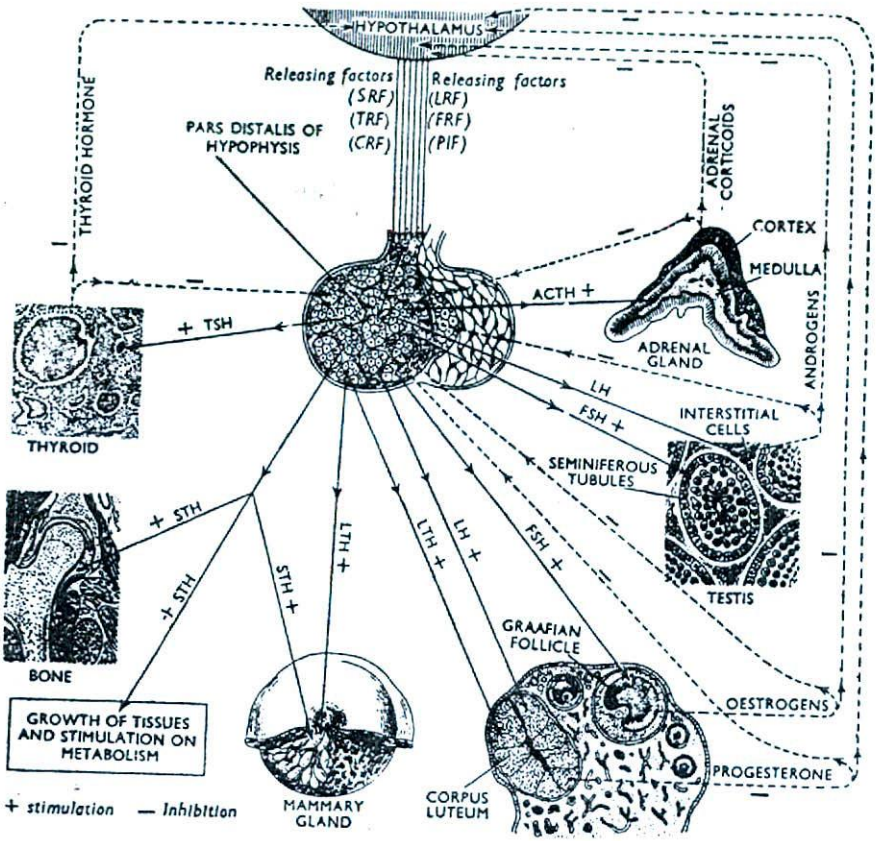


Fig. 4.21. Diagrammatic representation of central and peripheral organisations of anterior pituitary hormones.

(ACTH) control the secretion of the thyroid and adrenal cortex respectively. It has been observed that the hypothalamus controls the activity of pars distalis through individual releasing factors corresponding to different trophic hormones. So there are FSH-RF, LRF, TRF and CRF for the trophic hormones. On the other hand, the hormones elaborated by different endocrine organs named above have inhibitory influence over both the anterior pituitary and hypothalamus in releasing the pituitary trophic hormones and hypothalamic releasing substances respectively. Thus under normal conditions a perfect optimal endocrine balance is maintained in the body (Fig. 4.21).

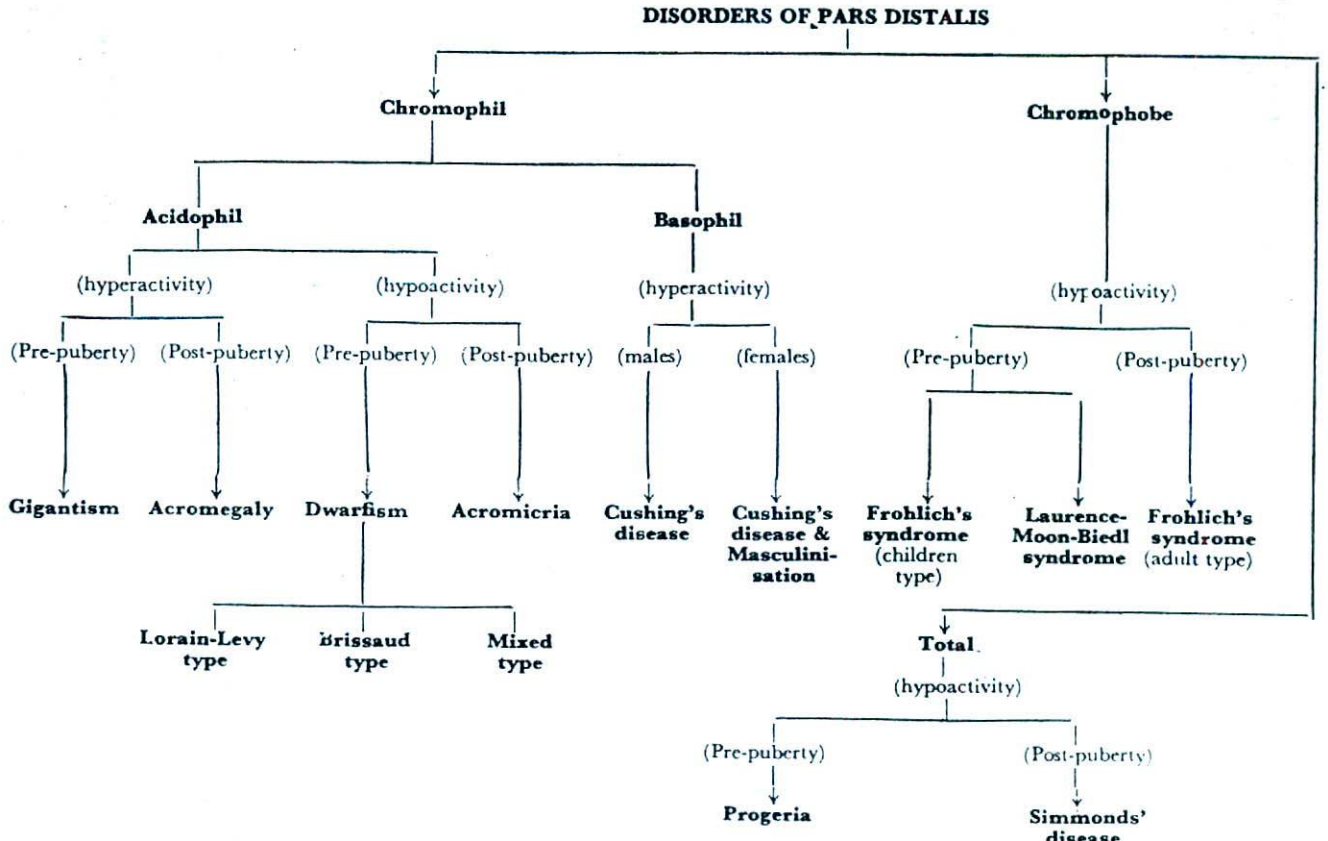
Mechanism of releasing factors

Commonly tissue content of a hormone produces a balance between rates of synthesis and release. An increase in hypothalamic content of releasing factor represents increased synthesis and secretion if there are changes of the corresponding hormone in the anterior pituitary and target gland. In such cases if there is no change in the hypothalamic content of releasing factor, target gland hormones may act directly at the pituitary level. Majority of stimuli may act through the hypothalamus and produces changes in secretion rates of releasing factors. Experimentally it has been observed that the *long-loop feedback mechanism* which involves peripheral target hormones and the *short-loop feedback mechanism* which involves pituitary hormones acting directly on the hypothalamus, can modify secretion rates. For example, the long-loop feedback mechanism in the control of FSH and LH secretion increases hypothalamic LRF and FSH-RF content after castration specially in males. After administration of testosterone or oestrogen to a castrate male there is a marked depletion of FSH-RF storage. So testosterone decreases the secretion of pituitary FSH by way of the hypothalamus. In case of short-loop feedback mechanism, systemic administration of FSH to castrated male rats produces a significant drop in pituitary FSH and hypothalamic FSH-RF, but injection of LH or ACTH shows no such effect. This experiment represents FSH acts directly back on the hypothalamus to prevent secretion of FSH-RF. In case of implantation of LH in the hypothalamus, there is a decrease of LRF and LH and in case of implantation of ACTH into the median eminence depresses markely blood conticosterone levels. It has been observed brain catecholamines take part in the regulation of gonadotrophin secretion. Histochemically as axons from dopamine-containing neurones project to the external layer of the median eminence in close proximity to the portal blood vessels, dopamine may participate in gonadotrophin secretion by way of directly stimulating release of pituitary FSH and LH, or by potentiating the effect of gonadotrophin-releasing factors or by functioning as a synaptic transmitter to produce the release of FSH-RF or LRF.

DISORDERS OF PARS DISTALIS

Introduction. The following facts will be helpful to understand the clinical manifestations of pituitary disorders:

1. Lesions may be of two types: (a) **hypoactive** (degenerative), (b) **hyperactive** (tumour, irritation, etc.).
2. Lesions may affect the (a) acidophil cells, (b) basophil cells, (c) chromophobe cells, or (d) total gland.
3. Tumour of a particular type of cell will cause the signs of hyperactivity of those cells *plus* either the signs of hyperactivity of other neighbouring cells (due to irritation) or the signs of their hypoactivity (degeneration due to pressure).
4. Anterior lobe, being the **leader of endocrine orchestra,** any disorder of this gland will lead to corresponding changes in the other endocrine glands and the ultimate result will be a pluriglandular dysfunction.
5. A hyperactive condition may ultimately end in corresponding hypoactive state, possibly due to exhaustion and degeneration.


DISORDERS OF PARS DISTALIS
Chromophil
Chromophobe
Acidophil
Basophil
(hyperactivity)
(hypoactivity)
(hyperactivity)
(hypoactivity)
(Pre-puberty)
(Post-puberty)
(Pre-puberty)
(Post-puberty)
(males)
(females)
(Pre-puberty)
(Post-puberty)
Gigantism
Acromegaly
Dwarfism
Acromicria
Cushing's disease
Cushing's disease & Masculini-sation
Frohlich's syndrome (children type)
Laurence-Moon-Biedl syndrome
Frohlich's syndrome (adult type)
Lorain-Levy type
Brissaud type
Mixed type
Total
(hypoactivity)
(Pre-puberty)
(Post-puberty)
Progeria
Simmonds'

The chief clinical disorders are briefly described below:

I. DYSFUNCTION OF THE ACIDOPHIL CELLS

(i) **Hyperactivity**
- (a) *In the young*—**Gigantism.**
- (b) *In the adult*—**Acromegaly.**

(ii) **Hypoactivity**
- (a) *In the young*—**Dwarfism.**
  Three types:
  (1) **Lorain-Levy type.**
  (2) **Brissaud type.**
  (3) **Mixed type.**
- (b) *In the adult*—**Acromicria** (very rare).

These conditions are briefly described below:

GIGANTISM (Fig. 4.22)

Caused by hyperactivity of the acidophil cells, in the young.

1. **Skeleton**—very tall (2·15 to 2·50 metres or 7-8 feet.; minimum 2·0 metres or 6 feet 8 inches).
2. **Muscles and Viscera**—proportionally large.
3. **Metabolism**—same as in acromegaly (*vide* below).
4. **Changes in other glands**—same as in acromegaly.

Note. (1) If hyperactivity continues in the adult life the case will be gigantism *plus* acromegaly. (2) Often the hyperactive condition ends in hypopituitarism, due probably to exhaustion or degeneration.

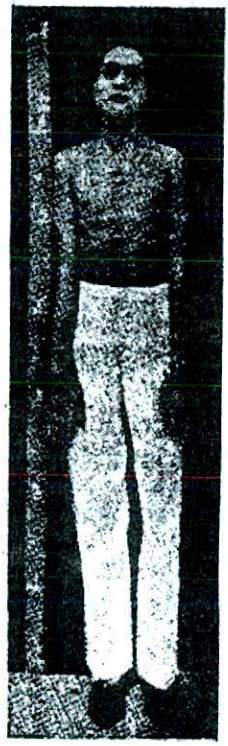


Fig. 4.22. Gigantism.

ACROMEGALY

Caused by hyperactivity of acidophil cells in the adult.

1. **Skeleton.** (a) Overgrowth of the two jaws, the malar bones, the supraorbital ridges, etc.

(b) Hands and feet (acral parts)—enlarged.

(c) Bowing of the spine (kyphosis). Anteroposterior diameter of the chest increased.

Due to above-mentioned skeletal causes a, b, c, the subject assumes a *gorilla-like* appearance. [Reversion to the 'gorila' type.]

2. **Subcutaneous tissues.** The subcutaneous tissues of the hands, feet, scalp, nose, lips and the skin increase in amount, producing deep furrows. Tongue also enlarged due to same cause.

3. **Metabolism.** (a) Hyperglycaemia, reduced sugar tolerance and may be, glycosuria (due to oversecretion of STH and ACTH).

(b) **BMR**—raised.

(c) **Sweating**—increased (to help heat loss).

4. **Viscera.** Organs concerned with metabolism are all enlarged, such as, heart, lungs, liver, kidneys, pancreas, spleen, etc., (*splanchnomegaly*).

5. **Other endocrines.** (a) Thyroid—enlarged and hyperactive, hence, may cause additional *Graves' disease.* (b) *Adrenal cortex*—hyperactive and enlarged. (c) *Thymus*—enlarged. (d) *Gonads*—At first hyperactive then may atrophy (overwork and exhaustion). Almost all patients have increased production of lactogenic hormone.

Other manifestations are—lantern jaw, papilledema, rhinorrhoea, deepening of voice, visual disturbances, drowsiness and lethargy, complete amenorrhoea, cutaneous pigmentation, concomitant presence of hypertension.

In males, diminished libido and sometimes impotence.

In females, development of sterility, menstrual disturbances, failure of breast to develop, persistant lactation (lactorrhoea).

DWARFISM (Fig. 4.23)

Caused by hypoactivity of the acidophil cells in the young.

Three types: (1) **Lorain-Levy.** (2) **Brissaud.** (3) **Mixed.**

1. **Lorain-Levy type** (Infantilism):

(a) Stunted growth (adult about 85 cm or 3 feet high).

(b) Sex organs and secondary sex characters do not grow. Hence, an adult man resembles a normal child.

(c) *Intelligence—normal* and *proportional to age.*

(d) Metabolism—normal.

[The difference with *thyroid cretins* should be noted, where everything is below normal.]



Fig. 4.23. Pituitary Dwarf—Lorain-Levy type. On the left, a normal subject for comparison (height 1·65 metres or 5 feet 6 inches). On the right, a Pituitary Dwarf.

2. **Brissaud type** (Fat boy of Dickens). Same as Lorain-Levy type *plus* excess deposition of fat in the body, round chubby face, no beard or moustache, sleepy and slothful nature.

3. **Mixed type.** There are mixed histological nature and combined clinical manifestations, and may be called dyspituitarism. The subject may be fat and hairless, and yet may have large accessory nasal sinuses, prominent supraorbital ridges, and other stagmata of acromegaly. In some, dysfunction of acidophil cells may develop pressure symptoms on neighbouring structures and behaves like a dysfunction of chromophobe cells—hypopituitarism along with acromegaly or gigantism.

ACROMICRIA

Caused by hypoactivity of the acidophil cells in the adult. It is the antithesis of acromegaly. Bones of face, hands and feet are small. Loss of hair and of sexual function. Very rare.

II. DYSFUNCTION OF THE BASOPHIL CELLS

Hyperactivity. Causes **Cushing's disease** (Pituitary basophilism). Harvey Cushing found out a disease in 1932. In this disease basophil adenoma of the pituitary gland occurs. So it is a primary pituitary tumour. After his name it was called *Cushing's disease*. Similar clinical changes are also found in *adrenal tumour* and it is called **Cushing's syndrome.** Due to mechanical pressure on the active cells, signs of hyperactivity develop.

CUSHING'S DISEASE

1. There is excessive deposition of fat over the supracondylar fossae, face (rounded or moon face in a well-developed case), back of the neck and abdomen. Extremities are usually spared. Fatty deposits are tender and painful. Purplish striae are found over the abdomen, thighs, etc. This is due to loss of protein matrix. Women are affected 4 times more than men.
2. *In males* excessive hair-growth (hirsutism). *In females* masculinisation with growth of bread, moustache, etc.
3. Asthenia and wasting of the muscles of the limbs. Obesity of the trunk, not of the limbs.
4. Osteoporosis of bones due to decalcification and loss of protein matrix.
5. Hypertension in a few patients.
6. Mental derangement.
7. *In males* impotence with atrophy of testis. *In females* amenorrhoea, sterility, etc.
8. Hyperglycaemia and glycosuria.
9. Retention of sodium and diminution of potassium level in the plasma.
10. Eosinopenia, lymphocytopenia, and polycythaemia.
11. Increased excretion of 17-oxogenic steroids and 17-hydroxycorticosteroids.

III. DYSFUNCTION OF THE CHROMOPHOBE CELLS

Usually caused by a tumour of the inactive chromophobe cells. Due to mechanical pressure on active cells produces signs of both irritation and hypoactivity. Hence, the following effects:

1. **In children:**

A. **Frohlich's syndrome** (*Adiposogenital dystrophy*). (a) Stunted growth (dwarfism). (b) Idiotic. (c) Sexual infantilism. (d) Generalised obesity (Fig. 4.24).

B. **Laurence-Moon-Biedl syndrome** (often familial). This is same as Frohlich's syndrome (children type) *plus* (a) *polydactylism,* and (b) *retinitis pigmentosa.*

2. **In adults Frohlich's syndrome**--(adult type).

A. **In males:** (a) Adiposity of feminine distribution. (b) Mental disposition and appearance resemble females. (c) Degeneration of sex. (d) Hands and feet are small and pretty, fingers delicate and tapering. (e) Skin of face and body is smooth and hairless.

B. **In females:** (a) Extreme adiposity. (b) Degeneration of sex.

IV. TOTAL DYSFUNCTION OF THE PARS DISTALIS

Hyperactivity. Not known as a pure clinical type. Often appears in a mixed form.

Hypoactivity. (Degenerative changes)
(i) *In children*—**Progeria.**
(ii) *In adults*—**Simmonds' disease.**

PROGERIA

Appearance of early senility—often seen in pituitary dwarfs.

SIMMONDS' DISEASE

It is due to degeneration of the anterior lobe of the pituitary.

1. **Cachexia**—loss of weight and shrunken appearance.
2. **Sexual degeneration**—amenorrhoea, sterility, loss of axillary and pubic hairs, impotency (in males), etc.
3. **Asthenia**—extreme weakness.
4. **Anorexia**—loss of appetite.
5. BMR—lowered.
6. Mental deterioration.
7. Atrophy of viscera.
8. Hypoglycaemia.
9. Gonads, thyroid, adrenal cortex, etc., degenerate.
10. Anaemia.
11. Diminished urinary excretion of 11-oxycorticoids, 17-oxogenic steroids and gonadotrophins.
12. PBI in the blood falls.



Fig. 4.24. Frohlich's syndrome—children type (From Halliburton's).

PARS INTERMEDIA

The **pars intermedia** is anatomically associated with the neural lobe and is a thin strip of tissue, separated from the anterior lobe by the **interglandular cleft.** It invests the pars nervosa and with it, forms the posterior lobe. It develops from the posterior wall of Rathke's cyst. In man, the pars intermedia forms only 2% of the hypophysis, but in mouse it constitutes about 19%.

In some species it is absent. In others, it consists of vesicles filled with **colloid.** In man, it consists of basophilic **polygonal** or **prismatic cells** (Fig. 4.25). Under light microscope the cytoplasm of the cell contains barely visible small granules, but under electron microscope these granules are often polarised toward the basal lamina and they consist of glycoprotein. The pars intermedia cells contain Golgi apparatus.

In man, numerous anastomoses, between superior and inferior hypophyseal arteries, traverse the pars intermedia and it receives some supply from a rich capillary network. The plexus is continuous with the capillary bed of the neural lobe and it possesses some connections with sinusoids of the pars distalis.

Nerve fibres enter the pars intermedia from the neural lobe and ramify among its cells, originated from the hypothalamus. These nerves appear to have a mainly inhibitory effect. In amphibians and some mammals, neurosecretory materials have been demonstrated in some of the nerves of the pars intermedia but not in man as yet.

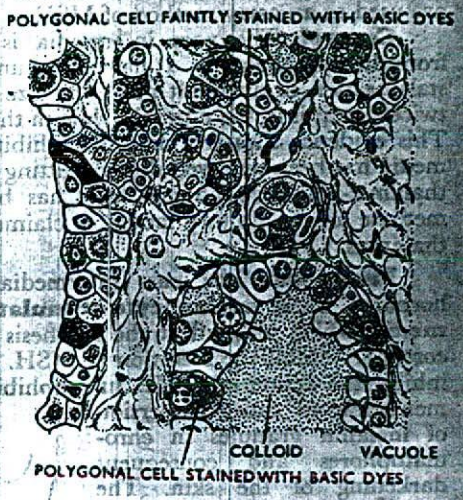


Fig. 4.25. Diagrammatic representation of pars intermedia showing basophilic cells and vesicles with colloid.

Chemistry. The melanocyte-stimulating principles are distinct for the MSH activity of ACTH and have been referred to α- and β-MSH.

α-MSH is a single chain polypeptide of 13 amino acids (Fig. 4.26) present in bovine, porcine, ovine, simian, equine and human pituitaries and has a C-terminal amide and N-terminal acetyl. As the amino acid sequence of α-MSH is identical to the N-terminal 13 amino acids of ACTH, the ACTH has a little melanin-dispersal activity. The synthesis of α-MSH has been carried out.

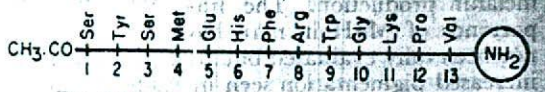


Fig. 4.26. Amino acid sequence of α-MSH.

The amino acid sequence of β-MSH from beef, pig, sheep, monkey, horse and human pituitaries has been established. Human β-MSH is a single chain polypeptide of 22 amino acids (Fig. 4.27), whereas this hormone from other species is a single chain of 18 amino acids (Fig. 4.28). The synthesis of β-MSH has been accomplished.

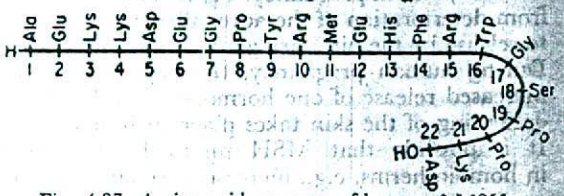


Fig. 4.27. Amino acid sequence of human β-MSH.

ACTH, α- and β-MSH and β-LPH have a similar heptapep-



Fig. 4.28. Amino acid sequence of bovine β-MSH.

tide amino acid sequence from position 4 to 10.

Assay. Changes in the individual pigment cells of amphibians or reptiles or measurement of the amount of light reflected from skin surface are used as an assay method of MSH.

Control. The pars intermedia is not deprived of all influences from the hypothalamus. It has been found recently that after the pituitary stalk section in an adult goat, the size of the pars intermedia increases twice that it is in the normal, while in the young, its size increases slightly. This evidence supports that the inhibitory influence on the pars intermedia has been removed due to cutting of the pituitary stalk, since hypothalamic-neurohypophyseal tract has been transected. MIF (the intermediate lobe-inhibitory factor) is claimed to be secreted from the hypothalamus.

Functions. The pars intermedia secretes a simple polypeptide hormone, called **melanocyte-stimulating hormone (MSH)** or **intermedin** which may affect the synthesis of melanin. Hydrocortisone and cortisone inhibit the secretion of MSH. Epinephrine and norepinephrine inhibit the action of MSH. In amphibian skin, MSH effects upon pigmentation to cause dispersion of melanin granules in chromatophores and consequent darkening of the skin. The gathering-in of granules is stimulated by **melatonin,** a hormone from the pineal body (Fig. 4.29). In mammals whose melanocytes are not subject to expansion and contraction, the effect of MSH appears to be on melanin production. The importance of MSH in man has not been fully evaluated but an increase in production may account for the increased pigmentation seen in Addison's disease, in some cases of thyrotoxicosis, and in pregnancy. Pigmentation that occurs in humans suffering from deterioration of the adrenal cortex (Addison's disease) is mainly due to release by the pituitary gland of excess blood ACTH and urinary MSH. During human pregnancy, the darkening of the skin may result from increased release of one hormone or both ACTH and MSH. Temporary darkening of the skin takes place in humans on administration of MSH. It is possible that MSH might have some extrapigmentary effects in homoiotherms, e.g., increase in synaptic potential.

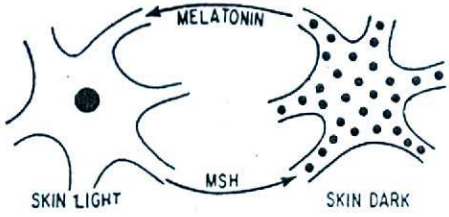


Fig. 4.29. Schematic representation of hormonal control of melanocytes in amphibian skin.

PARS TUBERALIS OR INFUNDIBULARIS

The pars tuberalis develops from the top of Rathke's pouch or cyst. Like the pars intermedia, the pars tuberalis is adjacent to and continuous with the pars distalis. The pars tuberalis is about 25 to 60 μm in thickness and forms a sleeve around the infundibular stalk. The thickest portion is on the anterior surface of the stalk.

The pars tuberalis is traversed by the major arterial supply for the pars distalis and the hypothalamo-hypophyseal venous portal system.

A thin layer of connective tissue which is continuous with the pia, separates the pars tuberalis from the infundibular stalk and the outside of the pars tuberalis contains a typical arachnoidal membrane of the connective tissue. Between these, the reticular fibres support the blood vessels and groups of epithelial cells.

The main component of the pars tuberalis is a cuboidal-columnar cell which contains numerous small granules or fine colloid dorplets. The mitochondria are short rods, and small lipid droplets may be present. Its epithelial cells contain undifferentiated and some small acidophilic and basophilic cells. In adult hypophysis, these cells are found and contain large amounts of glycogen. Sometimes the cells form a follicle-like structure. Despite the occurrence, the hormonal function of the epithelial cells of the pars tuberalis is not yet known.

STRUCTURE AND FUNCTIONS OF NEUROHYPOPHYSIS

Anatomically the posterior lobe includes the *pars nervosa* and the *pars intermedia*. But **physiologically** the term 'posterior lobe' commonly means the pars nervosa, which is the chief part of the neurohypophysis.

According to the terminology recommended by the International Commission on Anatomical Nomenclature, the neurohypophysis is made up of: (a) **Pars nervosa (Lobus nervosus)**—the true posterior lobe. (b) **Infundibulum**—pituitary stalk.

In some lower species the stalk and the pars nervosa are hollowed out by a canal, continuous with the third ventricle being the remnant of the original lumen of the nervous downgrowth. It is absent in mammals except cat.

Development—*Vide* pages 4-14, 4-15.

Pars nervosa. Pars nervosa consists of the following: (a) **Pituicytes.** The pituicytes are chief cells and contain fusiform or polygonal supporting **neuroglial cells** with delicate processes and make up the bulk of the gland. They are large, branching, spindle-shaped cells containing yellow-brown granules of neurosecretion due to presence carrier mucoprotein, stained with chrome-alum-haematoxylin. The pituicytes consist of *adenopituicytes, micropituicytes, fibropituicytes* and *reticulopituicytes* and do not actually secrete any hormone. (b) **Non-medullated nerve fibres,** (c) **Mast cells,** (d) **Herring (hyaline) bodies** which are densely staining accumulations of stored neurosecretory substance, and (e) **Blood vessels** (Fig. 4.30).

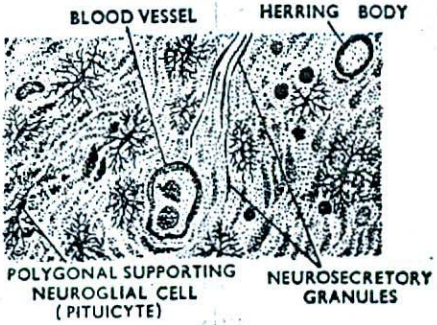


Fig. 4.30. Pars nervosa with nuclei of the pituicytes, neurosecretory granules and Herring (hyaline) body.

Pituitary stalk or **infundibulum** consists of clusters of numerous large sinusoidal vessels which are surrounded by neuroglial tissue, stained with H. E. From the hypothalamus neurosecretory cells contain **Nissl**

substance and smaller neurosecretory granules in the axons of the cells (Fig. 4.31), stained with chrome-alum-haematoxylin.

Blood supply. The neural lobe (pars nervosa) gets blood supply from inferior hypophyseal arteries originating from the internal carotid arteries. The vessels while ending in the pars nervosa do not form any sinusoids but with capillary network.

Nerve supply. Two tracts of non-medullated nerve fibres (containing at least 1,000,000 fibres in man) arise from the hypothalamus and supply the pars nervosa (Fig. 4.32). They are as follows:

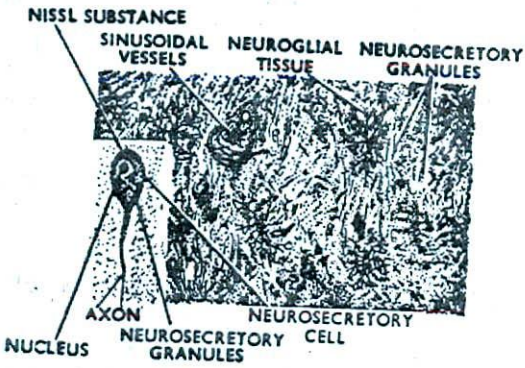


Fig. 4.31. Diagrammtic represention of microscopic structures of pituitary stalk with a neurosecretory cell *(inset)*.

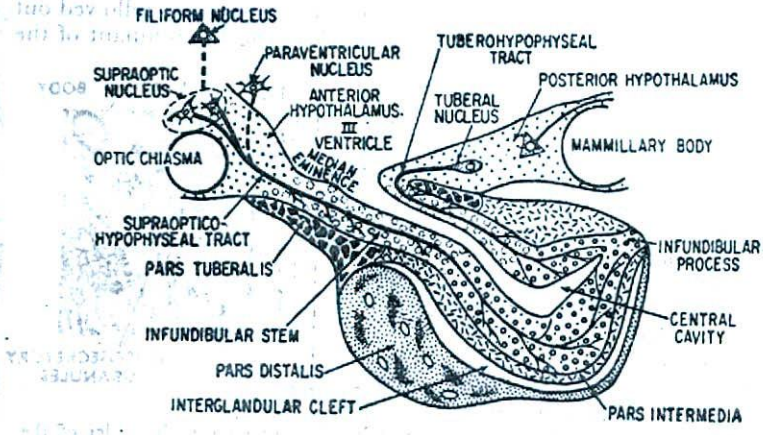


Fig. 4.32. Diagrammatic representation of the hypophysis cerebri and its connections with the hypothalamus (animal).

(a) **Tuberohypophyseal tract** arises from the median eminence of the tuber cinereum which is the protuberance at the floor of the third ventricle near the root of the stalk, passes along the posterior wall of the stalk and enters the gland.

(b) **Supraoptico-hypophyseal tract** arises from the supraoptic and paraventricular nuclei in the hypothalamus, passes down the anterior wall of the stalk, enters the pars nervosa and ends round the blood vessels and cells of the gland. *A few fibres have been traced to the pars intermedia and fewer still to the pars distalis or pars anterior.*

Synthesis, transport and storage of the neurohypophyseal hormones in the hypothalamo-hypophyseal system

Vasopressin and **oxytocin** are the two neurohypophyseal hormones. These hormones have been extracted and isolated from the hypothalamic nuclei in higher concentrations than those present in other areas. Both the supraoptic and paraventricular nuclei contain distinctive dense granules.

It is possible that supraoptic nuclei mainly forms vasopressin (ADH) while the principal hormone synthesised in the paired paraventricular nuclei and to a lesser extent in the paired supraoptic nuclei is oxytocin.

Posterior pituitary hormones are not synthesised in the gland itself, but these are synthesised in the supraoptic nuclei and paraventricular nuclei of the hypothalamus. Hormone synthesis presumably is initiated on the ribsomes *via* pathways common to the biosynthesis of other proteins and peptides, involving tRNA, mRNA, etc. (Fig. 4.33). The hormones are then transported from their origin to the posterior pituitary through axons of the hypothalamo-hypophyseal tracts and stored in association with 2 proteins—**neurophysin I** (mol. wt. of 19,000) and **neurophysin II** (mol. wt. of 21,000) (Hollenberg and Hope, 1968). Either vasopressin or oxytocin can be bound by each neurophysin. When liberated in the blood, vasopressin and oxytocin are transported to the target organs like kidneys, liver, mammary glands, etc., in association with serum proteins (Fig. 4.34).

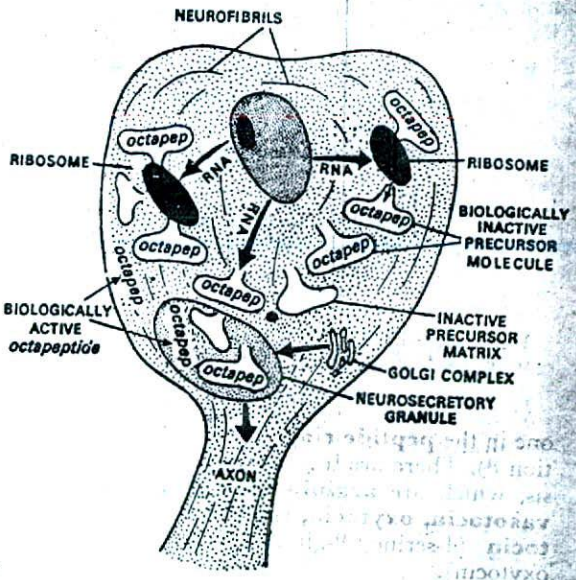


Fig. 4.33. Diagrammatic representation of octapeptide synthesis in the hypothalamic cell body of the neurone. mRNA seems to trigger the synthesis of peptides on ribosomes. Hormone precursor and removed biologically active peptide are incorporated into neurosecretory granules which are transported along the axon into the posterior pituitary for storage.

If the posterior pituitary is removed keeping its hypothalamo-hypophyseal tracts intact then hormonal functions remain unaffected and the nerve tracts show accumulations of neurosecretory materials.

Chemistry. Vasopressin and oxytocin, the two secretory hormones of the neurohypophysis, are octapeptides because they consist of 8 different amino acid residues arranged with a five-member S-S-bonded

ring and a tail composed of 3 amino acids. The amino acid sequence of oxytocin differs from that of vasopressin in two locations (Figs.4.36 & 4-38),

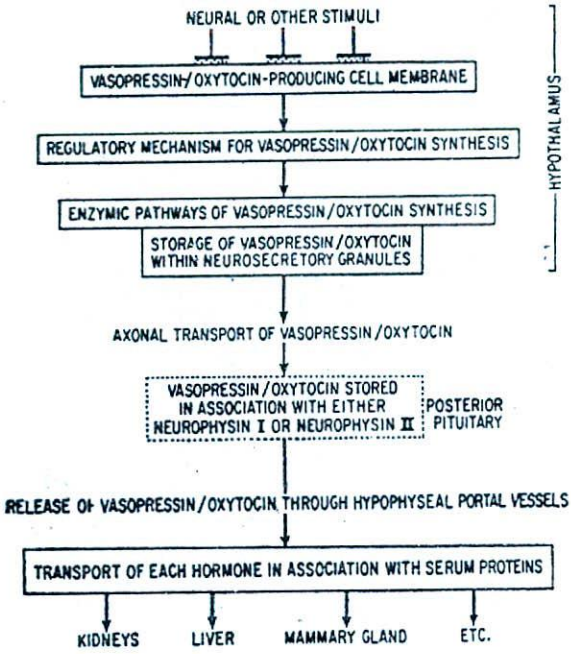


Fig. 4.34. Scheme showing intermediate stages which are involved in the neurosecretory function of the vasopressin-/oxytocin-producing cells of the mammalian hypothalamo-neurohypophyseal complex.

one in the **peptide ring** (position 3) and one in the **tripeptide tail** (position 8). There are seven naturally occurring principles in neurohypophysis, which are **arginine vasopressin, lysine vasopressin, arginine vasotocin, oxytocin, isotocin** (4-serine, 8-isoleucine oxytocin), **glumitocin** (4-serine, 8-glutamine oxytocin), and **mesotocin** (8-isoleucine oxytocin).

Phylogenetic distribution. Neurohypophyseal hormones are distributed in all classes of vertebrates having changes in amino acid composition which makes them pharmacologically different. Phylogenetic distribution of neurohypophyseal hormones has been presented in Fig. 5.35. Arginine vasotocin is the most widely distributed neurohormones and is the only principle present in cyclostome, the most primitive of all vertebrates. In elasmobranch fish, octapeptide resembling vasotocin is also present. Though it is not present in mammals, yet it is regarded as a very ancient molecule and is present in the four major lines of evolution leading of cyclostomes, chondrichthyes (shark-like fishes), the actinopterygians (ray-finned fishes) and the tetrapods. Arginine vasopressin along with oxytocin is found in the pituitaries of teleostean fishes, amphibians, reptiles and birds.

Two types of vasopressins are found in mammalian pituitary of which arginine vasopressin is most widely distributed. Lysine vasopressin has been found in the domestic pig and hippopotamus. Oxytocin present in bony fish as well as in reptiles, birds and amphibia.

increases facultative reabsorption of H_2O and thus reduces the volume of urine formed. Hence, vasopressin is now commonly called **antidiuretic hormone (ADH).** Vasopressin rises blood pressure by its vasopressor effect on peripheral blood vessels.

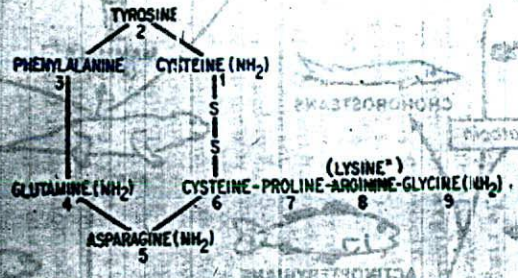


Fig. 4.36. Scheme showing the arrangement of amino acids in a molecule of arginine [beef] vasopressin (lysine [hog] vasopressin).

Evidences

1. **Clinical:** Diseases of the posterior pituitary, hypothalamus or injury to the nerve tracts of the gland produces a condition in which large amount of very dilute urine is produced. The condition is called **diabetes insipidus.** *Diabetes insipidus is the syndrome which results from the failure of the neurohypophyseal system to produce or to release a quantity of ADH (vasopressin) sufficient to bring about the normal homeostatic renal conservation of free water.* This syndrome is now called **vasopressin-sensitive diabetes insipidus** to distinguish it from the *nephrogenic diabetes insipidus* and is characterised by diuresis—up to 28-30 litres of urine per day. Subcutaneous administration or nasal instillation of posterior pituitary extract may control the disease.

2. **Experimental:** (a) Injection of the posterior pituitary extract diminishes urine volume in man.

(b) Diuresis that follows intake of large volume of water, is delayed by 4-5 hours after injection of the crude pituitary extract.

(c) When an isolated kidney is perfused with blood, from a heart-lung preparation, large amount of dilute urine is formed. If the pressure and volume of blood passing through the kidney be kept constant, addition of small amounts of pituitrin reduces urine volume and makes it more concentrated. The output of chloride per minute markedly increases. These results show that the antidiuretic effect is not caused by renal vasoconstriction but is due to the *action of antidiuretic hormone on the epithelium of the renal tubules.*

Units of vasopressin

The international standard of vasopressin has an activity of 2000 units per g and the unit is defined as the activity present in 0·5 mg of the dried material.

Assay

(a) Pressor activity is assayed by comparing rise in blood pressure following intravenous injection in anaesthetised rats, dogs and spinal cats, with that produced by known amounts of a standard preparation. (b) Other one depends upon the antidiuretic activity by comparing the

The **function of the neurohypophyseal hormones** in the phylogenetic sequence has also been studied. *Arginine and lysine vasopressins are*

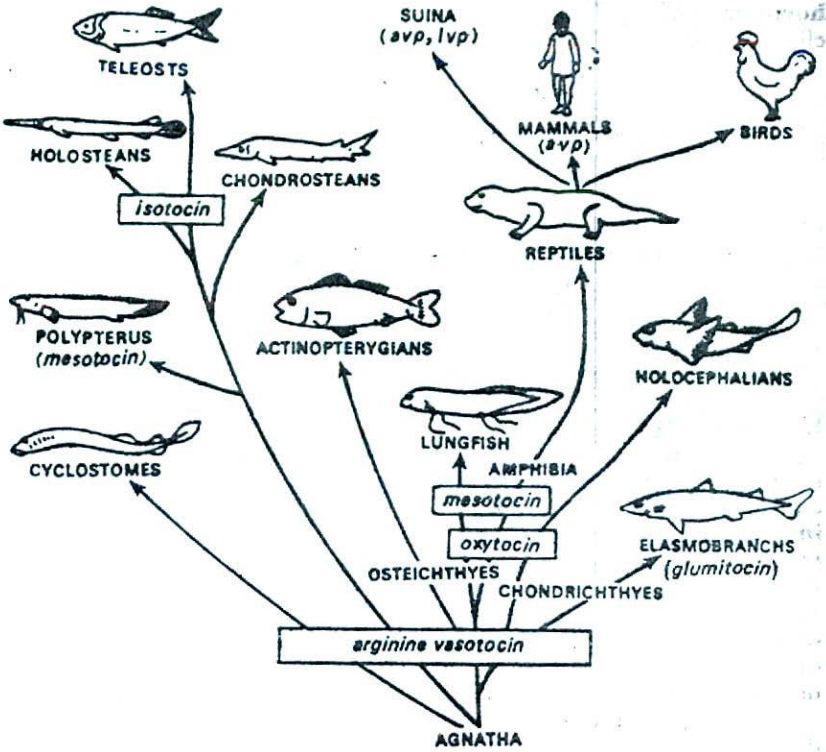


Fig. 4.35. Diagrammatic representation of the phylogenetic distribution of neurohypophyseal principles among the various major groups of vertebrates.

antidiuretic in mammals, but arginine vasotocin performs the such function in amphibians, reptiles and birds. Neurohypophyseal hormones have got no effect on water conservation in fishes and probably these hormones may take part in regulating the scretion of adenohypophyseal hormones. In mammals oxytocin takes part in milk ejection, parturition and in sperm transport. In birds arginine vasotocin acts like oxytocin. If vasotocin is administered in mammals then it acts like oxytocin. During evolution, vasotocin is discarded and clear separation appears between the octapeptides—vasopressin having water-conserving power and oxytocin having control over uterine contractility and milk ejection.

I. Vasopressin (Pitressin)

Chemistry. It is octapeptide (Fig. 4.36). Vasopressin has molecular weight of about 1100.

Vasopressin (ADH) is released in response to stress, and also in response to dehydration. Vasopressin has got antidiuretic property and

reduction in urine volume following intravenous injection in hydrated unanaesthetised rats or hydrated rabbits with that produced by a standard preparation. (c) At present a radioimmunoassay has been developed to detect ADH, utilising rabbit-produced antisera to either lysine or arginine vasopressin.

Mode of action

1. It stimulates reabsorption of water by the distal convoluted and the collecting renal tubules and thus regulates urine volume. The action of this ADH may be due to an increase in the enzyme *hyaluronidase* in the renal tubules and thus increase in the permeability of the intercellular ground substance. ADH increases the concentration of cyclic AMP in the renal epithelium and its antidiuretic action is mediated through cyclic AMP while addition of cyclie AMP mimics effects of vasopression on water and sodium transport.

2. It reduces chloride absorption and thus increases chloride loss. [Antagonistic to adrenal cortex.]

3. It causes contraction of all the plain muscles of the body excepting those of heart and uterus. Its action is directly on the muscles, independent of nerves. It should be noted that the following actions of antidiuretic hormone are observed only at a pharmacological dose level, which is much higher in concentration than that is observed in circulating blood.

Actions (*with a high dose level*)

1. **On blood vessels and blood pressure**—constriction of arterioles and capillaries *in animals* but only of capillaries *in man*. Generally, it raises blood pressure. But in human beings often a fall is noticed. The fall is due to cardiac depression. The portal venous pressure is reduced due to constriction of the splanchnic vessels.

2. **On heart.** Rate is reflexly reduced due to high blood pressure. Coronary vessels constricted.

3. **On respiration.** Hyperpnoea with occasional apnoea. This is due to changes in blood pressure which reflexly acts on the respiratory centre.

4. **On kidneys.** Due to renal vasoconstriction, urine volume is reduced. In this way it also acts as an *antidiuretic*.

5. **On plain muscles.** The muscular walls of the urinary bladder and ureter are stimulated.

6. The **intestinal muscels** contract and **movements of stomach, large and small intestines** are increased.

7. **Metabolism.** It produces glycogenolysis, hyperglycaemia and glycosuria. Sugar tolerance is reduced. The effect is only a secondary one.

Under physiological conditions, posterior pituitary secretes only small amount of vasopressin which regulates the urine volume only and has no vascular effects. ADH shows pressure effects only at pharmacological dose level, concentration so high that has not been observed in plasma any time.

One should note that the vasopressin is not responsible for the whole amount of water reabsorbed. Only a fraction of the total is reabsorbed with its help. In man about 170 litres of filtrate are formed by the two

kidneys daily. Of this about 168·5 litres are reabsorbed and only 1·5 litres pass out as urine. In extreme cases of diabetes insipidus (i.e., absence of antidiuretic hormone) the maximum urine output is about 40 litres only. This shows that the antidiuretic hormone is responsible for the absorption of about 38·5 litres of water only. The remaining 130 litres are reabsorbed without the help of this hormone.

Control of the secretion of vasopressin (ADH)

1. **Water deprivation.** Moderate or severe dehydration causes increased secretion of vasopressin. In response to water deprivation and to maintain the total plasma volume, vasopressin is liberated from the neurohypophysis.

2. **Plasma volume.** When a large quantity of water is taken, blood becomes diluted and its osmotic pressure falls. This acts through hypothalamus and reduces the secretion of the hormone. Consequently, less water is reabsorbed, urine volume increases and the excess water is got rid of. On the other hand, in conditions of dehydration, the concentrated blood with raised osmotic pressure stimulates the secretion of the hormone by acting on the **osmoreceptors,** present in the supraoptic nuclei of the hypothalamus and thus increases reabsorption of water. So that urine volume is reduced and more water is conserved in the body. In such conditions vesopressin may even escape in the urine. Thus the secretion of this hormone is adjusted according to the water requirements of the body.

3. **Plasma crystalloid osmotic pressure.** Raised crystalloid osmotic pressure of the blood produced either by water deprivation, or increased chloride, urea or glucose concentration in the blood increases secretion of ADH. Lowered crystalloid osmotic pressure produces diuresis by inhibiting the secretion of ADH.

4. **Role of atrial receptors.** Stimulation of atrial receptors causes inhibition of secretion of vasopressin and thus urine flow is increased. Henry and Pearce (1956) and also others have demonstrated the increase of urine flow after inflation of balloon in the left atria of anaesthetised hydrated dogs.

5. **Negative pressure breathing.** Gauer and Henry (1963) have demonstrated the inhibition of secretion of vasorpressin during negative pressure breathing. This manoeuvre causes water diuresis through the stimulation of atrial receptors.

6. **Positive pressure breathing.** On the other hand, Gauer and Henry (1963) have demonstrated the increased secretion of vasopressin during positive pressure breathing.

7. **Haemorrhage and other changes in blood volume.** Decrease of blood volume due to haemorrhage causes release of vasopressin. Haemorrhage being as little as 10% of blood volume causes an effect of inhibition of water diuresis due to an increase in the plasma vasopressin level (Fig. 4.37). This response depends upon the degree of blood loss. The vasopressin release in response to haemorrhage is impaired in animals whose vagi are cut. Vagotomy interrupts afferent pathway from low pressure receptors in the left atrium and probably others portions of the low-pressure side of the circulation. In course of haemorrhage, the fall of atrial pressure causes an effect due to a drop in the central venous pressure and a fewer impulses arise from the low-pressure receptors.

8. **Role of baroreceptors.** Bilateral carotid occlusion in anaesthetised dogs causes release of vasopressin. Lemaire *et al.* (1962) and Perlmutt (1963) have suggested that receptors at the thyrocarotid junction may mediate vasopressin release following carotid occlusion.

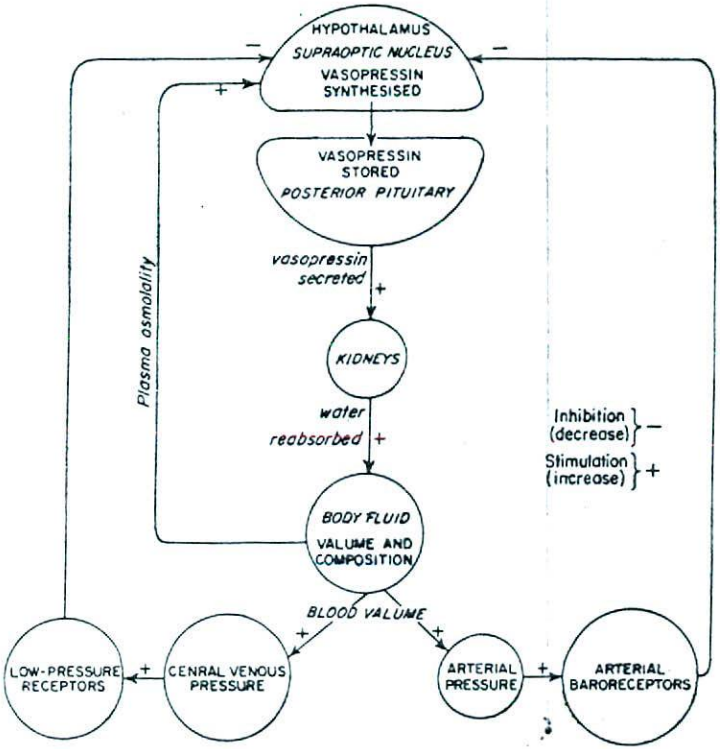


Fig. 4.37. Schematic diagram shows control of vasopressin secretion by plasma osmolality and blood volume (after Share, 1968).

9. **Hypoxia.** It is suggested that hypoxic stimulation may cause vasopressin release. But this is not settled whether this release of vasopressin has got any relation with the cardiovascular regulation during asphyxia.

10. **Pain.** Painful stimulation may cause vasopressin release. Mills and Wang (1964) have observed inhibition of urine flow during stimulation of sciatic nerve in anaesthetised animals. This response has been considered to be due to the release of vasopressin.

11. **Cholinergic mechanism.** Acetylcholine may stimulate the secretion of vasopressin, acting either directly on the neurones in the hypothalamus or the neural lobe.

12. **Adrenergic mechanism.** Adrenergic mechanism in the secretion of vasopressin is not clear. O'Connor and Verney (1945) have observed

epinephrine to cause a brief antidiuresis in conscious dogs probably by reducing renal blood flow.

13. Emotional and physical stress, electrical stimulation, nicotine and morphine increase ADH secretion. Alcohol inhibits ADH secretion.

II. Oxytocin (Pitocin)

Chemistry. It is octapeptide (Fig. 4.38). Molecular weight of oxytocin is about 1000. Oxytocin shares some of the activities of ADH or vasopressin and vice versa, and thus oxytocin is minimally antidiuretic. It produces vasodilation and a decrease in peripheral resistance and blood pressure, an effect which is directly opposite to that seen with ADH and one which is blocked with small doses of ADH.

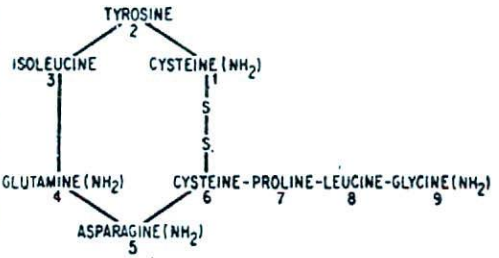


Fig. 4.38. Scheme showing the arrangement of amino acids in a molecule of oxytocin.

Oxytocin is an important uterus-contracting hormone of the posterior pituitary. This hormone gives rise to contracting of smooth muscle in the mammary gland, resulting in milk excretion. Oxytocin may exert a physiological action on the myometrium postpartum. Since during suckling an increase in contractility of the postpartal uterus is observed, a relationship between suckling, release of oxytocin and involution of uterus may exist.

Unit of oxytocin. One unit of oxytocin is equivalent to the activity of 0·5 mg of the standard dried posterior lobe, as tested on an isolated guinea-pig's uterus under standard conditions. A good preparation may have 225 units per mg. A dilution of 1 in 550 million causes strong contraction of the uterus.

Assay. (a) Oxytocic activity is assayed by comparing the degree of contraction of the isolated virgin guinea-pig uterus, in vitro, with that produced by known amount of a standard preparation. (b) Another method depends upon the blood pressure lowering effect of oxytocic principle in the anaesthetised chicken.

Action of oxytocin (Gr. *Oxys*=swift, *tokos*=childbirth)

1. **Uterus.** The action of oxytocin on uterus depends upon the species and pretreatment of the animal with oestrogen.

In the guinea-pig, it causes strong contraction of the virgin uterus, suspended in Ringer-Locke solution, This fact is made use of for assaying the oxytocic action of an unknown extract.

In the human beings, there is no conclusive evidence as regards the role of oxytocin in normal parturition process. On the virgin uterus its action is negligible. There is slight action in the first half of menstruation and a little more in the second half. During pregnancy, the action in the first half is very little. The action increases as pregnancy advances and becomes maximum during labour (2nd stage). Oxytocin thus takes part in the onset of parturition, expulsion of the foetus and placenta. The effect persists to some extent during puerperium and then gradually dies out.

Its action is inhibited by progesterone and increased by oestrogens. In the later months of pregnancy corpus luteum degenerates. The proportion of oestrogens increases and thus makes the uterine muscles more sensitive to circulating oxytocin. This explains why oxytocin acts on uterus in late pregnancy.

2. **Milk ejection.** It causes contraction of the myoepithelial cells around the mammary alveoli. The hormone also is responsible for maintaining the function of such tissue by releasing prolactin from anterior pituitary.

3. **Metabolism.** It produces hyperglycaemia in large doses, specially in dogs.

4. **Sperm transport.** Oxytocin facilitates the transport of sprem in the female genital tract.

5. **Water metabolism.** Oxytocin given in large doses and in abundant fluid volumes may cause water intoxication due to antidiuretic effect of oxytocin on the nephron.

6. Another action of oxytocin, probably of physiological significance, is to stimulate contraction of gall-bladder, intestine and urinary bladder.

Control of secretion of oxytocin

Hypothalamus through supraoptico-hypophyseal tracts controls the secretion. It forms the hypothalamo-neurohypophyseal unit. The mechanism of secretion has been studied by special staining method and the neurosecretory mechanism has been confirmed. According to Cross (1966) the major stimuli for release of oxytocin are (i) sucking and (ii) distension of cervix and vagina. Increased milk ejection following administration of oxytocin does not cover increased milk secretion but rather ejection of milk already present in the mammary glands. Oxytocin is released into the systemic blood stream which causes contraction of the myoepithelial cells surrounding the alveoli of the mammary glands and helps in the ejection of accumulated milk. Oxytocin level is increased after stimulation of nipple by suckling of the baby. It is reported that level of oxytocin has been measured in blood of the jugular vein before, during and after suckling or milking and have been found that there is an increase during suckling than before suckling. A specified unit of oxytocin injection duplicated the normal milk-ejection response to suckling. Tactile stimuli evoked by suckling and milking young from temperature and pressure receptors in the nipple are conducted via afferent nerve fibres to the central nervous system. These impulses compete with those from inhibitory centres for the final common path whose cell bodies are the paraventricular nuclei of the hypothalamus (Fig. 7.39). The role of oxytocin in normal labour is not fully evaluated. Labour is a complicated process which depends upon softening and dilatation of the cervix, loosening of pelvic ligaments and contraction of abdominal muscles. There is no effect of oxytocin in these events. But it has been found that there is an increase of blood levels of oxytocin in ewes during delivery of the lamb than during pregnancy. Similarly it has been reported that there is an increase in oxytocic activity of human systemic blood during second stage of labour than during pregnancy. Lesions in the supraoptico-hypophyseal tract in the anterior part of the median eminence impair labour grossly. So oxytocin seems to

serve a major role in labour. The frequency and duration of contraction in myometrial cells of the uterus are stimulated. For this reason many of obstetricians frequently give oxytocin in order to induce or intensify contractions of the uterus.

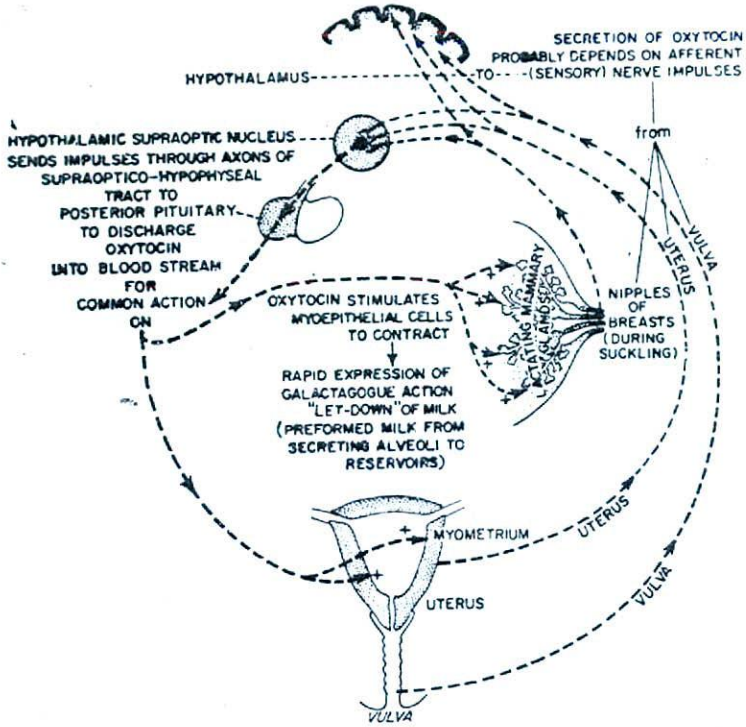


Fig. 4.39. Diagrammatic representation of functions of oxytocin in the female; but its function in the male is unknown.

Though the function of oxytocin is still unclear plasma levels of oxytocin are more or less same in women and men. It has been proved that the transport of sperms in rabbits to the epididymis is accelerated.

Disorders of Function of the Posterior Pituitary. Destruction of the neurohypophysis causes diabetes insipidus (*insipid*=tasteless). This is a condition in which large quantities of urine of very low sp. gr. 1·002 to 1·006 and low chloride content are excreted. But further development of lesion may destroy the pars distalis with amelioration of the diabetes.

In diabetes insipidus fluid intake is increased and thirst is often intense. This condition frequently accompanies tumours of the pituitary or hypothalamic region, and is essentially due to absence of posterior pituitary hormone. It has been shown that lesions in the supraoptico-hypophyseal tract in the hypothalamus in cats developed a permanent polyuria. A polyuria may be observed in human patients with an expanding lesion

developed in the sella turcica. A general diuretic effect which is necessary for the development of a marked polyuria, is exerted by the pars distalis. When animals are deprived of the pars distalis of the adenohypophysis, as well as the neurohypophysis causes only a slight development of polyuria if any at all.

Diabetes insipidus may be controlled with the administration of the hormone vasopressin except some cases. Such individuals may possess an inherent defect of the renal tubules with respect to renal reabsorption of H_2O.

NEUROENDOCRINOLOGY

The subject neuroendocrinology describes the interactions of nervous and endocrine systems. The nervous system is more concerned with adjustments that are rapid, whereas the endocrine system is concerned with slower chronic metabolic adjustments. The chemical substances released at the nerve endings are named as neurosecretions and are of several types.

The effect of the internal secretions on the nervous system can be seen from the following observations: (1) Physiological changes are found in association with changes in reproductive hormone titers during sex cycle. (2) Injection of sex hormones in certain parts of the brain stem changes the behavioural pattern. (3) Hyperactivity and hypoactivity of the thyroid affect brain activity and nervous tissue excitability. (4) Adrenal cortex and parathyroid glands affect nervous tissue excitability indirectly through changes in electrolyte concentration. (5) Anterior pituitary activity is influenced by injections into the hypothalamus of minute quantities of certain hormones of the pituitary target organs.

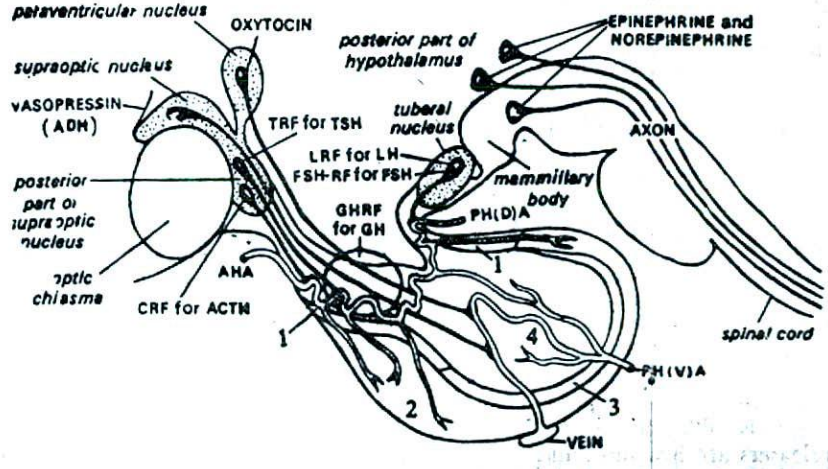


Fig. 4.40. Diagrammatic representation of hypothalamic control areas for the hormone secretions of anterior pituitary and others.
1=pars tuberalis, 2=pars distalis, 3 =pars intermedia, 4=pars nervosa. AHA=anterior hypophyseal artery, PH(V)A=ventral branch of posterior hypophyseal artery, PH(D)A=dorsal branch of posterior hypophyseal artery.

At the nerve endings of synapses, glands (exocrine and endocrine) and in many other organs nor-epinephrine and acetylcholine are liberated as a result of nerve impulses arriving on them. The above two neurosecretions are responsible for the activity of the organs concerned. Although the different endocrine organs have nerve connections also, they do not have any major control over secretion.

The secretions of the *neurosecretory cells of the hypothalamus* control the activity of both the anterior and posterior pituitary. The paired supraoptic and paraventricular nuclei of the hypothalamus synthesise the hormones of the posterior pituitary, oxytocin and vasopressin (ADH). The neurosecretory granules which can be stained by suitable staining methods, travel *via* the hypothalamo-hypophyseal tract to pars nervosa. The morphology of neurohypophysis permits a ready release of oxytocin or vasopressin (ADH) into the circulation. Neurovesicles lie very close to the basement membrane of the perivascular space, and the released hormones can generally reach it and enter the capillary bed through its thin fenestrated endothelium. Since processes of neurosecretory cells may reach third ventricle, a direct release of oxytocin and vasopressin in the cerebrospinal fluid (CSF) may also be possible.

In response to afferent impulses, chemical or neural, received from all over the body, different areas of the hypothalamus elaborates different neurosecretory-releasing factors. The releasing factors are *corticotrophin-releasing factor* (CRF), *thyrotrophin-releasing factor* (TRF), *growth hormone-releasing factor* (GHRF or SRF), *FSH-releasing factor* (FSH-RF) and *LH-releasing factor* (LRF), which are responsible for the release of the pituitary trophic hormones ACTH, TSH, STH or GH, FSH or ICSH and LH rexpectively. These neurosecretory-releasing factors travel from nerve cells to axons and then to capillaries which drain the sinusoids of the adenohypophysis (Fig. 4.40). Two inhibitory factors: (1) Prolactin-inhibitory factor (PIF) and intermediate-lobe-inhibitory factor (MIF) which inhibit the release of prolactin and MSH respectiaely, are similarly elaborated by the hypothalamic nuclei. A species specific prolactin-stimulating factor (PSF) is suggested to be present in pigeon hypothalamus. Thus secretion of prolactin by pigeon pituitary is not inhibited but stimulated by the hypothalamic secretion.

Chemistry of Releasing Factors. Chemical isolation and purification of releasing factors has been determined in different laboratories. The common purification procedure is to extract frozen tissue or acetone powders of lyophilised hypothalamic tissue commonly with *glacial acetic acid*. Such extracts are exposed to preparative gel filtration on columns of Sephadex G-25. Another purification method is commonly determined by chromatography on ion exchange cellulose. Lastly common usage are electrophoresis, partition chromatography, or counter-current distribution methods. Peptides are usually isolated by this procedures and the releasers are low molecular weight peptides. Proteolytic enzymes inactivate all the releasers save TRF.

The structure of Thyrotrophin Hormone RF. Ovine and procine TRF is (pyro) Glu-His- Pro (NH_2). 2-pyrrolidone-5-carboxylic acid (Pyroglutamic acid) forms N-terminus whereas protein amide forms C-terminus.

Although synthetic and natural TRF possesses usually identical biological activities, but the administration of tri-iodo-thyronine inhibits synthetic TRF in vivo and in vitro responses. The decrease or elimination of biological activity of TRF can also be modified in the chemical structure.

The structure of Growth Hormone RF. About 98% of dry weight of GRF consists of Lys-His-Ammo-Ser-Glu-Ala-Val-Leu. *GRF is an acidic polypeptide.* Its molecular weight is about 1600. Purified GRF is active in vivo determined by the pituitary depletion assay method. GIR (growth hormone inhibitory factor) was also determined during the course of screening fractions for GRF activity in vitro assay.

The structure of Gonadotrophin Hormone RF. Until recently release of gonadotrophin hormones LH and FSH was controlled by LRF and FRF (Fig. 4.9). According to Schally and others a single hypothalamic factor controls both FSH and LH secretions. The structure was confirmed by chemical synthesis. Purified preparation of both LRF and FRF activity is only one amino acid sequence (pyro) Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2. Like the natural substance the synthetic decapeptide apparently raises the concentration of LH in the plasma of ovariectomised rats pretreated with oestrogen and progesterone, stimulates release of FSH and LH from the in vitro pituitary and raises plasma levels of FSH and LH in castrated male rats pretreated with testosterone propionate.

The structure of Prolaction Hormone IR. Essentially the chemistry of PIF is not known. Commonly the used assay procedures have postulated either the inhibition of prolactin release from the in vitro pituitary or prevention of suckling-induced depletion of pituitary prolactin. Prolactin secretion is inhibited by epinephrine and nor-epinephrine in vitro; however there are many reasons that, catecholamines themselves are not PIF.

The structure of Corticotrophin RF. Untill now pure form of CRF is not prepared. CRF activity seems to decrease rather than increase. It is also difficult to prepare bioassay. Yet many evidences indicate that the active molecule of CRF is a small polypeptide, but not vasopressin. According to Saffran et al., substances in neurohypophyseal extracts, distinct from vasopressin would stimulate release of ACTH from the anterior pituitary. From porcine prosterior pituitary α- and β-CFR were available. β-CRF contains serine and histidine in addition to the amino acids of lysine vasopressin. It is almost doubtful α- or β-CRF forms a true physiological corticotrophin-releasing factor.

Role of Hypothalamic Releasing Factor in Human beings. Although active extracts have been prepared, yet highly purified porcine and ovine releasing factors are active in human beings. In a amenorrheic or anovulatory women / after administration of FRF plasma FSH levels rises; LH level rises rapidly following infusion of LRF in adult patients or children. After injection of small amounts of TRF in a human athyreotic cretins produces a marked elevation in plasma TSH after a few minutes and urinary and circulating 17-hydroxycorticoids cause to raise following intravenous perfusion of hog and beef CRF extracts. According to Schally in human beings the release of pituitary hormones is regulated by hypothalamic hormones and the molecular weights, iso-

electric points, etc., and perhaps the structure of TRF of human hypothalamic hormones are almost similar like those of domestic animals. After administration of synthetic TRF, there is an elevation of serum TSH and a significant rise of TSH levels. Pretreatment with T_3 before administration of TRF partially or completely inhibited the response to TRF.

Neuroendocrine interrelations

Earlier the anterior pituitary gland commands all other peripheral endocrine glands (with the unhappy exception of parathyroids and perhaps the pancreas) and a reciprocal equilibrium between concentrations of circulating *peripheral* hormones and quantities of the corresponding pituitary hormones (feedback or push-pull theory) maintained endocrine homeostasis. Nowadays the centre of control of the adenohypophyseal functions is to be found in the hypothalamus, and the feedback relationships between peripheral hormone levels and adenohypophyseal secretions are for the most part transhypothalamic, and the hypothalamic control over the pituitary functions is exerted through the secretion of hypothalamic hormones or releasing factors, which may be called hypophyseotrophic substances or hormones.

Hypothalamic-hypophyseal portal system. Connections between the hypothalamus and the pars distalis are not provided through tracts or nerve fibres (as in the case of hypothalamus to the pars nervosa), but rather through a vascular link in the form of a system of portal vessels. The *primary plexus* of this vascular system is to be found in a contact area between the ventral hypothalamus and the pituitary stalk (median eminence). Axon terminals from the cells of the hypothalamic

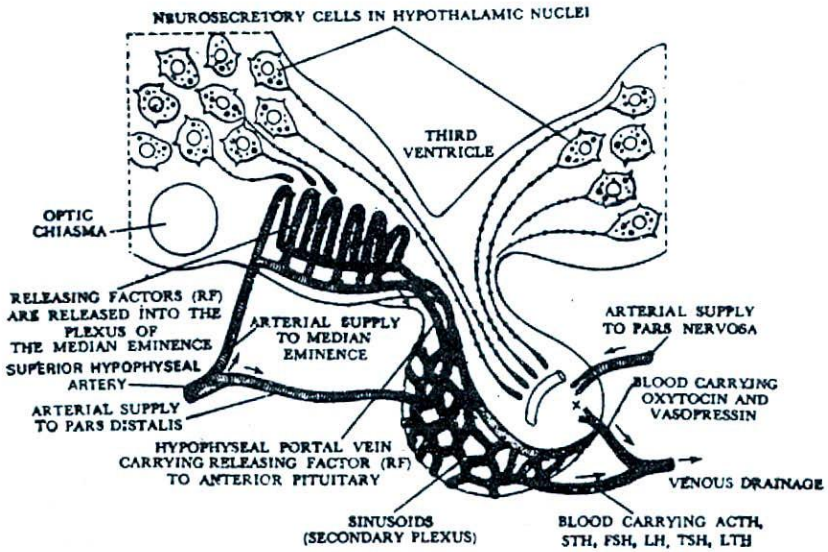


Fig. 4.41. Anatomical organisation showing hypothalamic and hypophyseal neurovascular link for the control of hormonal secretion from the hypophysis cerebri.

nuclei come into close contact with the capillaries of this primary plexus, and perhaps there is passage of the hypothalamic-releasing factors into the blood stream of these capillaries. The substances are distributed through collecting veins in the pituitary stalk into the adenohypophyseal parenchyma by the secondary plexus of capillaries of the hypothalamo-hypophyseal portal system (Fig. 4.41). The neuroendocrine areas in the hypothalamus appear to be somewhat diffuse if the considerable overlapping of the hypophyseotrophic activities related to one area or another is considered. These hypophyseotrophic areas do not coincide with the classic nuclei of the hypothalamus.

Control circuits

1. **Hypothalamic feedback.** The feedback mechanisms between adrenocortical or ovarian hormones, on the one hand, and ACTH or gonadotrophins (LH, FSH) secretions, on the other, are mediated by the hypothalamus. This feedback effect disappears after placement of the hypothalamic lesions or after peripheral transplantation away from the hypothalamus. Administration of crude hypothalamic extracts or purified preparations of releasing factors (CRF, LRF and FSH-RF) will still stimulate pituitary secretion of ACTH, LH and FSH in subjects pretreated with large doses of glucocorticoids or gonadal steroids. In the case of GH, acute secretion will not take place in animals with a hypothalamic lesion and occurs reflexly when hypoglycaemia is acutely produced by insulin injection. Conversely, local perfusion of the hypothalamic region with glucose solution will inhibit the reflex secretion of GH induced normally by hypoglycaemia or desoxyglucose administration.

2. **Pituitary feedback.** In the case of thyroxine or tri-iodo-thyronine and TSH secretion, most of feedback effects may take place at the level of the pituitary tissue, increased levels of throxine or tri-iodo-thyronine rapidly inhibiting the secretion of TSH by the isolated pituitary and also inhibiting the stimulation of TSH secretion produced by administration of TRF.

3. **Short-loop feedback.** The ACTH, LH and TSH may directly depress their own secretion by acting at the hypothalamic level.

Neuroendocrine integration

Hypothalamus receives a good number of afferent fibres, and hypothalamo-telencephalic as well as hypothalamo-adenohypophyseal afferents constitute the basis for reverberating circuits and feedback mechanisms. The hypothalamic afferent fibres come usually from the limbic system (hippocampus and amygdala) and globus pallidus, i.e., from the oldest part of the cortex and the basal ganglia. This system sends fibres into the medial forebrain bundle of the hypothalamus in addition to more certain specific projections upon hypothalamic nuclei. The hypothalamus also has connections with reticular activating substance and midbrain.

The integrity of these systems is necessary for a harmonious function of the neuroendocrine mechanisms involved in maintaining homeostasis. In the case of ACTH, LH, FSH and TSH, certain lesions in the amygdala, the hippocampus or the midbrain will inhibit, prevent or facilitate the release of these hormones as if some of the higher centres exerted stimulatory as well as inhibitory influences on neuroendocrine functions of the

hypothalamus, eventually resulting in corresponding modifications of secretions of the adenohypophysis. Electrophysiological studies have shown that these same extra hypothalamic systems influence the electrical activity of hypothalamic centres in the same directions. Thus the neuroendocrine activity of the hypothalamus is modified and modulated by the same neurophysiological components which modify and modulate the other autonomic functions of the hypothalamic centres.

Lesions of areas of the limbic system or of the midbrain will inhibit the circadian or diurnal variations inherent in the secretion of ACTH and TSH. Peripheral hormones (e.g., corticoids and gonadal steroids) can alter the electrical activity of several formations of the CNS including the hypothalamus. After electrical stimulation or electrical coagulation by stereotaxically implanted electrodes chemoreceptor areas for steroids related to sexual behaviour and pituitary gonadal functions appear to be the same as the hypothalamic *sex centres*.

The threshold of cerebral activity can be altered by local implantation or systemic administration of gonadal steroids. It is also observed that *EEG* changes is observed with the behavioural changes induced in males or females by gonadal steroids. EEG-arousal is easily measured by chronically implanted electrodes in the frontal cortex and certain parts of the limbic system following electric stimulation of the reticular activating system. *EEG-after-reaction* is observed in the hypothalamic and limbic areas of oestrogen-treated female rats.

Moreover, EEG-after-reaction also occurs spontaneously in the female rabbit following coitus and vaginal stimulation. Synthetic steroids (like norethindrone and norethynodrel) when administered orally to experimental animals elevate differentially EEG-after-reaction threshoed and block the ovulation induced by copulation without inhibiting the sexual behaviour or modifying the EEG-arousal threshold. Such antifertility steroids may block the pituitary secretion of ovulating hormones by preventing limbic-hypothalamic stimuli from reaching the area of median eminence thereby preventing release of hypothalamic hormone LRF. Hence there is the release of LH. Glucocorticoids as well as exposure to acute nonspecific stress also modify neuronal electrical activity in the mibrain, the limbic system and the hypothalamus.

In summary, the nature of neuroendocrine reciprocal relationships between the central nervous system and endocrine organs can be illustrated by the following facts: (a) The neurohypophysis being an extension of diencephalon is partly composed of nerve fibre tracts from the hypothalamic nuclei and the release of the stored posterior pituitary hormones is under the control of these nuclei. (b) The adrenal medulla is also under the hypothalamic influence by way of its extensive sympathetic nerve supply that effects the release of adrenal medullary hormones. (c) Since the nervous system monitors environmental changes in light and temperature, these alter reproductive, adrenal and thyroid activities. (d) Emotional states can alter the reproductive cycles in animals and man. (e) Transplantation of the pituitary away from its normal relationship to the hypothalamus results in varying degrees of functional loss of the thyroid, adrenal glands and gonads. (f) Stimulation of specific areas of the hypothalamus excites certain pituitary functions whereas lesions of these areas result in reduction of these functions (thyroid, adrenal and

reproductive). (g) Nonspontaneous ovulators require neural stimulation to effect a release of the ovulating hormone. These examples demonstrate that CNS is in part responsible for the maintenance of the activity of certain endocrine glands at the normal level and for the regulation of endocrine gland activity in adjustments to changes in the external environment.

Mechanism of action of releasing factors. Although physiological and chemical studies on the synthesis and release of hormone by the pituitary cell have been described, yet it appears to describe a little scope due to availability of pure or purified factors and the cellular heterogenity of the anterior pituitary.

The primary action of the releasing factor is the stimulation of hormone release due to administration of purified releasing substance causing a rapid release of hormone in circulation. Acetylcholine or high [K^+] controls the secretion of catecholamine from the adrenal medulla on the anterior pituitary. Therefore in the adrenal medulla, catecholamine release requires Ca^{++} availability, the uptake of ^{45}Ca is stimulated by acetylcholine; whereas Ma^{++} inhibits the stimulatory action. Since the transmembrane potential appears to lead to increased membrane permeability allowing a net flux of Ca^{++} into the cells, catecholamine release is diminished in the transmembrane potential of the cell. So hormone release is promoted by an increase in the intracellular Ca^{++}. So stimulation-secretion coupling is nothing but excitation-contraction in muscle. It was found that if membrane depolarisation be induced by high [K^+) in a Ca^{++}-free medium; there would be blockage of hormone release. Calcium is essential for *normal* as well as high [K^+]-induced hormone release. It was found that high [K^+] causes a reversed positive transmembrane potential in the majority of the cells. These positive transmembrane potential appears when a preferential transmembrane permeability to Ca^{++} triggered by an initial depolarisation of the cell membrane to high [K^+]. So stimulus-secretion coupling is the problems of hormone release from the anterior pituitary.

Cyclic AMP may be involved in the releasing factor-induced pituitary hormone secretion. Although calcium ion is required for hormone release but it is not required for releasing factor-induced stimulation of pituitary cyclic AMP.

The secretion of a number of stored peptides can be mediated by cyclic AMP and Ca^{++} is essential for the action of this nucleotide. Because the conversion of a small pool of ATP to cyclic AMP diminishes calcium chetation, making more clacium available for stimulation of peptide secretion. Cyclic AMP acts to promote hormone release and both Ca^{++} and energy are required for this action. An intracellular cyclic nucleotide phosphodiesterase hydrolyses cyclic AMP. Addition of target hormone to pituitaries *in vitro* may inhibit high [K^+]-induced hormone release for thyroxine and corticosterone on release of TSH and ACTH respectively. Thyroxine may inhibit TRF-induced release of TSH. So it may be added that certain hormones may influence additional feedback control directly on the pituitary cell membrane.

In general, hypothalamic factors stimulate the synthesis of hormone. Peptide-bond cleavage occurs in the conversion of proinsulin to insulin because of the activation of a precursor hormone molecule rather than

its *de novo* synthesis. Since a number of specific proteases are found in the pituitary tissue and since their levels fluctuate depending on the physiological state, a same nature of conversion may occur under the influence of a certain releasing factor. For example, glycoprotein hormones in which attachment of carbohydrate to inactive polypeptide may be sufficient for activation.

The effects of releasing factors on the synthesis of hormone can be measured by the incorporation of radioactive amino acid into a pituitary hormone. It has been proved that when an ovariectomised rat is pretreated with oestrogen and progesterone pituitaries are incubated in presence of hypothalamic extracts on D-^{14}C-glucosamine or L-^{14}C-leucine within a few hours. It is still debatable whether a releasing factor can enhance hormone synthesis a primary or secondary occurance or synthesis of new hormone is triggered by simple mass action. However it has been observed by some, the hypothalamic releasing factors may all be members of a closely related series of small polypeptides, probably with an amidated C-terminus and a protected N-terminus.

THE THYROID

Anatomy (Fig. 4.42). The thyroid situated at the root of the throat,

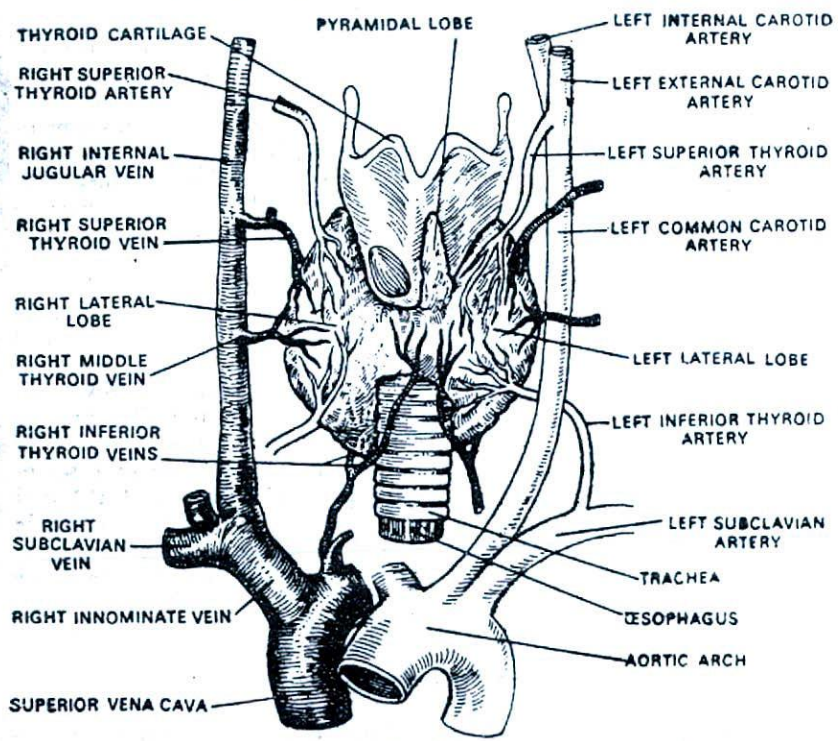


Fig. 4.42. Diagrammatic representation of the anatomical position of the human thyroid in relation to major great vessels.

having two fairly symmetrial lateral lobes, each about 5×2×2 cm, one on either side of trachea, joined by a thin portion of thyroid tissue called the isthmus (middle lobe) crossing in front of the second, third and fourth tracheal rings. As a normal variant the left lobe is often smaller than the right lobe. From the isthmus of the thyroid a variable sized lobe extends upwards in the neck. This is known as the pyramidal lobe which is clinically obvious in certain thyroid diseases. The thyroid is closely attached by the pretracheal fascia. So it is a well-known clinical sign that the thyroid moves upwards on swallowing. It possesses no duct. *Accessory thyroid*—similar aberrant bodies, situated below the main thyroid or along the thyroglossal duct. *Weight* in adults—The gland weight in the adult normally varies from 20-25 g but is influenced by age, sex, reproductive state and diet. *Highly vascular*—paired superior and inferior thyroid arteries supply the gland with a less important supply directly from the aorta or the innominate artery via the thyroidea ima artery. The venous drainage is to the internal jugular and innominate veins and the lymphatic drainage is to the lateral lymph nodes of the neck commonly; there is, however, some drainage down into the anterior mediastinal lymph nodes.

Blood flows from 3·5-6·0 ml per g of thyroid per minute. This high flow rate probably serves to supply the tissue with sufficient inorganic iodide. *Nerve supply*—sympathetic fibres are derived from the superior, middle and inferior cervical ganglia and parasympathetic fibres from the superior and inferior recurrent laryngeal branches of the vagus. They regulate the blood flow rather than secretion of the thyroid gland.

Development. In the human embryo the thyroid gland develops from the primitive foregut. In the adult this site of origin as the foramen caecum in the floor of the mouth at the junction of posterior one-third and anterior two-thirds of the tongue. During the early embryonic life the primitive thyroid gland descends in the midline of the neck in order to occupy the normal adult position in front of the thyroid cartilage. There the thyroid gland derives from the fourth pharyngeal pouch and other elements which go to form part of the lateral lobes of the thyroid gland. In the human embryo the thyroid gland can be recognisable in its final site from about the seventh week of the intra-uterine and it develops its characteristics follicular structure round about the 12th to the 14th week of foetal life. With the changes in structure, the thyroid develops its capacity to secrete thyroid hormone. The functional capacity of the thyroid gland appears in a stepwise fashion, the ability to accumulate iodine being followed by the development of other stages of thyroid hormone synthesis.

Histology (Fig. 4.43). When the thyroid is examined microscopically it is found to consist of follicles or vesicles lined in the resting state by a low cuboidal epithelium. The height of this epithelium depends upon the degree of stimulation of the thyroid. Under appropriate stimulation by thyroid-stimulating hormone (TSH) the low cuboidal epithelium may be converted into a tall columnar epithelium. (1) The thyroid follicles or vesicles—spherical, oval or irregular size—15-150 μm in diameter, (2) lined by a single layer of granular cubical cells with (3) mitochondria and distinct Golgi apparatus. (4) The bases of the cells are in contact with fine basement membrane which also encircles each follicle. The follicles are filled with a protein material which consists largely of thyroglobulin which, in fact, constitutes 75% or more of the soluble proteins of the

normal thyroid gland and is the main storage form of the thyroid hormones. The thyroid is unique in this way amongst the endocrine glands in having a large store of preformed hormone in the organ. The follicles are surrounded by a very vascular stroma which also contains the lymphatic channels. The border of the thyroid epithelial cells which impringes on the central colloid of the follicle has a regular formation of microvilli. The base of the cell is reparated from interfollicular stroma by a well-formed basement membrane. The cell nucleus of the epithelial cell is commonly situated in the basal part rather than the apex and the cell is well-supplied with the usual subcellular bodies concerned in protein and carbohydrate synthesis, namely rough endoplasmic reticulum and Golgi complex. (5) In addition, within the cell cytoplasmic vesicles are found and many of these at least are absorption vesicles, the colloid being absorbed from the follicles by the process of engulfment by the thyroid cell processes. This mechanism is called *pinocytosis*. Vesicles are filled up with a colloidal material secreted by the epithelium. (6) According to the staining reaction of the colloid, vesicles may be of three types—*acidophilic or eosinophilic* (pink), *basophilic* (blue), or *mixed*. (7) Using electron microscopic and histochemical methods, two types of cells have been identified in the thyroid vesicle. The numerous *principal cells* have lesser number of mitochondria and contain the different proteolytic and oxidative enzymes which are responsible for the synthesis of thyroid hormones. A lesser number of *mitochondrial-rich*, high cuboidal cells are also present in between the follicular cells. These are known as parafollicular cells (C-cells —C for calcitonin or clear cells or light cells which are larger) than the typical follicular cells, containing particular fluorogenic amines and high concentration of α-glycerophosphate dehydrogenase (Fig. 4.44), and synthesise a hormone *thyrocalcitonin* (TCT) which reduces the calcium level of blood. The calcium-lower hormone does not seen to be of

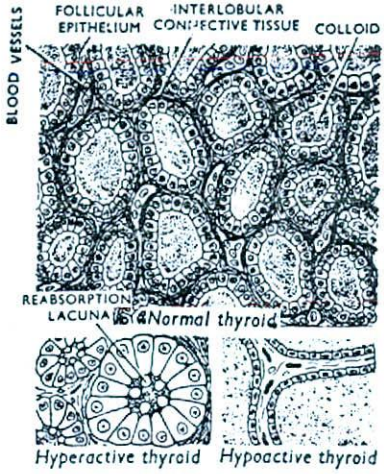


Fig. 4.43. Histological structure of thyroid gland showing normal, hyperactive, hypoactive thyroid cells.

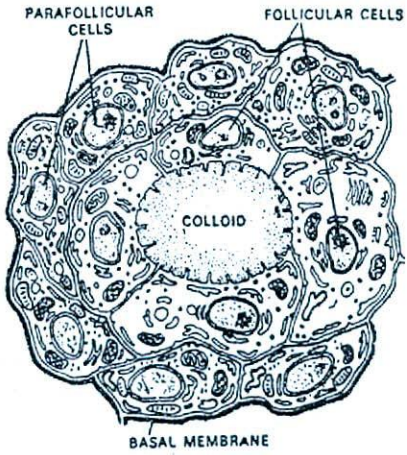


Fig. 4.44. Diagrammatic representation of the parafollicular cells of the mammalian thyroid gland.

great physiological importance in normal man but it is produced in excess in certain types of thyroid tumours and in this condition assay of the serum calcitonin can yield valuable diagnostic information. (8) In between the vesicles, solid clumps cells, lymphocytes and macrophages are seen. In **hyperactive** conditions of thyroid: (a) Cells may become more columnar. (b) The lining epithelium increases and becomes folded. (c) Mitochondria increase in number. (d) Golgi apparatus englarges (diagnostic feature) and colloid droplets appear near it. (e) Vesicles become irregular—some hyperdistended and others collapsed and they are basophilic in staining reaction. In **hypoactivity** the epithelium becomes flat or squamous type and the staining reaction of the vesicles becomes acidophilic. After puberty colloid store increases and as suggested by the histological changes, activity slowly decreases. This explains decrease in basal metabolic rate (BMR) and susceptibility to cold in old age.

In early foetal life, thyroid consists of a solid clump of cells. Vesicles start appearing at the fourth month. Each group of cells secrete colloid at a common centre and in this way the redimentary vesicles form.

THYROID HORMONES

The thyroid gland produces three hormones in man, *thyroxine, tri-iodo-thyronine* and *calcitonin.* Only the homones thyroxine and tri-iodothyronine will be considered at present.

Thyroid glands synthesise and release iodinated thyronine molecules. These molecules greatly influence metabolic processes and growth. Thyroid hormones are stored in colloid-containing vesicles enclosed by thyroid epithelium as thyroglobulin, a precursor protein. Within the thyroglobulin molecule, tyrosine residues are iodinated to form MIT and DIT which then combine to form the iodothyronines. All vertebrates have a follicular arrangement of thyroid tissue and produce similar thyroid hormones by iodination and subsequent degradation of thyroglobulin.

Chemistry. Thyroid hormones, thyroxine (T_4) and tri-iodothyronine (T_3) as monoiodotyrosine (MIT) and di-iodotyrosine (DIT) are stored within the thyroid follicle in binding with a highly specialised storage protein called the **thyroglobulin.** Thyroglobulin is an *iodinated glycoprotein* having a molecular weight of about 680,000. In normal condition, this thyroglobulin is too large to diffuse from the follicle into the blood stream. Peptide bonds of the thyroglobulin are hydrolysed by proteolytic enzymes, catheptases, from lysosome and free thyroxine, tri-iodothyronine are released and enter the thyroid cells, cross them and are ultimately discharged into the capillaries. Mono- and di-iodotyrosines are also released but do not leave the follicle. They are rapidly deiodinated by enzyme,deiodinase or dehalogenase within thyroid cells and iodine is reutilised for a recycling synthesis of thyroglobulin. MIT and DIT are biologically inactive. Thyroxine is 3,5,3′,5′-tetraiodothyronine and tri-iodothyronine is 3,5,3′-tri-iodothyronine (Fig. 4.45). These two iodine-containing amino acids are biologically active. Secretory processes of thyroid hormones consist of several complicated steps, such as (i) synthesis of large molecular protein—thyroglobulin, (ii) iodination of tyrosine molecules of the thyroglobulin, (iii) release of thyroglobulin into the

follicular lumen, (iv) reabsorption of thyroglobulin from the follicular lumen into the follicle epithelial cell, (v) hydrolysis of thyroglobulin to

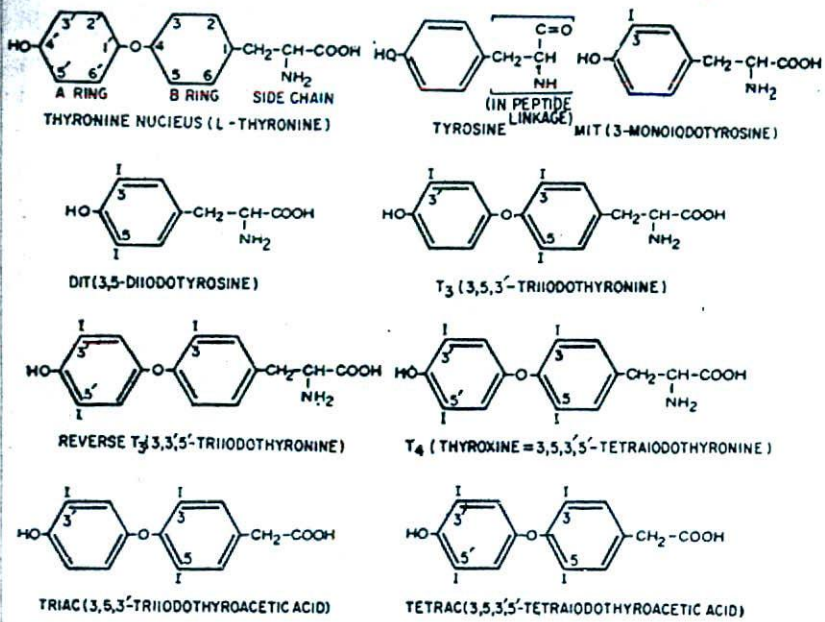


Fig. 4.45. Structural formulas of tyrosine, MIT, DIT, T_3, T_4 and some analogues of thyroxine having varying hormonal action.

release thyroxine and tri-iodothyronine, and (vi) release of thyroxine and tri-iodothyronine in the blood capillaries.

Thyrocalcitonin (Fig 4.46) which is secreted from parafollicular cells or C-cells (C for calcitonin) of the thyroid gland is a polypeptide having molecular weight of about 3600.

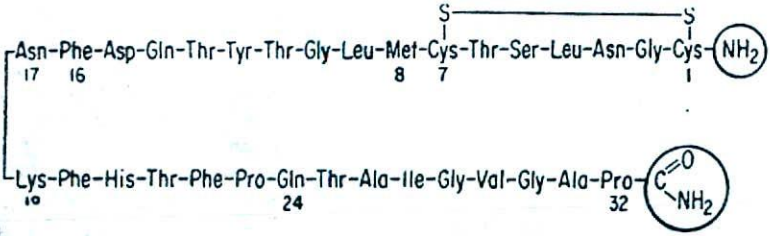


Fig. 4.46. Amino-acid sequence of human thyrocalcitonin.

Synthesis, Storage, Release and Transport of Thyroid Hormones

The thyroid gland is the main iodine-functioning organ in the body. The normal adult takes in about 100-150 μg of iodine per day in food (Fig. 4.47). The iodine is broken down in the gastro-intestinal tract and is commonly absorbed as iodide, which passes into the blood stream where

it circulates in the systemic circulation and is actively accumulated by the thyroid from the blood passing through it.

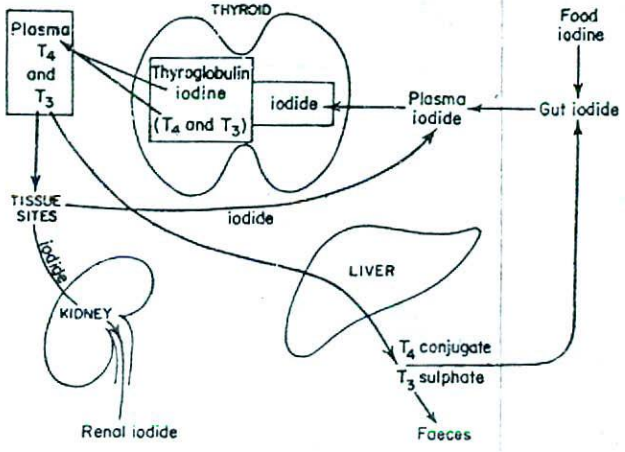


Fig. 4.47. Schematic representation of the iodine cycle.

Therefore, the first step in the synthesis of the thyroid hormones is that the thyroid gland has to accumulate inorganic iodide. This is done by an active process which is generally called trapping (Fig. 4.48). Usually the resting thyroid appears to accumulate iodine in the ratio of about 20 : 1 from the plasma. This can be increased markedly under the

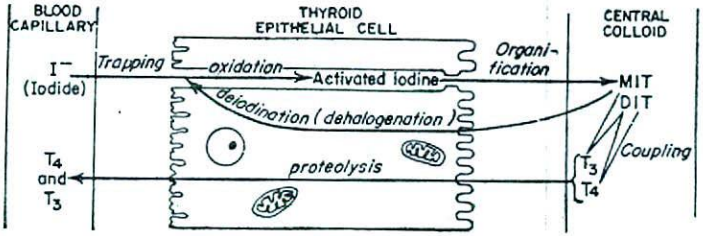


Fig. 4.48. Simplified diagram of the synthesis and degradation of the thyroid hormone.

appropriate condition of stimulation. This ability to trap iodine is not unique to the thyroid gland but is shared by the other derivatives of the embryonic foregut, viz., salivary glands, gastric mucosa and the lactating brest. This process can be established readily by the *in vivo* and *in vitro* demonstration and is stimulated by TSH and also by abnormal thyroid stimulators, viz., LATS (long-acting thyroid stimulator). *In vitro* it can be shown to be inhibited by processes interfering with enzyme function, e.g., boiling of the tissue. The process is also inhibited by perchlorate and thiocyanate. These may act by having a similar molecular size to iodide and they do not appear to act as competitive inhibitors for the enzyme process.

Once the iodine has been accumulated in the thyroid gland it undergoes a process of oxidation. A peroxidase enzyme system is present in a large amount in the thyroid gland and this may be involved in the oxidation process by the local manufacture of tiny amounts of hydrogen peroxide within the cell. By the mechanism of this oxidation process either free iodine is momentarily produced in the thyroid gland or another unstable iodine ion is produced.

The next stage of thyroid hormone synthesis is called *organification*, since it involves attachment of the iodine to tyrosine residues. This is believed to occur on preformed thyroglobulin which has been taken to the full *stage* of protein synthesis but which has not been iodinated.

Synthesis. Synthesis of thyroid hormones involves through several complicated processes, such as (a) synthesis of thyroglobulin, (b) trapping of iodide, (c) iodination (dehalogination) and condensation of tyrosine molecules that are attached to thyroglobulin, (d) release of thyroglobulin into the follicular lumen, (e) reabsorption of thyroglobulin from the follicular lumen into the follicular epithelial cell, and (f) hydrolysis of thyroglobulin to release thyroxine and tri-iodothyronine, MIT and DIT.

(a) SYNTHESIS OF THYROGLOBULIN. Thyroglobulin is only found in the plasma. Thyroglobulin is an iodinated glycopeptide of molecular weight about 680,000. On analysis in the ultracentrifuge it contains an 'S' value of 19 and is recognised as the main protein present in the thyroid. The synthesis and degradation of thyroglobulin have been established with the view that this large protein contains smaller polypeptide subunits which coalesce to form 6S and 12S subunits. The 12S subunits combine to form larger units, namely 17S, which is the largest non-iodinated part of the thyroglobulin molecule. The polypeptide part of this glycoprotein is synthesised by the rough-endoplasmic reticulum of the thyroid cell. The carbohydrate moiety is added possibly mainly in the Golgi apparatus, which produces the formation of a fully synthesised carbohydrate-containing protein of 17S size (Fig. 4.49). This protein is then iodinated. Then the thyroid cell passes iodine rapidly through itself into the colloid.

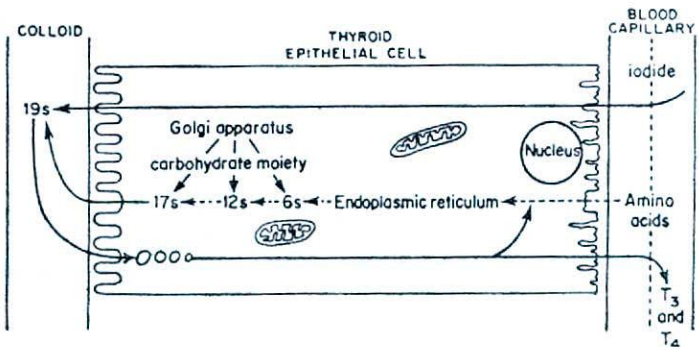


Fig. 4.49. Schematic representation of stages of formation and degradation of thyroglobulin.

Though there is controversy whether iodination takes place at the apical site of the thyroid epithelial cell or whether it takes place within the colloid.

But in normal circumstances iodination commonly takes place in the colloid at the edge of the follicle.

It should be mentioned here that the process of synthesis of the basic thyroglobulin molecule and the process of iodination are quite different in time relationship and in anatomical site within the cell. In the experimental animal iodine enters the colloid within seconds, but several hours are required for amino acid to reach the colloid. Thiouracil interferes with iodination but it does not interfere with amino acid incorporation in the short term and conversely actinomycin affects amino acid incorporation but does not interfere with short term iodination experiments.

(b) TRAPPING OF IODIDE. **Trapping of iodide** from circulation is an important phenomenon in the synthesis of thyroid hormones. The cells of the thyroid follicles have the unique abilities to trap iodide from the circulating blood. The thyroid gland concentrates the iodide by actively transporting it from the blood. This transport mechanism is often called **iodide-trapping mechanism** on iodide pump. For this active transport energy is required. The resting membrane potential of the acinar cell is —50 mV with respect to interstitial fluid and colloid. Iodide pump into the cell occurs against this negative potential and then diffuses down at the electrochemical gradient into the follicular lumen. The ratio of thyroid iodide to plasma iodide is about 20: 1 to 100: 1. This iodide pump is also dependent upon ATP-ase system. Uptake of iodide can be inhibited by cyanide or dinitrophenol. TSH stimulates iodide uptake by the gland. The normal daily intake of iodide is 100-150 μg and mainly absorbed from the small intestine and subsequently transported in the plasma in loose attachement to protein.

Salivary gland, gastric mucosa, placenta and the mammary glands can transport iodide against concentration gradients but this process is not stimulated by TSH.

(c) IODINATION AND CONDENSATION OF IODOTYROSINE MOLECULES THAT ARE ATTACHED TO THYROGLOBULIN (Fig. 4.51). Within the thyroid, the iodide that is taken up the follicular cells is quickly oxidised to an active form of iodine, by a reaction, catalysed probably by a particulate-formed peroxidase in presence of H_2O_2 as a source of oxygen.

Oxidation of iodine to nascent form of iodine: $2I^- \rightleftharpoons I_2$ or $I^0 + 2e$.

Peroxidase system: $H_2O_2 + 2I + 2H^+ \xrightarrow[\text{peroxidase}]{\text{iodide}} 2$ 'oxidised (active) I' $+ 2H_2O$.

TSH is active in stimulating this reaction.

The receptors of the activated iodine are the tyrosine residues of the thyroglobulin. Each molecule of thyroglobulin (19S proteins) contains 115 tyrosine residues and consists of four 8S subunits. The iodination of tyrosine in thyroglobulin takes place first at 3 position and then at 5 position of aromatic nucleus, forming respectively the monoiodotyrosine and di-iodotyrosine. Simply, the addition of one atom of iodine per tyrosine residue results in the formation of MIT and the addition of a further atom of iodine per tyrosine residue results in the formation of DIT. Normally monoiodotyrosine (MIT) and di-iodotyrosine (DIT) molecules are present in equal concentration. In human beings, deficiency of iodine causes an increase in the ratio of MIT/DIT and preferential production of T_3, leading to a euthyroid (*eu*=normal) condition in the presence of

low PBI. But in rats, iodine deficiency is associated with a fall in the residues of DIT and T_4 per TBG molecule and a rise in MIT and T_3 formation. It is unlikely that free tyrosine is iodinated and incorporated in the thyroglobulin.

Two di-iodotyrosine molecules thus undergo oxidative condensation with the liberation of alanine residue and the formation of thyroxine (T_4), still in peptide linkage to thyroglobulin. It is claimed that tri-iodothyronine (T_3) is formed by condensation of monoiodotyrosine with di-iodotyrosine. This process of condensation is known as **coupling.** Under normal conditions, coupling occurs readily between adjacent iodotyrosine residues on the surface of TBG molecules. Loss of iodine atom from thyroxine molecule may possibly produce T_3.

There are two opinions regarding the site of iodination of thyroglobulin in the thyroid gland. The site of iodination is one of the important problems in morphology and biochemistry. Some scientists are of opinion that iodination of thyroglobulin takes place within the cytoplasm of the follicular cells but some are of opinion that this takes place within the colloid. In the adult mouse, rat, frog and fish, the iodination

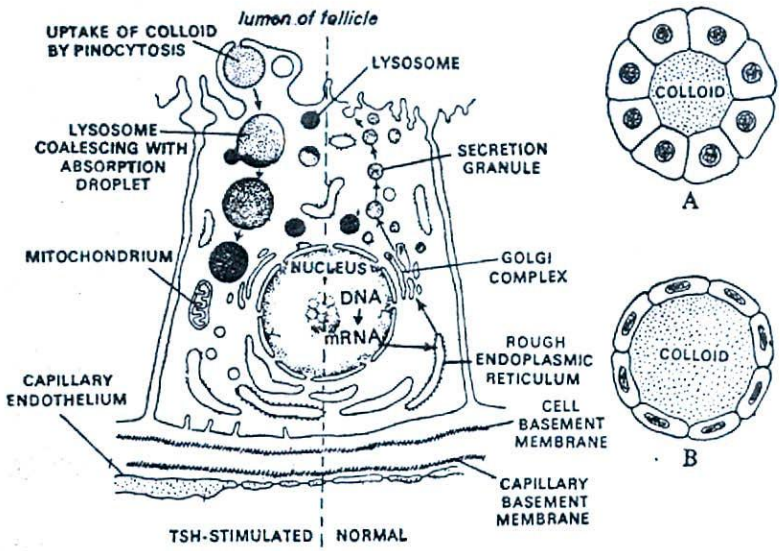


Fig. 4.50. Diagram shows normal thyroid and TSH-stimulated epithelial cells. In the normal cell secretory droplets are formed in the Golgi complex and discharged into the lumen of the follicle. In the TSH-stimulated cell, colloid droplets are taken up by micropinocytosis and coalescing with lysosomes. Degradation of TG by hydrolytic enzymes is followed by release of T_3 rnd T_4. *Inset A* shows thyroid follicular cell just becoming active and *inset B*, inactive, with accumulation of large amounts of colloid.

takes place almost entirely in the follicular lumen or apical cell region. Through autoradiography of ^{125}I (half-life, 60 days), they have shown that the silver grains appear almost entirely in the follicular lumen or in the apical cell surface region within a few minutes or several hours after the injection. From these observations, they have considered that iodina-

tion of thyroglobulin might occur mostly in the follicle colloid. It has been reported earlier that free thyroid cells dissociated from the thyroid follicles are capable of iodinating the thyroglogulin. But Fujita (1970) has claimed that in the adult animals, non-iodinated thryroglobulin synthesised in the rough-surfaced endoplasmic reticulum and transported to the Golgi apparatus are iodinated slightly in the cytoplasm and almost entirely in the follicular lumen including the cell surface region. He has considered that a number of thyroglobulin molecules which are synthesised in the rough-surfaced endoplasmic reticulum, transported to the Golgi apparatus and extruded into the follicular lumen without being sufficiently iodinated and have numerous spaces for binding iodine molecules and so iodination takes place almost entirely in the apical cell membrane region. Peroxidase necessary for iodination is localised in the cisternae of granular endoplasmic reticulum, perinuclear cisternae, a few Golgi lamellae, apical small vesicles and associated with the external surfaces of the microvilli.

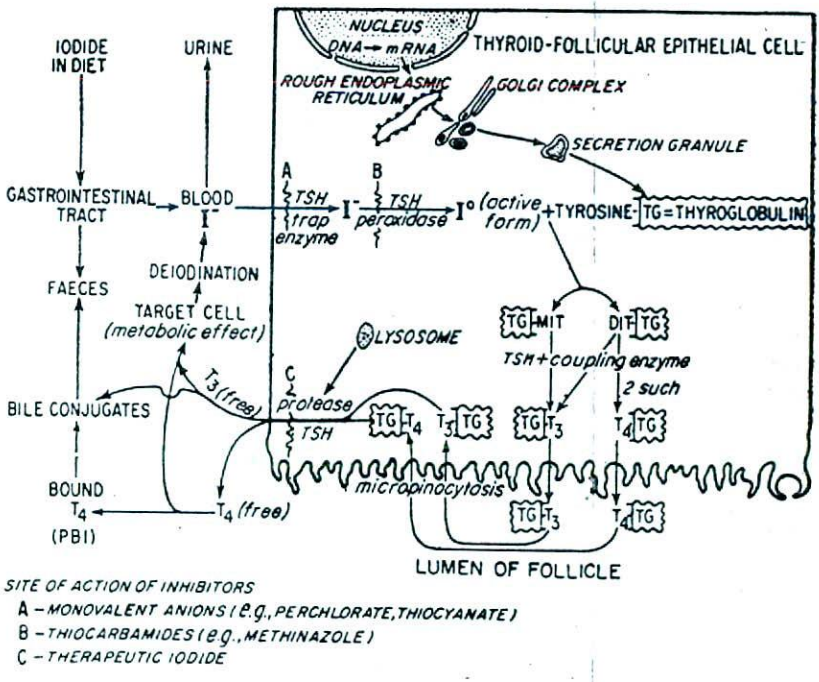


Fig. 4.51. Schematic representation of the metabolism of iodine and synthesis, storage, release and transport of thyroid hormones.

(d) RELEASE OF THYROGLOBULIN INTO THE FOLLICULAR LUMEN. Thyroglobulin with thyroid hormones that are formed in the follicular epithelium are discharged within the follicular lumen and stored there until these are required.

(e) REABSORPTION OF THYROGLOBULIN FROM THE FOLLICULAR LUMEN INTO THE FOLLICULAR CELLS. If TSH is administered then numerous large droplets appear in the thyroid follicular cell. These large droplets have been considered to be the reabsorbed materials from follicular lumen. These droplets are reabsorbed by a micropinocytosis as a small vesicle (Fig. 4.50).

(f) HYDROLYSIS OF THYROGLOBULIN TO RELEASE THYROXINE AND TRI-IODOTHYRONINE, MIT AND DIT. Final steps in the formation of thyroid hormones are the hydrolysis of thyroglobulin by the proteolytic enzymes from lysosomes or vesicle membranes. It is claimed that lysosomes coalesce with the droplets, releasing hydrolytic enzymes which degrade thyroglobulin to liberate thyroxine (T_4), tri-iodothyronine (T_3), MIT and DIT. Thus "the surface of the cells in contact with the colloid sends off narrow streamers which parcel out small amounts of colloid; the colloid droplets thus formed are then carried into the cytoplasm, where they combine with lysosomes; and under their influence they disintegrate and disappear with the release of thyroid hormones and MIT and DIT." TSH stimulates the process greatly.

Regarding the site of breakdown of thyroglobulin in the thyroid, De Robertis has described more earlier that the degradation possibly takes place in the colloid of follicles. He has claimed that there is an enzyme present in the colloid that can hydrolyse the thyroglobulin. But it is believed that this type of hydrolysis, possibly takes place in condition of thyrotoxicosis (exophthalmic goitre) and is not possible in normal functional gland.

MIT and DIT that are released from the thyroglobulin do not escape from the epithelial cells but are deiodinated by a specific deiodinase enzyme that plays a vital role in an intrathyroidal iodine cycle in which iodine and tyrosine are recirculated for fresh hormonal synthesis. This deiodinase has got affinity toward the globulin-bound MIT and DIT.

Transport of thyroid hormones. The hormones which are released in the blood are transported as such but in binding with plasma proteins. Thyroxine (T_4) is bound much more firmly than tri-iodothyronine and for this reason tri-iodothyronine is quick acting and short lasting but the thyroxine is slow acting but long acting.

Thyroxine is transported entirely with two plasma proteins: (i) **thyroxine-binding alpha-globulin (TBG**—about 60%) and (ii) **thyroxine-binding prealbumin (TBPA**—about 30%). When the binding capacity of these specific carrier protein is exceeded, thyroxine is bound with **serum albumin** and approximately about 10% of circulating thyroxine remains in the free and unbound state. This free thyroxine makes up only 0·1% and is the metabolically active hormone. TBG is stable reservoir for both T_4 and T_3. Thyroxine is less tightly bound with TBPA.

Degradation and excretion of thyroid hormones. Half-life of ^{131}I labelled thyroxine is about 8 days. In hyperthyroidism the half-life is 3-4 days and in myxoedema it is about 9-10 days. Half-life of T_3 is about 2 days.

The deiodination of thyroid hormones occurs in all peripheral tissues and probably is the most important degradation process. The iodide released is recirculated and is excreted (urine) or is recycled through

thyroid hormone synthesis due to the thyroid iodide-concentrating mechanism. Since the renal tubules reabsorb more than 95% of filtered iodide, thus body economy of iodide is achieved (Fig. 4.47)

Dehalogenase (iodothyronine-deiodinating enzyme) exists in muscle and other tissues. This enzyme is active only with non-protein (free) material. Thus cellular protein binding of thyroxine minimises deiodination and in this way provides for some reserve except the cells of the brain, spleen and gonads. Thyroxine dehalogenase activity of human leucocytes is increased in hyperthyroidism and decreased in hypothyroidism.

The thyroid hormones conjugate with **glucuronic acid** to form *β-glucuronide metabolite*. A considerable fraction of thyroxine and only a small portion of T_3 is handled as glucuronide and T_3 conjugates primarily as sulphate. Glucuronide derivatives being formed in the liver and passed *via* bile into tfle gut, where they are reabsorbed after hydrolysis by β-glucuronidase of the intestinal bacteria or excreted in the faeces.

Alanine side chain of T_4 and T_3 undergoes oxidative deamination and transamination to form **tetra- and tri-iodothyropyruvic acid** and **tetra- and tri-iodothyrolactic acid derivatives** and further **decarboxylation** leads to **tetraiodothyroacetic acid** (TETRAC) and **tri-iodothyroacetic acid** (TRIAC) derivatives (Fig. 4.45). These transformation appears to be the mode of renal metabolism of thyroid hormones.

Decarboxylation occurs to give rise to the amine derivatives. Administration of radio-active T_4 or T_3 can lead to recovery in the bile of some iodinated constituents: (a) iodide, (b) free hormone, (c) glucuronide or sulphate conjugates, and (d) pyruvates. When liver function is impaired by injury or disease, thyroid hormones are removed from circulation at a slower rate. The products of liver degradation are passed via the bile into the intestine where free amino acids, iodide, and hormone metabolites are partly reabsorbed. In such way an enterohepatic circulation exists to effect a body economy of amino acids, iodide and thyroid hormones. This type of circulation may vary among different species. It is evident that the enterohepatic circulation of thyroid hormone metabolism has some importance.

Metabolism of iodine and synthesis, storage, release and transport of thyroid hormones have been presented in Fig. 4.51.

Biological test for thyroxine

Presence of thyroxine can be detected by this test: If tadpoles are kept in a solution containing thyroid hormone for three days, metamorphosis begins, a few days later, much earlier than in untreated controls. The forgs, thus produced, are very small and die very soon. In these animals, thyroxine stimulates differentiation and not growth.

Plasma protein-bound iodine gives a measure of the circulating thyroid hormone. The most sensitive bioassay depends upon the release of labelled thyroid hormones from the thyroid of mice previously treated with radio-iodine. This assay detects TSH in plasma and also the long-acting thyroid stimulator (LATS) which is associated with thyrotoxicosis.

Role of iodine

The synthesis of thyroid hormones requires a regular and adequate supply of iodine. The daily requirement is about 100 to 150 μg, but

considerably more may be needed during puberty, pregnancy or stress of any kind and during residence in cold climate. Deficiency of iodine leads to hyperplasia and goitre.

Iodine is a relatively scarce element in the crust of the earth. Ultimately all of the iodine taken in the body is derived from soil and water and it is the content of iodine in these natural sources which determines the amount commonly ingested. Iodine is taken in with food (i) as the free element, (ii) as iodide (I^-) and (iii) as iodine bound to or part of an organic molecule. As a general rule, ingested iodine must be reduced to iodide before it can be absorbed, besides thyroid hormones themselves and certain radio-opaque drugs (e.g., those are used in gall-bladder investigations) which are absorbed intact. The speed of iodine absorption must be an active process.

Iodide in the blood has a concentration of about 3·5 to 8·0 μg per 100 ml (275·8 to 630·4 n mol/l). It is freely distributed in the extracellular fluid, but it is also concentrated in the cells of salivary and mammry glands, stomach, choroid plexus, placenta, skin and thyroid. These cells can concentrate iodide against a concentration gradient and an electrical gradient, the interiors of cells being negative.

Iodide is secreted in the sweat, saliva and in other gastro-intestinal secretions. Most of the alimentary iodide is reabsorbed and some is lost in faeces. Iodide is filtered freely at renal glomeruli and is only passiaely reabsorbed so that some are lost in the urine (Fig. 4.47).

In the mountainous region there is deficiency of iodine in food and water. So the incidence of goitre is higher in mountainous regions. Iodine remains in blood as (a) inorganic iodide in the plasma and corpuscles and varies from 0·5 μg to 1 μg per 100 ml. (b) Bound iodine—thyroxine and tri-iodothyronine in the plasma in combination with α-globulin. This iodine may be precipitated out. It is called **protein-bound iodine** or PBI. The normal range of PBI varies from 3·5 μg to 8 μg per 100 ml. It is a determining factor of thyroid secretion. In thyroidism the PBI level rises and it may reach to 15 μg and in hypothyroidism (myxoedema) the PBI level falls below 3·5 μg per 100 ml.

Selective affinity of iodide

1. The gland has remarkable affinity for inorganic iodine. Usually, it stores about 2 mg of iodine per gram of the dry gland in man and about 5 mg in other mammals.
2. If radio-active iodine ^{131}I with a half-life of 8 days is injected it is found that within a few hours one-third of it is taken up by the thyroid gland, The acinar cells of the thyroid gland selectively absorb inorganic iodide. There is a maximum limit of iodide intake by the cells and this is called *iodine absorption maximum.*
3. The thyroid gland contains about 20% of the total iodine content of the body.
4. Iodine is also present in the thyroid gland in the form of mono- and di-iodotyrosines which act as the precursor of thyroxine.
5. *Adult thyroid contains on the average 20 mg of thyroxine and secretes about 1 mg of thyroxine per day.*
6. Thyroxine remains stored in the gland combined with a protein —froming *thyroglobulin.*

7. Action of thyroxine shows a *long latent period.* Even after intravenous injection of pure thyroxine, a period of about 48 hours may pass before any appreciable effect is produced.

Role of anterior pituitary

After hypophysectomy thyroxine metabolism is disturbed as follows: (a) Thyroid takes up *less* inorganic iodide from the plasma. (b) Formation of monoiodotyrosine and di-iodotyrosine is reduced. (c) Formation of tri-iodothyronine and thyroxine is reduced and discharge of thyroxine into the blood is markedly reduced. Consequently, the iodine of plasma remains mostly as inorganic iodide and slightly as mono- and di-iodotyrosines, but not as thyroxine.

Administration of thyrotrophic hormone increases (1) thyroglobulin synthesis, (2) the uptake of iodide by the gland, (3) the rate of enzymatic transformation of iodide with tyrosine into thyroid hormones, (4) the rate of release of thyroxine in the blood, and (5) the basal metabolic rate via thyroid hormones. *These observations prove that the thyrotrophic hormone of the anterior pituitary controls the synthesis and secretion of thyroxine.* It is indicated that the action of TSH is mediated through increased formation of cyclic AMP. Breakdown of glucose through pentose phosphate pathway is also increased and this helps in hormone synthesis.

Physiological actions of thyroid hormones

Principal function of the thyroid gland is to act as a *catalyst*—of the nature of a **spark** for the maintenance of oxidative metabolism in most tissues. It is also necessary for normal growth and maturation and tissue differentiation. Thyroid hormones activate energy-producing respiratory processes within the cells of the body, perhaps by stimulating, in turn, the cyclic AMP system, transcription of mRNA, synthesis of protein in the mitochondria and microsomes and, lastly, cellular respiration and metabolism,

After acting on the cells of the body, T_4 and T_3 are degraded and the iodine which is released rejons the iodine pool in the plasma. The orderly sequence of these complex sceps (Fig. 4.51) is controlled by one or more enzymes, a failure of any one of them results in impairment of thyroid hormone synthesis, release or utilisation. As a result, the body attempts to compensate for the defect and the thyroid may enlarge. Thyroid hormones (T_4 and T_3) influence calcium metabolism to a much smaller extent, but the parafollicular (C) cells secrete calcitonin (thyrocalcitonin), a hormone primarily concerned in the blood calcium-lowering control, and are not under the control of TSH. The actions of the hormones in different bodily processes are described categorically.

1. **Calorigenic.** Thyroid hormone accelerates energy production, oxygen uptake and basal metabolic rate (BMR). T_3 is 3-4 times more potent than T_4 and increases the oxygen consumption more rapidly. Each milligram raises BMR to about 1000 C and increases the oxygen consumption of all tissues except anterior pituitary, brain, testes, spleen, uterus. The calorigenic effect of thyroxine is due to its direct effect on the cells.

2. **Metabolism**

(a) **Carbohydrate metabolism.** (i) Stimulates absorption of glucose from the intestine. (ii) Mobilises glycogen from liver and heart.

(iii) Promotes gluconeogenesis. (iv) Causes hyperglycaemia. (v) Reduces sugar tolerance.

(b) **Protein metabolism.** In *moderate concentrations* the thyroid hormone has an **anaebolic effect,** causing an increase in RNA and protein synthesis, an action which precedes increased BMR not only is RNA synthesis increased, but there is increased translation of the message contrained in messenger RNA at the ribosome where protein synthesis occurs. In hypothyroidism, the hormone has an anaebolic effect on plasma and tissue proteins but catabolic action on extracellular proteins. In *high concentrations,* negative nitrogen balance is observed and protein synthesis is depressed.

(c) **Lipid metabolism.** The concentration of most of the lipids of the serum specially cholesterol varies inversely with the level of thyroid activity. The thyroid hormone increases both the synthesis and the catabolism of cholesterol and other lipids. In hypothyroidism, the balance between the two is disrupted; there is less lipid catabolism, hence serum lipids increase.

(d) **Calcium and phosphorus metabolism.** Removes Ca and P from the bones leading to osteoporosis. Its action differs from that of parathyroid in (i) causing no rise of serum Ca, and (ii) increasing Ca loss both in faeces and urine. Similar changes are seen in Graves' disease.

3. **Kidneys.** (a) Increases nitrogen excretion. (b) Increases urine volume along with increased elimination of salt probably not by a direct effect on the kidneys but by raising the general metabolism and thus increasing nitrogenous endproducts which act as diuretics. (c) Increases the excretion of creatine.

4. **Growth and metamorphosis.** Thyroid is essential for normal growth and also for metamorphosis in tadpoles. In thyroidectomised animals there is retardation of growth. Growth in these animals is again initiated after administration of thyroxine.

5. **Mammary glands.** Increases the output and fat content of milk.

6. **Heart rate.** Thyroxine accelerates the rate of the normal as well as the denervated heart. It acts directly on the heart (S. A. node). The raised BMR may be an additional factor.

7. It is necessary for normal emotional responsiveness, cerebral activity, sensory activity, etc.

8. **Nerves and Muscles.** Thyroid hormones influence the levels and activity of the central, peripheral and autonomic nervous systems and of the voluntary muscles. Hyperthyroid patients are nervous and irritable, and exhibit muscular tremors. Catabolism of muscles may cause wasting, weakness and sometimes frank myasthenia. Autonomic stimulation causes sweating, gastro-intestinal hypermotility and vasomotor instability. In hypothyroidism, the patients are apathetic, mentally retarded. Electroencephalogram may show slow waves of diminished amplitudes and sometimes absence of α waves. Contraction and relaxation of voluntary muscles are delayed and the gut may be sluggish.

9. It increases the tolerance to some type of drugs, e.g., morphine, digitalis.

Variations in the functional states of the thyroid gland

The activity of the thyroid varies mostly at different **ages,** specially in the female. It is not functionally adequate until towards the end of the

first year. It shows increased activity at puberty, during menstruction, pregnancy and lactation; after the menopause it tends to atrophy.

Increased activity of the thyroid is generally associated with a tall, columnar epithelium (Fig. 4.50, inset A) and the epithelium may become folded to increase the secreting surface. There is usually little colloid and vacuoles often occur round the periphery of such colloid masses.

Decreased activity of the thyroid is generally associated with a low flat epithelium (Fig. 4.50, inset B) and an excessive amount of colloid which is deficient in iodine content.

However all parts of the gland do not exhibit the same degree of activity at any one moment. It is usual to find in sections that one microscopical field shows both active and resting follicles; on the other hand in a single follicle there may be a segment of high, active epithelium while the remaining cells are of low inactive type. The iodine content of the colloid material may vary very considerably and independently of the amount of the colloid itself.

Heat induces inactivity of the thyroid, the mitochondria and Golgi complex shrink and the colloid material accumulates.

On the other hand, **exposure to cold** stimulates the gland to activity, the mitochondria and Golgi complex are enlarged, the cells swell and the stored colloid material is resorbed.

INVESTIGATION OF THYROID FUNCTIONS

1. **Experimental.** (a) Removal of the gland. (b) Experimental hypothyroidism by administration of various anti-thyroid substances, viz., methylthiouracil, etc. (c) Thyroid feeding or administration of thyroxine. (d) *Onset of metamorphosis of tadpoles in a nutrient fluid containing thyroxine.* [This is a dependable test for detecting the presence of thyroxine in an unknown extract.]

2. **Clinical.** (a) Observing the effects of hypo- and hyperfunction in man. (b) Effects of radio-iodine on goitre, Graves' disease, etc. (c) Determination of basal metabolic rate. (d) Estimation of protein-bound iodine (PBI) in the plasma. (e) Measurement of thyroidal radio-iodine accumulation. (f) Tests that assess the turnover and release of thyroidal iodine. (g) Tests for thyroid antibodies. (h) Thyroid suppression test. (i) TSH-stimulating test. (j) Thyroid biopsy.

Hypothyroidism

Hypothyroidism produces (a) **cretinism** in young and (b) **myxoedema** in adults. The brief features are as follows:

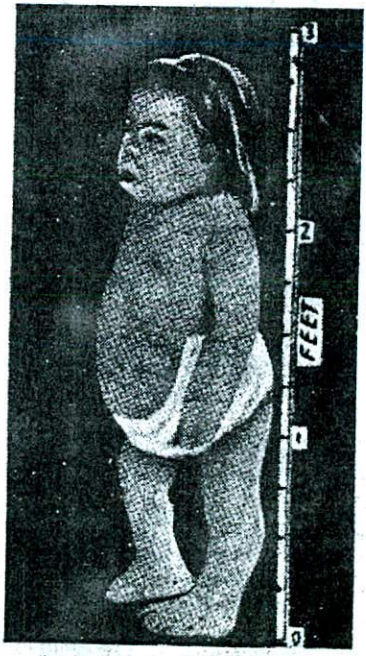


Fig. 4.52. Thyroid cretin. (From Samson Wright)

Cretinism (Fig. 4.52). Symptoms do not appear till after six months, because enough hormone is present in mother's milk. The chief features are the following:

1. The **milestones** of child's development, such as holding up the head (3 months), sitting and dentition (6-7 months), closure of anterior fontanelle (20 weeks), standing, walking, speech (12-18 months), etc., are all delayed.
2. **Skeleton.** Stunted growth, short club-like fingers, deformed bones and teeth.
3. **Skin.** Rough, thick, dry and wrinkled. Hairs scanty.
4. **Face.** Bloated, idiotic look, thick-parted lips, large-protruding tongue. Saliva dribbling. Broad nose with depressed bridge.
5. **Abdomen.** Pot-bellied, umbilicus often protruding.
6. **Sex.** Sex glands, sex. organs and secondary sex characters retarded.
7. **Mental growth.** Idiocy of varying degrees. Often deaf and dumb.
8. **Gastro-intestinal tract and metabolism.** (a) Appetite is reduced. Motility of the gastro-intestinal tract is reduced and there is often constipation. (b) BMR lowered by 20 to 40%, (c) low body temperature, (d) irregular deposit of fat specially above the clavicles.
9. **Blood.** *(a)* Law blood sugar. *(b)* High sugar tolerance. *(c)* High serum cholesterol. *(d)* Low blood iodine.
10. **Resistance.** Lowered. *Susceptible to cold,* toxins and intercurrent infection.
11. **Urine.** Creatine excretion less. Normal output (on a meat-free diet with 2 g of protein per kg) is 0·6-7·8 mg daily. In cretinism it falls to 0-3·8 mg.
12. **Vitamins.** Carotene accumutates sufficiently to cause yellowing of the skin but not the sclera.

Myxoedema or **Gull's disease** (Fig. 4.53). The disease occurs about 7-8 times more frequently in females than in males. Genetic factors (recessive gene) are also of some importance in the genesis of some hypothyroid conditions.

1. **Face, skin and body.** Swollen puffy oedematous look of the face (Mongoloid appearance) and the whole body, due to the deposition of myxomatous tissue. *Myxomatous tissue* consists of a semifluid substance rich in proteins and mucopolysaccharides. Parchment-like cheeks, malar flush, hairs fall out from axilla, pubis, head and *outer third of the eyebrows. Swelling* of the tongue and larynx causing hoarseness and slow-slurring speech. Irregular deposit of fat in the body, etc.



Fig. 4.53. Myxoedema.

2. **Sex.** Degenerates; impotency, amenorrhoea, etc.
3. **Mental condition.** Impaired; dullness, loss of memory, somnolence, etc.
4. **Gastro-intestinal tract and metabolism.** (a) Appetite is reduced. Motility of the gastro-intestinal tract is reduced and there is often constipation. (b) BMR lowered by 30 to 45%. (c) Body temperature low. (d) Increased susceptibility to cold. (e) Body weight increases.
5. **Blood.** (a) Low blood sugar and iodine, (b) Increased sugar tolerance. (c) Raised serum cholesterol—*above 300 mg* per 100 ml (if below 275 mg/100 ml—not myxoedema). (d) Secondary anaemia. (e) Rise of plasma proteins (albumin part).
6. **Heart, circulation and respiration.** (a) Slow heart rate. (b) Stroke volume and minute volume less. (c) Transverse enlargement of heart. (d) *QRST*—low voltage. (e) Fall in cardiac output and blood pressure. (f) Increase in circulation time. Respiratory rates are reduced.
7. **Urine.** Nitrogen excretion less—similar to cretinism.
8. **Thought** processes slow down—lethargy, apathy.

Goitre

It is non-inflammatory and non-neoplastic enlargement of the thyroid gland. In simple goitre usually there is no constitutional features of hypofunction or hyperfunction of the gland. There may be only pressure symptoms due to enlargement of the gland. The simple goitre may be of the following types: (a) **colloid,** (b) **diffuse parenchymatous,** (c) **nodular** or **adenomatous,** and (d) **toxic goitre.**

(a) **Colloid goitre** (Endemic goitre, Benign goitre). It is a deficiency disease caused by an inadequate supply of iodine in the diet.

The alveoli are distended with colloid lined by cubical or flattened epithelial cells. There is no hypertrophy or hyperplasia. Use of iodised salt reduces the incidence of simple goitre.

(b) **In diffuse parencymatous goitre** the alveoli are not distended with colloid like the colloid goitre. The cells lining the alveoli are of columnar type. There is hypertrophy and multiplication of the alveolar epithelial cells. The lumens of some of the alveoli are almost obliterated.

(c) **In nodular or adenomatous goitre** there is nodular swelling of part of the thyroid gland.

In all these types the iodine content is low.

Causes. (1) Iodine deficiency. (2) Presence of goitrogenic substances in the diet, viz., excess of cabbage, brassica seeds, etc. (3) **Drugs**—like methyl- or propylthiouracil and carbimazole. These drugs inhibit formation of thyroxine, hence used in hyperthyroidism (page 4.84). (4) Faecal contamination of drinking water (McCarrison). (5) Trypanosome infection (Brazil).

(d) **Toxic goitre.** Enlargement of the thyroid gland along with excessive secretion of thyroid hormones. The epithelial cells are hypertrophied and hyperplastic. Toxicosis does not mean excessive secretion but toxic symptoms developed due to hyperthyroidism. Graves' disease is always associated with toxic goitre.

(e) **Hokkaido goitre.** While deficiency of iodine may cause endemic goitre or endemic cretinism, a very high concentration of iodine may also cause hypothyroidism by inhibiting iodine organification—the **Wolff-**

Chaikoff effect. This may also be named as a **Hokkaido goitre** because in Hokkaido the Japanese people consume large amounts of seaweeds providing 8 to 25 mg of iodine per day or more produced a good number of endemic goitre with hypothyroidism but no case of cretinism. Iodine in high doses may also interfere with release of T_4 and T_3 from the thyroid gland. Parenchymal hyperplasia is very much marked and majority of patients become hypothyroidism.

Hyperthyroidism or Thyrotoxicosis

Hyperthyroidism is always associated with hypersecretion of thyroid hormones along with numerous clinical manifestations characterised by weight loss, increased BMR and sensitivity to catecholamines, tremor, increased vascularity of the gland, and also goitre and exophthalmos. More heat is produced and the skin becomes hot and sweaty. The heart beats at a faster rate and this increase is maintained during sleep. The pulse rate during sleep may, however, be difficult to quantify since the brain is stimulated by thyroxine and the subject becomes a very little sleeper. The increased excitability of the heart muscle may result in ectopic pacemakers arising in the atria leading to atrial fibrillation. In this condition, thyroid hormones secretion is tremendously increased, sometimes more than 10 times normal. Thyroid gland is enlarged and the enlargement is of nodular (toxic nodular goitre—Plummer's disease) type or of more commonly, the diffuse (toxic diffuse goitre) goitre—Graves' disease. The Graves' disease is always associated with (a) hyperthyroidism, (b) exophthalmos, and (c) goitre.

In thyrotoxicosis, a person with hyperthyroidism becomes clinically ill with the condition. Mental stimulation makes the subject very nervous, irritable and very difficult to nurse. Food is rapidly converted to heat, subjects frequently lose weight although their appetite is still good. Thyrotoxicosis can be treated by medicine and surgery. Carbimazole and methimazole prevent iodine uptake by the thyroid gland and thus may reduce thyroxine formation. However, the gland may increase in size during this treatment. In older patients, thyrotoxicosis may be treated by radio-actiae iodine by mouth. The radioactivity destroys the glandular tissue and thus reduces thyroxine formation.

Graves' disease (Basedow's disease or **Exophthalmic goitre).** Due to excessive secretion of thyroxine. Main features are as follows:

1. **Enlarged thyroid** (hypertrophy and hyperplasia).
2. **Increased BMR.** 50 to 100%, body temperature raised.
3. **Eye signs.** (a) *Exphthalmos**—protrusion of eyeball with a 'staring' look—less twinkling of the eyelids. *Causes*—due to deposition of fat in the retro-ocular region. It is neither due to contraction of Muller's muscle (rudimentary in man) nor due to sympathetic stimulation, as formerly believed. People with paralysis of cervical sympathetic also show it.**

* Similar changes in the muscles and retro-ocular tissues, leading to exophthalmos can be produced by repeated injections of thyrotrophic hormone in animals but this is due to oedema in retro-ocular region. But it is difficult to justify that thyrotrophic hormone is responsible for exophthalmos in Graves' disease. In Graves' disease increased blood thyrotrophin is not found but rather in myxoedema and in cretinism there is increased blood thyrotrophin.

** The pupils are not dilated—a fact which goes against sympathetic stimulation.

(b) *Retraction* of the upper eyelids, caused by infiltration of fat in the levator palpebral superioris which leads to spasm of this muscle. (c) Ophthalmoplegia or weakness of the external ocular muscles. The weakness of the muscles is due to excessive deposition of fat in these muscles. The muscles also show round cell infiltration and degeneration.

4. **Body weight.** Decreased. Fat stores depleted.

5. **Mental condition.** Sharp, emotional, restless, easy fatiguability.

6. **Skeleton.** Osteoporosis due to excessive loss of Ca and P.

7. **Skin.** Soft, moist and flushed—due to vasodilation—helping heat loss.

8. **Blood.** (a) Blood sugar raised, may lead to glycosuria. (b) Lowered sugar tolerance. (c) Increased iodine content. (d) Lowered cholesterol content.

9. **Liver.** (a) Efficiency depressed (low galactose tolerance). (b) Glycogen reduced (also from heart).

10. **Heart and circulation.** (a) Heart rate increases—may be up to 140 per minute. Cardiac output and work of heart increased. Increased irritability of heart—causing extra systoles and even fibrillation. Heart consumes more O_2 and requires more thiamine. (b) Systolic blood pressure increases. (c) Fall in circulation time.

11. **Voluntary muscle.** Fine tremor, increased ankle jerk reflex and muscular weakness.

12. **Electroencephalogram.** Shows abnormal α-waves.

13. **Increased vitamin need.** Due to rise of BMR, vitamin requirement rises—specially for vitamins A, B and C. Unless these are supplied in excess amounts, deficiency signs will appear.

14. Sensitive to heat and susceptible to infection (possibly due to increased protein breakdown).

Thyroxine for slimming. To treat obesity thyroid tablets by mouth has been used. If one has a calorie requirement of 2250 calories per day and takes 2500 calories, the intake exceeds requirement and there is fat deposition under the skin leading to obesity. If there is an increased calorie requirement of 2750 calories per day by taking thyroxine, the intake will now be less than the calorie requirement and the body fat will be used up. Care must be taken to ensure that the increase in thyroxine in the blood does not lead to atrial fibrillation.

Investigation of thyroid activity. Since metabolism is affected by thyroid gland activity, the determination of metabolic rate under basal conditions (BMR) gives a guide to thyroid activity.

The blood cholesterole level is high in myxoedema (600 mg per 100 ml) and is low in thyrotoxicosis (100 mg per 100 ml). The normal level is 200 mg cholesterol per 100 ml of blood. The blood cholesterol level is lowered by thyroxine by increasing the excretion of cholesterol in the bile.

It is difficult to measure the plasma thyroxine level clinically. However PBI is determined instead of plasma thyroxine level and may be used as an index of thyroid activity. Protein-bound iodine (PBI) is a measure of thyroxine plus other iodine protein compounds. The circulating tri-iodothyronine can not be included because it is not protein bound. PBI is low in myxoedema and high in thyrotoxicosis.

The use of radio-isotopes of iodine is used greatly to investigate thyroid activity. Rado-active iodine was first used in 1942. ^{132}I (half-life 2 hours) and ^{131}I (half-life 8 days) are employed. But the body cannot distinguish between these isotopes of iodine and normal iodine. These are incorporated in thyroxine in proportion to their relative amounts in blood.

It is advantageous to measure radio-isotopes with their radioactivity. An oral dose of radio-active iodine gives a measurable concentration in the neck region after 4 hours later and this can be measured using an external radiation counter. The uptake is commonly low in myxoedema and high in thyrotoxicosis.

Thyroid activity may be determined by the ankle jerk. In thyrotoxicosis, this reflex is brisk. But in myxoedema this reflex is sluggish and there is a delay before the muscle relaxes.

Pathogenesis of Graves' disease

Pathogenesis of Graves' disease is claimed to be due to a circulating TSH-like abnormal stimulator, the γ-globulin. This stimulator has got long action and is known as **long-acting thyroid stimulator** (LATS). Adams (1958) detected this long-acting thyroid stimulator in the plasma of patients with thyrotoxicosis. The LATS is γ-globulin and produced by lymphocytes of patients with thyrotoxicosis. It may be an autoantibody to a thyroid antigen. Solomon and Beale (1967) have been able to produce hyperthyroidism by immunising animals with thyroid microsomes. LATS activity may be neutralised by antiserum to human γ-globulin but not by antiserum to TSH. Anterior pituitary TSH has got no role and practically blood level of TSH remains low in thyrotoxicosis. If the heavy chain of LATS molecule is separated, then it only retains **short-acting thyroid stimulating property,** but on recombination of chains, it regains the original long-acting property. Thymus and lymphoid tissue may be related with the formation of LATS, because severity of Graves' disease is decreased after removal of enlarged thymus and lymph nodes.

LATS biological activity may be transferred through transplacental passage and neonatal hyperthyroidism may be the cause of transfer of LATS from the thyrotoxic mother to the foetus. However, the presence of LATS does not always provide adequate explanation for all aspects of Graves' disease. Neither LATS nor thyroxine is responsible for the exophthalmos. **Exophthalmos-producing substance** (EPS) has been extracted from the plasma of patients with the disease. This EPS is capable of producing exophthalmos experimentally in animals. This substance is not TSH but it may be of pituitary origin and a derivative of TSH.

Thyrotoxicosis can be controlled by blocking thyroid hormone synthesis with anti-thyroid drugs like propylthiouracil and carbimazole (Neo-Mercazole) or by ablation of the hyperactive gland by surgery or radio-iodine. Anti-thyroid drugs block thyroid hormone synthesis and about 50% of patients are cured during a course of anti-thyroid drug treatment for a year. Treatment of thyrotoxicosis through ablation is more effective and rapid but in about 40% of patients it is followed by hypothyroidism associated with hypoparathyroidism. Hypothyroidism seems to be the only complication of radio-iodine therapy, whereas surgery, which is followed less often by hypothyroidism, has other

complications such as hypoparathyroidism and damage to the recurrent laryngeal nerves. For rapid symptomatic remission in thyrotoxicosis, treatment with a β-adrenergic blocking agent (propranolol) may be used before treatment with anti-thyroid drugs, radio-iodine or surgery.

Genetic factors and thyroid disorders

Genetic factors may have a role in the precipitation of thyroid disorders due to iodine deficiency or other causes. Because in iodine deficient areas a certain family may be affected with thyroid disorders hereditarily. Endemic cretinism or goitre occurs in children of parents having goitres.

Hereditary defects of the thyroid hormone biosynthesis may cause sporadic goitrous cretinism. Such metabolic defects are: (a) abnormality in iodine trapping mechanism, (b) inability to incorporate iodine into thyroglobulin, (c) failure of iodotyrosine coupling, (d) abnormality in deiodination of MIT and DIT, and (e) impairment in the proteolysis of thyroglobulin.

Functions of the thyroid gland

1. **On metabolism**

(a) GENERAL METABOLISM. Thyroid stimulates the metabolism in the tissues. BMR is increased. O_2 consumption and heat production are also increased. In myxoedema BMR is diminished by 30% or more below normal. After administration of thyroxine in myxoedema BMR rises. In hyperthyroidism BMR is increased by 50% or more. Due to diminished BMR in myxoedema O_2 consumption, CO_2 output, etc., are diminished. One milligram of thyroxine increases the basal metabolic rate, equivalent to the oxidation of 250 g of glucose (1000 C). Thyroxine stimulates O_2 consumption of all the tissues except possibly brain, gonads and spleen. The mitochondria of the cells play an important role in O_2 consumption. In hypothyroid subjects O_2 consumption of the mitochondria of the cell is decreased. Thyroxine alters the permeability of the mitochondrial covering. Administration of thyroid hormones increases some enzymes like cytochrome oxidase, succinic dehydrogenase, etc.

(b) CARBOHYDRATE METABOLISM. Thyroxine stimulates the absorption of monosaccharides from the intestine. It stimulates glycogenolysis and produces hyperglycaemia. It reduces sugar tolerance. It also stimulates the utilisation of glucose in the tissues thus decreasing the hyperglycemic effect. Thyroid hormones enhance the breakdown of insulin. Hypothyroidism, on the other hand, increases hepatic storage of glycogen.

(c) PROTEIN METABOLISM. Thyroxine in physiological doses has a protein anaebolic effect including enhancement of growth of all bodily tissues. This effects has been observed in absence of pituitary hormone. Toxic amount does not show such effect. Hyperactive states of thyroid gland increase breakdown of proteins and so elevate nitrogen elimination leading to wasting of tissues. In hyperthyroidism or after administration of thyroxine in large doses there is impairment of the formation of creatine phosphate and muscular contraction becomes weak. Excessive accumulation of mucoprotein in the skin and other tissues is one of the characteristic features observed in cretinism and myxoedema, has been found to be corrected by thyroid hormones.

(d) FAT METABOLISM. The serum cholesterol and phospholipid rise in hypothyroid state, e.g., cretinism and myxoedema. In hyperthy-

roidism the serum cholesterol falls. This is partly due to the increeased excretion of cholesterol in the bile and partly due to destruction in the liver. In hyperthyroid state, after depletion of carbohydrate reserve due to increased oxidation, there is an enhanced breakdown of depot fat and so a rise in ketone body formation. On the other hand during thyroid deficiency, increase in depot fat is usually not observed.

(e) IODINE METABOLISM. Thyroxine helps in the absorption of inorganic iodide from plasma, oxidation of the iodide into iodine, formation of monoiodotyrosine and di-iodotyrosine and subsequent transformation into thyroxine. Thyrotrophic hormone of the anterior pituitary controls the formation and the synthesis of thyroxine.

(f) MINERAL METABOLISM. Hyperthyroidism mobilises Ca and P from bones making them porous. But small amounts of thyroxine increase calcium retention in growing animals as a secondary effect. In hypothyroidism retention of sodium, chloride and water has been noted, administration of the hormone under such condition causes loss of sodium and water.

2. **Relation with vitamins.** Thyroxine helps in the conversion of β-carotene into vitamin A in the body. In hypothyroidism blood carotene level rises. Thiamine requirements and urinary excretion are increased in hyperthyroidism. Hyperthyroid rats appear to have an increased need for riboflavin. Lack of vitamin B_{12} appears to affect the thyroids of chick embryo. Vitamin C requirement may be increased in hyperthyroidism because urinary vitamin C level is below normal.

3. **Growth and differentiation.** The growth of the body is influenced both by somatotrophic hormone (STH) of the anterior pituitary and thyroid hormone or thyroxine but the process of differentiation of tissues is influenced only by thyroid hormones.

(a) SKELETAL GROWTH. Somatotrophic hormone helps in general bony growth but thyroxine helps in the maturation and differentiation of the epiphysis, etc. In hypothyroidism before puberty, i.e., in cretinism, there is retardation of skeletal growth.

(b) MUSCULAL GROWTH. In cretinism there is retardation of muscular growth. In hyperthyroidism the formation of creatine phosphate is impaired and muscular contraction becomes weak.

(c) SEXUAL GROWTH. Thyroid controls the normal functions of gonads. In cretins there is retardation of the gonadal growth and secondary sex characters do not appear. In myxoedema due to hypofunction of the gonads, amenorrhoea occurs in women. It is not definitely clear if gonadal hypofunction during hypothyroidism is due to reduced secretion of pituitary gonadotrophins. Androgens and oestrogens cause alteration of thyroxine-binding globulin (TBG) and thyroxine-binding prealbumin (TBPA) in the plasma.

(d) MENTAL GROWTH. Thyroid plays an essential role in mental growth and development of the central nervous system. In cretins there is retardation of mental growth and of development of the central nervous system. The child remains mentally backward and he may be an idiot. Speech, walking, etc., are all delayed. The development of the cerebral cortex shows various abnormalities, e.g., myelination of axons, arrangement of dendrons, etc. Electroencephalographxic study also reveals a lowering of alpha waves.

4. **Cardiovascular system.** During hyperthyroidism a characteristic feature is an increase in systolic blood pressure without any alteration in the diastolic, and also an increase in stroke volume, cardiac irritability and output, heart rate and dilatation of peripheral vessels. Thyroid hormones (T_3 and T_4) increase sensitivity of catecholamines to the tissue. The effect of thyroid hormone on circulating titers of catecholamines may affect the cardiovascular system. In hypothyroid condition, the heart becomes oedematous, sluggish and contracts less forcefully resulting in reduced cardiac output. O_2 consumption of myocardial tissue of experimental animals is increased by thyroid hormones.

5. **Nervous system.** Thyroid hormone increases the sensitivity of the nervous system. This system is profoundly affected by deficiency of thyroid function. Deficiency of the hormone in the young animals results in decrease in myelin, neurone size, cerebral water content, number of axons, etc. Thyroid therapy repairs several neural effects of hypothyroidism if begun before a critical period. Effect of thyroid hormone on brain excitability is associated with alteration in electrolyte distribution.

6. **Maturation of red cells.** In hypothyroidism, e.g., in myxoedema, anaemia is a common feature. The anaemia is of megaloblastic type and probably due to deficient absorption of vitamin B_{12}.

7. **Heat regulation.** In hypothyroidism there is an increased susceptibility to moderate cold. The calorigenic effect of thyroxine is due to its direct effect on the cells.

8. **Secretion of milk.** Thyroid stimulates galactopoiesis, i.e., it stimulates and maintains the secretion of milk during lactation. It also increases the content of milk.

Mode of action of thyroid hormones

The mode of action of the thyroid hormones is not definitely clear. It has been suggested that the thyroid hormones stimulate metabolism through increased activation of the citric acid cycle. Enzymes taking part in this cycle are present in the mitochondria and the thyroid hormones stimulate the enzyme system by changing the permeability of the mitochondrial membrane. There is also a possibility that the hormones act to uncouple oxidative phosphorylation. Inhibition of the phosphorylation of ATP as a causative factor for increased oxygen consumption during hyperthyroidism has also been advocated. The protein-stimulating effects of thyroid hormones are possibly mediated through increased synthesis of ribosomal RNA, as these effects are inhibited by actinomycin D. Stimulatory effects of thyroid hormones on RNA synthesis might augment the activity of lysosomal enzymes thus causing involution of a tadpole tail. Thyroid hormones are capable of influencing the formation or metabolism of cyclic AMP under certain conditions, but the role of cyclic AMP in the physiologically important effects of these has not been established.

Control of thyroid secretion and endocrine interrelationship

1. **Role of anterior pituitary** (Fig. 4.54). The thyrotrophic hormone of anterior pituitary controls the formation and secretion of thyroid hormones. *On the other hand, the circulating level of thyroxine controls the secretion of thyrotrophic hormone or thyroid-stimulating hormone (TSH) or thyrotrophin.* Rise of thyroxine level in blood depresses, whereas fall of thyroxine level increases secretion of thyrotrophin. After thyroidectomy

more thyrotrophin is secreted. In this way, the TSH and thyroxine control each other. In other words, through anterior pituitary, thyroid controls its own secretion. Excess secretion of thyrotrophin stimulates the thyroid hormone formation in the following ways: (1) Increase in size and secretory activity of follicular epithelial cells. (2) Increase in number of the follicular epithelial cells. (3) Increase in the uptake of iodine. (4) Increased enzymatic processes for the transformation of iodide and tyrosine into thyroid hormones. (5) Incease in the proteolysis of thyroglobulin (Fig. 4.51). In myxoedema there is increased secretion of thyrotrophin. In some patients suffering from thyrotoxicosis, an abnormal protrusion of eyeball, exopthalmos, has been observed. It is not clearly known if this "exopthalmos-producing substance" is a second type of an abnormal kind of TSH.

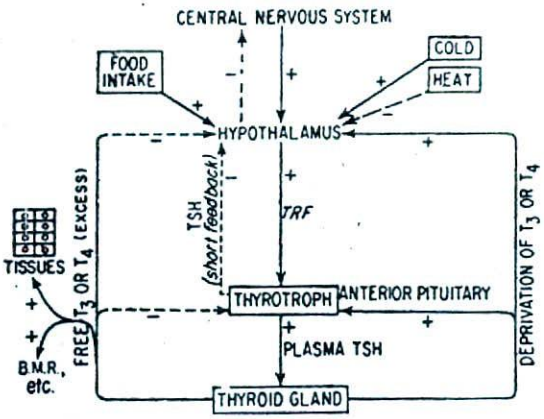


Fig. 4.54. Diagrammatic representation showing interaction of feedback mechanism and neurohumoral control in the regulation of thyroid hormone secretion.

2. **Influence of the central nervous system and role of hypothalamus.** Thyroxine may reach hypothalamus and excerts its inhibitory influence on secretion of pituitary thyrotrophin. Hypothalamus controls the secretion of thyrotrophic hormone or thyrotrophin of the anterior pituitary. The neural mechanism which controls TSH secretion is possibly located in the median eminence of the hypothalamus through liberation of TRF (thyrotrophin-releasing factor) into the hypothalamico-hypophyseal blood vessels. Lesion of this part causes reduction in secretion of thyrotrophin. The size of the thyroid gland is reduced along with diminution of secretion of thyroid hormones. Stimulation of the hypothalamus with fine electrodes causes liberation of TRF which indirectly increases secretion of thyroid hormones by acting directly on the specific thyrotroph cells of the anterior pituitary (TSH). TRF release from hypothalamus is controlled by the environmental temperature. In cold the anterior hypothalamus and its adjacent areas are stimulated with TRF which then activates the liberation of the TSH from the anterior pituitary. It is claimed that TSH secretion from anterior pituitary is controlled by blood thyroid hormones at dual levels, both pituitary and hypothalamus. The "long-loop feedback" control at the hypothalamic level acts both in an excitatory and inhibitory way and requires the presence of an intact hypophyseal portal system. A direct feedback regulation at the pituitary level is also operative.

3. **Role of sympathetic and parasympathetic nerves.** [Vasomotor regulation]. Thyroid is supplied both by sympathetic and parasym-

pathetic nerves. They regulate thyroxine secretion through vasomotor effects only. No direct secretory activity has been observed. Thyroid can secrete thyroid hormones even after complete denervation.

4. **External temperature.** Cold (moderate) climate stimulates and hot climate depresses thyroid hormone secretion. This is a great help for regulation of body temperature. This regulation of body temperature through the alteration of thyroid hormone secretion may be due to (a) modification of reflex TSH secretion, (b) vasomotor reflex, causing vasodilation or constriction in the thyroid and thus increasing or decreasing thyroxine formation respectively. It is possible that through a nervous pathway cold stimulates hypothalamus for secretion of TRF which helps in excess secretion of TSH.

5. **Stress phenomena.** Physical and emotional stress, peripheral electric stimulation, haemorrhage, laparotomy, injection of turpentine, abscess formation, etc., inhibit the thyroid gland due to a reduction of thyrotrophin output.

6. **Thyroid-adrenal interrelationship.** Administration of ACTH or cortisone inhibits the thyroidal accumulation and release of radioiodine. It is probable that TSH secretion is interfered with, since injury to adrenal cortex results in a rise in heat production provided the thyroid is intact. There is a reciprocal relation between ACTH and TSH, and hence between cortical and thyroid hormone effects.

7. **Thyroid-gonadal interrelationship.** Normal reproductive functions in both the male and the female requires normal amount of thyroid hormones to be present. *Female reproductive system*—Hypothyroidism results in prolonged or arrested oestrous cycle, reduced fertility, increased foetal resorption, etc. It is suggested that the placenta secretes a thyroid-stimulating factor which may account for the increased thyroid-stimulating activity of the sera of pregnant women and of patients with tumours of chorionic tissue. *Male reproductiae system* — Adequate thyroid function is presumably necessary for normal development and function of the male reproductive system. Thyroid may exert effects on sperm number and motility and testicular DNA. It is not clear if the abnormal reproductive functions as observed in thyroid hormone deficiency are due to reduced ability of the pituitary to secrete gonadotrophins or defective response of the target organs.

Anti-thyroid compounds

Anti-thyroid compounds are used experimentally to produce hypothyroidism, and clinically in the treatment of hyperthryoid conditions (Fig. 4.55). The most active group like thiourea, thiouracil, prophylthiouracil, etc., and also sulpha drugs, para-aminobenzoic acid (Fig. 4.56)



para-amino-salicylic acid, etc., act in a similar manner to produce hyperplastic goitres. In such condition although the gland can accumulate iodine normally, the synthesis of thyroid hormones does not occur, due to interference in the iodination of tyrosine in the gland, Low thyroxine level in blood increases secretion of TSH from the anterior pituitary. The size

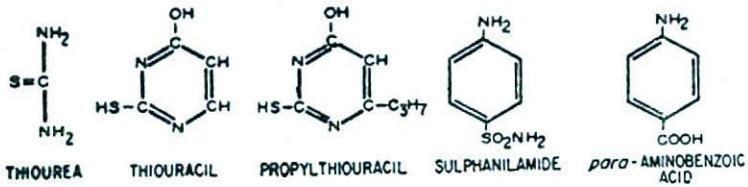


Fig. 4.56. Structural formulae of some anti-thyroid drugs.

of the thyroid gland may be reduced by removing the pituitary but such a gland does not produce any hormone. External administration of thyroid hormones relieve the goitres. The second group of compounds like thiocyanate, periodate, perchlorate, etc., do not block the synthesis of thyroid hormone as in the previous group, but inhibit in the concentration of iodide inside the gland through an unknown mechanism. A high dietary intake of iodide, enough for the synthesis of normal amount of thyroxine prevents the occurence of such goitre.

Compounds occurring in some natural foods like cabbage, turnips, kale, etc., also produce goitre. They contain a compound **progoitrin** and an activator which converts it into **goitrin.** The activator is also present in the intestine.

Thyrocalcitonin

It has been observed recently that thyroid gland produces another hormone, the **thyrocalcitonin** (TCT). It is secreted from the mitochondrial-rich parafollicular cells of the thyroid gland (Fig. 4.44) in response to hypercalcaemia, and lowers plasma calcium and phosphate. The hormone is polypeptide in nature. These parafollicular cells arise from the neural crest and migrate with the ultimobranchial body.

Source. Copp (1961) was the first to propose the presence of a hormone in the parathyroid gland which is capable of lowering the blood calcium (Fig. 4.56). He termed the name **calcitonine.** But subsequent work has shown that this hormone is secreted from the thyroid and



not form the parathyroid and at present known as **thyrocalcitonin.** Mammalian parafollicular cells (Fig. 4.44) of the thyroid contain the secretory granules of thyrocalcitonin and vary in abundance according to the blood level of calcium. Other tissues like thymus and adrenal gland may elaborate this hormone, but it seems to secrete mainly from the mitochondri- rich parafollicul cells or C cells (C for calcitonin) of the thyroid gla

Chemistry. Potts nd (1969) have determined the amino-acid sequence of porc hy. onin (Fig. 4.58) containing 32 amino aci . Molecular w of ine thyrocalcitonin is about 3600. In all

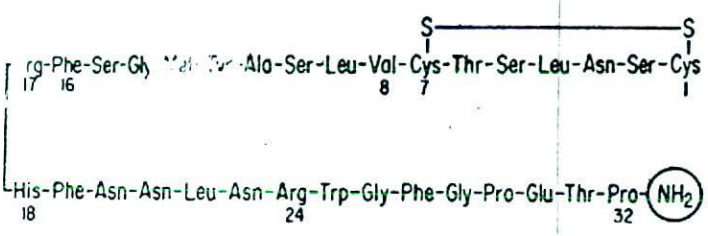


Fig. 4.58. Amino-acid sequence of porcine thyrocalcitonin.

species, thyrocalcitonin is a linear peptide. Bovine and porcine thyrocalcitonins are similar but human hormone (Fig. 4.46) shows some differences in structure (Potts and Deftos, 1969). Fish thyrocalcitonin is more potent and may be the useful form of the hormone for therapeutic use.

Regulation of secretion. It has been observed by radio-immunoassay and bioassay studies that the thyrocalcitonin is secreted continuously in normal animals, with elevation during hypercalcaemia (Fig. 4.59) and decrease of secretion during hypocalcaemia. The direct infusion of Ca^{++} either intravenously or directly into the thyroid arteries leads to a rapid increase of blood level of the thyrocalcitonin. This secretion is directly proportional to the blood Ca^{++}, above the level of about 9·5 mg per 100 ml of blood. Below this level, hyrocalcitonin is not detected in the blood (Fig. 4.59).

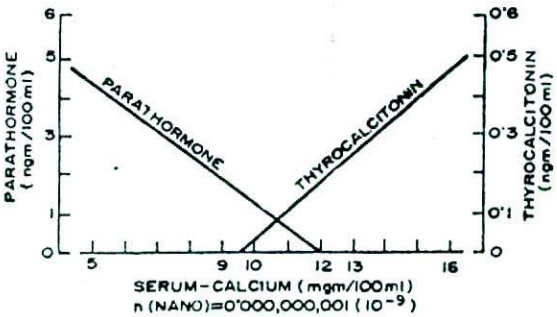


Fig. 4.59. Effects of serum-calcium changes in the levels of parathyroid hormone and thyrocalcitonin in the peripheral blood.

On the other hand, serum-parathormone concentration is inversely related to serum-calcium, falling to 0 when serum-calcium exceeds about 12 mg/100 ml. Secretion of parathormone is stimulated when edetic-acid (EDTA) infusion lowers serum-calcium, and is greatly increased during lactation. The parathormone is rapidly cleared from circulating blood.

There is no such evidence that cyclic AMP is required for its release. But it is indicated from the observation of Care *et al.* (1970) that dibutyryl derivative of cyclic AMP can stimulate thyrocalcitonin release, Ca^{++} is required for this effect and theophylline also enhances this process. Care and Bell have described that pancreatic glucagon also stimulates the release of thyrocalcitonin.

Role of β-adrenergic catecholamines in the secretion of thyrocalcitonin has been observed by Care *et al.* (1970). Cyclic AMP may have some role in the above process.

There is at present no evidence that the parathyroid elaborates thyrocalcitonin-releasing factor. TSH or other pituitary hormones ha got no role in controlling the secretion of thyrocalcitonin. Hypophysectomy does not affect the parathyroids or ultimobranchial glands.

Physiology. It is the calcium-lowering hormone of the thyroid. It acts on the circulating calcium levels faster than the parathormone and half-life of thyrocalcitonin is 4-12 minutes, which is shorter than the parathyroid hormone. The hypocalcaemic action of this hormone is due to inhibition of bone resorption and calcium release. Studies which isotopic Ca^{++}, it has been observed that the hormone blocks the release of bone calcium rather than facilitating calcium uptake. Thyrocalcitonin might affect the metabolism and transport of phosphate in addition. Intravenous administration of TCT (thyrocalcitonin) into young animals leads to rapid fall of blood calcium and phosphate. Major and probably main site of action of TCT is upon bone, where it inhibits bone resorption. Thus TCT may have a considerable therapeutic usefulness in protecting the resorption of bone as in the case of osteoporosis. TCT infusion blocks the metabolism of Ca^{++} from mature bone induced by parathormone and the dibutyryl derivatiae of cyclic AMP (Klein and Raisz, 1970). Considering these effects, it has been postulated that thyrocalcitonin might act by stimulating phosphodiesterase, thereby reducing the level of cyclic AMP in the same cells affected by PTH. But this hypothesis does not hold good when it is observed that thyrocalcitonin does not prevent the increase in cyclic AMP produced by PTH in bone (Chase and Aurbach, 1970). Thyrocalcitonin rather increases the level of cyclic AMP in bone. It is noteworthy that the action of TCT is not suppressed by actinomycin D, an inhibition of RNA synthesis. Human synthetic calcitonin has produced appreciable biochemical and clinical remission in cases of Paget's disease of the bone. Skin temperature overaffected limbs comes down to normal and bone pain is relieved. Elevated total urinary hydroxiproline level and serum alkalime phosphatase level come down to normal or near normal values. The new bone is formed during the course of treatment as a normal lamellar structure.

THE PARATHYROID

Anatomy. Four small oval bodies ($6 \times 3 \times 2$ mm) embedded in the posterior surface of the thyroid—one pair arranged vertically behind each lobe. Total weight about 140 mg. Highly vascular. Blood supply is derived from paired superior and inferior thyroid arteries. Nerve supply same as the thyroid, probably purely vasomotor. **Accessory parathyroids** may be found in the thyroid, thymus or near the arch of aorta.

Development. Upper pair are formed from the fourth and the lower pair from the third branchial pouch.

Histology. Masses or columns of epithelioid cells, with large blood sinuses in between them. Two types of cells: (1) **Chief cells** or **principal cells**—small faintly staining non-granular cells and completely clear cytoplasm, with comparatively large vesicular nucleus. Sometimes these cells enlarge and possess numerous cytoplasmic vacuoles; they are then called *water-clear* cells. The cells contain glycogen. Majority in number, remain throughout the life. (2) **Oxyphil cells** or **eosinophil cells**—large polyhedral granular cells and acidophilic cytoplasm. Granules stain with eosin and other acid dyes. Electron microscopic observations have indicated the granules to be mitochondria. The oxyphil cells are few in number (Fig. 4.60). Both types contain fatty granules or minute spherical globules, increasing in number with age. Principal cells persist up to 10th year of life in human beings. Small colloid vesicles (like those of the thyroid) are also found, but without any iodine. Variable amounts of adipose tissue may be observed within the gland.

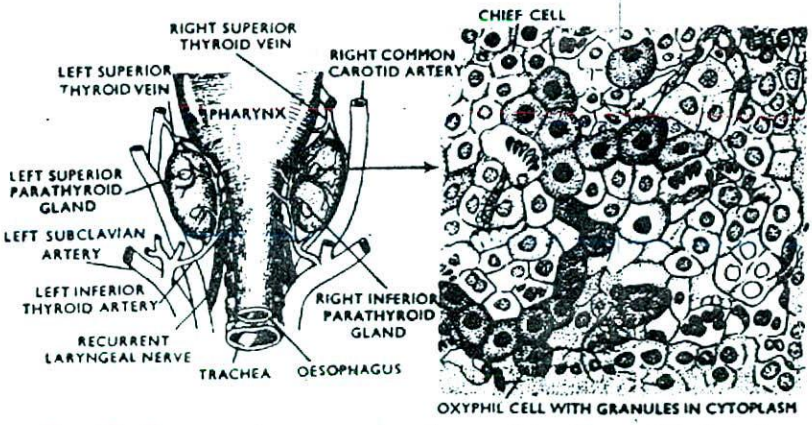


Fig. 4.60. Diagrammatic representation of anatomical position and histological structure of parathyroid gland.

Methods of investigation of parathyroid

(1) **Extirpation**—(a) in *animals*, (b) in *man*.
(2) **Administration of parathormone.**
(3) **Clinical**—(a) *hypoactivity*, and (b) *hyperactivity* in man.

Effects of extirpation. Both in animals and man, it causes a condition of **tetany** characterised by: (1) Increased neuromuscular irritability. (2) Twitchings or convulsions. (3) Low Ca, high P and increased guanidine bodies in blood. (4) Reduced Ca and P excretion. *If untreated the condition is fatal.*

Effects of administration of parathormone. (1) In *tetanic* infants and animals, serum Ca is raised and the condition is cured. (2) In *normal* animals, serum Ca raised and inorganic phosphate lowered. This state of *hypercalcaemia* causes neuromuscular depression and various other ill effects (page 4-96).

Clinical study. Hyperactivity of parathyroids in man (parathyroid tumour) mobilises Ca from bones causing rarefaction and cyst formation (*osteitis fibrosa cystica*). The serum Ca is raised and inorganic phosphate lowered. Hypoactivity (idiopathic or accidental removal during thyroidectomy) causes tetany as described above.

Functions of parathyroid gland

1. The parathyroids are essential for life, as they are important for the regulation of the concentration of calcium ions in the body fluids. After parathyroidectomy the blood calcium level falls and causes a neuromuscular irritability—the *tetany*.

2. The active principal of the parathyroid is parathormone (PTH) and acts in response to a lowering of blood calcium by stimulating the **osteoclasts** to resorb bone and free calcium ions from the reserve skeletal depots. After administration of the hormone, blood calcium level rises due to mobilisation of calcium from the bones. With continued administration there is increase of osteoclastic activity in the bones.

3. It regulates the excretion of inorganic phosphate in the urine. After administration of parathormone there is increased phosphate excretion in the urine and serum phosphate level falls. This effect is most probably mediated by an action of PTH on the kidneys, which causes the tubular reabsorption of phosphate to be inhibited. Hence this ion is then lost in the urine.

4. DeLuca (1968) has described that vitamin D is not metabolised to its functional form in absence of parathyroid hormone. He provides a new light in the interrelationship between vitamin D and PTH. Elevation of serum calcium by PTH is through the 1, 25-$(OH)_2D$ mechanism.

PARATHORMONE (PTH)

The chief cells of parathyroids secrete parathyroid hormone (PTH) Parathormone is not stored in the parathyroid gland but it is synthesised and secreted continuously. This hormone isolation is difficult due to absence of storage granules and very low content of tissue.

Chemistry. *Parathormone* is the active principal of parathyroid gland, prepared by Hanson in 1924 and by Collip in 1925 by extracting with 5% HCl. Structure of bovine parathyroid hormone has been determined. It is a polypeptide having a linear chain of 84 amino acids (Fig. 4.61).

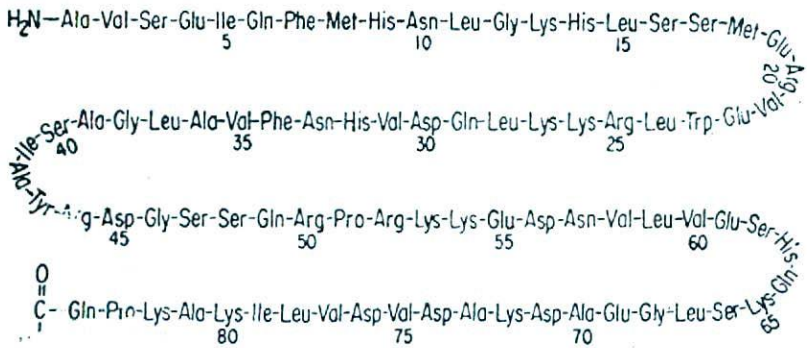


Fig. 4.61. Amino-acid sequence of parathyroid hormone.

Potts and others (1971) have been able to synthesise a peptide of 34 amino acids having amino acid sequence similar to first and 34 amino-acid residues of the naturally occurring hormones, i.e., the amino acids from N-terminal alanine to the 34th amino acid phenylalanine. This synthetic hormone possesses the same biological potency as it is observed in natural hormones, suggesting that the biological potency of the hormone remains with the 34 N-terminal amino acids. Half-life of parathormone is about 18 minutes. It is rapidly destroyed in the circulation into smaller fragments. Mol. wt. about 9500. Freely soluble in water and 80% alcohol, but not soluble in ether and acetone. Easily destroyed by pepsin and trypsin, hence not effective by month.

Unit of parathyroid activity. There is no international unit. Due to absence of a standard preparation, the activity of parathyroid preparation has been considered in terms of biological response. The US parathormone unit is defined as 1/100 of the amount of an extract required to raise serum calcium of a dog weighing 20 kg by 5 mg/100 ml within 16 to 18 hours after administration of the preparation.

Bioassay. Following methods have been developed for the bioassay of parathyroid hormones:

(a) **Method based on the elevation of serum calcium.** This method was originally described by Collip and Clark (1925) and involves the measurement of a rise in serum calcium after dosage with parathyroid extracts in groups of 10 dogs.

(b) **Method based on the prevention of magnesium anaesthesia in mice by a rise in serum calcium produced by parathyroid hormones.** Calcium ions have got depressive actions on the narcotic effects of magnesium. This effect is produced equally by parathyroid hormones. Simon (1935) and subsequently Dyer (1935) described a bioassay method on the basis of the above effect.

(c) **Assay method using a fall of serum phosphate.** Tepperman *et al.* (1947) described this method, based on the fall in serum inorganic phosphorus in the rat after administration of the hormone.

(d) **Method based on the urinary excretion of phosphate.** Davies and her colleaques (1955) described this assay method, involving phosphate excretion in the urine. Radiophosphorus (^{32}P) may be used for this assay method.

(e) **Method based on the urinary excretion of calcium.** This method was originally described by Dyer (1932).

(f) **Based on the increased height of leg muscle contraction by parathyroid hormone.** Based on the observation of Gellhorn (1935), a very useful assay method has been developed. Skeletal muscle is highly sensitive to calcium ions while in a hypodynamic state. In hind limb preparation of *Rana esculenta,* parathormone gives a graded response.

(g) **Immunoassay by complement fixation.** Since the observation of Tashjian *et al.* (1962) that bovine parathyroid hormone can produce a precipitating antibody in the rabbit, an assay method has been developed by them (1966). This method is very sensitive and as little as 24 ng can be detected.

Effects of administration of parathyroid extracts or parathormone

In tetanic infants and animals serum Ca is raised, P diminished and their excretion is rectified. Thus the condition is cured. In normal animals, if given subcutaneously every two hours, serum Ca is raised to about 15-20 mg/100ml (hypercalcaemia) within 24 hours and inorganic phosphates fall. Continued administration keeps the blood calcium high and produces the ill effects of hypercalcaemia, viz., (a) Loss of appetite, diarrhoea, flaccidity of muscles. (b) Drowsiness and dullness. (c) Increased viscosity of blood, diminished blood volume, Circulation slowly fails. (d) Urea and non-protein nitrogen (NPN) increase in blood due to renal failure caused by impaired renal circulation. (e) Rarefaction of bones and metastatic calcification. (f) Finally coma ending in death.

So low blood Ca leads to hyperexcitability of the nervous system while hypercalcaemia depresses the nervous system, circulation and kidney function.

Physiological functions of parathyroid gland

The current idea is that the principal target organ for PTH is bone and kidneys. Contradictory evidences indicate that the hormone may possibly accelerate intestinal absorption of calcium. PTH also exerts its effects in absence of kidneys. Some exchange of Ca and PO_4 between bone and plasma can take place in absence of parathyroid gland. The physiological functions of the gland are as follows:

1. It increases rate of bone resorption with consequent mobilisation of calcium and phosphate (Fig. 4.62). An early effect on *osteocytes* leads to release of calcium from crystals of mature bone into blood, occurs within 15-30 minutes and does not initially depend upon the synthesis of RNA by osteocytes. A slower effect occurs on the turnover of bone and remodelling during maintained hypersecretion.
2. It directly increases renal tubular reabsorption of calcium.
3. PTH increases direct renal excretion of phosphate.
4. It may increase the rate of calcium ion absorption in the gut.
5. PTH may decrease the rate of Ca^{++} secretion by the lactating mammary gland.
6. It may control the intracellular disposition of phosphate.
7. In case of *mitogenic activity*, PTH may be capable of stimulating DNA syntheis and mitosis in (and hence proliferation of) the thymic lymphocytes of rats.

Mechanism of action of parathormone

The mechanism of action of parathyroid hormone is not definitely understood but there are some postulations:

1. The action of PTH is dependent upon the formation of DNA-dependent RNA in some way.
2. The action of PTH on bone and kidneys is stimulated by adenyl cyclase. PTH may act directly on *osteocytes* to cause the release of lysosomal enzymes, probably by *exocytosis* and this effect can be mimicked by dibutyryl derivative of cyclic AMP. PTH may also act on certain mesenchymal cells to stimulate their transformation to *osteoclasts* and this may be mediated by cyclic AMP, although an increase in number of

osteoclasts in response to exogenous cyclic AMP. The effect of PTH may be mediated by an increase in the level of cyclic AMP in the *renal proximal tubules* to stimulate the secretion (or to inhibit the reabsorption) of inorganic phosphate, PTH and dibutyryl derivative of cyclic AMP stimulate gluconeogenesis from lactate in *renal cortical tubules.*

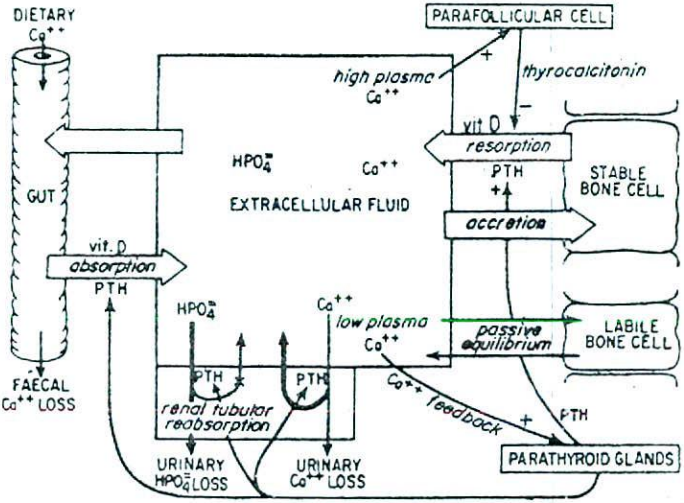


Fig. 4.62. Schematic representation of parathyroid control of extracellular calcium and the sites of PTH action.

3. The 'solubilising' effect of the hormone might possibly be due to destruction of the organic matrix by some enzyme secretion.

4. An effect of the hormone in increasing membrane transport has also been advocated.

5. According to a controversial hypothesis, parathyroid hormone, possibly due to its regulatory control over either permeability changes or enzyme systems, increases the utilisation of glucose and pyruvate by bone resulting in increased production of citrate. Due to deficiency of the enzyme isocitric dehydrogenase in bone, there is an accumulation of citrate in this organ. The citrate replaces phosphate from bone followed by dissolution of calcium citrate formed. Thus both calcium and phosphate are removed from bone. The renal threshold for calcium is raised but that of phosphate is lowered, resulting in hypercalcaemia and hypophosphataemia.

Control of parathyroid secretion

Secretion of PTH is controlled by a negative feedback mechanism, relating to the level of calcium in the plasma (Fig. 4.62). Abrupt decrease of PTH concentration occurs after administration of Ca^{++} and rises after administration of EDTA (ethylenediaminetetra-acetate) when circulating ionised calcium is lowered. EDTA lowers the blood calcium by chelation.

By Ca content of blood. The relation is reciprocal. Parathyroids control Ca metabolism while blood Ca controls parathyroid secretion (Fig. 4.59).

Evidences. (a) Intravenous injection of 40 mg of sodium oxalate lowers serum Ca by precipitating Ca oxalate. In normal animals rapid recovery occurs, but if parathyroids are removed before, no recovery occurs. (b) If decalcified blood be perfused through the parathyroids (along with thyroid) and then this blood be infused into another animal, serum Ca in the latter increases. Both these experiments show that, *low blood Ca stimulates parathyroid secretion.* (c) Low calcium in the diet causes hypertrophy of the parathyroid.

There is possibly no endocrine or nervous control.

TETANY

Parathyroid gland is essential for life. Experimental removal of the gland in animals or accidental removal during thyroid surgery is followed by a gradual fall in plasma calcium, neuromuscular hyperirritability (tetany) and death.

Tetany in animals. In animals symptoms appear one to three days after extirpation. The features are as follows:

I. **Increased neuromuscular irritability.** (a) *Peripheral nerves*—hyperexcitable. not a central effect but due to alteration of the fluid which surrounds peripheral nerves. PROOF—If isolated limb be perfused with the blood of a tetanic animal, tetany occurs in the limb. (b) *Spinal cord*—twitching occurs in the muscle—at first fine, then coarse. The centre is in the cord. PROOF—occurs in spinal animal but not when motor nerves are cut. (c) *Midbrain*—extensor stiffness in the legs. Convulsions alternate with paralysis; at first tonic spasm, then clonic twitchings. Death may be due to asphyxia—caused by spasm of the respiratory muscles or due to exhaustion. The centre for the convulsion lies in the midbrain. (d) *Muscles*—Creatine phosphate content less and resynthesis slower.

II. **Blood.** (a) Fall of serum calcium from 10 to 6 mg/100 ml or less. (b) Rise of inorganic phosphate from 5 mg per 100 ml to 6 or 8 mg/100 ml or more.
(c) Increase of guanidine bodies. (d) Defective blood clotting.

III. **Urine**—calcium and phosphorus excretion reduced.
IV. **Respiration**—rapid and noisy.
V. **Heart rate**—increased.
VI. **Salivation**—increased.
VII. **Temperature**—raised.

Tetany is more severe (a) in young than in old, (b) in carnivora than in herbivora, (c) in pregnancy, lactation and with high protein diet.

Tetany in man—Due to removal of the parathyroid during thyroidectomy. Features same as in animal but less intense. Rapid respiration and high temperature, not seen. The features of hypoparathyroidism—tetany develops. Tetany may be manifest or latent.

Features

I. **General convulsion** (children).

II. **Carpopedal spasm (Trousseau's sign**—Fig 4.63). Slight pressure on the limbs may elicit this: Elbow and wrist flexed; fingers flexed

at the metacarpophalangeal joints but extended at the interphalangeal joints. Thumb in the palm and finger-tips drawn together (Accoucheur's hand), feet extended and plantar flexed.

III. **Laryngismus stridulus.** Spasm of the glottis with inspiratory stridor.

IV. **Chvostek's sign.** Tapping the facial nerve near the styloid process causes facial spasm.

V. **Erb's sign.** Increased excitability of motor nerves to galvanic current.



Fig. 4.63. Trousseau's sign (diagrammatic).

Causes of tetany. Lowered ionic calcium in blood and tissue fluid is the immediate cause. Fall of Ca increases the proportion of neuro-excitatory factors, viz., Na, K, etc., and causes tetany. Total calcium has no relation. Lowering of total calcium due to diminished plasma protein does not cause tetany. This shows that calcium which remains bound with protein is not physiologically active.

Other forms of clinical tetany

Apart from parathyroid deficiency, hypocalcaemic tetany is also found in the following conditions:

I. **Tetany from alkalaemia.** Alkalaemia may be produced from (a) excess intake of alkali, (b) profuse vomiting, (c) increased breathing, etc. It causes tetany without reducing total calcium. Two explanations: (1) Alkalaemia alters ionic balance and decreases the amount of ionic calcium without affecting the total calcium. (2) Alkalaemia increases the proportion of the neuro-excitatory ions, and makes the nerve fibres and centres more excitable.

II. **Tetany in rickets** (*Infantile tetany*). Owing to vitamin D deficiency, serum calcium is lowered due to lack of absorption, hence tetany. In *osteomalacia* (*adult rickets*) the same thing happens.

III. **Tetany in renal failure.** Reabsorption of calcium by renal tubules is diminished and serum calcium level is lowered.

IV. **Tetany due to impaired absorption of calcium** from the intestine as in coeliac disease and *sprue*.

V. **Tetany due to increased alkaline phosphate.** Injection of large amounts of alkaline phosphate lowers serum calcium and produces tetany.

VI. **Magnesium-deficiency tetany.** It has been produced experimentally in dogs, rats and young cattle by giving magnesium deficient diet. Serum phosphorus and calcium are normal but magnesium is lowered. Not known in man.

Disorders of parathyroid secretion

Hyperparathyroidism occurs due to parathyroid tumours or diffuse hyperplasia of the parathyroid glands.

Features. (a) Weakness, loss of muscular tone, renal disorders (calculi, nephrocalcinosis, renal failure), nausea, vomiting, reduced appetite, thirst, mental symptoms, polyuria, etc. *Polyuria* is due to damage of the distal renal tubules inhibiting the reabsorption of water and there is in-

creased excretion of calcium in the urine. Stones, containing calcium salts, are formed in the kidney. (b) Rarefaction of bones (specially *subperiostial resorption*) due to increased mobilisation of calcium and phosphate from the bones, sometimes there is also formation of many bone cysts, a disease known as **osteitis fibrosa cystica.** Increased plasma alkaline phosphatase level. (c) Increased plasma calcium level and reduction of plasma phosphate level. Sometimes only ionic calcium level is raised without increasing total calcium level.

Hypoparathyroidism is uncommon due to idiopathic atrophy (sometimes accompanied by evidence of an autoimmune disorder) of the parathyroids but it occurs most commonly after the surgery of the thyroid gland. Hypoparathyroidism causes tetany. When PTH is secreted in excessive amounts, a target-organ resistance to PTH causes pseudoparathyroidism which shows all the features of hypoparathyroidism. There is a defective indication in the membrane-receptor/adenyl-cyclase system in kidneys and bone, and cyclic AMP formation in response to PTH is deficient.

CALCIUM

Calcium is the principal component of the human skeleton and the *fifth* most common inorganic element of the body. A total calcium content of normal adult humans is 20-25 g per kg of fat-free body tissue.

Sources. Water, specially hard water, eggs, milk (about 1 g per litre), cheese (5-10 g per kg) and green vegetables are the chief sources. Fish and meat are poor sources.

Distribution of calcium in the body. Total quantity is 2% of the body weight, of which bones and teeth contain 99%. The remaining 1% is distributed in different tissues as follows: **muscles**—8 mg per 100 g of fresh muscle; **plasma** or **serum**—9-11 mg per 100 ml; RBC—minute traces; **lymph** and **aqueous humour** (also ascitic and oedema fluid)—slightly less than in plasma; **cerebrospinal fluid**—5·3 mg per 100 ml. Before the fifth month of intra-uterine life, very little calcium is found in the foetus. Because bone formation is only starting. During the last two months of intra-uterine life, when rapid and extensive ossification takes place, over 60% of the total deposition occurs.

Blood calcium. Total quantity (*vide* above)—varies from 9 to 11 mg (average 10 mg) per 100 ml of blood. This level is kept fairly constant. It remains in the following forms: (a) **Diffusible calcium** remains in the (i) ionised form—4·8 to 6·3 mg per 100 ml of blood and (ii) non-ionised form—remains in combination with citrate, bicarbonate and phosphate 0·25 to 0·5 mg per 100 ml of blood. (b) **Non-diffusible calcium** remains in combination with plasma proteins, specially albumin —4 to 5 mg per 100 ml of blood. **Levels of blood calcium** depends on the following: (1) **Amount of soluble calcium ingested.** After ingestion of large doses of soluble calcium salts, the serum calcium rises, being maximum in about 2 hours and coming back to normal level in about further 3 hours. *It is not possible to maintain a constantly high calcium level by giving calcium salts alone.* Calcium salts, given intravenously, quickly disappear from circulation. (2) **Amount of calcium absorbed.** (3) **Parathyroid activity.** Hyperactivity increases blood calcium. (4) **Thyrocalcitonin secretion.** This hormone lowers blood calcium

Hydrogen ion concentration of plasma. A rise of hydrogen ion concentration raises serum calcium whereas alkalaemia, although does not produce a definite decrease in the total serum calcium, yet produces symptoms of hypocalcaemia. Two explanations are advanced: (a) Alkalaemia reduces the amount of active ionised calcium, (b) Alkalaemia excites the nervous system in the same way as low blood calcium. (6) **Concentration of plasma proteins.** Raised concentration of proteins increases blood calcium, because some calcium remains in combination with the proteins. But this is in an inactive form. The variation of the amount of calcium content in different fluids is mainly due to their difference of protein concentration. (7) **Plasma phosphate.** It varies inversely as the plasma calcium. An increase in the phosphate ion causes a corresponding decrease of calcium ion and *vice versa. The product of calcium and inorganic phosphate of blood* (10 mg Ca × 5 mg PO_4) *is kept constant and is about* 50. This inverse relation may be due to increased excretion or deposition in the bones. (8) **Sex hormones.** Women during menopause sometimes suffer from negative calcium (and also phosphorus) balance. Either oestrogens or androgens are effective in correcting the above conditon, a combination of both hormones is most effective.

Calcium content of lymph and cerebrospinal fluid. Lymp, having a lower protein content, contains less calcium than blood. **Cerebrospinal fluid** which has traces of proteins contains still less. It contains only about 5·3 mg per 100 ml, but practically the whole of it is in a diffusible active form. Calcium of cerebrospinal fluid is constant and generally runs parallel to the ionic calcium of plasma.

Absorption. It is always incomplete. The ability to utilise calcium of different foods varies greatly. On a high protein diet, 15% of dietary calcium is absorbed; on low protein diet only 5%. The following facts are important about calcium absorption.

(a) **Site of absorption.** Absorption takes place mainly from the food (specially diary products) in the upper part of small intestine (maximal in the duodenum) under the influence of vitamin D (present in food or produced by action of ulta-violet light on 7-dehydrocholesterol in the skin); excess intestinal lipids reduce calcium absorption.

(b) **Form of absorption.** Soluble inorganic forms are much better absorbed. It is probable that the organic calcium of food is converted into inorganic form, before it can be absorbed. Insoluble calcium compounds are never absorbed. Thus presence of phytic acid in cereals produces formation of calcium phytate which is insoluble. Oxalates may have a similar effect. Calcium phosphates are not absorbed.

Recently, a specific calcium-binding protein has been observed in intestinal mucosa whereby vitamin D leads to enhancement of intestinal transport of calcium. In addition, isolation and biological characterisation of active metabolite of vitamin D, 25-hydroxycholecalciferol (25-HCC), has provided additional insights in the intestinal mucosa. DeLuca and his associates have shown that administration of 25-HCC leads to stimulation of transport *in vitro* within 3-4 hours. Wassermann has also reviewed that the calcium-binding protein is important in mediating the action of vitamin D in the intestine. Recently DeLuca and his associates have reported presence of a calcium-dependent ATP-ase in intestinal brush borders; synthesis of this protein is also under the control of vitamin D.

Inhibitor of protein synthesis such as actinomycin D blocks the stimulatory effects of vitamin D on transport of calcium in the intestine; blocks the restoration of serum calcium to normal concentration in animals made deficient in vitamin D and then repleted with the vitamin; and blocks the facilitating effect of reinstitution of vitamin D administration to vitamin D-depleted animals with parathyroid hormone-induced hypercalcaemia.

(c) **Source of calcium.** Calcium from different sources is differently absorbed. Milk calcium is highly absorbed but vegetable calcium much less. Certain vegetables, such as spinach, owing to its high oxalic acid content, converts soluble food calcium into insoluble calcium oxalates. Hence, these oxalic acid containing vegetables reduces calcium absorption.

(d) **Reaction of the intestine.** A high acidity favour calcium absorption, because the calcium salts become soluble in acid medium. Alkalinity produces insoluble calcium salts and reduces absorption. Sugars, specially lactose, which increase acidity due to its conversion into lactic acid, favour absorption. An excess of unabsorbed fatty acids if present in the intestine, converts soluble calcium into insoluble calcium soaps and reduces absorption. Phytic acid which is commonly present in cereals, precipitates calcium in the bowel as the insoluble *calcium phytate,* and thus reduces its absorption.

(e) **Phosphorus content of diet.** High phosphorus content of food forms insoluble calcium phosphate and reduces calcium absorption.

(f) **Bile salts** help calcium absorption by their hydrotropic action on calcium soaps.

(g) **Vitamin D.** It is essential for calcium absorption in man but it lowers the renal reabsorption of calcium.

(h) **Protein content of diet.** Increased protein intake enhances the absorption of calcium. The amino acids, lysine and arginine, are most effective.

Control of calcium metabolism (1) **Parathyroid** exerts the strongest influence on calcium metabolism. It mobilises Ca from bone and this effect is mediated by cyclic AMP. Adenyl cyclase from bone is stimulated by this hormone. Lysosomal enzymes have been claimed to be involved for the dissolution of bone matrix and solubilisation of bone mineral. (2) **Prostaglandin E_1** (PGE_1) which increases the level of cyclic AMP in foetal rat bone, stimulates the resorption of bone in tissue culture. Prostaglandins E_1 and E_2 are a class of C_{20} fatty acids containing a cyclopentane ring. (3) **Thyroid gland** synthesises the hormone *thyrocalcitonin,* which lowers blood calcium level, and antagonises the effect of PTH (parathyroid hormone). But thyroid hormones—thyroxine (T_4) and tri-iodothyronine (T_3) if given in parathyroidectomised animals then stimulate bone resorption causing hypercalcaemia. (4) **Adrenal cortex** through phosphorylation helps indirectly calcium metabolism. (5) **Growth hormone**—GH has got also effect on calcium metabolism. Its effects on renal excretion of phosphate and calcium are opposite to those of the parathyroid hormone. Major effects of GH upon skeleton are to increase the rate of bone formation that leads to an increased rate of bone remodelling. (6) **Pancreatic hormones**—Insulin has been claimed to act on bone metabolism in conjunction with pituitary GH. Very little work has been done

on the relationship of glucagon and Ca^{++} metabolism. Hypocalcaemic effect of glucagon has been suggested. (7) **Vitamin D** also controls calcium metabolism. It is essential for the hypercalcaemic action of parathyroid hormone but not for the hypocalcaemic action of thyrocalcitonin. Dietary vitamin D is converted into an active 25-hydroxylated form of vitamin D, which influences the transcription of mRNA in the intestine and bone, leading to formation of calcium transport systems in these tissues. This function of vitamin D operates in conjugation with parathormone and thyrocalcitonin (Fig. 4.64).

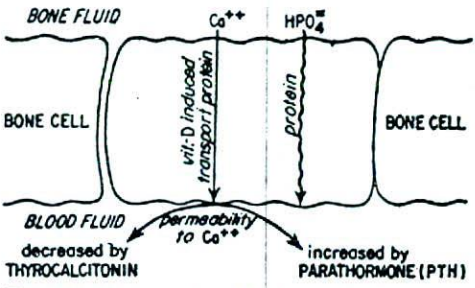


Fig. 4.64. Diagram shows the interaction between parathormone, thyrocalcitonin and vitamin D in regulation of bone resorption. Vitamin D directs the protein synthesis concerned with calcium transport to the extracellular fluid, and PTH increases and TCT decreases the permeability of the cells to calcium.

Excretion. (a) Through the faeces. (b) Through urine.

With an ordinary diet, the calcium content of human faeces is about 200 mg daily. This is believed to be fully exogenous (i.e., unabsorbed calcium). After intravenous administration of the radioactive isotope ^{44}Ca it is found that about 10% of the amount injected is excreted in the faeces within 8 to 10 days. About 150-200 mg (25% of the total excretion) are excreted through the urine.

Calcium balance. In the adult, the calcium intake and loss are same. This is called **calcium balance.** When calcium is retained in the body (i.e., more intake than loss) the balance is called **positive.** When the loss is more than the intake, the balance is called **negative.** The positive balance is found during growth, pregnancy, acromegaly, or recovery after calcium starvation. In a growing child the retention is according to the size of the child and not according to its age (0·01 g per kg per day). A negative balance is found during hyperactive condition of thyroid and parathyroid, in calcium deficiency and in certain other diseases, such as rickets, sprue, osteomalacia, coeliac disease, etc.

Functions. Calcium is of enormous importance for the body. The following is a brief summary: (1) **Essential for the fomation of bones and teeth.** (2) **Essential for the coagulation of blood.** (3) **Essential for the coagulation of milk.** (4) **Essential for the contraction of the heart muscle** and the rhythmicity of heart. (5) **Maintains normal neuromuscular excitability.** In this respect it is the reverse of sodium and potassium. These are neuro-excitatory whereas calcium is neuro-sedative. Consequently, in hypocalcaemia, the action of sodium and potassium becomes unopposed, nervous system becomes highly excitable so that convulsion, tetany, etc., occur. (6) **Controls the permeability of the capillary endothelieum.** High blood calcium diminishes and low blood calcium increases the permeability. (7) **Activation of enzymes.** Certain enzymes like succinic dehydrogenase, ATP-ase, lipase, etc., are activated by calcium.

Effects of deficiency. Rickets, tetany, etc. A deficiency of calcium in the diet on a defective absorption of calcium due to low vitamin D content results in faulty calcification of bones. This condition is known as rickets and is usually found in children maintained on poor diets specially, lacking in calcium and phosphorus content. **Renal rickets** is caused by a tubular defect in the kidney in which normal reabsorption of calcium from the tubular filtrate is inhibited, and large amounts of calcium are excreted in the urine. As the defect lies in the tubules, vitamin D administration does not correct this phenomenon.

Daily requirement. 1·0-1·5 g. 0·63 g is minimum for a subject of 70 kg of body weight. In childhood (from 3-14 years) and pregnancy the daily requirement is about 1·5 g. During lactation the daily requirement is more than 3 g.

PHOSPHORUS

Sources. (1) **Inorganic**—As salts in milk, muscles, vegetables. (2) **Organic**—(a) Nucleoproteins—in all cell nuclei. (b) Phosphoproteins—milk, eggs, etc. (c) Phospholipids—brain, liver, yolk of eggs, etc. (d) Phosphagen and adenosine triphosphate (ATP)—in all muscles.

Distribution. Total quantity is about 1·1% of the total body weight. Occurs in the form of orthophosphoric and pyrophosphoric acids. Present in all tissues, both in organic and inorganic forms. In the tissues the concentration is higher than in blood. Bones, brain, liver, pancreas, etc., are very rich in phosphorus.

Blood phosphorus. (a) **Inorganic phosphate**—distributed equally between plasma and corpuscles—2·5 to 4·5 mg (average 3 mg) per 100 ml. Higher values are found in infants. (b) **Organic phosphate**—large quantities in the form of **phospholipids** (lecithin, etc.) and also as **ester phosphorus** (glycerophosphates, etc.) are found in the red cells. **Calcium/Inorganic phosphorus ratio** in blood is on the average 2:1. *Anything that raises calcium, lowers phosphate and vice versa. Their products remain constant.* Inorganic phosphorus is diminished in rickets, hyperparathyroidism and after insulin injections. Lipid phosphorus content of blood varies in various conditions. *In most cases it is found that lipid phosphorus and cholesterol run parallel.* Blood contains phosphatases which hydrolyse ester phosphates into inorganic phosphates chiefly in the red cells.

Absorption. Usually incomplete. Absorbed from the upper part of the small intestine (specially in the jejunum), chiefly in the form of inorganic phosphate. Organic phosphates are believed to be converted into inorganic forms before absorption. The **amount absorbed** depends upon the following: (a) **Amount of intake.** (b) **Reaction of the intestinal contents.** Acidity favours absorption. (c) **Calcium/Phosphorus ratio of diet.** High calcium content diminishes phosphorus absorption. In the diet the optimum ratio should be 2 P: 1 Ca. (d) **Bile salts** favour phosphorus absorption by their hydrotropic action. (e) **Vitamin D** does not appear to stimulate phosphate transport directly. However dietary level of vitamin D intake does affect phosphate transport indirectly since phosphate appears to be transported in association with calcium. Parathyroid hormone and dihydrotachysterol cause a direct stimulation of phosphate transport in the jejunum. (f) **Fatty acids in diet.** The effect of fatty acids on phosphorus absorption is opposite to that upon calcium. Fatty acids

combine with Ca and liberate phosphorus for absorption. Hence, excess fatty acids hinder calcium absorption but favour phosphorus absorption. But under such condition the increased absorption is without any value. Because phosphorus cannot be retained without calcium and since calcium absorption is low the extra amount of phosphorus is quickly excreted. It is to be noted that the effect of cod-liver oil is due to both vitamin D and the fatty acids. (g) **Excess cereals in diet.** The cereals are rich in phytic acid (inositol hexaphosphate) or phytin (Ca-Mg-phytate), which are insoluble, indigestible and hence are not absorbed. In this way large amounts of Ca and P are lost. In some cereals, such as oat-meal, the phytic acid content is so much that not only its own Ca and P are lost but also the phytic acid combines with these elements of other constituents of diet and prevents their absorption. Care should be taken in giving excess cereals to a subject.

Control of phosphorus metabolism. (1) **Endocrines.** Probably all the endocrine glands that are mentioned under calcium take part also in the phosphorus metabolism. (2) **Kidneys** regulate the phosphate buffer system of blood. (3) **Vitamin D** also takes part.

Excretion of phosphorus. The total daily output is about 3·5 g of phosphoric acid. Two-thirds of it (2·5 g) are passed in the urine and rest in the faeces. The loss in the faeces is due to non-absorption, and not due to actual secretion through the large gut. Anything that hinders phosphate absorption reduces urinary phosphate but increases faecal phosphate. It is believed that inorganic phosphate of urine is derived from two sources: (a) directly from plasma, (b) formed by the kidneys by hydrolysing the organic phosphates of blood. The phosphate content of urine is mainly responsible for its reaction. Phosphate excretion is increased mainly by PTH and oestrogens; and reduced by GH, glucocorticoids and vitamin D.

Functions. (1) **Essential constituent of all cells,** for instance, nucleoproteins in the nuclei and phospholipids in the element constant of the cells. In these forms they play an essential role in the function of the cells. (2) **Essential for the formation of bones and teeth.** (3) Phosphoric acid compounds, such as ATP, phosphagen, hexose phosphate, etc., **take an essential role in the chemical changes underlying muscular contraction.** (4) **Phosphorylation of lipids** is important for —(a) lipid absorption, (b) lipid transport through blood, and (c) lipid metabolism, (5) **Phosphorylation of sugars** is essential for—(a) absorption of sugar from intestine, (b) reabsorption of glucose from kidneys, and (c) the metabolism of glucose and glycogen. (6) **Takes part in blood clotting.** Kephalin (cephalin) is a phospholipid which initiates blood clotting. (7) **Regulates H-ion concentration** of cells, blood and urine. In the blood the acid and alkaline phosphates act as buffers. In the urine the relative proportions of these salts are responsible for the reaction of the urine. Inside the cells there are also phosphate buffer systems which regulate intracellular reaction. (8) **Helps in the enzymatic functions of vitamin B complex.** Thiamine, riboflavin, nicotinic acid, etc., act as coenzymes in combination with phosphoric acid. (a) **Other metabolic actions.** Phosphate ion plays a critical role in maintaining an adequate ion solubility product and thereby maintaining sufficient metastability of extracellular fluid to sustain an adequate mineralisation of bone. An adequate level of blood phosphate may be essential to provide inorganic

phosphate for enzymatic coupling of phosphate to elements in the organic matrix of bone, perhaps as an essential step in the initiation of calcification. Phosphate ion may also play a role in inhibition of bone resorption. The intracellular concentrations of blood phosphate, particularly in the kidneys, may influence renal transport of calcium. Increased levels of inorganic phosphate in the diet have a direct effect on renal clearance of calcium, as calcium excretion is decreased by a phosphate load. However, unlike calcium ion, phosphate concentration in the plasma has no direct effect on controlling secretion of parathormone and thyrocalcitonin, the peptide hormones concerned with regulating the rate of bone mineral turnover.

Effects of deficiency. Rickets and **osteomalacia. Phosphate diabetes** is one of the most common forms of osteomalacia. **Hypophosphatasia** is a recessive disorder with deficiency of alkaline phosphatase in tissues and serum, which interferes with the calcification of osteoid tissue. **Renal tubular dysfunction** is usually a low serum phosphate due to reduced renal tubular reabsorption of phosphate and generally a genetically determined primary defect of the renal tubule.

Daily requirement. About 1 g is the minimum need for a man of 70 kg. Pregnant and lactating women and growing children require more. On the average, a child requires 1 g, pregnant female 1·5 g. *Usually enough is present in a normal adequate diet.*

Relationship between plasma ionised calcium and phosphate. When one is increased the other tends to fall, which accounts for the importance of lesions of phosphate metabolism in disorders of calcium utilisation. Bone mineralisation does not take place satisfactorily unless the product of calcium and phosphorus is fairly high (normally over 25, but above 60 there is a possibility of metastatic calcification).

ENDOCRINE PANCREAS

The human pancreas is a large *retroperitoneal gland* and is both exocrine and endocrine in its secretory functions. The **exocrine cells** of the pancreas responsible for the enzyme-rich pancreatic secretion are the *pancreatic acinar cells*. The **endocrine cells** of the pancreas are found in scattered groups throughout the organ and are commonly designated as the **islets of Langerhans,** or simply as *pancreatic islets or small islands*. The endocrine tissue of the pancreas makes up about 1-2% of the total gland. In 1869, Langerhans demonstrated that the islets were unconnected with the duct system of the pancreas.

Histology. Islets of Langerhans are groups of epithelioid cells, situated between the pancreatic alveoli or acini pervaded by large tortuous blood vessels and having no duct. Islets are more numerous in the tail of the pancreas than in the head and body. The number of islets in the adult human beings varies from about 200,000 to 2,000,000 and in the new-born infant from 100,000 to 500,000. Adult human pancreas has about 1·7 units of insulin per gram. Islet tissue contains about 150 units/gram of it. The islet cells (Fig. 4.65) can be stained intravitally with *neutral red.*

Lacy and Greider (1972) have described the presence of three distinct islet cell types in a large number of mammals. These are (i) α-cells or A_2-cells, secreting glucagon; (ii) β-cells, secreting insulin; and (iii) δ-cells

or A_1-cells, secreting gastrin. Other islet cell types have been identified in a few species, but their specific functional importance has not been observed.

1. α- or **A_2-cells** (10-15%)—larger, granular and *oxyphil*. Opaque and spherical granules are relatively uniform in size, are distributed throughout the cytoplasm, enclosed in a smooth membranous sac, stained *red* with Mallory-Azan, insoluble in alcohol. A_2-cells contain high electron density of secretory granules. The cytoplasm contains a well-developed Golgi complex, a moderate amount of granular endoplasmic reticulum and occasional free ribosomes. A few small filamentous mitochondria are encountered in the cytoplasmic matrix. The nucleus of the A_2-cell tends to be deeply indented or lobulated. **Glucagon** is secreted from these cells which have got hyperglycaemic effect. Caramia *et al.* (1965) on the basis of the morphology of the cells' secretory granules have divided the A_2-cells into Aa, Ab, Ac in guinea-pigs. Hoyos-Guevara (1969) also described similar types of cells in the mouse.

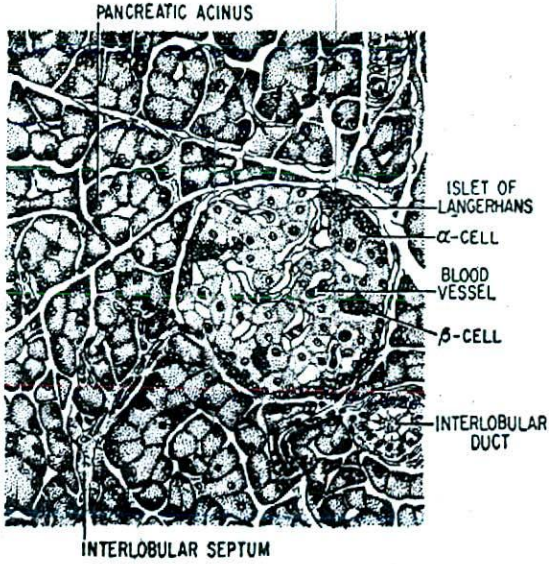


Fig. 4.65. A section of the pancreas showing a pancreatic islet cell.

2. β- or **B-cells** (30-40%)—also granular but *basophilic*. They are stained *orange* with Mallory-Azan. Granules are generally similar in size but less opaque than those of A_2-cells. They are soluble in alcohol. *The β-cells are smaller in size, remain in the periphery and are believed to secrete* **insulin.** The granules in them represent stored insulin which produces hypoglycaemia. Electron microscopy shows that each granule within the β-cells is enclosed in a sac. The sac has got a double membrane. A large clear space is present between the β-cell secretory granules and its limiting membrane. The sac is derived from the reticulum of the cell. Under normal conditions the sac will coalesce with the cell membrane. The β-cell has a more prominent Golgi complex than that of an α-cell, contains more granular endoplasmic reticulum and free ribosomes. The granules in the β-cells tend to be located between the round or ovoid nucleus and the plasma membrane bordering the capillary. This then ruptures and the granules containing insulin are extruded. β-cells can be classified into β_1 and β_2. In the yellow-tail fish, some β_1-cell granules are hexagonal in shape, but other granules are cuboidal, needle-like or amorphous; and β_2-cell granules are less

dense amorphous resembling secretory granules of the so-called δ-cell of islets of other species.

In the higher vertebrates, the α : β ratio of the islet cell population is generally 1 : 4, whereas in the lower vertebrates (e.g., reptiles, fishes) it is almost 1:1 except the snake and yellow-tail fish.

3. Another type of cells—the δ- or **A_1-cells** (5%) are stained *blue* with Mallory-Azan stain. In the rat pancreas they contain numerous, small, membrane-enclosed granules of moderately low density. These cells were first found in human pancreas. Recently it has been described that δ-cells contain the hormone **gastrin.** The exact function of these cells is not known except that they are a source of gastrin. In the **Zollinger-Ellison syndrome** the non-β-cells (A_1-cells) appear to secrete gastrin. The syndrome is due to a tumour or hyperplasia arising in the islets, which certainly does not secrete insulin and glucagon, but does produce enormous amount of gastrin.

4. **Other cell types**

(a) **X- or F-cells** (50%). Bencosme and Leipa (1955) first described the presence of an argyrophil cell type other than A_1- (δ-) cells and β-cells in the uncinate process of dog through light microscopic studies. They called them the X-cells. The dog uncinate process does not contain A_2-cells. Munger *et al.* (1965) through electron microscopic studies suggested the term F-cells. Functional importance of this cell has not been established.

(b) **E-cells.** Thomas (1937) and afterwards Munger *et al.* (1965) described the presence of this cell type with very large granules in islets of opossum.

(c) **C-cells.** Until recently, the presence of C-cell in guinea-pig's pancreas was identified as a distinct cell type and considered as functional phase of α- and β-cells. But recent electron microscopic studies fail to show these cells. Fujita (1968) has provided sufficient evidences that C-cells are nothing but δ-cells or A_1-cells. But Watari, Tsukagoshi and Honma (1970) have obseraed in the yellow-tail fish that C_1-cells contain a large number of vacuoles whose diameter is almost the same of β_1-cell granules. As some vacuoles of C_1-cells contain filamentous structures similar to β_1-cell granules, so C_1-cells may be degranulated β_1-cells and transitional cell type between β_1. It is also suggested that β_1, β_2 and C_1 might represent only different functional stages of one cell type.

Development. The islets of Langerhans develop as buds from the ducts and remain as solid clumps of cells. During development in most cases, the connections with the duct disappear and the islets become completely isolated. But in a few cases the connections persist as solid chords.

Nerve supply. Richly supplied with branches of the right vagus (secretomotor) and stimulation of the right vagus causing incressed secretion of insulin.

Insulin

Insulin is *hypoglycaemic antidiabetic factor* and the protein hormone which regulates the blood glucose. It increases the deposition of glucose in the liver and muscles as glycogen. It also increases the oxidation of glucose to CO_2 in the tissues and depresses gluconeogenesis, i.e., formation of glucose from sources other than carbohydrates.

Evidences of internal secretion by the β-cells

1. Extirpation of pancreas in animals causes hyperglycaemia, glycosuria and ketosis, resembling diabetes mellitus in man. If untreated, the condition becomes fatal in a few days.

2. Administrations of **alloxan,** a pyrimidine derivative, **diazoxide,** a sulphonamide derivative (Fig. 4.66) and **streptozoticin,** an N-nitroso derivative of glucosamine, cause damage to the β-cells and produce a condition similar to diabetes mellitus.

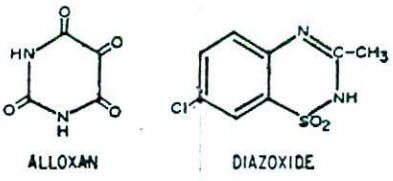


Fig. 4.66. Structural formulae of alloxan and diozoxide.

3. Administrations of pilocarpine intramuscularly and of DHA (dehydroascorbic acid) intravenously in dogs cause majority of β-cell granules turned to amorphous or empty vacuoles and the two chemicals are known to stimulate the parasympathetic nerves which accelerate the function of the pancreatic islets.

4. Ligation of the pancreatic duct causes degeneration of the alveoli of the pancreas but leaves the islet tissues intact. No metabolic disturbance is seen in this case. If then, this atrophied pancreas be removed, diabetes mellitus follows at once. *This shows that the islets are responsible for certain endocrine secretion.*

5. An active principle (insulin) can be extracted from the islet tissues. When it is administered (or when pancreas is grafted) to the experimental diabetic, the condition is cured. But when administered to a normal animal, it causes hypoglycaemia.

6. In both experimental and clinical diabetes mellitus, the β-cells are found to be degenerated.

From these facts it has been concluded that the β-cells of the islets produce an antidiabetic hormone, called **insulin.**

Active principle. *Insulin* is the active principle of the β-cells of the islets.

Unit of insulin

The third International Reference Standard of insulin, a preparation of zinc insulin crystals, contains 24·5 units/mg. 1 unit is equivalent to 0·0408 mg. One unit is defined as one-third of the amount of insulin that will lower the blood sugar level of a 2-kg fasting rabbit to the convulsion value in 3 hours. Crystalline insulin from different sources has the same potency.

Assays

Various insulin assays have been established. Among these assays, the endpoints of a few are as follows:

1. Blood sugar-lowering potency in a fasted rabbit.
2. Induction of hypoglycaemic convulsions in fasted mice.
3. Enhancement of glucose uptake, $^{14}CO_2$ production from ^{14}C glucose, glycogen deposition by the isolated rat diaphragm. The method measures all of the activity attributable to insulin, referred to as **insulin-like activity** (ILA) where apparent insulin content of plasma progressively increases as the plasma is more and more diluted.

4. Enhanced potassium movement from the medium in the isolated diaphragm.

5. Hypoglycaemic action in the adrenal demedullated, depancreatised (or alloxanised) and hypophysectomised subject.

6. Radioimmuno-assay method is capable of detecting microgram amounts.

7. Ability of insulin to suppress lipolysis in adipose tissue.

Chemistry. Various attempts were made to extract the principle from the pancreas, and the first potent extracts were obtained by Banting and Best in 1922. Crystalline insulin was obtained by Abel *et al.* in 1926. It consists of two open polypeptide chains (A and B). There are 21 amino acids in the A-chain and 30 amino acids in the B-chain. The two chains are interlinked by disulphide bridges. There is, in addition, a disulphide bridge between the 6th and 11th amino acid residues of the A-chain. The complete unit contains 51 amino acids (Fig. 4.67). Breaking the

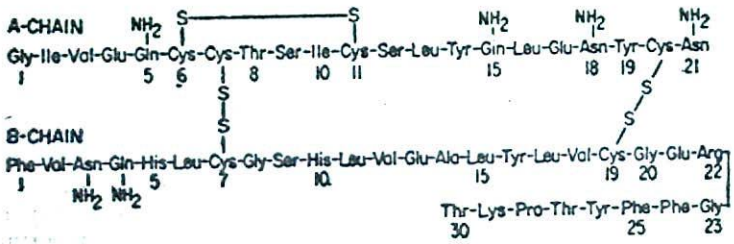


Fig. 4.67. Amino-acid sequence of human insulin.

disulphide bonds inactivate insulin. When insulin is in solution, its molecular weight is affected by its concentration, pH, protein concentration, etc. The minimal molecular weight is about 5800, iso-electric point 5·35 and is assumed to be the molecular weight of the monomar. It is rich in cysteine, leucine and glutamic acid. Tryptophan, methionine and hydroxyproline are absent. Soluble in dilute alcohol, stable in acid but not in alkaline solution, destroyed by proteolytic enzymes, hence, inactive by mouth. Isolated in pure crystalline form, as salts of Zn (0·5%), cobalt, cadmium or nickel. The fact that normal pancreatic tissue is rich in Zn, probably indicates that insulin remains stored in it as a Zn salt. The insulin unit is composed of 6 insulin molecules and 2 zinc atoms (Hodgkin).

Recently from the pancreas, a precursor of insulin, **proinsulin** has been isolated. This compound begins with the normal B-chain sequence but contains a linking polypeptide of 33 amino acids, which connects to the amino terminals of A-chain sequence (Fig. 4.69). The molecule weighs 50% more than insulin. It is almost devoid of biological activity, but can cross-react with antisera prepared against insulin. The conversion of proinsulin to insulin occurs in the granule package. Since proinsulin usually appears in plasma, some part of IRI (immune-reacting insulin) is not true insulin.

Synthesis, storage and release of insulin. Biosynthesis of insulin in β-cells of the islets of Langerhans is presumably similar to protein synthesis in other cells. At first, raw material such as amino acid must be

ingested into the islet cells from blood penetrating through some barriers, i.e., endothelial cells of blood capillary, two basement membranes and plasma membrane of the islet cell (Fig. 4.68).

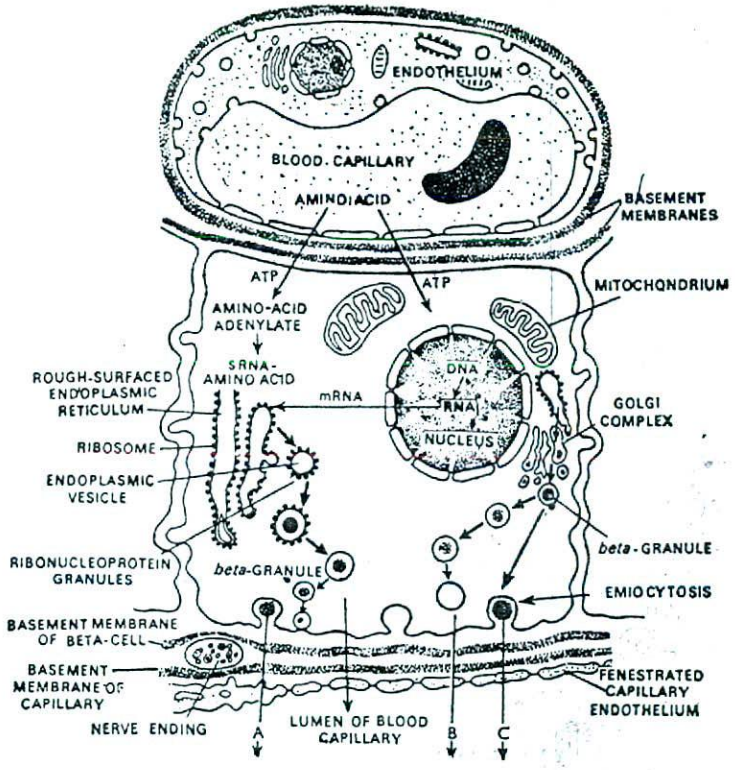


Fig. 4.68. Diagrammatic representation of course of granular synthesis, storage and extrusion in the pancreatic β-cell. A and C denote eruptocrine mechanism, whereas B donates diacrine process (read mitochondrion for mitochondrium)

Insulin is synthesised by the ribosomes of the granular endoplasmic reticulum (GER) under the control of triplet nucleotide sequences of mRNA. It is known that the amino acids participating in the synthesis of insulin are apparently activated by an enzyme system requiring ATP and go with the soluble RNA constituents, where through the influence of mRNA and enzyme systems, they are arranged in proper amino-acid sequences. The proteinous secretory substance is condensed into a visible core within the cisternae of granular ER and studded ribosomes are released from the outer surface of the cisternae with the formation of endoplasmic vesicle (EV) and surrounded by ribonucleprotein granules (RNG). Eventually beta-granules (BG) are then formed from EV in the region of Golgi bodies and RNG disappears. BG becomes dense and remains enclosed within the smooth membranous sacs. BG is the storage form of insulin.

The insulin with Zn may be easily crystallised *in situ* and may make a visible crystalline granule in the cytoplasm of β-cell granules for the storage type insulin. When the appropriate stimuli for insulin secretion are applied, the storage type insulin may be extruded in the blood flow after dissolving in free type insulin and losing its Zn atoms (Hodgkin, 1969). Histidine, cysteine, citric acid, oxalo-acetic acid, etc., may participate in separating Zn atoms from insulin (Maske, 1960). During secretion of insulin, the β-granules enclosed in smooth membranous sacs are released from the Golgi complex into the cytoplasm and become attached to the microtubular-microfilamentous system (Fig. 4.75). Glucose metabolism initiates calcium entry and this calcium ion then triggers the change in physical conformation of the microtubules resulting migration of β-gran-

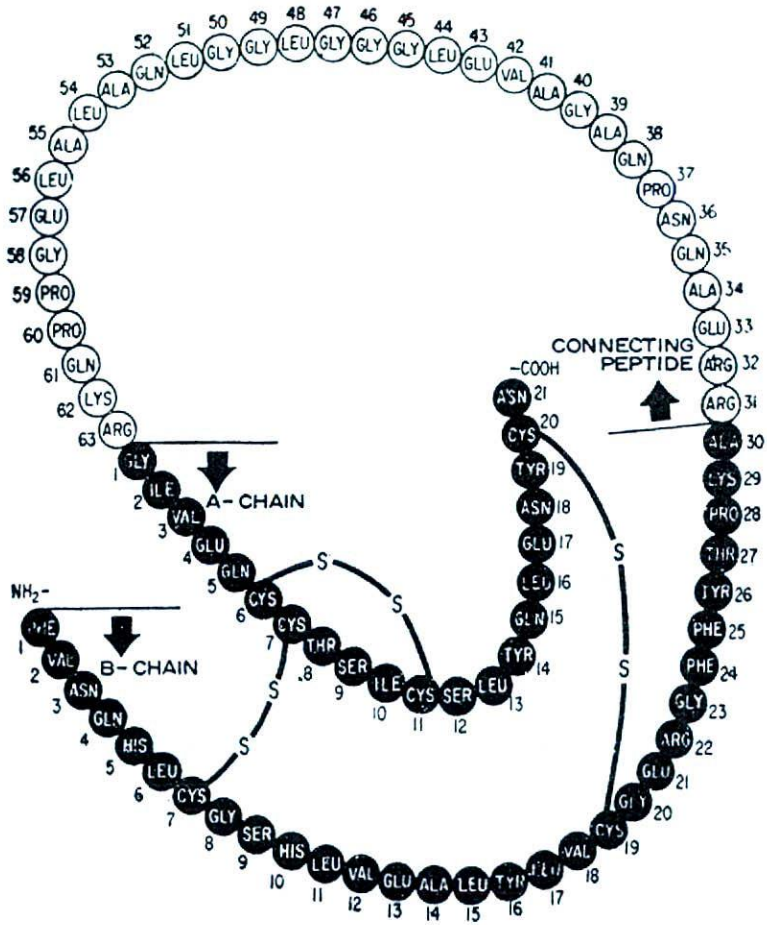


Fig. 4.69. Amino-acid sequence of porcine proinsulin (after Chance and Others).

ules along the plasma membrance of the β-cells and wall of the sac of the β-granules fuse with plasma membrane of the β-cells and rupture, liberating the granules within the extracellular space. This eruptocrine process of ejection is known as **emiocytosis.** With the disappearance of granules by emiocytosis, cytoplasmic projections remain on the surface of the plasma membrane and is called microvilli. Villi actually are the remnants of the membranous sacs that encased the granules.

There are two kinds of insulin secretions: (a) basal secretion may regulate the normal blood sugar level and (b) extrasecretion acts when the level of blood sugar is increased in an emergency. There are also two kinds of insulin extrusion mechanism—(a) eruptocrine and (b) diacrine.

There is controversy whether A- and B-chains of the insulin are synthesised separately. Initially it was suggested that the A- and B-chains were synthesised separately and had been joined through sulphide bridges afterwards. But Steiner and Cunningham (1967) have been able to isolate a precursor of insulin—**proinsulin.** The proinsulin as it is described earlier, is a long-chain polypeptide having 84 amino acids and molecular weight 9082 which is about 50% greater than that of insulin. This long-chain (Fig. 4.69) begins with the normal B-chain sequence at its amino terminals but contains a linking polypeptide of 33 amino acids, that connects the carboxy terminals of the B-chain to the amino terminals of the A-chain amino-acid sequence. Proinsulin on incubation with trypsin liberates the biologically active product, the insulin. Thus for the activation of proinsulin to insulin, it requires proteolysis in presence of enzymes. From this it is suggested that initially proinsulin is synthesised in the ribosomes of the endoplasmic reticulum and conversion of proinsulin to insulin occurs in the vesicle package or endoplasmic vesicle by lysosomal proteolytic enzymes. After conversion the insulin in the vesicle package becomes condensed and forms a typical β-granules enclosed in the membranous sacs.

Big insulin. In addition to insulin and proinsulin, there are at least two other immuno-reactive substances. These may be present in the plasma and interfere with the determination of true plasma insulin concentration by the immune reaction technique. (1) **Big insulin** is a large fraction, molecular weight around 9000, reacts with antiserum against true insulin and can be separated from insulin by gel filtration. It is secreted by the pancreas and is increased in the plasma during starvation. After a glucose meal or intravenous administration of *tolbutamide,* (2) **little insulin** increases during the first half-an-hour and falls thereafter. On the other hand, big insulin rises only from 45 to 90 minutes. The secretory pattern of big insulin is the opposite of that of *true insulin* (little insulin). Therefore it is likely that big insulin is proinsulin or some similar molecule. It is also observed that the ratio of big to little insulin in plasma is not abnormal in thin diabetics, but obesity is associated with increased proportions of big insulin in the plasma. But in myotonic dystrophy and acromegaly, the proportions are normal.

Non-suppressible insulin-like activity (NSILA)

For many years the presence of NSILA in the serum has been indicated. Molecular weight has been indicated to be within 6000-10,000.

It acts like insulin on adipose tissue. It stimulates the glucose uptake and formation of glycogen in diaphragm, and also increases glucose uptake and lipogenesis in adipose tissue. Its action is not suppressed by insulin antibody.

Origin of NSILA is not known. Some are of opinion that it is the reconjugation products of degraded insulin. More studies are required to establish the origin and physiological role of NSILA.

The route by which insulin entering the general circulation. It is not definitely clear by which route the insulin enters the general circulation. It is of general assumption that insulin after being synthesised in the β-cells, secreted into the pancreatic veins from which through hepato-portal system it is carried to the liver (Fig. 4.70). By this route, high concentration of isulin reaches the liver. But when the insulin reaches the general circulation after passing through the liver, its concentration is decreased due to hepatic utilisation and subsequent degradation. So the liver being vital organ for glucose utilisation gets high concentration of insulin but other peripheral tissues get lower concentration.

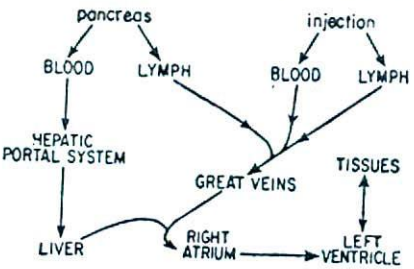


Fig. 4.70. Schematic representation of possible route by which insulin after being liberated from the pancreas or after intramuscular administration, reaches the different target organs.

Another possibility is presumably expected which is through lymphatic system. In this route, insulin ultimately carried to the blood (Fig. 4.70). By this route insulin is evenly distributed all throughout the body, because it is not degraded. But due to dilution effect in general circulation, the liver gets lower concentration of insulin. If insulin is administered from external source through intramuscular injection then it is diluted through whichever route (Fig. 4.70) it follows. So for adequate requirement of insulin by the liver, how much quantity may be injected should be considered.

Preparation of insulin

1. *Soluble insulin.* Watery solution of insulin, crystalline or otherwise. Acts quickly but duration of action is 6 hours only.

2. *Lente insulin.* It is a 3 : 7 mixture of regular and ultralente insulin. **Ultralente insulin** is prepared by controlled crystallisation in presence of high concentration of Zn and acetate due to production of large crystals. Insulin Zn suspension. Slow absorption, hence action prolonged. One injection is effective for 24 hours.

3. *Protamine insulin.* Insulin chemically united with protamine (extracted from the nuclei and containing arginine or lysine). Slow absorption and more than 24-hour duration of action.

4. *Protamine and Zinc insulin.* Protamine insulin *plus* zinc chloride solution. Remains stable for months. The action starts within 6 to 8 hours and lasts for 48-50 hours.

2, 3 and 4 are collectively known as *retard* insulin due to prolonged action.

5. *Regular* insulin *plus* a *retard* insulin. This has the double advantage of both *quick* and *prolonged* action.

6. *Globin zinc insulin.* Combination of insulin and globin obtained from haemoglobin, together with a small proportion of zinc chloride. It acts within 2 to 4 hours, intermediate between regular and protamine zinc insulin (a 18- to 20-hour duration of action).

7. *Hybrid insulins.* Wilson *et al.* (1962) have been able to prepare hybrid insulins by thiol treatment of the mixtures—cod A-chain with ox B-chain, and cod B-chain with ox A-chain. The former resembles *native* cod insulin and the latter resembles ox insulin.

8. *Monocomponent insulin* may be a highly purified insulin with greatly reduced immunogenicity.

Functions of insulin

In condition of insufficient insulin, there are increased gluconeogenesis, glycogenolysis, hyperglycaemia, hyperosmolalaemia, ketogenesis, polyuria and etc. (Fig. 471).

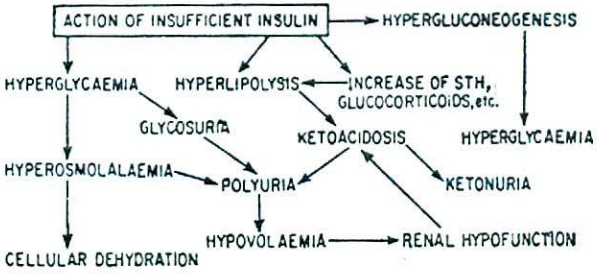


Fig. 4.71. Diagram shows the different metabolic derangement in the liver due to insufficient insulin.

From experimental and clinical studies insulin is found to have the following functions:

I. On Carbohydrate Metabolism

(a) **Oxidation of sugar.** Insulin increases combustion of sugar in the tissues and also helps in the transport of glucose into the cells. Since insulin is effective on the skeletal muscle mass, this is quantitatively an important effect.

Evidences. (a) In advanced diabetes, RQ is about 0·7, showing that mostly lipids are burnt. The arteriovenous difference of blood sugar is very low, showing that tissues are not utilising sugar. When insulin is administered, RQ increases and arteriovenous difference of sugar rises. (b) Isolated living tissues when perfused with sugar solutions, exhibit a diminished power of burning glucose. The rate increases if insulin be added to the perfusing fluid. (c) Administration of glucose labelled with radio-active ^{14}C and studies thereafter have shown that insulin directly increases the rate of glucose oxidation.

(b) **Glycogen formation and storage.** Insulin increases synthesis of glycogen from sugar and lactate both in the liver and muscles. This is called the **directive effect** of insulin. Generally, in animal tissue, glycogen synthesis takes place *via* the uridine diphosphate glucose pathway.

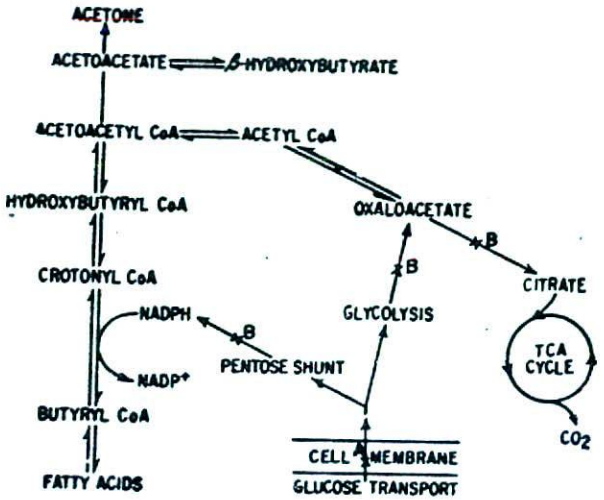


Fig. 4.72. The scheme represents influence of insulin deficiency on carbohydrate metabolism, lipogenesis and ketone body formation showing primary influence at *A* and secondary influence at *B*

Insulin increases the activity of UDGP-transglucosylase enzyme also known as synthetase to synthesise amylase, the acceptor being glycogen. Insulin promotes the uptake of glucose inside the cells and the intracellular phosphorylation of glucose to glucose-6-phosphate (Fig. 4.72). Glucose-6-phosphate itself also appears to be a specific activator of glycogen synthetase.

EVIDENCES. (a) In the diabetic, glycogen content of liver is very low, and its power of forming glycogen is also diminished. (b) Similarly, in the diabetic muscles, the glycogen store and the power of synthesising glycogen (during recovery after exercise) are very low. Hence, fatigue easily sets in. When insulin is administered, the glycogen content and the power of its synthesis increase both in liver and muscles. The rate of glycogen formation is increased after administration of insulin. (c) Insulin causes fall in the inorganic phosphate content of blood plasma. This change is due to quick entry of hexose inside the cells. After administration of insulin, hexose monophosphate of muscle rises. It has been suggested that insulin increases the permeability of cells to glucose.

II. ON PROTEIN METABOLISM

(a) **Prevents gluconeogenesis.** Glucose is normally formed from proteins and lipids in the liver. In diabetes, this process is enhanced. High blood sugar level in diabetes is due to overproduction of glucose (Fig. 4.73). In the starving diabetic, dextrose/nitrogen ratio (D/N ratio) is

fairly constant, about 3·6. This shows that both sugar and nitrogen are coming from the same source—proteins. Administration of glucose labelled with radio-active ^{14}C to normal and diabetic subjects has shown that there is overproduction of glucose, marked diminution of glycogen storage in the liver and muscles, and glucose dissimilation. When insulin

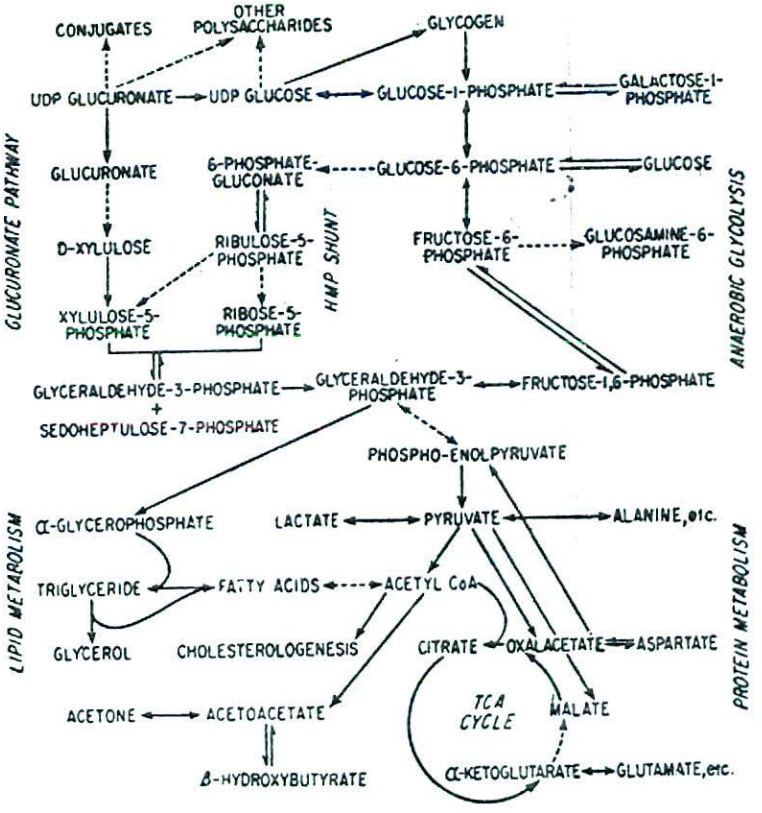


Fig. 4.73. Diagram shows the integrative mechanism of carbohydrate, fat and protein metabolism indicating the pathways of gluconeogenesis, lipolysis, ketogenesis, etc., in diabetes.

is given, both sugar and nitrogen excretion falls, showing that formation of new glucose from proteins has been checked.

(b) Insulin stimulates protein synthesis (proteogenesis) and growth, e.g., nitrogen retention, bone formation, etc. It increases the incorporation of amino acids into peptides. Insulin, somatotrophic hormone and testosterone operate in close co-ordination, since in the absence of either of the three protein synthesis is retarded.

(c) Insulin increases the synthesis of messenger RNA. It is possible that insulin is concerned with the activity of intracellular structures, e.g., ribosomes involved in protein biosynthesis.

(d) During insulin lack, amino acids are catabolised (proteolysis) providing source of energy.

III. ON FAT METABOLISM

(a) **Prevents formation of ketone bodies.** (Anti-ketogenic). In advanced diabetes, excess ketone bodies are formed in the liver, due to incomplete combustion of fatty acids. The RQ is lowered. After administration of insulin, more sugar burns and liver glycogen increases displacing the lipids. Hence, lipid combustion is discouraged and ketosis disappears. After administration of glucose labelled with radio-active ^{14}C it has been found that insulin stimulates the rate of conversion of glucose into fatty acids in the liver. Normally about 1/3rd amount of glucose is converted into lipids and is deposited as such in the body. Insulin helps in the deposition of lipids (lipogenesis) in adipose tissue from glucose. It inhibits lysis and mobilisation of depot lipids (anti-lipolysis). On diabetic subjects due to lack of insulin, lipid deposition is markedly decreased.

(b) Insulin decreases the cholesteraemia and lipaemia. It also prevents accumulation of excess lipid in the liver and breakdown of lipid in adipose tissue.

Mechanism of action of insulin

Insulin promotes the transfer of glucose, amino acids and electrolytes across cell membranes, the target tissues being hepatic, muscle and fat cells. The cell membrane has got a specific transport system. This transport system helps in the transfer of glucose across the cell membranes into the interior. Insulin helps this transport system. Insulin helps glucose uptake in muscle and adipose tissue due to its influence on the specific transport system, cell permeability and phosphorylation process. The transport system in the neurones and erythrocytes does not require the help of insulin. Intestinal absorption or renal reabsorption is not affected by this hormone. The liver also does not require insulin for transport of glucose, as there is no barrier in extracellular and intracellular glucose in the liver (Williams *et al.* 1968). However, it still stimulates uptake of amino acids and their incorporation into protein by hepatic cells.

Insulin activates the intracellular enzymes. It antagonises the phosphorylase activation in response to glucagon and epinephrine and increases the muscle and hepatic glycogen synthetase activity. Insulin inhibits the action of the growth or somatotrophic hormone of anterior pituitary and glucocorticoids of adrenal cortex. The latter hormones enhance the release of fatty acids from depot lipid and inhibit the uptake of glucose in muscle and adipose tissue whereas insulin produces opposite effect.

It is also known that some effects of insulin are the result of a fall in the level of cyclic AMP. Cyclic AMP decreases glycogenesis, proteogenesis and lipogenesis. All these processes are stimulated by insulin. Under normal physiological conditions, insulin apparently exerts a continuous damping effect on the hepatic level of cyclic AMP. It is suggested by Exton and Park (1968) that glucose output by liver is dependent on a balance between agents such as glucagon and the catecholamines, which increase the level of cyclic AMP, and insulin that lowers it. This has been proved

that hepatic cyclic AMP increases in response to the injection of anti-insulin serum (AIS). On the other hand, hepatic cyclic AMP levels increase in fasted and alloxan-diabetic animals.

The mechanism by which insulin lowers the cyclic AMP is not clear but it is suggested that adenyl cyclase activity may be decreased by insulin. Another possibility is the formation of a metabolite, other than 5'-AMP from cyclic AMP due to the action of insulin, which reduces the cyclic AMP level.

Factors influencing insulin secretion

The islets secrete insulin continuously. That the hormone is secreted continuously, is proved by the fact that the removal of pancreas is always followed by hyperglycaemia. In normal individuals about 50 units of insulin are secreted by the pancreas daily. Numerous factors influence the secretion of insulin (*vide* table in pages 4-121 & 4-122). These are discussed below:

1. **Control by anterior pituitary.** A pituitary trophic hormone for the pancreas has not been isolated so far. Yet it exerts an important influence on the secretion of insulin through the effects of growth hormone, prolactin, ACTH and TSH. It is believed that the growth or somatotrophic hormone of anterior pituitary opposes the action of insulin. The growth hormone inhibits the uptake of glucose into muscle and adipose tissue and enhances the release of fatty acids from depot lipids. In addition it increases the release of glucose from the liver. Furthermore, it causes the release of a blood sugar elevating substance into the portal vein of depancreatectomised dogs, which contributes to the initial hyperglycaemia. This hyperglycaemia stimulates β-cells to synthesise more insulin and the level of this hormone in plasma increases. Insulin opposes all these actions of growth hormone. Excess of the growth hormone and glucocorticoids may exhaust the β-cells. This is not due to any direct action on the β-cells but to hyperglycaemia caused by their demand of more insulin.

2. **Control by blood sugar level.** It has been shown that excess sugar of arterial blood entering the pancreas controls the rate of insulin secretion. The rate of insulin release by the pancreatic β-cells is controlled mainly by the glucose concentration of the fluids that bathe the β-cells. High blood sugar stimulates (Fig 7.74), whereas low blood sugar depresses it. This action is directly on the islet tissues independent of nerves. Because, in denervated or in the transplanted pancreas, the same effect is seen. It has been indicated that extracellular Ca^{++} is required for glucose-induced insulin release (Fig. 4.75). Influence of Na^{+} on glucose-induced insulin release is mostly through altered movements of Ca^{++} across the membrane of the β-cells. In complete absence of Na^{+}, glucose can stimulate insulin release but not in the absence of Ca^{++} Therefore Ca^{++} uptake repre-

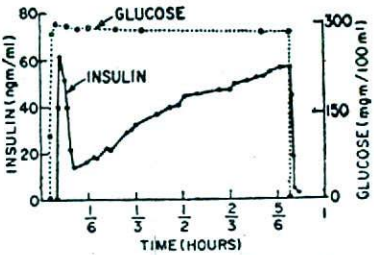


Fig. 4.74. It shows the effect of glucose infusion on insulin release (after Grodsky and others).

sents the trigger for insulin release through emiocytosis of secretory granules by causing the contraction of the microtubular-microfilamentous system.. Blood sugar level also acts on the central nervous system (hypothalamus, pons and medulla) and adjusts insulin secretion by modifying the vagal tone. Thus blood sugar controls insulin secretion in two ways: (a) by directly acting on the pancreatic islets and (b) through the right vagus. In addition, glucose also stimulates insulin syntheis, an effect which requires a longer period.

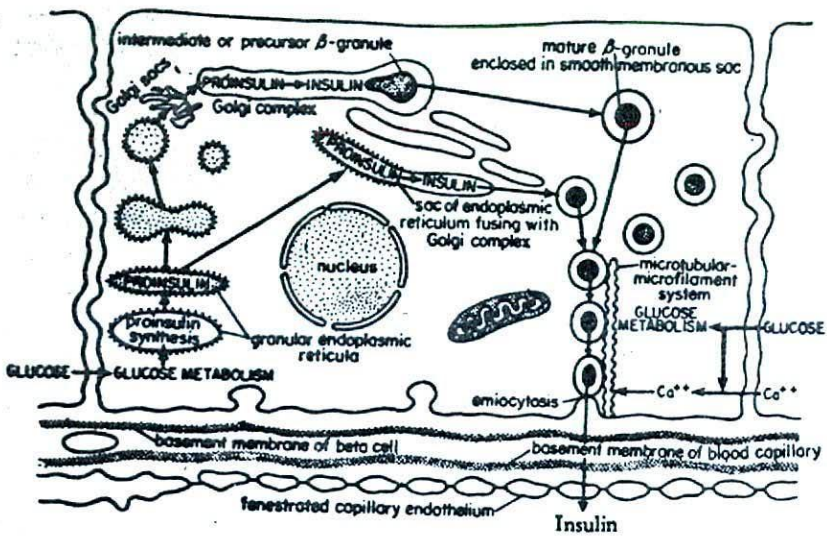


Fig. 4.75. It is based on the hypothetical model of glucose-induced insulin release as proposed by Lacy & Greider (1972); and Malaisse (1972).

3. **Control by adrenal cortex.** Glucocorticoids of adrenal cortex also stimulate the secretion of insulin. Glucocorticoids prevent the utilisation of glucose in the muscle and adipose tissue and stimulate gluconeogenesis in the liver and thus they oppose the action of insulin. But these corticoids and insulin have got some similar functions, i.e., both of them promote glycogenesis.

4. **Glucagon,** secreted by A_1-cells of the islet tissue has got antagonistic effect against insulin. It produces hyperglycaemia, which is responsible again for increased secretion of insulin. Glucagon seems to have a direct stimulatory effect on insulin release through activation of adenyl cyclase. But at time it helps insulin in the uptake of glucose by the peripheral tissue.

5. **Thyroxine** has got hyperglycaemic effect and thus opposes the effects of insulin. It stimulates the secretion of insulin. The thryroid effects are probably related to the effects of thyroid hormone on carbohydrate metabolism—(i) acceleration of glucose oxidation in tissues, and (ii) increase in gluconeogenesis.

6. **Epinephrine, norepinephrine, and role of α- and β-adrenergic receptors** cause glycogenolysis and hyperglycaemia. When the blood sugar level becomes low, epinephrine secretion is stimulated. Coore and Randle (1964) observed that inhibition of glucose-induced insulin release by catecholamines like epinephrine and norepinephrine. Epinephrine is more potent inhibitor than norepinephrine. Recently it is described by Malaisse (1972) that α-adrenergic receptor of β-cells are concerned with epinephrine-induced inhibition insulin release by glucose. Because α-adrenergic blockers are capable of inhibition of such effect. β-adrenergic blockers are not useful. Activation of α-adrenergic receptors by epinephrine lowers the cyclic AMP in the β-cells. Thus epinephrine inhibits glucose-induced insulin release possibly through lowering of the cyclic nucleotides.

Whereas β-adrenergic receptors of the β-cells when activated, cause increased liberation of insulin. This effect is possibly mediated through the activation of adenyl cyclase of β-cells resulting increased concentration of cyclic AMP.

Mono-amine-oxidase inhibitors stimulate insulin release through activation of β-adrenergic receptors.

7. **Oestrogen** also stimulates secretion of insulin.

8. **Gastro-intestinal hormones.** Secretin, pancreozymin and gastrin stimulate insulin secretion. Whether they stimulate through activation of β-cell adenyl cyclase is not clear.

9. **Neural factors.** Stimulation of the right vagus increases insulin secretion. Normally, blood sugar level adjusts the vagal tone and modifies insulin secretion. High blood sugar stimulates and low sugar depresses the vagus. But nerves are not essential for the process (*vide* pages 4-119—4-120). The vegal mechanism represents a 'fine adjustment'.*

10. **Serotonin** inhibits insulin release indirectly by glucose.

11. **Vasopressin** and **oxytocin** do not exert any marked influence on insulin release.

12. **Other factors.** (a) Factors that *stimulate secretion* are fatty acids, ketone bodies, insulin antibodies, calcium, magnesium (low concentration), potassium, adenosine triphosphate, cyclic AMP. (b) Factors that *inhibit secretion* are insulin itself, starvation, hypoxia, glucosamine, phene-ethyl-biguanide, diazoxide and magnesium (high concentration).

Above factors affecting insulin secretion have been presented in the following way:

FACTORS AFFECTING INSULIN SECRETION

Stimulation	*Inhibition*
Glucose	Epinephrine
Fructose	Norepinephrine
Mannose	Insulin
Ribose	Starvation
Amino acids	Hypoxia
Ketones	2-Deoxyglucose

***Role of sino-aortic nerves.** It may be that the sino-aortic nerves reflexly adjust the rate of insulin secretion through the vagus, just as they regulate epinephrine secretion through the sympathetic.

Stimulation	*Inhibition*
GH	Mannoheptulose
ACTH	Diazoxide
Glucosteroids	Vagotomy
T_4	Phene-ethylbiguanide
Glucagon	Glucosamine
Placental lactogen	Hypoxia
Oestrogen	Serotonin
Magnesium (low conc.)	Magnesium (high conc.)
Insulin antibodies	
Potassium	
ATP	
Cyclic 3′,5′-AMP	
Vagal stimulation	
Secretin	
Pancreozymin	
Gastrin	
Sulphonylureas	
Isoprophyl norepinephrine	
Phentolamine	
Mono-amine-oxidase inhibitor	

Cyclic AMP and adenyl cyclase system of the β-cells

The β-cells of prancreatic islets are well equipped with adenyl cyclase—cyclic AMP phosphodiesterase system. Activation of adenyl cyclase system causes excess release of insulin. This effect has also been observed after *in vitro* addition of cyclic AMP. Thus increased level of cyclic AMP is always associated with increased release of insulin.

Mode of action of cyclic AMP in the release of insulin is not clear.

Fate and degradation of insulin

Insulin administered from external source or released internally undergoes the same fate like other proteins. It is destroyed in blood and tissues by degradation process.

Mostly all the tissues can degrade insulin. Liver, kidneys, pancreas, testes and placenta can degrade the insulin greatly. Glutathione-insulin transhydrogenase activity is of great importance in insulin degradation by liver. This enzyme is specially essential for reductive cleavage of disulphide bonds of insulin to form reduced A-chain (ASH) and reduced B-chain (BSH). This is the first step of degradation. There are certain compounds which inhibit degradation of insulin such as α-corticotrophin, growth hormone, oxytocin, vasopressin, prolactin, insulin itself, glucagon, etc.

Insulin hypersensitivity. It is found in Addison's disease and Simmonds' disease. The dose of insulin which produces slight fall of blood sugar level in normal subjects, causes severe fall of blood suger level in these diseases.

GLUCOSE TOLERANCE TEST

Subjects for glucose tolerance test are required not to take carbohydrate more than 300 g a day at least for 3 days and to report on 4th day's morning without having breakfast. Venous blood is drawn for analysis and immediately 100 g of glucose dissolved in 300 ml of water are given orally. Blood samples for analysis are drawn at half-an-, one, two and three hour intervals after taking sugar. Fig. 4.76 shows the typical glucose tolerance curves, i.e., (a) normal, (b) mild diabetes, and (c) severe diabetes. The following facts are seen:

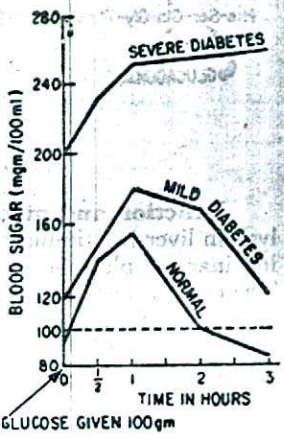


Fig. 4.76. Glucose tolerance curves of diabetics (severe and mild) and normal subjects after oral administration of glucose.

I. IN THE NORMAL SUBJECT

The maximum rise of blood sugar level takes place between half-and-one hour but never beyond the renal threshold (180 mg per 100 ml). In the one-and-half hour sample the sugar level may go below the fasting value due to stimulation of insulin secretion. But the level goes back to normal by the end of 2nd hour. There is no glycosuria.

Role of previous diet. If the diet was poor in carbohydrates, blood sugar level may go up to 200 mg per 100 ml and the rate of fall is very slow. It may not come to normal even after 3 hours (*poor tolerance curve*). But if the diet was rich in carbohydrates—less variation occurs (*good tolerance curve*).

II. IN DIABETES MELLITUS

1. **Lag curve.** Fasting level is normal, between half and one hour the blood sugar shoots sharply above the renal threshold causing glycosuria. But the level comes back to normal by 2 hours. It is due to delayed insulin mobilisation which now requires a stronger stimulus (i.e., higher sugar level) than normal. It is seen in early diabetes.

2. **Diabetic curve.** Fasting blood sugar level is high; after administration of glucose it becomes higher and takes several hours to come down to the fasting level.

Insulin has no effect on the utilisation of fructose. For this reason this sugar is given to persons suffering from diabetes as a source of energy.

Glucagon

Chemistry and source. *Glucagon* (also known as hyperglycaemic-glycogenolytic factor, HGF) is secreted by the α-cells (A_2-cells) of the islets of Langerhans. It is a polypeptide hormone with 29 amino acids having molecular weight of 3,485 (Fig. 4.77). This polypeptide has been completely synthesised. Unger, Samols and others have obtained glucagon from different portions of the gastro-intestinal tract, having similar biologic and immuno-reactive characteristics. This glucagon-like substance (**gut glucagon**) has been extracted from the mucosa of the stomach, duodenum, jegunum and colon.

The proteinous secretory substance that is elaborated at the ribosomes and stored in the cisternae of granular ER may be transferred to Golgi apparatus *via* budding of the cisternae or detachment of ribosomes from the outer surface of the cisternae, which is faced to the Golgi apparatus. The α-cell granules are released mainly by so-called emiocytosis.

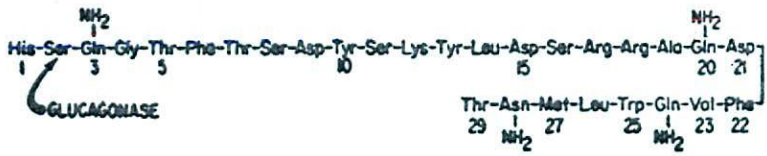


Fig. 4.77. Amino-acid sequence of glucagon.

Function and mechanism of action. Glucagon causes glycogenolysis in liver by stimulating the enzyme **adenyl cyclase** and thus activating inactive phosphorylase. The action is similar to that of epinephrine. The enzyme cyclase, activated by glucagon and catecholamines causes an accumulation of cyclic 3′, 5′-AMP by catalysing the cyclisation (Fig.4.78). The cyclic 3′, 5′-AMP in turn activates the kinase system. Difference in action that exists between glucagon and insulin is that the former does not

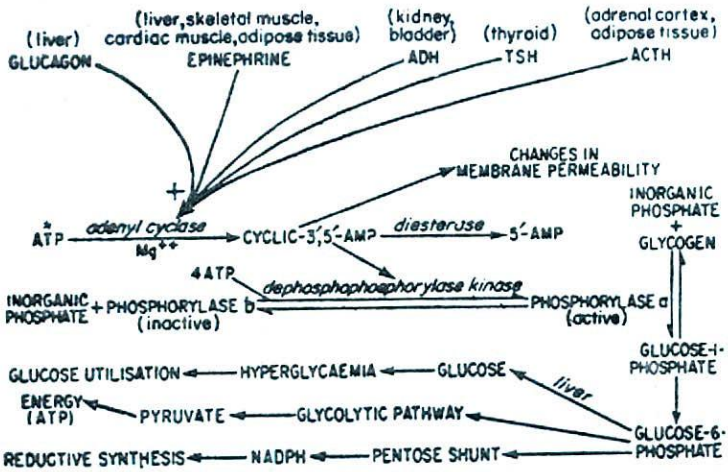


Fig. 4.78. Scheme showing hormones which activate adenyl cyclase and some possible consequences on carbohydrate metabolism where end-result depends upon responsive tissue affected.

cause breakdown of muscle glycogen as evidenced by its failure to increase blood lactate. But epinephrine causes glycogenolysis in extrahepatic tissue also, in contrast to the action of glucagon on the hepatic tissue only. Glucagon also increases breakdown of amino acids and increases gluconeogenesis from the deaminated part. Continued treatment with the hormone causes negative nitrogen balance. Evidences accumulated, so far, indicate that glucagon is involved in lipid metabolism. It increases liver

lipids in mice and promotes fatty acid oxidation. Glucagon causes a reduction both in volume of gastric juice and its hydrochloric acid content. Pancreatic juice is markedly reduced as also the movements of gastrointestinal tract.

Recently it has been shown that gluconeogenesis and ketogenesis are stimulated by enhanced rate of lipolysis within the liver causing increased levels of short- and long-chain fatty acid CoA esters (Fig. 4-79). It has been considered further that these esters possibly provide the basis for the gluconeogenic action of glucagon since they stimulate indirectly the carboxylation of pyruvate to oxaloacetate and inhibits the conversion of pyruvate to acetyl CoA.

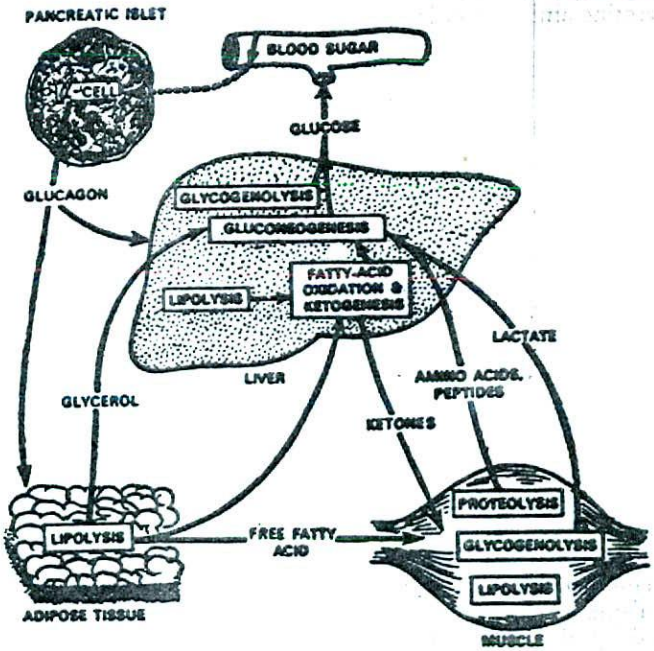


Fig. 4.79. It shows the effects of glucagon on metabolic changes involving adipose tissue, muscle and the liver.

Factors influencing secretion. Glucagon secretion is regulated by the blood sugar level. Hypoglycaemia stimulates and hyperglycaemia inhibits release of glucagon from the islets of Langerhans. This hormone is also secreted in increased amounts during muscular activity. Growth hormone has been implicated as an agent capable of stimulating glucagon release, but other workers suggest that there are some other agents which resemble growth hormone. Pancreozymin also stimulates release of glucagon. Glucagon secretion is also enhanced by protein meals and amino acids. Amino acids also stimulate insulin and hypoglycaemic action of insulin is prevented by concomitant release of glucagon.

Glucagon and cyclic AMP

The hyperglycaemic response to glucagon resembles that of epinephrine. Cyclic AMP acts as a second messenger for both hormones. Glucagon stimulates the adenyl cyclase activity. The stimulatory effects of glucagon have been presented in Figs. 4.80 & 4.81. Glucagon causes a prompt increase in the intracellular level of cyclic AMP. It has been observed that for any given effective concentration of glucagon, the level of cyclic AMP in the liver remains high so long the glucagon is present in the purfusing medium, and begins to fall as soon as the hormones is removed. Accumulation of cyclic AMP by glucagon precedes glycogenolysis, gluconeogenesis, amino acid uptake, urea formation and the increase in tyrosine amino transferase activity.

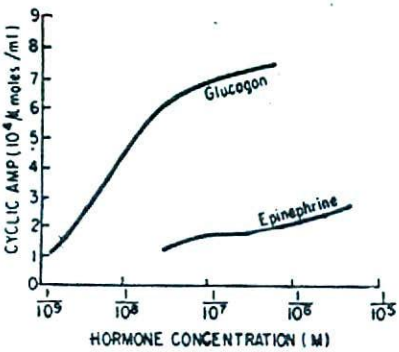


Fig- 4.80. It depicts the effects of glucagon and epinephrine on the activity of liver adenyl cyclase (after Makman & Sutherlands.)

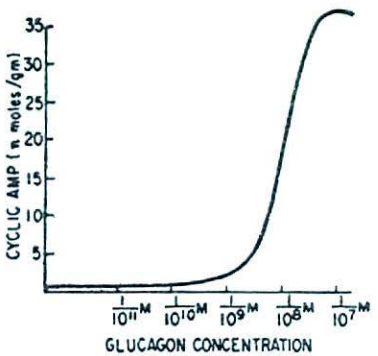


Fig. 4.81. It shows the effects of increasing concentration of glucagon on the level of cyclic AMP in the isolated perfused rat liver (after Exton *et al.*).

Gluconeogenesis is the process by which glucose is formed from a variety of small molecules other than carbohydrate. This process can be stimulated by glucagon, catecholamines and exogenous cyclic AMP. It is presented that glucagon stimulates the gluconeogenesis process through cyclic AMP. The molecular mechanism by which the cyclic AMP produces these effects is not known.

The stimulatory effect of glucose on insulin release is possibly mediated through cyclic AMP. A stimulatory effect of glucagon on the formation of cyclic AMP by pancreatic islets has been reported.

Glucagon and diabetes

In alloxan diabetic animals, the β-cells are damaged but the α-cells from which the glucagon is secreted, remain intact or unharmed. With prolonged treatment with glucagon, β-cells are exhausted (**exhaustive degeneration**) and pancreatic reserve is diminished. In diabetic animals, α-cells may remain unharmed or functionally normal. Whether the pathogenesis of diabetes is partly related with α-cells' functions it remains for further study.

Degradation of glucagon

Similar to insulin, glucagon is also degraded in different tissues. Liver and kidneys take the major part in its degradation. It is indicated from many studies that the degradation of glucagon is associated with cleavage of peptide linkage of the hormone. Inactivation of the hormone in the liver is inhibited by insulin, ACTH, GH, and cysteine-activated insulin. Reduced glutathione has got no role in degradation of glucagon.

REGULATION OF BLOOD SUGAR LEVEL

Normal blood sugar level varies from 80-100 mg per 100 ml (fasting) to 100-120 mg per 100 ml (after meal). Large quantities of sugar are constantly entering the blood stream (*absorption, glycogenolysis* and *gluconeogenesis*) and are constantly being removed from it (*glycogenesis, oxidation of sugar, synthesis of lipids*, etc.). In spite of those opposing forces, blood sugar level remains fairly constant within this limited range. This indicates that there must be a strong machinery for blood sugar regulation. The mechanism involves the following factors:

I. Alimentary Mechanism

1. **Assimilation limits of glucose.** (a) When glucose is given by mouth up to 200 g, no sugar is found in the urine in the next 24 hours. (b) When given up to 300-500 g, a large amount of water is osmotically drawn in and stomach becomes distended. Its movements are inhibited and consequently, it slowly empties into the small intestine. In this way, rapid absorption causing a very high rise of blood sugar is prevented. Blood sugar rises to some extent but as a rule, does not go beyond the renal threshold. Yet, some subjects develop a little glycosuria. This is probably due to the fact that, kidneys being unaccustomed to work under such high sugar pressure, fail to reabsorb glucose completely from the renal tubules and cosequently, a small amount leaks out. (c) If more than 500 g be given at a time, the subject develops nausea and the glucose is vomited out.

2. **Digestion of starch.** It is a slow and long process. Necessarily, absorption becomes slow. So that a sharp rise of blood sugar is prevented.

3. **Rate of absorption.** It is believed that there is a maximum limit of glucose absorption. It is about 1·84 g per kg per hour. Whatever be the amount of sugar given, the rate of absorption does not go beyond it, hence blood sugar cannot have a sharp rise. It is interesting to note that utilisation of sugar by tissues has also nearly the same rate (*vide* p. 4-129).

II. Role of Liver

This organ takes an important part in blood sugar regulation. It helps in two opposite ways, e.g., (a) when blood sugar tends to rise, liver stores it as glycogen and thus rise of blood sugar is checked. (b) When blood sugar tends to fall, liver mobilises its glycogen store and speeds up the rate of gluconeogenesis (other than carbohydrates) and thus restores the level to normal. Both these processes are under the control of hormones—to be decribed below.

III. Role of Muscles

Muscles also help in the same two ways as liver. It draws in glucose from the blood stream and sotres it as glycogen, thus tending to reduce blood sugar. When blood sugar becomes low (hypoglycaemia) or after

severe muscular exercise, lactic acid is mobilised from the muscles, converted first into glycogen and then into glycose in the liver and discharged into the blood stream. Thus blood sugar is raised. Through this 'Cori cycle' (Fig. 4.82) live and muscle co-operate in maintaining blood sugar.

IV. Role of Endocrines

Endocrines are the chief regulators of blood sugar level. The following endocrines take part:

1. **Insulin.** *It is the stongest blood sugar-reducing factor.* It lowers blood sugar in three ways: (a) By increasing glycogenesis. (b) By promoting glucose uptake in muscles and adipose tissue due to its influence on the cell permeability and phosphorylation process and by stimulating glucose combustion. (c) By preventing gluconeogenesis.

2. **Anterior pituitary.** A member of anterior pituitary hormones increases blood sugar: (a) *Growth hormone* decreases peripheral utilisation of glucose and the resulting hyperglycaemia stimulates the β-cells to overproduction of insulin and to exhaustion, Growth hormone also has lipolytic action. (b) *Adrenocorticotrophic hormone* (ACTH) through adrenal cortex, and (c) *Thyrotrophic hormone* (TSH) through thyroid, increase blood sugar level.

GH, ACTH and TSH have got opposing actions to that of insulin.

3. **Posterior pituitary.** A large does of *vasopressin* and *oxytocin* raise the blood sugar level temporarily. In rabbits vasopressin is more effective in raising the blood sugar level, whereas in dogs oxytocin has greater hyperglycaemic effect.

4. **Adrenal cotex.** Glucocorticoids work in the following ways: (a) Depresse the peripheral utilisation of glucose due to retardation of phosphorylation. (b) Increase gluconeogenesis in the liver due to retarded amino acid incorporation into protein, thus making more gluconeogenic material available. Administration of glucocorticoids produces temporarily diabetes in a number of animal species. Partially pancreatectomised animals may be made permanently diabetic by administration of cortisol or contisone.

5. **Epinephrine and norepinephrine.** It raises blood sugar by: (a) stimulating glycogenolysis from liver, and (b) converting muscle glycogen into lactic acid, which, through Cori cycle (Fig. 4.82), increases the blood sugar level. Epinephrine increases basal metabolic rate (BMR) by 20%, and increases the oxidation of glucose in the tissues. Norepinephrine has siimilar effects, although to a much lesser degree.

6. **Thyroid.** The following effects are exhibited by *thyroxine* on carbohydrate metabolism: (a) Increase in the peripheral utilisation and combustion of glucose in the

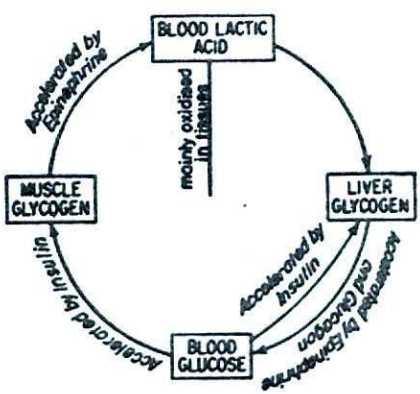


Fig. 4.82. It represents the hormonal control of blood sugar level in the body.

tissues. (b) Stimulation of glycogenolysis and gluconeogenesis. (c) On continued administration the animals become resistant to epinephrine and more sensitive to insulin. Severity of diabetes increases in hyperthyroidism.

7. **Glucagon.** Glucagon increases blood sugar due to glycogenolysis in liver and gluconeogenesis.

Thus, one will find that, almost all the important endocrine glands take part in blood sugar regulation. *Insulin tries to reduce blood sugar, whereas the other glands try to raise it.* The normal sugar level is the optimum blance between these two opposite forces.

V. Role of Nervous System

Hypothalamic lesion causes disturbances of carbohydrate metabolism, namely hypoglycaemia, increased sensitivity to insulin, etc. Autonomic nervous system takes a great part in blood sugar regulation. For instance, (a) stimulation of the right vagus reduces blood sugar level by increasing insulin secretion. (b) Stimulation of the sympathetic increases blood sugar level by mobilising liver glycogen (direct action on liver) and by stimulating epinephrine secretion. Autonomic action is controlled by blood sugar level (*vide* below).

VI. Role of Blood Sugar

Blood sugar regulates its own level. Hyperglycaemia stimulates insulin secretion by—(a) directly acting on the β-cells, and (b) stimulating the right vagus. (c) It also increases the rate of oxidation of sugar in the tissue independent of hormones and probably (d) depresses the secretion of growth hormone. In this way, the raised blood sugar is brought down to normal. On the other hand, hypoglycaemia depresses insulin secretion by—(a) directly action on the pancreatic islet tissues, and (b) inhibiting the right vagus. (c) It stimulates epinephrine secretion, and (d) that of the growth hormone. In this way, the low sugar level is raised to normal. In this way, blood sugar level controls itself.

VII. Role of Tissues, Tissue Fluid and Skin

(a) The tissue fluid (nearly 30 litres), having nearly the same glucose content as plasma, can store a large amount of glucose. Any rise or fall of blood sugar is at once compensated by appropriate exchange with tissue fluid. (b) The skin and subcutaneous tissue can store a large amount of glucose temporarily. (c) The tissues in general use up sugar in a number of ways—such as, conversion into lipids (adipose tissue), synthesis of other substances, oxidation of glucose and so on. The rate of oxidation is controlled by endocrines, blood sugar level, vitamins, etc. *The tissues can remove sugar from the blood stream at a maximum rate of 2 g/kg/hour* (nearly the same as the maximum rate of sugar absorption from the intestine—page 4-127).

VIII. Role of Kidneys

Kidneys act as the last outposts. When blood sugar goes above the renal threshold (180 mg per 100 ml), it leaks out through the kidneys. It is an obvious attempt on the part of the kidneys to check further rise of blood sugar.

From the above considerations, it will be seen that, glucose being the ready source of energy, the body has developed a very elaborate and an efficient machinery to control its supply, storage and utilisation.

Hypoglycaemia

Hypoglycaemia is a condition in which blood sugar level is present below the normal level, i.e., below 80 mg per 100 ml. Generally, hypoglycaemic symptoms start somewhere between 70-50 mg per 100 ml. In diabetic subjects whose tissues are accustomed to high blood sugar, hypoglycaemic symptoms may start at a blood sugar level much above normal. Hypoglycaemic symptoms depend on three factors: (1) The actual blood sugar level. (2) The rapidity of blood sugar reduction. (3) The previous blood sugar level, i.e., that level with which the tissues were accustomed.

Since nerve cells have very little stored food and since they use sugar mostly as the sole source of energy. Hypoglycaemia will, therefore, affect the nerve cells first. Hence, the earliest manifestations will be nervous in origin. For instance: (1) A feeling of fatigue, weakness and hunger. (2) Extreme anxiety and irritability. (3) Abnormal behaviour as in alcohol poisoning. (4) Tremors develop and fine movements are not possible. (5) Vasomotor disturbances, such as flushing or palor, perspiration and chilliness. (6) Later on, there may be delirium, diabetic coma and convulsions and loss of deep reflexes.

Hypoglycaemic symptoms are relieved by the administration of glucose. Other monosaccharides are less effective.

Hyperinsulinism. A rare clinical condition association with hyperfunctioning islet tissues (Tumours) due to excessive production of insulin of β-cells. The symptoms are those of paroxysmal attacks of typical hypoglycaemia. Only a few cases have been recorded uptil now.

Compensatory reactions of hypoglycaemia. Hypoglycaemia stimulates hypothalamus which in its return promotes the secretion of ACTH and other hormones which oppose the actions of insulin and restore the blood sugar level to normal. Hypoglycaemia also stimulates the secretion of epinephrine which stimulates glycogenolysis and raises the blood sugar level.

Hyperglycaemia

Hyperglycaemia is a condition in which blood sugar increases above the normal level, i.e., above 120 mg per 100 ml. When the blood sugar level exceeds the renal threshold (180 mg per 100 ml), sugar appears in the urine. Persistent hyperglycaemia occurs when there is diminished utilisation of glucose, and discharge of excess sugar from the liver. Hyperfunction of some of the endocrine glands, viz., anterior pituitary, adrenal cortex, etc., causes hyperglycaemia. Lack or diminished secretion of insulin is the main factor which produces hyperglycaemia and glycosuria as in diabetes mellitus (vide below).

Factors causing hyperglycaemia vide glycosuria.

Glycosuria

Glycosuria is a condition when the blood glucose level exceeds 180 mg glucose per 100 ml blood above the normal blood glucose level (60-100 mg) glucose per 100 ml blood. At this time the renal tubule cells are not able to reabsorb all the glucose. Some glucose reaches the urinary bladder and glycosuria results. In normal condition, the renal tubule cells are able to transfer all the glucose back into the blood by active

transport, and no glucose passes to the urinary bladder. But the tubule cells are limited in the quantity of glucose that they can transport back to the blood in a given time (tubular maximum for glucose).

For details see elsewhere in the chapter of Excretion.

DIABETES MELLITUS

The term *diabetes* means that a large volume of urine is passed. The term *mellitus* (=sweet) dates from the time when the urine was tested by tasting and the urine in this condition is sweet to the taste.

Hyperglycaemia, glycosuria, ketosis, acidosis, diabetic coma (unconsciousness), polyuria, weight loss in spite of polyphagia (condition of increased appetite) and polydipsia (condition of increased thirst) are the abnormal characteristics of diabetes. But the principal abnormalities are an increased liberation of glucose in circulation from the liver and a reduced entrance of glucose in peripheral tissues due to deficiency of intracellular glucose and excess of extracellular glucose. Diabetes mellitus is a disorder of metabolism characterised by high blood sugar level and excretion of sugar in urine.

Causes of diabetes mellitus

1. Due to insulin lack:

(a) *Juvenile diabetes* has its onset in childhood or adolescence—frequently complicated by ketoacidosis.

(b) *Maturity onset diabetes* is mild, develops late in life and occurs much more frequently in obese persons. Ketoacidosis is uncommon. Reduction of weight improves glucose tolerance.

In **juvenile diabetes** β-cell pathology is common and the insulin content of the pancreas is low. On the other hand, **maturity onset diabetes** (adult type) β-cell morphology and the pancreatic insulin content are generally normal.

2. Hyperpituitarism—gigantism and acromegaly (due to hypersecretion of GH).

3. Hyperthyroidism—Graves' disease.

4. Hyperfunction of adrenal cortex—Cushing's syndrome (due to hypersecretion of adrenal glycocorticoids).

Metabolism in diabetes mellitus

Diabetes mellitus is a condition of *glycosuria accompanied with hyperglycaemia,* primarily due to lack of insulin, caused by the degeneration or hypoactivity of the β-cells of the islets of Langerhans. Serious derangement of carbohydrate, fat and protein metabolism takes place. Almost all the ill effects can be explained from two standpoints: (a) Absence of insulin. (b) Unopposed activity of the insulin antagonists. The metabolic disturbances (Fig. 4.83) are briefly described below:

I. Carbohydrate Metabolism

1. **Glycogen metabolism.** Insulin stimulates *glycogenesis* and prevents *glycogenolysis.* Absence of insulin, in this disease, will, therefore, cause reverse effects, viz.: (a) Glycogen content of liver will be low and glycogen formation depressed. (b) In the muscles, glycogen content

diminishes and its synthesis during recovery is depressed. (c) Curiously enough, *in the heart, the glycogen content increases.* Insulin rectifies all these defects.

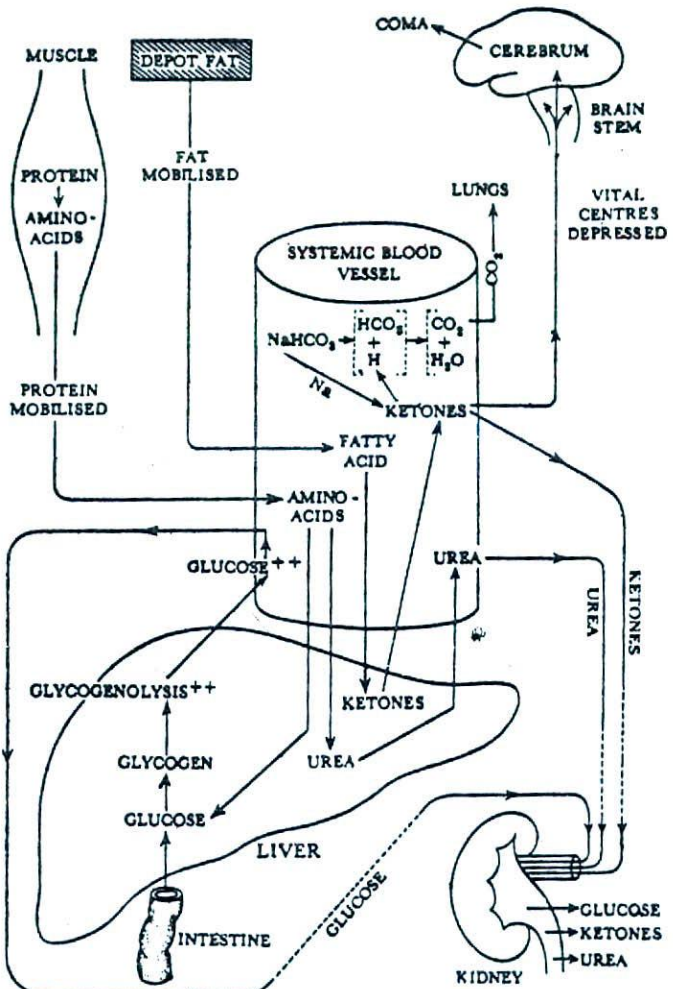


Fig. 4.83. Diagrammatic representation of the metabolism in diabetes mellitus in the body.

2. **Combustion of glucose.** Insulin stimulates oxidation of glucose. Hence in diabetes mellitus, glucose combustion will be depressed. There is an impairment of glucose oxidation, not a complete interruption. This is shown by the following facts: (a) RQ falls to about 0·7 and arteriovenous glucose difference is low. This shows that tissues are utilising very little

glucose and fats are being burnt. (b) Isolated organs or limbs, if perfused with glucose *plus* insulin, remove glucose much more quickly than when perfused with glucose alone. Studies after administration of glucose labelled with radio-active ^{14}C have shown that insulin directly increases the rate of glucose oxidation.

3. **Gluconeogenesis.** Normally insulin checks this process. In diabetes mellitus the rate of gluconeogenesis increases, because insulin is absent. The activity of this group of enzymes, called transaminases, which are responsible for the conversion of glucogenic amino acids to carbohydrate intermediates, is increased in the liver of a person suffering from diabetes. The liver helps in the formation of more glucose and hyperglycaemia is enhanced.

4. **Glucose tolerance**—lowered. [*Vide* page 4-123.]

II. FAT METABOLISM

The following changes occur:

1. Depot fats mobilised.
2. Liver loaded with fats.
3. There is formation of excess ketones due to lack of insulin. In the liver, fatty acids are converted into *active acetate* units—acetyl CoA. Under normal condition these two-carbon units pass through Krebs cycle for oxidation and are completely oxidised. In diabetes mellitus carbohydrate metabolism is deranged and all the two-carbon units do not pass through the Krebs cycle, rather they condense with each other and form four-carbon aceto-acetic acid. Ketone bodies are produced at a faster rate than can be used up by the tissues causing *ketosis*. [They can be used up by the tissues even in absence of insulin.] Hence, *ketosis is due to overproduction of keton bodies and not due to their non-utilisation.* Increase in blood ketone bodies leads to **metabolic acidosis** which causes deep and rapid breathing; a characteristic feature of **diabetic acidosis** and finally *ketotic* (diabetic) coma (unconsciousness) and death. *It should be noted that the chief danger in diabetes mellitus comes from the derangement of fat metabolism and consequent ketosis.*
4. Glycerol—quantitatively converted into sugar.
5. Blood cholesterol rises. It is due to active acetate being converted into more cholesterol instead of fatty acids. The increased cholesterol level of blood produces atherosclerotic condition of the blood vessels in diabetic subjects.
6. RQ. Since glucose is not burning, energy is derived chiefly from the combustion of fats. Hence, RQ falls to about 0·7. After administration of insulin it rises to normal. [Fall of RQ may also mean conversion of fats into glucose, vide Respiratory Quotient.]

III. PROTEIN METABOLISM

This is also seriously distrurbed. Insulin stops gluconeogenesis. In diabetes mellitus, insulin being absent, the growth hormone acts unopposed. Hence, glucose is formed from the non-carbohydrate sources at a faster rate in the liver. About 60% of the non-nitrogenous residue of proteins is converted into sugar. If enough protein is not given in the diet, the tissue proteins will be mobilished, deaminated in the liver and be converted into sugar. That this sugar is coming from proteins, is proved by the fact that, in a starving diabetic, D/N ratio is constant and is about 3·6, showing that both dextrose (D) and nitrogen (N) are coming from

the same source—the protein. There are certain amino acids, called antiktogenic amino acids which are all converted into sugar. In juvenile diabetes there is marked loss of weight and protein synthesis is impaired leading to negative nitrogen balance.

IV. Blood Changes

1. Blood sugar rises due to 4 causes—(a) Increased glycogenolysis. (b) Lack of glycogenesis. (c) Increased glyconeogenesis. (d) Depressed sugar combustion. When it goes beyond 0·18%—glycosuria results.
2. Increased blood lipids (Lipaemia), may go up to 12-24%.
3. Increased blood cholesterol—even up to 350 mg per 100 ml.
4. Ketosis and acidosis—Alkali reserve lowered.
5. Blood phosphates—rise.
6. **Haemoconcentration.** Decrease in blood volume, peripheral circulatory failure and the resulting reduction in O_2 supply to tissues in general.

V. Urine Changes

(a) Presence of sugar and acetone. (b) Increased urine volume—diuresis. Presence of sugar increases osmotic pressure, retards water absorption, hence polyuria. [Consequently, increased thirst—polydipsia.] (c) Loss of electrolytes. (d) Dehydration. (e) Increased loss of nitrogen. (f) Raised ammonia coefficient (acidosis). (g) Increased PO_4 excretion.

VI. Infections

Characteristically, there is reduced resistance to infections. It is probably related to altered immunological response circulating impairment, altered metabolism, etc.

Action of insulin in diabetes mellitus. When insulin is given, reverse changes will take place and the condition will be restored to normal. (a) The rate of dissimilation of glucose becomes normal. (b) Blood sugar comes down to normal level and glycogenesis is stimulated. (c) Excess of gluconeogenesis is checked. (d) Ketosis disappears and ketone formation in the liver stops. (e) RQ becomes normal. (f) Sugar and ketone bodies disappear from the urine. (g) Rate of protein synthesis augmented. (h) Increased conversion of glucose to fat. (i) Fall in blood the level of potassium and inorganic phosphate.

Oral anti-diabetic agents

In recent years, several hypoglycaemic agents effective orally, have been used in the treatment of diabetes, as a substitute for insulin. These agents include the sulphonamide drugs, glybenzcyclamide, tolbutamide and chlorpropamide. Sulphonamides act possibly by stimulating the production of insulin and so their activity depends on the presence of functional β-cells. Action of exogenous insulin is also potentiated by these agents possibly due to inhibition of insulinase, an enzyme responsible for degradation of insulin. Sulphonylureas and biguanides may destroy the α-cells decreasing the production of glucagon. It has also been suggested that they reduce glycogenesis by the liver.

Conclusion. Until recently diabetes used to be regarded as a disease of carbohydrate and fat metabolism and this disease was complicated by accelerated atherosclerosis and other vascular abnormalities. These abnormalities could be delayed or even prevented with insulin and diet. Although the control of the vascular disease may be intensified

with the uncontrolled diabetes, the onset and progression of blood vessel abnormalities are not usually dependent of the degree of diabetic control. Considering these, diabetes may be regarded as a congenital disease where disordered carbohydrate metabolism is a relatively late development. However, diabetes may be divided into the following stages:

STAGE I—**Prediabetes.** It is observed from birth. These patients are considered to have occult, potential or suspected diabetes.

STAGE II—**Latent chemical diabetes, subclinical diabetes or latent diabetes.** Glucose tolerance of this subject is mostly normal but under stress (pregnancy or major disease) abnormalities of carbohydrate metabolism becomes precipitated.

STAGE III—**Overt diabetes.** In this type of diabetes, full-fledged syndrome of clinical diabetes is present.

STAGE IV—**Chronic diabetes.** Members of this group show abnormal glucose tolerance test and abnormal fasting blood sugar. Symptoms of different clinical manifestations are encountered. Vascular lesions are also observed.

Heredity and diabetes

Considering the greater incidence of diabetes in the same family generation after generation thorough analysis has been made. It has been described by Joslin that 41% of diabetics have a definite family history of disorder. It has been observed that diabetics are homozygous for a recessive gene. The children of two diabetics (parents) are always potent diabetics, Even diabetics may appear simultaneously in monozygotic tuins.

ADRENAL (SUPRARENAL) GLANDS

Anatomy. Two in number, roughly triangular in shape, one situated on the upper pole of each kidney (hence, the name suprarenal). The right gland is smaller and looks like a cocked hat; and the left one is roughly crescentic and usually larger. Each gland in the adult usually

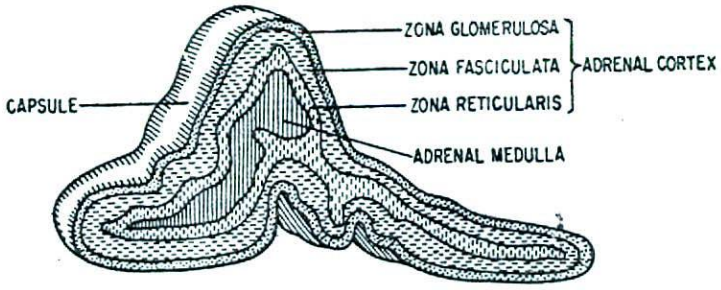


Fig. 4.84. It shows section through adrenal (*ad*=to) gland.

measures about 50 mm × 30-40 mm × 10 mm. The average weight of each gland is about 5-9 g in adults (medulla being about one-tenth of the total weight). Consists of two parts (Fig. 4.84): (a) Outer part—the

cortex. (b) Inner part—the **medulla.** Whole enclosed in a capsule. The two parts are structurally, functionally and embryologically different. At birth the adrenal glands weigh about 8 g, which is proportionately 16-20 times greater than in the adult. They gradually decrease in size.

Development. (a) **Cortex** is derived from the mesodermal coelomic epithelium covering the fore part of the mesonephros (on either side of the root of mesentery). During foetal life in human, an inner layer, called *provisional cortex,* is formed by chains of large, eosinophilic, granular cells and is highly developed. It comprises 80% of the foetal adrenal cortex. This layer is structurally and functionally different from the outer *permanent cortex,* which is very ill-defined at this time of life and represents only 20% of the gland. The provisional cortex rapidly degenerates after birth and the permanent cortex begins to develop and descends over the medulla. A major function of this foetal adrenal is to secrete sulphate conjugates of androgens, which become converted to active androgens and oestrogens in the placenta, entering the maternal circulation. Laboratory animals have no structure comparable to the foetal adrenal. The hormones of adrenal cortex are derivative of cholesterol and contain *perhydro-cyclopentano-phenanthrene nucleus.* [From the tissues immediately behind it, arises the germinal epithelium, from which the sex glands develop. This embryological similarity may explain close relationship between gonads and adrenal cortex and their metabolic similarity—both being concerned with sterioid hormone production.]

(b) **Medulla** is ectodermal in origin and is derived from the same tissues from which the sympathetic ganglia develop. The primitive tissue (*sympathogonia*) separates out from the *neural crest* and differentiates on two lines. Some develop into sympathetic ganglion cells, others into *phaeochromocytes.* The latter stains brown with chromic acid, hence, called the *chromaffin tissues.* The adrenal medulla is the largest mass of the chromaffin tissue. Hence, the medullary cells should be regarded as modified sympathetic ganglion cells (Fig. 4.87).

The primitive medullary tissue at first lies close to the posterior root ganglion, then migrates laterally to be invested by the cortical tissue. The migration starts in the *fourth week* of foetal life and is completed about the *tenth year* after birth.

Accessory cortical tissues are very small in amount in man and higher mammals. When present, they are commonly found in the retroperitoneal tissues, kidneys, liver, gonads, broad ligaments, etc.

Accessory medullary tissues are more abundant. They are found in and near the sympathetic ganglia and in the nerve plexuses over the aorta. On the whole, accessory cortical tissue is very little in amount but accessory medullary tissue is abundant. Hence, bilateral removal of cortex is fatal but not that of medulla.

Blood supply. It is one of the most vascular organs of the body, receiving 6-7 ml of blood per g per minute. Blood vessels enter the gland through the surface and form a rich vascular plexus in the cortex. From these plexuses blood runs into dilated sinuses in the medulla. The latter join up to form the central vein which passes out through the hilus of the gland. Thus blood passes, first to the cortex and then to the medulla. Few arterioles pass direct to the medulla through the cortex (Figs. 4.85 & 4.86).

Nerve supply. From the greater splanchnic nerves. Fibres pass through the suprarenal plexus, pierce the surface of the gland, pass through the cortex and end in the medulla. They are medullated fibres without any cell station in their course. In other words, they are entirely

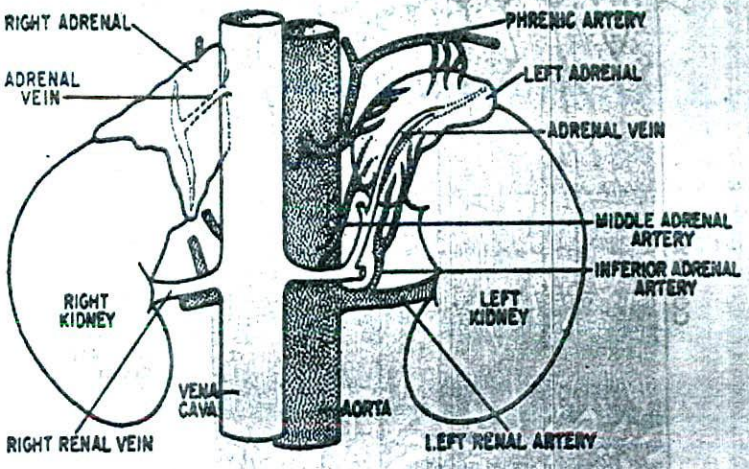


Fig. 4.85. It shows major arterial supply and venous drainage of the human adrenal gland.

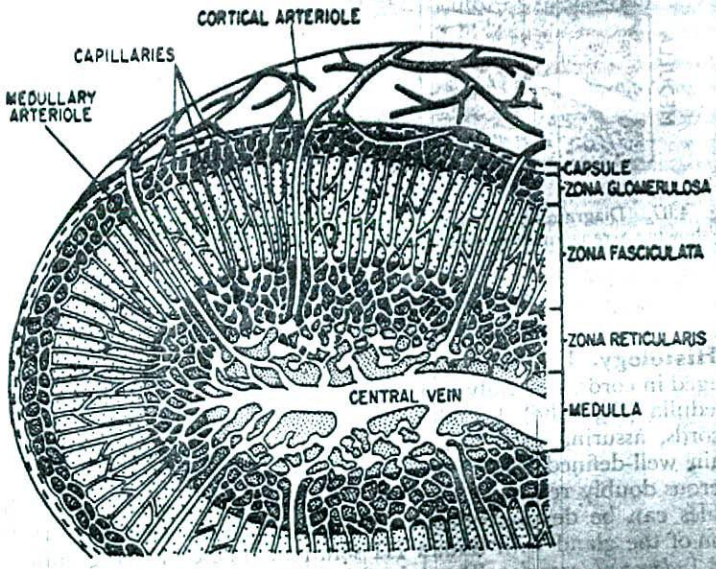


Fig. 4.86. Schematic representation of the circulation in a mammalian adrenal gland.

preganglionic; the medullary cells representing the whole of the third neurones of the sympathetic ganglia. *These nerves control adrenal medulla only. They are believed to have no action on the cortex.*

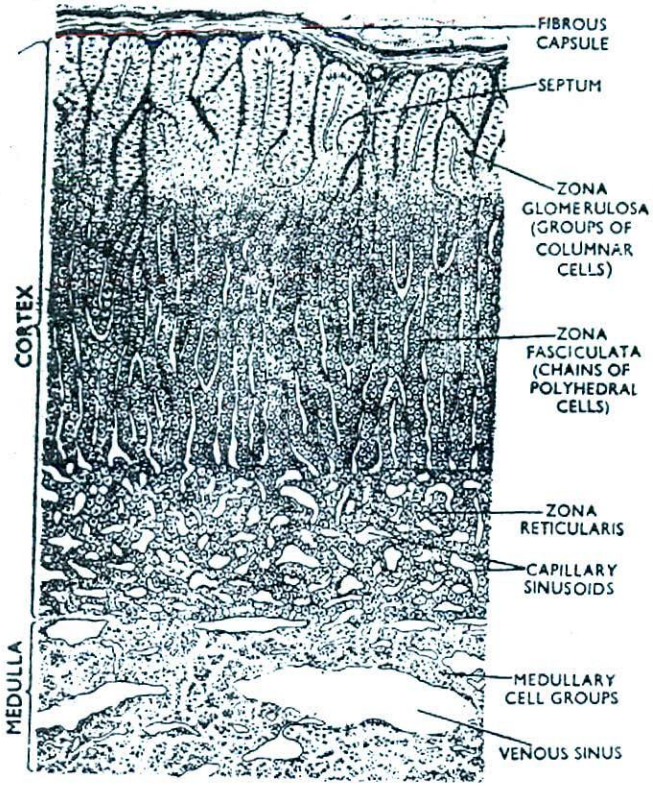


Fig. 4.87. Diagrammatic representation of section of adrenal (suprarenal) gland showing arrangement of cells in three layers of the cortex and part of medulla at the bottom

ADRENAL CORTEX

Histology. The parenchyma consists of polyhedral epithelioid cells arranged in cords, generally two cells thick, that run radially from capsule to medulla (Fig. 4.88). Capillaries form an anastomotic network around the cords, assuring each cell of direct contact with a vessel. The cells contain well-defined nuclei, typical mitochondria, Golgi apparatus and numerous doubly refractile lipid granules in the cytoplasm. The lipid granules can be demonstrated as red granules after staining the fresh section of the gland with Sudan III solution. These stained granules are called *Sudanophil granules*. The adrenal cortex is covered by a capsule from which trabeculae pass into the gland, carrying blood vessels and

nerves. The cells are arranged in the following *three layers from outside inwards* (Fig. 4.89).

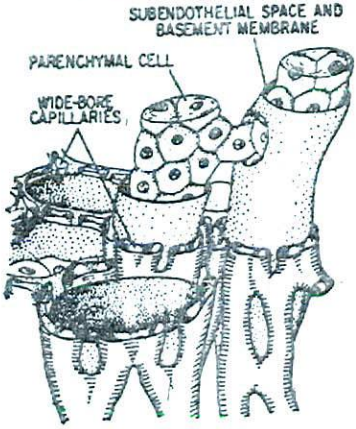


Fig. 4.88. It depicts the relationship of parenchymal cords to capillaries in the zona fasciculata of the human adrenal gland.



Fig. 4.89. Diagrammatic representation of histological structure (magnified) of zona glomerulosa, zona fasciculata and zona reticularis of cortex of the adrenal gland.

1. **Zona glomerulosa** (outer). There are groups of columnar cells which are comparatively small and thickly set with their long axis *parallel* to the surface. Zona glomerulosa secretes mainly aldosterone, and a small amount of glucocorticoids and sex hormones.

2. **Zona fasciculata** (middle). It is the widest layer. The polyhedral cells present in this layer are proportionally larger, containing pigment granules upon which the brownish-yellow colour of the cortex depends. The cells are arranged in radiating columns, *perpendicular* to the surface and also contain lipid droplets **(liposomes).**

Nuclei are larger and less dense than those of the zona glomerulosa. Cytoplasm under H and E staining shows extensively vacuolated indicating presence of lipid droplets in life. The cells of the zona glomerulosa are so pronounced that sometimes these are called as *spongiocytes*. Cholesterol and vitamin C are present in considerable amount in the cells. ACTH if administered then ascorbic acid and cholesterol are depleted rapidly from these cells. The cells are rich in mitochondria and these mitochon-

dria are different in having tubular villi instead of relatively flat shelves. This layer secretes predominantly glucocorticoids. The cells of both the zona glomerulosa and zona fasciculata contain large amounts of smooth-surfaced endoplasmic reticulum, which are supposed to be related to the synthesis of steroid hormones.

3. **Zona reticularis** (inner). Made up of an *irregular* network of rows of cells. The cells contain lipid droplets. The meshes of the network are filled up with sinusoids lined by reticulo-endothelial cells.

Zona reticularis secretes sex hormones and a small amount of glucocorticoids, but no aldosterone.

Electron microscopic structure of the adrenal gland, as described by Fawcett *et al.* (1969), indicates that the structure of the steroid-secreting cells is very similar. The cells of the adrenal cortex contain large amounts of smooth endoplasmic reticulum. This smooth endoplasmic reticulum is presumably involved in the synthesis of steroid hormones (Fig. 4.90).

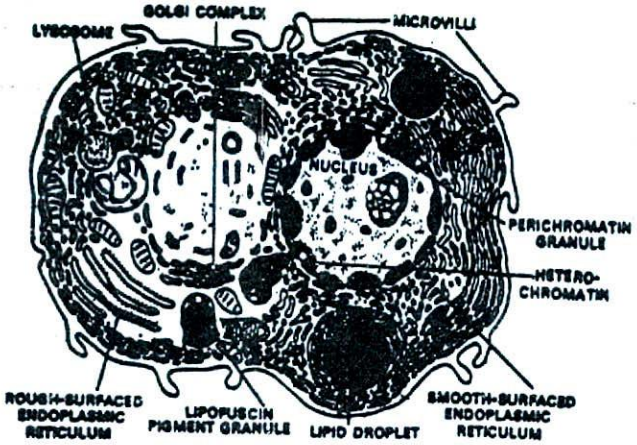


Fig. 4.90. Diagram representing fine structures of a steroid-secreting cell where mitochondria are characterised by tubular cristae and large amounts of smooth endoplasmic reticulum is actually a meshwork of interconnected tubules throughout the cytoplasm.

Extirpation of both adrenals, *leads to death within a week,* if kept on ordinary diet. Hence, *it is essential for life.* Administration of a suitable cortical extract, preferably with added amounts of NaCl in the diet, rectifies the condition and keeps the animal normal and alive for indefinite period (Fig. 4.91). Administration of large amounts of NaCl only prolongs life and corrects some of the symptoms but not all. The functional disturbance is observed in experimental animals after bilateral adrenalectomy resembles the patients suffering from Addison's disease (hypofunction of adrenal cortex).

Clinical features appear within 42 hours of extirpation, and are briefly as follows:

1. **General features.** (1) Weakness and prostration; (2) Nausea

and vomiting; (3) Anorexia—specially for lipids; (4) Rapid loss of weight; (5) Diarrhoea; (6) Dehydration; (7) Hypotension; (8) Low BMR (by 20%); (9) Subnormal temperature; (10) Convulsions (hypoglycaemic and uraemic); (11) Coma, followed by (12) death.

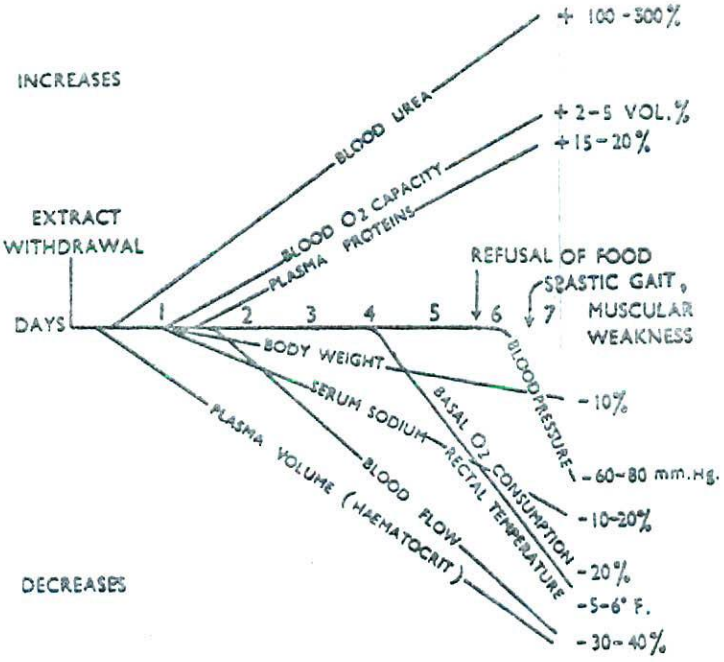


Fig. 4.91. Graph showing the various changes caused by acute adrenocortical deficiency, following withdrawal of cortical extract with which a group of adrenalectomised animals were kept in good health previously.
(Harrop, G.A., Weinstein, A., Saffer, L.J., and Throscher, J.H., 1933, F .*Exper.Med.*,58,1).

2. **Renal changes.** (1) *Excretion of NaCl and water—increased;* (2) *Retention of K through reduction of K excretion;* (3) Excretion of Ca, Mg, PO_4—*reduced;* (4) *Nitrogen excretion—lowered;* leading to (5) renal failure (urea clerance, etc.,—lowered).

3. **Blood changes.** (1) Haemoconcentration, plasma protein—increased. Percentage of haemoglobin may not rise, even there may be anaemia. Because adrenal cortex helps haemopoiesis. (2) Plasma volume, blood flow and blood pressure—lowered. Both (1) and (2) are mostly due to increased loss of water. (3) *Sodium chloride* and *sugar contents—fall.* (4) *Nitrogen retention, urea, non-protein nitrogen,* etc.,—*increased.* (5) Phosphates, Ca, Mg and *specially* K—raised (increased Mg ion may partly explain muscular weakness, because Mg ions inhibit the breakdown of ATP). (6) Increased loss of bicarbonate, fall in blood pH leading to acidosis.

4. **Liver and muscles.** (1) Glycogen stores diminished in the muscles and liver, (2) Less gluconeogenesis in the liver.

5. **Adaptation reactions.** Very much limited. Easily succumbs to heat, cold, infection, shock and such other stresses.

Active principles of adrenal cortex and their functions

Active principles

The crude extract **cortin** contains a number of steroids. It can keep up an adrenalectomised animal in normal health. About 50 steroids have been isolated from adrenal gland of which only 7-8 have physiological activity. The characteristic structure of steroid hormones is that of a relatively flat four-membered ring group carrying attached functional groups ($-H$, $-CH_3$, $\cdots OH$, $=O$) which project α (below) or β (above) the plane of the ring structure. Commonly two essential features are: (i) an α, β-unsaturated carbonyl group in ring A at 3C, usually written as Δ^4-3-ketone, and (ii) a 2-carbon side-chain (20C and 21C) at the 17C with a ketone group at the 20C and a hydroxyl group at the 21C. Differences in physiological activity are related to the presence or absence of an α-hydroxyl group at the 17C and of a ketonic or β-hydroxyl group at the 11C. Although numerous variations of structures occur in the precursors and metabolites of active steroid hormones, the configurations of major steroids are relatively few and quite well-defined (Fig. 4.92). The active substances can be divided into:

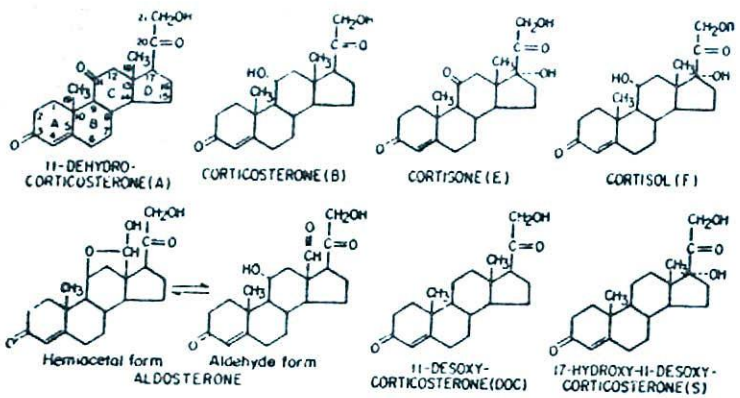


Fig. 4.92. Structural formulae of adrenal corticoids.

Adrenal corticoids (glucocorticoids and mineralocorticoids) containing 21 carbon atoms and sex steroids containing 19 such atoms. They asre as follows:

I. ADRENAL CORTICOIDS: (1) **Glucocorticoids** (C_{21})—*Steroids* with $=O$ or $\cdots OH$ at the 11C position.

(a) 11-Dehydrocorticosterone ($=O$ at the 11C position)—Compound A of Kendall. Moderate sodium and carbohydrate activity.

(b) Corticosterone ($\cdots OH$ at the 11C position)—Compound B of Kendall. Moderate sodium and carbohydrate activity.

(c) Cortisone ($=O$ at the 11C position) - 17-Hydroxy-11-dehydrocorticosterone—compound E of Kendall. Weak sodium retention; Potent carbohydrate activity: Potent anti-inflammatory activity.

(d) 17-Hydroxycorticosterone (···OH at the 11C position)—Hydrocortisone or cortisol or compound F of Kendall. Weak sodium retention; Potent carbohydrate activity; potent anti-inflammatory activity.

(2) **Mineralocorticoids—**

(a) *Steroid with* ...OH or =O at the 11C position:

Aldosterone (Electrocortin, 18-Aldocorticosterone)—(18-Aldehyde form and 11-Hemiacetal form). Characteristic feature—the methyl group at 18C position is replaced by an aldehyde group. It can remain both as aldehyde and hemiacetal forms in solution. Very potent sodium retention; Very weak carbohydrate activity.

(b) *Steroid* without =O or ···OH at the 11C position:

Deoxycorticoid—11-Deoxycorticosterone (DOC)* or deoxycortone. High sodium retention; Slight carbohydrate activity.

(c) 17-Hydroxy-11-deoxycorticosterone (···OH at the 17C position)—Compound S of Reichstein. Only slight activity.

II. **Sex steroids** (C_{19}) with =O or ···OH or a side-chain $CO\text{-}OH_3$ at the 17C position:

(a) Androgen—androsterone;

(b) Dehydroepiandrosterone (DHEA);

(c) Oestrogen—oestradiol;

(d) Progesterone.

Biosynthesis of adrenal steroid hormones. Adrenal corticosteroids are synthesised from cholesterol or from acetate. There is good evidence that mineralocorticoid secretion is largely confined to the zona glomerulosa and glucocorticoid secretion to the zona fasciculata. It has been observed in some species that the androgen synthesis occurs predominantly in the zona reticularis. It is suggested that the several steps

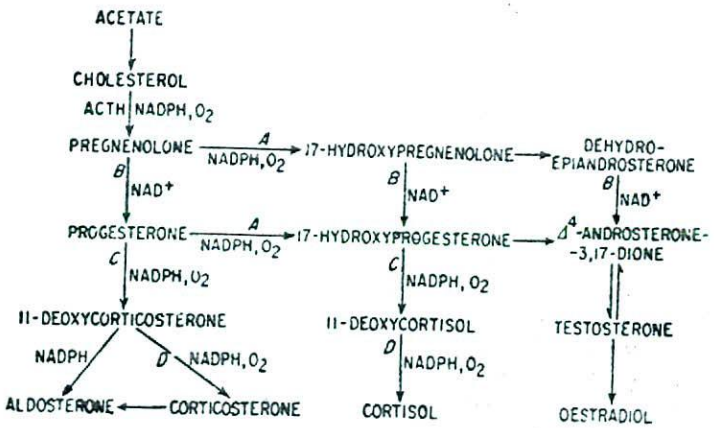


Fig. 4.93. It represents biosynthetic pathways in the adrenal gland to the major steroid hormones. D is blocked by metyrapone causing transient fall in glucocorticoids like corticosterone and cortisol. A = 17 α-hydroxylase (*zona glomerulosa* and *zona reticularis*), B = 3β-dehydrogenase-Δ^5, Δ^4-isomerase, C = 21β-hydroxylase, D = 11β-hydroxylase

* Aldosterone is the chief mineralocorticoid secreted from the adrenal cortex. Deoxycorticosterone seems to be secreted in abnormal situation.

of biosynthesis *in adrenal steroid formation take place in the mitochondria.* The synthesis of any type of steroid hormones—whether gonadal steroids, mineralocorticoids or glucocorticoids—depends upon the presence and activity of special enzymes in the endocrine tissues. ACTH has got predominant effects upon lipid content and mitochondrial morphology in the zona fasciculata. Mitochondria of the zona glomerulosa are affected by the alteration of plasma-sodium deficiency. Other than ACTH, GH is also required for the maintenance of the adrenal mass. Role of vitamin C in steroidogenesis is not clear.

Main pathways for the synthesis of glucocorticoids, mineralocorticoids and sex hormones have been presented in Fig. 4.93. It shows that pregnenolone acts as pivot for the synthesis of all the adrenal steroids from cholesterol, and NADPH acts as a coenzyme for hydroxylation reaction in different steps. Haynes and Berthet (1957) postulated that ACTH involved in steroidogenesis (**Haynes-Berthet hypothesis**) through the activation of phosphorylase by cyclic AMP—resulting increased generation of NADPH. This NADPH in presence of O_2 takes part in the conversion of cholesterol to pregnenolone. Haynes *et al.* (1960) has postulated (Fig. 4.94) that ACTH activates adenyl cyclase causing excess formation of cyclic AMP from ATP. C_{21} hydroxylation is necessary for the synthesis of both glucocorticoids and mineralocorticoids.

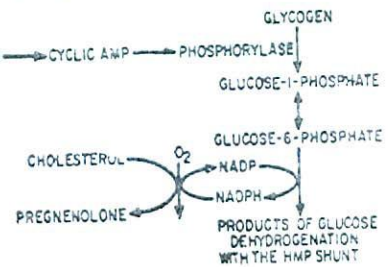


Fig. 4.94. The Haynes hypothesis (after Haynes *et al.*).

The major adrenal androgens are synthesised by side-chain cleavage of 17-hydroxy pregnenolone. A little amount of oestrogen is also produced either from dehydroepiandrosterone (DHEA) or 17-hydroxy progesterone.

Transport, metabolism and excretion. Normally steroid hormones circulate in association with the specific binding proteins and only a small quantity is free. In man daily secretion rate of cortisol is about 14-15 mg, of 17-ketosteroids (17-oxosteroids) is 18-20 mg and of aldosterone 50-200 μg according to Na^+ balance. About half of the hydroxycorticosteroids are transported in the blood, bound loosely to plasma proteins. Glucocorticoids are bound specifically to an **α-globulin—corticosteroid-binding globulin** (CBG) or **transcortin** which binds cortisol and corticosterone and **β-globulin—sex-hormone binding globulin** (SHBG) or **gonadal steroid-binding globulin** (GBG) which transports oestradiol and testosterone. CBG binds steroids with Δ^4-3-ketone and 20-ketone groups, while SHBG or testosterone-oestradiol binding globulin binds oestrogen and androgens with a 17β-OH group. A minor degree of binding to albumin also takes place. The bound hormone is essentially inactive. Normally, very little 'free' cortisol is present in plasma, but if secretion increases, there is rise in unbound fraction.

CBG and SHBG are synthesised in the liver and are increased by oestrogen. CBG and SHBG levels are elevated during pregnancy and

depressed in cirrhosis, nephrosis, etc. When CBG level rises, more cortisol is bound, producing a decrease in free cortisol level, which results in ACTH secretion.

Normally plasma contains 5-15 μg /100 ml of cortisol. Aldosterone content is 3-15 ng /100 ml. The cortisol level is higher in early morning (6-8 a. m.) and very low near midnight in man.

Major metabolities of adrenal steroid hormones are formed in the liver, kidneys and in the gut. During metabolism, ring structure remains intact, the adrenal steroid being inactivated by reduction of double bond and ketone groups, and rendered water-soluble by conjugation to form glucuronide and sulphate derivatives (Fig. 4.95). Adrenal corticoids

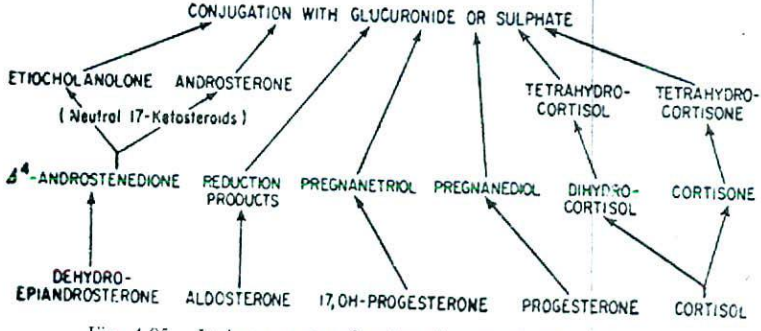


Fig. 4.95. It shows routes of major adrenocorticoid inactivation.

are inactivated mostly in the liver very rapidly. They are conjugated with glucuronic acid and the inactivated and conjugated products are excreted in the urine as tetrahydro forms coupled to glucuronide, DHEA as sulphate, progesterone as pregnanediol, testosterone as reduced compound such as androsterone and etiocholanolne which appear in the urine with a large quantity of 17-ketosteroids (recently called 17-oxosteroids, 17-OS) secreted by the adrenal, oestradiol as conjugated form after hydroxylation to oestriol or oxidation to oestrone. Inactivation takes place by bond reduction catalised by NADPH requiring hydrogenases and by reduction of the 3-ketone group by NADH or NADPH. Other tissues do not inactivate since the enzyme system necessary for inactivation are absent from them. About 10% of the secreted cortisol is converted in the liver to the 17-ketosteroids (17-oxosteroids) (*vide* urinary 17-ketosteroids). These are mostly conjugated to sulphates and excreted in the urine. In all cases, small amount of 'free' cortisol are excreted in the urine.

Mechanism of action of adrenal steroids. Many of the mechanisms of action of steroid hormones are explained by their action as a 'repressor' or 'de-repressor' of mRNA synthesis in their respective target tissues.

Cyclic AMP is known to be involved in the mechanism of actions of steroid hormones. There is evidence that the steroid may enhance the sensitivity of the responding system to the action of cyclic AMP. Cyclic AMP may be involved in the stimulation of thymocyte proliferation by glucocorticoids.

As glucocorticoids are important to the production of glucose from short-chain precursors by gluconeogenesis, so in gluconeogenesis they act by inhibiting protein synthesis in peripheral tissue and thus releasing free amino acids from muscle and other tissues to the liver. They also act by stimulating the breakdown of lipids in adipose tissue, resulting in the release of free fatty acids and glycerol. Thus glucocorticoids inhibit the activity of DNA-dependent RNA polymerases, directly protein synthesis from ribosomal RNA in thymocytes, and directly suppressing transcription or synthesis of RNA from DNA template. It is observed that injection of cortisol into rats leads to decreased glucose uptake by the thymocytes. Glucose or some other energy source is necessary for an inhibitory effect of cortisol, specially on the inhibition of uridine incorporation into RNA. According to Munck, a primary physiologic action of glucocorticoids is to decrease glucose utilisation by certain extrahepatic tissues. Suppression of glucose uptake may or may not be related to the decreased uptake or utilisation of amino acids.

Cyclic AMP may be involved in quite a different fashion in some of the effects of gonadal steroids. Intravenous injection of oestradiol causes rapid transient increase in the uterine level of cyclic AMP.

Many of the effects of steroid hormones are known to involve the stimulation of protein synthesis. Like other steroids, according to Edelman *et al.*, aldosterone specifically stimulates the transcription from nuclear DNA of a unique mRNA, regulating the synthesis of a protein enzyme that is rate-limiting for the production of ATP by the mitochondria (Fig. 4.96). Na^+ reabsorption mechanism of aldosterone is dependent on

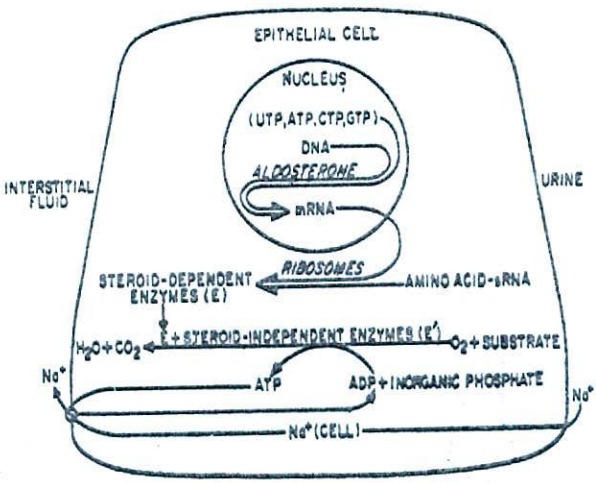


Fig. 4.96. It shows mechanism of action of aldosterone according to Edelman and others. The hormone stimulates the transcription from nuclear DNA of mRNA, regulating the synthesis of a protein enzyme concerned in the production of ATP by the mitochondrion. Na^+ is pumped out of the cell by an ATP-dependent process.

active transport mechanism. ATP required for active transport comes from the metabolic processes in the TCA cycle. It is proposed that

aldosterone apparently initiates an increased synthesis of mRNA which, on the other hand, stimulates protein synthesis at the level of ribosome. Newly formed proteins induced by aldosterone then enhance substrate oxidation and coupled ATP synthesis. This ATP is then utilised for the active Na^+ transport.

Functions. The chief functions of the active principles of adrenal cortex are briefly described below.

Active Principles	Chief Physiological Functions
I. Adrenal Corticoids	
1. GLUCOCORTICOIDS—Steroids with = O or ···OH at the 11C position Cortisol. Cortisone. Corticosterone. 11-Dehydrocorticosterone.	1. *On carbohydrate metabolism—Chief action.* Hence, called **glucocorticoids**—(a) stimulate formation of glycogen in the liver and muscles (same as inxulin), (b) increase gluconeogenesis in the liver (specially from proteins), (c) depress glucose uptake and oxidation by tissues (anti-insulin), the enzyme hexokinase helps in the phosphorylation of glucose to glucose-6-phosphate. Glucocorticoids together with STH interfere with the phosphorylation of glucose. (d) Help absorption of sugar from the intestine and possibly from renal tubules. (e) Cortisol raises the blood pyruvate level and helps in the synthesis of glucose from pyruvate in the liver. (f) Deficiency of cortisol produces hypoglycaemia and increases sensitivity to insulin. Excesss of cortisol produces hyperglycaemia and depresses sensitivity to insulin. 2. *On protein metabolism*—Glucocorticoids (a) increase the rate of deamination and breakdown of tissue proteins to amino acids. Body proteins are lost, increasing nitrogen excretion. They also decrease protein synthesis which might be due to interference in nucleic acid metabolism. (b) Increase gluconeogenesis in the liver (anti-insulin). (c) Excess of cortisol causes wasting of muscles, osteoporosis, dissolution of lymphoid tissue and increased excretion of creatine and uric acid in the urine. 3. *On lipid metabolism*—Glucocorticoids (a) stimulate lipid absorption from the intestine, (b) mobilise lipid from the depot and disintegrate to form ketone bodies in the liver. (c) Excess of cortisol causes redistribution of lipid in the body, increased lipid and cholesterol level in the blood. (d) Depress the synthesis of lipid from carbohydrate. 4. *On mineral metabolism—Minimum action.* There may be some retention of NaCl and water, and the increased excretion of K and PO_4. 5. *On muscle*—Cortisol and cortisone relieve muscular weakness found in hypofunction of adrenal cortex. 6. *Blood*—Anti-lymphocytic and anti-eosinophilic action—causes dissolution of lymphocytes in blood and lymphoid tissues, producing (a)lymphopenia in blood, (b) involution of thymus, lymph gland, spleen, etc., (c) eosinopenia in blood. 7. *Blood pressure*—Hypotension found in deficiency of the adrenal cortex is rectified after administration of cortisol. 8. Protect the body against stress. 9. *Bones*—Excess of cortisol causes osteroporosis due to decalcification and interference in formation of protein matrix.

Active Principles	Chief Physiological Functions
1. Glucocorticoids	10. *Central nervous system*—In hypofunction of the adrenal cortex, e.g., in Addision's disease, the function of the central nervous system is deranged. It is rectified after administration of cortisol. 11. *Cortisone*—has a special action in curing certain types of arthritis. [ACTH has same action probably through cortisone—*vide* page 4-27.] 12. *Anti-inflammatory effect*—At high concentrations, glucocorticoids decrease cellular protective reactions and in particular, retard migration of leucocytes into traumatised areas. 13. *Immunosuppressive effect*—Cortisol also decreases immune responses involved in infections allergic states and anaphylactic shocks. There is decreased synthesis of DNA and DNA-dependent RNA polymerase in lymphocytes. The action may be thus at the sites of nucleic acid and protein synthesis in immunologically active cells. 14. *Exocrine secretory effect*—Chronic treatment with glucocorticoids causes increased secretion of HCl and pepsinogen by the stomach and trypsinogen by the pancreas. Effects of cortisol on different biological processes have been presented schematically in Fig. 4.97.
2. MINERALOCORTICOIDS— (a) **Steroid with —OH or =O at the 11C position, and presence of an aldehyde group at the 18C position** Aldosterone. (b) **Steroid without =O or ···OH at the 11C position** Deoxycorticoid— Deoxycorticosterone or Deoxycortone.	1. *On mineral and water metabolism—Greatest action.* Hence, called **mineralocorticoids:** (a) retention of NaCl and water, (b) increased excretion of K, and (c) intracellular K lowered and Na raised. 2. *On carbohydrate and protein metabolism—Little action.* 3. More effective in protection against stress. 4. Does not inhibit the secretion of ACTH. [No reciprocal relation.] 1. *On mineral and water metabolism—Ponounced action, but less than Aldosterone. Also named as* **mineralocorticoids:** (a) retention of NaCl and water, (b) increased plasma volume, (c) increased excretion of K, and (d) intracellular K lowered and Na raised. 2. *On carbohydrate and protein metabolism*—Action much, less than glucocorticoids. 3. Regulates renal function. [*Vide* below.] 5. Most potent in maintaining life of adrenalectomised animals.
II. Sex steroids with =O or ···OH or a side-chain $CO\text{-}CH_3$ at the 17C position Androgen—androsterone. Oestrogen—oestradiol. Progesterone.	1. Action on sex organs and secondary or accessory sex characters similar as sex steroids.

Functions of adrenal cortex

Essential for life. From clinical and experimental studies, functions seem to be as follows:

1. **Control of mineral metabolism.** Mineralocorticoids, e.g., deoxycorticosterone, aldosterone, etc., increase reabsorption of NaCl and bicarbonates and depress that of potassium and phosphates by renal

tubules. Thus, by controlling the excretion of these salts, the mineralocorticoids keep an adequate balance of inorganic ions in blood, other body fluids and tissue cells. The *seat of action* is on the renal tubules where these hormones raise *the threshold for NaCl and bicarbonates and diminish that for K and* PO_4. Thus, not only increasing sodium reabsorption but also concomitantly increasing potassium excretion, but to a more variable degree.

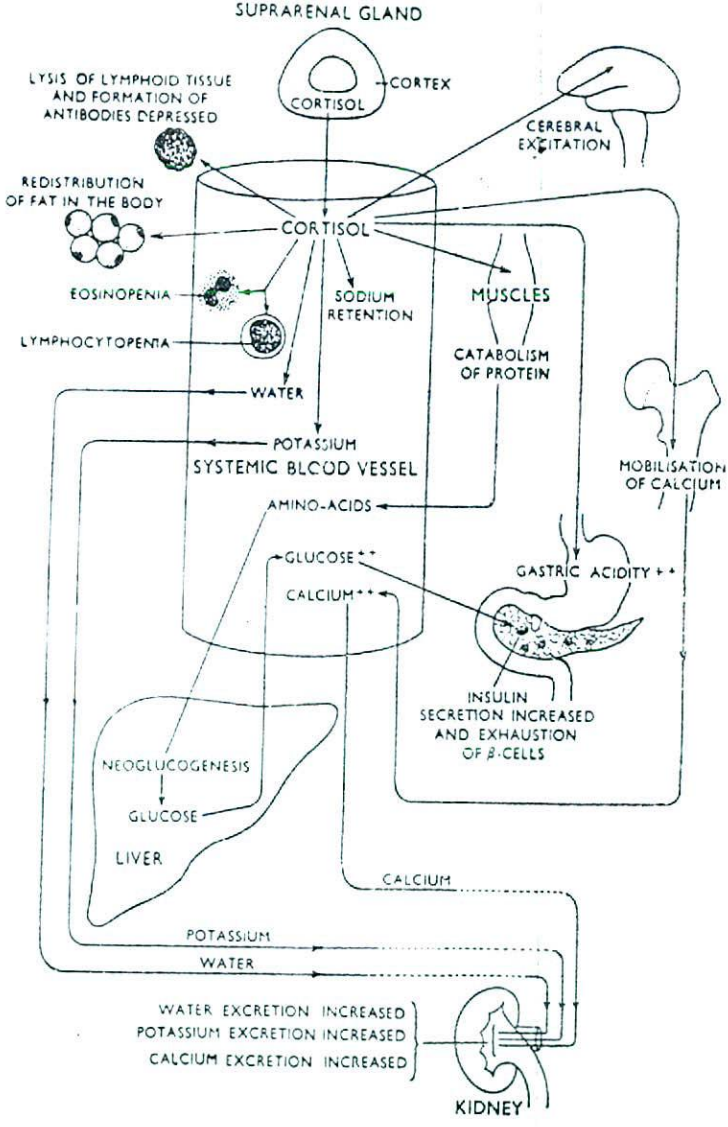


Fig. 4.97. Diagrammatic representation of the effects of cortisol.

Aldosterone is most effective in controlling the electrolyte balance, the site of action being the distal renal tubules.

2. **Control of water balance.** Adrenal corticoids stimulate water absorption by the renal tubules and thus regulate water balance. After adrenalectomy there is increased water loss by the kidneys. Despite the continued salt wasting, a defect in water excretion occurs as a consequence of which water loss lags behind salt loss, the patient becoming oliguric. Water may move into cells to produce cellular overhydration and water intoxication may occur. This diuresis is partly due to associated loss of NaCl and partly due to reduced reabsorption of water by the renal tubules.

3. **Control of carbohydrate, protein and fat metabolism:** (A) CARBOHYDRATE METABOLISM. Glucocorticoids produce the following effects: (a) Stimulate formation of glycogen in the liver and muscles (same as insulin). (b) Increase gluconeogenesis in the liver, specially from the proteins (antagonistic to insulin). (c) Depress glucose uptake and oxidation by tissues. The action of glucocorticoids to decrease oxidation of glucose by tissues is possibly mediated through its inhibitory action (along with STH) on glucose phosphorylation (antagonistic to insulin). (d) Help absorption of sugar from the intestine and possibly from the renal tubules. (e) Cortisol raises the blood pyruvate level and helps in the synthesis of glucose from pyruvate in the liver. (f) Excess of cortisol produces hyperglycaemia and depresses sensitivity to insulin. Deficiency of cortisol produces hypoglycaemia and increases sensitivity to insulin. The release of glucose from glycogen by epinephrine or glucagon depends on the presence of cortisol. This effect of cortisol is known as the **permissive action.**

Consequently, *the action is partly similar and partly antagonistic to insulin.* Aldosterone has got very little effect on carbohydrate metabolism.

(B) PROTEIN METABOLISM. Glucocorticoids (a) increase the rate of deamination and breakdown of tissue proteins to amino acids indicating increased liver transaminase activity. Body proteins are lost, increasing nitrogen excretion. Synthesis of protein is also reduced. (d) Stimulate gluconeogenesis from the amino acids. (c) They by antagonising the effects of insulin decrease the rate of synthesis of protein. The effect may be mediated through the metabolism of nucleic acids. (d) Excess of cortisol causes wasting of muscles, osteoporosis, dissolution of lymphoid tissues and increased excretion of creatine and uric acid in the urine.

Aldosterone has got very little effect on protein metabolism.

(C) FAT METABOLISM. Glucocorticoids (a) stimulate lipid absorption from the intestine. (b) Stimulate mobilisation of lipid from the depots and its disintegration in the liver to form ketone bodies through their ability to mobilise free fatty acid (FFA). In the intact animal, insulin stimulates *lipogenesis* hence ketosis is rarely found in Cushing's syndrome. (c) Excess of cortisol causes redistribution of fat in the body with increased deposits on the trunk and shoulders at the expense of fat in the extremeties while the fat in the arms and legs is diminished. There is increased lipid and cholesterol level in the blood. (d) Depress the synthesis of lipid from carbohydrate. Chronic overproduction of cortisol causes lypolysis, hyperlipidaemia and hypercholesterolaemia and increases the incidence of atheroma.

STH and ACTH also accelerate the breakdown of fatty acids, all acting antagonistically to insulin.

Aldosterone has got very little effect on protein metabolism.

4. **Control of phosphorylation.** Glucocorticoids are believed to help the action of *phosphatase, phosphorylase,* etc., and thus control all the processes involving phosphorylation. The metabolic functions of adrenal cortex, specially on carbohydrates and fats are supposed to be due to its influence on phosphorylation.

5. **Relation with digestive functions.** Adrenal corticoids may have some relations with the normal digestive process. In deficiency (a) diarrhoea, (b) vomiting, (c) anorexia, etc., have been observed. Glucocorticoids and ACTH increase gastric acid and pepsin secretion in some species. In man there is altered resistance of the mucosa to the irriant actions of the gastric secretions leading to formation of ulcers. Water-insoluble lipid absorption from the intestine is facilitated. Cortisol is concerned in the secretion of hydrochloric acid and pepsin by the stomach.

6. **Control of basal metabolic rate.** In some way adrenal corticoids control BMR; a fall even 25% below normal has been noted in adrenal cortical deficiency.

7. **Anti-lymphocytic abd abci-eosinophilic action.** Cortisone, cortisol, etc., cause rapid destruction of the number of lymphocytes in the blood, reduce the blood eosinophils without affecting the marrow eosinophil count (page 4-147) and also cause involution of thymus and other lymphoid structures (page 4-160). Hypofunction of adrenal cortex is generally associated with lymphocytosis, eosinophilia, neutropenia and anaemia. In tropical eosinophilia treatment with adrenocortical steroids is indicated.

Aldosterone has about half eosinopenic activity.

8. **Control of normal composition, volume and pressure of blood.** Adrenal corticoids regulate the volume, composition and pressure of blood. In adrenal deficiency—(a) blood volume and pressure fall; myocardial weakness develops. In hyperfunction of the adrenal cortex the blood pressure rises, which may lead to the formation of angiotensin II. Angiotensin II in its turn enhances the secretion of aldosterone. (b) Haemoconcentration takes place—specific gravity, cell count, percentage of haemoglobin and concentration of plasma proteins in blood—increase. Both (a) and (b) are due to increased water loss by the kidneys and passage of more water from the blood stream into the tissue spaces. (c) Retention of nitrogen, increased excretion of potassium and PO_4, reduced NaCl and bicarbonate occur. (d) *Vascular reactivity*—In adrenally insufficient animals, vascular smooth muscles become unresponsive to norepinephrine and epinephrine. The capillaries dilate and ultimately lead to vascular collapse. Glucocorticoids restore the vascular reactivity.

9. **Control of kidney functions.** Adrenal corticoids help to regulate normal kidney function. After adrenalectomy kidney function is depressed. At first, excretion of water increases, then urine volume diminishes. This is partly due to low renal circulation and partly to a direct depression of kidneys. Due to low renal circulation and fall of glomerular pressure there is less glomerular filtration and diminution of the quantity.

of urine. Renal efficiency falls, nitrogen retention takes place, leading to uraemia. Diuretic response to water drinking is not seen after adrenalectomy.

10. **Effects on nervous system.** Adrenal corticoid hormones specially glucocorticoids are in some way related to the normal functioning of the nervous system. In deficiency personality changes and slower EEG waves have been noted. Both excess and deficiency of cortisol may cause mental depression and psychosis

11. **Relation with sex.** Sex hormones of the adrenal cortex are believed to control the differentiation of sex in the foetus, and growth of sex glands, sex organs and secondary sex characters after birth. *Proof:* (1) *Cortical tumours* cause—(a) pseudohermaphroditism—in foetal life, (b) precocious sexual development—in children, (c) reversal of sex characters, adrenal virilism—in adults. (2) In Addison's disease—depression of sex. (3) Cortex produces sex hormones. (4) Sex hormones in large doses exert similar actions as corticoid sex hormones. (5) Adrenal cortex and gonads develop from the same parent tissues. (6) Persistence of 'libido' after castration—is supposed to be, at least partly, due to the sex hormones from the adrenal cortex.

12. **Provides resistance against various stress.** Adrenal corticoids help the body to resist against various physical and mental stress, viz., H-substances, low temperature, low oxygen pressure, mental and physical strain, etc. Subjects with Addison's disease easily succumb under such conditions. The glucocorticoids are effective in protecting the body against stress.

13. **Relation with melanin formation.** In Addison's disease excess melanin is deposited leading to bronzing of skin and mucosa. It is due to increased secretion of the melanocyte-stimulating hormone from the pars intermedia of the pituitary gland. So there might be some relation between adrenal cortex and secretion of melanocyte-stimulating hormone (MSH) from pars intermedia of the pituitary gland and it is suggested that cortisone and hydrocortisone inhibit the release of the hormone.

14. **Storage of vitamin C.** The cortex is very rich in vitamin C about 200 mg per 100 g of cortical tissue; it is stored here. When cortical secretion is stimulated its ascorbic acid content (also cholesterol content) falls. This is used as an index for stimulated cortical secretion.

15. **Bone metabolism.** Excess cortisol impedes the development of cartilage and causes thinning of the epiphyseal plate and interruption of growth in children. Cortisol being antagonistic to vitamin D prevents absorption of calcium from the gut, when given in excess. There is also decreased deposition of calcium in protein matrix of bone.

16. **Effects on cardiac and skeletal muscle.** Glucocorticoids and mineralocorticoids have a positive inotrophic effect on cardiac muscle *in vitro*, but it is not known whether this digitalis-like action is effective *in vivo*. The P-R interval of ECG is prolonged in adrenal insufficiency. In adrenalectomised animals, skeletal muscles become fatigued rapidly and glucocorticoid treatment is necessary for restoration of muscle to normal.

17. **Anti-pyretic effect.** Cortisone as well as ACTH are antipyretic in that, they reduce an elevated body temperature to a normal level and reduce any toxemia, that may be present. They are particularly

effective in inhibiting the effects of the endotoxins of Gram-negative organisms. The mode of operation is not known as yet.

18. **Anti-inflammatory and anti-allergic response.** Glucocorticoids have an effective therapeutic influence against any type of inflammatory and allergic syndrome. These glucocorticoids have profound effects on allergic and immune responses triggered by foreign antigens such as those in pollen or bacteria. Because, glucocorticoids suppress the formation of antibodies and reduce the response to histamine and other inflammatory agents.

HYPOFUNCTION OF ADRENAL CORTEX

Acute insufficiency of adrenal corticoids sometimes occurs when the subject is exposed to sudden stress or failure of secretion of ACTH after prolonged administration of glucocorticoids. In acute phase adrenal cortex fails to secrete cortisol.

Chronic insufficiency of adrenal corticoids occurs in Addison's disease.

ADDISON'S DISEASE

Addision's disease is usually due to tuberculosis of the gland, involving both cortex and medulla. But the effects are mainly due to cortical damage and consequently, insufficient secretion of cortisol and aldosterone.

Clinical features

Muscular weakness and easy fatiguability: Muscular weakness is due to loss of sodium chloride and defective power of glycogen formation.

Vomiting, anorexia, hypochlorhydria and gastro-intestinal disturbance.

Low blood pressure: The systolic blood pressure remains at about 90 mm Hg, and the diastolic, at 60-65 mm Hg.

Pigmentation (bronzing) of the skin (specially in the exposed areas) and mucous membrane: Catecholamine production is deranged with the formation of the dark pigment **melanin** instead of adrenaline and noradrenaline. The skin becomes pigmented. It has been tentatively attributed to an increased secretion of MSH (Lunce *et al.*, 1956) or probably by excess ACTH (by feedback mechanism) possessing the melanocyte-stimulating property. [It is not seen in adrenalectomised animals, because the animals die within a week and the time is not sufficient for the formation of pigment. In some animals however a brownish colour has been reported following adrenalectomy.]

Low *BMR* and subnormal temperature.

Disturbed ionic balance, viz., (a) increased excretion of NaCl and decreased excretion of potassium in the urine. This leads to (b) fall of NaCl (and bicarbonate) and rise of K in the plasma. *The primary action is on the renal tubules.*

Decreased blood volume and haemoconcentration: Sodium in the extracellular fluid falls. The cell membranes are impermeable to Na^+. The crystalloid osmotic pressure inside the cells thereby becomes relatively higher than the extracellular fluid. Water is withdrawn from the extracellular into intracellular fluid. Due to all these changes the blood cell count, percentage of haemoglobin and concentration of plasma proteins increase.

Increased capillary permeability: More water passes out of the capillaries and accumulates in the tissues, causing oedema.

Deficiency of kidney function: Glomerular filtration is decreased causing low urine volume, nitrogen retention and ultimately uraemia. Large intake of NaCl and restriction of potassium improve the condition.

Disturbance of carbohydrate metabolism: (a) Absorption of sugar from the small intestine (and probably from the renal tubule) is slowed down, due to depressed *phosphorylation.* (b) Glycogenesis (from glucose or lactate) and gluconeogenesis (specially from proteins) in the liver are depressed. (c) Hypoglycaemia may occur. *Hypoglycaemic unresponsiveness* is also seen. (d) Skeletal muscles show defective power of glycogen formation. (e) There may be glycosuria (uncommon) due to depressed reabsorption of sugar (defective phosphorylation).

Absorption of lipid from the intestine and its metabolism is diminished.

Restlessness, insomnia, lack of mental concentration, etc., also occur.

Excretion of 17-ketosteroids (17-oxosteroids) in urine is much reduced and in females it almost becomes nil.

Depression of sex functions.

Terminal changes: (a) Rate of deamination of amino acids, (b) rate of ketone formation in the liver and their utilisation, (c) the process of phosphorylation, (d) the rate of O_2 consumption, (e) BMR and body temperature, etc., fall. Ultimately causes, (f) circulatory collapse, and (g) uraemia. These changes are probably secondary to the disturbed ionic balance. Because, by appropriate salt therapy the condition clears up.

HYPERFUNCTION OF ADRENAL CORTEX

A. Cushing's Syndrome.
B. Hyperaldosteronism.
C. Adrenogenital syndrome.

A. Cushing's Syndrome

Cushing's syndrome is found in adrenal tumour or adrenal hyperplasia and there is excessive secretion of cortisol. Pituitary tumours causing excess secretion of ACTH may be the reason of this syndrome. [*Vide* Cushing's disease—page 4-41.]

Clinical features

1. There is an increased deposition of fat on the trunk (but not the limbs), face (rounded or moon-face—the plethoric *moon-face* of the textbook is often absent in Cushing's syndrome, although a thick neck and fat below the chin is usually seen), characteristic pad of fat at the back of the neck (a buffalo hump) and abdomen. Extremeties are usually spared. Fatty deposits are tender and painful. The skin bruises easily and shows purple striae with hirsutism in the female. Purple striae are usully found over the abdomen, thigh, etc. This is due to loss of protein matrix.
2. *In males* excessive hair-growth (hirsutism). *In females* masculinisation with growth of bread, moustache, etc.
3. Asthenia and wasting of the muscles of the limbs.
4. Wounds heal poorly and minor injuries cause bruises and echinoses.

5. Osteoporosis of bones due to decalcification and loss of protein matrix.
6. Hypertension.
7. Mental derangement.
8. In males importance with atrophy of testis. In females amenorrhoea, sterility, etc.
9. Hyperglycaemia and insulin-resistant diabetes with glycosuria.
10. Retention of sodium and diminution of potassium level in the plasma.
11. Eosinopenia, lymphocytopenia, and polycythaemia.
12. Increased excretion of 17-ketosteroids (17-oxosteroids) and 17-hydroxycorticosteroids.

B. Hyperaldosteronism

Hyperaldosteronism may be primary or secondary. An excessive production of aldosterone commonly due to a tumour of the zona glomerulosa tissue of the adrenal cortex is termed **Conn's disease** or *primary aldosteronism* Hypertension, muscular weakness, retention of sodium, alkalosis, etc., are found. There is no oedema. Prolonged potassium depletion damages the kidneys, resulting in polyuria. The potassium depletion causes muscle weakness, metabolic alkalosis which may lower ionised calcium level resulting in tetany. *Secondary hyperaldosteronism* occurs in congestive cardiac failure, cirrhosis of liver with ascites, etc.

C. Adrenogential Syndrome

The main characteristic of **adrenogenital syndrome** is the overproduction of adrenal androgens. It is associated with tumours of the adrenal cortex.

Clinical features

In foetal life: Produces pseudohermaphrodites (Eunuchs). Both male and female sex characters are present in the same subject, but both incomplete.

In childhood: (a) *Females*—Precocious sex development, viz., early menstruation, breast formation, etc. (b) *Males*—Precocious growth of sex (puberty) and body—producing the so-called 'Pocket Hercules' or 'Infant Hercules'.

In adults: Reversal of sex characters is the main feature. (a) In females—virilism. Female changes into male. Face, body appearance, voice—become of male type. Beard and moustache grow. Menstruation stops. Uterus and ovaries degenerate. Clitoris enlarges. (b) In males—*feminisation* may occur (but not common).

Similar changes may also result from congenital inborn errors of metabolism which derange the production of cortisol by the gland.

Laboratory studies for adrenocortical functions. A Urinary 17-hydroxycorticosteroid. The level of urinary 17-hydroxycorticosteroid gives an indication about the functional status of the adrenal cortex. The normal twenty-four-hour excretion of these corticosteroids in adult males is 10 ± 3 mg (approx.) and in adult females 7 ± 2 mg. The level of this urinary 17-hydroxycorticosteroid (17-OHCS) is increased after administration of ACTH. If it is abnormally increased then it is strongly suggests that the patient has got hyperactive adrenal cortex.

Metyrapone (metopirone) may be used as an diagnostic test for adrenocortical function. It is an enzyme inhibitor and decreases the formation of glucocorticoids through inhibition of 11β-hydroxylase. After infusion of metopirone if urinary cortical excretion is decreased then favours the diagnosis of adrenal neoplasm rather than hyperplasia. **Amphenone** and **dichloro-diphenyl-dichloroethane** (DDD) block all steroid secretions. The former causes adrenocortical hypertrophy and the latter produces necrosis of cortical cells (Fig. 4.98).

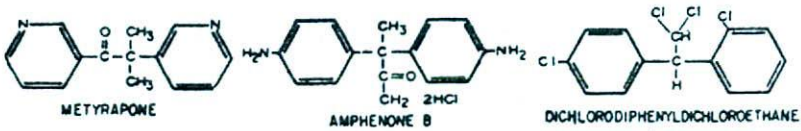


Fig. 4.98. It showing some drugs which inhibit adrenocortical secretion.

Porter-Silber colour reactions using phenylhydrazine and sulphuric acid are often followed for the estimation of urinary corticoids. This reaction is not specific because it gives colour reaction for 17,21-dihydroxy-20-ketosteroids such as cortisone, cortisol, 11-deoxycortisol and also reduced tetrahydro derivatives. For specific assay, prior chromatographic separation is required.

B. **Urinary 17- ketosteroids.** There are three types of 17-ketosteroid (17-oxosteroids,) such as (a) phenolic, (b) acidic and (c) neutral. Acidic compounds are derived from bile acid, phenolic from oestrogen and neutral mainly from adrenal cortex and gonads.

Neutral urinary 17-ketosteroids (17-oxosteroids) are androsterone and its isomers, etiocholanolone, iso-androsterone and dehydro-epiandrosterone (DHEA). 17-ketosteroids (17-oxosteroids) may be further divided into α- and β-fractions. β-fractions are precipitated by digitonin whereas α-fractions are not.

Androsterone is α and DHEA is β.

Urinary 17-ketosteroids (17-oxosteroids) may be estimated by Zimmermann reaction, involving the reactive 17-keto group to react with the *m*-dinitrobenzene.

Normal urinary excretion of neutral 17-ketosteroids in 24 hours is 10-20 mg (approx.) in adult male and 5-15 mg (approx.) in adult female. The ratio of α- to β-ketonic fraction is 9:1.

Urinary 17-ketosteroid gives an indication about the androgenic function of the subject. It is produced both from the adrenal cortex and testis in adult male and entirely from adrenal cortex in adult female. Thus urinary 17-ketosteroid (17-oxosteroid) does not represent the absolute measurement of adrenocortical activity in male.

C. **Urinary aldosterone.** Determination of urinary aldosterone level is of great importance in the diagnosis of mineralocorticoid activity of the adrenal cortex.

NATURE OF CORTICAL SECRETION

Methods of study. (1) To note the cholesterol and vitamin C content of adrenal cortex. During cortical secretion they fall, during rest they rise. (2) Blood from

suprarenal vein, injected in adrenalectomised animals—to see the presence of hormone in it– in various ways, e.g., (*a*) lymphopenia, eosinopenia, (*b*) maintenance of life, etc.

From such studies the following facts are known: (1) **Continuous secretion.** Adrenal cortex is continuously secreting. In 24 hours it secretes hormones equivalent to the amount extractable from 17,300 g of gland. (2) **Rate of secretion does not depend upon blood pressure, blood flow or nerves.** (3) **Rate of secretion increases** in muscular exercise, trauma, cold, low atmospheric pressure (high altitude), certain toxic substances (all included by the term *stress and strain*).

CONTROL OF CORTICAL SECRETION

I. Hormonal control. A. **Anterior pituitary.** ACTH is the chief controller. *Proof:* (a) Addition of ACTH to the perfusing blood, even in the isolated gland, stimulates secretion. (b) Hypophysectomy causes degeneration of adrenal cortex and marked diminution of secretion of corticosterone, cortisone, hydrocortisone, but *slight* diminution of secretion of aldosterone. The secretion of ACTH in turn is regulated by corticotrophin-releasing factor (CRF) which is released from the hypothalamus during stress. CRF acts hurriedly on the anterior pituitary, causing ACTH release within a minute or two. Normal secretion of ACTH is cyclical, leading to a circadian or nycterohemeral rhythm in steroid secretion by the adrenal gland, with very low plasma-cortisol level at or soon after midnight and the highest level at about 6 to 8 a.m. and thereafter fall again slowely (Fig. 4.99). The daily rhythmic secretion of ACTH and cortisol may appear to be related to the sleep pattern and may be modified by alterations in daily activity. **Adrenal cortex** regulates its own secretion. B. **Feedback control.** There is evidence to prove that the concentration of cortical hormones (glucocorticoids) in blood regulates ACTH-formation. This is the *feedback mechanism*. Rise of cortical hormones depresses, whereas a fall stimulates ACTH secretion by anterior pituitary. In the hypothalamus, secretion of CRF is influenced by negative feedback control from the plasma-tree cortisol level, a mechansim imposing diurnal variation, and stressful stimuli mediated by CNS. There is presumably also direct feedback control by cortisol to the corticotrophin-producing cells in the anterior pituitary. The 39-amino acid ACTH peptide chain is secreted by specific basophil cells in response to these stimuli and combines with membrane receptors on the zona fasciculata cell, leading to the formation of cyclic AMP. This cyclic AMP stimulates pregnenolone synthesis in the mitochondria (Fig. 4.100).

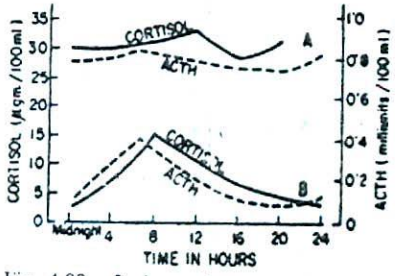


Fig. 4.99. It shows plasma-cortisol and ACTH levels at B in a normal subject and a patient with Cushing's syndrome at A.

In such feedback control cortisol and cortisone are most effective, aldosterone regulates the secretion to some extent whereas deoxycorticosterone (DOC) has only one-tenth activity. Hyperplasia of the gland occurs after repeated injections of ACTH.

II. **Nervous control.** Stimulation of the hypothalamus (stress, excitement, cold, etc.) releases a chemical mediator, *corticotrophin-releasing*

factor (CRF), which is carried through local blood circulation to the anterior lobe of the pituitary gland and stimulates secretion of ACTH which in its turn causes secretion of adrenal cortical hormones.

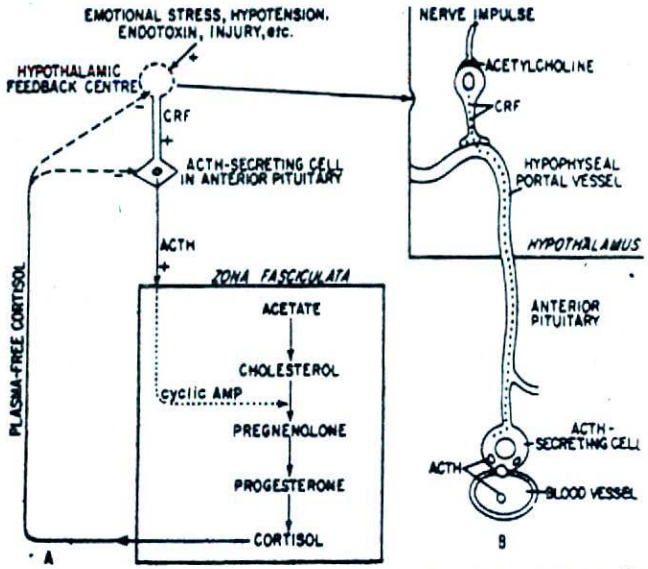


Fig. 4.100. Scheme shows pathways for secretion of cortisol. A shows those factors which can affect the hypothalamus directly to bring about the secretion of cortisol. In B the increased secretion of cortisol in response to injury comes about as a consequence of nerve impulses from the injured area to the hypothalamus where CRF is released and carried to the anterior pituitary *via* the hypophyseal portal system.

III. **Glomerulotrophin.** Secretion of aldosterone is controlled by chemical mediator known as *glomerulotrophin* liberated from the **pineal gland.** This hormone exerts no influence on liberation of cortisol. An *anti-ACTH factor* which inhibits secretion of both cortisol and aldosterone, has also been isolated from the pineal gland.

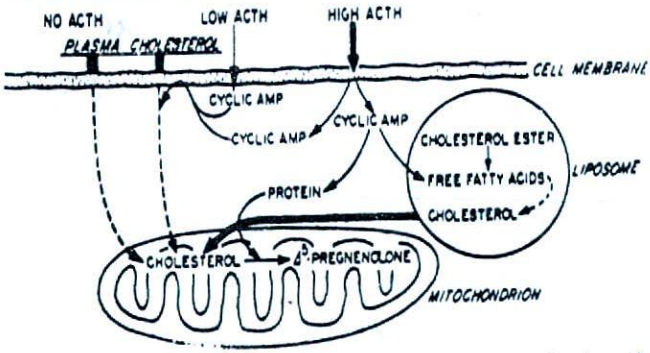


Fig. 4.101. Diagrammatic representation of mechanism of action of adrenocorticotrophin.

IV. **Inorganic control.** Diet low in sodium or high in potassium stimulates secretion of aldosterone.

V. **Blood volume.** Diminished blood volume increases the section of aldosterone whereas increased blood volume produces opposite effect.

Mechanism of action of ACTH

Since a major source of NADPH is the oxidative degradation of glucose-6-phosphate *via* phosphogluconate shunt, ACTH might increase corticosteroid synthesis by increasing the activity of phosphorylase and since cyclic AMP induces increased phosphorylase activity in liver, it also increases phosphorylase activity in the adrenal cortex.

According to Haynes, ACTH produces the accumulation of cyclic AMP in bovine adrenal slices, and cyclic AMP is itself, in turn, capable of stimulating steroidogenesis. It was also shown that ACTH increases adenyl cyclase activity in rat adrenal homogenates. Thus ACTH acts by stimulating adenyl cyclase activity, leading to accelerated formation of cyclic AMP from ATP. Cyclic AMP then serves as an intracellular mediator of the effect of ACTH on steroidogenesis. The involvement of protein synthesis along with increase in liver RNA in the process of steroidogenesis by cyclic AMP has been suggested by many investigators. Actinomycin inhibits protein synthesis, induced by cortisol but not the effects on hepatic glycogen or blood sugar

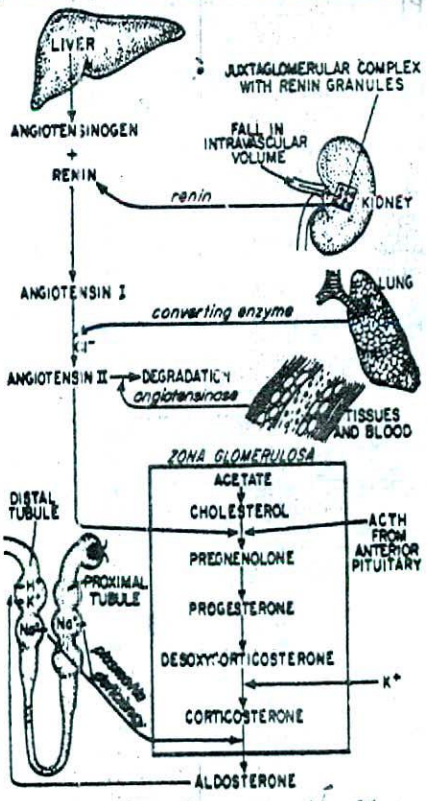


Fig. 4.102. Schematic representation of factors stimulating aldosterone secretion in the zona glomerulosa.

In hypophysectomised animal, plasma and adrenal cholesterol are not used for steroid biosynthesis. At normal, non-stimulated concentration of ACTH, plasma cholesterol is available for biosynthesis. At abnormal, high concentration of ACTH, there is an added effect on steroid synthesis depending on the cyclic AMP. In order for increased steroid synthesis to occur, a protein must be produced (probably by the mitochondria) which facilitates the movement of free cholesterol into the steroid synthetic pathway (Fig. 4.101). Thus, acute stimulation by ACTH causes mobilisation of steroid cholesterol and cholesterol ester stores from the lipid droplets. Long-term administration of ACTH continues the acute mechanism, but also produces general stimulation of protein synthesis, e.g., hyperplasia and hypertrophy, of the gland.

Renin-angiotensin mechanism

The main regulatory control of aldosterone secretion is mediated through *renin* (mol. wt. 57,000) liberated from juxtaglomerular cells. These cells respond to changes in the diameter of the renal arteriole and the renin liberated, converts into plasma α_2-globulin ultimately to *angiotensin II* (half-life in the circulation of only one minute). The latter compound stimulates zona glomerulosa cells to release aldosterone and also other adrenal cortical hormones to some extent. Recent experiments have shown that a specific aldosterone-stimulating hormone (ASH) is also an immediate effector of aldosterone production by the adrenal cortex (Fig. 4.102). A relation of aldosterone secretion to potassium has also been established. Oral contraceptive pills increase renin and aldosterone secretion.

INTERRELATION OF ADRENAL CORTEX WITH ANTERIOR PITUITARY, THYROID GONADS, ENDOCRINE PANCREAS, THYMUS AND PINEAL GLAND

1. **With anterior pituitary.** Reciprocal relation. Anterior pituitary through ACTH – stimulates growth and secretion of adrenal cortex, while level of corticoid hormones in blood stream controls ACTH secretion. Rise depresses, fall stimulates. Administration of ACTH in normal animals causes hypertrophy of zona fasciculata and zona reticularis, while hypophysectomy causes degeneration of these layers. Since ACTH affects the metabolism of carbohydrate, protein and fat—it is believed that the inner two layers secrete *glucocorticoids* while the outer layer (zona glomerulosa) secretes *mineralocorticoid*, e.g. aldosterone.

2. **With thyroid.** Injury to the adrenal cortex increases basal metabolic rate by 60%. This is not found after thyroidectomy. After thyroidectomy cortex enlarges. The interrelation is complex. May be through anterior pituitary.

3. **With gonads** (page 4-151).

4. **With insulin.** Peripheral action partly similar and partly antagonistic (*vide* pages 4-120, 4-147, 4-150).

5. **With thymus.** Adrenal cortex takes some part in causing the normal thymic involution. *Proof:* (a) Administration of corticosterone produces thymic atrophy in animals. (b) Cortical hypofunction causes enlargement of thymus in man. (c) When adrenalectomised male rats are kept alive for 45 days by adequate NaCl therapy, thymic involution fails to occur. This shows that mineralocorticoids, i.e., those controlling inorganic metabolism, are responsible for this effect on thymus.

6. **With pineal gland.** Pineal gland probably controls aldosterone secretion through *glomerulotrophin*. It also liberates an anti-ACTH factor.

GENERAL ADAPTATION SYNDROME

It is known that after adrenalectomy or in adrenal deficiency (Addison's disease) the animals fail to react quickly and effectively to environmental changes. They easily succumb under *stress and strain*, viz., infection, toxin, *cold*, anoxia, etc., which a normal animal can easily withstand. Selye has shown that if the strain be continued for some length of time

certain morphological and functional changes take place in various glands and tissues which enable the animal to adapt itself successfully to the changed environment, and that, adrenal cortex takes a prominent role in the process.

It is no wonder that adrenal cortex, with its immense influence on organic and inorganic metabolism, on kidney functions, on sex, on immunity mechanism, etc., will play an essential role in the process of adaptation. After all, adaptation means the calling-up of the reserves of the body and to set up a new metabolic balance at a new gear and if that be so, then adrenal cortex, with its all-abiding influence on every cell of the body must naturally play a very prominent role.

The **mechanism** as to how the stress, strain, toxin, etc., act, is also getting somewhat clearer, Roughly, it may be as follows: Stress and Strain, etc.,→Cerebrum→Hypothalamus. From Hypothalamus, three possible ways: (1) One way is Hypothalamus→Anterior pituitary→to other glands and organs. But specially ACTH→Adrenal cortex. (2) Another way is Hypothalamus→Sympathetic→Adrenal medulla (adrenaline)→to other tissues, organs and glands specially to anterior pituitary→increased ACTH secretion→Adrenal cortex. (3) The third possible way is Hypothalamus→ →Parasympathetic→Insulin, etc.,→Peripheral action of corticoid hormones checked.

The whole process of adaptation is called by Selye as **general adaptation syndrome.** When an animal is kept in *cold,* three stages are described during adaptation:

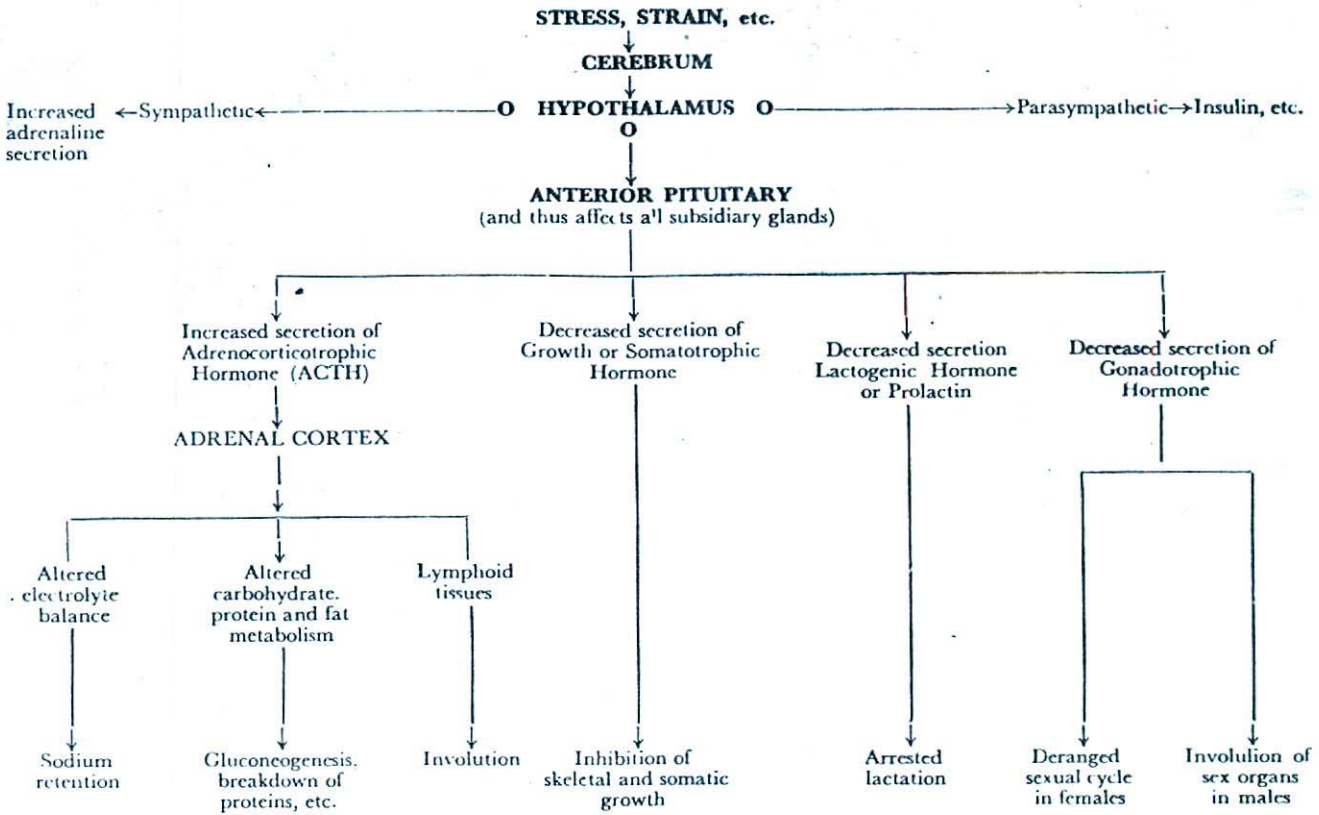
1. **Alarm reaction.** This is the first stage and comes within 6-24 hours. The stress is at first too severe and the animal seems to bend under the below. (a) *General changes*—fatigue, loss of muscle tone, diminished urine. (b) *Blood changes*—fall of NaCl and sugar. Lymphopenia. (c) *Changes in glands.* (A) Adrenal gland—both medulla and cortex—shrunken. Granules disappear from the cells. (B) Thymus and lymphoid tissues—involution. If the strain be too severe the animal may die in shock. If not, the second stage appears.

2. **Stage of resistance.** The animal recovers and gains back a good deal of its normality. Blood sugar and chloride become normal. Urine output increases and there is large amounts of adrenocorticoid hormone in it. The adrenal cortex enlarges and granules reppear in the cells. Other organs and glands regain their normal structure and functions. Thus the animal gradually adapts itself against ssress. Such adaptive mechanism may, on the other hand, induce a number of pathological changes like arthritis, arteriosclerosis, ulceration of digestive tract, etc. Excess of ACTH and adrenocortical steroids produced as a result of such stress causes such "adaptation disease" described by Seyle.

3. **Stage of exhaustion.** If the stress is maintained and increased further, the resistance gradually fails after one or two months. All the signs of failure as described under first stage, reappear and the animal dies in prostration.

The chief features of **general adaptation syndrome** (Selye) are summarised in the scheme given on page 4-162.

GENERAL ADAPTATION SYNDROME



ADRENAL MEDULLA

Histology. Adrenal medulla consists of irregular masses of polyhedral granular cells, surrounded by blood sinuses. Granules represent stored adrenaline, disappearing during secretion and reappearing after rest. They stain black with osmic acid, green with iron salts and brown with chromic acid, For the last property, they are called *chromaffin (glanglion) cells.* (Fig. 4.103). Some of the chromaffin cells secrete epinephrine and some norepinephrine. Small lymphocyte-like cells with dark nucleus and scanty cytoplasm, called *sympathogonia* are also present. Staining reactions help to determine the limit between cortex and medulla.

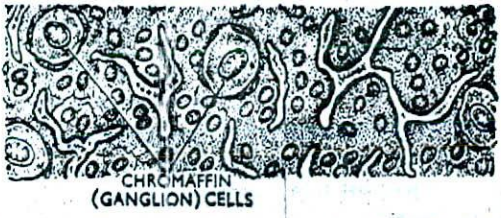


Fig. 4.103. Diagrammatic representation of histological structures (magnified) of adrenal medulla of the suprarenal gland.

Electron microscopic structure of adrenal medullary cells shows dark granules (Fig. 4.104). Each granule is enclosed by a smooth-surfaced membrane and is denser in its core than in its periphery. Average diameter of the granules are about 200 nanometres. Number of granules in different cells may vary probably in different stages of secretory activity.

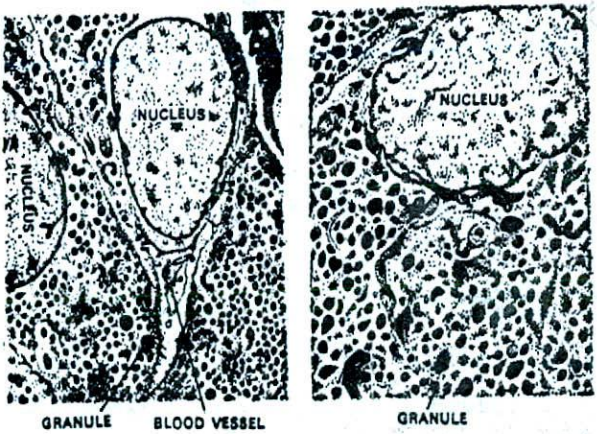


Fig. 4.104. Diagrammatic representation of electron micrographs of adrenal medullary cells showing abundant membrane-limited dense granules which are sites of storage of catecholamines.

Besides this, there are two types of cells: one is specialised to secrete norepinephrine and the other to secrete epinephrine. Granule formation does not involve linking of amino acids in specific segments but merely bringing about successive changes in an amino acid. The granules are enclosed by a smooth-surfaced membrane and thus packaging process does take

place in the Golgi apparatus. Membrane-enclosed granules move towards the cell surface during discharge. The medullary cells do not have many rough-surfaced vesicles. Moderate content of free ribosomes is also present.

Accessory medullary tissues (*vide* page 4-136).

Development, blood supply and **nerve supply** (*vide* pages 4-136, 4-137)

Adrenal medulla produces *catecholamines*, e.g. *epinephrine, norepinephrine* or *levarterenol* and dopamine.

Chemistry and biosynthesis of adrenomedullary hormones

The principal catecholamines (Fig. 4.105), found in the body, are norepinephrine, epinephrine and dopamine. These catecholamines are formed from amino acids, tyrosine and phenylalanine by hydroxylation and decarboxylation. Steps and enzymatic processes involved in

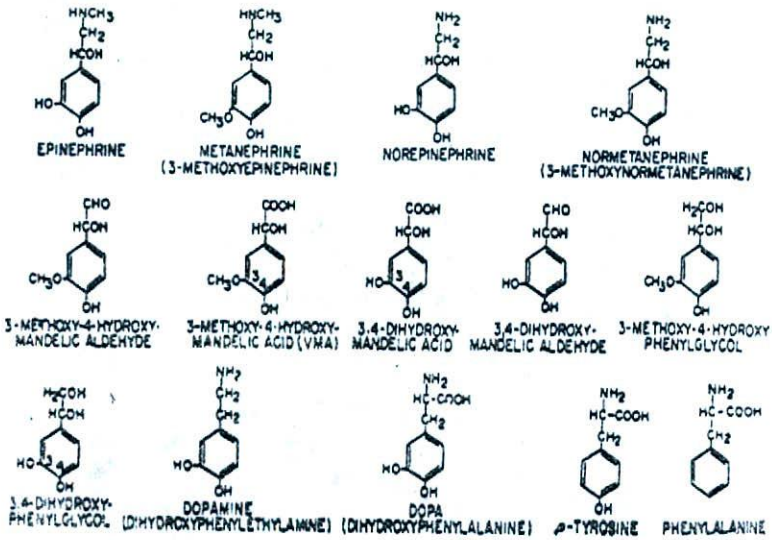


Fig. 4.105. Chemical structures of catecholamines.

the synthesis of catecholamines from phenylalanine and tyrosine have been presented in Fig. 4.106. *Phenylalanine hydroxylase* takes place in the conversion of phenylalanine to tyrosine, which is formed in the liver because this specific enzyme is present in the liver. Tyrosine thus formed from phenylalanine and also of the dietary sources are transported to the adrenal medulla. There it is converted to DOPA by tyrosine hydroxylase. DOPA on decarboxylation by enzyme aromatic L-amino acid decarboxylase, dopamine is formed. Dopamine thus formed enters the granulated vesicles (storage vesicles), within which it is converted to norepinephrine by the enzyme dopamine β-oxidase. The rate-limiting step in the synthesis of catecholamines is the conversion of tyrosine to DOPA.

Synthesis in adrenergic nerve endings is similar up to this stage. In the adrenal medullary cells there is cytoplasmic enzyme phenylethanolamine-N-methyl transferase (PNMT) in high concentration. This enzyme converts the norepinephrine to epinephrine. Thus appreciable amount of

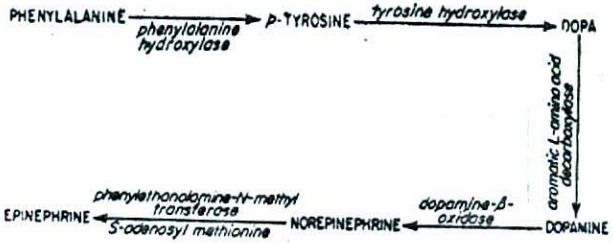


Fig. 4.106. Schematic representation of biosynthesis of catecholamines. PNMT is only present in high concentration in the adrenal medulla. That is why excess amount of adrenaline is being formed in this organ.

epinephrine is formed in the adrenal medulla. The amines are held in the storage vesicles by an active transport system. In the storage vesicle norepinephrine and epinephrine are bound to ATP and protein.

Release of catecholamines. Very little is known about the mechanism of release of catecholamines from the storage granules by acetylcholine.

Catecholamines are released from the adrenal medulla by a process known as **exocytosis** (Axelord and Weinshieboum, 1972). After chemical stimulation, followed by membrane depolarisation, the **chromaffin granules** discharge catecholamines including ATP and specific proteins. It is presumed that this process occurs through the cell membrane rather than by diffusion through the cell cytoplasm. The vesicle membrane is not released with the catecholamines. Calcium ion is intimately related to the exocytosis process. Similar process runs for the mechanism of release of norepinephrine in the sympathetic neurone.

It is described by Hillarp that hydrolysis of ATP in the granules by an enzyme adenosine triphosphatase might play a role in hormone release. According to his analysis, the chemical composition of granules is 68% water, 6·7% catecholamines, 11·5% protein, 4·5% adenine nucleotide and 7% lipids. On stimulation of the gland there is marked fall in both ATP and catecholamines. It is presumed that ATP helps in keeping the granules intact. Thus, hydrolysis of the ATP causes breaking of the string that holds the package granules together. Acetylcholine does alone cause the release of the hormones, but component of intact cell is required.

Mechanism of action of catecholamines. It is claimed that a good number of catecholamine responses are mediated by an increase in the intracellular level of cyclic AMP, while certain others are mediated by a fall in the levels of cyclic AMP. Catecholamine effect through the increased levels of cyclic AMP are always associated with β-adrenergic receptors. Recently it is described that β-adrenergic effects in general are mediated by cyclic AMP.

The effects of catecholamines that are mediated by the increase in the level of cyclic AMP include several effects on carbohydrate metabolism, lipolytic effect, positive inotropic effect in the heart, the relaxation of smooth muscle, etc.

The mechanism by which catecholamines cause fall of cyclic AMP, may be associated with adrenergic α-receptors but this is still doubtful.

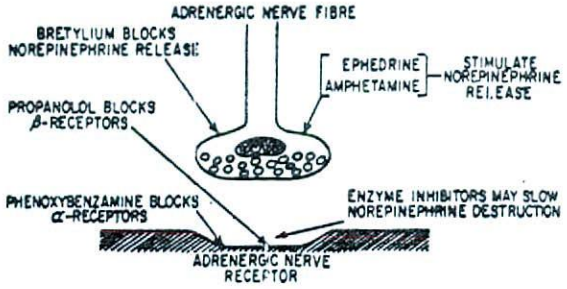


Fig. 4.107. It shows the different adrenergic receptors and the modification of their activities under respective blockers.

The effects of catecholamines which are mediated through fall of cyclic AMP are the inhibition of insulin release by pancreatic β-cells, reduced permeability to water and electrolytes in several epithelial tissues, stimulation of platelet aggregation and inhibition of melanophore dispersion in forg skin.

Alpha and beta receptors. It is possible that there are two types of receptor substances in the target organs that respond differentially to epinephrine and norepinephrine. The *alpha receptor* in the cells responds to norepinephrine and both *alpha* and *beta receptors* to epinephrine. The α-receptor and β-receptor functions of the two catecholamines can be studied by using respective blockers (Fig. 4.107).

Principal actions of some drugs with affect sympathetic activity

Nature of action	α-receptors	β-receptors
Stimulation	Norepinephrine Epinephrine Metaraminol Methoxamine Phenylephrine	Isoproterenol Epinephrine Norepinephrine
Inhibition	Phenoxybenzamine Phentolamine Ergot alkaloids	Dichloro-isoproterenol Pronethalol Propranolol

Catabolism of catecholamines. Catecholamines, released from the adrenal medulla or from the sumpathetic neurones, are metabolised in the liver by two enzymes —mono-amino-oxidase (MAO) and catechol-O-methyl transferase (COMT). Metabolic inactivation takes place by oxidation where MAO catalyses this reaction and also by methyla-

tion where COMT catalyses this reaction. MAO is present in the mitochondria and is widely distributed in different parts of the body such as liver, brain and kidneys. Mitochondria of the adrenergic nerve endings also contain high amount of MAO. COMT is also distributed widely in the body with high concentration in the liver and kidneys. COMT is not present in the adrenergic nerve ending (Fig. 4.108).

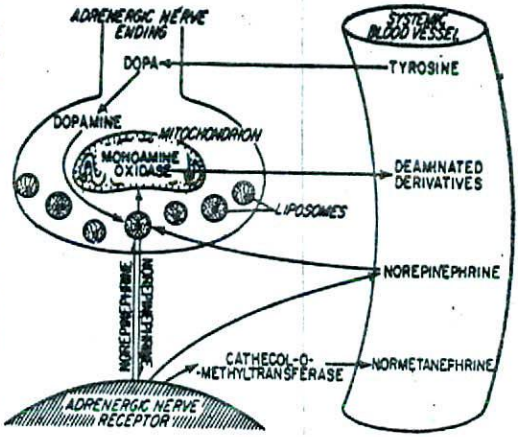


Fig. 4.108. Schematic representation of formation uptake and metabolism of norepinephrine at the adrenergic nerve ending (read *catechol* for cathecol)

Epinephrine and norepinephrine of blood are for the most part methylated by COMT and then oxidised in the liver by MAO. Epinephrine on methylation by COMT gives rise to metanephrine, whereas norepinephrine on methylation by COMT gives rise to normetanephrine. Both metanephrine and normetanephrine are ultimately converted into 3-methoxy-4-hydroxymandelic aldehyde by MAO. 3-methoxy-4-hydroxymandelic aldehyde is excreted as 3-methoxy-4-hydroxymandelic acid or vanil (vanilly) mandelic acid (VMA) (Fig. 4.109).

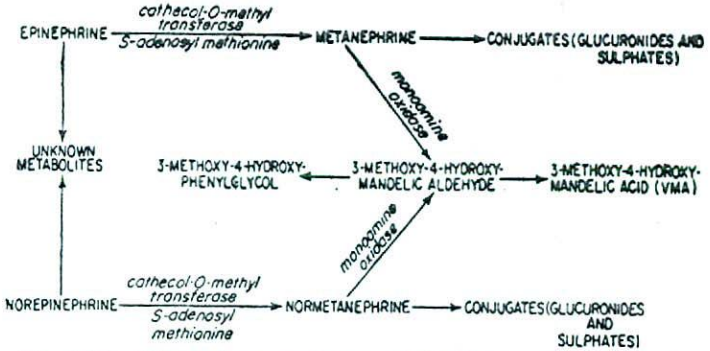


Fig. 4.109. Scheme shows catabolism of circulating epinephrine and norepinephrine. The chief site of catabolism is the liver. The conjugates are mainly glucuronides and sulphates (read *catechol* for cathecol).

Principal metabolic product either of epinephrine or of norepinephrine that is excreted through urine is the VMA. Normetanephrine and metanephrine are also excreted. All these metabolic products are excreted as sulphate or glucuronide. Preferably sulphate of the products is excreted through the urine.

Urinary products of epinephrine are:

1.	Metanephrine	40%
2.	VMA	41%
3.	3-methoxy-4-hydroxyphenyl glycol ..	7%
4.	3,4-dihydroxymandelic acid	2%
5.	Unchanged epinephrine	6%
6.	Miscellaneous	4%

In sympathetic neurone, norepinephrine is constantly oxidised by MAO into 3,4-dihydroxymandelic acid and its corresponding glycol. These two inactive products thus formed, enter the general circulation and then subsequently is converted into their corresponding O-methyl derivatives, the VMA and 3-methoxy-4-hydroxyphenyl glycol (Fig. 4.110).

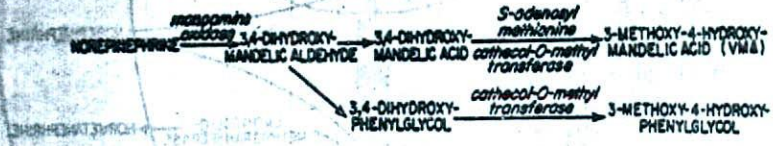


Fig. 4.110. Schematic representation of catabolism of norepinephrine in the adrenergic nerve endings. The acid and glycol enter general circulation (read *catechol* for cathecol).

EPINEPHRINE (ADRENALINE)

Epinephrine is one of the active principles of adrenal medulla. Epinephrine content of the resting gland is about 0·1 mg % of its moist weight. Total store in both glands is about 10 mg in man.

Chemistry. Epinephrine is a tyrosine derivatiae. [*Vide* page 4-164.] In the adrenal medulla, the epinephrine is soted in granules bound to ATP and protein. Its secretion is initiated by acetylcholine, released from the preganglionic neurones that innervate the secretory cells. The acetylcholine increases the permeability of the cells and the Ca^{++} entering the cells from ECF triggers exocytosis.

Natural epinephrine is laevorotatory and is about 12-15 times stronger than the dextro form. Both forms have been synthesised. It is rapidly oxidised on exposure to light, producing a brown substance having a different pharmacological action.*

Mode of action. Epinephrine acts through modifying the cyclic AMP level in the tissue (pages 4-164—4-165). It is a *sympathomimetic* hormone, exerting the same effects as sympathetic stimulation throughout the body. The exact nature of action on a particular tissue (viz., stimulation or depression, constriction or relaxation) will be same as the local sympathetic effect. The strength of action on a particular tissue will depend on the richness of sympathetic supply.

Site of action. Site of action of epinephrine is on the α-receptors and β-receptors of effector organs. Due to the α-receptor activity of epinephrine, blood pressure is raised, cardiac muscle is excited, smooth muscle of spleen and epididymis of rat are contracted and so on. But due to the β-receptor

* The enzyme tyrosinase can convert tyrosine into DOPA (dihydroxyphenylalanine). In the animal body, ascorbic acid or ultra-violet rays, in presence of Fe, can convert tyrosine into DOPA. The rich ascorbic acid content of adrenal cortex may have some part to play.

activity, blood pressure is decreased (vasodilatation), bronchial muscle is relaxed, force of contraction and frequency of heart beat are increased. *The main effects of α-receptors are excitatory whreas those of β-receptors are inhibitory.*

Action of epinephrine

Its action on different tissues and systems are briefly summarised below:

on circulation:

A. **Heart:**

(1) Rate, force and output increase. [In the intact body, the rate may be slowed down by sino-aortic reflex—caused by raised blood pressure.]

(2) Myocardium—excitability increased.

(3) Bundle of His—conductivity raised.

B. **Blood vessels.** *All constricted except coronary vessels and those of skeletal muscles.* Although splanchnic vessels constrict, yet the intestinal vessels are believed to dialate.

1. **Constriction** of (a) skin and splanchnic vessels mainly—(volume of limb, kidneys, intestine, etc., diminishes—as observed by Plethysmograph) and of (b) cerebral and pulmonary vessels. But these are less affected due to poor sympathetic supply. They passively may dilate due to raised blood pressure.

2. **Dilation** of (a) coronary vessles, (b) vessels of skeletal muscles.

Evidences: (1) Volume of intact limb diminishes but that of skinned limb increases. (2) Minute amounts of epinephrine, when given I.V., does not produce any appreciable change in the pulse rate, blood pressure, skin vessels, etc., but dilates the vessels in the skeletal muscles, raising the blood flow through them.

These facts partly prove that epinephrine actively dilates the muscle vessels.

C. **Blood pressure.** Rises sharply and comes down slowly and even below the basal level. Systolic blood pressure rises. Diastolic blood pressure may fall. But mean arterial blood pressure is raised. Total peripheral resistance is increased due to vasoconstriction in the skin and splanchnic area —causing decrease of total vascular capactiry of the body.

It has been discussed in preceding section that epinephrine has α-receptor and β-receptor activities. If small amount of epinephrine is administered then β-receptor activity (dilatation of blood vessels) being predominant, blood pressure falls but with higher dose the α-receptor activity being predominant, rise of pressure is observed due to vasoconstriction. If suitable blocker each for α- and β-receptors are used (I.V.) then the discriminating actions of epinephrine can be studied.

on respiration:

A. Bronchial muscles relax causing dilatation of bronchioles and cause shrinkage of the mucosa and diminution of secretion of mucus (hence, its therapeutic use in Asthma). The rate and depth of respiration are increased. The metabolic rate is accelerated, hence RQ is increased.

B. **Epinephrine apnoea.** This is not seen in man. In animals respiration becomes shallow and even may cease at the height of raised blood pressure following epinephrine injection.

on anterior pituitary:

Epinephrine stimulates anterior pituitary to liberate ACTH, which again helps in the release of glucocorticoids mainly. Glucocorticoids increase blood sugar through a process of *gluconeogenesis*. The stimulation of anterior pituitary might either be a direct one or mediated through the hypothalamus.

on skeletal muscles:

A. Excitability and contractility raised.

B. Onset of fatigue delayed.

C. Muscle glycogen converted into lactic acid and discharged into the blood stream (Fig. 4.82).

D. Exert *anti-curare* action (curare paralyses myoneural junction).

on liver:

Glycogen is mobilised due to increased breakdown of liver, glycogen (Fig. 4.82).

on blood:

A. *Blood sugar* raised due to: (1) Glucogenolysis from liver, (2) Formation of glucose from lactic acid of muscles through Cori cycle. (3) Increased gluconeogenesis due to increased liberation of glucocorticoids (through increased ACTH liberation caused by epinephrine).

B. Blood lactate increases due to breakdown of muscle glycogen.

C. Coagulability raised (coagulation time reduced).

D. A rise in serum potassium.

E. Red cell, white cell, platelet count, percentage of haemoglobin, as also blood volume raised, due to contraction of spleen. It causes a fall in the number of circulating eosinophils (indirectly through ACTH).

The increased cell count is usually believed to be due to mobilisation from the depots, specially due to contraction of spleen. But small changes occur after splenectomy. Since, plasma proteins are also concentrated to the same degree, it is more likely that the haemoconcentration is due to increased filtration of fluid from the blood stream into the tissue spaces—caused by raised blood pressure.

F. Plasma proteins concentrated.

on kidneys:

(a) Urine volume reduced. (b) Renal circulation diminished (caused by constriction of renal vessels, specially the efferent glomerular vessels). (c) May cause glycosuria (due to hyperglycaemia).

Even if renal circulation is cut down, the volume of glomerular filtrate remains the same. This is because, plasma is more extensively filtered. In other words, *filtration fraction* (i.e., glomerular filtrate/plasma flow) increases. These facts prove that epinephrine selectively constricts the efferent glomerular vessels, thus reducing the glomerular flow but increasing the glomerular pressure. Consequently, filtration increases. Bute in spite of it, urine volume is reduced. This is because rate of reabsorption of water by the renal tubules increases.

on metabolism:

A. Basal metabolic rate is increased by moderate doses of epinephrine, large doses cause a fall.

B. Respiratory quotient: O_2 consumption rises by 20-40% and CO_2 production by 30-50%. Hence, respiratory quotient rises.

C. Carbohydrate metabolism : Blood sugar is raised due to breakdown of glycogen (glycogenolysis) in the liver and muscles (through Cori

cycle) Fig. 4.82). Epinephrine activates phosphorylase in the liver and skeletal muscles, and hence blood sugar rises. The blood lactic acid also rises. Plasma potassium rises coincident with the glycogenolysis. The primary effect is probably due to increase in cyclic AMP by activating the enzyme adenyl cyclase. Cyclic AMP in turn activates the phosphorylase. This effect is similar to that of glucagon but the latter is only effective in the liver tissue. Epinephrine also stimulates ACTH release and thus indirectly rises blood sugar by gluconeogenesis. Depressed utilisation of glucose by the tissues is another causative factor for hyperglycaemia.

D. Adipose tissue: Causes hydrolysis of neutral lipid through cyclic AMP.

on smooth muscle:

A. Intestine: Movements inhibited, sphincters closed.

B. Gall-bladder: *Contraction* of gall-bladder.

C. Urinary bladder: Relaxation of bladder and constriction of sphincter.

D. Uterus: Effects not uniform. In labour and puerperium, inhibited.

E. Spleen: Contraction (smooth muscles in the capsules contract).

F. Eye: Dilatation of pupils, due to contraction of dilator pupillae muscle and retraction of the lids due to contraction of the smooth muscle of the lids.

on skin:

A. *Contraction of arrectores pili* causing standing of the hairs. Other smooth muscles of the skin also contract. [The spreading of bird's feathers, peacock's plumes, porcupine's 'thorns', etc., are due to epinephrine secretion during excitement.]

B. *Sweat gland:* In human beings sweating can be induced by intradermal administration of epinephrine in small amount. But in some animals, through the glands are innervated by sympathetic nerves, yet sweating cannot be induced.

on nervous system: Epinephrine but not norepinephrine produces a sense of restlessness, anxiety and fatigue.

on spinal cord: Large doses of epinephrine diminishes muscle tone and somatic reflexes (knee jerk, etc.). This is due to a direct depressant action of the cord, independent of any circulatory or other changes.

on salivary gland: Salivary glands have got both sympathetic and parasympathetic innervations. The two nerves act *synergistically* and for the same reason their respective neurohumours, norepinephrine, likewise epinephrine and acetylcholine have got synergistic effects on the secretion of salivary glands. Epinephrine or norepinephrine stimulates thick mucinous secretion and acetylcholine stimulates profuse watery secretion. Thus total volume of salivary secretion is increased when epinephrine and acetylcholine are administered at a time.

on lacrimal gland: Lacrimal glands receive secretory fibres from the parasympathetic but not from the sympathetic. Stimulations of the parasympathetic always cause secretion, but sympathetic stimulation has got no such effect. Epinephrine likely has got no effec on lacrimal secretion.

on melanophores. The effects of MSH on the dispersion of melanin granules within melanophores are antagonised by catecholamines through

adrenergic α-receptors. This effect seems to be mediated through the decreased level of cyclic AMP.

Control of epinephrine secretion

Nervous control. *Hypothalamus* is the higher centre which controls the sympathetic and epinephrine secretion. This centre may be affected in two ways: (a) *directly,* and (b) *reflexly.*

I. The following factors affect the centre **directly:**

1. **Higher centre.** Excitement generally stimulates secretion.

2. **O_2 lack, CO_2 excess, increased H-ion concentration,** etc., stimulate. They also exert reflex effects through sino-aortic mechanism.

3. **Blood sugar level.** Hypoglycaemia stimulates, hyperglycaemia depresses the centre.

II. The following factors affect the centre **reflexly:**

1. **Sino-aortic reflexes**

(a) Raised blood pressure depresses, lowered blood pressure (haemorrhage, etc.) increases epinephrine secretion.

(b) O_2 lack, CO_2 excess, increased H-ion concentration—stimulate.

The sino-aortic nerves exert a tonic inhibitory control over adrenal medulla, Section of these nerves stimulates secretion.

2. **Exposure to cold** stimulates epinephrine secretion reflexly. This helps in two ways: (a) *Vasoconstriction of the skin*—reducing heat loss. (b) *Increased metabolic rate*—raising heat production. Thus in cold climates, epinephrine plays a great part in heat regulation.

3. **Any acute sensation,** viz., pain, heat, etc., stimulates reflexly.

Tests and assay of epinephrine

Presence of epinephrine in an unknown extract can be tested in various ways. Quantitative assay (standardisation) can be made by comparing the effect of the unknown extract with that of another solution of known strength.

Assay methods are based on the different principal actions.

A. **Bioassay:**

1. *A segment of rabbit's intestine*—movements inhibited.

2. *Pregnant rat's uterus*—movements stimulated.

3. *Non-pregnant rat's uterus* (sensitive test)—relaxation (placed in Tyrode solution with excess (0·2%, KCl). Can detect up to 1 in 10^{11} million.

4. *Isolated arterial ring*—constriction of the ring.

5. *Denervated iris*—pupil dilated, particularly after the removal of superior cervical ganglion.

6. *Denervated heart* (sensitive test)—rate increases by as little as 1 in 1,400 million dilution of epinephrine.

7. *Decapitate cat* (sensitive test)—sharp rise of blood perssure.

8. *Caval pocket method* (Stewart and Rogoff)—Inferior vena cava is clamped above and below the entrance of the adrenal vein. Other veins entering the pocket are ligated. After a definite period the upper clamp is removed. If epinephrine has been secreted in the mean time, it will enter

the general circulation and the puhils of the animal will dilate. Quantitative estimation can be done by removing the lower clamp and collecting blood from the adrenal vein and then testing it by one of the above methods.

B. **Chemical assay:**

1. *Fluorimetric*—fluorescence of epinephrine in alkaline solution.
2. *Colorimetric*—oxidation of epinephrine to pink adrenochrome and other pink compounds.

NOREPINEPHRINE (NORADRENALINE) OR LEVARTERENOL OR LEVOPHED

Norepinephrine is another hormone, discovered from adrenal medulla (Goldenburg and his associates). It is the immediate precursor of epinephrine. It is supposed to be the actual sympathetic transmitter produced at the endings of the adrenergic fibres.

Commercial epinephrine, as usually extracted from the adrenal medulla, contains about 18% norepinephrine. Hence, the effects of the commercial epinephrine is due to the presence of both. Normally, when adrenal medulla secretes, it liberates both the hormones. Consequently, the effects of stimulation of adrenal medulla (or sympathetic) in the body is due to both the hormones. Paroxysmal hypertension, due to tumour of adrenal medulla is believed to be due to an increased secretion of norepinephrine.

Chemistry. It is same as epinephrine except it is devoid of one methyl group. [*Vide* page 4-164.]

Action. Except for a few instances, the actions of epinephrine and norepinephrine are very similar. Norepinephrine is more closely sympathomimetic than epinephrine. When norepinephrine is infused slowly in normal animals or humans, the systolic and diastolic blood pressure rise. This hypertension stimulates the carotid and aortic pressoreceptors, producing reflex bradycardia which overrides the direct cardioaccelaratory effect of norepinephrine. The chief differences are as follows:

Human Systems	Epinephrine	Norepinephrine
1. Heart		
(a) Rate	Increased.	Slightly increased.
(b) Output	Raised.	No change.
(c) Blood Pressure		
Systolic Pressure	Raised.	Raised.
Diastolic Pressure	No change.	Raised.
Mean Arterial Pressure	Increased.	Raised.
2. **Vessels**	Dilator for some (muscle), constrictor for others.	Entirely constrictor.
3. **Respiration**	Stimulated.	Stimulated.
4. **Kidney**	Constrictor of renal vessels.	Constrictor of renal vessels.
5. **Uterus**		
Non-pregnant in rat or cat	Inhibited.	Inhibited.

Human Systems	Epinephrine	Norepinephrine
6. **Central nervous system**	Increased mental anxiety.	No effect.
7. **Metabolism**		
(a) O_2 consumption	Increased.	Increased.
(b) Blood sugar	Increased.	Increased.
(c) Free fatty acid release	Increased.	Increased.
8. **Eosinophil count**	Increased.	No effect.
9. **Intestine**	Inhibited.	Inhibited.

Site of action. Norepinephrine acts on the α-receptors of effector organs. The α-receptors are mostly excitatory. Due to α-receptor activity, blood vessels are constricted (rise of pressure), cardiac muscle is excited, smooth muscle of spleen and epididymis of rat are contracted and so on.

Control of norepinephrine secretion

Adrenergic endings continuously secretes norepinephrine which is essential for the normal control of vascular tone and so, blood pressure. It is believed that changes in the blood pressure level regulate the liberation of norepinephrine from both the adrenergic endings and the adrenal medulla. The increased liberation of this hormone in emergent condition is not of primary importance.

Bioassay of a mixture of epinephrine and norepinephrine

1. *Rat's uterus* or *chick's caecum* is more sensitive to epinephrine, while denervated cat's nictitating membrane is more sensitive to norepinephrine.
2. It is also possible to separate the two hormones by *chromatography* and then assay them separately on rat's blood pressure, etc.
3. Small doses of epinephrine usually cause a definite *fall of blood pressure* in anaesthetised animals, due to predominant vasodilator effect in the muscles. But norepinephrine only produces constrictor effects, hence, *raises blood pressure.*

FUNCTIONS OF ADRENAL MEDULLA

The idea of **sympathetico-adrenal system** has now changed. It has now been observed that the norepinephrine which is liberated after stimulation of sympathetic nerves plays an important role in the regulation of circulation, whereas epinephrine which is secreted mostly from adrenal medulla is mainly concerned in metabolic adjustments. Both hormones are involved in emotional expression. Presence of normal amount of adrenocortical hormones are necessary for majority of actions catecholamines.

Developmentally, medullary cells represent the nerve cells of sympathetic ganglion. *Functionally,* they also resemble each other by secreting epinephrine. The only difference is that, the ganglion cells secrete epinephrine at the nerve endings whose effect is limited to a particular locality. While the medullary cells secrete the same epinephrine directly into the blood stream and thus affect the whole body. Thus adrenal medulla and the sympathetic ganglia should be regarded as the two divisions of a common sympatho-epinephrine mechanism—one division reinforcing the activity of the other.

Methods of functional study of adrenal medulla

Functions of adrenal medulla have been studied by the following methods:

I. **Experimental:**

1. **Extirpation**—effect negligible. Hence, not an essential gland.
2. **Administration of epinephrine**—*vide* 'Action of Epinephrine'.
3. **Histological study.** The effect of a particular factor on the gland can be studied by noting the change in the granules. Anything that stimulates epinephrine secretion, causes the disappearance of the granules.
4. **Denervated heart.** Such a preparation is sensitive to epinephrine even in 1:1,400 million dilution. If a particular factor accelerates such a heart and if the acceleration is abolished after denervation of adrenal glands, it is reasonable to conclude that the increased rate is due to epinephrine secretion caused by the factor.
5. **Cross-circulation experiments.** Venous blood from the adrenal gland of a dog is made to pass into the circulation of another adrenalectomised dog. In the latter, blood pressure and the volume of an organ (spleen, intestine, etc.) are noted. A rise of blood pressure and diminution of volume of the organ, will indicate epinephrine secretion by the former dog. The reverse effects will indicate inhibition of epinephrine secretion.

II. **Clinical:**

1. **Hyperactivity**—causes *paroxysmal hypertension* in man.

Paroxysmal hypertension is usually due to a tumour of adrenal medulla (phaeochromocytoma). *Features*—(1) temporary attacks of high blood pressure (300 mm Hg), (2) hyperglycaemia, glycosuria, (3) dyspnoea, sweating, tremors, extreme nervousness.

2. **Hypoactivity**—occurs in Addison's disease, where a low blood pressure is usual.

From these observations the following **facts about epinephrine secretion** are known:

1. **Continuous tonic secretion at rest.** Nerve impulses from different parts of the body are constantly stimulating adrenal medulla and so it is believed that small amounts of epinephrine are continuously being secreted.
2. **Emergency secretion.** Cannon and his associates have shown that adrenal medulla liberates its secretion in emergent condition which disturbs the mental or physical state of the subject, e.g., in times of stress, emotion, threatened dangers, etc. Any other emergent condition which disturbs the normal mental or physical state of the subject stimulates epinephrine secretion, for instance—physical exercise, exposure to cold, fall of blood pressure, asphyxia, anoxia, cerebral anaemia, hypoglycaemia, anaesthesia, stimulation of afferent nerves, acute sensations like pain, heat, cold, etc., and so on.

Epinephrine secreted under such conditions, reinforces the already mobilised sympathetic action and prepares the whole body in such a way that the subject can cope with the situation successfully. For this reason, it is called the gland for *fright, fight* and *flight*. During muscular exercise the secreted epinephrine causes the following changes: (1) Redistribution of blood from one part to another. (2) Increases coronary flow, rate and force

of heart beat; raises blood pressure and cardiac output—thus assuring adequate blood supply to the muscles and enabling the heart to deal effectively with the increased venous return. (3) Increases pulmonary circulation for carrying more oxygen (there is relatively less constriction of the pulmonary vessels). (4) Increases cerebral circulation to maintain an adequate cerebral control. (5) Raises the red cell count and thereby the O_2 carrying capacity of blood, thus ensuring more oxygen supply to the muscles. (6) Increases coagulability of blood assuring quicker coagulation in case of bleeding. (7) Delays the onset of fatigue. (8) Dilatation of bronchioles—helping pulmonary ventilation. (9) Mobilises liver glycogen and raises blood sugar, so that tissue cells may get enough fuel. (10) Stimulates tissue oxidation and raises basal metabolic rate.

In this way epinephrine prepares the subject in such a way that, muscular activity can be carried on with advantage for a longer time.

The secretion of norepinephrine from adrenal medulla during emergency has secondary importance only.

Summary of the functions of adrenal medulla

From the above observations, functions of adrenal medulla can be summarised as follows:

1. **Reinforce sympathetic action** (sympathomimetic).
2. **Helps to keep the normal resting blood pressure** at a steady level by adjusting the rate of epinephrine secretion. Fall of blood pressure stimulates, rise of blood pressure depresses secretion *via* sino-aortic nerves.
3. **Secretes more epinephrine during emergency** and enables the subject to fight out the situation successfully.
4. **Takes part in heat regulation.**
5. **Takes an important part in metabolism**—specially in carbohydrate metabolism.

HYPERFUNCTION OF ADRENAL MEDULLA

Phaeochromocytomas are catecholamine-producing tumours that arise from chromaphin cells most commonly in the adrenal medulla. **Clinical manifestations:** (1) Paroxysmal or permanent hypertension—excessive effects of epinephrine or norepinephrine. (2) Spells or crises. (3) Extreme elevations in blood pressure associated with headache, angina pectoris or blanching of face and extremeties—these reflect primarily α-receptor stimulation. (4) Facial flushing or sweating, rapid palpitations, fever and, sometimes, hypotension—β-receptor effects seem to dominate. (5) There may also be nervousness or anxiety, diabetes mellitus, thyrotoxycosis, various types of emotional disturbance, primary haemorrhagic lesions of G. I. tract, biliary colic, primary renal disease, acute adrenal insufficiency, toxaemia of pregnancy, hyperglycaemia along with transient glycosuria, a high haematocrit, weight loss, anorexia, increased BMR, increased O_2 consumption, etc. Myocarditis is sometimes observed; the cause is unknown.

GASTRO-INTESTINAL HORMONES

From a localised area of the gastro-intestinal tract, certain hormones are secreted. These are generally known as **gastro-intestinal hormones** (Fig. 4-111). Some of the hormones, of course, are not exclusively secreted from the gastro-intestinal areas but also from other areas too. These hormones regulate the motor and secretory activities of the digestive

organs. The hormones are polypeptide in nature, a structural resemblance between the chemical nature of these hormones themselves, and also other polypeptide hormones like glucagon and caerulein (amphibian skin secretion) has been noted. Their secretions are mostly conditioned by the presence or absence of specific food materials in the lumen of the gastro-intestinal tract, rather than by other glandular products in the circulation. The specific cells responsible for the synthesis of these hormones are not known with certainty. By appropriate stimuli, these hormones are secreted quickly and also destroyed rapidly following withdrawal of the appropriate stimuli.

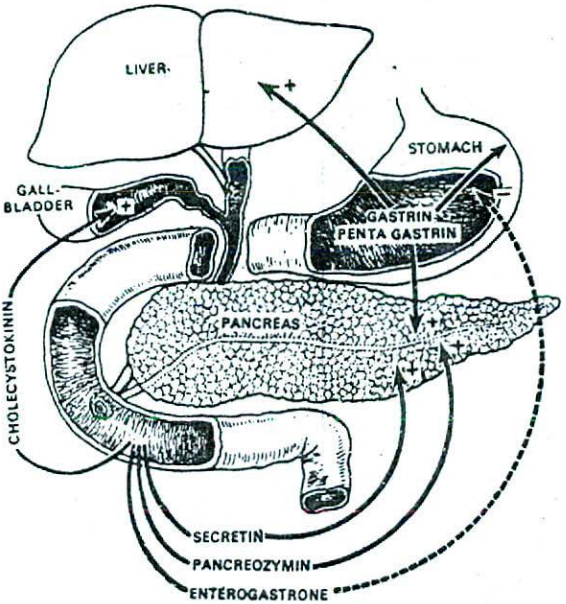


Fig. 4.111. It illustrating source and action of the gastro-intestinal principles.

Following are the gastro-intestinal hormones:

A. **Gastrin**

It stimulates the secretion of HCl and pepsin by the stomach.

Two pure gastrins (gastrin I without sulphate and gastrin II with an ethereal sulphate) have been isolated from the antral portion of the gastric mucosa. These are produced in the modified epithelial cells of the pyloric glandular mucosa.

Gastrin I and **gastrin II** are identical except that gastrin II contains a $—HSO_3$ group on the tyrosine residue in the 12 positions, and the other gastrin I does not contain this $—HSO_3$ group (Fig. 4.112). The structure of gastrin II, extracted from human tissue has been presented

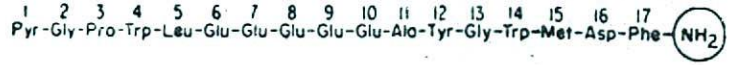


Fig. 4.112. Amino-acid sequence of gastrin I.

in Fig. 4.113. It is a heptadecapeptide and its N-terminus is protected from digestion by aminopeptidases by a pyroglutamyl residue (Pyr) and the C-terminus is protected from digestion by carboxypeptidases by ammination. Both the gastrins are equally active.

The gastrins have been synthesised from the L-amino acids and are equally effective.

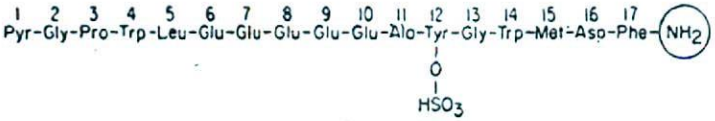


Fig. 4.113. Amino-acid sequence of gastrin II.

There are structural similarities and functional resemblance between gastrin, cholecystokinin-pancreozymin and caerulein. The last one is more active than the other two.

A synthetic product—**pentagastrin** (Fig. 4.114) having c-terminal tetrapeptide with substituted β-alanine possesses all the physiological properties of natural gastrin and is used in man to test gastric secretory capacity.

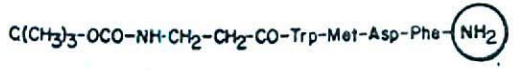


Fig. 4.114. Pentagastrin structure.

Recently, it has been observed that some gastrin is secreted from duodenal mucosa and from δ-cells (A_1-cells) of the islets of Langerhans.

The most effective chemical stimuli for gastrin release are meat extracts, amino acids, ethanol (10%). Gastrin release is stimulated by moderate distention of the antrum. Vagal stimulation also leads to the secretion.

Acid inhibits the secretory gastrin. Over distention of the antrum also inhibits secretion.

Gastrin is secreted in response to vagal impulses of the cephalic phase and to distention and secretagogues of the gastric phase.

Main **functions** of gastrin are as follows:

It stimulates

(i) gastric motility (Fig. 4.111).
(ii) oxyntic glandular cells to secrete pepsinogen and acid (HCl),
(iii) pancreas to secrete bicarbonate containing fluid and enzymes,
(iv) duodenal mucosa for the release of secretin.

B. Cholecystokinin-Pancreozymin (CCK)

Until recently it was known that cholecystokinin causing contraction of the gall-bladder and pancreozymin causing enzyme secretion by the pancreas, were separate hormone. But it is now clear that it is a single hormone and is secreted from the mucosa of the upper small intestine when foodstuffs (especially dilute acids, lipids, fatty acids, peptones, etc.) come in contract with it. This hormone has got action on the contraction of the gall-bladder and also on enzyme-rich pancreatic juice. CCK is polypeptide in nature containing 33 amino acids (Fig. 4.115) and has structural resemblance to that of gastrin. This accounts for some gastrin-like activities of CCK.



Fig. 4.115. Amino-acid sequence of CCK.

C. Secretin

It is secreted by the duodenal mucosa due to presence of chyme from stomach; the release of pancreatic juice but not enzymes is stimulated. Bayliss and Starling (1902) first reported the presence of a blood-borne factor which is responsible for the secretion of pancreatic juice. In addition to hydrochloric acid, fatty acids, hydrolysed protein, etc., even water are effective stimulant. Removal of the vagus does not affect release of secretin, but hypophysectomy lowers it. Secretin is ineffective by mouth and is destroyed in the body due to the action of an enzyme "secretinase". The cells responsible for its synthesis as also the mechanism of its action is not definitely known.

It contains 27-amino acid residues (Fig. 4.116) and is a polypeptide hormone. It has got similarity with glucagon regarding the position of

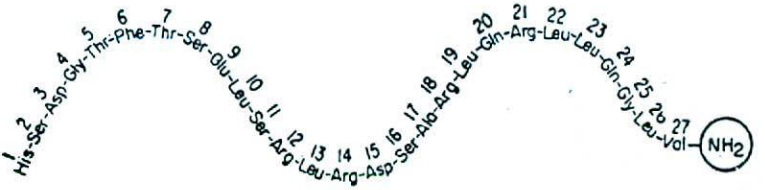


Fig. 4.116. Amino-acid sequence of secretin.

amino acids in the chain. Of the 27-amino acid, a total of 14-amino acid residues occupy the same position, counting from N-terminus of the two hormones—glucagon and secretin. The secretin molecule is a helical structure between the positions 5 and 13. For this reason the whole chain is required for its activity. Until recently a hormone **"enterogastrone"** released in the duodenum has been considered responsible for inhibition of gastric secretion. Purified secretin has similar effect and is possibly the real inhibitor.

D. Glucagon-like immunoreactive factor (Gut glucagon)

This is also known as intestinal glucagon (GLI). This is secreted from the wall of the stomach and small intestine in response to glucose ingestion. It is not identical to pancreatic glucagon but its other physiological properties are mostly similar to pancreatic glucagon. It stimulates the β-cells of islets of Langerhans to secrete insulin (Fig. 4.117).

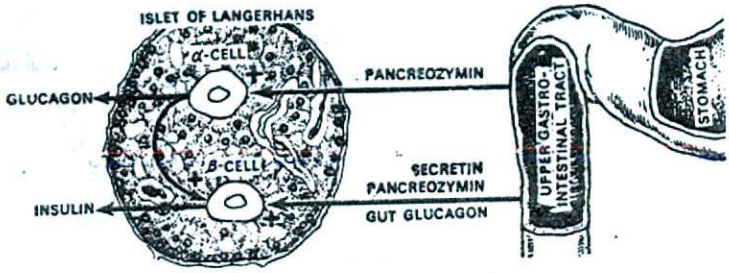


Fig. 4.117. It shows gastro-intestinal hormones which stimulate glucagon and insulin release.

E. Caerulein

It has both structural and functional similarities with gastrin and cholecystokinin-pancreozymin (CCK). Caerulein is a decapeptide (Fig. 4.118) and has been isolated from the skin of Australian frog, *Hyla caerulea*. It has got the strong stimulating effects on the contraction of gall-bladder and it is generally used in humans, for the contraction of gall-bladder during cholecystography.

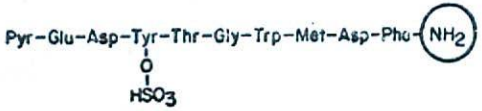


Fig. 4.118. Structure of caerulein.

F. Gastrone

Mucous membrane of human stomach probably contains a substance called gastrone. It inhibits secretion of acid, stimulated by histamine and gastrin. It is present in the highest concentration in condition of gastric atrophy.

G. Urogastrone

This is a polypeptide and has been isolated from urine of men and dogs. It can inhibit the gastric secretion in response to cholinergic drugs. Source of this hormone is not known.

H. Villikinin

This hormone is believed to be secreted from the mucosa of the upper small intestine by chyme. It is known to stimulate movement of villi in the small intestine. Chemical nature of this specific intestinal hormone has not been established.

I. Enterocrinin

This hormone is also postulated to be secreted from the intestinal lumen. It is known to increase the secretion of intestinal juice.

ERYTHROPOIETIN

Erythropoietin is a polypeptide hormone. It acts on bone marrow. It stimulates the production of red blood cells, but unlike haemopoietin, does not affect the white blood cells or platelets.

Chemistry. Erythropoietin appears to be a glycoprotein. Its mol. wt. of between 20,000 and 30,000. It can be measured by bioassay in mice. An antibody has been prepared which neutralises biological activity of human erythropoietin, but a sensitive immunoassay awaits the isolation of pure hormone.

Physiological basis. Erythropoietin is secreted largely, but not exclusively, by kidneys, but its exact site of origin is still unknown. Erythropoietin or more probably, an enzyme precursor (erythrogenin) is found in the light-mitochondrial fraction of kidney extracts and in epithelial cells of the glomerular tuft when examined by immunofluorescence. Erythrogenin may act on erythropoietinogen (plasma substrate) to form erythropoietin, in the same sort of way as renin produces angiotensin. A lipid inhibitor to erythropoietin in the kidney and urine has been described.

Action. Erythropoietin acts on the bone marrow to promote the development of the erythroblast stem cells, which then mature into reticulocytes and red blood cells without the need for further hormonal action. It can augment the production of haemoglobin within red cells, increasing cell volume and haemoglobin content.

Interrelationships. Hypophysectomy causes anaemia which may be reversed probably by GH, ACTH and TSH, by stimulating an increase in erythropoietin secretion. Androgens increase erythropoiesis in a variety of disorders. Cobalt, thyroid hormones and adrenaline enhance erythropoietin production. Oestrogens impair the bone marrow's response to erythropoietin.

Erythrocytaemia results from increased erythropoietin secretion. Hypoxia and anaemia are the main factors known to increase erythropoietin secretion.

HISTAMINE AND HEPARIN

From physiological point of view histamine (an amine) and heparin (a polysaccharide) cannot be clearly designated as hormones. But the pathological effect of both, when produced in excess, resemble those of hormones. Both histamine and heparin are synthesised by the tissue mast cells, stored there and released under certain circumstances. The mast cells belong to the APUD series which shows a high amine content, amine precursor uptake, aminoacid decarboxylase and marked metachromia and are described widely in tissues close to the walls of small blood vessels. In some species, but probably not in man, the mast cells also contain 5-HT.

Histamine is probably responsible for some of the effects of the atypical carcinoid syndrome and both are probably involved in the mastocytosis syndrome which proliferaties mast cells giving rise to diffuse or nodular infiltration of many organs including the skin, liver, spleen, bone marrow, lymph nodes, pancreas, thymus, lungs, meninges, etc. Excessive release of histamine causes urticaria pigmentosa, dermographism, duodenal ulceration and probably pyrexia. Heparin may cause defects of coagulation.

THE PROSTAGLANDINS

Introduction. Prostaglandin was the name given by von Euler in 1935 to a substance isolated from human seminal fluid and thought to be secreted by the paostate gland.

The name prostaglandin given by him was a lipid-soluble, acidic compound, which lowered blood pressure and stimulated various isolated smooth muscle preparation. Bergström and Sjövall (1960) isolated the crystalline form of an unsaturated nitrogen-free hydroxy fatty acids—the PGF from extracts of sheep seminal vesicle. This PGF has got stimulating action on the isolated rabbit duodenum and jejunum but no effect on the rabbit blood pressure. Another compound—called PGE was subsequently isolated. This substance has got both vasodepressor and intestinal smooth muscle-stimulating property. Within recent years, thorough chemical analysis of the prostaglandins have been made and as many as 14 types of naturally occurring prostaglandins have been isolated. Among the fourteen, eight are metabolites of the remaining six and 13 of the 14 are found in human. They are classified into four main groups, viz., PGE, PGF, PGA and PGB—with each individual compound being designated by a subunit.

Chemistry. Prostaglandins are a class of C_{20} fatty acids containing cyclopentane ring. These biologically active lipids are derivatives of a hypothetical fatty acid—the prostanoic acid (Wolfe, 1970). All natural prostaglandins with the exception of certain metabolites contain a hydroxyl group at C-15 (R) and a trans double bond at C-13-14 (Δ^{13}). The chemical

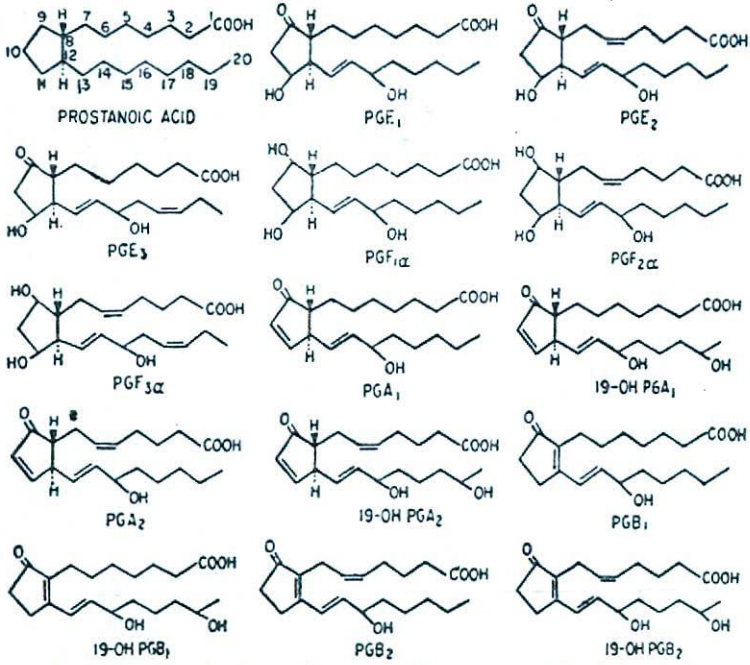


Fig. 4.119. The absolute configuration of prostanoic acid and chemical structures of prostaglandins of E, F, A and B groups.

name of PGE_1 is 9-keto-11(α), 15(R)-dihydroxy-prost-13 (tr)-enoic acid, $PGR_{1\alpha}$ is 9(α), 11 (α), 15(R)-trihydroxy-prost-13 (tr)-enoic acid, etc. Structural configuration of certain prostaglandins have been shownin

Distribution. Prostaglandins are widely distributed. Almost each tissue contains small amount of prostaglandin. Seminal plasma and seminal vesicles, menstrual fluid, endometrium, amniotic fluid, decidua, placenta, spleen, skin, iris, lung, thyroid, thymus, submaxillary salivary gland, gastro-intestinal tract, pancreas, kidney, adrenal medulla, cerebrospinal fluid (CSF), brain, spinal cord, phrenic nerve, vagus, etc., contain prostaglandins.

Mode of action. Generally, members of the A and E groups of prostaglandins lower blood pressure and members of F group raise blood pressure. They also inhibit gastric secretion, dilate bronchi, constrict the pupil, inhibit lipolysis, increase intra-ocular pressure and produce contraction of uterus. These mimic the actions of some hormones and inhibit the actions of other hormones, possibly by an action on cyclic AMP.

According to Flack *et al.* (1969) the prostaglandins stimulate steroidogenesis and it is suggested by Kuehl *et al.* (1970) that a prostaglandin may actually mediate the effect of LH on cyclic AMP in ovarian tissue. PGE_1 inhibits the permeability response of toad bladder to vasopressin or theophylline. PGE_1 may antagonise the actions of not only catecholamines but also of polypeptide lipolytic hormones including TSH, ACTH, glucagon and LH on cyclic AMP accumulation in isolated fat cells. PGE_1 stimulates bone resorption in tissue culture and increases sodium transport probably by a mechanism similar to that of neurohypophyseal hormones or catecholamines in frog skin.

Prostaglandins may take part in locally produced feedback systems. For example, catecholamines increase the production of cyclic AMP in fat cells and cause lypolysis. At the same time prostaglandins, which inhibit the formation of cyclic AMP and depress lipolysis, are released. A similar mechanism may operate in the kidneys, where the action of ADH (mediated via cyclic AMP) may be held in check by local release of PGs.

Release. Prostaglandins may be released from different organs by nerve stimulations, venoms and other treatments.

Antidromic stimulation of the trigeminal nerve in the rabbit causes an intense and prolonged miosis and vasodilation. This is unaffected by atropine. In the rat's stomach, prostaglandins (PGE_1, PGE_2, $PGF_{1\alpha}$, $PGF_{2\alpha}$) are present in higher concentration. Stimulation of vagus nerve markedly increases the release of prostaglandins and it is dependent upon intensity of stimulation. It has been observed that stimulation of periarterial sympathetic nerves does not affect the spontaneous release but inhibits the effect of vagus stimulation. There occurs selective increase of release of the prostaglandins after stimulation. Prostaglandins are not released from preformed stores but are formed during the stimulation. Increased release from nerve stimulation is due to increased biosynthesis in the tissue. Stimulation of sympathetic nerves to the spleen perfused with blood from a second donor animal release PGE_2 into the venous effluent. Phenoxybenzamine blocks the codtractile response to stimulation and release of prostaglandins. Application of catecholamines also release prostaglandins. Acetylcholine added to the perfused adrenal gland also releases prostaglandins along with adrenaline. Thus both adrenergic and cholinergic stimulations initiate biosynthesis and relase of prostaglandins. Cyclic AMP may have some role in the release mechanism of prostglandins.

Functions. The physiological functions of prostaglandins are not clear. These substances are synthesised in many tissues from essential fatty acids and are unlikely to be a part of a classical endocrine target system. They possess a broad spectrum of pharmacological properties including contraction and relaxation of smooth muscle, effects all leevls of the reproductive system and other varieties of cell function. Prostaglandins may be regarded as local tissue hormones that are involved in the regulation of the action of neurohormones. Robison, Butcher and Sutherland (1971) described that prostaglandins like catecholamines possibly acts through the alteration of cyclic AMP level in a number of tissues.

Possible functions of prostaglandins are as follows:

A. **Prostaglandins in reproduction**

(a) Prostaglandins have got significant role in sprem transport. Prostaglandins deposited in the vagina during coitus may act locally on the cervix and body of the uterus so as to help in sprem transport.

(b) *Menstruation.* There is some evidence that certain prostaglandins ($F_{2\alpha}$ and E_2) are related with the onset of menstruation.

(c) *Parturition.* Prostaglandin $F_{2\alpha}$ possibly takes part during labour as evident from high concentration of prostaglandin $F_{2\alpha}$ in amniotic fluid during labour.

(d) *Placental blood flow.* As umbilical cord contains high concentration of prostaglandins, it is suggested that prostaglandins may have some role in regulation of placental blood flow.

B. **Central nerve transmitters**

There are certain evidences suggesting the role of prostaglandins as neurotransmitters in the CNS. When administered by microelectrophoresis on to nerve cell, they alter the firing rates of neurones. But more work is required for substantiating this postulation.

C. **Lipolysis**

Prostaglandin is potent inhibitor on lipolysis. Lipolytic effects of catecholamines, ACTH, glucagon, TSH, vasopressin, sympathetic nerve stimulation, cold stress, etc., are reduced by prostaglandin E_1. Lipolytic actions of epinephrine, vasopressin, LH and gastrin are due to formation of cyclic AMP through the activation of adenyl cyclase on ATP. It is claimed that prostaglandin E_1 behaves like a competitive inhibitors of the different hormones that increase lipolysis. As antilipolytic actions of prostaglandin E_1 may result from inhibition of adenyl cyclase (Fig. 4.120), it has been suggested that the apparent competitive antagonism of adenyl cyclase by prostaglandin E_1 may be due to an action in preventing the adsorption of ATP by the enzyme.

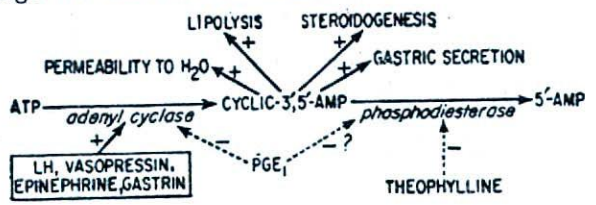


Fig. 4.120. It represents lipolytic effects of PGE_1 through inhibition of adenyl cyclase activity.

D. **Gastric secretion**

Considering the involvement of cyclic AMP in gastric secretion in response to a variety of secretagogues, it is predicted that prostaglandins must inhibit gastric secretion. This has been observed true in rats. Oral administration of prostaglandin E_1 causes inhibition of gastric secretion.

E. **Permeability to water**

Water permeability of the isolated toad bladder and tubule by vasopressin can be inhibited by prostaglandin E_1 through acting on the adenyl cyclase.

F. **Steroidogenesis**

Prostaglandin E_1 and E_2 do not inhibit the increased secretion of progesterone and dihydroprogesterone in response to gonadotrophins. Prostaglandin E_2 potentiates the formation of dihydroprogesterone in response to gonadotrophins.

G. **Vascular smooth muscles**

Prostaglandins (E and A groups) are potent vasodilators. This action is not abolished by atropine.

H. **Respiratory smooth muscle**

Human bronchial smooth muscle *in vitro* is relaxed by prostaglandim E_1 and E_2 but contracted by $F_{2\alpha}$.

THYMUS

Anatomy. Thymus is partly an endocrine gland and partly a lymphoid structure. It is located in the anterior and superior mediastina of the thorax behind the sternum. It extends from the pericardium up into the neck to the lower border of the thyroid gland. The thymus consists of two fused asymmetrical, elongated and flask-shaped lobes. The left lobe is smaller than the right. Each lobe is composed of innumerable *lobules*. Each lobule is made up of small follicles of about 1 mm in diameter. Thymus is largest relative to body weight in the foetus and in childhood up to the age of puberty, after which it undergoes gradual and continuous

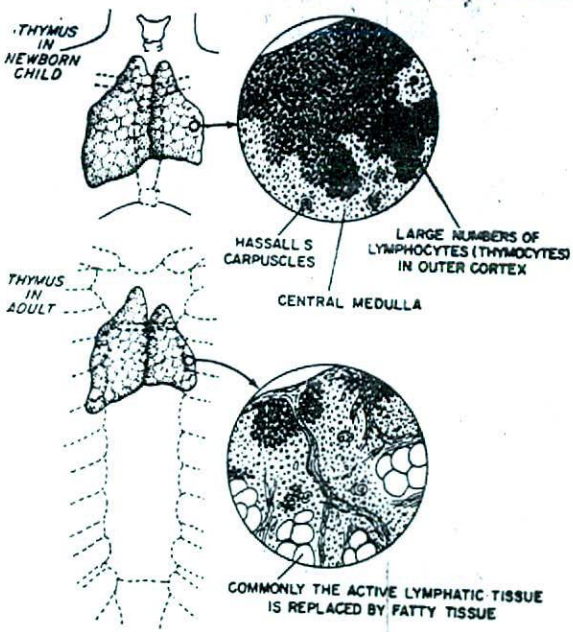


Fig. 4.121. Diagrammatic representation of involution of the thymus.

involution* (Fig. 4.121). This process may be greatly accelerated in the course of many infections and in wasting diseases **(accidental involution).** During regression the thymic tissue is generally replaced by fat, so that the adult thymus is composed largely of fat and connective tissue.

Development. The thymus develops from the *endoderm* of the third and probably of the fourth branchial clefts on both sides, and in mammalian embryos it is the primordial lymphoid organ, in which lymphocytes can first be identified. The outgrowths coalesce to form a single mass in the midline. Later on, these endodermal elements become secondarily invaded by surrounding mesodermal tissues from which the lymphoid tissues develop. **Hassall's corpuscles** (Fig. 4.122) develop from the remnants of the original endoderm. In birds there is a second primary lymphoid organs, the **bursa of Fabricius,** situated near the cloaca but also arising from the epithelial tissue. The thymus is probably the main source of lymphocyte production in mammalian embryonic life; the rudimentary spleen, for example, does not contain the primordial cells necessary for lymphoid differentiation. At birth, when the spleen and lymph nodes are still poorly developed, the thymus is already a prominent lymphoid organ.

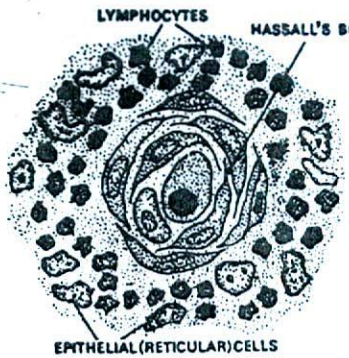


Fig. 4.122. Diagram shows thymic medullary tissue.

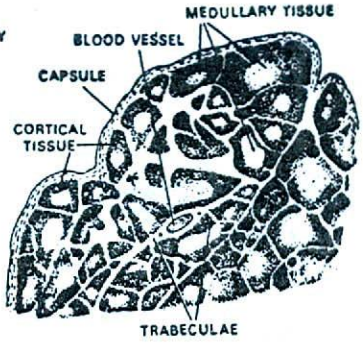


Fig. 4.123. Diagrammatic representation of histological structure of the thymus.

Histology. The organ has a connective tissue **capsule** and consists of two lobes—each with numerous lobules. Each lobule has a dense, darkly-staining peripheral **cortex** and a looser lightly-staining central **medulla** (Fig. 4.123). Actually, thymic lobules are not separate. The medullary tissue constitutes a central strand with projections at intervals and these projections are surrounded by cortical substance. Normally the thymus contains neither germinal centres nor plasma cells.

(a) **Capsule.** It is dense white connective tissue variably rich in macrophages, plama cells, mast cells, granular leucocytes, and fat cells. The connective tissue continuous with the capsule dips into the organ

*The weights at different ages are as follows (Hammar): At birth—13 g; 1-5 years—23 g; 6-10 years—26 g; **12-15 years—37 g; (maximum);** 16-20 years—25 g; 21-25 years—25 g; 26-35 years—20 g; 35-45 years—16 g; 46-55 years—13 g. In old age it decreases to 3-6 g.

separating lobes and lobules, and forms a trabecular or septal system. Large blood vessels, lymphatic vessels and nerves run in the capsule and septa. When the thymus ages and undergoes involution, lobes diminish in size, and the septa and capsule become correspondingly broader and fatty.

(b) **Cortex.** It looks very similar to the lymphoid tissue of the ordinary lymphatic nodes, but lack of primary follicles. The cortex consists of densely packed masses of cells that morphologically look like lymphocytes. Of the lymphocytes in mouse thymus, according to Metcalf's report, about 1% has cell diameter more than 11 μm (**large lymphocytes**), about 10% have cell diameter about 7 to 11μm (**medium-sized lymphocytes**) and the rest are **small lymphocytes** (also called **thymocytes**) having cell diameter less than 7 μm. The large lymphocytes transform into the medium and the latter in turn into small lymphocytes. The large and medium lymphocytes probably undergo 3-4 cycles of cell division a day, fulfilling the role of primitive lymphoid cells actively engaged in *lymphopoiesis*. Scattered among these cells, are elongated reticular cells with pale nuclei. Cyclic AMP appears to stimulate the rate during which time DNA synthesis takes place. These cells are certainly of epithelial origin.

(c) **Medulla.** It is a broad, branched band of thymic tissue. The medullary branches provide the lobar and lobular patterns of the organ. The medulla consists of epithelial (reticular) cells like those in the cortex. These cells are easily visible due to much less numerous lymphocytes than in the cortex. There are variable numbers of plasma cells, mast cells, eosinophil cells and melanocytes, generally near blood vessels. **Hassall's concentric corpuscles** or **thymic corpuscles** are organisation of flattended epithelial (reticular) cells and are the characteristic features of the thymus. They are formed from hypertrophied and degenerating reticular cells, concentrically arranged cells of Hassall's bodies stain with acid dyes. The innermost cells show signs of degeneration and hyalinisation (Fig. 4.122). Central cells may degenerate completely and cysts or calcareous deposits appear. According to the report of Blau, these corpuscles may be active structures of phagocytosis, which become sites of aggregation of γ-globulin and antigens. Calcium may be deposited in them in other cases.

Blood vessels and nerve supply. The thymus receives arterial supply from the internal thoracic and the inferior thyroid arteries. Large venules arise in the medulla, then combine with larger veins which empty into the left innominate and thyroid veins.

The thymus itself is not the site of antibody formation. Epithelial-reticular cells in the thymic vessels constitute an element in the **blood-thymus barrier** just as the glial cell contributes to the *blood-brain barrier*. In the thymus the antigen appears to be unable to pass through the *blood-thymus barrier* to reach to the lymphoid tissue from the blood vessels.

The thymic capsule is moderately rich in small unmyelinated and myelinated nerve fibres from the vagus, cardiac plexus, first thoracic ganglion, and ansa hypoglossi. Unmyelinated fibres enter the organ with blood vessels and are probably of vasomotor nature.

Experimental and clinical evidences of thymic function

I. **Effects of extirpation.** In young animals: (a) At first adiposity, then cachexia and coma. (b) Defective deposition of mineral salts on the

bones. (c) A wasting disease with a great fall in blood and tissue lymphocytes and interference in immunity reactions.

II. **Clinical evidences**

1. In myasthenia gravis—thymus enlarges. Thymectomy often improves.
2. In Graves' disease, Addison's disease and acromegaly—thymus enlarges.
3. In eunuchs or after castration in young—thymus does not involute.
4. Sex hormones or maturation of gonads cause thymic involution.
5. In cancer thymus—features almost same as Cushing's syndrome (page 4-154).
6. Status thymicolymphaticus—slight injury, infection, anaesthesia, etc., causes sudden death in many young people. In such cases, enlarged thymus and swelling of the lymphoid structures of the body are found at autopsy. It is held that the thymic enlargement is somehow related to the occurrence of sudden death. Since subjects of status thymicolymphaticus die during anaesthesia, operation, etc., and since adrenal cortex protects against such *stress and strain* (*vide* pages 4-160, 4-161), it seems all the more reasonable that the fundamental defect in status thymicolymphaticus may be an adrenocortical dysfunction. It has also been observed that either any kind of stress or administration of adrenal cortical steroids causes shrinkage of the thymus in young animals or human beings.

Thymosin or thymin

A polypeptide, thymosin has been isolated from the thymus gland. This substance has been shown to increase the number of lymphocytes in the circulation and accelerate rejection of skin grafts from other mammals.

Functions of the thymus

The thymus attains its largest relative size during childhood, gradually involuting after sexual maturity and being eventually replaced by fat and connective tissue, so the thymic functions are, in some way, concerned with events in early life.

1. **Source of lymphocytes.** The thymus is a derivative of branchial epithelium, and at a relatively early stage in embryonic life, lymphocytes develop appearing after about 12 weeks of uterine life in man from thymic epithelium. These are the first lymphocytes to be found in the body and the primary colonisation of lydphoid organs with the cells forming lymph follicles is from these thymic lymphocytes. So the thymus may be the main source of over 90% of short-lived (3-5 days) and about 5% of long-lived lymphocytes in the foetus and it may be the main source of long-lived lymphocytes in the adult.

2. **Production of lymphocytes.** At birth, when the spleen and lymph nodes are still poorly developed, the thymus is already a prominent lymphoid organ. The mitotic rate of thymic lymphocytes is greater than that in the spleen, lymph nodes and Peyer's patches by a factor of 3 to 5 as long as cortical tissue persists. Despite the high mitotic rate of its lymphocytes the thymus normally contains no germinal centres or plasma cells. Removal of the thymus in neonatal life in animals is followed by a profound depletion of small lymphocytes.

3. **Development of immunologic competence.** Immunologic mechanism represents a mechanism of recognising genetic relatedness. Experimental use is made of genetic differences to test immunologically competent tissue exchange by autograft, isograft, homograft and heterograft. When an organ (or skin) is transplanted from one animal to anothe of the same species, this transplant or homograft is rejected by the reci pient after a few days. This rejcdtion is due to the transplant acting as an antigen which stimulates the formation of antibody against foreign protein in the host. The antibodies are believed to be made in the lymphoid tissue by certain lymphocytes.

At birth, most mammals only have passive type of immunisation. Within a few days of birth, the newborn's own immunologic mechanism becomes active for differentiation and proliferation of primitive lymphoid cells etering it from the blood stream but originating in the bone marrow and elaborating a humoral factor that induces immunologic competence in lymphocytes. Immunologic tolerance may be induced in mature individauls by damaging lymphoid tissue with X:ray or radiomimetic drugs.

4. **Control of immunologic phenomena.** The thymus controls over immunologic mechanism without itself participating directly in significant antibody production. After intravenous, intraperitoneal or subcutaneous administration of antigen, the spleen, lymph nodes or other lymphoid tissue respond by the production of antibody, but the thymus does not. Thymic lymphocytes with antibody-forming properties (*immunocytes*) migrate *via* the thymic vein into the blood stream and form colonies in other lymphoid sites. Thymectomy removes a thymus hormone which normally stimulates lymphocyte production in other centres and enables lymphocytes to react with antigen and form antibodies. Current hypothesis is that the thymus produces *migrant cells* from the marrow which are possibly 'bone marrow small lymphocytes' that can display immunological competence.

5. **Role in leukaemia.** In certain pathological conditions, the thymus is associated with the leukaemia, probably as a bridge between the leukaemia stimulus and the diseases. In addition to the genetic predisposition the presence of a virus and the presence of the thymus are required for appearance of leukaemia. Thymectomy prevents the occurrence of this diseases. In leukaemic strains, the mitotic rate in the preleukaemic period may exceed that of non-thymic lymphocytes by a factor of 7 to 10.

6. **Humoral factor.** Metcalf observed that a cell-free extract of the thymus contains a factor capable of stimulating a lymphocytosis (LSF). The thymus normally secretes a polypeptide hormone, **thymosin** or **thymin** which is responsible for the development of sensitised lymphocytes. It is postulated that thymosin is involved in the regulation of neuromuscular transmission and can reduce the *quanta* of acetylcholine at the neuromuscular junction of the skeletal muscle. Acetylcholine is responsible for muscular contraction, when access of acetylcholine to motor-end plates is blocked it leads to the paralysis of voluntary muscles. In **myasthenia** there is an abnormal response of motor-end plate to acetylcholine, resulting in extreme fatigability. The **relationship between the thymus, neuromuscular transmission and myasthenia gravis** may involve an autoimmune mechanism. In myasthenia gravis an antibody stimulates the epithelial (reticular) cells of the thymus to secrete excessive amount of

thymosin, with the consequent reduction of acetylcholine in the neuromuscular junction and the thymic epithelium reacts apparently with the formation of same antibody that combines with A band of voluntary muscle. Adrenal glucocorticoids make dissolution of thymic lymphocytes and atrophy of tumours. Sex hormones may elicit inhibitory influences in thymic lymphocytes. ACTH of the pars distalis influences lymphatic system through adrenal cortex. GH stimulates the thymus.

7. The apparent rejuvenation in health of a pregnant woman during the second half of the period of gestation may be due to great hormone-producting activity of the thymus of the embryo.

8. Animals lacking this organ seem to be abnormally sensitive to environmental stress and particularly to malnutrition of suboptimal vitamin intake.

9. It helps in **deposition of mineral salts on the bones.**

Control of thymus. (a) Anterior pituitary—stimulates. After administration of growth hormone of anterior pituitary in experimental animals enlargement of thymus has been observed. In Graves' disease thymus is enlarged. (b) Gonads—inhibit, and this is possibly the cause of reduction in the size of the organ at sexual maturity. (c) Adrenal cortex also causes inhibition and was used as an assay method of corticosteroids. In Addison's disease thymus is enlarged.

Thymic disorders and genesis

In the neonatal myasthenia, the thymus-stimulating antibody passes through the placenta and acts on the foetal thymus. This is responsible for excessive production of thymosin. The pattern and the genesis of neonatal myasthenia gravis resemble neonatal thyrotoxicosis, as a result of the action of LATS (long-acting thyroid stimulator) transferred to the foetus from the blood of the thyrotoxic mother.

THE PINEAL BODY

Anatomy. The pineal body (**epiphysis cerebri** or **conarium**) of the human brain is a somewhat flattened, cone-shaped, grey body measuring about 5-8 mm in length and 3-5 mm in breadth. It is attached by a short hollow stalk (Fig. 4.124) to the roof of third ventricle. Pia mater covers the body (except where it is attached to the habenular and posterior commissures) and gives rise to connective tissue septa that carry numerous blood vessels into the organ. These septa separate cellular element into cords.

Histology. The cells of the pineal are neural in origin, but bear little semblance to nerve cells when fully differentiated. There are at least two types of major cells: (i) **parenchymal** or **chief cells (pinealocytes)** have large irregular nuclei and a moderately basophilic cytoplasm. By specific silver impregnation methods, these pinealocytes have long processes with club-like endings apparently terminating in close proximity to the endothelia of the capillaries. Under E/M, the cytoplasm of chief cells are rich in microtubules which penetrate to the tips of the extended cell processes. A moderately well-developed endoplasmic reticulum

and Golgi apparatus, and dense membrane-bounded vesicles also typify

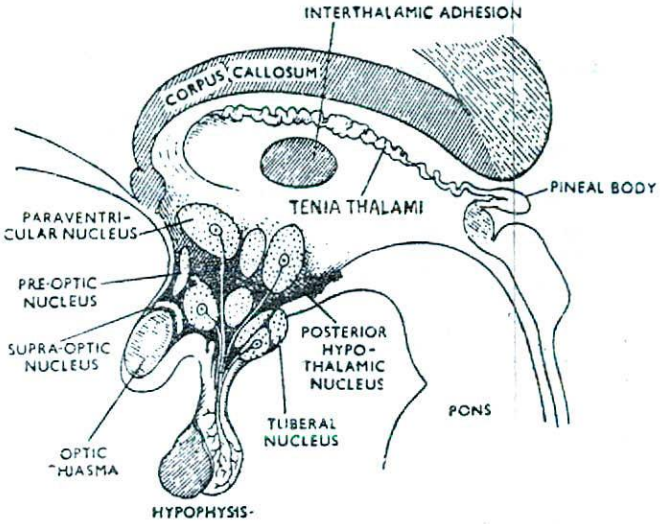


Fig. 4.124. Segittal section of human brain stem showing the anatomical position of pineal body.

these cells (Fig 4.125). Lipid droplets, lipochrome pigment droplets and lysosome-like structures are often present. Histochemically, the paren-

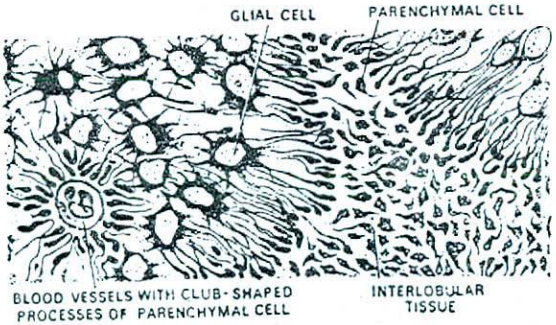


Fig. 4.125. Semidiagrammatic representation of an adult human pineal body showing two lobules.

chymal cells contain acid phosphatase, succinic dehydrogenase, and some non-specific esterase and lipases. (ii) **Interestitial** or **supportive cells.** Pineal interstitial cells (Fig. 4.126) are markedly stellate in appearance and lie between the clusters of pinealocytes and in perivascular spaces. The cytoplasm is somewhat more basophilic. These cells have a more

dense nucleus, fewer mitochondria, granular reticulum, and free ribosomes. There are occasional deposits of glycogen. These cells constitute approximately 5% in the pineal. These cells are regarded by many authorities as *atypical glial elements*. Some species variation exists in the form and number of interstitial or supportive cell elements.

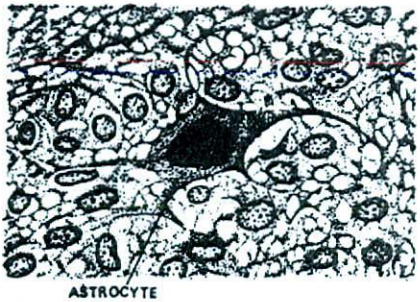

ASTROCYTE

Fig. 4.126. It shows astrocyte in lobule of an adult human pineal body.

Connective tissue cells, mast cells, Schwann cells, and axons of autonomic nerves are also present in the pineal.

Blood supply and **Nerve supply.** Blood supply in rats is nearly as great relatively as that of adenohypophysis. It is also observed that stained after intravitam injection of trypan blue or ingestion of silver nitrate, blood vessels of the organ, like those of neurohypophysis act as peripheral vessels in their permeability to these substances and the pineal can be regarded as lying outside the **blood-brain barrier.** In the wall of pineal blood vessels, alkaline phosphatase and mono-amine-oxidase are present.

Principal innervation of the gland is *via* autonomic fibres originating in *superior cervical sympathetic ganglia* and entering the pineal substance through two large tracts—*nervi conarii* and coursing through *tentorium cerebelli.*

Concretions. The human pineal body shows the presence of extracellular concretions known as **corpora arenacea** (*acervuli cerebri,* or *brain sand*). These concretions are composed of a mineralised organic matrix and usually appear in the capsule and septa. They consist of laminated calcareous nodules (carbonates and phosphates of calcium and magnesium).

Changes with age. The pineal body first appears at about 36 days of gestation in the posterior region of the roof of the diencephalon. This body attains its maximum development by about 7 years of age and then undergoes a very slow involution. This involution continues to about 14 years of age and is characterised by a relative increase of interestitial tissue, and by hyaline changes in the septa and in the cells. *Brain sand* increases as involution proceeds.

Functional evidences of the gland

1. Enlargement of reproductive organs and early onset of puberty—due to pinealectomy in rats.

2. Delayed puberty and reduced ovarian weight followed by injection of pineal extracts.

3. Application of isotopic methods shows that the pineal body of rodents and birds displays a rapid uptake and turnover of radio-active phosphorus and a high rate of amino-acid incorporation.

Melatonin

Pineal gland contains high concentration of melatonin. It is N-*acetyl-5-methoxytryptamine*. It has been named melatonin because it lightens the skin of tadpoles by an action on the melanophores. Melatonin is synthesised from *5-hydroxytryptamine*—the **serotonin.** The pineal glands of primates and certain rodents contain high concentration of serotonin. Mammalian pineal gland if properly oxygenated in organ culture then it is capable of synthesising serotonin and melatonin from tryptophan without the addition of cofactors. For the synthesis of serotonin from tryptophan requires two enzymes—the tryptophan hydroxylase and aromatic L-amino-acid decarboxylase. These two enzymes are present in high concentration in this gland. For melatonin synthesis, serotonin is first acetylated by acetylating enzymes into N-acetyl serotonin. N-acetyl serotonin is converted into melatonin in presence of s-adenosylmethionine and enzyme HIOMT (*hydroxyindole-O-methyl transferase*) (Fig. 4.127).HIOMT is only present in the pineal gland. High concentration of HIOMT in the tissue serves as a good indicator for pineal tissue. In mammals the melatonin is released as hormone and acts at the level of brain and other tissues so as to influence the development and functional activity of the gonads, pituitary, thyroids and other organs.

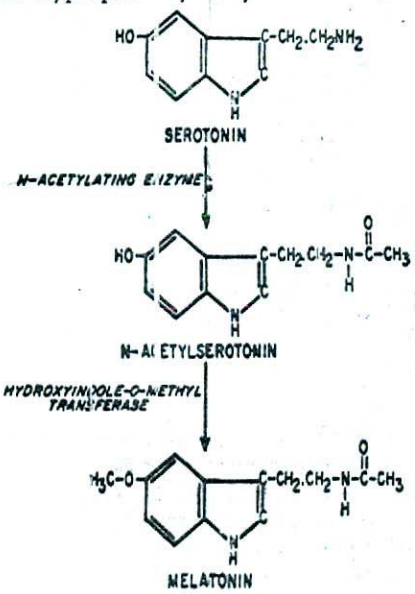


Fig. 4.127. Synthesis of melatonin from serotonin.

Fate of melatonin has been studied in rats and cats. Circulating melatonin is destroyed in the liver by 6-hydroxylation and followed by conjugation with sulphuric or glucuronic acid.

Control of synthesis and release of melatonin

Exogenous norepinephrine and other catecholamines or the sympathetic nerve fibres increase the melatonin content in the pineal body. This effect is brought about from the stimulating rate of melatonin synthesis from serotonin and of serotonin synthesis from trytophan. Since catecholamines stimulate adenyl cyclase in the pineal gland and since dibutyryl derivative of cyclic AMP can mimic these effects of catecholamines on the synthesis of serotonin and melatonin, so these effects are mediated by cyclic AMP. But that effect of norepinephrine is not mimiced by exogenous cyclic AMP in the pineal body, leads to an increase in the rate of tryptophan uptake. It is suggested that cyclic AMP stimulates melatonin synthesis not by changing the properties of HIOMT but rather by making more substrate available to it.

A typical β-receptors can only block the stimulation of pineal adenyl cyclase, but not by α-adrenergic blocking agents. Norepinephrine (or perhaps some other factors) released from sympathetic nerve endings normally acts to inhibit the synthetic rate of adenyl cyclase.

Environmental lighting affects the melatonin formation and its release from the pineal body and these effects of light are mediated by the sympathetic nervous system. It is proved that the level of melatonin and the activity of enzyme HIOMT rise during periods of darkness and fall during periods of light. According to Wurtman *et al.*, the inhibitors of protein synthesis do inhibit the increase in HIOMT activity which normally responses in continuous darkness.

Functions

(a) The pineal body shows a quick phosphorus metabolism as measured by the uptake of radio-active phosphorus.

(b) Pineal body of young goats displays a high rate of glucose utilisation with much of glucose being employed in the formation of amino acids. But this capacity diminishes with advancing age.

(c) As photic stimulus relayed for optic pathways to the pineal *via* sympathetic nerve fibres is translated by pineal activity into humoral gonadal control, melatonin plays an active role in the light-influenced reproductive cycle of rodents and birds.

(d) The HIOMT is responsible for the production of a number of hydroxyindoles. This specific enzyme possesses a photoreceptor-type pineal system in the brain and retina of lower vertebrates. But the activity of HIOMT is reduced in constant light.

(e) Histochemically, pineal phospholipid content is highest during dioestrus and lower during oestrus. During the oestrous cycles, serotonin content and HIOMT activity of the pineal fluctuate. Injections of melatonin and other methoxyindoles reduce ovarian weight and alter the stages of oestrus

(f) The ability of melatonin to modify gonadal function suggests that its secretion is associated with the timing of the oestrous and menstrual cycles, probably mediated through the decrease in gonadotrophic-releasing factor of the hypothalamus.

(g) It is postulated that **adenoglomerulotrophin** of the pineal may regulate the secretion of aldosterone and it also liberates an anti-ACTH factor.

(h) It is a source of growth-inhibiting factor.

(i) Pineal tumour in children generally is concerned with delayed puberty as a result of increased pineal activity.

(j) The pineal body seems to have a neuroendocrine function, although the physiology of this gland is a subject of intensive investigation. The pineal seems to participate in the regulation of the rhythmic activity of the endocrine system, by the elaboration of specific hormone or compounds such as methoxyindoles. The prime stimulus for this secretion may well be mediated by visual reflexes.

(k) It is a source of a compound that cures schizophrenia.

GONADS AND REPRODUCTION

Introduction. The **male generative** organs consist of testes, epididymis, vas (ductus) deferens, seminal vesicles, ejaculatory ducts, prostate, Cowper's (bulbo-urethral) glands and penis. The **female reproductive** organs include ovaries, Fallopian tubes, uterus, Bartholin's (greater vestibular) glands, vagina and breasts. Of these, testes and ovaries are called the **primary sex organs** or the **gonads.** The remaining structures are collectively known as the **secondary** or **accessory sex organs.**

It should be noted that both males and females are fundamentally *bisexual.* In males, the masculine features predominate, the feminine features remain rudimentary. In females, it is just the reverse. The urine of both sexes contains both male and female hormones but in different proportions. It is more than probable that the physical and mental get-up of the *male* or *female* does not depend on the activity of the male or female hormone only—but on the combined action of both the hormones. It seems that an optimum balance of these two hormones is the more important factor than any one of them.

GONADAL FUNCTION. Testis and ovary possess two functional components. One component secretes hormones and the other produces gametes. Interstitial cells of Leyding produce testosterone in the male; and in the female follicles of the ovary secrete oestrogen, 17α-OH progesterone, and variation of progesterone secretion depends upon the time of the ovarian cycle. This small amount of progesterone which is present in the female blood are formed mostly by peripheral conversion from precursors, e.g., androstenedione which is secreted by the ovary. The steroids which are formed in the gonads and adrenals follow a common biosynthetic pattern except 11β-hydroxylatioh characteristic of corticosteroid synthesis in the adrenal gland.

REPRODUCTION. It depends upon hormonal control in all aspects. Gonads fail to develop completely without pituitary gonadotrophins and in the adult, removal of the pituitary causes the failure of reproduction. Most of the functioning of the hypothalamic-pituitary system control the gonadal development and generation of reproductive rhythms. Pituitary gonadotrophin secretion makes gonadal differentiation and development doing maturation of germ cells and gonadal steroid secretion. In turn, gonadal steroid hormones lead to genital development and appropriate libido regulating a cyclic fashion in the female. This cyclic fashion provides conditions suitable for conception and implantation. Moreover, when the function of placenta is established, the foetal development becomes independent of pituitary and ovarian support. But ovulation, implantation and early development of zygotes are controlled by the hormones.

Puberty

The onset of reproductive life is called **puberty.** *It is the time when the gonads develop both endocrine and gametogenic functions.* It occurs about two years earlier in females. The usual age of onset lies between 12 and 16 years. In old age, reproduction ceases. In females, the limits of reproductive life are very sharp. It begins with the first appearance of menstruation (**menarche**) and ceases with *menopause female* (*climacteric*). Menopause usually comes between 45th and 55th years and at this stage both the primary and the secondary sex organs degenerate. In males the limits of sex life are not so sharp. Its beginning and termination are gradual and indefinite.*

*From puberty to menopause, the human female is a continous breeder. But in the lower mammals there are definite reproductive periods—*the mating* or *breeding season* —during which only conception is possible. The duration of the season and its recurrence in the year, vary in different species. It may come once or twice a year (Dogs—*spring* and *autumn*) or more frequently.

Puberty changes

At puberty, the following changes take place:

1. **Physical changes.** In the males, the voice breaks, beard and moustache grow. The body becomes taller and more muscular, assuming the 'male type'. In the females, breasts develop. In both the sexes axillary and pubic hairs grow. [In females, the pubic hairs are concave forwards, in males convex forwards.]
2. **Sexual changes.** The gonads develop, producing mature *gametes*. The secondary sex characters appear. In the females, the most characteristic feature is the *appearance of menstruation.* [In animals—Oestrous cycle.]
3. **Mental changes.** The appearance of sexual desire, etc.

Development of gonads

They arise from two sources: (1) The **germinal epithelium.** (2) **Mesoblastic genital ridge.** The former is derived from that part of coelomic epithelium which covers the inner surface of the Wolffian body. The latter originates from a mesoblastic thickening lying just underneath the germinal epithelium. Both these structures send finger-like processes into each other in an interlocking manner. Those from the germinal epithelium are called the *genital cords.* They gradually become cut off from the surface and give rise to the *seminiferous tubules* of the testes. In the females, the proliferating sex cords are less prominent and the germ cells are larger and arranged in clusters, giving rise to the *Graafian follicles* of the ovaries. The stroma cells and probably the interstitial cells develop from the mesoblastic genital ridge.

Delayed puberty

In some individuals there is failure of development of accessory sex organs and also lack of development of secondary sex characters though the pubertic age is already attained. This condition if occurred in males is called **eunuchoidism** and in females is called **primary amenorrhoea.** This condition may occur primarily due to pituitary disorder or due to hypofunctioning in either of the gonads.

GENETIC BASIS OF SEX DIFFERENTIATION

Similar to somatic cells, the male spermatogonia and the female oögonia also contain 23 pairs of chromosomes. Of the 23 pairs of chromosomes observed in human beings, 22 pairs are *autosomes* which do not play any part in the determination of sex. The last pair is named as **sex chromosomes** as they have a major contributory role in sex determination. *The sex chromosomes in women are homologous because of having two X chromosomes (XX pattern), whereas in men they are of a heterologous type, consisting of one X and one Y chromosome (XY pattern),* the last one being smaller in size (Fig. 4.128).

During *meiosis* or reduction division of the primary spermatocyte or oöcyte, half of the number of chromosomes pass to the daughter cell. As a result of this, each daughter cell contains 22 autosomes and one sex chromosome. In the female, the sex chromosomes in all the daughter cells are X chromosomes, but in the male half of the normal sperms will contain

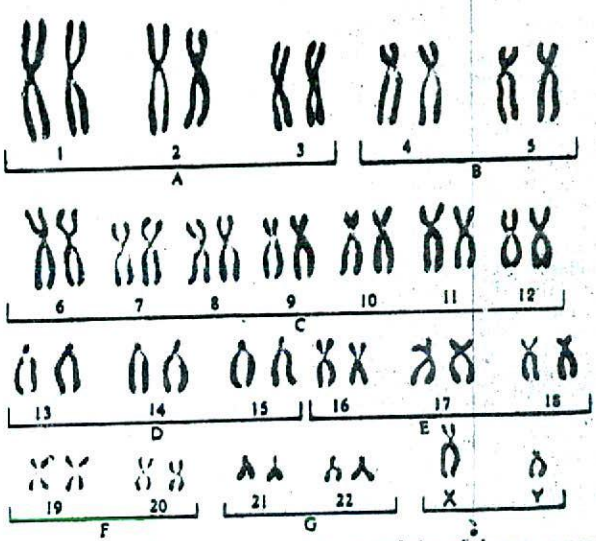


Fig. 4.128. The present-day conception of shape and size of chromosomes and their classification was accepted in Paris Conference of 1971. Autosomes in A to G groups and sex chromosomes XY are shown separately, The Y chromosome and X chromosome will be in G group and in C group respectively. In females both X chromosomes will be in G group.

an X chromosome and the other half will contain a Y chromosome. Hence each daughter cell (gamet) will possess either X or Y sex chromosomes (Fig. 4.129).

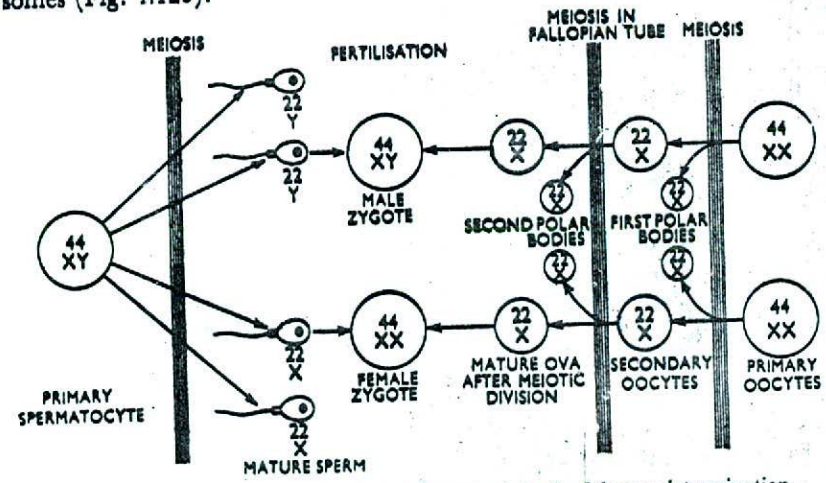


Fig. 4.129. Schematic representation of the genetic basis of the sex determination.

In the process of fertilisation between sperm (22 autosomes and either X or Y sex chromosome) and ovum (22 autosomes and X sex chromosomes); the resulting zygote would contain 23 pairs of chromo-

somes, of which 22 pairs are autosomes. The remaining 23rd pair determine the sex chracter of the offspring. *If the sperm containing X sex chromosome unites with ovum (X), the two sex chromosomes would be identical (X and X) and the resulting sex will be a female offspring. But if Y sex chromosome of the male unites with ovum (X), the two sex chromosomes will be different (X and Y) resulting in a male offspring.*

In the process of oögenesis (maturation of ovum) the germinal cell is differentiated into *primary oocyte* which contains deploid number of chromosomes (44XX). Primary oöcyte thus enters into the meiotic division and separates into (i) first polar body (22X —*haploid*) and (ii) secondary oöcyte (22X—*haploid*).

Secondary oöcyte then undergoes second meiotic division only after being fertilised (after penetration of *zona pellucida* by the sperm) within the Fallopian tube. Under this meiotic division the chromosomes split longitudinally, the cytoplasm divides unequally and the nucleus divides equally so that the secondary oöcyte is transformed into (i) matured ovum (22X) and (ii) second polar body (22X).

In case of spermatogenesis, the epithelium lining the seminiferous tubules undergo series of mitotic division to give rise to spermatogonial cells. The spermatogonial cell then divides further mitotically into two resting primary spermatocytes (44XY). The primary spermatocyte then undergoes first meiotic division (heterotypical) to give rise to two secondary spermatocytes (22X or 22Y). Secondary spermatocyte then undergoes second meiotic division (homotypical) to give rise to two permaids (22X or 22Y). The spermatid then is simply transformed into mature sperm (22X or 22Y) (Fig. 4.129).

Sex chromatin

Cells of individuals who have two X chromosomes contain an extra bit of chromatin, the 'sex chromatin' (Fig. 4.130) or 'Barr body'. It is seen in the nuclei, usually attached to the nuclear membrane. In almost all cells of females except the ova, an X chromosome is partially inactive and condensed and it is this which is visible as the sex chromatin. It is easily visible in over 25% of the cells in smears of the buccal mucosa and skin biopsies, and is well-developed in many other cells as well. There is also a small drumstick of chromatin (Fig. 4.131) projecting from the nuclei of 1-15% of the polymorphonuclear leucocytes in females but not in males. Normal females thus have sex chromatin and are said to be chromatin positive.

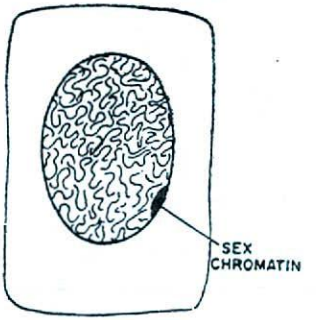


Fig. 4.130. Sex chromatin in a cell from a buccal smear.

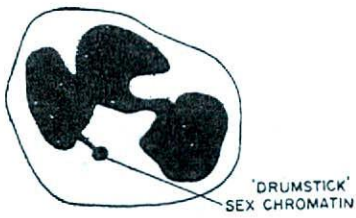


Fig. 4.131. Drumstick sex chromatin in a polymorphonuclear leucocyte.

Chromosomal abnormalities in sex differentiation

The major defect in chromosomal abnormalities is **non-disjunction,** a phenomenon in which sex chromosomes fail to separate; one gamet gets both sex chromosomes, but the other gets none.

ABNORMALITIES DUE TO MATERNAL NON-DISJUNCTION

Due to maternal non-disjunction during oögenesis four abnormal zygotes may be formed following fertilisation with sperm having either 'X' or 'Y' chromosome. These are as follows.

1. **Gonadal dysgenesis** (44XO chromosomal pattern) due to union of female gamet having no sex chromosome with male gamet having X chromosome. In this chromosomal defect, gonads are rudimentary or absent. Because normal number of sex chromatin is absent. This syndrome is known as **ovarian agenesis** or **Turner's syndrome** (Fig. 4.132) or **gonadal dysgenesis.** The affected female is sterile, does not menstruate, is short of stature, and often has various congenital abnormalities such as a web of skin on either side of the neck, coarctation of the aorta, and when the arm is extended the forearm tends to be displaced outward away from the body (cubitus valgus).

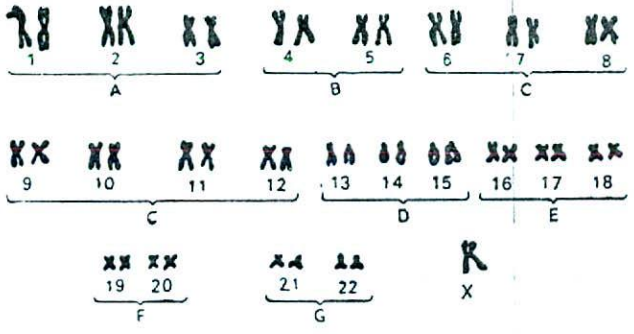


Fig. 4.132. Karyotype with Turner's syndrome (45, XO).

2. **'Super-females' (44XXX)** due to union of female gamet having XX chromosomes with male gamet having X chromosome.

3. **Klinefelter's syndrome** or **seminiferous tubule dysgenesis (44XXY)** due to union of female gamet having XX chromosomes

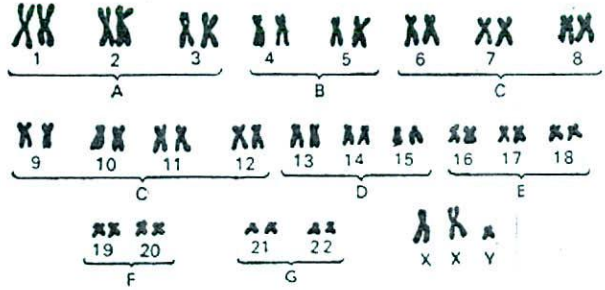


Fig. 4.133. Karyotype with Klinefelter's syndrome (47, XXY).

with male gamet having Y chromosome. In this pattern seminiferous tubules are not developed and there is defect in gametogenic function of the testis. In this syndrome, affected men are sterile, have very small

testes, sometimes have gynaecomastia, and are often mentally defective. They are sterile because their testes fail to produce spermatozoa (Fig. 4.133).

4. **44YO pattern.** It is mostly **lethal** and caused by fusion of male gamet having Y chromosome with female gamet having no chromosome.

ABNORMALITIES DUE TO PATERNAL NON-DISJUNCTION

Due to the paternal non-disjunction during spermatogenesis, two abnormal zygotes may be formed following union with ovum having X chromosome:

1. **Klinefelter's syndrome or seminiferous tubule dysgenesis (44XXY).** If male gamet having XY chromosome units with female gamet having X chromosome, then this type of syndrome may occur. This type of syndrome is also observed due to defect in female non-disjunction.

2. **Gonadal dysgenesis (44XO).** Gonadal dysgenesis also occurs due to abnormalities in disjunction of chromosomes in male gamet. Here male gamet having no sex chromosome unites with female gamet having X chromosome:

ABNORMALITIES DUE TO MOSAICISM

Chromosomal abnormalities are also observed due to defects in the separation of sex chromosome during mitosis after fertilisation of the ovum. This type of defect in the separation of chromosome is known as **mosaicism** and these abnormalities are very common. True **hermaphroditism,** in which the individual has both ovaries and testes is probably due to XX/XY mosaicism and related mosaic patterns, although other genetic aberrations are possible.

Down's syndrome or Mongolism

The term mongolism is commonly obsolete and Down's syndrome has replaced this. In this syndrome there are 47 chromosomes instead of normal 46 chromosomes. The extra chromosome is small acrocentric chromosome similar to chromosome 21. This syndrome is usually detected autosomal disorders. Down's syndrome has continued to be accepted as trisomy 21. Down's syndrome is generally common in children with mental retardation. Flattened head becomes anterior and posterior. There is an upward slant of eyes and bony orbit becomes smaller than the normal. There are speckling of iris (Brush-field spots) and conspicuously small external ears. The younger child possesses an epicanthic fold. The nose becomes short with flat bridge and tongue protrudes from a small mouth. The foot is broad with a deep grove on the sole of the foot between 1st and 2nd toes. The most characteristic features are palmar and plantar dermatoglyphic analyses. There is an incidence of leukaemia (10-20% more than normal). There is rudimentary condition in the second phalanx of the fifth finger. In Down's syndrome there is a transverse simian crease on the palm of the hand. There is furunculosis, skin infection and incidence of congenital heart disease. Hypotonia of abdominal muscles is frequently seen. Other defects may be observed in the children with extrachromosomal numbers 13, 14, 15 and 17 to 18.

Abnormal sex differentiation due to hormonal abnormalities

Though the sex differentiation is taken place genetically, yet several factors may alter the embryonic sex development. The gonads are developed from the mesodermal primordia in the genital ridges, which are bipotential organs and become either testes or ovaries. The origin of the genetic sex is the balanced activity between the testicular hormones and ovarian hormones. It has been observed in amphibia and birds that administration either of the hormones (testicular or ovarian) in embryonic life may give rise to reversal gonadal development.

Sex hormones, particularly the androgens, have got immense role in sex differentiation and subsequent development in mammals. Male differentiation of the internal genitalia depends upon the action of an **inductor** substance elaborated by the embryonic testes. This substance which may be a polypeptide exerts its effect unilaterally. The male differentiation of the external genitalia, on the other hand, occurs in response to androgen secreted by the Leydig cells in the embryonic testes. This effect, unlike that of the inducers, is bilateral. Testicular hormone suppresses the female primordia and thus male genital organs are developed in genetic male. If the functional status of the embryonic testes is defective then female internal genital organs are developed in genetic male. The syndrome that results in the genetic male is known as **male pseudohermaphroditism.** In this syndrome female internal genital organs along with testes (rudimentary) are present in genital male. In **female pseudohermaphroditism,** ovaries only are present and it shows male genital development. Sex chromosomes are female type (genetic female—XX). The female pseudohermaphrodite is the cause of excessive androgenic activity in embryonic life due to adrenal virilism in the mother. This type of syndrome is also observed in some cases where the mother is treated with excessive androgens. In **true hermaphroditism,** both the testes and ovaries are present and secondary sexual characters are variable. This syndrome is generally due to defect in the separation of the chromosomes during mitosis after fertilisation of the ovum (mosaicism).

Development of brain

In some species the development of brain as well as the external genitalia is affected by androgens early in the life. A brief exposure to androgens in the first days of life in rats causes the male pattern of sexual behaviour and male pattern of hypothalamic control of gonadotrophin secretion to develop after puberty. It is suggested that androgens exert similar effects on human, but no firm conclusion has yet been achieved.

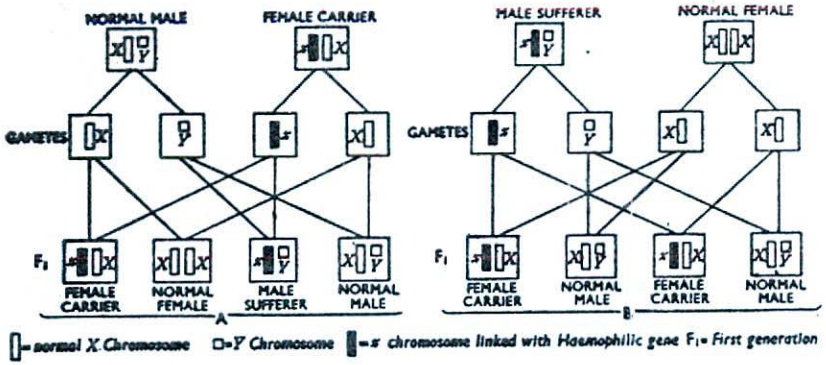
HEREDITY

Heredity, according to Oxford Dictionary, is *the property by virtue of which offspring inherit the nature and characteristics of parents and ancestors.* G. Mendel (1865) postulated that chromosomes contain the hereditary characters, which is now known to be ultra-microscopic DNA particles.

Genes. Genes are ultra-microscopic DNA particles present inside the chromosomes, which are carried from one generation to other, carrying the hereditary characters along with it.

Depending upon the effectiveness of the gene to influence the character of the offspring; the genes from the parents might be shown prominently in the offspring, in which case it is called **dominant gene.** It

is also possible for the characters not to be exposed, and then the gene is named as **recessive gene.** If one of the gene is dominant and the other recessive, the character of the dominant gene is indicated in the offspring.



Some of the hereditary diseases are transmitted from parents to offspring due to dominant gene. Here at least one of the parents has the particular disease. The diseases inherited are, certain types of retinitis pigmentosa, sickle-cell anaemia, night-blindness, a number of metabolic disorders (pentosuria, lipomatosis), etc. There are some diseases which may be carried over to the offspring through recessive gene. Here the parents are apparently healthy but simply act as carriers. Children born of closely releated parents have a greater chance of these disease to be transmitted to them. Diseases transmitted, are albinism, hepatolenticular degeneration, certain metabolic disorders (alcaptonuria, cystinuria, fructosuria), etc. Some of the hereditary diseases may be due to pathologic characteristics of one of the sex chromosomes, only the male sex usually suffer from some hereditary diseases like haemophilia, colour-blindness, night-blindness (nyctalopia), etc., the female act as carriers.

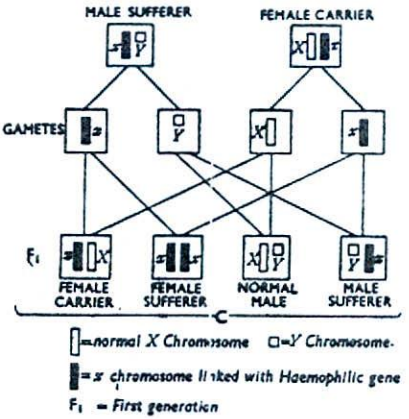


Fig. 4.134. Schematic representation of the transmission of haemophilia by union between female carrier and normal male (A), between male sufferer and normal female (B) and between male sufferer and female carrier (C).

Haemophilia. In this disease which is due to hereditary disorder, the blood does not coagulate extravascularly. This disease is transmitted by 'sex-linked' transmission. The haemophilic gene is linked with recessive x chromosome in female which only act as carrier of the disease but do not suffer from it. This is due to the other X chromosome in female being dominant. If any female carrier (xX) marries a normal male (XY), some

of their male offspring (xY) will suffer from the disease and again some of the female offspring (xX) will act as carrier in the first generation (Fig. 4.134-A). In case of marriage of male sufferer (xY) with a normal female (XX). male offspring of the first generation will have no abnormalities (XY). But the female offspring will be the carrier (xX) (Fig. 4.134-B). In case of marriage between female carrier (xX) and male sufferer (xY), a certain percentage of both male and female offspring of the first generation will suffer from the disease and some of the female will be carrier only. According to Mendel's law of heredity dominant gene always characterises over the recessive sex-lined gene. The female carrier (xX) does not suffer only for having dominant gene 'X' from the male. On the same ground the female may suffer when the individual possesses the recessive sex-linked gene (xx) (Fig. 4.134-C).

MALE REPRODUCTIVE ORGANS

TESTIS

Anatomy (Fig. 4.135). The testis consists of two somewhat flat, oval bodies, one on each side, remaining inside the scrotum. In early foetal life, they remain within the abdomen. During the third week of gestation, the testes move caudally from their position high in the abdomen, then gradually migrate and descend in the scrotum by the time the child is born. The exact cause of descent is not known, but it is suggested that the hypophyseal hormones probably initiate the process. Failure of descent results in the testes being retained somewhere along the route of descent and the condition is known as **cryptorchism** (hidden testes). This produces sterility as spermatogenesis does not occur at the higher temperature of the abdominal cavity.

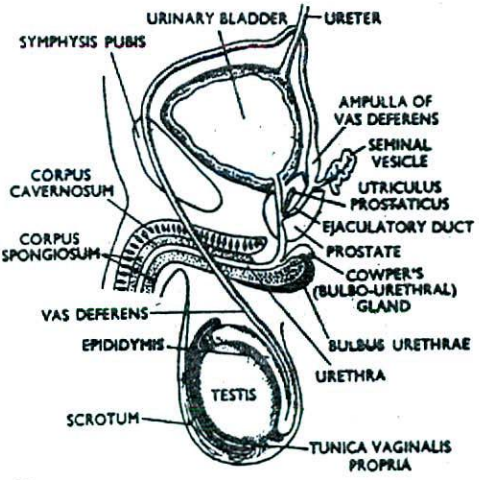


Fig. 4.135. Diagrammatic representation of the anatomical organisation of the male reproductive organs (median view).

The testis is covered by a tough, compact, fibrous capsule (*tunica albuginea*) from which trabeculae descend and divide the gland into a number of pyramidal lobules. The lobules are filled up with convoluted **seminiferous tubules,** each about 500 mm long. Several tubules unite to form a **straight tubule** which again again unite forming the **rate testis.** These join up to form the **vasa efferentia** which finally combine to form the **duct of epididymis.** The whole epididymis consists of a single convoluted tube about 6 metres long remaining coiled up together with the entwining connective tissue and surrounding tunic (Fig. 4.136) at the back of the testis and is ultimately continued as the **vas deferens.**

The epididymis is lined with pseudostratified epithelium with tall columnar cells and rounded basal cells (Fig. 4.137). The surface cells contain secretory granules and, sometimes, pigment. The epididymis acts as a storehouse for spermatozoa until ejaculation occurs. Spermatozoa are produced whether ejaculation takes place or not, those not ejaculated are reabsorbed in the vas deferens. The vas deferens is joined by the duct of the **seminal vesicles**—a musculoglandular sac — to form the ejaculatory duct which opens into the prostatic urethra. The secretion of the prostate and seminal vesicles makes up a considerable part of the semen. The seminal vesicles do not store spermatozoa but act as an activator for them. The secretion of seminal vesicles contains fructose, ascorbic acid, citric acid, inorganic phosphorus, acid-soluble phosphorus, electrolytes, protein, and trace amounts of ergothioneine, among other constituents and a number of enzymes like creatine phosphokinase. These vesicles contribute about 60% of total volume of human semen. The secretion of prostate contains spermine, citric acid, cholesterol, phospholipids, fibrinolysin, fibrinogenase and has important functions for the nourishment of the sperms. The prostate contributes about 20% of total volume of human semen (*vide* prostate).

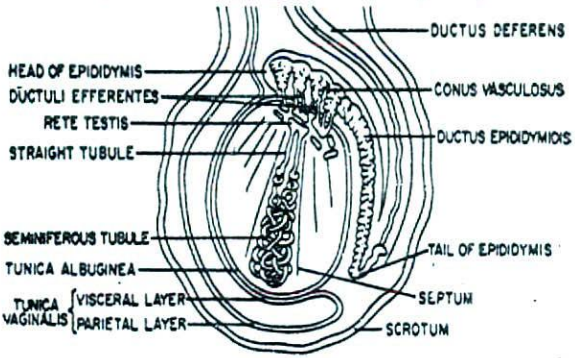


Fig. 4.136. The vertical view of the testis showig arrangement of ducts and the scrotal relation.

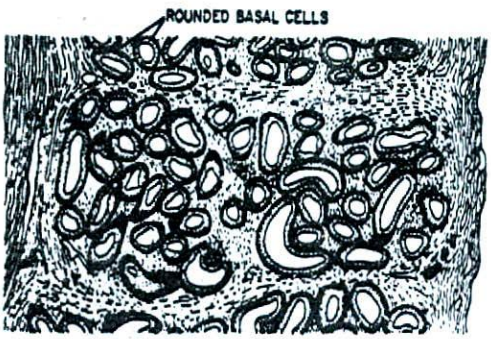


Fig. 4.137. Vertical section through body of human epididymis (diagrammatic).

Histology (Fig. 4.138). The testis is composed of three elements: (1) The fibrous covering, **tunica albuginea,** from which trabeculae descend. Tunica albuginea is composed of collagenous connective tissue with an admixture of elastic tissue. There is a thin layer of squamous cells on the outer surface of this coat, which is mesothelial lining of the scrotal sac reflected over the testes. The *tunica vasculosa* is the inner aspect of the tunic and contains a number of blood vessels. The tunic becomes thickened at the posterocephalic margin of the testis forming **mediastinum**

testis. Ducts, blood vessels and nerves enter or leave the testes through the mediastinum. (2) **Seminiferous tubules**—where spermatogenesis takes place. (3) **Interstitial cells** of Leydig, which secrete the male hormone. The last two *essential elements* are briefly described below.

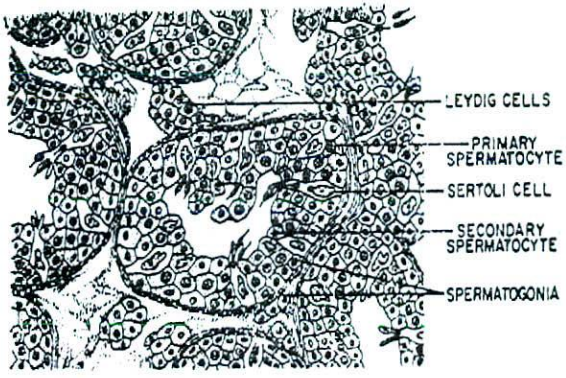


Fig. 4.138. Diagram shows the section of normal human testis.

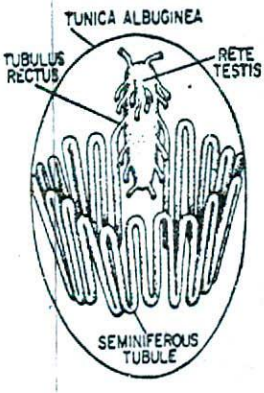


Fig. 4.139. Schematic representation of arched nature and connections of the seminiferous tubule with the rete testis.

Seminiferous tubules

Each testicular lobule contains one to three greatly convoluted, sperm-producing tubules, seminiferous tubules. These exist mainly in the form of arches (Fig. 4.139) which connect at each end with a space in the mediastinum, the *rete testis*. They are lined by four to eight layers of cells, each layer representing a particular stage in the development of spermatozoa (sperma). From outside inwards the following *five* layers are found: the *spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids* and *spermatozoa* (Fig. 4.140). The **spermatogonia** divide into **spermatocytes**

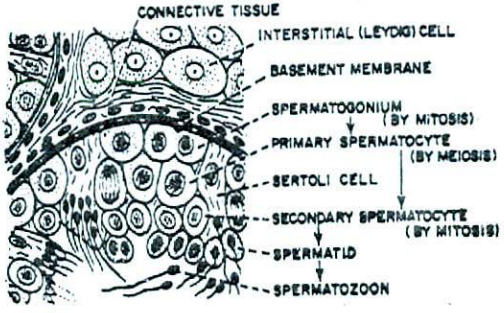


Fig. 4.140. Showing portions of two seminiferous tubules, stages of spermatogenesis and group of Leydig (interstitial) cells.

—**primary** and **secondary.** The secondary spermatocytes undergo further division and form the **spermatids.** The spermatids, therefore, contain only half the number of chromosomes (i.e., 23). The spermatids do

not divide any more. They gradually mature into **spermatozoa.** The whole process of proliferation from primary to mature cells is known as **spermatogenesis.** This includes one reduction division from diploid to haploid chromosomes and one of differentiation from spermatid to spermatozoa **(spermiogenesis).** Elongated *supporting cells* are found in the outermost layer among the spermatogonia. They are called the **cells of Sertoli** (L. *sustento*—I support) in which sperms bury their heads. These glycogen-containing cells **(sustentacular cells)** can be distinguished by the presence of lipid droplets, small fibrils, elongated mitochondria and reticular cytoplasmic appearance. Electron microscopic view of the Sertoli cells indicates that they are possibly steroidogenic. They may have a role in the process of differentiation of spermatids to spermatozoa. These cells supply nourishment to the germ cells and are not affected by different factors which destroy the spermatogenic cells. The maintenance of the structure and the spermatogenic function of the seminiferous tubules, is, on the other hand, dependent upon the action of FSH. FSH stimulates the metabolic activity of Sertoli cells. FSH secretion is controlled by the feedback of a hypothalamic substance **(inhibin)** which is secreted by certain element in seminiferous tubules (Fig. 4.141). These cells may also secrete oestrogens. The release of spermatozoa from the Sertoli cells is known as **spermiation.**

In humans, the combined length of seminiferous tubules is *estimated at about* 200-400 *metres*. They are ensheathed by a heavy basement membrane. Electron microscopy has revealed that each seminiferous tubule is enclosed in a limiting membrane composed of two concentric lamellae of collagenous fibres and an interlamellar space occupied by flattened cells. These cells contain numerous extremely fine cytoplasmic filaments.

Interstitial cells (cells of Leydig)

Inside the stroma and between the seminiferous tubules, groups of polyhedral cells are found which are called **interstitial cells of Leydig.** They are abundant in early foetal life, then gradually diminish during childhood, increase again at puberty and diminish in old age. These cells are large, polyhedral ones with an eccentric nucleus contain one to three nucleoli and possess scanty, poorly staining cytoplasm. They contain abundant agranular endoplasmic reticulumn (rich in ascorbic acid), vacuoles containing fats, phospholipids and cholesterol, resembling in such features, the interstitial cells of the ovary, the luteal cells of the corpus luteum and cells in the adrenal cortex, and mitochondria, Golgi apparatus, pigment granules, many lysosomes, refradtile globules, protein crystals of Reinke. Cells are arranged round the blood vessels. The presence of highly developed abundant agranular endoplasmic reticulumn is responsible for the postulated region of steroid hormone synthesis. Leydig cells appear to involve increased formation of cyclic AMP and increased protein synthesis.

Functions of Leydig Cells. Leydig cells comprise less than 10% of the testicular volume and secrete about 7-8 mg of testosterone per day. At puberty, the rising secretion of pituitary ICSH (identical with LH) favours the transformation of androstenedione to testosterone. Testosterone is known to be converted in certain androgen-responsive tissues to a more potent androgen, dihydrotestosterone, which is localised in the nuclei of target cells and appears to be active intracel-

lular androgen. ICSH stimulates development and differentiation of Leydig cells at puberty, leading to about 20-fold rise in plasma testosterone level. ICSH secretion is suppressed, rather slowly, by the feedback effects of testosterone and oestradiol on the hypothalamus (Fig. 4.141). The prominent effect of ICSH on the synthesis of steroid hormones is a rapid increase in testosterone secretion, mediated by cyclic AMP and apparently due to accelerated conversion of cholesterol to pregnenolone in the mitochondria of Leydig cells.

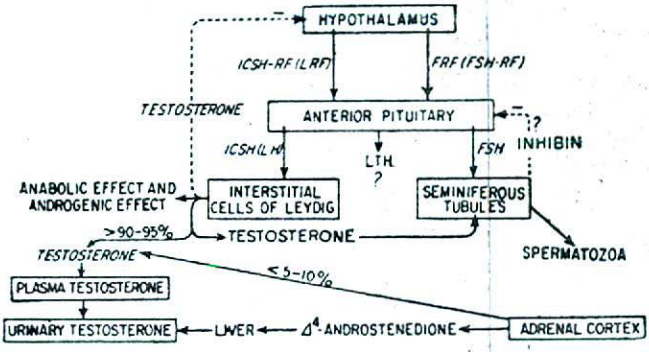


Fig. 4.141 Schematic representation of pituitary-gonadal axis of normal male. In contrast to the females, function of LTH in the male is unknown.

In absence of ICSH, the Leydig cells undergo severe atrophy and cease functioning. The completely atrophic Leydig cells are spindle-shaped, the amount of cytoplasm is greatly reduced, and the nucleus is extremely pycnotic. Lipid droplets also disappear. However, it is commonly accepted that the interstitial cells of the testis do secrete not only testosterone, but also oestrogens such as oestradiol. They are themselves stimulated into the activity by the interstitial cell-stimulating hormone (ICSH—identical with LH) and possibly follicle-stimulating hormone (FSH) of the anterior lobe of hypophysis cerebri.

Functions of testis

I. **Spermatogenesis.** This takes place at the seminiferous tubules.

Evidence: (a) In a histological section of the testis, the process of spermatogenesis can be seen in the seminiferous tubules (Fig. 4.140). (b) Degeneration, hypoactivity or malformation of these tubules disturb the production of spermatozoa.

II. **Secretion of testosterone.** The interestitial cells, which represent the endocrine tissue of the testis, synthesise the hormone.

Evidence: 1. *Effects of tying vas deferens.* (a) Degeneration of tubules with ceasation of spermatogenesis. [Sometimes spermatogenesis may continue. The spermatozoa undergo degeneration and liquefaction and are absorbed. Fresh sperms take their place.] (b) The interstitial cells remain intact and liberate their hormone.

2. *Cryptorchidism (Undescended testis).* The tubules in the undescended testis are non-functioning. Spermatogenesis fails to occur. If the condition

is bilateral, the subject is sterile but *the accessory sex organs and the secondary sex characters are fully developed,* indicating normal production of the hormone. The high abdominal temperature inhibits spermatogenesis. If the active normal testes be artificially warmed—spermatogenesis, but not hormone synthesis is inhibited.

3. *Effects of extirpation.* (a) *Before puberty*—The puberty changes (page 4-195) do not appear and the accessory sexual organs, e.g., penis, prostate, seminal vesicles, etc., do not develop. The subject is sterile. There is abnormal deposition of fat on breasts, hips, buttocks, etc. There is poor development of the muscles and delayed union of the epiphysis. (b) *After puberty*—The subject becomes sterile, and the sex organs degenerate. Secondary sex characters usually, but not always, disappear. The sex hormones secreted by the adrenal cortex probably maintain the latter to some extent.

4. *Administration of testicular extracts* (testosterone). Prevents the changes in the secondary sex organs and characters, etc., following castration after puberty. If given to immature animals, puberty changes are hastened.

These experiments show that in addition to spermatogenesis the testes elaborate a hormone on which the growth of secondary sex organs and the secondary sex characters depend. This hormone has been isolated and is known as the *testosterone.*

ANDROGENS

Androgens are substances having masculinising properties. Administration causes—(1) growth of the accessory sex organs in the castrated males, (2) growth of the comb, wattles and the ear lobules of the castrated male birds. These methods are employed for assaying the androgenic potency of an unknown substance.

Chemistry and Varieties. Androgens are C-19 sterol compounds. Their structures (Fig. 4.142) are as follows:

Two varieties: (a) **natural,** and (b) **synthetic.** The chief natural androgen is called **testosterone.** Of the synthetic products, *methyl testosterone* and *testosterone propionate* are important members. *Synthetic androgens are effective by mouth being freely absorbed and unaffected by liver. Natural androgens are mostly inactivated by liver. Hence, not much effective by mouth.* Activity increases if combined with fatty acids, such as propionic acid. The chief degradation products of androgens are the **17-ketosteroids** (17-KS), **androsterone** and **dehydroepiandrosterone** (DHEA).

The peripheral conversion of testosterone to oestrogen accounts for a large proportion of oestradiol production in the male. Testosterone originates almost entirely in the testis; small amounts of ketosteroids secreted by the testis do not possess significant activity, either directly or as precursors of testosterone. However, testosterone, ketosteroids and much greater quantities of DHEA secreted by the adrenal gland after puberty contribute substantially to urinary 17-ketosteroid excretion.

Sources. Two sources: (1) **Testis**—the interstitial cells secrete testosterone. The testosterone is synthesised from the cholesterol ester content of these cells. (2) **Adrenal cortex** (pages 4-143, 4-148)—traces.

International unit for androgens is defined as the activity of 0·1 mg of androsterone. Since pure androgens are available, one can use only and express activity on a weight (mg) basis.

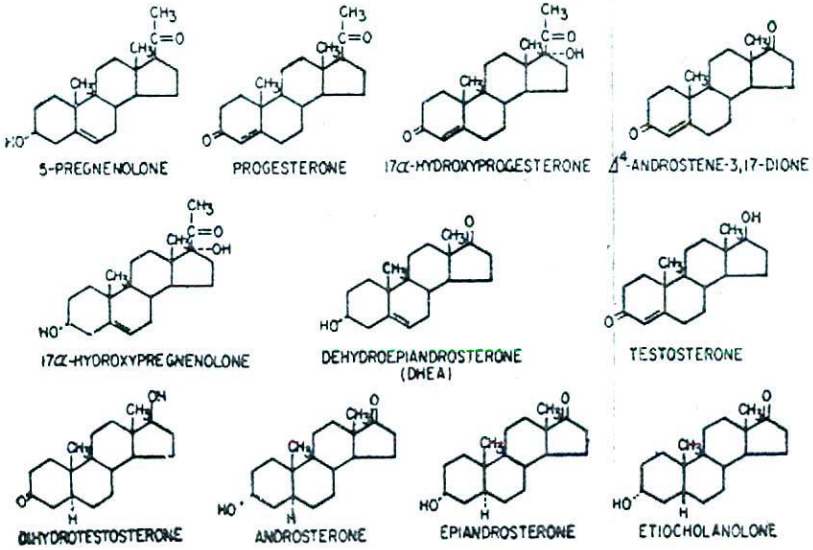


Fig. 4.142. Chemical structures of naturally-occurring androgens.

Bioassay method for assessing androgen concentration in human blood and urine is the capon comb test. Fluid being tested is injected for 3-5 days and then the comb is weighed or measured (*sum of height + length*). Other bioassay methods utilise such responses as chick comb growth, enlargement of prostate or seminal vesicles, and darkening of sparrow bill (0·07 γ sensitivity).

Biosynthesis of androgens in the testis

Interstitial cells of Leydig are the target cells that are stimulated by gonadotrophins for an increased synthesis of androgens. The chief products which are secreted into the spermatic venous blood of adult testes are testosterone and smaller quantities of Δ^4-androstenedione and dehydroepiendrosterone (DHEA). Major pathways for synthesis of androgens are presented in Fig. 4.143.

The major route for the formation of androgens from 5-pregnenolone is 17 α-hydroxypregnenolone to DHEA and thence to Δ^4-androstenedione and is the same as for reactions in the ovary and in adrenal cortex.

The other pathway is 5-pregnenolone to progesterone and thence to Δ^4-androstenedione. Probably similar mechanisms exist in Leydig cells for the synthesis of androgens as occur for androgen synthesis in the adrenal cortex, because mitochondrial and microsomal function, metabolic pathways, source of reducing equivalents, mechanism of electron transport, etc., have not been studied in detail as in the adrenal cortex.

There is an increase in activity and amount of 17-dehydrogenase in the male at puberty. This enzyme leads to a marked increase in the formation of testosterone from Δ^4-androsteronedione.

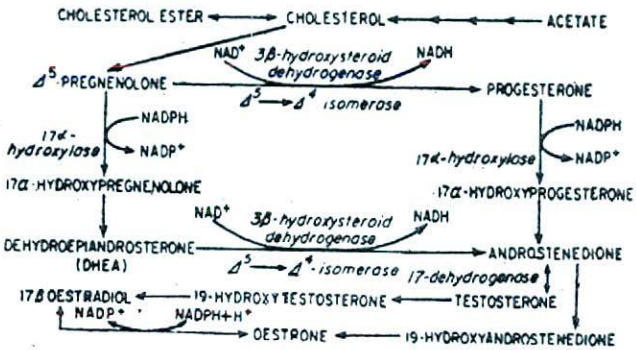


Fig. 4.143. Steroids synthesis in the testis. Although about 95% of testosterone is secreted from Leydig cells, some of the intermediates may be in the circulation.

According to Dorfman and his associates, the pathway of progesterone to 17α-hydroxyprogesterone to testosterone is more important in man and other primates.

Normally, the testis and the adrenal possess similar qualitative capacities to synthesis androgens. Since the testis lacks 11-hydroxylase activity, the adrenals can only synthesise glucocorticoids and mineralocorticoids.

In the Leydig cells, 11- and 21-hydroxylases are absent and pregnenolone is hydroxylated in C-17. Side-chain cleavage forms 17-ketosteroids and these ketosteroids are converted to testosterone.

Mode of action

Androgens promote protein synthesis in the male accessory glands by direct stimulating effect on DNA and RNA polymerase by increasing the syrthesis of mRNA in the nucleus and increased amino-acyl transferase at the ribosomal level. Androgens increase the activities of glycolytic enzymes, phosphofructose kinase and of hexokinase due to a probable results of those actions. Androgens also act at the mitochondrial level to increase respiration rate, synthesis of mitochondrial membranes and number of mitochondria.

Transport, metabolism and excretion of testosterone

About 60-65% of testosterone in plasma are bound to albumin and β-globulin. The latter protein binds also oestradiol and hence is known as testosterone-oestrogen-binding globulin (TEBG), sex-hormone-binding globulin (SHBG) or gonadal-steroid-binding globulin (GBG). Free and bound testosterone levels in plasma are about 0·6 μg/100 ml in normal men and 0·1 μg/100 ml in normal women. In men it somewhat declines with age.

Large amounts of secreted testosterone are converted in the liver in 17-ketosteroids and a small amount of secreted testosterone is converted to oestrogen somewhere in the body. Recent evidence indicates that testosterone is converted in target tissues to the more potent dihydrotestosterone, which may be an active intracellular androgen.

Principal metabolites of testosterone are androsterone and etiocholanolone and the major 17-ketosteroids (17-oxosterbids, 17-OXOS) in urine. In addition a small quantity of DHEA is also excreted as sulphate (Fig. 4.144). Androsterone and DHEA are weaker than testosterone. About 60-65% of urinany 17-ketosteroids (17-OXOS) have adrenal origin and the rest are of testicular origin. But not all 17-ketosteroids are androgens and not all androgens are 17-ketosteroids.

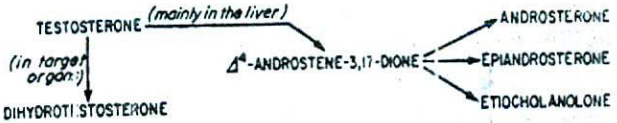


Fig. 4.144. Schematic representation of metabolic pathway of testosterone.

The major pathway of degradation of testosterone in the liver involves oxidation to androstenedione and subsequent saturation of double bond in ring A (Δ^4) and reduction of ketone groups.

Testicular steroid secretion can be stimulated by ICSH known in female physiology. LH and by the administration of other gonadotrophin, such as human chorionic gonadotrophin. These hormones stimulate

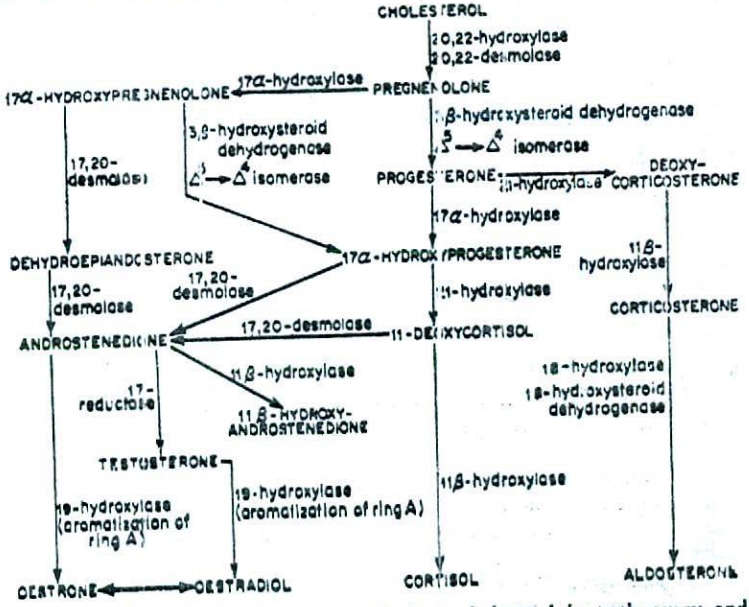


Fig. 4.145. Biosynthesis of main steroids from cholesterol in testis, ovary and adrenal. The enzymes 20, 22 desmolase and 11β-hydroxylase are situated in the mitochondria, whereas 3β-hydroxysteroid dehydrogenase, 17α-hydroxylase and 21-hydroxylase are microsomal in origin. Isomerase is contained in the cell cytoplasm. The sequence of events resulting in the production of the individual steroid depends on the enzymes being attached in the correct order on the mitochondrial surface within the cell.

luteotrophic action. In the male regulating androgen secretion ICSH is the principal gonadotrophin. In the case of ovary and adrenal cortex, cyclic AMP is an intermediate in the mechanism of hormone action. Mitochondrial conversion of cholesterol to 5-pregnenolone is a rate-limiting step, especially 20-hydroxylation reaction of cholesterol for the adrenal cortex and the ovary (Fig. 4.145). Gonadotrophins may also influence this step.

Functions of androgens

1. Growth of accessory male sex organs, the seminal vesicles, prostate, epididymis, vas deferens, penis, etc., and maintenance of the morphologic and functional activity of these seminal vesicles enlarge and fructose begins to be secreted from them.

2. Normal development of male secondary sex characters. These the following: (a) the growth of hair on the face, in the axillae, incude on the chest and in the pubic region at puberty, responsible for the upward growth of hair towards the umbilicus in the midline (male escutcheon: Δ), (b) development of typical masculine voice, (c) activity and emotional make-up of characteristic man. In the female, androgens cause the growth of sexual hair and may influence other aspects of sexual activity and libido. In experimental animals androgens provide boi-oestrous and o. aggressive nature.

3. Effect on sperm production. Androgens facilitate spermatogenesis. Due to absence of blood vessels at the germinal epithelium, androgens produced by the Leydig cells under the influence of ICSH diffuse inside the tubules and exert their action. There is also a possibility that androgens may be produced by the Sertoli cells regulated by FSH.

4. Androgens have a direct stimulating effect on DNA and on RNA polymerase and increase the synthesis of mRNA and the incorporation of amino acids into protein.

5. Protein anabolic action. Testosterone is a protein anabolic hormone (nitrogen-retaining effect). It causes increased passage of amino acids inside the cell and positive nitrogen balance. This effect is a direct one, independent of the presence of endocrine organs.

6. Muscular development. Selective stimulation of certain muscles by androgens indicates that the hormone has myotrophic effect.

7. Effect on growth of bone. Due to the protein anabolic nature of testosterone it causes increase in bone matrix and thus increases deposition of clacium salts. It also leads to closure of epiphyses of long bones, thus arrests growth.

8. Effect on BMR. Testosterone increases BMR. This effect might also be secondary to the protein anabolic nature of the hormone.

9. Effect on RBC. Total number of RBC in males is higher than in females. Castration causes reduction of RBC which comes back to control level on androgen treatment.

10. Effect on water and mineral metabolism. Testosterone causes retention of sodium, potassium, calcium, phosphate and water to some degree. This is probably related to increased protein anabolism.

Control of testicular activity

1. **Hormonal and neural control.** (a) **Anterior pituitary** (Fig. 4.146). Anterior pituitary releases the gonadotrophins: (1) follicle-

stimulating hormone (FSH), (2) lutenising hormone (LH) or ICSH and (3) prolactin, similar to that in females. Of these FSH controls spermatogenesis and ICSH or LH initiates and maintains the activity of the interstitial cells and controls the secretion of androgens and the latter activate the seminiferous tubules. Prolactin is reported to have synergistic

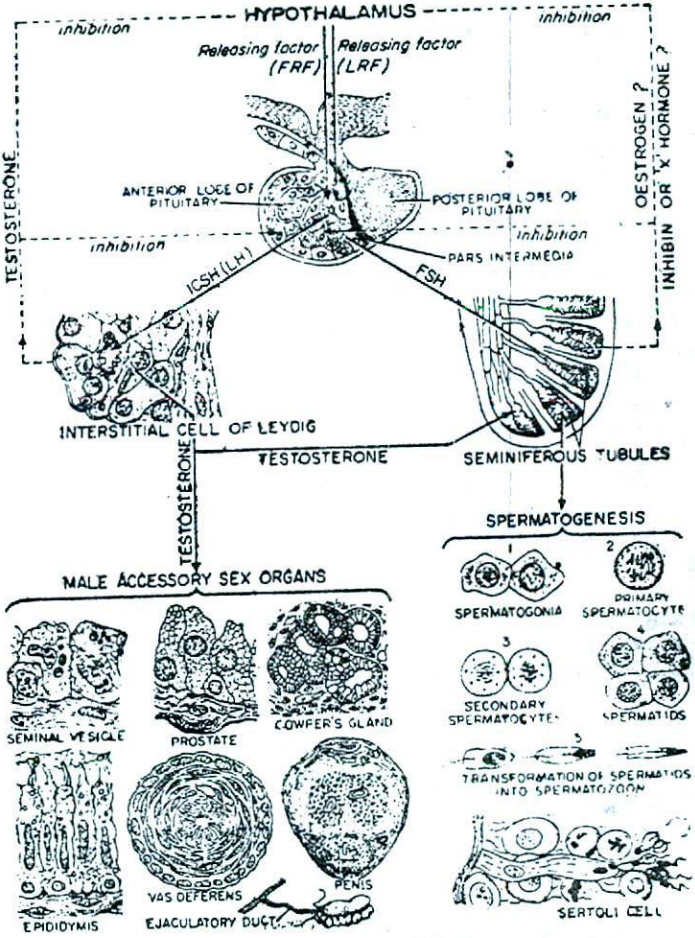


Fig. 4.146. Diagrammatic representation of the hormonal control of testicular activity.

action with androgens in stimulating growth of male accessory sex organs. FSH is also called 'gametokinetic' as it controls formation and maturation of gametes, i.e., sperm in males and ovum in females. FSH not only helps in the formation of spermatozoa to complete maturation, but androgens which are secreted by interstitial cells under the influence of ICSH is also required. It has been observed

that when FSH and ICSH are induced together in hypophysectomised animals, spermatogenesis is completely restored. If hypophysectomy is done in immature male animals, testes and accessory sex organs do not develop due to lack of androgens. ICSH stimulates the interstitial cells to produce androgens which are responsible for the maturation and activity of secondary or accessory sex organs and characters. The mechanism by which LH stimulates the Leydig cells appears to involve increased formation of cyclic AMP and increased protein synthesis. There is reciprocal relation between testis and anterior pituitary. Testosterone inhibits the secretion of ICSH (LH). Secretion of FSH is possiby controlled by the feedback of a substance called **inhibin** or X-hormone which is secreted by some element in the seminiferous tubules. Alternative pathway is that the seminiferous tubules remove FSH from the circulation in the specific process of being stimulated by it. The fact that large dose of androgens inhibits spermatogenesis suggests that FSH secretion might also be controlled by the androgens. Oestrogen inhibits gonadotrophin (LH) and thus reduces androgen level. Female hormone oestrogen secreted from testis also takes part in the regulation of FSH secretion. (b) **Hypothalamus.** The release of pituitary gonadotrophins is regulated by the releasing factors FSH-RF and LRF liberated from hypothalamus. Hypothalamic disorder causes testicular degeneration. It has also been established that the feedback inhibition of gonadotrophin released by testosterone is at the hypothalamic level. (c) **Adrenal cortex** (page 4-152). (d) **Thyroid** (page 4-86). (e) **Thymus.** Testosterone depresses thymus. At puberty it involutes.

2. **Temperature control.** Higher temperature inhibits spermatogenesis (*vide Cryptorchidism*—page 4-207). Undescended testis, being under higher abdominal temperature, is sterile, Scrotum, with the help of dartos muscle, adjusts its own surface area and therefore, the heat loss: In this way it maintains an optimum temperature in the sac and acts as a perfect incubator for the testes. Impairment of testicular function has been noted in febrile condition, when testes are subjected to higher temperature.

3. **Dietetic control.** Malnutrition has a deleterious effect on testicular physiology. Insufficient intake of calorie, protein or vitamins affects adversely the accessory sex organs and also semineferous epithelium. Either androgens or pituitary gonadotrophins are able to restore them to normal condition. This indicates the primary defect to be in gonadotrophins. Some type of infertility in adults might result from malnutrition during prepuberal life.

Antiandrogens. Subsctances with strong antiandrogenic properties, which probably compete with androgens for receptors in target organs, have been discovered recently. They include 17, α-methyl-B-nortestosterone, free cyproterone (an alcohol) and its acetate. Cyproterone acetate differs from the free alcohol in possessing, in addition, progestational and antigonadotrophic properties. Both compounds have been used in man, but neither has been tested adequately.

LIFE HISTORY OF SPERMATOZOA (SPERMA)

Histology. The spermatid is converted into a spermatozoon by the process of metamorphosis and is the last descendent of the male germ cell.

The spermatozoon is a specialised cell and differs from an ordinary cell in various aspects. It is much smaller compared to the ovum and its total length, about 60 μm, is equal to that of the nucleus of the ovum. The mature spermatozoon can be commonly devided into **head** and **tail.** The tail again consists of a **neck, middle piece** or **engine room** or **body, main piece** and **end piece** on the basis of slight differences in thickness along its length. There are significant differences in the internal structure of these segments. The **head** of the human spermatozoon is oval on surface view and pear-shaped on side view (Figs. 4.147 & 4.148). It is elastic and measures about 4-5 μm in length and 2·5 to 3·5 μm in diameter. The head is a condensed nucleus and contains about 40% of DNA and is rich in arginine. It is suggested to be refractory to deoxyribonuclease. The nucleus is characterised by its affinity for basic dyes, absorption of ultra-violet rays and by its chemical composition. The nucleus with its nuclear membrane is surrounded by a continuous plasma membrane without any intervening cytoplasm. The nucleus is covered on its anterior two-thirds by acrosome **(galea capitis)** or **head cap proper.** The head cap has anterior and posterior parts in most animals. But the posterior head cap is said to be absent in the human spermatozoon. The posterior head cap, known as *postnuclear part,* lies

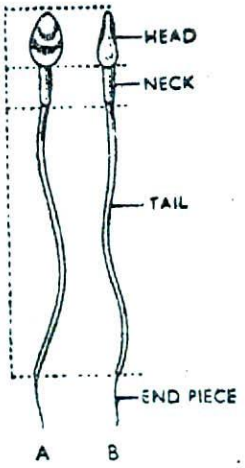


Fig. 4.147. Diagram of spermatozoa. A = front view, B = side view.

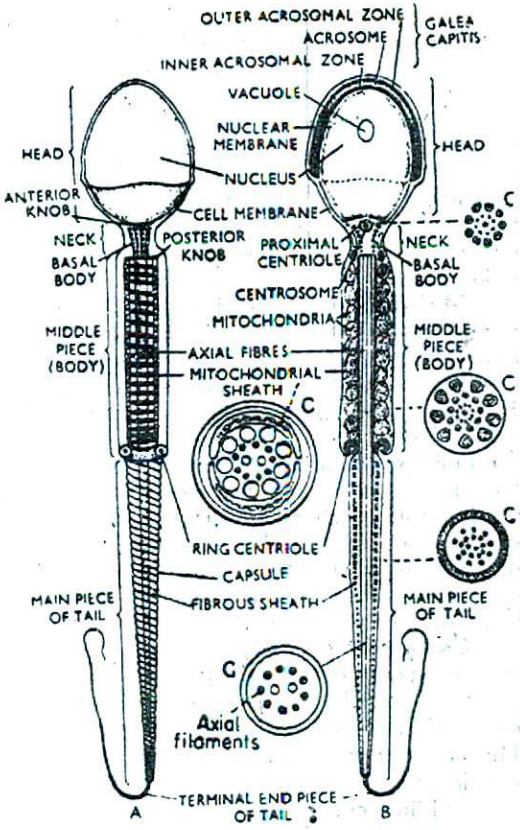


Fig. 4.148. Diagrammatic representation of spermatozoon. A = front view; B = longitudinal section, C = cross-section of the respective parts.

behind the nucleus. The acrosome is formed out of the Golgi apparatus of the spermatid and can liberate a chemical substance which enables the sperm to pierce the *zona pellucida* of the ovum. The **neck** is a short, weak segmen and connects the head with the middle piece (body). The proximal centriole (*anterior knob*) fits in the depression in the head and is the junction of the head and neck whereas distal centriole (*posterior knob*) lies between the neck and the middle piece. In the neck the axial filaments consist of three bundles of fibrils being connected with the three basal granules in the anterior centriole.

Middle piece or body. It is the *engine room* of the spermatozoon and is cylindrical in form. It has a length of 5 to 7 μm and a thickness of somewhat more than 1 μm. It extends from a slender connecting piece immediately behind the posterior pole of the head to a ring-like structure called the **annulus** or *terminal ring*. In the middle piece the *helical mitochondrial sheath* consists of 10-14 turns in which cisterne mitochondrialis are found to be fused with its limiting double membrane with lipid-rich substance. The axial fibre bundle is the central filamentous body which begins from the proximal centriole and ends in the end piece. Except in the end piece the axial filaments are surrounded by the axial sheath which is formed out of condensation of a thin layer of the cytoplasm.

Main piece of tail. It is about 45 μm long and about half a micrometre thick at the base, gradually tapering towards the end piece. The main piece consists of a core or *axoneme* of longitudinal filaments surrounded by a fibrous sheath of circumferentially oriented dense fibres. In the human sperm tail, the outer coarse fibres taper rapidly and extend only a short distance into the principal piece. In the tail the fibrils of the axial filament are made up a pair of central filaments which are surrounded by concentric cylinders each being consisted of nine fibrils. The fibrils, peripherally arranged, contract and relax at times producing shortening and lengthening and the spermatozoon moves forwards by means of bending waves. The *ATP-ase* is believed to be present throughout the whole length of the tail. This enzyme helps in the movement of spermatozoon.

End piece of tail (Flagellum). It consists of the *terminal* portion of the axial filament and is about 5 μm long. Its axial filament is embedded in a minimum matrix of the cytoplasm being enclosed only by the plasma membrane that invests the entire sperm. The cross-section through the end piece of the sperm tail is essentially identical to that of a cilium. Formerly it was believed to be composed of naked filaments.

The total length of the cell is about 60-62 μm. There are 100,000 sperma per cu. mm in healthy seemen.

Spermatogenesis

Befor puberty, mature spermatozoa are not formed. It takes place actively following puberty and is maintained with the advancing age, although the same is slowed gradually. There is no such clear-cut male menopause (**male climacteric**) as it is characterised in women with advancing age. In many species it occurs during the breeding season only. At other times, development remains suspended at the spermatocyte stage. **Spermatogenesis is the process of formation of spermatozoa in-**

cluding spermatocytogenesis and spermiogenesis. The **spermatocytogenesis** in the first stage of formation of spermatozoa in which the spermatogonia develop into spermatocytes and then into spermatids, whereas the **spermiogenesis** is that stage in the formation of spermatozoa in which spermatids transform to spermatozoa.

The seminiferous tubules, which are long, convoluted structures about 150 to 200 μm in diameter and 30 to 70 cm in length, are responsible for the main bulk of the organs. There are several hundreds of them in each testis. The tubule is surrounded by a thin basement membrane where lies a compound epithelium composed of supporting cells and spermatogenetic or germ cells. Each supporting cell (cell of Sertoli) extends from the basement membrane to the lumen. Between the Sertoli or sustentacular cells lie the spermatogenetic cells in a series of development, i.e., **spermatogenesis** (Fig. 4.140).

Near the basement membrane occur the most immature of the male germ cells known as the spermatogonia or parent cells which are capable of a limitless number of mitotic divisions. Occasionally, a daughter cell, formed as a result of one of these mitoses, is pushed towards the lumen of the seminiferous tubules and enters a growth phase to become a **primary spermatocyte.** At this stage this germ cell reaches its greatest size. The primary spermatocyte undergoes a meiotic cell division whereby the number of chromosomes in the nucleus of each daughter cell becomes half that of the parent cell. After exchanging components, however, they do not split lengthwise as in mitosis, but separate, one from every pair passing to each daughter cell. The **secondary sepermatocytes,** which are formed as a result of meiosis of the primary spermatocytes, are smaller than their parent cell and lie nearer the lumen. They are very short-lived, since after their formation they pass through mitosis to produce cells, known as **spermatids** which are still smaller. The spermatids are converted, without further division, into spermatozoa. Spermatogenesis proceeds in waves along each tubule, so that not all stages are seen in any one cross-section.

The Sertoli cells are larger than the spermatogonia and rest on the basement membrane. They project towards the lumen of the tubule, tapering as they do so. As the spermatids are formed they become attached in clusters to the Sertoli cells and actually penetrate into their cytoplasm, where they develop into spermatozoa. The fully formed germ cells are then displaced into the lumen by the immature cells passing between them. The Sertoli cells aid the development of the spermatozoa and may also have an endocrine function. The spermatozoa pass down the seminiferous tubules, through the network of the rete testis, along the efferent ductules and into the duct of the epididymis.

The mechanism of their release from the Sertoli cell is unknown; when first liberated they are non-mortile. As there is no muscle in the wall of the seminiferous tubule, their passage along the lumen towards the rete testis is probably attribute to fluid currents caused by the tension of the fibrous capsule of the testis and passive, induced by the pressure of the new spermatozoa behind and possibly by the actions of the cilia and smooth muscle in the walls of the efferent ductules. During their passage through the epididymis, however, they become actively motile and attain their maximum capacity for the fertilisation of ova. When they enter

the vas deferens they are ready for ejaculation. The fluid part of the semen is contributed by the seminal vesicle, prostate and bulbo-urethral glands.

For convenience of description spermatogenesis can be discussed under (1) spermatocytogenesis, (2) meiosis and (3) spermiogenesis. **Spermatocytogenesis:** *Spermatocytogenesis* is the first stage of formation of spermatozoa in which the spermatogonia develop into spermatocytes and then into spermatids. For continuation of spermatogenesis without draining the supply of germinal cells, the spermatogonia must perpetuate themselves and also produce generation after generation of spermatocytes. If the testicular tissue preserved in Zenker-formol fixative at least two types of spermatogonia can be distinguished.

(i) *The type A spermatogonia* are the primitive cells in length and lie close to the basal membrane. These cells have two types: (a) *The dark type A spermatogonium* has dark nucleoplasm and a large pale-stating nuclear vacuole; (b) *the pale type A spermatogonium* has a spherical or ellipsoid nucleus with very fine chromatin granules and one or two irregular nucleoli attached to the interior of the nuclear envelop. Its cytoplasm is homogeneous and pale-staining.

The type A spermatogonium may remain dormant for a considerable period and then undergo a series of changes which give rise to the other type A spermatogonia. Here certain spermatogonia remain

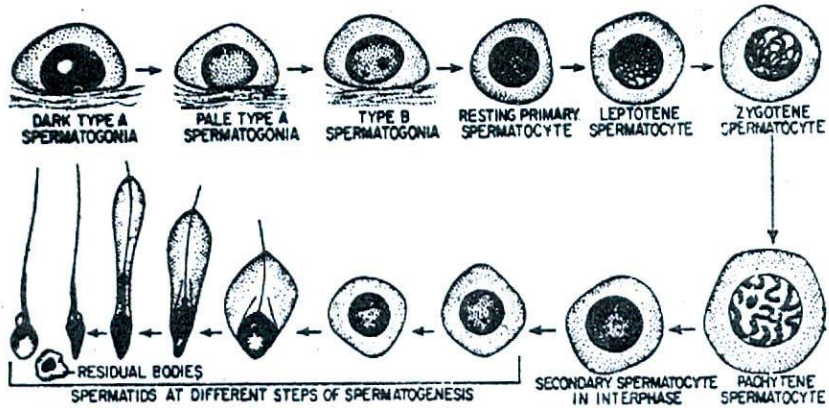


Fig. 4.149. Schematic representation of evolution of human spermatozoa from spermatogonia.

dormant to serve as stem cells for future cycles of spermatogonial renewal and spermatocytogenesis. Others proceed to transform through recognisable intermediates into the type B spermatogonia. The type B spermatogonium has a spherical nucleus with the chromatin granules of varying size along the nuclear envelop and contains centrally located single nucleolus often with chromatin granules. The division of the type B spermatogonia produces *primary spermatocytes* (Fig. 4.149).

(2) **Meiosis.** The primary spermatocytes at first resemble the spermatogonia from which they arise and pass through prophase, meta-

phase, anaphase and telophase of meiotic cell division and the resulting daughter cells are called *secondary spermatocytes* which again devide mitotically to form *spermatids*. In human spermatogonium there are 46 chromosomes of which 22 pairs are autosomes and one pair sex chromosomes.

Therefore one of the secondary spermatocytes contains 22 autosomes and a Y sex chromosome, and other contains 22 autosomes and an X sex chromosomes. After meiotic division in females an ovum contains 22 autosomes and X chromosome, and the spermatozoon containing 22 autosomes and X chromosome fertilises the ovum, the resulting sex will be *female determining*. On the other hand, when an ovum is fertilised by a spermatozoon containing 22 autosomes and one Y chromosome, the resulting sex will be *male determining* (Fig. 4.150).

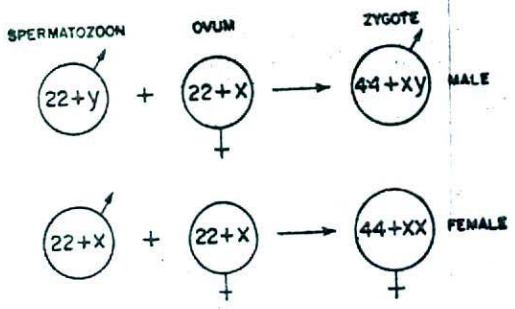


Fig. 4.150. At fertilisation the nuclei of two gametes (a spermatozoon and an ovum) unite to form a zygote (or fertilised ovum). Since the normal ovum always contains an X chromosome, the sex of the offspring depends on whether an X-bearing or a Y-bearing spermatozoon effects fertilisation. Although equal numbers of X-bearing and Y-bearing spermatozoa are probably formed, for some unknown reason rather more males are conceived than females.

It has been traditionally known that each spermatogonium developed into a primary spermatocyte that ultimately devided into two secondary spermatocytes, and these in turn devided to form four individual spermatids. Recent studies under electron microscope reveal that the type B spermatogonium when devides, the division of cell body (cytokinesis) is incomplete and the daughter cells remain connected by a protoplasmic bridge at the site where the constricting cleavage furrow encounters the spindle remnant. Such spindle bridges occur as transient structures in mitosis of somatic cells, but in the seminiferous epithelium they remain, after resorption of the spindle, as sizable communications between the daughter cells. The cytokinesis of the last spermatogonial division is incomplete, resulting in two conjoined primary spermatocytes, which again devide into four interconnected secondary spermatocytes which devide into clusters of eight conjoined spermatids (Fig. 4.15I). This condition persists upto a late stage in the differentiation of spermatids into spermatozoa. Occastionally partial failures of this process may account for the frequency of abnormal double spermatozoa in the ejaculated semen.

Spermiogenesis. It is the sequence of developmental events by which spermatids are transformed into mature sperm. The main features

of this process involve elaboration of a nuclear cap from the Golgi complex, condensation of nucleus, formation of motile flagellum, and extensive shedding of the cytoplasm. Each spermatid is the relatively small spherical on polygonal cell resulting from division of the secondary spermatocyte and has a nucleus 5-6 μm in diameter with pale-staining finely

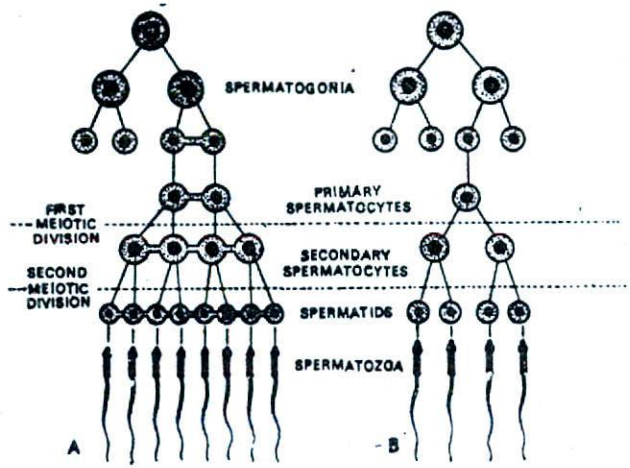


Fig. 4.151. Diagram of mammalian spermatogenesis. **A**=showing as revised on the basis of electron microscopic observations of intercellular bridges connecting the developing germ cells. In most species primary and secondary spermatocytes and spermatids seem to be joined by bridge. Late in their differentiation the spermatids separate to produce individual spermatozoa. **B**=shows conventional spermatogenesis (after D.W. Fawcett).

granular chromatin and several darker chromatin masses. A small Golgi apparatus is present in the juxtanuclear cytoplasm. The sequence of changes converting the spherical spermatid into an elongated spermatozoon with complex intracellular reorganisation can generally be described under *Golgi phase, cap phase, acrosomal phase* (Fig. 4.152) and *maturation phase.*

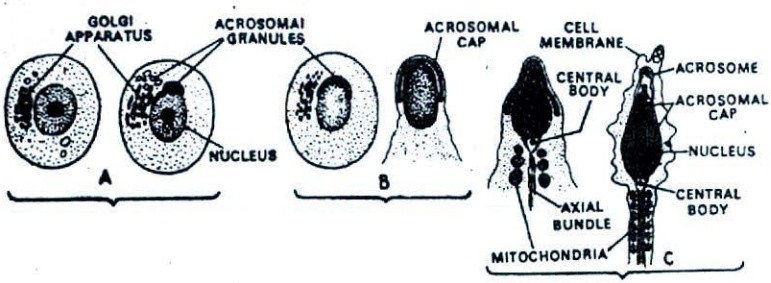


Fig. 4.152. Showing different phases of spermatogenesis. A=Golgi phase. B=cap phase. C=acrosomal phase.

In the first or Golgi phase the first sign of differentiation of a specific component of the spermatozoon is the appearance of several small granules within the Golgi apparatus. It is believed that in some

species these are first observed in the spermatocytes whereas in others these are not observed until the spermatid stage. These are called **pro-acrosomal granules** which are rich in carbohydrate and are commonly stained by the periodic acid-Schiff reaction. The general features of spermiogenesis can be examined under light microscope, but electron micrographs show finer details. As development progresses the several separate granules coalesce into a single large globule, known as **acrosomal granule,** contained within a membrane bounded acrosomal vesicle or vacuole. This **acrosomal vesicle** becomes adherent to the outer aspect of the nuclear envelops. This adherent point marks the future anterior tip of the sperm nucleus. The Golgi apparatus still continues to form further small vesicles that conglomerate with acrosomal vesicle and thus a hemispherical acrosomal granule is seen to be formed from the appearance of the proacrosomal granules and remain fixed to the sperm nucleus. The Golgi phase passes on to the cap phase.

In the **cap phase.** the limiting membrane of the acrosomal vesicle increases its area of adherent to the nuclear membrane, forming a thin fold that is seen to spread outwards from the pole of the nucleus. Ultimately it covers the anterior half to 2/3rds of the nucleus as a membranous **head Cap,** the acrosome being localised at the pole of the nucleus.

In the third or **acrosomal phase** there occurs redistribution of the acrosomal substance, elongation of the spermatid and condensation of the nucleoplasm. The bulk of the acrosomal substance which remains condenced at the anterior pole of the nucleus now gradually spreads into a thin layer into the membranous fold comprising the head cap untill the acrosomal vesicle and head cap are coextensive. Thus the **acrosomal cap** or **acrosome** is formed. The spermatid nucleus becomes elongated and slightly flattend during the acrosomal phase. The uniformly dispersed fine granules of the nucleoplasm become condensed into thin strands or filaments which subsequently shorten and then thicken into coarse dense granules.

During the **maturation phase** of spermiogenesis there is little further change in the relatively simple acrosome of the human sperm. But in other species it becomes continuous undergoing further alterations. The dense granules in the condensing nucleus become further coarse and large, and lastly they coalesce together, and the nucleus is converted into a homogeneous dense mass devoid of visible substructure. As the nucleus begins to elongate and condense the pair of centrioles now migrate to the opposite end of the spermatid which has acquired definitive form and size. The distal centriole becomes oriented perpendicular to the cell surface and gives rise to a slender flagellum which grows out extracellularly in the cleft between the spermatid and surrounding supporting or Sertoli cell. Then the pair of centrioles and the base of the flagellum recede from the surface and occupy a position at the caudal pole of the nucleus. Cytoplasmic microtubules now arise and become laterally associated to form a roughly cylindrical shape known as **caudal sheath** or **manchettle.** The caudal sheath extends caudally from a ring-like specialisation of the cell membrane at the posterior margin of the acrosomal cap. There is now a marked elongation of the spermatid, the bulk of the cytoplasm is displaced caudally and surrounds the proximal part of the flagellum which at this time consists only of the **axial filament complex (axoneme)** with

two central fibrils and nine peripheral doublets being continuous with the wall of the distal centriole. Under E/M the centriole seems to be surrounded by a ring of annular dense granular substance which is a derivative of **chromatoid body,** was called the *ring centriole.* In further differentiation of the tail, nine longitudinally oriented segmented columns develop around centrioles and are joined to each other proximally forming **connecting piece** at the nuclear base. Concurrently nine thick longitudinal fibres develop just peripherally to the doublets of the axial filament complex being joined with nine structural elements. The distal centriole and large ring around it disappear and outer fibres of the tail develop. In the further elongation of tail the smaller, dense ring which is fixed to the flagellar membrane moves back and the manchette disappears. The mitochondria gather around the flagellum between the annulus and nucleus and wrap helically around the flagellar segment and thus the middle piece is formed completely.

In further progress, circumferentially oriented hoops or ribs, deposited around the tail fibres distal to the annulus, constitute the **fibrous sheath** of the main piece. Lastly, membrane-bound excess cytoplasm containing fine granules, lipid droplets and degenerating organelles is cast off as the residual body of Regaud being separated clusters of conjoined spermatids into individual sperm. Soon thereafter sperm heads being released from the supporting (Sertoli) cells the spermatozoon is set free in the lumen of the seminiferous tubule.

Stages of spermatogenesis

Spermatogonia → primary spermatocyte → secondary spermatocyte → spermatid (maturation) → **spermatozoon.** In man, this process begins during adolescence. On an average, the maturation process takes about 74 days—from a primitive germ cell to a mature sperm.

Control of spermatogenesis (pages 4-212—4-213) The following factors regulate it:

1. FSH and LH of anterior pituitary (pages 4-31-4-32).
2. Temperature—The process of spermatogenesis takes place at a temperature which is considerably lower than that of the body. Normal position of the testes in serotum ensures this lowered temperature. The temperature within the serotum is 1·5° to 2° C below that in the abdomen, and normal development and function of the seminiferous tubules can take place only at this lower temperature. Nowadays adopting methods of preservation and storage for artificial insemination in cases of infertile human marriage are utilised. Humen semen can be stored at a temperature of —70° C for weeks and months. The motility and fertility of the suspended spermatozoa reappear when the frozen suspension is unfrozen.
3. Vitamins—E, A, ascorbic acid and several members of vitamin B complex.
4. Thyroid (page 4-86).
5. Adrenal cortex (vide 'Relation with Sex' on page 4-152).
6. Testosterone secreted from interstitial cells of testes also has regulatory control on spermatogenesis.

It is inhibited by deep X-ray, radium, alcoholism, etc. Close confinement also inhibits the process in animals. Wild animals do not breed in captivity. Probably mental depression may inhibit anterior pituitary

through hypothalamus. But these factors do not affect the secretion of testosterone from interstitial cells.

Motility, span of life and fertilising power. The motility of the spermatozoa depends upon undulatory and tortional movements of the tail of spermatozoon of which tortional movement is attributable to the unequal size of the coarse fibrils of the tail. In the seminiferous tubules and the proximal part of epididymis, the spermatozoa are not motile and their fertilising power is low. In the distal end of the epididymis motility develops and the fertilising power becomes double. The secretion of the epididymis has got spermatotrophic effect. If the epididymis be tied at the both ends, the spermatozoa inside remain alive for 60 days but their fertility lasts only for 30 days. If the same experiment is repeated after castration, both the periods are reduced by half. This proves that *the internal secretion of the testes increases both the span of life and the fertilising power of the spermatozoon. Full fertilising power* of the sperm is achieved only after travelling within the uterine tube. This power is known as **capacitation.** This capacitation is acquired only after staying in the uterine cavity for a few days because freshly ejected sprem into the vagina has got a little fertilising power. The spermatozoa swim through the vas deferens aided by the ciliary movement of the vas epithelium and probably by the contraction of its muscular wall. In the female genital tract the spermatozoa do not live more than 72 hours.

Fate of spermatozoa. After their entry into the vagina, the spermatozoa travel at the rate of about 1-3 mm per minute and takes about 45 minutes to pass from the opening of the cervix to the ovarian end of the Fallopian tube (about 16-20 cm). The onward progress of the male gametes in the female genital organ is helped by the following factors: (1) the inherent movement of the gametes, (2) the positive chemotaxis exerted by the alkaline secretion of the cervix, and (3) the negative pressure created due to contraction of the uterus during coitus. (4) Prostaglandin present within seminal plasma helps in the movement of sperms. The cervical opening dilates and the uterus moves making its axis parallel to vaginal canal—thus facilitating the entry of the gametes in the cervix. Although contraction of the female genital organs in human is unknown, but in some species contractions of female organs facilitate the transport of the sperm to the Fallopian tube.

After traversing the body of the uterus, the gametes enter the Fallopian tube, where—if the ovum be present—fertilisation takes place. If the ovum be not there, the spermatozoa die, degenerate and disappear generally after 72 hours.

Metabolism of spermatozoa. While the spermatozoa are present inside the epididymis, they are metabolically inactive due to the absence of O_2 and sugar to be metabolised. After ejaculation they normally metabolise fructose in contrast to other tissues which use mainly glucose. Fructose is present in the accessory sex glands, and regulated by androgens. Under *anaerobic conditions* fructose is broken down to lactic acid which is oxidised with liberation of energy in presence of O_2. Sperm motility depends upon the breakdown of ATP which provides energy for contraction of the fibrils present in the tail. **Fructolysis** helps in the regeneration of ATP. For maximum motility of sperm, both fructose and O_2 should be present.

Hyaluronidase. Although fertilisation is done *by a single spermatozoon,* yet the presence of myriads of spermatozoa in the seminal fluid appears to be an apparent prodigality of Dame Nature. But it is seen that, *if the semen contains spermatozoa less than* 20 *million per ml, the subject is usually sterile.* In other words, the enormous number is an essential factor for successful fertilisation. The explanation of this curious paradox is that the ovum remains covered up by a layer of cells (corona radiata) derived from the discus proligerus. These cells remain cemented together by *hyaluronic acid.** Unless this cell layer be penetrated, the spermatozoon cannot reach the ovum. It is found that the spermatozoa contain an enzyme, *hyaluronidase,* which breakes up the cementing acid, so that the discus cells fall away. In this way, the ovum is made accessible for fertilisation up to next layer, the zona pellucida of the ovum.

Fertilisation of the ovum. During the chemical warfar for removal of the corona radiata a large number of spermatozoa die and only the strongest survivor ultimately reach the ovum. One sperm, the strongest one enters the zona pellucida of the ovum by *side-to-side jerky movements.* The physical characteristics of the zona pellucida are changed due to direct contact with the sperm. Here the sperm loses its head cap (acrosome) which contains an enzyme that depolarises the zona pellucida in a very restricted zone. It is further claimed that the penetration in the zona pellucida is taken place by a specific mucolytic enzyme present in the sperm and for penetration in this layer *capacitation* is required by the sperm. As soon as one spermatozoon enters the ovum, a stiff membrane develops around the latter preventing the entrance of any other spermatozoon. After crossing through the zona pellucidal layer and the perivitelline space, the sperm touches the vitelline membrane (Fig. 4.160). At this stage the second meiotic division of the secondary oocyte takes place. The sperm penetrates vitelline membrane by flagellary movement of its tail. At the point of contact of the vitelline membrane with the sperm head, an outward bulging in the *ooplasm* occurs which helps in the sperm penetration. However, the head and probably a part of the neck enter the ovum. The rest of the gamete drops out and degenerates. Fusion of the male and female pronuclei takes place (Syngamy), which acts as a powerful stimulus for multiplication. The fertilised ovum starts multiplying at a terrific speed. The mass of cells thus formed (Morula) is carried slowly along the Fallopian tube by the ciliary movement of its epithelium and reaches the body of the uterus in about *eight days.* At this time it has a diameter of about 0·2 mm. The fertilised ovum is embedded in the already prepared decidua and pregnancy starts.

Semen (Seminal plasma or fluid)

Semen is a suspension of spermatozoa in the secretion of the epididymis, prostate, seminal vesicle, Cowper's glands. *Volume* at each emission is about 2-4 ml containing 100-200 million spermatozoa, 80% of which should remain actively motile for 45 minutes but not later than 3 hours. They should not contain more than 20% abnormal forms. Volume and

***Hyaluronic acid** molecule is made up of acetyl glucosamine and glucuronic acid with molecular weight 2,00,000-4,00,000. The viscosity of the vitreous humour, synovial fluid, Wharton's jelly of the umbilical cord, etc., is chiefly due to hyaluronic acid. Hyaluronic acid gel serves as the cementing material by which cells are held together.

sperm count in semen are decreased with repeated ejaculation. About 50% of men contains 20 to 40 million sperm/ml of semen. But containing lower than 20 million/ml is generally sterile. Semen contains fructose, sorbitol, spermine, citrate, acid phosphatase, lipids, fibrinolysins and prostaglandins. Its specific gravity 1·028 and pH 7·35-7·50. Seminal buffers (phosphate and bicarbonate) serve to protect the sperm against low pH of the vagina. Its colour is highly viscid opalescent, greyish white. Prostaglandins possibly help in the movement of spermatozoa to the Fallopian tube through uterus (page 4.184).

Human semen after ejection coagulates immediately due to conversion of fibrinogen into fibrin, but after 15 to 20 minutes there is liquefaction by the enzyme fibrinolysin or plasmin contained in it. The substrate for gel formation (coagulation) is the protein-like material which is secreted by seminal vesicles, whereas the gelating enzymes make contact with the substrate only during ejaculation, being elaborated distally to the seminal vesicles by the prostate.

Functions of the constituents of seminal fluids

1. **Fructose.** One of the most characteristic constituents of human semen is the reducing and yeast-fermentable sugar fructose. Fructose arises from glucose *via* glycogen → phosphohexose pathway, phosphohexose isomerase and alkaline phosphate bringing about the final steps to form seminal fructose. Seminal vesicles are the chief site of fructose formation in men. Fructose is produced by the dorsolateral prostate and coagulating gland in rats. As the storage capacity of seminal vesicles in men is small, consecutive ejaculations within several days result in a low semen fructose content. About 2-4 days are required for replenishment. Seminal vesicle epithelial activity and therefore seminal vesicle fructose elaboration and semen fructose content depend on the level of androgens. Therefore fructose content of semen is an index of testicular activity (fructose test). Vitamin deficiencies (A, E, B-complex), malnutrition, and high fat diet markedly diminish accessory gland secretory activity (decreased fructose) by a direct effect on the accessories or indirectly *via* altered pituitary or testicular function.

Fructose is also the principal source of energy for ejaculated sperm. In mammals internal fertilisation is the rule, survival and motility of the sperm are probable under *anaerobic conditions,* provided there is presence of seminal plasma. Since mammaliam sperms contain little intracellular glycogen, they should depend upon an extracellular energy source, viz., fructose. Fructolysis thus provides energy for motility. Epididymal sperm (like washed sperm) cannot survive anaerobic conditions and are immotile.

2. **Phosphatases.** Human semen contains a high amount of acid phosphatase that arises from prostatic secretion. This is directly influenced by androgen titer. Acid phosphatase is normally found in male urine in large amounts due to admixture of prostatic secretion. It promptly acts to dephosphorylate phosphorylcholine contained in semen to free choline and orthophosphate. Action of acid phosphatases allows phosphric acid to accumulate after ejaculation. The phosphoric acid can then react with spermine to form spermine phosphate. Alkaline phosphatase is also present in low concentration in human semen.

3. **Spermine.** It is a nitrogenous base secreted by the prostate and found in large amounts in human semen. When human semen is allowed to stand for a short period, crystals of spermine phosphate begin to appear. The presence of spermine distinguishes semen from other body fluid. At ejaculation, acid phosphatase liberates orthophosphate from phosphorylcholine which reacts with spermine. The reaction of spermine with picric acid is the basis of a test of diagnostic value in forensic medicine (Barberio test).

4. **Choline.** It is also a nitrogenous base and is found in high concentration in human semen. Physiologic functions of choline in semen involve lipotrophic activity, stimulation of phospholipid turnover, transmethylation and possibly an acetylcholine-like activity. Choline is secreted as phosphoryl compounds (bound choline) and begins to appear as free cfioline only after ejaculation through the action of acid phosphatase. Choline plays some role in phospholipid metabolism of male accessory organs or of the sperm. Choline may have an action on female genital organs or on sperm motility.

5. **Ergothioneine.** It is also a nigrogenous base and is found in the semen of certain forms, including humans. It ensures sperm motility and fructose utilisation by protecting essential sulphydryl groups in the sperm from sulphydryl group-binding substances and agents which are capable of oxidising the sulphydryl groups. This sulphydryl-reducing groups of ergothioneine act as protection.

6. **Citric acid.** Citric acid which is found in human semen is derived from prostatic secretion. But it originates in seminal vesicles in bull, ram, boar, etc. Citric acid content in human semen is influenced by androgenic level, being increased by androgen and decreased after castration. The citric acid content in human semen varies from 140-630 mg/100 ml. Although the physiologic role of citric acid in semen is not clear, some experiments support a beneficial effect on sperm motility, facilitation of semen coagulation and liquefaction by virtue of its *calcium*-binding capacity, activation of prostatic acid phosphatase, protective action against antinvasin to promote hyaluronidase action and involvement in the maintenance of osmotic equilibrium in semen.

7. **Lipid.** It is found in the semen and derived from prostatic secretion. This lipid is composed mainly of phospholipids and cholesterol. Lipid globules (not *licithin*), macrophages containing lipid ganules and corpora amylacaea, being prostatic origin, account for opalescence of semen.

8. **Protein materials.** Seminal plasma of the male contains about 3·5-5·5 g of protein-like substance/100 ml. This substance is responsible for the coagulation of semen being acted upon by a gelating enzyme doing contact during ejaculation.

9. **Hyaluronidase.** It is commonly associated with the sperm. It is also found in semen as a consequence of its release from the sperm and is derived from the tubule area of testes. It is mucolytic enzyme which depolymerises and hydrolyses hyaluronic acid. Although hyaluronidase is involved in the process of spermatogenesis, yet it may facilitate fertilisation by liquefying a passage for each sperm through cumulus cells which surround the ovum.

10. **Other substances.** These include creatine, creatinine, epinephrine, norepinephrine and inositol. These substances have been identi-

fied in the semen of experimental forms. Their function in seminal fluid is not clear.

IMPOTENCE

Impotence may be defined as the failure to achieve or to maintain an erection sufficient to accomplish intromission and coitus. If an erection is achieved it usually subsides without ejaculation. Primary impotence implies that a male has never been successfull in any attempt at intromission. Impotence may be 'constitutional', 'organic' or 'psychogenic' (Cooper). Constitutional impotence is associated with a defective sexual drive without evidence of organic disease. Organic impotence develops in a previously normal subject and is the result of some pathological process. Psychogenic impotence implies that there is no organic basis for the condition and that it results from psychological causes. Patients with endocrine disorders rarely complain of impotence unless questioned directly. Those with hypogonadism have diminished libido as well. In humans, various degrees of impotence are commonly found, but it is rare to find definite organic cause for the complaints.

The development of the *male* sexual attitude and power depends upon the presence of normal androgenic secretion at the tide of puberty and the development of a male-oriented sexual attitude based on sociologic and psychogenic influences.

The ability to gain and maintain an *erection* that culminates in timed normal orgasm and *ejaculation* requires an *intact* nerve and blood supply to the lower genito-urinary tract. Both autonomic and somatic (voluntary) nerves control various aspects of such potency. Erection requires arteries of such caliber that an adequate blood flow can be delivered to the penis.

Coitus. Coitus, by which the sperms are introduced into the vagina, depends upon the presence of testosterone and on the integrity of many parts of the nervous system. The higher centres usually initiate the act, but may also inhibit it. Erection and ejaculation, however, can be induced reflexly in a paraplegic man.

Erection. This function is initiated by psychic or local influences. Erection of the penis is brought about by the distention with blood of venous sinuses in the erectile tissue, in response to sexual excitement. Afferent stimuli pass from the glans penis, via the parasympathetic pudendal nerve (S_2—S_4), and from other areas to the spinal cord. A centre for erection in the lumbosacral part of the spinal cord sends impulses, via the nervi erigentes (S_2—S_4) which cause vasodilatation of the arterioles of the corpora cavernosa and spongiosa of the penis to relax and constriction of the main dorsal vein and the blood pressure within them approaches that of the carotid arteries. The corpora cavernosa and spongiosa of the penis fill with blood under high pressure and the penis changes from a small flabby organ to a rigid elongated organ. If the sacral parasympathetic outflow is interrupted such as will occur after a bilateral lumbar sympathectomy operation, ejaculation no longer occurs although the quality of erection of the penis is still present. Lack of psychic stimulus can impair the quality of erection.

Emission. Emission is the movement of the semen into the urethra and is a sympathetic response. Secretory nerve impulses from the glans

penis reach the sacral cord and travel to the integrating centre in the upper lumbar segments of the spinal cord. These impulses set off a massive stimulus through the hypogastric sympathetic nerves with the contraction of smooth muscles of the vasa deferentia and seminal vesicles. These nerves cause secretions from the prostate, seminal vesicles and ejaculatory ducts to enter the prostatic urethra. Interruption of these nerves may preclude emission.

Ejaculation. Ejaculation proper is the propulsion of semen out of the urethra at the time of orgasm. The centre for ejaculation is in the upper lumbar region of the spinal cord. It sends impulses, via the lumbar sympathetic trunks (L_1–L_2), which cause the muscles of the vasa deferentia, the seminal vesicles and the prostate to contract rhythmically and eject the semen into the urethra. At the same time sympathetic impulses relax the detrusor muscle and cause the internal sphincter to contract, thus inhibiting micturition and preventing the reflux of semen into the bladder. Ejaculation from the urethra is effected by rhythmic contractions of the perineal muscles, which are supplied by the perineal branch of the pudendal nerve (S_2—S_4). Hence, a lower motor neurone lesion in this zone could impair ejaculation and the sensation of orgasm.

Subsidence of erection. The arterioles to the penis then contract and erection subsides.

PROSTATE

Histology (Fig. 4.153). Prostrate is a male accessory sex organ. It surrounds the first part of urethra, and is covered by vascular capsule. It is composed of fibro-elastic tissue, plain muscles and mucosal glands. The glands are tubulo-alveolar, containing a *low cubical* or *pseudostratified columnar epithelium*, highly folded upon itself. The cytoplasm of the cells has a high amount of acid phosphatase activity. The cells shrink and become non-secretory in deficiency of androgens.

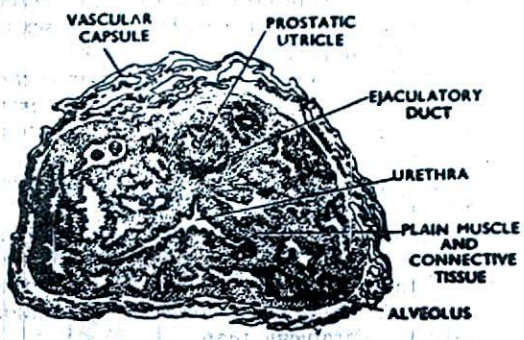


Fig. 4.153. Diagrammatic representation of histological structure of prostate gland.

Functions. The secretion of the prostatic glands increases the volume of semen. Probably, it acts as a nutritive fluid for the male gametes, prolonging their life and helping their function. The prostatic fluid is acid in reaction, contributes 20% of total volume of human semen, and contains citric acid, spermine, phospholipids, fibrinolysin, fibrinogen, thromboplastin, cephalin and cholesterol. In addition, prostatic secretion contains epithelial cells, a few leucocytes, protein and electrolytes. It is rich in enzyme fibrinogenase and acid phosphatase which hydrolyses organic phosphates (glycerophosphate, hexose phosphate, etc.) in an acid medium and liberates inorganic phosphates. [Bones contain *alkaline phosphatase*, being active in alkaline medium.] The acid phosphatase

content in the prostatic fluid is a measure of its activity. This enzyme probably helps and nutrition and function of the spermatozoa. A small amount of the enzyme enters the blood stream, where the upper normal limits and below 3·25 units per cent. Its level fluctuates according to the degree of activity of prostate. In cancer of prostate the figure goes above 10 units% (Diagnostic). The prostatic secretion is opalescent and has an approximate pH of 6·4 in human.

Control of prostate. Prostate is under the dual control of both androgens and oestrogens (both present in the male body).

Proof. 1. Castration causes atrophy of the prostate with degeneration of the glands. Injection of androgens restores it to normal, showing that optimum androgen level is necessary for prostatic activity.

2. Injection of oestrogens causes degeneration and keratinisation of the glandular epithelium (same as vaginal epithelium),but enlarges the non-glandular part of prostaste. [Specially utriculus masculinus—homologue of uterus.]

This shows that prostate is controlled by both the hormones—an optimum balance between them is responsible for its normal structure and functions. In old age, prostate hypertrophies with the degeneration of its glands.This is believed to be due to the *declime of androgens and relative rise of the oestrogens.* In simple senile prostatic hypertrophy administration of androgens is of great therapeutic value. But in cancer of prostate oestrogens are very useful—probably by checking the growth of the epithelium and trying to keratinise it.

FEMALE REPRODUCTIVE ORGANS

Anatomy (Fig. 4.154). The important female reproductive organs consists of (i) two ovaries, one on each side; (ii) two connecting Fallopian (uterine) tubes; (iii) uterus and (iv) vagina. The *Fallopian tube* is a muscular one, having an inner mucous membrane. From attachment at the uterus, the two Fallopian tubes extend laterally, one on either side to the wall of the pelvis, then turn downwards and terminate near the ovaries. Each tube enlarges forming *ampulla* and *infundibulum,* and at last bends downwards to end in a *fimbriated margin.* One of the fimbriae is attached to the ovary (ovarian fimbria).

Histology. The *infundibulum* is formed by a number of fimbriae and is funnel-shaped. The thin-walled *ampulla* is the largest segment of the tube and its terminal short segment extends to the *isthmus* which is thicker-walled and shorter in diameter than that of the ampulla. The mucous membrane of the ampulla is composed of complicated foldings and these folds, in progression towards the uterus, decrease in size.

The wall of the Fallopian tube is composed of mucous membrane, muscularis and serous membrane. The epithelial lining of the mucous membrane consists of a layer of simple columnar cells some of which are ciliated but other are not. The cilia blow towards the uterus and have a mechanical function. The non-ciliated cells are secretory in nature. The height of ciliated cells differ with phases of the cycle. They are highest during the follicular phase and lowest during terminal part of the luteal phase and also during pregnancy. The muscular layer becomes progressively heavier towards the uterus and consists of a well-developed inner circular layer and a thinner outer layer arranged longitudinally.

The *uterus* is a thick-walled, pear-shaped muscular organ, the inside of which is hollow. It has a *body* and its lower part is *cervix,* which is continuous with the body above by the *internal os* add with the vagina below by the *external os.* The muscular wall of the uterus *(myometrium)* made up of smooth muscle fibres is arranged in three layers, an inner longitudinal

layer, a thick oblique middle layer and a thin outer longitudinal layer. It responds to the posterior pituitary hormone, *oxytocin*. The inner muscous layer *endometrium* is lined by epithelial cells and contains many tubular coiled glands penetrating deeply into the underlying loose connective tissue (tunica propria). The uterine endometrium undergoes cyclic changes during different phases of menstrual cycle.

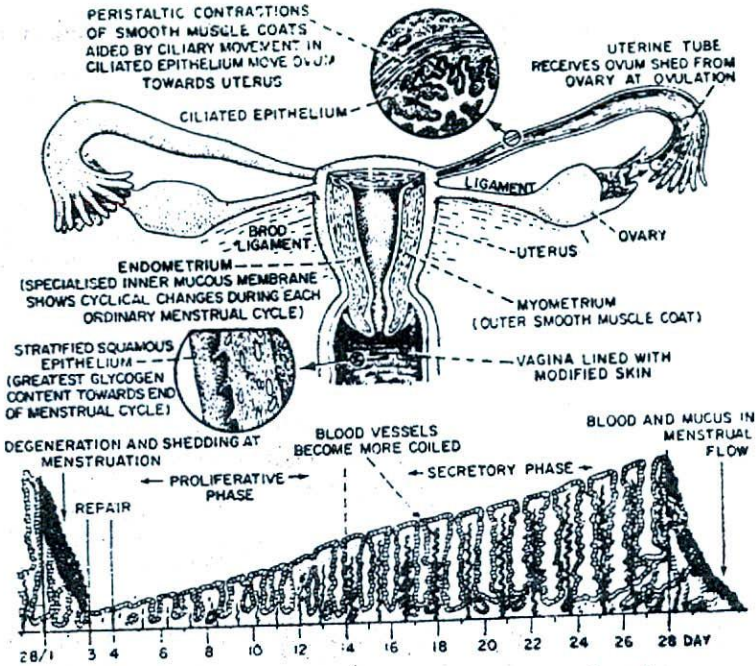


Fig. 4.154. Diagram showing the vagina, uterus, uterine tubes.

Functionally, the *uterus* plays an important role in maintaining the growth and development of the embryo. The ovum which is discharged from the ovary is transported to the uterus along the Fallopian tube. The fertilised ovum after segmentation and division is embedded in endometrial bed previously prepared by the actions of both oestrogen and progesterone. Placenta is then formed with the dual shares of the embryonic and maternal tissues. The placenta, thus formed, maintains the nutrition, respiration and excretion of the embryo until parturition.

The *vagina* is a musculomembranous tube lined by non-keratinising stratified squamous epithelium. The lining is thrown into numerous transverse fold or rugae. No glandular structure is present here.

OVARY

More or less 7 million oocytes in the ovary of 30-week female foetus are enclosed by granulosa cells by about 40 weeks, forming functional units, the follicles, containing ova already in the prophase of meiotic division. These follicles undergo successive stages of development and atresia

during foetal life and childhood, the number of primordial follicles declining from about 2 million just before birth to about 3,00,000 in the adult. The accumulation of atretic follicles forms most of the ovarian cortical stroma. Either ovulation or corpus luteum formation does not occur before puberty. Cyclic secretion of gonadotrophins takes place at puberty, leading to the full development of an adult ovary. The functional units (follicles) each consist of an ovum and its *two* surrounding cell layers. The *avascular inner layer of granulosa cells* synthesises progesterone. Progesterone is secreted into the follicular fluid during the first half of the menstrual cycle, and may also serve as precursor for oestrogen syntheis by the surrounding layer of theca-interna cells. Oestrogen is synthesised in the luteinised cells of the theca interna. The main pathway is from pregnenolone and 17OH-progesterone, but also from progesterone produced by the granulosa cells. The theca interna is highly luteinised and vascular during the first half of the menstrual cycle, and the sunthesis and secretion of 17OH-progesterone and oestradiol in the circulation reflect a peak hormonal activity of the follicle—oestrogen secretion.

The earliest stages of follicle development may be independent of gonadotrophic activity, but the mature follicle, along with antral cavity surrounded by the granulosa cells and vascular theca layer, responses to gonadotrophic stimulation. FSH initiates the development of a group of follicles. One group matures and becomes responsive to the ovulatory stimulus of LH. At ovulation, ovum and surrounding cumulus of cells becomes extruded. Secretion of oestradiol temporarily declines and afterwards rises during luteal phase. Granulosa layer is vascularised and luteinised, forming *yellow corpus luteum.* This luteum secretes progesterone (maximum 20 mg/day) and involutes after about 2 weeks unless supported by stimuli of chorionic gonadotrophin which is secreted by trophoblast of the embedded zygote.

The ovum is released at midcycle and is viable for only about 12 hours. Once the ovum is fertilised the zygote remains free for a further 5-6 days before implanting in the endometrium. The endometrium has already been prepared by actions of progesterone and oestradiol secreted by the corpus luteum. If there is implantation, the vascularisation of developing zygote is established by the 7th-9th day and chorionic gonadotrophin is secreted into maternal circulation if there is appropriate stimulus for continued development of the corpus luteum. If there is no occurrence of implanation, the corpus luteum ceases to function after 10-12 days and menstruction follows.

The development and decline of the corpus luteum run parallel by the rise and fall in the secretion of progesterone, 17α-OH-progesterone and oestradiol.

Anatomy. Two somewhat bean-shaped bodies (4×2·5×1·5 cm), one on each side, near the free end of the Fallopian tube, hanging from the broad ligament by a fold of peritoneum, called the **mesovarium.** They are richly supplied with blood vessels and nerves (vasomotor).

Histology (Fig. 4.155). There is no organ in the female body which shows so much histological variations at different phases of life. In childhood, puberty, pregnancy and menopause, the ovarian structure shows characteristic variation. The following is a brief description of a normal ovary. Ovary consists of the following *six* elements.

1. **Germinal epithelim.** It is the outermost covering by a single layer of cuboidal cells, continuous with the peritoneum, derived from the coelomic epithelium. It is the parent tissue from which the primitive Graafian folicles develop.

2. **Tunica albuginia.** Thin layer of eosinophilic collagenous connective tissue of low cellularity under germinal epithelium.

3. **Stroma.** It is a connective tissue network continuous with the tunica albuginia and containing spindle-shaped cells with a few involuntary muscle fibres. It supports the essential ovarian tissues and carries blood vessels, lymphatics and nerves.

4. **Vesicular follicles** or **Graafian follicles.** Small islands of cells in various stages of development and scattered mostly at the peripheral part of the ovary. The immature ones are called the *primordial follicles.* The central cell is the ovum. The remaining cells surround the ovum in a single layer forming a sort of capsule (page 4-234).

Function. It forms the female *gamete* (ovum) and secretes *oestradiol.*

5. **Corpus luteum.** When the Graafian follicle ruptures, corpus luteum develops on the remnants of the ruptured follicle.

Function. It is a temporary gland secreting *progesterone.*

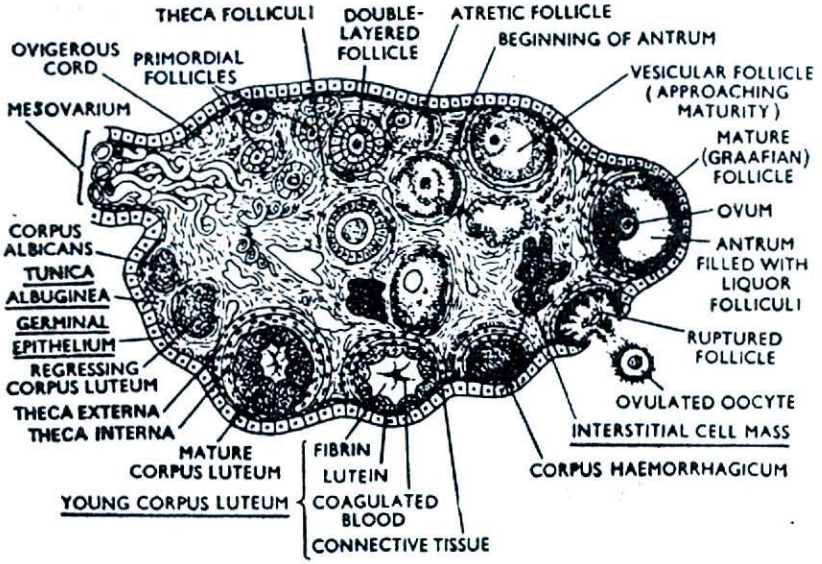


Fig. 4.155. Composite diagram of ovary showing development of follicle, formation of corpus luteum, atretic follicle, mature follicle, etc.

6. **Interstitial cells.** Groups of polyhedral cells containing lipid granules representing stored active principle. They develop from the stroma cells or from the cells of the unruptured follicles. Probably they secrete oestrogens.

In **infancy,** the ovaries are made up of spindle-shaped stroma cells. a cubical-celled germinal epithelium and a large number of primordial

follicles. The follicles do not mature, there is no ovulation and no corpus luteum. At **puberty,** the germinal epithelium contains flattened cells, a large number of maturing follicles—ruptured or unruptured—and corpora lutea of small size. During **pregnancy,** the characteristic additional feature is the presence of a large corpus luteum. At **menopause,** the ovaries atrophy, become smaller in size, the follicles disappear and are replaced by fibrous scars. The interstitial cells degenerate and very little oestrogen is formed.

Methods of studying ovarian functions

I. **Histological study.** To observe ovarian structure at different phases of woman's life.

II. **Effects of extirpation of ovary** (ovariectomy, oophorectomy, spaying, castration). Following effects are seen:

A. **Before puberty.** All puberty changes—physical, mental and sexual (page 4-196) fail to appear. Secondary sexual characters are not developed.

B. **After puberty.** (a) Degeneration of accessory sex organs. (b) Menstruation ceases. (c) Breasts atrophy. (d) Generalised obesity. (e) Mental disturbances—irritability, depression, etc. (f) Disappearance of sexual desire (but sometimes it persists, showing that anterior pituitary or more probably adrenal cortex is at least partly responsible for it).

III. **Active principles.** From the ovary four hormones have been extracted. (a) Oestrogen (Follicular), (b) Progesterone (Luteal or Progravid), (c) Androgen (Ovarian tumour) and (d) Relaxin (Polypeptide). Judicious administration of these hormones prevents or rectifies the ill effects produced by oophorectomy. When given to immature animals—puberty is hastened.

Functions of ovary. From the above observations, the functions of ovaries may be summarised as follows: (1) **Exocrine function**—formation of mature ova. (2) **Endocrine functions**—secretion of four hormones: (a) *Oestrogen*—secreted by the Graafian follicles (membrana granulosa or theca interna). (b) *Progesterone*— secreted by the corpus luteum. (c) *Androgen.* (d) *Relaxin.* With the help of these four hormones, the ovary controls the whole reproductive life of the female. For instance, it is responsible for—

1. **All the puberty changes,** such as, (a) growth and development of uterus, Fallopian tube, vagina, (b) menstrual changes, (c) appearance of secondary sexual characters.

2. **Pregnancy and the changes associated with it,** such as, embedding of ovum, development of placenta, further growth of mammary glands, etc.

3. Due to release of relaxin, **it helps in parturition.**

LIFE HISTORY OF VESICULAR OR GRAAFIAN FOLLICLES: OVARIAN CYCLE

Development and histology. The surface layer of the ovary—the *germinal epithelium*—sends down finger-like processes called the *genital cords (egg tubes of Pfluger)*. The cords become cut off from the surface and are

broken up into small islands of cells of which one cell becomes enlarged and differentiated from its neighbouring cells into primary oocyte. The rest cells surround the oocyte forming the primordial follicles. The primary oocyte is thus lined by a homogeneous membrane, called the zona pellucida. These are small spherical structures, about 0·5 mm in diameter, having a single cell at the centre representing the immature ovum. All the other cells remain surrounding the ovum in a single layer forming a sort of capsule (*follicular epithelium*). The number of immature primordial follicles varies with age. In the *new-born,* the number varies 2,50,000 to 5,00,000; at *puberty*–1,00,000 to 2,00,000; at *menopause*—nil. Of these, only about 400-500 follicles undergo maturation and ovulation during the whole sex life of the female. Others degenerate.

Maturation of the Vesicular follicles or **Graafian follicles** (Fig. 4.156). In childhood the follicles do not mature. After childhood maturation begins but fails to be complete. It proceeds up to some extent and then the follicles degenerate and undergo fibrosis (*Atretic follicles*) (Fig. 4.155). At puberty, the follicles undergo complete maturation and ovulation takes place. *Only one follicle matures and only one ovum is discharged at each menstrual cycle*—probably alternately from each ovary.* **The stages of the maturing process** are briefly as follows: Under the influence of follicle-stimulating hormones (FSH) the primordial follicular epithelium multiplies forming several layers of cell surrounding the ovum. The ovum and the follicle gradually enlarge. Droplets of fluid appear between the cells, gradually run together and form a cavity (antrum formation) containing *liquor folliculi.* At this stage only the primordial follicle can be said as Graafian follicle. The cavity thus formed, separates the surrounding cells into two layers: (a) the peripheral layer—called the **membrana granulosa** (*multi-layered follicular granulosa*), and (b) the central layer—**discus proligerus** or **cumulus oophorus (ovaricus).** The innermost *cumulus cells* are disposed about the ovum in radial fashion, forming **corona radiata.** The zona pellucida is conspicuously

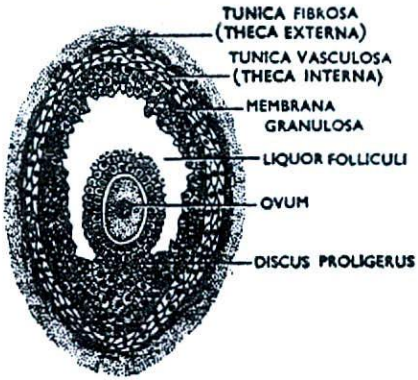


Fig. 4.156. Vesicular or Graafian follicle.

* Although there are numerous Graafian follicles, yet, at each ovarian cycle, only one follicle matures and undergoes ovulation. In other words, at each cycle, only one mature ovum is presented for fertilisation. [Occasionally, two ova may be discharged producing binovular twins.] But this is not the case with lower animals, where a number of ova are usually discharged. How this unitary relation—one ovum for one cycle—is regulated in human female is not understood. Injection of suspension of anterior pituitary in adult female animals is followed by ovulation of a large number of follicles, formation of multiple corpora lutea and in case of pregnancy—multiple foetuses. These observations show that probably there is a quantitative relation between the amount of pituitary gonadotrophins secreted and the number of follicles matured. It is probable that the amount of FSH secreted by the anterior pituitary of the human female is so nicely regulated that it becomes just sufficient for the maturation of a single Graafian follicle.

thickened and crossed by processes from the cells of corona radiata (Fig. 4.157). Microvilli from the ovum extend into the substance of zona pellucida. The thecal layers keep pace with the growing follicle and come to form quite a thick outer layer. The cumulus oophorus surrounds the ovum which remains attached eccentrically to the outer pole of the follicle. In the mean time, the stroma cells surrounding the follicle become differentiated into two layers. The inner vascular layer—called **tunica vasculosa (theca interna)** and outer fibrous layer known as **tunica fibrosa (theca externa)** (Fig. 4.156). At this stage the Graafian follicle is not fully matured. It is matured only when the primary oocyte undergoes meiotic division to loose one polar body and becomes secondary oocyte.

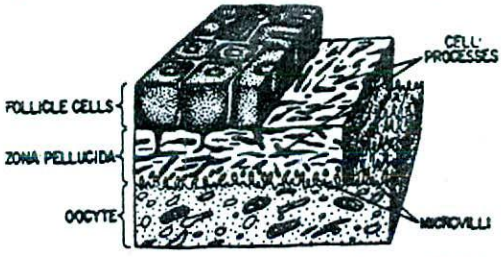


Fig. 4.157. Schematised representation of zona pellucida and structural relationship between follicle cells and oocyte. Cytoplasmic extensions of follicle cells permeate and penetrate zona pellucida, but do not establish contact (or syncytical continuation) with the ovum.

Maturation of the ovum. Before ovulation, the ovum must undergo the process of maturation. The oocyte increases in size, fat globules appear in the cytoplasm and a thin transparent structure develops between the follicular cells and the oocyte, known as the **zona pellucida.** The primary

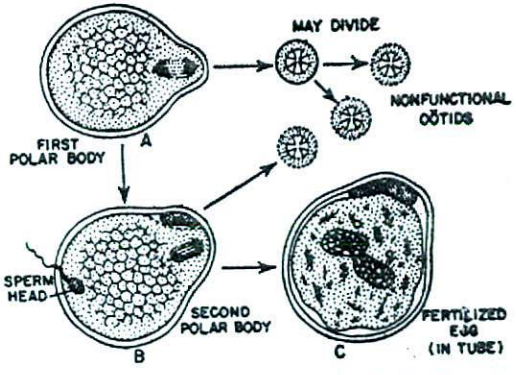


Fig. 4.158. Diagram showing the liberation of the polar bodies from the maturing ovum. Note that although it may divide again, as suggested in the diagram, the first polar body ordinarily degenarates without so doing. The presence of a sperm within the ovum (B) is indicative of the fact that sperm penetration is ordinarily necessary before the second polar body is formed.

oocyte of the Graafian follicle undergoes first meiotic division (heterotypical) giving rise to secondary oocyte and one polar body. This is the full maturation of the Graafian follicle and the ovum is discharged from the Graafian follicle (ovulation) as a secondary oocyte. The secondary oocyte contains half the number of standard chromosome (22X).

Further differentiation of the secondary oocyte is not possible until it is fertilised by the sperm in the Fallopian tube. Second meiotic division (homotypical) of the secondary oocyte takes place only after penetration of the sperm in the zona pellucida (Fig. 4158). With this second meiotic division matured ovum (22X) is formed by losing another polar body (Fig. 4.159).

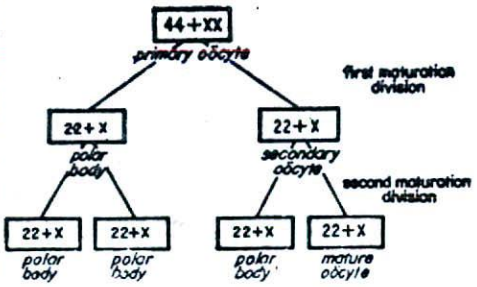


Fig. 4.159. Scheme shows two maturation divisions in the female germ cells where single ovum is formed from one primary oocyte.

Covering of the ovum. The ovum is covered by the vitelline membrane which is again covered by the zona pellucida. In between the vitelline membrane and the zona pellucida there is a narrow space which is known as *perivitelline space*. During the discharge of ovum (secondary oocyte) from the Graafian follicle, several layers of epithelial cells from the *cumulus oophorus* (cumulus ovaricus) adhere to the ovum. These epithelial cells are arranged radially over the zona pellucida and are collectively known as *corona radiata* (Fig. 4.160).

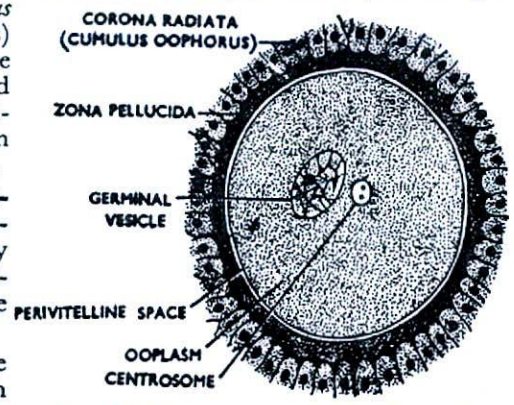


Fig. 4.160. Ovum surrounded by discus cells.

Control of maturation of ovum. Early maturation is chiefly controlled by FSH, but for full maturation both FSH and LH are necessary.

Ovulation. The follicle becomes fully matured in 10-14 days and measures about 10 mm in diameter. It becomes progressively larger and gradually migrate to the surface. More *liquor folliculi* accumulates, the pressure within the follicle increases, the surface layer of the ovary undergoes necrosis and the enlarged follicle protrudes from the surface of the ovary. Due to increasing pressure the follicle ruptures. The ovum, surrounded by the discus cells, is discharged into the peritoneal cavity near the mouth of the Fallopian tube. *This process of rupture of the Graafian follicle is called* **ovulation.**

Ovulation occurs usually between 13th and 17th (average 14th) days after the first day of last menstruation.* The ovum (Fig. 4.160) is taken up by the free end of the Fallopian tube, probably aided by a positive chemotaxis exerted by the tubal secretions. It is then carried along the tube by

* Knaus and Ogino suggest that ovulation bears a more constant relation with the menstrual period that follows it and not the one which precedes it. It always takes place 12-16 days before the onset of the next menstruation.

the ciliary movement of the tubal epithelium and also by the movements of the muscular wall of the tube.

It has been estimated that it takes the ovum approximately 72 hours to arrive at the uterus after ovulation. The exact span of life of the ovum is not known. But it does not remain functionally active after a few days. *The best chance of pregnancy lies in the third week and the least chance in the first week of the menstrual cycle.*

Fertilisation. If spermatoza is recently deposited in the genital tract then fertilisation of ovum occurs usually at the ampullary-isthmic junction of the Fallopian tube. The penetration of the ovum by the sperm is brought about by the lysosomal enzymes present in the acrosome of the sperm. Only one sperm is allowed to penetrate the ovum. Once the ovum is fertilised, it causes a barrier for other sperms. After fertilisation blastocyst —the developing embryo is formed and moves down the uterine tube into the uterus for implanation (Fig. 4.161).

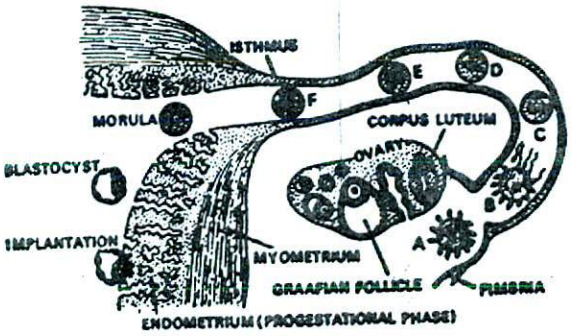


Fig.4.161. Diagrammatic representation of the female genital tract showing the probable site of fertilisation in the ampula and the stages through which an embryo goes before fertilisation and the site where the embryo is implanted A=oocyte immediately after ovulation, B=fertilisation about 12-24 hours after ovulation, C=stage of male and female pronuclei. D=spindle of 1st mitotic division during which diploid number of chromosomes is restored, E=two-cell stage (about 48-50 hours old), F=morula containing blastomeres.

Implantation. The mechanism that determines the location of blastocyst and the formation of placenta in the human has not been fully studied. The rate of transport of the blastocyst influences the success of pregnancy as because fertilised ovum and endometrium should achieve their maturity equally for implantation. It is claimed that probably the secretory efficiency of the endometrium controls the location as well as the effectiveness of implantation and thus optimally secreting endometrium would tend to attract the blastocyst more firmly, causing implantation in the fundus. Thus a less fully developed endometrium will be incapable of accepting the blastocyst for implantation.

Implantation, or uterine attachment, of the blastocyst presumably occurs between 7th and 9th days after ovulation. It is believed that the penetration and erosion of epithelial cells of the uterine mucosa necessary for implantation result from combined effects of proteolytic enzymes which are produced by the trophoblast and by vascular changes in the endometrium (Fig. 4.162).

Mechanisms of implantation has been studied in rabbits by Boving (1959). From his observation in such animals it can be described that mechanism of implantation is likely the same in other mammals. He has described that possibly 'chemotropism' plays in the attraction and subse-

quent penetration of the blastocyst in the specific site of the endometrium. He has postulated that rabbit blastocyst is surrounded by fluid of relatively high concentration of calcium bicarbonate. Progesterone increases the concentration of carbonic anhydrase in endometrium, particularly in the area surrounding the capillary. This tends in the transfer of bicarbonate from the blastocysts to the maternal circulation, causing alkalinity by conversion of bicarbonate to carbonate.

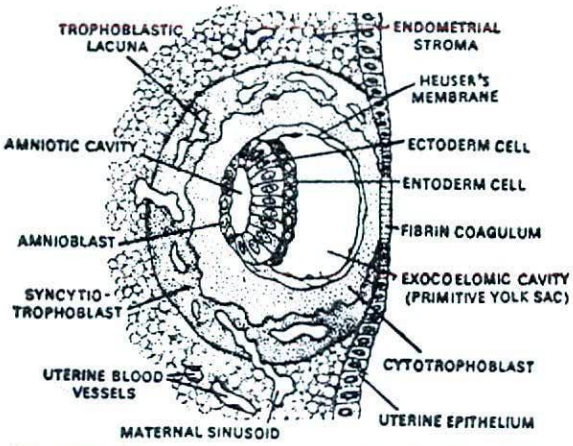


Fig. 4.162. Diagrammatic representation of a 9- to 12-day blastocyst after implantation to show developing ectoderm, entoderm, layers of the trophoblast, beginning of the uteroplacental circulation, etc. Note flat mesothelial cells which have delaminated from the inner surface of the cytotrophoblast to form Heuser's membrance. Amniotic cavity is well delineated and original uterine surface defect is closed by a fibrin coagulum.

This alkalinity favours adhesion to and dissociation of the uterine epithelium so as to permit penetration by the blastocyst. Once the blastocyst comes in contact with the endometrium, it becomes surrounded by an outer layerof syncytiotrophoblast, and by an inner layer of cytotrophoblast. The blastocyst then makes it space within the endometrium through the process of erosion by the syncytiotrophoblast and a placenta is gradually developed.

Abnormal implantatation. When the blastocyst implants abnormally in close proximity to the cervical internal os, this condition is known as placenta previa which causes severe bleeding in the latter part of pregnancy and during delivery. When implantation occurs in uterine tube, this condition is known as tubal pregnancy, which is dangerous because it causes severe internal haemorrhage, rupture of the tube and death of the embryo during the 2nd or 3rd month of pregnancy. However, implantations outside the uterus are known as ectopic or extrauterine pregnancies.

The **ovarian cycle,** in brief, is that at puberty, under the influence of the pituitary gonadotrophins, the follicles start to grow regularly, release ova and form corpora lutea. The first ovum

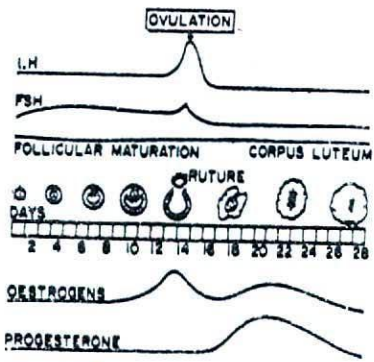


Fig. 4.163. Diagrammatic representation of ovarian cycle and associated hormonal secretion.

is probably shed at about the time of the first menstrual period, but regular ovulation is not commonly established until the age of 16 or 17 years. The adult ovary goes through a recurrent cyclical process which results in ovulation and menstruation and occupies about 28 days. The cycle starts on the **first** day of the menstruation, and consists of two pheses : the **follicular phage** during which ovum ripens and discharges; this lasts about 14 days and is followed by (2) the **luteal phese**. Ovulation takes place normally between 13th and 17th days of the cycle and separates the two phases (Fig. 4.163). The regularity of the cycle is usually controlled by the time of ovulation. Menstrual bleeding occurs 14 days after this event unless fertilisation takes place.

Control of ovulation

Certain species have one or two oestrous cycle per year, at which time ovulation and receptivity to the male coincide with the male's sexual interest in the female. In rabbits, ferrets and cats, ovulation is the result of a compound neuroendocrine reflex which is intiated by the intravaginal tactile stimulation and involves the secretion of pituitary LH. Only primates possess menstrual periods, others have oestrous cycles. In some species, viz., baboon, the sexual skin provides an interesting and colourful exterior indication of waning and waxing of the sex cycle. The central stimulus to ovulation is affected to only a minor degree by environmental stimuli, being primarily regulated by an internal hormone cycle mediated by a hypothalamic clock of characteristic frequency —4-6 days in rat and mouse, 14 days in guinea-pig, 16 days in sheep, 20 days in cow, 21 days in sow, 27 days in macacus monkey, 28 days in women, 36 days in chimpanzee, 2 cycles a year in bitch, and 1 cycle per year in bat and marmot.

During the menstrual cycle, which is conventionally dated from the first day of bleeding, there is a gradual thickening of the lining of the uterus and the proliferation of straight glands; then in the postovulatory half of the cycle the glands become complicated and tortuous, the submucosal layer of tissue becomes very vascular and edematous and the leucocytes infiltrates this layer. The formation of this progestational or secretory type of endometrium is important for reception, nourishment and enccouragement of the fertilised ovum. The menstrual bleeding of primates is an external marker of the end of the ovarian cycle and is due to endometrial necrosis and shedding following withdrawal of ovarian hormone support for the vascular hypertrophic endometrium characteristic of the luteal phase of the cycle.

Ovulation occurs at midcycle and there is wide variation from one individual to another. There is also no guarantee that the day of ovulation will remain contant for the same individual from cycle to cycle.

The statistics of the *rhythm method* of birth control have little meaning for individuals who deviate significantly. Fertilisation may occur as a result of insemination during or immediately after the menstrual time in a few individuals. Fertility is at its peak during two days following ovulation.

Ovulation may also occur in any given cycle. Anovulatory cycle occurs frequently not only in adolescent girls but also in mature women. In anovulatory cycles the rise in basal body temperature does not occur.

During the preovulatory half of the cycle basal body temperature taken in the morning immediately on awakening varies between 36·3°C and 36·8°C and at the time of ovulation it increases by 0·3-0·5°C. It continues at the higher level throughout the latter half of the cycle, falling to initial level at the period of menstruation.

At about the time of ovulation other changes are: (a) an increase in urinary gonadotrophins, (b) an increase in the number of large acidophilic squamous cells with small nuclei in vaginal smear, (c) a peak in oestrogen output just before ovulation, (d) 3-4 days after ovulation a rise in pregnanediol excretion. In anovulatory cycle none of these events occurs.

If fertilisation does not occur, the endometrial degeneration and sloughing take place. At the period of sloughing, marked vasoconstriction of arterioles, a slowing of circulation with extravasation and pooling of blood in the stroma layer occur. Submucosal blood pools coalesce, and superficial layers of the endometrium, leucocytes and mucus are shed as menstrual discharge (Fig. 4.151). The discharged blood does not normally clot and may vary in amount from 20-200 ml in volume for a single period. This flow lasts 4-6 days in 95% of women.

Premenstrual endometrium loses about half its thickness in about 3-4 days and reparative processes begin to be apparent.

A complicated reciprocal relationship exists between two main ovarian hormones (Fig. 4.163)—oestrogen and progesterone on the one hand and three pituitary gonadotrophins—FSH, LH and prolactin (rats and mice only). It is very difficult to assess the role of these hormones in the control of ovulation and in the preparation of the endometrium for nidation and nourishment of the fertilised ovum. From observations on ovariectomised women and animals; from the effects of oestrogen and progesterone administration separately and in combination to ovariectomised individuals; from studies in intact women of the urinary excretion of gonadotrophins and steroid hormone excretion products correlated with major events of the cycle, ovulation and menstruation; from studies on hypophysectomised animals in evaluating the relative roles of FSH and LH in ovulation; from studies on the concentration of steroids in blood and from radioimmunoassay methods for the gonadotrophins many important insights are promised.

The ovarian interstitial cells and the thecal cells of the developing follicle can make oestrogen. Oestrogens have essential local effects in the ovary and may indeed prepare the ovary for stimulation by gonadotrophins.

At the earliest stage of the cycle, certain follicles can be seen to be undergoing differentiation and growth, but when the hypophyseal gonadotrophic stimulation begins with FSH, a single follicle is selected for maturation and ovulation (or rarely 2) and other follicles undergo regression or atresia. This survival mechanisms of one follicle and the failure of developing all others are unknown. But it is probable that the earlier development of many follicles may play a physiological role in sending signals to the hypophysis because small amounts of oestrogen elicit the release of increasing amounts of pituitary gonadotrophins. The early preovulatory phase is characterised by a gonadotrophin mixture which is FSH. As this mixture stimulates the ovary to produce more and more oestrogen, information in ovaries is fed back to the hypothalamic

centre which regulates composition of gonadotrophin recipe. At this stage there is a fall in the release of FSH and facilitation in the release of LH. Both FSH and LH are required for the production of oestrogen by Graafian follicle. The ovum is extruded from the ruptured follicle to the site of fertilisation—in the human the Fallopian tube. (Fig. 4.164)

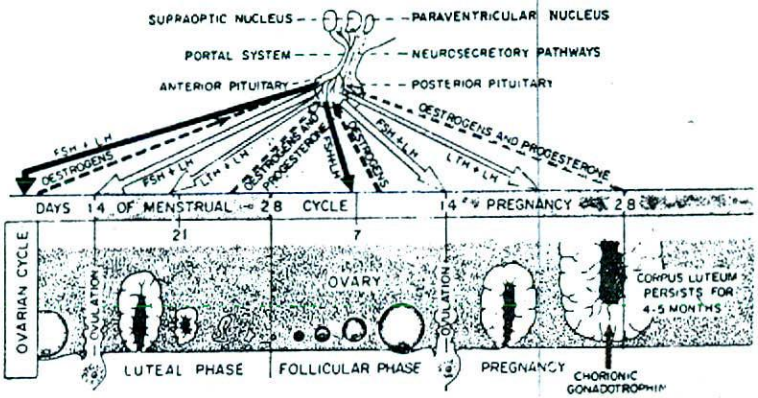


Fig. 4.164. Diagram shows female reproductive cycle.

Under the strong stimulus of LH, the ruptured follicle cells change their characteristics to typical lutein cells and their enzymes profile at the same time. This is nothing but a sort of physiological and cyclically recurring form of biochemical differentiation, because in the act of making the rapid change from follicular cells to lutein cells, these miniature steroid factories change the character of their major end product. They produce progesterone, for pregnanediol is excreted within 3-4 days after ovulation. On the other hand, during the preovulatory phase of the cycle small amount of pregnanediol is found in the urine, because progesterone is such a prominent intermediate in the steroid biosynthesis that some of it may diffuse out of the cells. But corpus luteum also produces oestrogen and a secondary oestrogen excretion peak can be often detected during the second half of the postovulatory phase.

In rats and mice prolactin is necessary for persistance of corpora lutea and secretion of progesterone by them. This is not applicable for other mammals including man. An administration of HCG (or CG) can extend the life of functioning corpus luteum, but injections of prolactin do not prolong the luteal phase of the menstrual cycle in monkeys.

If fertilisation does not take place, large amounts of oestrogen and progesterone instruct the pituitary through the hypothalamus to cut down the production and release of gonadotrophin. Being deprived of the gonadotrophic stimulus the corpus luteum withers and dies, and there is abrupt withdrawal of progesterone and then of oestrogen. In its turn, the endometrium, which has grown with lavish amounts of oestrogen and progesterone, abruptly finds itself with an insufficient hormonal stimulus to sustain it, and the endometrium promptly deteriorates. Sloughing and bleeding begin and the cycle resumes 3-4 days later.

If fertilisation takes place, CG (or HCG) that appears urgently prevents the degeneration of the corpora lutea and sustains it until the placenta can assume the burden of producing large amounts of steroids required during pregnancy. The relationship of the hypophyseal-ovarian system is summarised below (Fig. 4.165).

FSH stimulates ovary→Ovary secretes oestrogen→Oestrogen stimulates the release of **FSH**→LH→Ovary secretes large amounts of oestrogen →Rising level of oestrogen stimulates the release of FSH→**LH**→occurrence of **ovulation**—development of corpora lutea begins and corpus luteum secretes progesterone and oestrogen→(LTH is elicited in some species)→

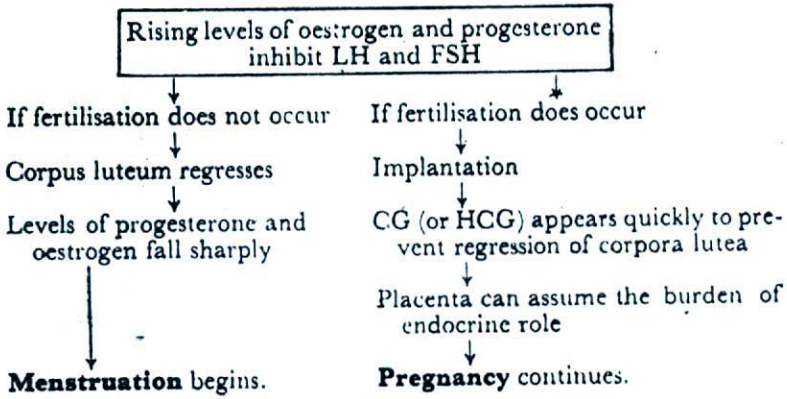


Fig. 4.165. Summarised representation of hormonal control of ovulation, menstruation and pregnancy.

Although FSH and LH are not differentiated in most assays, there is a marked rise in urinary gonadotrophins, especially FSH following menopause and radiological or surgical castration. At this time oestrogen injection causes a fall in gonadotrophin secretion. Intravenous injection of high doses of progesterone shows the same result. As progesterone may be a biosynthetic precursor of oestrogen, this inhibition is accomplished by oestrogen obtained from injected progesterone.

On the other hand, small amounts of oestrogen stimulate LH release. The pituitary content of both FSH and LH is decreased following the administration of synthetic oestrogen, stilboestrol, and small doses of oestrogen injected into ovariectomised rats. Administration of oestrogen to immature rats may lead to the formation of corpus luteum. But this may not occur in hypophysectomised animals due to stimulation of hypophyseal LH release. It is commonly seen that small doses of oestrogen stimulate the output of FSH, large doses of estrogen inhibit FSH output and in moderate doses oestrogen stimulates the release of LH (Fig. 4.165)

Ovulation can be inhibited by both progesterone and testosterone, but synthetic compounds, e.g., *norethindrone* (Norlutin) and *norethinodrel* (Enovid) are extremely effective and produce fewer undesirable side effects. Gemzell has used gonadotrophin mixtures in the management of infertility due to ovulation failure. *Clomiphene* has also proved to be [illegible] in inducing ovulation in women with a history of infertility and

works by cancelling out inhibitory influences which play upon the GRF (gonadotrophin-releasing factor) complex in the hypothalamus.

Fate of Graafian follicles. The follicles have four fates: (1) *Before puberty*—Maturation is incomplete. The ovum dies and is phagocytosed by the granulosa cells. The follicles thus become transformed into fibrous scars. Even during active sex life large numbers of follicles undergo this fate. (2) *Between puberty and menopause*—About 400-500 follicles undergo complete maturation and ovulation. (3) *After ovulation*—Corpus luteum grows on the ruins of the ruptured follicles. (4) *After menopause*—All the remaining follicles degenerate.

Functions of Graafian or vesicular follicle

1. Forms mature ova.
2. Secretes oestrogen (oestradiol).
3. Provides a medium for the growth of corpus luteum.

Atretic follicles. If the Graafian follicle instead of going on to ovulation, degenerates, there is death to the ovum being replaced by a ring of hypertrophied thecal cells. Such follicles are called atretic follicles.

LIFE HISTORY OF CORPUS LUTEUM

Formation and histology. Corpus luteum grows on the remains of the ruptured follicles. When ovulation occurs, the sudden release of tension causes rupture of blood vessels in the tunica vasculosa, leading to haemorrhage in the cavity of the follicle. A blood clot forms at the site of the ruptured follicle forming what is known as *corpus haemorrhagicum*. Although this is definitely present in lower animals, it is not readily found in human females or in monkeys. The granulosa cells and the stroma cells from the tunica vasculosa rapidly multiply and fill up the cavity, forming the *corpus luteum*. The cells enlarge and the fully formed corpus luteum is found to consist of columns of large conical cells with distinct nucleus and yellowish pigment granules—the **lutein** (Fig 4.155). These granules represent the internal secretion of the gland.

The corpus luteum attains its maximum size on the 19th day of the menstrual cycle. In absence of pregnancy, it persists up to 27th day and degenerates on the 28th day, when menstruation begins. This corpus luteum is called the *corpus luteum of menstruation* or the false corpus luteum. The degeneration takes place rapidly due to fatty infiltration and many of the cells are removed producing an appearance of a white fibrous structure, which is aptly termed **corpus albicans.** This structure atrophies, and after several months sinks deep within the stroma of the ovary as a tiny scar, called the **corpus fibrosum.** If pregnancy takes place, the corpus luteum continues to grow attaining its largest size about the 3rd or 4th month. It degenerates in the latter months forming a fibrous scar (Fig. 4.155). This is called the *corpus luteum of pregnancy* or the true corpus luteum.

Maintenance of corpus luteum

The growth and secretion of corpus luteum is controlled by (i) LH of anterior pituitary. (ii) Placental gonadotrophins. This is an additional but essential stimulus for continued growth of corpus luteum during pregnancy.

The corpus luteum is essential for the maintenance of pregnancy, at least during the first trimester (about 90 days) of human gestation. An

important factor in the functional persistence of the corpus luteum in human pregnancy is the production and secretion of chorionic gonadotrophin by the chorion cells of the placenta.

The corpus luteum at the earliest stage of human pregnancy provides oestrogen for the growth of the uterus and progesterone. Progesterone probably possesses a relaxing effect upon the uterus, which suppresses contraction. After the first trimester, the production of oestrogen and progesterone is taken over by the placenta itself.

For implantation to occur, migration of the blastocyst down the Fallopian tube should be integrated with the development of the uterine endometrium and the activity of the myometrium. Tubal migration of blastocyst is influenced by oestrogens, and both oestrogens and progesterone are necessary for the development of secretory endometrium. After implantation, continued secretion of progesterone is necessary for maintenance of the established pregnancy. Progesterone secretion originating at first from the corpus luteum has a functional life of 8-10 weeks during early pregnancy. Progesterone secretion shows an initial rise and a transient fall with the decline of function of the corpus luteum after the first few weeks of pregnancy and then rises again as the placental secretion of progesterone becomes established.

Since implantation depends upon the provision of an optimum steroid-hormone milieu by the corpus luteum, there is a probability that some cases of implantation failure are due to defective function of the corpus luteum. In certain species, in the absence of implantation, a luteolytic factor from the uterus has been postulated to cause the lysis of the corpora lutea. On the other hand, implantation leads to various hormonal mechanisms which are involved in maintaining the corpora lutea.

In most mammals other than primates, the ovary provides oestrogen and progesterone. This involves both persistent corpora lutea or the constant formation of new corpora lutea and probably also functional follicular activity. Several studies in animals have suggested theories as to the maintenance of corpus luteum. It is not established to what extent these might apply to the human.

1. Lysis of the corpus luteum by a luteolytic factor

In some animal species, a specific internal secretion from the uterine mucosa is responsible for shortening the life of the corpus luteum in absence of implantation of a fertilised ovum or ova. Hysterectomy (removal of the uterus) can extend the life-span of functional corpora lutea in rats, rabbits, guinea-pigs, etc. On the other hand, hysterectomy has little or no effect on the duration of the corpora lutea in the Rhesus monkey, etc. In the human being, the menstrual cycle is slightly prolonged if hysterectomy is carried out 3-4 days after the ovulation period. So there is some effect of the uterus in the woman on the corpus luteum function. But its significance is unknown.

In rats, rabbits, guinea-pigs, cows, sheep and pigs, where the lytic stimulus of the uterus is stronger than the luteotrophic stimulus, the action of the early embryo implantation is believed to antiluteolytic rather than luteotrophic. When the uterus is under progesterone stimulus, the uterus may produce a *luteolytic* substance which causes luteal regression in absence of an embryo. Oestrogen may depress the luteolytic stimulus.

It has been observed that granulosa cells from sow ovaries at the preoestrous stage possess the existence of such a uterine luteolytic factor. Flushing of the uterian horns of sows is carried out with tissue culture.

2. **Hypophyseal and other factors in luteolysis**

Luteolysis is considered to be functional and structural processes. **Functional luteolysis** decreases the secretion of progesterone in blood, coinciding with an increase in the 20α-steroid dehydrogenase. This enzyme makes a substantial decrease in the activity of progesterone by converting 20-keto group to 20α-hydroxyl. **Structural luteolysis** is morphologic regression of the corpus luteum. In the laboratory rat, hypophysectomy leads to functional luteolysis, but retards structural luteolysis.

Intravenous injection of LH can stimulate progesterone secretion, Prolactin (LTH) does not stimulate progesterone synthesis. But prolactin. not LH, maintains functional corpora lutea after hypophysectomy. Prolactin can inhibit the activity or synthesis of 20α-hydroxysteroid dehydrogenase and thus can prevent catabolism of progesterone to less active 20α-hydroxy derivative of progesterone.

According to Wiest and Kidwell (1969), 3β-hydroxysteroid dehydrogenase and glucose-6-phosphate dehydrogenase act to increase progesterone synthesis and 20α-hydroxy dehydrogenase acts to decrease progesterone in laboratory rat. Administration of FSH and LH stimulates steroidogenesis partly by increasing the amounts of glucose-6-phosphate dehydrogenase and 3β-hydroxysteroid dehydrogenase. These enzymes also increase in activity during functional period in the oestrous cycle and in pregnancy. After ovulation and in the absence of pregnancy, 20α-hydroxysteroid dehydrogenase increases concomitantly in plasma levels of 20α-hydroxy derivative of progesterone.

At the beginning of the oestrous cycle in rats, FSH stimulates follicle development with increases in oestrogen, in glucose-6-phosphate dehydrogenase, and pentose phosphate pathway. At this time, low level of plasma progesterone permits the release of LH necessary to initiate ovulation. LH further stimulates pentose phosphate pathway and increases 3β-hydroxysteroid dehydrogenase in progesterone synthesis. In an unmated rat, after ovulation the corpus luteum regresses markedly due to a lack of LTH as well as to an effect of LH in increasing 20α-hydroxysteroid dehydrogenase. However, when the stimulation of the cervix occurs either by mating or artificially, the pituitary LTH maintains the new corpus luteum both functionally and morphologically. An increasing activity of 20α-hydroxysteroid dehydrogenase is prevented through the action of LTH and a lack of sufficient LH, so that the reduction of progesterone to 20α-hydroxy compound is retarded. When *nidation* occurs, placental luteotrophins relieve the pituitary of this function. The activity of 20α-hydroxysteroid dehydrogenase continues to be suppressed, allowing continued secretion of progesterone. The secretion of placental luteotrophic hormone declines prior to parturition. This allows to drop in progesterome synthesis with a subsequent partial removal of the inhibition to LH release. The increasing level of LH and the declining level of LTH initiate the activity of 20α-hydroxysteroid dehydrogenase. Thus, a shift of progesterone to relatively inactive 20α-hydroxy compound occurs, and delivery of the foetuses follows shortly.

After about the first trimester (about 90 days), the ovarian steroid function in the human is replaced by *de novo* production of progesterone by the placenta and by the aromatisation of androgens reaching the placenta from the maternal and foetal adrenal cortex, where they are converted to oestrogens.

According to Hilliard *et al.* (1969) the rabbit is a reflex ovulator. Coitus in the rabbit in oestrus leads to a stimulus to the hypothalamus which initiates a release of pituitary LH. The initial release of LH stimulates ovarian interstitial tissue with an increased production of 20α-hydroxyl derivative of progesterone. This preovulatory release of 20α-hydroxy derivative of progesterone prevents progesterone inhibition of LH, which continues to be released and insures ovulation. At this time the ovary becomes relatively insensitive to LH apparently owing to a depletion of cholesterol stores for steroid synthesis. Pituitary LTH is released at this period causing hypertrophy of the ovarian interstitial cells. These cells then accumulate cholesterol and regain sensitivity to LH. Prolactin thus maintains the morphology of ovarian interstitial tissue and its capacity to secrete steroids. The secretion of oestrogens from the ovarian follicles is at least essential to maintain development and function of the corpus luteum. The uterus regulates the level of oestrogen and during gestation metabolises less oestrogen. This sparing effect does more available to sustain the corpus luteum. If there is no implantation, the rate of metabolism by the non-pregnant uterus is such that insufficient oestrogen is available to stimulate the synthesis of progesterone from the corpora lutea. When progesterone level falls, pituitary LH is released, 20α-hydroxy derivative of progesterone increases and there is occurrence of luteolysis.

Functions. Corpus luteum *secrets oestrogen and progesterone* which are essential for gestation and many other changes associated with pregnancy.

OESTROGENS

Definition. Oestrogens are compounds which can produce oestrus in ovariectomised animals.

Varieties. Two varieties: (1) **synthetic,** and (2) **natural.**

1. **Shnthetic oestrogens.** Several have been synthesised (Fig. 4.166). They are benzanthracene compounds and are not sterols. They are effective by mouth (not destroyed by liver). Important members known in this group are **diethy stilboestrol** and **hexoestrol. Ethinyl oestradiol** is another synthetic oestrogen which is a modified form of oestradiol.

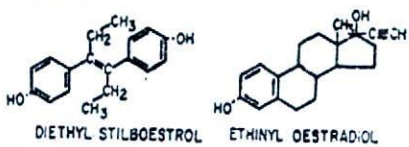


Fig. 4.166. Chemical structures of synthetic oestrogens.

2. **Natural oestrogens**

Chemistry. They are all sterol derivatives. They are less effective by mouth. Their structures (Fig. 4.167) are as follows:

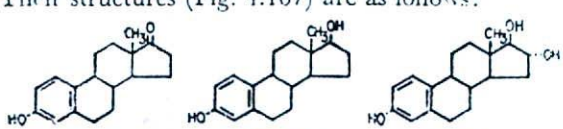


Fig. 4.167. Structural formulae of naturally occurring oestrogens.

Varieties

1. **Oestradiol** (with —OH at the 17C position)—It is the hormone secreted by the ovary. **Oestrone** (with =O at the 17C position) is the possible circulating hormone.

2. **Oestriol** (with —OH at the 17C position and an additional ···OH at the 16C position)—It is found in adult female urine and increased during pregnancy, is also liberated from placenta. They are believed to be derivatives of oestradiol and are excreted in the urine as glucuronates. Increased excretion of oestriol occurs after administration of oestradiol or oestrone. There is close relationship between oestradiol, oestrone and oestriol in the body such as oestradiol $\rightleftharpoons$ oestrone $\rightarrow$ oestriol.

Sources: 1. **Ovary is the chief source.** (a) From the *Graafian follicles*—The liquor folliculi and the follicular epithelium are rich in oestrogens. Histologic and histochemical evidence supports the view that the primary source is the theca interna of the follicle. As the follicle matures oestrogen secretion increases, maximum being at the time of ovulation. (b) From the ovarian *interstitial cells*—These cells also seem to secrete oestrogen. Though the follicles may be destroyed by deep X-ray or radium, yet the ovaries secrete oestrogens and the animals get normal oestrus. (c) The oestrogen secreted during the luteal phase of the cycle is formed by the theca-lutein cells of the corpus luteum.

2. **Adrenal cortex.** Oestrogens have been extracted from this gland.

3. **Testes.** Small amounts of oestrogens have been extracted from testes, being locally synthesised.

4. **Placenta.** In cow and many other mammalian species it is the principal source of oestrogens during pregnancy.

5. **Plants.** Oestrogenic activity has been noted in about 50 species of plants. In some the amount is sufficient to cause reproductive abnormality in grazing animals.

Distribution. In addition to the above sources, oestrogens are widely distributed in animal tissues. They are found in the blood, muscles, urine of both pregnant and non-pregnant females and in the urine of adult males. The urine of stallions has a very high oestrogen content and is one of the richest known sources. Small amounts have also been obtained from spermatozoa.

International unit for oestrone is defined as the activity of 0·1 γ of standard oestrone preparation; for oestradiol, as the activity of 0·1 γ of standard oestradiol preparation.

Assays. 1. Induction of vaginal cornification (oestrous smear) in ovariectomised rats or mice (*Allen-Doisey test*). The mouse or rat unit is defined as the amount required to elicit the oestrous smear in a specific % of the particular subjects used.

2. Induction of an increased uterine weight in ovariectomised immature rats or in intact immature rats and mice.

3. The induction of vaginal opening and removal of lipid from the rat uterus.

Chemical methods for oestrogen determination are based on the development of colour as in Kober's reaction—(pink colour develops when oestrogens are heated with phenol and sulphuric acid, then cooled and reheated) and the improved modifications of Brown and Bauld (1956). Fluorimetric methods are also available.

Synthesis of oestrogens

The naturally occurring oestrogens are secreted by the theca-interna cells of the ovarian follicles, by the corpus luteum, by the placenta, and, in less amounts by the testis and adrenal cortex. The ovarian stromal tissue also has the potential to make androgens and oestrogens, but they presumably do so in negligible amounts in *normal premenopausal* women. The first half of the menstrual cycle, or the follicular phase, is concerned primarily with secretion of oestrogens. The rise in the progesterone occurs in the second, or luteal, phase due mostly to the formation of a corpus luteum. These patterns of steroidogenesis agree with the concept that oestrogens are largely secretory products of the theca-interna cells, and progesterone is secreted largely from granulosa cells of late follicle phase, about midcycle, and from cells of the corpus luteum derived from granulosa cells.

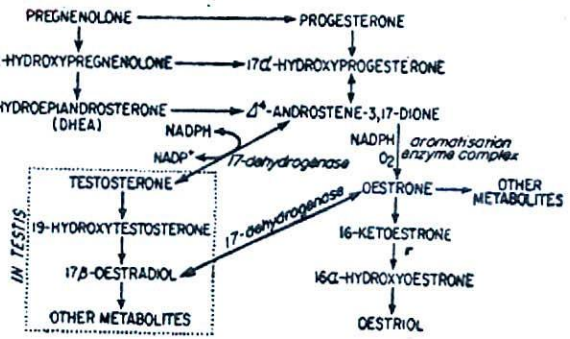


Fig. 4.168. Schematic representation of biosynthesis and metabolism of oestrogens.

The principal reactions that occur in the transformation of 5-pregnenolone to progesterone and other progestational compounds, and, *via* androgens, to oestrogens are represented in Fig. 4.168. In the theca cells oestrogen production is for the most part through pregnenolone. The granulosa cells of the follicle first prior to ovulate and the corpus luteum after ovulation have an active series of enzymes for the conversion of 5-pregnenolone to progesterone, and thus progesterone is a chief secretory product. 17α-hydroxy progesterone is secreted to a lesser extent A more limited conversion of progestins to oestrogens occurs in these cells.

Formation of progesterone from 5-pregnenolone involves hydrogen removal from 3-hydroxyl group of 5-pregnenolone by 3β-hydroxysteroid dehydrogenase, which utilises NAD as a hydrogen acceptor. NADH is reoxidised by mitochondria and also probably by extramitochondrial lactic dehydrogenase in which pyruvate is reduced to lactate. The oxidation process of 3-hydroxyl by 3β-hydroxysteroid dehydrogenase is followed by shifting double bond from 5 position to 4 position catalysed by an enzyme or an enzyme complex ($\Delta^5 \rightarrow \Delta^4$ isomerase). 5-pregnenolone and progesterone can be hydroxylated in the 17α-position forming the corresponding 17α-hydroxysteroid. A desmolase enzyme is able to cleave 17β-side chain of pregnenolone or progesterone to give either DHEA or 4-androstenedione respectively. Androstenedione is converted to oestrone

by aromatisation. Before DHEA is aromatised to oestrogen, DHEA is converted to its 5-androstene-3,17-dione (3-keto derivative) by enzyme Δ^5, 3β-hydroxysteroid dehydrogenase (3β-hydroxysteroid dehydrogenase). However, there is a separate enzyme Δ^5, 3-ketosteroid isomerase for the conversion of 5-androstene-3,17-dione to 4-androstenedione.

Aromatisation of androgens to oestrogens occurs greatly in the microsomes of the cell and also requires NADPH and molecular O_2. A 17-dehydrogenase enzyme can lead to an interconversion of androstenedione and testosterone and the interconversion of oestrone and oestradiol (Fig. 4.145).

Histochemistry of the ovary. Available histochemical tests provide evidence as to the distribution of and changes in lipids, mucopolysaccharides, and enzymes. In human ovaries, the presence of free lipids and hydroxylating or NADPH-generating enzymes known to play a role in steroid hormone biosynthesis has been demonstrated in the theca interna and in the parenchymal cells of the corpus luteum. In functional corpora lutea the parenchyma is rich in acid phosphatase, and the vascular endothelium in alkaline phosphatase. With the onset of follicular atresia, fats and acid phosphatase appear in the granulosa and aid in the identification of early stages of the process.

Metabolism and excretion. The major secreted oestrogen, 17β-**oestradiol,** is in equilibrium in circulation with **oestrone.** Oestrone is again metabolished to **oestriol** probably in large amounts in the liver. Oestradiol is the most potent oestrogen of the three and oestriol the least. About 60-70% of the circulating oestrogens are protein-bound. The rate of secretion ranges from 200-500 μg/day in women at the time of ovulation. [In males, oestrogen-secretion rate is about 50 μg/day.] Oestrogens are secreted throughout the menstrual cycle, but there are two peak times, one at the time of ovulation and the other during midluteal phase. These are then destroyed in the liver being transformed into other products or are inactivated by conjugation with sulphates and glucuronates, in which form they are found in the urine. Traces may be found in *uncombined form.* Appreciable amounts are secreted in the bile and reabsorbed into the blood stream (enterohepatic circulation). For this reason, *the natural oestrogens are less effective by mouth.* The synthetic oestrogen, *stilboestrol,* is not destroyed in the liver, hence, highly effective by mouth. **During menstrual cycle**—Oestrogen excretion starts rising from the resting phase and becomes maximum at the time of ovulation (14th day) and then falls (Fig. 4.164). **During pregnancy**—Excretion starts rising from the first week and reaches the maximum just before the onset of parturition. After delivery it falls but traces may still be found in the *free uncombined form.*

Mechanism of action of oestrogens

Oestrogen is bound to specific cytosol receptor proteins in the cells. The cells transfer these proteins into the nucleus. These proteins act to increase RNA synthesis there. Oestrogen may act on the end-organ tissues at the level of RNA-synthesising enzymes in ovariectomised rats. After an administration of oestrogen by 2 hours, RNA polymerase activity in the nuclear fraction of uterine homogenates (not liver homogenates) is increased.

Oestrogen may also act as a cofactor in the transhydrogenation reaction. In this reaction hydrogen ions and electrons are transferred from reduced NADP to NAD.

Oestrogen-dependent transhydrogenase catalyses the transfer of hydrogen from NADPH to NAD producing an increased rate of biological energy: (a) In the cells, concentration of NADP is commonly very low, increased oxidation of NADPH may maintain availability of NADP for the activity of dehydrogenases. These enzymes may require the oxidised form of this cofactor. (b) An indirect method of deriving high-energy phosphate is made available by transfer of H to NAD, thus NADH is formed. Now NADH is then oxidised through respiratory chain. An increased rate of production of biologically useful energy stimulates growth in those tissues affected by oestrogens. The mechanisms partly explain ability of oestradiol to stimulate the rate of O_2 consumption, conversion of acetate to CO_2 and incorporation of acetate into protein, lipid, and the adenine and guanine of nucleic acid fraction of target tissues. In human placenta, soluble 17β-hydroxysteroid dehydrogenase catalyses interconversion of oestrone and 17β-oestradiol.

Actions of oestrogen

In general, oestrogen stimulates the development and maintains morphologic and functional state of the female reproductive system and secondary sexual characteristics. Oestrogen also contributes to the regulation of nitrogen and electrolyte metabolism, capillary reactivity, water balance, bone metabolism, etc. In the following discussion the general term oestrogen is used.

On ovary. Physiologic levels of oestrogen promote the pituitary release of LH. As a new crop of follicles begins to grow during and just after menstruation, the theca interna either spontaneously or in response to a constant low level of LH begins to secrete small amounts of oestrogen. The oestrogen can then act to increase LH release which in turn further increases oestrogen secretion in the first half of the menstrual cycle. Cyclic re-enforcing mechanism acts to promote oestrogen secretion and LH release. The increasing oestrogen will, by increasing LH release, progressively establish a gonadotrophic ratio with an increased amount of ovulating hormone LH. Oestrogen has a direct stimulatory effect on follicle growth. Oestrogen in high doses is capable of stimulating follicular development to the medium-sized follicle stage in the hypophysectomised animal, even after ovarian involution. Oestrogen enhances (synergises) effect of administered gonadotrophins.

In prolonged, very high dosages, oestrogen has an inhibitory effect on the ovary both by a direct action and indirectly by inhibiting pituitary gonadotrophin release.

On Fallopian tube. Oestrogen stimulates the epithelium and musculature of the Fallopian tubes. Oestrogen promotes the motility of the uterine tube, an activity which may play an important role in sperm transport.

On uterus. In the absence of ovary, the uterus of the preadolescent remains infantile and that of the adult undergoes atrophy; an administration of oestrogen restores and maintains the adult morphology and functional activity. Oestrogen produces effects on the endometrium—

increase in size and weight, increase in cell number, changes from the cuboidal to columnar type of epithelium, growth stimulation of uterine glands marked increase in water content, increase in vascularity, increase in protein content, increase in alkaline phosphatase.

Oestrogen has physiologic effects on the myometrium. In absence of *normal* oestrogen titers, as in an oophorectomised subject, the uterine smooth muscle is relatively atrophic and inactive. Oestrogen treatment corrects these castration effects. Oestrogen brings about an increase in the contractile material, **actomyosin;** consequently, an increase in isometric tension is attained. The absolute and relative actomyosin content of the human uterus is greatest in the gravid uterus.

Oestrogen also stimulates the activity of uterine muscle—an increase in motility. Thus, in the human menstrual cycle one can correlate the stages of increased uterine motility with oestrogen titers.

Oestrogen increases the sensitivity of the myometrium to oxytocin. Uterine threshold to oxytocin is lower in the follicular phase than the luteal stage of the cycle. Human uterus shows a progressive increase in response to oxytocin from midgestation on to term. Action potentials are absent or rare following oophorectomy, but with oestrogen treatment they re-appear and myometrial activity becomes more synchronous. Oestrogen administration raises the membrane potential of the rabbit uterine muscle to a level (—43 mV) above that found in the uteri from the immature animal (insufficient oestrogen). This oestrogen effect is dependent on the presence of calcium ion. Oestrogen may influence uterine muscle cells by altering calcium-ions binding at the membrane.

After treatment with oestrogen there is an initial rise in RNA content of uterus. After 48 hours an increase in DNA, total phosphorylase and glycogen have been found to occur.

Certain adrenal steroids (cortisol and cortisone) antagonise the effects of oestrogen on the uterus.

Experimentally it has been noted that different oestrogens have varied types of effects on the uterus and the normal uterine response depends upon their combined actions.

On cervix. Under the influence of increasing oestrogen levels during proliferative stage the mucus which is secreted by the cervical glands becomes more acidophitic and thinner, and approaches its lowest viscosity at ovulation. These changes may promote sperm survival and transport.

On vagina. Oestrogen is responsible for vaginal enlargement to adult size which begins during adolescence. It is observed with oestrogen administration or with elevated endogenous oestrogen titers during follicular stage there occur proliferation *(increased mitoses)* and stratification (increased number of layers) of the vaginal epithelium, with subsequent cornification. This is a reliable index of oestrogenic function. Oestrogen stimulates glycogen and mucopolysaccharide deposition in squamous epithelial cells. The eventual conversion of this glycogen to lactic acid accounts for the pH-lowering effects of oestrogen on the vagina (pH=4·5).

On mammary glands. Oestrogen hypertrophises the mammary glands at puberty including areolar development and pigmentation. Oestrogen primarily stimulates duct growth and has little effect on lobular-alveolar system in the human female. Normal mammary growth requires

the action of both oestrogen and progesterone. High doses of oestrogen inhibit pituitary prolactin secretion and oestrogen is used to prevent lactation and to dry the breast in recent parturient if breast feeding is contra-indicated, or not desired.

On secondary sexual characteristics. Oestrogen is responsible for the development of the female secondary sexual characteristics that distinguish females from males.

On electrolyte and water metabolism. Salt and water retention correlates with great rise of oestrogen titer at midcycle and with the luteal rise in oestrogen. It is observed in dogs and in human beings that an administration of oestrogen results in sodium and chloride retention, decreased urinary volume, increased extracellular fluid and blood volume, decreased RBC and Hb, and increased body weight.

Effect of oestrogen on fluid and electrolyte balance is complecated by some oestrogen-adrenocorticosteroid interrelationships. In the humans, oestrogen has no influence on aldosterone secretion, but it retards metabolic degradation, increase plasma-protein blinding and decreases secretion of cortisol.

Other effects. Oestrogen promotes protein metabolism and affects morphogenesis of the skeleton. Oophorectomy (loss of oestrogen) abolishes mating response and other sexual behaviour patterns. Oestrogen may elevate the mitotic index of a number of tissues.

Carcinogenesis. Along with pronounced growth effects on accessory sex tissues, oestrogen has growth effects on other tissues also. This effect is due to stimulated cell division. This is correlated with the hypothesis that high rate of cell division predisposes a tissue to become cancerous. It has also been noted that some synthetic carcinogens are similar to oestrogen in chemical nature and can also cause typical oestrous changes in experimental animals.

On the basis of above actions on different biological processes, the physiological functions of oestrogens are described as follows:

Functions of oestrogen

1. **Responsible for all the puberty changes.** Such as, (a) growth of uterus, vagina, stratification of vaginal epithelium; (b) increased contractility, secretion and ciliary movement of the Fallopian tube; (c) development of breasts chiefly by proliferation of ducts; (d) menstrual changes (*vide* below); (e) appearance of secondary sex characters.
2. **Responsible for the proliferative stage of menstruation.**
3. **Causes growth of uterus during pregnancy.** The enormous growth of uterus is believed to be due to oestrogens aided by the mechanical stimulus of the growing embryo.
4. **Exerts synergistic action with oxytocin** (causes onset of parturition). It has been shown that oestrogens increase the sensitiveness of the uterine musculature to the action of oxytocin, while progesterone depresses it. At full term, progesterone level falls—due to degeneration of corpus luteum and to some extent, of the placenta. But oestrogen level still remains high. This enhances oxytocin effect and thus parturition starts.

5. **Relation with progesterone and corpus luteum**

(a) Oestrogen and progesterone often act synergistically, viz., (i) *Menstrual changes*—Progesterone can cause the premenstrual changes in the endometrium only after the proliferative changes has been already done by oestrogen. Progesterone alone will have no effect. (ii) *Breast formation*—Glandular (lobulo-alveolar) development of breasts, as seen during pregnancy, only occurs by their combined action.

(b) They may antagonists—in large doses, oestrogen antagonises the actions of progesterone and prevents the progestational changes in the uterine mucosa.

(c) Oestrogen is also necessary for the maintenance of corpus luteum during pregnancy. Probably this is brought about through the action of anterior lobe of hypophysis.

6. **Inhibits the secretion of anterior pituitary,** particularly the FSH. In this way, anterior pituitary and ovaries maintain a reciprocal relation ship (page 4-33). Due to this inhibitory action, administrationof large doses of oestrogens may cause atrophy of the ovaries, arrest menstruation and produce sterility. Oestrogens have no direct action on the gonads. They work by inhibiting the follicle-stimulating hormone (FSH) of anterior pituitary.

7. **Inhibits thymus.** Oestrogens depress thymus and causes its involution at puberty.

8. **Exerts synergistic action with androgen.** In males oestrogen in physiological dosage acts synergistically with androgen and helps in the development of secondary sexual characters.

9. **Stimulates oestrus** in female animals.

10. **Stimulates the secretion of ACTH from anterior pituitary** and causes hypertrophy of adrenal cortex.

11. **May help water balance.** Administration of oestrogens causes water sodium and chloride retention, increase of blood volume and of the water content of muscles. Oophorectomy, on the other hand, causes loss of water and diminishes blood volume. They are all restored to normal by administration of oestrogens.

12. **Effect on protein synthesis.** Oestrogens increase total body protein as indicated by positive nitrogen balance. The effect is however much less than that of testosterone.

13. **Effect on bone growth.** Oestrogens induce positive calcium balance and thus increases skeletal growth. However oestrogen (like androgen) hastens closure of epiphysis and thus stops growth.

14. **Effect on fat deposition.** Oestrogens cause increased deposition of fat in subcutaneous tissue and also in other particular regions to make a typical feminine body.

15. **Effect on cholesterol metabolism.** Possibly by its action on lipoproteins, oestrogens lower plasma cholesterol level.

16. The oestrogens are responsible for the development of the female secondary sexual characteristics that distinguish the female from the male, e.g., formation of narrow shoulders, broad lips, thighs that converge, etc.; the larynx retains its prepuberal proportions and voice stays high-pitched; there is less body hair and more scalp hair, etc.

High levels of testosterone-oestrogen-binding globulin (TEBG) in the blood stream, as occurs naturally in women, are associated with scant bo[dy]

hair. TEBG inactivates the hormones responsible for stimulating hair follicles. Large doses of oestrogens slow sexual hair growth in many hairy women; can now, be viewed as the result of a concomitant increase in TEBG.

17. Oestrogens are said to move sebaceous gland secretions more fluid and thus inhibit formation of **comedones** (black heads) and acne.

It is observed that oestrogens appear to aggravate the conditions of acne, excessive oiliness of the skin, and to modify the rate of sexual hair growth (hirsutism). Prolonged administration of large doses of oestrogens in women increases in plasma-testosterone concentration. It is believed that oestrogens may cause alterations in the peripheral metabolism by decreasing the metabolic clearance rates of testosterone.

18. For several decades, many investigators contended that oestrogens induced breast cancer. Prolactin is now suspect, not oestrogen. Oestrogens stimulate prolactin secretion and mammary development only in the presence of the pituitary gland. Injections of prolactin contributed in the rate of tumour growth in rats with breast cancer. On the other hand, oestrogens failed to maintain tumour growth after hypophysectomy. On this basis the temporary regression observed in human breast malignancies after hypophysectomy may be explained as resulting from lack of prolactin. Large doses of oestrogens produce comparable benefits in controlling breast cancer is attributed to interference with tumour-stimulating potential of prolactin at the level of target tissues.

PROGESTERONE

Progesterone is the active principle of corpus luteum.

Sources. (1) **Corpus luteum,** (2) **Placenta,** (3) **Adrenal cortex.**

Varieties and chemistry. (1) **Natural,** (2) **Synthetic.**

Natural. Called *progesterone*—It is a sterol derivative with a side chain at the 17C position. Its structure is shown in Fig. 4.169. It is found in two crystalline forms, e.g., α and β.

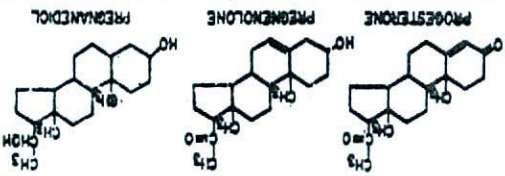


Fig. 4.169. Chemical formulae of naturally occurring progesterone.

Synthetic. Progesterone is comparatively ineffective by mouth. Synthetic progestational agents (Fig. 4.170) which are effective orally have been prepared. Important members of these are ethisterone, 19-norprogesterone, A-norprogesterone, 6α-methyl-17α-acetoxyprogesterone. These compounds, as they inhibit ovulation are also used as **oral antifertility agents.**

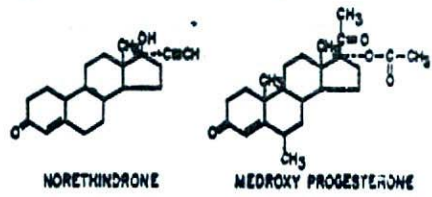


Fig. 4.170. Chemical formulae of some orally active synthetic progestins (21C position).

International unit for progesterone is equal to one mg of the standard preparation.

Blood progesterone may be **assayed** by biologic or chemical techniques. **Biologic method** involves induction of endometrial

changes. 'Hooker-Forbes' method involves the intrauterine injection in ovariectomised mice, with subsequent histologic examination of the endometrium for nuclear and nucleolar enlargement. **Chemical methods** involve blood extraction and determination by ultra-violet spectroscopy. Most of these are obtained from determinations of urinary-free or conjugated pregnanediol.

Synthesis of progesterone

Progesterone is secreted by the corpus luteum and the placenta. Secreted progesterone is possibly bound to protein.

The *de novo* synthesis of progesterone from cholesterol by the placenta is important to the maintenance of human pregnancy after first trimester when ovarian function declines. The placenta has enzymes for the synthesis of cholesterol from acetate and for the production of 5-pregnenolone from cholesterol. The formation of 5-pregnenolone may occur largely in the mitochondria and involves hydroxilations at 20 and 22 positions of cholesterol side chain, followed by a cleavage of the side chin between 20 and 22 positions by a *desmolase* enzyme. The formation of progesterone from 5-pregnenolone involves, the removal of hydrogen from 3-hydroxyl by 3β-hydroxysteroid dehydrogenase utilising NAD as hydrogen acceptor and a shift of double bond from 5 to 4 position by $\Delta^5 \rightarrow \Delta^4$-isomerase enzyme. 17α-hydroxyprogesterone is obviously secreted along with oestrogen from ovarian follicles. In the corpus luteum, 20α- and 20β-hydroxy derivatives of progesterone are formed. Progesterone is an essential intermediate in the steroid biosynthesis in those tissues which secrete steroids and small quantities leak into the general circulation from the testis and adrenal cortex.

Metabolism and excretion. Small quantities of progesterone are found in blood and urine. During the metabolic processes progesterone gets reduced into an inactive derivative known as pregnanediol. It is conjugated with glucuronic acid and sodium in the liver and appears in the urine as **sodium-pregnanediol-20-glucuronate** (Fig. 4.171). *It appears in the urine in the luteal phase of menstruation but not in the follicular phase.* The

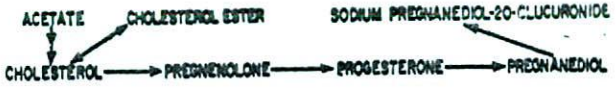


Fig. 4.171. Scheme showing biosynthetic and major metabolic pathways of progesterone.

excretion starts one or two days after ovulation (i.e., when corpus luteum has formed) becomes maximum about a week before the onset of menstruation and ceases 2-3 days before the period comes. During pregnancy much larger quantities are excreted, the amount being maximum (about eight fold) during the eight and ninth months. It falls before parturition. *Its presence in the urine is regarded as an index of progesterone secretion.*

Actions of progesterone

Actions of progesterone are normally effective only when the uterus has been under the acting influence of oestrogens.

On uterus. Following oestrogen-primed uterus, progesterone stimulates development, growth and activity of the endometrial secretory glands causing a thickening of the mucosa and continued water accumulation. Glands become highly developed and active, and a marked increase in glycogen deposition occurs. Glycogen droplets may be found in glandular cells. Highly secretory mucosa provides nutrients necessary for implanted blastocyst. Spiral arteries become slightly enlarged and tightly coiled. The endometrium becomes highly convoluted.

If fertilisation and implantation (*nidation*) occur, the corpus luteum, under the stimulation of CG, continues to secrete progesterone and oestrogen to maintain the gravid type of endometrium.

Progesterone diminishes spontaneous myometrial activity. The progesterone-dominated uterus will not conduct an excitation wave and so such a uterus could not participate in the organised contractions necessary for expulsion of the young. That progesterone effects this block by altering ionic gradients across the myometrial cell membrane is supported by the fact that progesterone increases resting membrane potential. This progesterone-induced hyperpolarisation results in the uterus showing poor conduction, lowered excitability, little spontaneous activity and reduced pharmacologic reactivity. Recently it indicates that progesterone and oestrogen may control membrane potential magnitude by their effect on calcium balance of myometrial cells. Resting membrane potential of rat myometrial cells increases with advancing pregnancy. The transmembrane potential of the rabbit myometrial cell is maximum at placental sites and progesterone administration to a normal rabbit can increase the potential up to the pregnancy value (—60 mV). The resting membrane potential of pregnant human myometrial cell has been recorded at about —31 mV. Progesterone lowers and may block the sensitivity of the uterus to oxytocin. Progesterone in appropriate amounts inhibits oestrogen-induced sensitisation to oxytocin. This inhibitory effect of oxytocin is readily demonstrated in all species except in rats and guinea-pigs.

On ovary. The effect of progesterone on the ovary is indirect through alteration in pituitary gonadotrophin release. At present this effect of progesterone, alone or in combination, is not well understood. It is suggested that progestational hormones at a specific level inhibit LH release and stimulate FSH release, but the explanation has not been substantiated. It is known that oestrogen and progesterone in combination can block gonadotrophin release.

On Fallopian tube. Progesterone may be responsible for diminished muscular activity during luteal stage.

On cervix. The cervical mucus is reduced in amount and becomes more viscous during luteal phase under the influence of progesterone.

On vagina. Progesterone induces cellular proliferation and increased mucous secretion of the vaginal epithelium. Cornified cells are reduced in number. Vaginal smear shows few cornified cells, various leucocytes and clumped epithelial cells having folded edges.

On mammary glands. Progesterone stimulates mainly lobuloalveolar growth following oestrogen action.

On kidneys. Large dosages of progesterone produce natriuresis, presumably by blocking action of aldosterone on the kidney.

On DNA. Progesterone does not possess a significant anaebolic effect. At least, some of its effects are owing to an actich on DNA to make synthesis of new mRNA.

Progesterone is responsible for a rise of 0·5 to 1·0°C in the **basal (waking) temperature** of the body after ovulation and through the luteal phase of the cycle.

On the basis of above actions, the physiological functions of progesterone are described as follows:

Functions of progesterone

Progesterone is essential for the maintenance of pregnancy and various other changes associated with it. It also takes part in menstruation. Its functions are briefly summarised below:

1. **Responsible for premenstrual changes of uterine mucosa.** EVIDENCE: In ovariectomised animals, if progesterone be injected after a course of oestrogen, typical premenstrual changes will take place in the uterus. If then progesterone be discontinued, bleeding will take place, identical with menstrual bleeding. Progesterone alone fails to cause these changes. It must be preceded by a course of oestrogens. The latter produces the initial change on which only progesterone can work.

2. **Takes an essential part in pregnancy,** for instance—

(a) *Embedding of ovum.* EVIDENCE: (i) Progesterone secreted by the corpus luteum is responsible for the premenstrual hypertrophy of the endometrium which is essential for the reception and embedding of the fertilised ovum. (ii) If corpora lutea be removed from the rabbits, 20 hours after mating, endometrial changes, as described above, will not take place and the embryo will die by the fourth day. In such a case, administration of progesterone will cause the necessary endometrial changes and normal embedding will take place.

(b) *Essential for the formation of placenta.* EVIDENCE: (i) If corpora lutea be removed after embedding of the ovum, placenta fails to develop, the embryo dies and abortion takes place. (ii) If then progesterone be administered, placenta formation continues and pregnancy proceeds up to full term.

(c) *Inhibits uterine muscles* (antagonistic to oxytocin). Progesterone desensitises the uterine muscle to the action of oxytocin. This is important for the following: (i) During menstruation corpus luteum degenerates, progesterone is absent, oxytocin works unopposed and uterine muscle powerfully contracts. This helps to expel the menstrual discharge. (ii) During pregnancy progesterone secreted by the corpus luteum and placenta neutralises oxytocin action, uterine contraction absolutely ceases and the growth of uterus is facilitated. (iii) At full term corpus luteum dies and placenta also degenerates to some extent. Hence, progesterone secretion falls. Oxytocin acts unopposed and parturition starts.

3. **Development of breasts.** Breasts develop further during pregnancy—chiefly due to the proliferation of the glandular elements. Experimentally, it is seen that progesterone alone fails to cause this change. But if progesterone and oestrogens be given simultaneously, typical

pregnancy changes are seen in the breasts. During actual pregnancy, both hormones are present in the body and are responsible for the breast changes.

4. **Inhibits oestrous or menstrual cycle and ovulation.** During pregnancy, maturation of follicles, ovulation, oestrous or menstrual cycle are inhibited also. The production of pituitary luteinising hormone (LH) is inhibited by large doses of progesterone. Experimentally, injected progesterone prevents ovulation. Its purpose is to prevent formation of further embryos (superfoetation) during pregnancy. Probably it acts by inhibiting FSH.

5. **Causes enlargement of birth canal.** At full term birth canal enlarges due to growth of vagina and relaxation of pelvic ligaments. The same effect can be experimentally produced by giving progesterone along with oestrogens—but not with the former alone.

6. **Relation with oestrogens.** It helps, completes and antagonises oestrogen action (*vide* Oestrogens – page 4-253).

7. **Protein catabolic activity.** Progesterone has slight protein catabolic activity.

8. **Water and salt metabolism.** In animals progesterone causes mild salt and water retention, but administered to men or women it causes salt loss. On the other hand, the synthetic progesterones have a mild sodium-retaining effect.

9. Progesterone stimulates respiration and this is confirmed by the fact that in women during the luteal phase of menstrual cycle, the alveolar PCO_2 is lower than in males.

RELAXIN

Relaxin is a water-soluble polypeptide hormone present in pregnant mammalian ovary, placenta and uterus. This non-steroidal hormone of pregnancy has not been isolated from blood of either non-pregnant women or men. Relaxin level of blood reaches maximum at the terminal stages of pregnancy. **Bioassy**: (1) *In vitro* inhibition of motility of mouse uterus. (2) Measurement of the length of interpubic ligament with a transilluminating device and ocular micrometer.

ANDROGENS

In the biosynthetic pathway of oestrogens, testosterone is an intermediate. A large amount of androgens present in pathological ovaries is possibly due to enzymatic defects. There is no definite evidence that normal mammalian ovary produces significant amounts of androgenic steroids. . The small amounts of testosterone in the plasma of women are formed peripherally, mainly from precursors, such as androstenedione.

Control of ovarian functions

1. **Anterior pituitary**—(pages 4-31 & 4-33). **FSH,** having half-life about 170 minutes, controls (a) maturation of the Graafian follicles, and (b) secretion of oestrogens. **LH,** having half-life about one hour, controls (a) full maturation of Graafian follicles; ovulation; formation, growth and maintenance of corpus luteum; and (b) secretion of progesterone.

The stimulating effect of LH on progesterone secretion by the corpus luteum is accompanied by an increased formation of cycle AMP. It is blocked by puromycin indicating that it depends upon the synthesis of protein. However, increase in cyclic AMP content of corpus luteum produced by LH is not blocked. This suggests that LH activates adenyl cyclase in corpus luteum. The increased cyclic AMP initiates a reaction involving protein synthesis and leads to steroid secretion. Lactogenic hormone or prolactin or luteotrophic hormone (LTH)—has got influence on the secretion of progesterone from corpus luteum and so it is known as *luteotrophic hormone*. Although prolactin is active in rats and mice, it has no influence on progesterone synthesis in most other mammals like guinea-pigs, rabbits, monkeys and women.

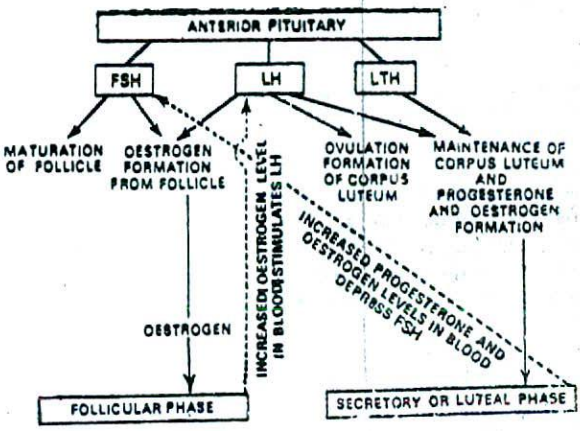


Fig. 4.172. Schematic representation of hormonal effects upon the ovary and the uterus.

There is reciprocal relation between ovarian hormones and pituitary gonadotrophins. Oestrogen and progesterone inhibit the secretion of gonadotrophins—FSH and LH from the anterior pituitary respectively (Fig. 4.172).

2. **Ovary.** In addition to its effects through the anterior lobe of the hypophysis, oestrogens directly affect the ovary. Oestrogen given to immature hypophysectomised rats, increases ovarian weights. Thus target organ for a hormone might be the same one that produces it.

3. **Uterus.** Progesterone released from corpus luteum is essential for the implantation of blastocysts, and is necessary during the early part of gestation. There is a clear evidence that stimuli from uterus influence ovarian function. It is possible that uterine endometrium synthesises a specific luteolytic factor named as **luteolysin,** which acting on the ovary, causes involution of corpus luteum. Removal of uterus (hyterectomy) maintains corpus luteum as during pregnancy. This uterine effect is not mediated through pituitary, it is a direct one. It is postulated that a natural prostaglandin present in the uterus is responsible for the luteolytic activity.

4. **Pineal gland.** It is postulated that melatonin or some other active principle present in the pineal gland has antigonadal activity. The action may either be a direct one or through anterior pituitary and central nervous system. Pinealectomy causes an increase in ovarian weight. On the other hand, administration of pineal extract decreases ovarian weight and reduces the incidence of oestrus.

5. **Adrenal cortex**—(page 4-152).

6. **Thyroid**—*vide* 'Sexual Growth' in page 4-86.

7. **Thymus.** Oestrogen inhibits thymus. At puberty it involutes.

8. **Hypothalamus.** In some mammals such as cat and rabbit, ovulation takes place only during coitus. Experimentally, it has been shown that sensory impulses arising from the female genitalia reach the hypothalamus and reflexly stimulate the secretion of gonadotrophins by the anterior pituitary. After stimulation of the hypothalamus, ovulation occurs, and this is due to the release of a releasing factor (LRF, *vide* p. 4-32) from the hypothalamus, which circulates in the blood stream and stimulates the secretion of gonadotrophic hormone (LH or ICSH) of the anterior pituitary. In other mammalian species such as rat and human, the median eminence and the tuberohypophyseal neurones of the hypothalamus release FSH-RF and LRF into the hypophyseal protal vessels which in turn control the secretion of FSH and LH from pituitary. Lesion of the hypothalamus causes abolition of gonadotrophic hormone secretion and atrophy of the ovary along with changes in the reproductive organs and stoppage of sexual cycle. The presence of another hypothalamic centre affecting gonadotrophin secretion in rats is indicated by the observation that lesions below the paraventricular nuclei are associated with development of multiple follicular ovarian cysts and constant vaginal cornification. **Feedback effects**—It is probably that secretion of FSH and LH is inhibited by circulating levels of oestrogen and progesterone during the luteal phase of menstrual cycle. The site is apparently the hypothalamus.

9. **Diet** should have enough caloric value. Starvation depresses ovarian functions.

10. **Vitamins**—E, A, ascorbic acid and some members of B complex are essential.

11. **Temperature.** The optimum temperature for the ovaries is a bit higher than that of testes. While undescended testis is sterile—the ovaries can only work when inside the abdomen at a higher temperature. [One curious finding is that—if the ovaries of rats be grafted at a cooler place, say, the pinna—not only ovulation stops but it manufactures male hormone instead of oestrogens.]

MENSTRUATION

Definition. *Cyclical discharge of blood, mucus and certain other substances from the uterus in the reproductive life of the females, at an average interval of* 28 *days* (24-32 *days*) *is called* **menstruation.** It occurs every month from puberty to menopause. It is **absent** (a) before puberty, (b) during pregnancy, and (c) after menopause (45-55 years). **Duration.** The flow lasts for 4-6 days without any appreciable pain. **Composition.** It is made up of (a) *blood* (30-40 *ml*), (b) *stripped of endometrium*, (c) *mucus*, (d) *leucocytes*, and (e) *an unfertilised ovum*. The menstrual blood which comes out from the uterus clots promptly due to rapid *formation of fibrin*. If the blood remains in the uterus for some time fibrin is deposited on the endometrium and as a result there is partial clotting. Intra-uterine clots if retained for a longer period dissolves due to the action of **plasmin.**

During each cycle the uterine mucosa (Fig. 4.173) gradually hypertrophies. The whole purpose is to prepare a suitable bed for the reception and implantation of the fertilised ovum. If pregnancy takes place, the proliferated mucosa becomes converted into *placenta*. If pregnancy does not

take place, the hypertrophied mucosa breaks down and is discharged as menstruation. Menstruation, therefore, may be described as the *funeral of the unfertilised ovum* or as the **weeping of the uterus for the lost ovum.**

The endometrial changes during the whole menstrual cycle has been divided into four stages: (i) the **resting** or **postmenstrual phase,** (ii) the **proliferative** or **reparative** or **oestrogenic phase,** (iii) the **secretory** or **progestational** or **luteal** or **premenstrual** or **progravid phase,** and (iv) the **menstrual phase.** The phases of menstruation and pregnancy are shown in Fig. 4.173.

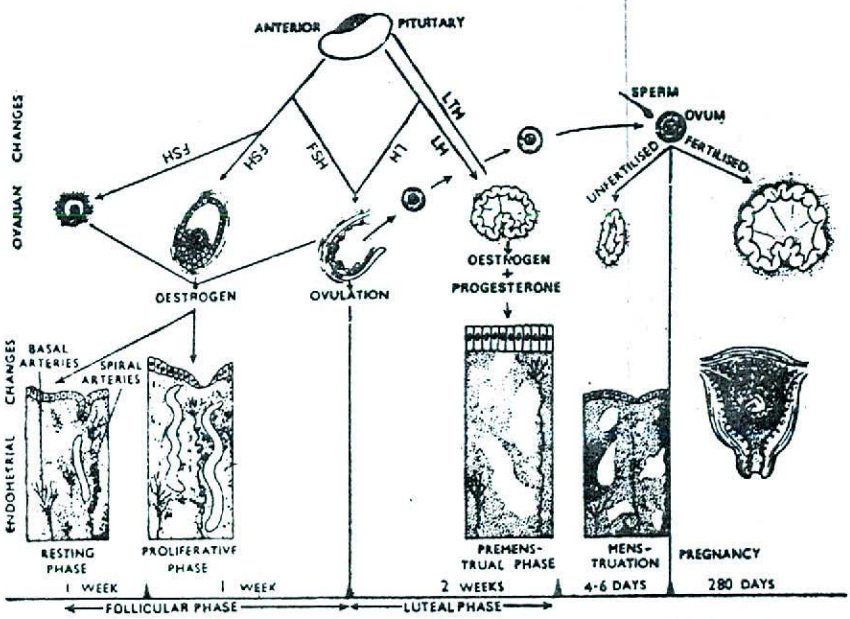


Fig. 4.173. Diagrammatic representation of the hormonal control during the menstrual cycle.

The 1st and 2nd phases may be called **follicular phase.** They are due to the action of gradually increasing amounts of oestrogen. In the beginning of the cycle theca interna of the vesicular or Graafian follicle secretes oestrogen and mediated through the secretion of follicular fluid by the granulosa, sensitise the follicle to FSH secreted by the anterior pituitary. FSH initiates the maturation of only one follicle (very seldom, two), which has been primed by oestrogen. The secretion of oestrogen reaches maximum at the time of ovulation, and such high level of the hormone inhibits the secretion of FSH but stimulates the secretion of LH. Anterior pituitary begins to secrete LH and LTH probably in increasing amounts at this stage. LH acting on sensitised follicle causes ovulation and then together with LTH helps in the development of corpus luteum. Progesterone secretion starts—causing the premenstrual changes —luteal phase. If pregnancy occurs, placental gonadotrophins stimulate

further growth of corpus luteum. More progesterone is secreted and the endometrium develops into a full-fledged placenta. But if fertilisation does not take place, the high level of progesterone inhibits the secretion of LH

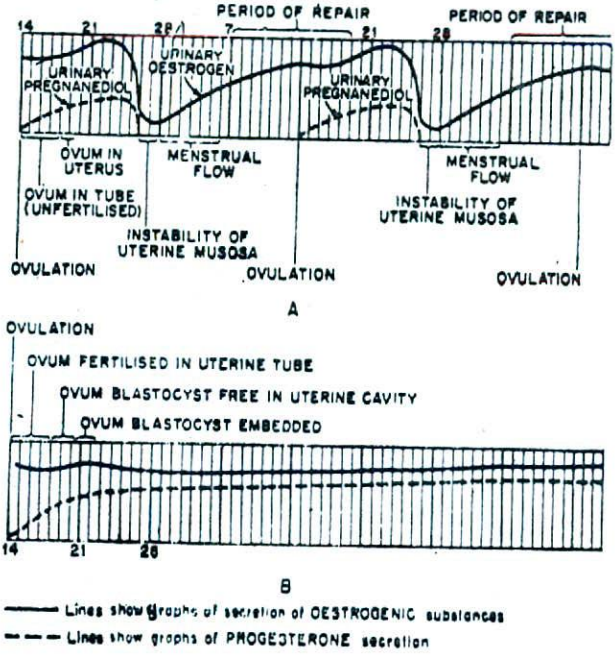


Fig. 4.174. Diagram A shows graphical representation of menstrual cycle in absence of fertilisation. Diagram B shows graphical representation following fertilisation, cleavage and blastocyst.

and LTH. This causes involution of corpus luteum, fall in progesterone secretion and dissolution of uterine endometrium. Above sequence of events in the ovary and uterus has been presented schematically in Fig. 4.174. The hypertrophied endometrium breaks down and discharged as *menstruation*. These phases are briefly summarised below:

Phases and Uterine Changes	Ovarian Changes and Excretion of Ovarian Hormones in the Urine	Cause and Control
1. **Resting phase** (Follicular phase) Duration — 1st-5th day (about 1 week). Endometrium heals and becomes normal. Slow proliferative changes begin.	Corpus luteum has degenerated. Inhibitory action of progesterone absent, hence, follicles slowly maturing and oestrogen secretion rising. *Urine*—oestrone and oestriol rising.	Proliferative changes are due to the action of oestrogens from the maturing follicles. Controlled by FSH of the anterior pituitary.

Phases and Uterine Changes	Ovarian Changes and Excretion of Ovarian Hormones in the Urine	Cause and Control
2. **Proliferative phase** Duration—6th-appx. 14th day, i.e.until ovulation. (*a*) Mucosa thickens (from less than 1 to more than 2 mm) and becomes more vascular. (*b*) Endometrial glands become longer, tortuous, narrow and straight. (*c*) Vessels slightly dilate. At the end of this phase basal secretory vacuoles appar beneath the nuiclei of glandular cells.	Graafian follicle maturing and oestrogen secretion rising. on the 14th day ovulation occurs and corpus luteum formation starts. *Urine*—maximum oestrogen excretion.	Caused by further action of oestrogens. Injection of oestrogens in immature or ovariectomised animals produces same changes. Controlled by FSH of anterior pituitary (page 4-33), which is finally inhibited by high oestrogen level.
3. **Premenstrual phase** (Luteal phase) Duration — 15th - 28th day (2 weeks) (*a*) Mucosa thickens further. (*b*) Glands more enlarged and distended with mucus. (*c*) Capillaries dilated like sinus. (*d*) Exudation of clear or blood-stained fluid. (*e*) Proliferation of stroma cells (as in early placenta). (*f*) Secretory vacuoles containing glycogen appear above nuclei.	Corpus luteum growing; progesterone secreted—inhibiting further maturation of follicles. Corpus luteum attains maximum size on the 19th day, lasts up to 27th day and degenerates on the 28th day. *Urine* — (1) Pregnanediol appears 2-3 days after ovulation, rises to maximum about one week before the period and falls 2-3 days before the flow starts, (2) Oestrogen falls.	Caused by progesterone. Only oestrogen or only progesterone cannot cause this. But if progesterone be given after a course of oestrogen these changes are seen. These hormones have got effect on the spiral arteries of the endometrium. Formation of corpus luteum and secretion of progesterone are controlled by LH and LTH of anterior pituitary. Discharge of menstrual fluid is aided by the presence of certain prostaglandins (page 4-184).
4. **Destructive phase** (Menstrual stage) Starts on the 28th day. Duration — 4 - 6 days. During this period capillaries rupture and haemorrhage occurs. Superficial endometrium, with pseudodecidual stroma and tortuous glands is sheded; basal layer remains intact.	Corpus luteum degenerates because placental gonadotrophins are essential for the further growth of corpus luteum. In absence of pregnancy no placenta forms, hence, corpus luteum degenerates.	Lack of progesterone is the cause. If a course of progesterone be given after a course of oestrogen, typical premenstrual changes occur in the endometrium. If then progesterone be suddenly witheld, bleedingdtakes place, identilcal with menstrual discharge.

Present knowledge about the nature of outputs of LH, FSH, oestradiol and progesterone is summarised in Fig. 4.175. Oestrogen output is at a minimum during menstruation, and then rises slowly during the follicular phase, the rate of rise being increased just before ovulation. After ovulation it falls to a plateau while the corpus luteum is active and then falls to menstrual levels as the corpus luteum degenerates. Progesterone output

appears to be at a very low level during the follicular phase. It begins to rise quickly just before ovulation occurs and reaches a peak soon afterwards, thereafter declining until next menstrual bleed. FSH stimulates follicular growth. In a normal oestrous or menstrual cycle there is a burst of LH release about the time of ovulation.

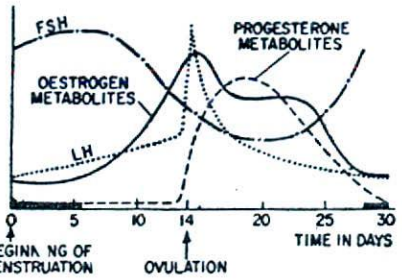


Fig. 4.175. It shows probable changes in blood levels of FSH and LH, and urinary outputs of oestrogen and progesterone metabolites during the menstrual cycle.

Gravid cycle. After ovulation, it takes about a week for the ovum to reach the uterus. If fertilisation has been taken place, the zygote will be undergoing rapid embryonic development and by the early part of second week of the luteal phase it will have become embedded in the uterine mucosa (*nidation, implantation*). By this time, the peripheral elements of the blastocyst (so-called *chorion*) begin to secrete a hormone (*human chorionic* gonadotrophin, HCG). This hormone helps in sustaining the life and function of the corpus luteum beyond the usual limits of a menstrual cycle. The cycle is not therefore interrupted by a menstrual flow and this curcumstance may be denoted as the **first skipped period.** A progestational or secretory endometrium becomes a *gestational endometrium* simply by the fact of pregnancy. It undergoes further progestational development during early weeks of pregnancy.

Anovulatory cycle. Anovulatory menstrual cycle is sometimes found in young girls and towards the end of menopause. In some animals, e.g., Rhesus monkey, anovulatory cycle is the usual feature. In anovulatory menstrual cycle no ovulation occurs and so no corpus luteum is formed. The endometrium changes up to proliferative phase and similar vascular changes as in ovulatory cycle also occurs. When there is bleeding its duration may be same as that of ovulatory cycle.

Vaginal cycle. Under the influence of oestrogens, the vaginal epithelium becomes cornified which can be identified in the vaginal smear, while under the influence of progesterone, a thick mucus is secreted and the epithelium proliferates becoming infiltrated with leucocytes.

Clinically it is often of importance to determine the time of ovulation. Biopsy of the endometrium indicates the functional level. A conaenient indication is to determine the basal body temperature. At the time of ovulation, there is a change, usually a rise. The cause of the change is not known.

Abnormalities of ovarian function. If menstrual periods are absent, the condition is known as **amenorrhoea. Primary amenorrhoea** is the condition when menstrual bleeding has never occurred. Cessation of the cycle in women with previously normal periods is known as **secondary amenorrhoea.** If during regular periods, the flow is scanty, it is called **oligomenorrhoea** and if profuse, it is known as **dysmenorrhoea.**

Menopause, or female climacteric. In women with advancing age (45-55 years) certain irregularities in sex life occur due to gradual irresponsiveness of the ovaries to the pituitary gonadotrophin (FSH). Under this condition the senility comes in the ovary, mostly in the corpus luteum, and oestrogen level in the blood stream decreases greatly through the pituitary gonadotrophin level in the blood stream is high. With these irregularities in gonadal functions, particularly in gametogenesis, the cyclic changes in endometrium are not observed. Finally the menstruation fails to occur and the women cannot bear children any more. It is usually marked by atrophy of breasts, uterus, uterine tubes and ovaries. This condition is known as **menopause.** Therefore, *menopause is the physiological cessation of menstrual flow in women.*

Certain complaints are generally encountered by the menopausal women. These complaints are flushing of the skin, feeling of warmth and also a marked increase in sweating especially about the head and the neck. In association with the above complaints certain emotional disturbances, arthralgias (associated with arthritis). Other menopausal problems may include obesity, believed to result from decreasing caloric expenditure as a result of oestrogen deficiency, *osteoporosis* due to decreasing protein anabolism, causing a loss of protein matrix particularly to the vertebral column. Osteoporosis causes decalcification and softening of bones which may result in compression fractures of bones.

Excess gonadotrophin secretion during menopause is not the cause of these syndromes as may be supposed but oestrogen therapy has got some satisfactory effects in ameliorating the certain symptoms.

Summary of changes and influences of hormones during the menstrual cycle

(*a*) During the period of menstrual flow, FSH is secreted from the anterior pituitary. This causes growth and development of follicles in the ovary. At the end of about 2 weeks, the maturity of *one* follicle occurs. Under the influence of FSH, the theca interna begins to secrete gradually increasing amounts of oestrogens. This may be called as proliferative or oestrogenic phase of the menstrual cycle. It lasts normally about 2 weeks.

(*b*) Oestrogens now help to repair and proliferation of the uterine endometrium. They make an increased thickening, keratinisation and glycogen storage of vaginal epithelium. They may also aid *libido.* The rising oestrogen-level in blood stimulates LH secretion towards the end of two weeks.

(*c*) LH somehow aids in the ovulation or rupture of mature follicle and release of ovum (about 13 to 15 days) before the beginning of next menstrual flow. LTH is now released from the anterior pituitary. Together with this LTH, LH causes the remains of ruptured follicles to be transferred into the corpus luteum which secretes increasingly large quantities of progesterone and small amounts of oestrogens during the secretory or progestational phase of the menstrual cycle.

(*d*) Progesterone possesses a secretory effect on the endometrium of the uterus. This effect makes increased secretion and nutrient storage within the mucosa of the uterus in preparation for the reception and nourishment of a fertilised ovum. Progesterone also effects quietly upon the uterine musculature which favours implantation of the embryo. When the progesterone level in blood increases towards the end of the menstrual cycle, there appears a *feedback effect* upon the anterior pituitary by causing it to secrete less and less LH and LTH. As the productions of LH and LTH are thus inhibited, there are involution of the corpus luteum and cessation of progesterone secretion.

(*e*) Menstruation may be a cause of the resulting low levels of progesterone and oestrogens in blood. Due to lack of appropriate amounts of oestrogens there may be an effect of vasospasm of the spiral arteries to the endometrium. This results in inadequate nutrition of superficial layers of the endometrium.

(*f*) Decreased progesterone level in blood is no longer enough to inhibit the pituitary FSH production, so FSH is again secreted and the menstrual cycle resumes (Fig. 4.172).

(*g*) If there are occurrence of fertilisation and implantation, the corpus luteum persists and secretes continuously large quantities of oestrogen and progesterone under the stimulating influence of CG (chorionic gonadotrophin) which is produced by the developing placenta and whose action is similar to that of LH and LTH.

OESTROUS CYCLE

In lower mammals, menstrual cycle is represented by a homologous condition—the *oestrous cycle*. It occurs only during breeding season. One cycle may occupy the whole breeding season, such animals are called *monoestrous* (dog). Many oestrous cycles may take place in the same mating

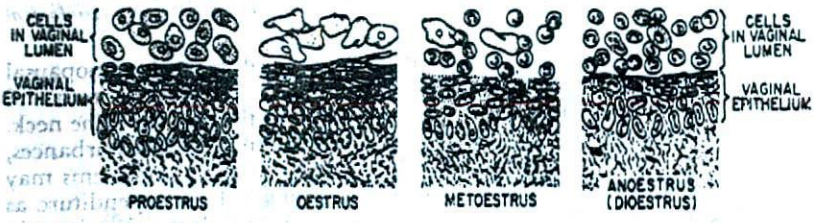


Fig. 4.176. Diagrammatic representation of the characteristic features of vaginal cell type—found in the vaginal smear during the oestrous cycle.

season with short resting intervals. Such animals are called *polyoestrous* (rats, mice, guinea-pigs, etc.). Stockard and Papanicolaou (1917) working on rats, guinea-pigs, etc., proved that characteristic changes take place in the vaginal epithelium at different phases of oestrous cycle, which can be followed accurately by examination of vaginal smears (Fig. 4.176). This test is made use of in assaying the oestrogenic potency of an unknown substance. The **Allen-Doisey test** be used for the detection of oestrogenic activity, is based on the ability of a compound to produce oestrus in ovariectomised, sexually mature rats. In the cat and some rodents, the follicles develop, but ovulation does not occur (ova are not released from follicles) until after copulation. In rabbits the follicles do not develop fully before copulation and a long oestrus may occur, ripening of the follicles and ovulation are initiated by copulation. The different phases of oestrous cycle are briefly described below:

1. **Proestrus** *(Preparatory stage)*—Uterus and vagina become congested and secrete a clear sanguinous fluid. Vaginal epithelium proliferates. This is caused by oestrogens secreted by the maturing follicles. *Vagilnal smear* shows a large number of nucleated cells, broken off from the proiferating vaginal epithelium.

2. **Oestrus** *(Heat period)*—This is the period of desire, only when the female will receive the male. This occurs at the end of the proliferation phase. This time of receptivity immediately prior to ovulation is termed oestrus. Congestion of the uterus becomes maximum, the vaginal epithelium thickens further and the superficial layers are fully keratinised. Ovulation takes place at this time so that impregnation is possible. If fertilisation takes place, placenta forms and pregnancy begins. If not, it passes on to the next phase. In some animals (e.g., cat, rabbit) ovulation does not occur in absence of copulation. *Vaginal smear* shows a large number of keratinised cells.

3. **Metoestrus** *(Luteal phase)*—Changes initiated in the previous stage proceed still further due to the action of progesterone, secreted by the newly formed corpora lutea. In absence of pregnancy, corpora lutea degenerate and the changes of the generative organs subside. In monoestrous animals (bitch) the hypertrophied mucosa breaks down and is discharged. In certain species of **monoestrous animals** (rabbit) changes alike pregnancy have been seen to occur even when the female has not been fertilised. These include changes in the uterus and mammary glands and it is named as **pseudopregnancy.** Activity of corpus luteum is very high and is prolonged in such animals. *Vaginal smear* shows a large number of WBC along with keratinised cells.

4. **Anoestrus or Dioestrus.** This is the resting asexual period. In monoestrous animals it lasts up to the next mating season, and is known as **anoestrus.** In polyoestrous animals the resting interval is short up to the next cycle and is called **dioestrus.** In rats it lasts for 4-6 days. *Vaginal smear* shows a good number of WBC, a few epithelial cells and mucus.

Studies on changes in the oestrous cycle of different experimental conditions have great help to understand the physiology of female reproductive system.

ROLE OF THE CENTRAL NERVOUS SYSTEM (CNS) IN HYPOPHYSEAL-OVARIAN AXIS

Many observations suggest that the central nervous system bears a link in the communications between the adenohypophysis and the ovary (Fig. 4.177). It is seen that destructive lesions of the CNS, especially at the base of the brain, cause either ovarian hypofunction or hyperfunction. It is said that emotional disturbances can cause profound irregularities of the menstrual cycle and can impair fertility in women. The hypophyseal-portal system of blood vessels represents a pathway of information transfer from the hypothalamus to the adenohypophysis. Gonadotrophin output is controlled by messages which reach it from the brain and the locus of the feedback action of oestrogen may be in the CNS. For example, in ferrets exposure to light induces a *constant* oestrus which is thought to be due to increased output of FSH induced by photic stimulation. In certain species, a special odour by its presence or absence, can have a profound effect on gonadotrophin output. In such cases, sensory afferents send information to the CNS, which then sends a humoral message to the pituitary through the hypophyseal-portal system. The light effect may be prevented by pituitary stalk section and placing a mechanical barrier to the regeneration of portal vessels across the section. FSH-RF and LRF have been prepared and they are virtually free from other releasing factor activity.

Ovulation is not produced in the hypophysectomised animal and is brought about by the release of pituitary LH (and FSH?) in the intact animal. Ovulation is used in experiments as an indicator of LH release.

Ovulation can be produced by electrical stimulation of very precisely demarcated areas of the hypothalamus (Fig. 4.178) in oestrus in those animals which normally ovulate only in response to coitus or to mechanical stimulation of the vagina. Ovulation does not occur in such species whose hypophysis is removed immediately following vaginal stimulation, but it does if the operation is delayed for a while after stimulation. Atropine, morphine and pentobarbital block the neuroendocrine reflex in rabbits.

In such drug-blockaded animals, electrical stimulation of the hypothalamus near the pituitary still produces ovulation (Fig. 4.178).

If the pituitary gland is grafted in a hypophysectomised animal at a site remote from the anterior chamber of the eye or beneath the renal capsule, in its newer site the tissue becomes revascularised and recovers a certain amount of its original capacity to secrete trophic hormones, but gonadotrophins are not secreted in appropriate amounts to maintain normal gonadal function for some weeks postoperatively. If such a transplanted gland is retransplanted back to the sella turcica, normal gonadal function may be resumed.

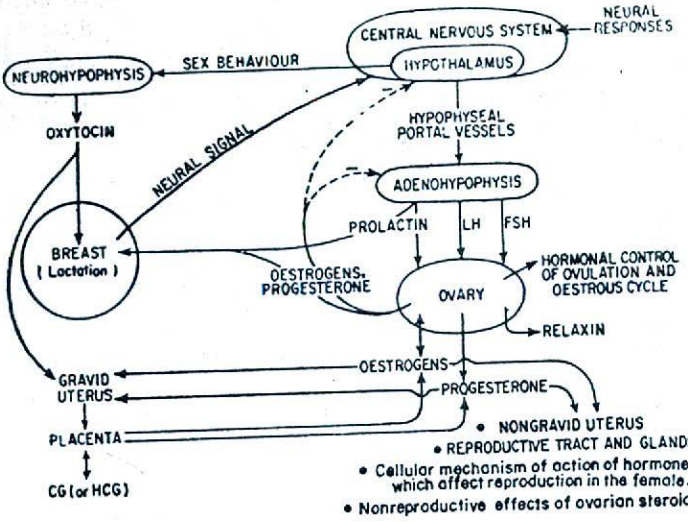


Fig. 4.177. Schematic outline of the neuro-endocrine control of reproduction in the female.

The brain is involved in the feedback inhibition of gonadotrophin secretion by oestrogens and androgens. Lesions in the hypothalamic paraventricular nuclei prevent gonadal atrophy which occurs due to administration of the steroid hormones. Therefore sex steroid hormones may alter the threshold of response to stimulation of brain centres which regulate gonadotrophin secretion. An oestrogen-treated rabbit may ovulate in response to vaginal stimulation alone if the rabbit has been progesterone-primed. Oestrogen treatment of intact rabbits might increase sensitivity of the brain to the sort of stimulation causing ovulation, but very high doses of oestrogen might rise the threshold of stimulation, i.e., might decrease the sensitivity.

These experiments are concerned with FSH and/or LH release. But the action of prolactin or luteotrophin is meager. If the hypophysis is transplanted to a site remote from the sella turcica, there is an increase in prolactin output rather than a decrease. If signals of hypothalamic origin inhibit prolactin output constantly, and transplantation constitutes a form of release from this inhibition. Therefore there is some sort of reciprocal

relationship between FSH and LH on the one hand and prolactin on the other, because those physiological conditions which increase prolactin secretion decrease FSH and LH.

HORMONES AND SEXUAL BEHAVIOUR

Patterns of sexual behaviour in animals are commonly regulated by the effects of gonadal steroid hormones secreted in response to hypothalamic-hypophyseal rhythms and related to seasonal factors or characteristic ovarian cycles.

In humans, steroid hormones are equally necessary as a background for sexual behaviour but they are relatively small effective on the frequency and timing of sexual activity except at the extremes of concentration (e.g., decline in potency and libido following failure of pituitary or testicular function, and enhanced libido accompanying androgen excess). In the male, testosterone is essential for the development and maintenance of libido and potency. Once established, libido may be retained despite the development of moderate androgen deficiency, though there is generally some decline in potency when androgen secretion is impaired. The decline in sexual potency which occurs with ageing in males, is not due to lack of androgen, because testosterone secretion remains almost constant with advancing ages. In humans with true androgen deficiency, testosterone treatment causes marked improvement in libido, but in psychogenic impotence the secretion of testosterone is generally normal and androgen administration has little effect. In humans, oestrogen treatment causes a striking decline in libido and potency due to inhibition of pituitary LH secretion and reduced secretion of testosterone.

In the female, gonadal steroid hormones have less clearcut effects on sexual activity, whereas androgen secretion by the adrenal gland may be a major factor in promoting libido. Activity of the pituitary or adrenal failure is followed by loss of libido, whereas ovarian failure is relatively less effective on sexual function. When a woman is treated with testosterone analogues, excess androgen is sometimes accompanied by enhanced libido, while oestrogen and progesterone are relatively little effective on the intensity of libido. However in the female, secretion of oestrogen maintains normal genital function and to this extent it is an important contributing factor to sexual activity. Although high rates of oestrogen secretion are associated with sexual receptivity and oestrous behaviour in most other vertebrates, there is little evidence in the women for oestrogen-programmed sexual activity. Normally psychic factors are important in the patterns of human sexual behaviour. On the other hand, steroid hormones maintain necessary genital development and libido which provide background for sexual function.

In certain species hormonal events are stimulated by coitus and influence the subsequent course of pregnancy.

In certain species, mating behaviour may be observed in the decorticate animal, but not in spinally transected animal. So, the neural integrative circuits for sexual behaviour in these species lie below the cortex and above the spinal cord. Although these circuits are specifically, complicated, there is a preprogrammed computer into which response is fed in the form of visual, tactile, olfactory and auditory stimuli. These stimuli elicit a response that involves many widely dispersed muscles and nerves

involved in mating behaviour. There are skeletal muscle reflexes which are associated with posture and movement. There are also particular certain parts of the autonomic nervous system, which are concerned with cardiopulmonary reflexes and which are associated with the act of copulation.

The central nervous integrative circuitry is essential for the mediation of both masculine and feminine sexual behaviour. This ambivalence is frequent in the female than in the male. Because one can commonly see male copulatory thrusting movement in an untreated spayed bitch. In various species *androgen-treated* female may show peculiar male mounting behaviour and assume dominance over the female—and even over the male.

Effects of castration on sexual behaviour may vary from species to species. In the male, *prepuberal castration* abolishes sexual behaviour, but *postpuberal orchiectomy* may only diminish sexual pattern. In the female, *oophorectomy* does not eliminate sexual activity and interest, while *adrenalectomy* does eliminate sexual activity and interest. Androgen treatment can restore full sexual activity to the prepuberally castrate male animal. On the other hand, full sexual activity and interest can be restored by oestrogen treatment to those female animals whose sexual activity is abolished by oestrogen deprivation.

Androgen treatment may increase sexual drive doing no alteration of its direction in the *homosexual human male,* who often shows no overt evidence of androgen deficiency, while in the heterosexually adjusted women, androgen administration may increase libido and responsiveness to sexual stimulation.

Specific regions of the hypothalamus are involved in the central integration of sexual behaviour. Posterior hypothalamic lesions in both male and female rats abolish sexual behaviour, but these lesions are not in the same place as are those which interfere with the production and release of hypophyseal gonadotrophin (Fig. 4.178). Recording electrodes in the hypothalamus of animals reveal brusts of electrical activity on artificial stimulation of the vagina.

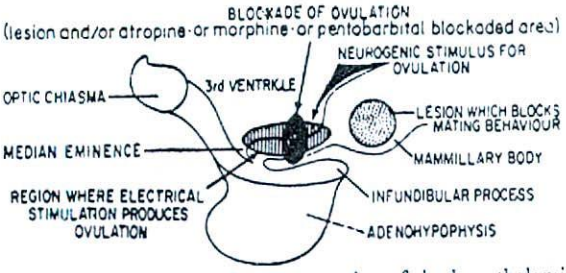


Fig. 4.178. Diagrammatic representation of the hypothalamic structures associated with sexual behaviour.

The central neural structures which are associated with mediating and integrating sexual behaviour, are under the influence of sexual steroids and while the gonadal hormones do not originate the circuitry, by their presence they permit circuitry to operate. It was Harris who performed the ingenious experiment of inserting *stilboestrol* into that part of a spayed cat's hypothalamus that had already been associated with sexual behaviour. This ester of synthetic oestrogen could diffuse for short distance locally, but not enough of it entered blood stream to affect the reproductive organs. On the other hand, implantation of similar synthetic oestrogen into the cerebellum, preoptic area, caudate nucleus, thalamus or amygdaloid nucleus do bot elicit normal sexual behaviour in similar experiments.

The gonadal hormones in the adult activate previously established patterns of sexual behaviour. In the morphologically and psychosexually undifferentiated mammals a different function of the male hormone (androgen) has been demonstrated. During this early developmental period, prenatal or postnatal depending upon the species, the male hormone organises the tissues, probably neural, that mediate the display of sexual behaviour in the adult.

Fundamental in establishing the form and extent of sexual behaviour displayed by the individual are the actions of genes and chromosomes. The pattern of sexual behaviour displayed, including the presence or absence of specific components of sexual behaviour of the vigour of display, has been shown to be heritable. Morphological abnormalities of the reproductive system in man are associated with chromosomal abnormalites, but disorders of sexual behaviour are not. The type of sexual behaviour associated with abnormal sex chromosomes depends more on functional characteristics of the developing gonad than on the chromosomal factors.

PREGNANCY

If the ovum be fertilised pregnancy begins. Normally, it lasts for 280 days (viz., ten menstrual cycles), at the end of which parturition takes place.

Physiological changes during pregnancy. Main changes are:

I. **Uterus and birth canal**

(a) Uterus enlarges due mainly to hypertrophy but partly to hyperplasia of uterine muscle fibres. The individual muscle fibre becomes wider by 6 or 7 times and longer by 10 or 11 times than non-pregnant uterus. Connective tissue in between the muscle fibres also increases. Weight of the uterus increases. Weight of the uterus increases up to 1000 g at full term. *Cause:* (a) Progesterone inhibits movement and facilitates growth. (b) Oestrogen stimulates growth directly. (c) Mechanical tension and irritation caused by the growing foetus also act as an important stimulus.

(b) Development of placenta.

(c) Enlargement of the birth canal and relaxation of the pelvic ligaments.

II. **Breast.** Mammary glands proliferate and development of breasts is completed. Lactation begins after parturition. *Pigmentation* of the areola and nipple occurs. The pigmentation may be due to ACTH or MSH (melanocyte-stimulating hormone).

III. **Ovaries.** (a) Formation and growth of corpus luteum in the early months and its degeneration in the later months. (b) Cessation of ovulation.

IV. **Blood.** (a) Blood volume and blood cholesterol are increased. (b) Plasma fibrinogen level also increases. There is an increase of plasma α- and β-globulins and there is a decrease of plasma albumin. Plasma iron level falls. There is an increased *erythrocyte sedimentation rate.*

V. **Circulatory system.** Cardiac output is increased. Heart may be enlarged. It may be due to enlarged uterus pressing the diaphragm and causing the change of position of the heart. There is slight fall of systolic blood pressure and greater fall of diastolic blood pressure. Blood flow in the forearm and hand is increased.

VI. **Respiration.** Vital capacity is increased. Tidal volume and pulmonary ventilation are increased.

VII. **Digestive system.** Nausea and vomiting occur in the early months of pregnancy. Hypochlorhydria and hypotonicity of the colon are often found.

VIII. **Excretory system.** There is an increased glomerular blood flow and glomerular filtration. Sometimes glycosuria is found. Excretion of the following sex hormones occurs in the urine—(a) oestrogen, (b) pregnanediol, and (c) placental gonadotrophins.

IX. **Endocrine system.** (a) *Thyroid gland* is enlarged and increased thyroid hormones secretion occurs. (b) *Adrenal cortex* is enlarged especially the zona fasciculata. Secretion of cortisol is increased. (c) Parathyroids are enlarged. Secretion of parathormone is increased.

X. **Metabolism.** (a) *Carbohydrate*—Renal threshold may be lowered and as a result glycosuria may occur during pregnancy. (b) *Protein*—There is positive nitrogen balance and more nitrogen is retained in the body provided there is intake of balanced diet. (c) *Lipid*—Lipaemia often occurs. (d) *Water*—Increased water retention usually occurs in later months of pregnancy. Water is retained in the amniotic fluid, placenta, foetus, breast, uterus, blood and other tissues. There is increased blood volume. The retention of Na and H_2O is probably due to effects of female sex hormones.

XI. **Other changes.** High outputs of protein and steroid hormones from the placenta produce a variety of maternal changex during pregnancy. Most of these effects occur owing to high levels of plasma-oestrogen, which stimulate the synthesis of a group of hormone-transport proteins by the liver. SHBG (sex-hormone-binding globulin) or GBG (gonadal-hormone-binding globulin) or TEBG (testosterone-binding globulin) which binds androgens and oestrogens is rapidly increased. It probably helps to minimise the circulating level of free oestrogen. CBG (cortisol-binding globulin) and TBG (thyroxine-binding globulin) are also increased, resulting in high levels of cortisol and thyroxine in blood. Because of increased binding of these hormones, their physiologically effective free levels are not markedly raised and forms of hormone excess do not unfold.

Formation of renin substrate by the liver is also increased by oestrogen, causing an elevation of plasma-renin activity during pregnancy and a high level in plasma-angiotensin II. As pregnancy advances, there is an accompanying rise in aldosterone secretion and excretion. This rise in aldosterone secretion may be due to elevation of plasma-angiotensin on the one hand, and due to inhibitory effect of high concentration of progesterone and its hydroxylated derivatives on Na retention by the renal tubules on the other hand. High concentration of renin appears in the pregnant uterus and amniotic fluid, but does not contribute to the maternal plasma-renin level and do not possess any local action upon the uterus and its contents.

Pregnancy tests

These tests are generally performed when confirmation of pregnancy is needed. Nost of the tests are carried out with the patient's urine and are based on the fact that the placenta produces gonadotrophins, which

are excreted in the urine. If urine contains such hormones and it is injected commonly into immature female test animals, the ovaries will undergo maturation. The first satisfactory test was done by Aschheim and Zondek in 1927-28. Most of the other tests are modifications of the original.

(1) **Aschheim-Zondek test** or **Mouse test**—0·4-0·5 ml of the morning urine from a woman thought to be pregnant is injected subcutaneously twice daily for 3 days into 3 or 4 immature mice (3-4 weeks old). On the 4th or 5th day, the mice are sacrificed and the ovaries are examined. The test is positive if blood-filled follicles (ovulated) or corpora lutea are found in the ovaries. The method is essentially 99% accurate. Disadvantages are the cost of animal maintenance and the 4 to 5 days required to perform the test. (2) **Friedman test** or **Rabbit test**—uses mature female rabbits that have been isolated from males for at least 6 weeks. The rabbits, normally two, are injected intravenously with 10-15 ml of test urine. One rabbit is examined 24 hours later, the second one in two days. A positive test is identified when the fresh corpora lutea appear in ovaries. The test is accurate in more than 99%, but it is used infrequently because of the greater cost of test animals. (3) **Hogben test**—Injection of 20-30 ml urine into the dorsal lymph sacs of South African female clawed toad *(Xenopus laevis)* will cause appearance of a large number of ova within 6-12 hours. (4) **Sperm-shedding test** or **Galli-Mainini test**—5 ml urine injected into dorsal lymph sacs of the male toad *(Bufo arenarum)* or male frog *(Rana pipiens)*. One to two hours later the toad's or frog's urine is aspirated from the cloaca with a pipette and examined under the microscope for spermatozoa. The presence of sperm cells indicates a positive test. (5) **Ovarian hyperaemic test** or **Rat test (Kupperman test)**—Two ml of the urine to be tested are injected intraperitoneally twice with an interval of one hour into prepuberal rats. Animals are then examined for hyperaemia or blushing of ovaries at 2, 6, 12 or 24 hours. This hyperaemic response may be used as a quantitative assay for CG. (6) An **immunological pregnancy test** is based on precipitation of latex particles coated with antibodies to chorionic gonadotrophin. Antibodies are produced after administration of *human chorionic* gonadotrophin in rabbits. Antiserum taken from these animals are used to detect the presence of chorionic gonadotrophin in the urine of pregnant women with the help of complement fixation tests. Abnormal proliferation of chorionic epithelial tissue, viz., *hydatidiform mole* or chorionepithelioma would give rise to false results in pregnancy tests.

[Most of the tests are more than 95-96% accurate when these are carried out 2 weeks after the first missed menses. The test urine should be the first urine voided in the morning. The patient should not be taken any drugs or alcoholic beverages for at least 12 hours before collection of urine.]

In addition to these, several physical signs associated with the generative organs are of diagnostic importance. During pregnancy (1) the vagina shows (a) vascularity, (b) softening and (c) increased secretion. The increased vascularity changes the colour of the vaginal wall from its usual pink mucosa to a bluish or cyanotic colour. This is known as **Chadwick's sign.** (2) Another physical sign that is most reliable is **Hegar's sign.** This is found as a softening of the isthmus or junction between the cervix and body of the uterus, which results in a greater mobility between two areas. This may be felt by the physician on bimanual pelvic examination.

PLACENTA

The mammalian placenta. A functional connection between the embryo and the uterus is necessary in animals in which development of the foetus occurs within the uterus, and in which nutrients for the foetus come directly from the uterus rather than from the yolk stored in the ovum. This connecting structure, the *placenta*, allows for nutritional, respiratory, and excretory interchange of material by diffusion between the embryonic and the maternal circulatory systems, and consists of both embryonic and uterian tissues. The placenta also functions as a barrier that excludes from the embryonic circulation bacteria and many large molecules. In addition, in eutherians, the placenta produces certain food materials and synthesises hormones important for the maintenance of pregnancy. Mammals are not unique in having a placenta. Among mammals, the major types of placentae differs sharply in structure and in the efficiency with which they facilitate the nourishment of the embryo.

Formation and Development. The fertilised ovum enters the uterus and eats its way into the hypertrophied endometrium. The gap closes over the ovum and it becomes embedded. This process is known as

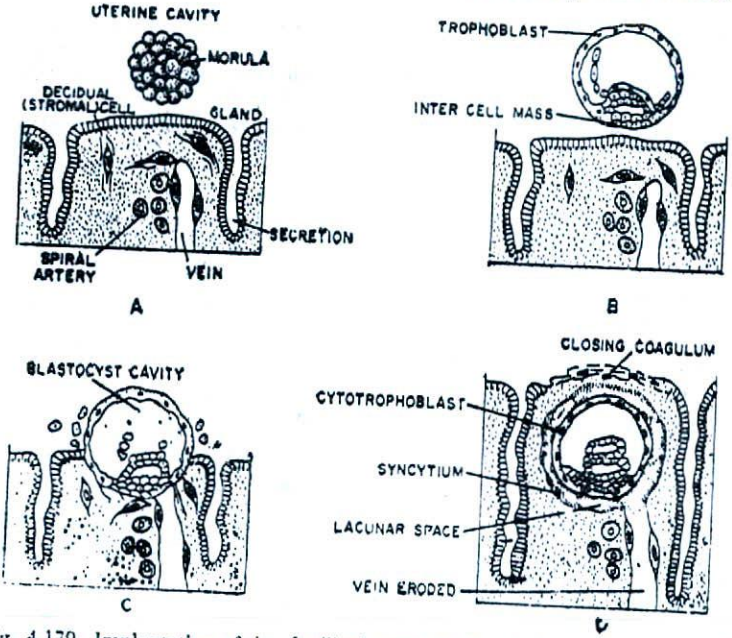


Fig. 4.179. Implantation of the fertilised ovum in the uterian cavity. A = cell division has led to the formation of the compact morula. B = reveals the cavity of blastocyst and the inner cell mass of a slightly later ovum. C = process of implantation. D = sealed coagulum shows the implanted ovum within the uterian wall.

implantation or **nidation** (Fig. 4.179). The site of implantation is commonly on the dorsal wall of the uterus. The portion of the decidua that covers the projecting ovum is called **decidua reflexa,** and that portion of the mucous membrane, which intervenes between the ovum and the mus-

cular layer of the uterus, is the **decidua basalis,** in which there is great dilatation of the maternal blood vessels and extensive proliferation forming the so-called *placenta.* The stimulus for the growth of the placenta comes from: (1) Increased secretion of progesterone, at first from the corpus luteum and then from the placenta itself. (2) Irritation—mechanical and probably chemical—exerted by the growing embryo.

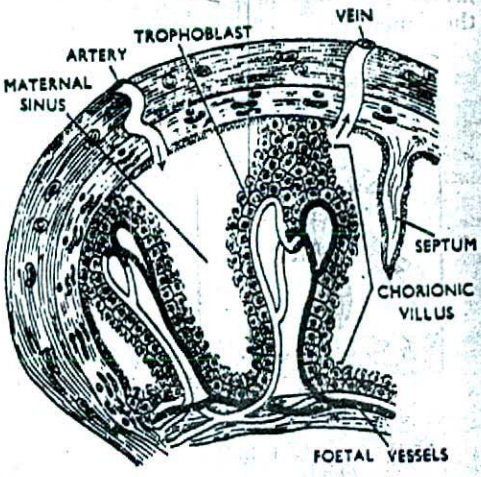


Fig. 4.180. Diagrammatic representation of the histological structure of placenta showing the circulation of maternal and foetal blood.

Histology. Placenta consists of two parts: (1) **maternal,** and (2) **foetal.** The **maternal part** (Fig. 4.180) consists of large blood sinuses, incompletely partitioned by fibrous septa. Maternal blood circulates through these sinuses—arterial blood entering through the uterine arteries and venous blood passing out through the uterine veins. The **foetal part** (Fig. 4.181) consists of numerous finger-like processes—the *chorionic villi*—projecting into the maternal sinuses and being bathed all round by the maternal blood. These villi contain foetal capillaries through which foetal blood circulates. They remain covered by a layer of syncytium, the *trophoblast.* Some villi float freely, others remain attached to the maternal decidua by thin prolongations of the trophoblast (Fig. 4.182).

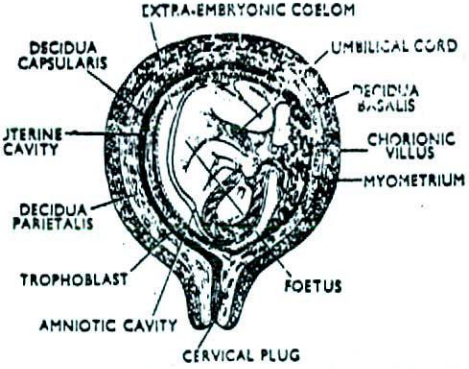


Fig. 4.181. Diagram represents the position of growing embryo within uterus and connecting link of the embryo with maternal parts through umbilical cord.

With the growing of the embryo the structure of the trophoblast becomes differentiated into two distinct cell layers—the *cytotrophoblastic layer (cellular trophoblast)* and *syncytial trophoblast.* The cytotrophoblastic layer is inner, well-defined and discrete layer. The cells of this layer is generally known as *Langhans cells* (Fig. 4.183). These cells generally elaborate chorionic gonadotrophin and chorionic growth hormone-prolactin (CGP). This layer presists up to first half phase of the pregnancy and then disappears gradually. At term it is nearly absent. The outer trophoblastic layer or syncytial trophoblast is outermost layer over the cyto-

trophoblast and is bathed into the maternal sinus. This layer persists all throughout the pregnancy. The cells of this layer elaborate oestrogen and progesterone mainly and also chorionic growth hormone-prolactin.

Thus, maternal blood is separated from the foetal blood by two layers —the cytotrophoblastic layer and the outer trophoblastic layer (syncytial layer). Exchange of gases, nutriment and metabolites take place through these membranes (Fig. 4.182).

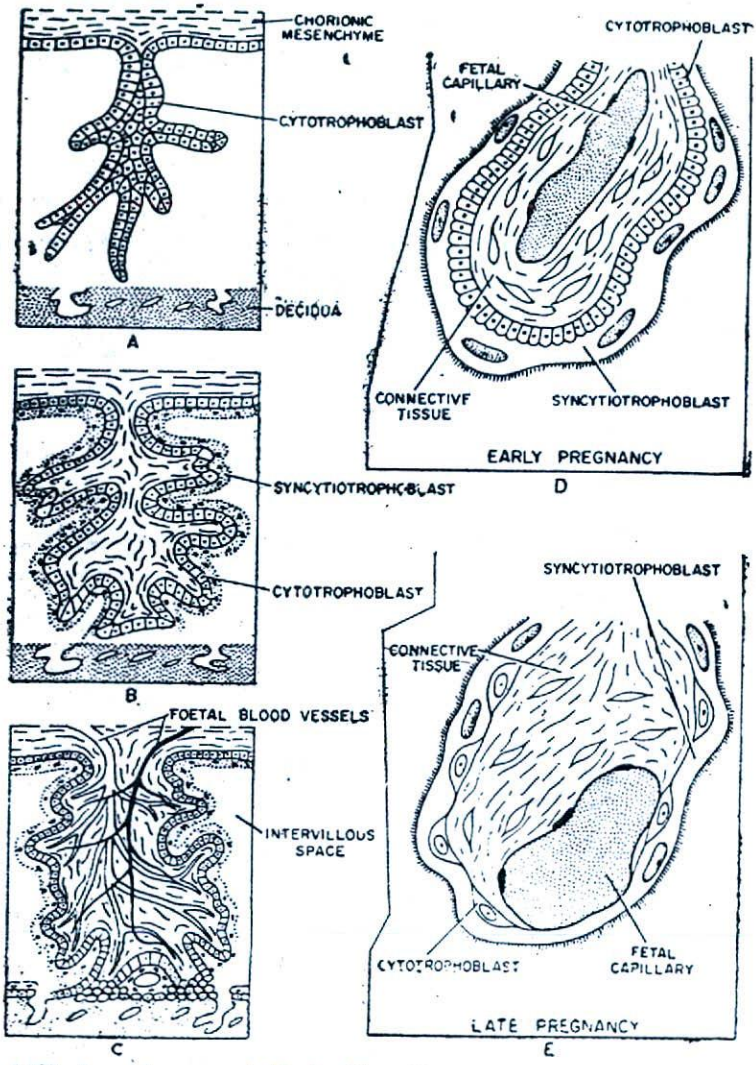


Fig. 4.182. A = primary trophoblastic villus with a central core of cuboidal cells. B = well-defined cuboidal cells covered by a second layer devoid of intercelluar boundaries. C = extended loose connective tissue into the villus from the foetus. Microscopic structure of chorionic villi in early pregnancy (D) and in late pregnancy (E).

When the human placenta becomes fully functional in the steriod synthesis, the steroid hormones secreted are mainly progesterone, 5-pregnenolone, large quantities of oestriol, smaller amounts of 20α- and 20β-hydroxy derivatives of progesterone, oestrone and oestradiol. The

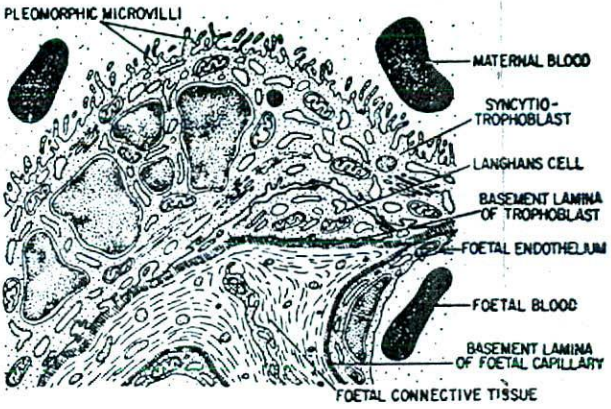


Fig. 4.183. Diagrammatic representation of human placental barrier near term.

placenta can synthesise progestational steroids from acetate or cholesterol, but oestrogens are derived by aromatisation reaction of androgen precursors from the foetal or maternal adrenal glands.

The placenta has enzymes for the synthesis of cholesterol from acetate and for the production of 5-pregnenolone from cholesterol. As far as is known, these transformations are identical to, or the same as, enzymatic mechanisms found in other endocrine tissues (p.4-255). The placenta is also very active in converting androgenic steroids, viz., androstenedione, DHEA, and 16α-OH DHEA, to phenolic oestrogens. By 'aromatisation reaction of C-19 steroids' the enzymes are found greatly in the microsomal fraction of the placental syncytial trophoblast, and require NADPH and O_2. The aromatisation reactions and the probable sources of reducing equivalents (NADPH) are identical as those described for the ovary. Unlike the ovary, the placenta does not synthesise oestrogens from acetate *via* cholesterol through pregnenolone or progesterone, but it is dependent upon androgens derived from the foetal adrenal cortex or from the maternal adrenal for conversion to oestrogens. The foetal adrenal cortex secretes greatly Δ^5-androgens, viz., DHEA and 16α-OH DHEA. The human placenta can convert 16α-OH DHEA to oestriol. Since the placenta does not possess a 16α-hydroxylase, oestriol secretion reflects the formation of 16α-hydroxylated androgen precursor from the foetal adrenal cortex. A portion of Δ^5-androgen secretion by the foetal adrenal cortex represents the conversion of 5-pregnenolone reaching the foetus from the placental secretion (Fig. 4.187).

Functions of the placenta

In the foetus, the respiratory system and the digestive system are not functioning. The kidneys and liver are also just having their functions

gradually. The functions of these systems and organs, as well as certain other important functions are carried out by placenta. The functions are summarised below:

1. **Nutritive.** All the nutritive elements from the maternal blood passes into the foetus through the placenta. It is generally assumed that substances of low molecular weight and dialysable, pass simply through diffusion. Placenta is permeable to glucose in both the direction. Placenta can store considerable amount of glycogen and lipid which are essential in case of emergency of the foetus. Besides these, placental tissue can form fructose from glucose. Fructose can pass from the mother to the foetus but not from the foetus to the mother (Huggett et al, 1951).

2. **Excretory.** The foetal metabolites partly diffuse into maternal blood through placenta and are excreted by the mother.

3. **Respiratory.** The placenta contains numerous blood sinuses and the chorionic villi remain in these sinuses. Oxygen passes from the maternal to the foetal blood, while CO_2 passes in the reverse direction (Fig. 4.184). The chorionic villi have got thicker and less permeable cellular layers and hence, rapid gaseous interchange is difficult. Moreover, the maternal blood in the placenta has a relatively low O_2 pressure. This also makes oxygenation of foetal blood more difficult. But in spite of it, adequate oxygenation takes place, as the *foetal haemoglobin has the property of taking*

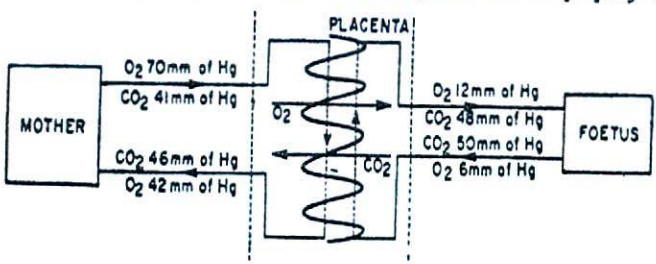


Fig. 4.184. Diagrammatic representation of gaseous interchanges in the placenta.

up oxygen at relatively low oxygen pressure and its oxygen dissociation curve shows a marked deviation to the left. It is 70% saturated at 20 mm and 90% saturated at 40 mm O_2 pressure. The arterial blood (in cow) is about 90% saturated with O_2 at an O_2 pressure of about 70 mm of Hg. The venous blood is about 70% saturated at an O_2 pressure of about 42 mm of Hg. In the foetus O_2 pressure of the blood in the umbilical artery is about 6 mm of Hg and O_2 pressure of the blood in the umbilical vein is about 12 mm of Hg. The arterial blood of the foetus is 50% saturated at an O_2 pressure of about 12 mm of Hg. The CO_2 pressure in the arterial blood of the mother is about 41 mm of Hg and in the venous blood is about 46 mm of Hg. In foetal arterial blood the CO_2 pressure is 50 mm of Hg and in the venous blood the CO_2 pressure in 48 mm of Hg.

Transfer of CO_2 is also carried out adequately, due to two reasons: (a) The pressure difference is enough. (b) CO_2, being several times more soluble and diffusible than O_2, can pass out more quickly than the latter.

4. **Storage.** Glycogen, fat, inorganic ion, etc.

5. **Endocrine functions.** Placenta secretes the following hormones:

(a) **Chorionic gonadotrophin** (CG) or **placental gonadotrophin** or **human chorionic gonadotrophin** (HCG). This non-pituitary gonadotrophin has also been named **pregnancy urine extract** (PUE), **chorionic luteotrophin, anterior pituitary-like hormone** (APL). But the last term is misleading since its action differs from that of the pituitary gonadotrophins. CG is found in the plasma and urine of the pregnant human female and also in that of individuals with certain gonadal tumours (chorionepithelioma, hydatidiform mole, teratoma). Gonadotrophin from gonadal tumour may not be similar with CG.

The HCG is a glycoprotein with a high content of sialic acid and other carbohydrate residues. Its mol. wt. is about 30,000 (according to some, 100,000).

Soon after implantation of the trophoblast, the growth of chorion cells leads to the secretion of protein hormone chorionic gonadotrophin. The placental gonadotrophin in human is called **human chorionic gonadotrophin.** This hormone stimulates the ovary and, along with pituitary hormones and other factors, maintains oestrogens and progesterone secretion and aids in the maintenance of the corpus luteum of pregnancy. Actions of the HCG are primarily luteinising and luteotrophic, but it has a little FSH activity. By about the end of first trimester the placenta provides necessary oestrogens and progesterones for the maintenance of pregnancy. The presence of CG in plasma and urine can be detected by radio-immunoassay as easy as 8-10th day of the first missed period, i.e., 22-24th day of pregnancy. The role of HCG in pregnancy seems to be confined to its transient stimulating effect on the corpus luteum. Ovariectomy before the 3rd month leads to abortion or miscarriage but after the third mouth has no effect on the pregnancy.

The **international unit** of CG is defined as the activity contained in 0·1 mg of standard preparation.

CG may be **assayed** by the induction of ovarian hyperaemia in the rat, expulsion of sperm in Amphibia, increase in uterine weight, development of an oestrous smear, increase in prostatic weight, and increase in seminal vesicle weight.

EVIDENCE: (i) Excreted in the urine during pregnancy. Appears in the urine from 8-10th day after the last missed period, i.e., when the conception is less than one month old. Maximum is reached between 50-60 days, then it gradually falls up to 80 days as the cytotrophoblast cells of the placenta become less numerous, after which it remains steady. The time of decreasing synthesis of the CG represents a period of full functional capacity for the synthesis of progesterone and oestrogens by the placenta, and a decreasing importance of the corpus luteum for steroid synthesis in human pregnancy. CG again falls towards the end of pregnancy and almost disappears a few days before the onset of parturition. If portions of placenta be retained, it persists in the urine during puerperium.* (ii) Disappears after placenta is expelled. (iii) If the foetus dies excretion ceases showing that secreted by the foetal chorion. Certain pregnancy tests depend on the presence of this hormone in the urine of pregnant women. Function differs according to the species. In women, its function

*In certain other pathological condition, viz., *chorion epithelioma* and *hydatidiform mole* in the females, this gonadotrophin is excreted in the urine.

is to help further growth brought about by nitrogen, postassium and calcium retention, and persistence of corpus luteum just like LH In the rodents, it stimulates all the ovarian processes, viz., growth of follicles, ovulation and corpus luteum formation. In the hypophysectomised rodents it only causes luteinisation.

Pregnant mare's serum (PMS). Another non-pituitary gonadotrophin has been demonstrated in the serum of pregnant mare and has been named the **pregnant mare's serum hormone (PMS).** PMS is secreted by the endometrial cups of the uterus. It is not found in the urine due to its large molecular size. It is a glycoprotein.

PMS behaves physiologically as if it were a mixture of potent FSH and a small amount of LH. PMS has potent follicle-stimulating activity. It is largely effective in the experimental and farm animals in inducing oestrus and ovulation. In the male, it maintains tubular function and induces hyperplasia of accessory sexual organs. Although PMS exhibits marked luteinising and follicle-stimulating activity in the normal woman and stimulates growth of the infantile male and female gonads, the results obtained by injecting it in human beings are not well established. However, in a general way, PMS acts like gonadotrophin in the human being.

(b) **Chorionic 'growth hormone prolactin' (CGP)** or **human chorionic somatomammotrophin (HCS)** or **human placental lactogen (HPL).** This hormone is secreted in increasing quantities by the syncytiotrophoblast cells from the first few weeks of pregnancy until term. HCS has no resemblance to HCG. HCS does not contain carbohydrate residues, being a simple protein chain (mol. wt. 18,500) with notable structural and physicochemical similarity to HGH, although it is a slightly larger molecule and is more acidic. There is also some similarity in the sequence of the 1st few amino acids from N terminal. HCS has no biologically significant growth activity but cross-reacts with antibodies to human growth hormone. It possesses significant prolactin-like activity in certain animal systems and causes impaired carbohydrate tolerance in animals with reduced insulin secretory capacity. HCS secretion rises significantly as pregnancy proceeds. Although its real function is not clear, yet it is believed to promote energy supply to the foetus by providing FFA for maternal metabolism and spearing glucose and protein for utilisation by the foetus. HCS stimulates the lobulo-alveolar growth of the mammary gland.

Other metabolic effects include enhancement of nitrogen and calcium retention (despite increased urinary calcium excretion), reduction in the urinary excretion of phosphorus and potassium, and an increase in the turnover of hydroxyproline, as reflected by increased urinary excretion of that amino acid.

Secretion-rate of HCS depicts an index of placental function and may be useful in the management of toxaemic and other pregnancies. A correlation takes place between placental mass and HCS level in plasma. Placental size may determine the quantity of HCS produced during pregnancy. There is insignificant transfer of HCS in foetal circulation. Very high level of HCS occurs in blood during late pregnancy. Although HCS possesses relatively small intrinsic growth hormone-like activity, this high plasma level may act indirectly as a GH for the foetus by sparing glucose utilisation in the mother. Since the foetus is actively dependent

on glucose for metabolism, HCS-induced insulin resistance and mobilisation of FFA for maternal utilisation will provide glucose to the foetus during the period of maximum growth in late pregnancy.

(c) **Oestrogens.** Placenta secretes oestrogens *(vide* p. 4-277*)*. As a matter of fact during pregnancy, oestrogen secretion takes place chiefly—probably only—in the placenta. Because, bilateral ovariectomy does not affect oestrogen excretion, but removal of placenta diminishes it considerably. This steroidal hormone is secreted from the syncytial trophoblastic layer of the chorionic villi.

(d) **Progesterone.** Placenta also secretes progesterone *(vide* p.4-277*)*. It is also secreted from the syncytial trophoblastic layer of the chorionic villi. In the later months of pregnancy, bilateral ovariectomy neither causes miscarriage or abortion nor any fall of progesterone excretion in the utine. But after removal of placenta, it is not found in the urine any more.

(e) **Chorionic thyrotrophin.** Recent works suggests that the placenta secretes a thyroid-stimulating factor which may account for the increased thyroid-stimulating activity of the sera of pregnant women and of patients with tumours of chorionic tissue. Its structure and physiological role are uncertain.

(f) **Relaxin** or **uterine-relaxing factor (URF).**. This hormone helps in relaxation of the ligament of the symphisis pubis and is secreted from the placenta, uteri and also from ovaries (p. 4-257). The cells of the placenta, which are responsible for secretion of this hormone are not yet clear. It is polypeptide and highly water-soluble with mol. wt. 9000. It can be inactivated by treatment with proteolytic enzymes or by reagents which convert (by reduction) disulphide linkages to sulphydryl groups.

In addition to this, placenta in some unknown way, *inhibits lactation.* It is claimed that high titers of oestrogen and progesterone during pregnancy inhibit the secretion of milk. Because milk secretion is increased after parturition when the titers of oestrogen and progesterone become low.

Growth hormone, ACTH and MSH have also been detected in the placenta. It is not known if they are produced or stored in the organ.

The placenta may also secrete renin.

Thus, with the help of its hormones, placenta—(a) stimulates the growth of mammary glands, (b) inhibits lactation, (c) stimulates the growth and persistence of corpus luteum, (d) inhibits ovulation, (e) controls anterior pituitary, (f) stimulates thyroid and adrenal cortex (*via* anterior pituitary), (g) stimulates growth of uterus and placenta itself, etc. In other words, all the important changes during pregnancy are carried out with the help of placenta.

HORMONAL REGULATION IN PREGNANCY

All of the uterine changes of the postovulatory phase of the menstrual cycle can be regarded as preparation for the participation of hormones in pregnancy before fertilisation and the implantation of the fertilised ovum. If fertilisation does not occur and there is a decrease in the production of oestrogens and progesterone by the corpus luteum, the appearance of endometrial shedding and menstrual bleeding occurs. If fertilisation and endometrial implantation of the fertilised ovum occurs there is no drop in production of sex steroid hormones by the corpus luteum

and both progesterone and oestrogen continue to exert their effects on the gravid uterus.

In some species, including the human, the corpus luteum provides oestrogen and progesterone during early pregnancy but later the placenta takes over the function of producing them. In other species, oestrogen and progesterone are produced by the corpus luteum which remains critically functional throughout pregnancy. Though the corpus luteum persists due to stimulation of placental gonadotrophin (CG or HCG), it is not essential for the successful continuation of pregnancy after about the 3rd month of gestation.

The presistence of functioning corpus luteum in the earliest pregnancy occurs because the chorionic tissue beings to produce CG at least as early as two weeks after ovulation. Thus, the corpus luteum is sustained although pituitary trophic influences are removed. The appearance of CG in the urine is the important basis of pregnancy tests, The CG, having same biological effect as pituitary LH, is capable of stimulating ovaries or the testicular Leydig cells to produce their respective steroids in sexually immature animals.

When the growth and differentiation of the placenta takes place, the placenta begins to make more and more oestrogen and progesterone and when the output of oestrogen and progesterone mounts, the output of CG diminishes. Both oestrogen and progesterone take part in the continuing structural growth of the uterus and mammary glands in pregnancy. Oestrogen, which acts as a specific growth hormone for uterine smooth muscle cells, stimulates the growth of the uterine muscle mass and thus contributes to the contractile force that will ultimately be needed to drive out the foetus at the time of delivery. Progesterone which exerts its inhibiting effect on the uterine smooth muscle, prevents the establishment of effective, co-ordinated contraction of the uterine muscle and insures the persistence of feeble, ineffectual, fibrillatory contraction until appropriate information for the expulsion of the foetus is given.

Progesterone in association with oestrogen, assists to prepare mammary glands for lactation by stimulating the formation of new glandular elements. The mechanism of suppression of lactation during pregnancy is not clear. Prolactin may be inhibited at its source or its effect may be blocked at the tissue level.

The placenta is a whole endocrine system in one tissue package in particular and it appears to combine the biochemical capability of the pituitary, the ovaries, the corpus luteum, the adrenal cortex and the other structures. There is no reason to doubt that the placenta produces CG, oestrogen (oestriol in the human) and smaller amounts of progesterone. Adrenal cortical steroid hormones may be produced in small quantities, so the placental cells may have at least vestigial enzyme systems for 11- and 21-hydroxylation. From the placental tissue of the human, trace amounts of ACTH, growth hormone, lactogenic hormone, androgens, vasopressin, and relaxin have been extracted. Of course, androgens could simply be metabolic intermediates in the synthesis of oestrogens, and others are all protein or peptide in nature or at least some of them might be artifacts of preparation. There is also the possibility that traces of hormones found in the placenta could simply represent elements present in maternal blood. However, the presence in the same tissue of a trophic hor-

mone (CG) and steroid hormones suggests that trophic hormone acts locally to stimulate the production of steroids. According to Velardo CG is made by one cell type in the placenta (cytotrophoblast) while the steroid hormones are produced by an adjacent cell type (syncytial trophoblast) and these adjacent cells have a relationship similar to that of a gonadotrophin-producing anterior pituitary cell and a corpus luteum cell.

Indirect evidence is that steroid hormones which are produced by the placenta, act locally within the uterus. The birth of twins at different periods from two horns of a bicornuate uterus may suggest a local action of progesterone. According to Csapo's view, the membrane potential of interplacental sites can be raised to the level of that of placental sites by local application of progesterone.

The mechanisms which are involved in parturition are not clear and stimuli which initiate the train of events *(labour)* are not fully understood. Just before the beginning of labour both progesterone and oestrogen levels fall, the *progesterone fall occurring before that of oestrogen.* There is an ageing process which occurs in the placenta and its impaired vascularisation is the cause of its diminished steroid hormone output at term. The decrease in availability of progesterone may be:

(i) The withdrawal of an inhibitory effect of progesterone on the oestrogen-primed uterine muscle causes development of co-ordinated squeezing movements of the uterus by oxytocin in aiding the expulsion of the foetus and placenta.

(ii) The withdrawal of the depressing effect of progesterone on prolactin release by the pituitary is essential for the initiation of the process of lactation in the mammary glands which have already been prepared for this event by the compensatory actions of progesterone and oestrogen.

During pregnancy, thyroxine and cortisol are produced in larger amounts than normal, and their concentration in blood gradually increases up to the period of parturition. In both cases, specific hormone-

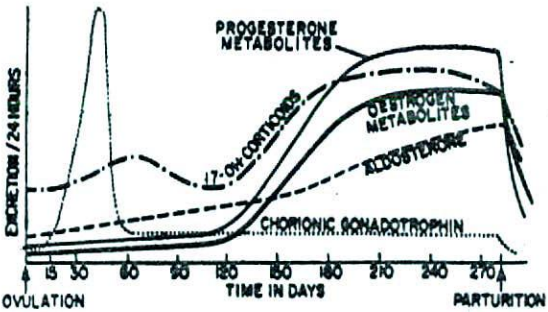


Fig. 4.185. Graphical representation shows urinary outputs of hormones during pregnancy.

carrying globulins, TBG and CBG (page 4-272), appear to be synthesised quickly in the pregnant women and they accumulate in blood. In the case of thyroid hormone, the level of activity of the thyroid gland itself is high because the uptake of tracer does of ^{131}I by the gland is elevated. Cortisol production is also increased in the pregnant women because the rate of

urinary excretion of 17-hydroxycorticoids rises. They may simply be sequelae of some modifications that occur in the rate of globulin production by the liver which is inundated with great amounts of oestrogen and progesterone for disposal in one way or another.

In the latter part of pregnancy, aldosterone excretion in the urine is significantly elevated. It is not impossible that the intra-abdominal pressure and the consequent inferior vena caval compression may set up a volume receptor *call* for aldosterone. A summary of excretion patterns of the hormones in pregnancy is given in Fig. 4.185.

Summary: (1) Changes in the uterus are controlled by oestrogens and progesterone. (2) Activity of the corpus luteum is controlled by chorionic gonadotrophin and hence oestrogens (and uterine contents later in pregnancy). (3) Activity of the ovary is controlled by the anterior pituitary. In pregnancy ovulation is inhibited. (4) Activity of the anterior pituitary affects uterine contents by an unknown mechanism. (5) The pituitary gonadotrophin secretion increases until just before parturition.

PARTURITION

Parturition is achieved by the effects of uterine muscle, assisted in the later stages by all the skeletal muscles which may be used to raise the intra-abdominal pressure.

Properties of uterine muscle

1. This muscle contains both actin and myosin and although slower and less strong, the basic mechanism of contraction is almost same as in the skeletal muscle.
2. During pregnancy, actomyosin content of the upper region of the uterus becomes double. This is hormonally stimulated since in aanimals with bicornuate nuclei with a pregnancy in only one horn, changes are the same in both horn.
3. In an ovariectomised animal, oestrogens increase the actomyosin content of uterine muscle but progesterone has no effect.
4. If an ovariectomised animal is treated with oessrogens alone, an electrically-stimulated excised strip behaves as follows: (a) a series of shocks give progressiaely increasing responses until a plateau is reached (positive staircase), (b) increasing the frequency raises the plateau, (c) oxytocin causes a chain of action potentials and contraction, (e) the resting membrane potential is —40-50 mV, (f) local electrical stimulation leads to contraction of the whole strip.
5. If an ovariectomised animal is treated with both oestrogen and progesterone behaves as follows (a) a series of stimuli gives progrewsively diminishing responses until a plateau is reached, (b) increasing the frequency lowers the plateau, (c) local electrical stimulation leads to a contraction confined to the part stimulated, (d) oxytocin fails to cause a contraction, (e) the resting membrane potential is —50-60 mV.
6. The cause of these differences is not certain but possibly has an ionic basis. Calcium and potassium-free solutions have little effect on progesterone-dominated uterus, but convert an oestrogen-dominated uterus into one of the progesterone type.

Towards the end of pregnancy, the following factors aid expulsion of the foetus:

1. Oestrogen raises the content of uterine actomyosin.

2. An increasing stretch tends to make the uterus contract like all smooth muscle.

3. Oxytocin tends to cause contraction of the uterus.

4. Relaxin softens birth canal and relaxes the ligament. Either stretch or oxytocin is not important for uterine contraction. In bicornuate uteri, contraction occurs even in the empty horn. Contraction may also occur in hypophysectomised animals and humans.

Progesterone may be the only factor which opposes uterine contraction.

EVIDENCE: 1. Progesterone output by the placenta falls converting progesterone-dominated uterus into an oestrogen-dominated uterus.

2. Probably stimulated by stretch, contraction of the uterus begins.

3. Weak contraction causes oxytocin release from the posterior pituitary and its strength increases. The foetus is gradually pushed towards the cervix.

4. Sensory stimulation of cervical dilatation further increases output of oxytocin.

5. As soon as the cervix is fully dialted, the woman feels an irresistible urge to use her skeletal muscle in the Valsalva manoeuvre and to push the baby out.

In certain animals, progesterone output falls rapidly immediately before the contraction of the uterus begins. In other animals and in humans, the fall in progesterone output is not fully clear.

Overall processes of parturition is summaried below:

Parturition takes place, normally, at about 280 days from the beginning of the last menstrual period and results in **child-birth.** It is brought about by the periodic contraction of smooth muscles of uterine wall, aided by contractions of skeletal muscles of abdominal wall. Various investigations into the causes of the onset of parturition at the end of ninth month and into the process of labour have been carried out. At present, however, the mechanisms involved in the process of *labour* is complex and not yet fully clear. It is not the phenomena of a single factor but of multiple factors are expected to play synchronously for smooth delivery of the baby. During gestation the placenta remains in a quiescent state but as the labour approaches, the uterine myometrium becomes irritable and excitable. It is claimed that at the end of gestation, the contractile protein (the actin and myosin) concentrations of the uterine muscles are increased greatly and help in forceful contraction during labour.

Hormonal factors which play as key roles in determining the onset of labour are the hormones elaborated from the ovaries and placenta. It is claimed that levels of progesterone and oestrogen in the blood as well as in the uterine muscles actually determine the onset of labour. Oestrogen stimulates the uterine contraction whereas progesterone inhibits it; and pregnancy is maintained by the dominant action of progesterone. It is due to **progesterone block** which maintains pregnancy and as soon as progesterone level is decreased during the end of gestation, labour starts. It has been further reported that during pregnancy the placenta secretes progesterone directly into the uterine tissue rather than to the general circulation. This effect tends to increase the progesterone concentration in the uterine myometrium so as to inhibit contraction.

During the onset of labour oestrogen level in the blood is increased and progesterone level is decreased. Oxytocin secreted from the posterior pituitary then brings about vigorous contraction of the uterus. Oestrogen level in the blood and uterine tissue, potentiates the oxytocin effect. **Relaxin** secreted from the ovaries and placenta enlarges the birth canal by relaxing the pubic ligments. The foetus is moved towards the cervix by the vigorous contraction of uterus, and stimuli from the cervix reflexly secrete oxytocin *(Ferguson reflex)*. Distention of the cervix and vagina stimulates the hypothalamus for the liberation of oxytocin from the posterior pituitary. So with the proper hormonal balance and timing, the smooth parturition takes place. Factors involving in the process of parturition have been presented schematically in Fig. 4.186.

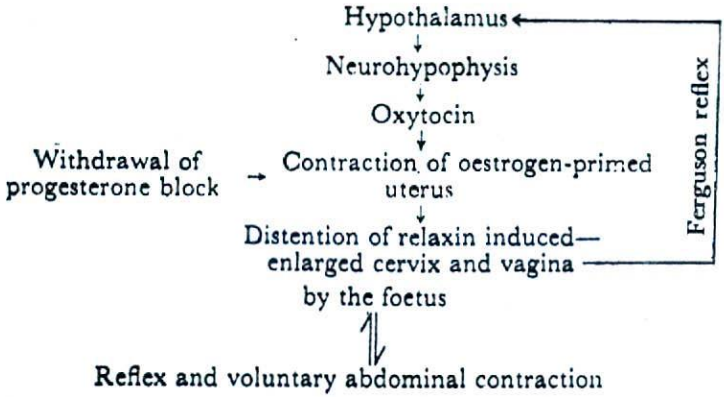


Fig. 4.186. Hypothetic scheme describing the factors involved in parturition.

Corticosteroid levels and local formation of angiotensin by the action of uterine renin may initiate the onset of labour, although there is no convincing evidence for either of these hypotheses, and the level of angiotensin II in amniotic fluid remains unchanged during the onset of labour.

Plasma leevl of certain prostaglandins rises during labour (p. 4-184) and may have some possible role in parturition.

Duration of labour is variable. However, the average length of time is about 12-18 hours. During **first stage** or **stage of dilatation** the cervix of uterus is dilated and as a rule the amnion is ruptured and the amniotic fluid is expelled. During **second stage** or **stage of descent** the child descends through the vagina and is expelled. During **third stage** or **placental stage,** the membranes of the foetus are expelled.

Involution. It is the process of rapid decrease in the size of the uterus. It is brought about by a gradual autolysis or self-digestion of the uterine wall and requires from 6-8 weeks. During this perod, the uterus again resumes its original position in the pelvic cavity and *approximately* its original size.

Twins and Multiple Births

According to the nature of origin, twins may be divided into two distinctly separate classes or groups which as follows: (1) *monozygotic* or *uniovular* (identical) and (2) *dizygotic* or *biovular* (fraternal).

1. **Monozygotic twins.** They occur from a single ovum and are fertilised by a single sperm. At the early stages of the development of the daughter cell following fertilisation up to the establishment of the axis and primitive streak, the zygotic material may be divided into two complete halves which give rise to two separate embryos. The chorionic and amniotic sacs may be either separate or common for both the embryos. In this case the twins are always of the same sex, i.e., either two male babies or two female babies having same blood groups and tissues of same antigenic potencies. So they are named as **identical twins.** Conjoined twins or double monsters are originated from incomplete division of the unizygote.

2. **Dizygotic twins.** These types of twins are developed from discharge of two ova at a time and fertilised by separate sperms. The embryo develops in separate chorionic sac in most cases. They may be of either same or opposite sexes having separate antigenic constitution. They have got no more similarity (or resemblance) to each other than do other children of the family. The type of twining may be hereditary.

Twining takes place once in about seventy-five births and about two-thirds of them are of dizygotic origin.

Multiple births, more than twins, e.g., triplets, quadruplets or quintuplets, may occur from any of the above origins or from a combination of both of them.

INTERRELATION OF STEROID HORMONE BIOSYNTHESIS AND METABOLISM IN THE FOETUS AND PLACENTA

During pregnancy the steroid-secretion patterns differ from species to species, and the human foetoplacental unit displays unique features of steroid metabolism and interconversion (Fig. 4.187). Both foetus and placenta are complementary to each

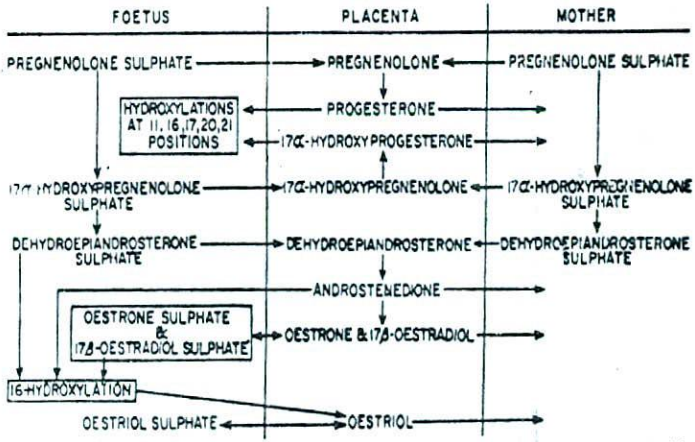


Fig. 4.187. Schematic representation of the foetoplacental unit in steroid biosynthesis and metabolism.

other in the biosynthesis and metabolism of progesterone, oestrogens, androgens and corticosteroids. So the foetus and the placenta possess incomplete pathways for steroid biosynthesis.

The human placenta synthesises large quantities of progesterone from maternal acetate or cholesterol (transferring about 70-75 mg of progesterone per day to the foetus), while the rest is metabolised by maternal tissues. The placenta supplies preg-

pregnenolone to the foetus and transfers steroids, viz., DHEA (dehydroepiandrosterone) from the maternal adrenal gland. Foetal tissues metabolises massive quantities of progesterone either to inactive derivatives or to steroids which are produced by foetal adrenal glands and testis. The foetal adrenal glands convert pregnenolone to Δ^5-androgens. These androgens again pass back to the placenta and possess major precursor for oestrogen formation during pregnancy.

In the first half of gestation, the human foetal adrenal has a deficiency of 3β-hydroxy steroid dehydrogenase and Δ^5, Δ^5-isomerase. The deficiency or inhibition of these enzymes brings about the secretion of Δ^5-androgens such as DHEA. Large quantities of androgens produced by foetal adrenal cortex represents a function of the foetal zone (a *pituitary-dependent hypertrophic inner cell layer)* to form androgens from 5-pregnenolone. The foetal adrenal gland is capable of *de novo* synthesis of5 -pregnenolone as well as being able to convert 5-pregnenolone from the placenta. The foetal adrenal is dependent on the placenta for progesterone for the synthesis of adrenal steroids. Large quantities of 5-pregnenolone are converted to androgen precursors, viz., DHEA sulphate in foetal adrenal and some of DHEA sulphate is hydroxylated in the foetal liver forming 16α-hydroxy-DHEA sulphate.

The placenta contains a very active sulphatase enzyme system. The placental hydrolysis of circulating androgen sulphates is followed by rapid aromatisation. Thus in the placenta Δ^5-sulphates are hydrolysed, converted to Δ^4-androgens and are rapidly aromatised to form oestradiol and oestriol. Large amounts of oestradiol which are formed from DHEA by the placenta, are extensively metabolised by the foetus, mainly by 16-hydroxylation to oestriol and by a 15-hydroxylation pathway. Most of the maternal excreted oestriol is formed from metabolism of oestradiol by foetal tissues and from 16-OH DHEA originating in foetal adrenal cortex.

Progesterone secreted by the placenta into the foetal circulation is extensively metabolised by foetal adrenals, liver and other tissues. In the foetal adrenals, progesterone can be converted into 6β-, 16α-, 17α-, and 20α-hydroxyprogesterone.

The foetal adrenal is capable of synthesising glucocorticoids using the progesterone from the placenta as precursor. These glucocorticoids include corticosterone, deoxycorticosterone, 11-dehydrocorticosterone, and 17α-hydroxycorticosterone. These are secreted both as free compounds and sulphate esters.

The foetal liver metabolises progesterone actively, forming 20α-dihydroprogesterone and pregnanediol. In the foetal liver, progesterone is conjugated to form glucuronides and sulphates. Other tissues of the foetus can also metabolise progesterone actively, forming 20α-dihydroprogesterone and certain derivatives. The placenta can reconvert 20α-hydroxyprogesterone back to progesterone, thus conserving progesterone, providing a mechanism whereby the steroid is recovered after passage through the foetus. This process probably allows high progesterone levels in the maternal tissues, with lower levels of the active steroid in the foetus. The foetus can also metabolise large amounts of oestrogen formed in the placenta substantially, by hydroxylation to inactive derivatives and sulphation to enhance H_2O solubility and excretion.

Even by midpregnancy the adrenal cortex of foetus still utilises progesterone from the placenta for the most part for the synthesis of mineralocorticoids such as deoxycorticosterone and aldosterone, and glucocorticoids such as corticosterone. The ready availability of progesterone from the placenta may by-pass necessities for *de novo* synthesis of glucocorticoids. Mineralocorticoids and glucocorticoids are available to the foetus from the maternal adrenal glands.

In late pregnancy, the foetal adrenal cortex can show an increased ability to form progesterone from 5-pregnenolone and an ability for *de novo* synthesis of aldosterone and glucocorticoids. As the neonate must possess a self-sufficient mechanism for the production of mineralocorticoids and glucocorticoids in order to survive. this ability is developed in the foetal adrenal from the fact.

FOETAL ENDOCRINE SYSTEM

Development of certain endocrine glands of the foetus has been briefly described. All endocrine organs of the foetus are well developed by the end of first trimester and at this time the secretion of foetal pituitary GH (STH) and ACTH has already begun. The foetal adrenal and gonads secrete steroid hormones and precursors during embryonic life. Sex chromosomes of the foetus determine the development of the gonad into functional testis or ovary. On the other hand, genital development depends upon the presence or absence of androgens and an additional testicular inhibitor of Mullerian-duct system. The formation of foetal gonads occurs only when the primordial germ-cells

invade the mesodermal gonadal ridges which contain primitive sex cords. At first male and female gonads are similar and the germ cells possess the potential to form either sperm or ova. Within 6-8 weeks, gonad differentiates into testis or ovary, and determines the subsequent development of the genital tract from the co-existing Wolffian-duct and Mullerian-duct systems.

In male embryos, the proliferation of medullary part of the indifferent gonad occurs and the development of immature seminiferous tubules takes place from sex cords. The inferstitial cells of Leydig develop from interstitial mesenchymal cells of the early testis and become functional by the 8th week of development. These Leydig cells are active during early foetal life, but the component of germ cells of the testis remains quiescent until puberty. Androgen production reaches the peak during 4th month. The action of the human chorionic gonadotrophin may participate in this activity that may be dependent on the pituitary control.

In the male genitalia early steroidogenesis of the foetal testis may be an important stimulus to the development of the Wolffian-duct system. The testis of the foetus suppresses the Mullerian-duct system where testosterone is not involved. This inhibition is caused by an inducer or organiser produced by the testis and acting locally to suppress the adjacent Mullerian duct.

Features of foetal ovarian development are almost opposite to those of the testis and occur several weeks later. The development of the ovary from the cortical part of indifferent gonad occurs and the ovary is mostly active in the multiplication of germ cells with little steroidogenesis. During foetal life, full complement of female gametes is present, whereas in the male these do not develop until puberty. Since the differentiation of the Mullerian-duct system takes place in the internal genitalia spontaneously and the regression of the Wolffian-duct system occurs in absence of androgen, the development of female genital system is not dependent upon the presence of the gonad.

The development of external genitalia from a common structural primordium (anlage) occurs due to the potential for differentiation into either female or male genitals. Here again, the paramountcy of androgen influence determines the direction and extent of differentiation from the innate female modes to that of the male pattern. The deprivation of androgen makes the male foetus to develop female external as well as internal genitalia; and removal of the ovary does not influence the differentiation of female genitalia, but the administration of androgen causes varying degress of masculinisation of foetal female genitalia.

FOETAL CIRCULATION

Peculiarities in foetal circulation

1. In foetal life, the two ventricles, right and left, have got common function in circulating blood to the periphery. With the functional differentiation following birth, structural differentiation in relation to functions becomes inevitable. Such changes are observed not only in heart but also in other essential organs of the body. In foetal life, the thickness of the right and left ventricular wall is almost same but following birth as the pulmonary and systemic circulations are gradually separated by the fusion of the ductus arterious, the thickness of the left ventricular wall is gradually increased with the increased work load. All throughout the adult life the left ventricle has to maintain the systemic circulation (high resistant circuit) whereas the right ventricle has to maintain the pulmonary circulation (low resistant circuit).

2. In foetal life the respiratory function of the lungs is absent and the lungs remain as solid organ. For this reason major part of the blood, coming from the right ventricle, is shunted through patent ductus arteriosus to the descending aorta, and small amount of blood to the lung only for its nutrition. But following birth, as the respiratory function is gradually resumed, the circulatory status is changed. Ductus arteriosus is fused and anatomical dissociation in between the pulmonary and systemic

circulation thus occurs. So in foetal life, right and left hearts work in parallel but in adult life they work in series.

3. Placenta takes the important part in maintaining the respiration, excretion and nutrition of the foetus. So exchanges of nutrient materials,

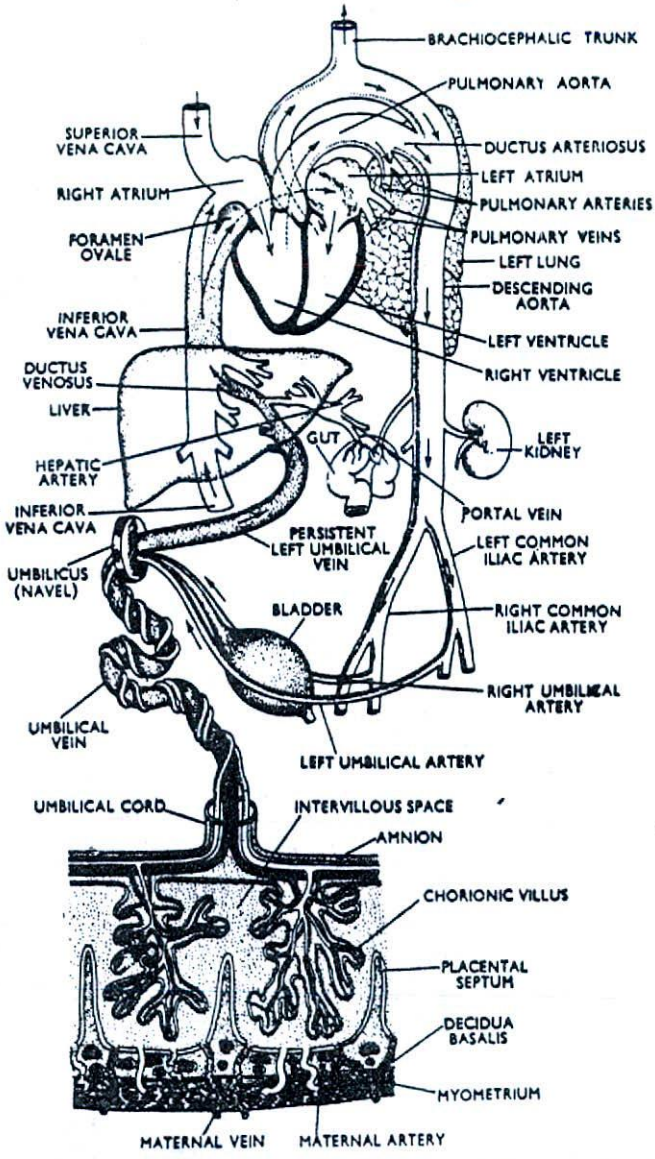


Fig. 4.188. Diagrammatic representation of the anatomical organisation of the foetal circulation including placenta (microscopic) showing blood vessels to run from and to the placenta.

gases and also of the waste products are taken place; and blood after loaded with O_2 and nutrient materials from the placenta returns to the foetus through the umbilical vein.

4. Unlike in adult life, the O_2 saturation in the arterial blood in foetal life is different from region to region and this is mostly related with the functional importance of the particular organs in comparison to the others. The vital organs, like brain and heart, get blood supply with higher saturation of O_2 (62%) wheras the periphery gets lower saturation of O_2 (58%).

5. Foetal haemoglobin has the higher holding capacity of O_2 than the adult haemoglobin. For this reason the O_2 dissociation curve of foetal haemoglobin is shifted more towards the left.

6. Through foramen ovale, connection between the inferior vena cava and left auricle is present in foetal heart only to avoid mixing with deoxygenated blood in the right auricle and also to supply well-oxygenated blood to the brain and heart. With the onset of respiration in the new-born baby, the circulatory pattern is abruptly changed and the foramen ovale is gradually fused against the interatrial septum.

7. In foetal heart capillary-fibre ratio is 1 : 5 whereas in adult heart it is 1 : 1.

8. Pulmonary veins carry deoxygenated blood, as the foetal lung behaves like a solid mass, gaseous exchange is out of question.

Course of circulation

It has been discussed earlier that the placenta is of paramount importance in foetus in maintaining different essential functions like respiration, excretion and nutrient exchange of the growing embryo. Left and right umbilical arteries supply foetal blood to the placenta and blood is returned from the placenta by two umbilical veins at early embryonic life and later it is returned only by the persistent left umbilical vein as the right one is fused afterwards. Umbilical impression (umbilicus) in adults is the position of the fused umbilical cord which makes blood-vascular link with the placenta in foetal life (Fig. 4.188).

Thus the blood loaded with O_2 and nutrient material leaves placenta by persistent left umbilical vein and ends in the liver. The umbilical vein carries blood, saturated with 80% O_2. Here, part of the blood is directly shunted to the inferior vena cava by ductus venosus and the rest is mixed with the portal blood. Inferior vena cava thus carries blood from the lower portion of the body, from the hepatic vein and also from ductus venosus. Here in the inferior vena cava, O_2 saturation of blood becomes 67% and major part of the blood is poured directly into the left auricle through foramen ovale, and smaller

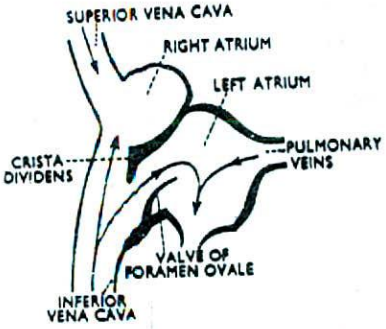


Fig. 4.189. Diagrammatic representation of the great veins in the foetus indicating the splitting of blood flow of inferior vena cava into two streams by the crista dividens of which one passing through the foramen ovale and other going into the right atrium.
(Modified after Dawes).

part to right auricle. By the edge of the interatrial septum—the crista dividens, the inferior vena caval blood splits into two streams, of which one goes to the left auricle through foramen ovale (discussed above) for avoiding further mixing with deoxygenated blood in right auricle and other goes to the right auricle (Figs. 4.189, 4.190 & 4.191). In the left auricle the blood losses its O_2 saturation further as it mixes with the venous blood from the lung which contains deoxygenated blood. From the left auricle blood enters the left ventricle and thence to the aorta. The ascending aorta actually carries blood, saturated with 62% O_2 and passes through brachiocephalic trunk and descending aorta. Thus brain and heart get blood, 62% saturated with O_2. But blood while passing through the descending aorta receives blood from the ductus arteriosus which carries deoxygenated blood (52% O_2) from the right ventricle. Right auricle receives blood from the lower part of the body and also from the upper part of the body. O_2 saturation of the blood in the right auricle becomes 52%. From here it is poured in the right ventricle and thence to the pulmonary arch of the aorta. Here, major portion (78%) of blood is shunted through ductus arteriosus to the descending aorta and lesser part (22%) is directed to lungs for its nutrition. Thus after mixing in the descending aorta, the O_2 saturation of the blood comes to 58% (Fig. 4.190). This blood while following the course through the abdominal aorta gives off branches to the neighbouring organs and returns to the placenta through two umbilical arteries.

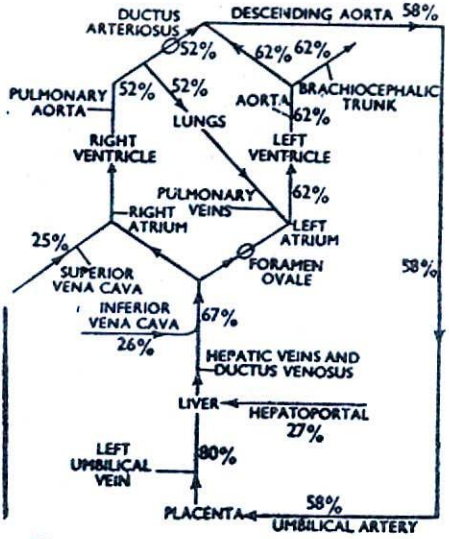


Fig. 4.190. Schematic representation of the mean percentage of oxygen saturation and distributing pattern of blood through the heart and blood vessels of the foetus. *(Modified after Dawes).*

In the placenta, the branches of the umbilical artery project like villi within the maternal sinus and gaseous exchange is taken place in between the foetal and maternal blood. O_2 is taken up by the foetal blood and CO_2 is discharged into the maternal sinus across the walls of the

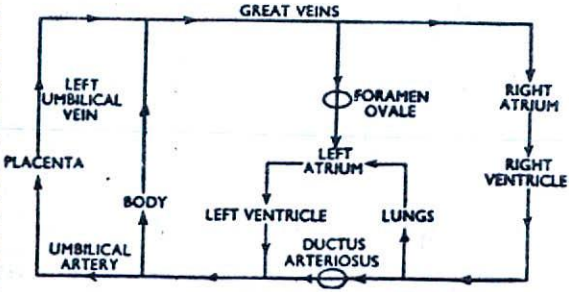


Fig. 4.191. Schematic representation of the foetal circulation. *(Modified after Dawes).*

villi. Nutritive materials for the foetus are also taken from the placenta and excretory products are discharged into the maternal sinus.

Of the total cardiac output of the two hearts, 57% is supplied to the placenta and foetal membranes, 10% to the lungs, 15% to the fore parts and 18% to the hind parts. Blood flow through foramen ovale and ductus arteriosus is also high and is 46% and 35% of the total cardiac output of both ventricles respectively.

CHALLANGES OF NEW EXISTENCE

The foetus in the uterus lives at a temperature of 33°C.—the temperature of the amniotic fluid. His lungs are solid. The pressure in the pulmonary artery is higher than the pressure in the aorta. In addition to this, the foetus arrives at a new world with myriads of sensory stimuli conveyed to its eyes, ears and skin. Adaptation to this new world requires a set of completely new physiological adjustments.

The process of physiological adjustment from the new-born infants to full-grown child is gradual and consists of changes especially in the mechanism of circulation, respiration, temperature regulation, functions of the kidney, intestine and liver.

A. Changes in circulation after birth

(a) CLOSURE OF THE FORAMEN OVALE

With tying of the umbilical cord, the inferior vena caval pressure falls and the systemic arterial pressure rises. Accordingly, the left atrial pressure exceeds the vena caval pressure because venous return from the pulmonary bed to the left auricle is increased tremendously within this period. Besides this, venous return through the inferior vena cava is curtailed greatly following tying. The valves of the foramen ovale is fused against the wall of the interatrial septum (Fig. 4.192).

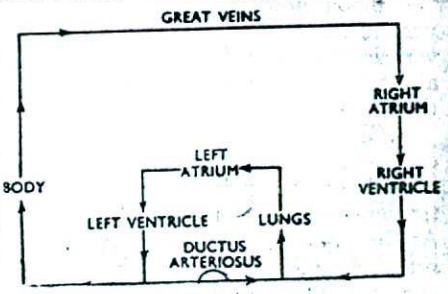


Fig. 4.192. Schematic representation of neonatal circulation after closure of foramen ovale and of still functioning ductus arteriosus. This occurs within a few minutes after birth. *(Modified after Dawes).*

(b) CLOSURE OF THE PATENT DUCTUS ARTERIOSUS

Within an hour or two, O_2 saturation of the blood is restored up to 90%. Ductus arteriosus is then gradually constricted with the increase of O_2 saturation of the blood and finally it is obliterated. Certain amines also have got relation with prompt obliteration of the ductus arteriosus (Fig. 4.192).

(c) CLOSURE OF THE DUCTUS VENOSUS

With the cessation of blood flow though the umbilical cord after birth, the ductus venosus is closed within an hour or three, because the pressure drop is such that cannot keep the vessel open. So functional closure is observed very sharply (Fig. 4.192). In some cases where the portal venous pressure is raised, this vessel may remain open.

(d) CHANGES IN THE CARDIAC MUSCLE AND ITS BLOOD VESSEL

In foetal life, thickness of the wall of the right and left ventricle is more or less same and even the total mass of the right ventricle exceeds that of the left by 25% at term (Hort, 1966). The number of fibres in the two ventricles is approximately same and is not changed with ages though the major changes are observed in length and thickness of the fibres.

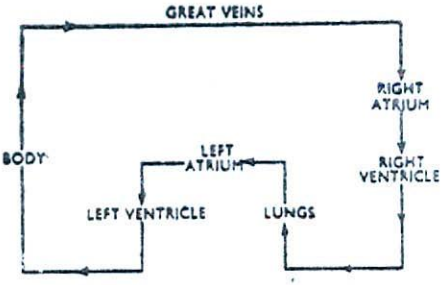


Fig. 4.193. Schematic representation of the adult circulation. *(Modified after Dawes).*

Just after birth, functional status of the two ventricles are changed abruptly. The work load in the left ventricle is increased whereas the same is decreased in the right ventricle. To cope with the increased work load, the left ventricular wall grows rapidly and this growth is related with the rapid growth of the infant and the increase in peripheral resistance. The growth of the right ventricle is decreased gradually. The mean diameter of the muscle fibres of the human right ventricle decreases from 5·6 to 5·1 μm while that of left ventricle rises from 4·5 to 6·0 μm over the first 6 weeks from birth (Boellaard, 1952). RNA concentration in the left ventricle is increased from first day of birth and there is little change in right ventricle.

Blood flow in the two ventricles are obviously changed. The left ventricular flow is increased greatly with simultaneous decrease in right ventricular blood flow. Capillary-fibre ratio in both ventricles is also altered. It becomes 1 : 1 in adult life.

(e) CIRCULATION

Circulatory changes are observed following occlusion of the umbilical cord and the cessation of umbilical blood flow. When the umbilical cord is tied, the peripheral vascular resistance of the foetal blood vessels is tremendously increased. Not only that, blood pressure is also increased. As the placenta is cut off from the foetus, the new-born baby begins to suffer from O_2 lack. CO_2 is accumulated in the blood and when the condition becomes severe, the baby begins to have first respiratory effort. The lungs are then inflated for the first time (Figs. 4.192 & 4.193).

B. Changes in the lungs and respiration

With the onset of respiration, pulmonary vascular resistance is greatly decreased with associated increase in pulmonary blood flow. These changes occur within a minute or two. With the fall of pulmonary resistance and along with the increase of resistance in the abdominal aorta direction of blood flow through the ductus arteriosus is changed. Blood begins to flow from the aorta towards the lungs through the patent ductus areteriosus (Fig. 4.192).

C. Temperature regulation

In an infant the body temperature is unstable especially during the first 48 hours. The function of the sweat glands and skin circulation do not develop immediately after birth to control the heat loss. In an adult severe cold is combated by increase of heat production by voluntary

exercise or by shivering. Infants when subjected to cold do not shiver. The metabolic rate in an infant is doubled or tripled in a different way. The new-born infants have a large storage of dark-yellow 'brown fat' widely distributed in the body of the infant with an abundance in between the scapulae. This fat is rich in blood and sympathetic adrenergic nerve supply with plenty of mitochondria. When an infant is exposed to cold, the body temperature rises by increased oxygen consumption, by the yellow 'brown fat'. The temperature in the fat rises and the heat is supplied to the rest of the body—non-shivering thermogenesis.

D. **Kidneys**

An infant immediately after birth is not capable of secreting urine whose osmolarity is more than twice that of plasma. This may be due to the hypofunction of tubular reabsorption or the absence of normal concentration gradient between cortex and medulla of the kidney. Infants are particularly capable of eliminating an excess of water.

E. **Intestine**

During the first week, neonates lose weight. This is due to inadequate water intake and poor quality of the maternal milk. Until breast feeding ceases, most infants utilise its own iron-store, built up during the last part of foetal life. It takes some time for the intestine to be cleared of meconium. The brown colour of the stool is an important clinical feature which indicates the patency of the elementary tract.

F. **Liver**

At birth the liver is rich in glycogen. Within 24 hours liver becomes depleted of glycogen storage and the level of blood glucose falls. The enzymes of glycogen breakdown are not fully active in the foetal liver.

Conjugation of bilirubin with glucuronic acid takes place in the liver. The conjugated bilirubin is water-soluble and is excreted with the bile, down the intestinal tract. The enzyme which is responsible for the conjugation of the bilirubin with glucuronic acid is very low in concentration during the first week of life.

Resistance to infection

γ-globulin and γ-macroglobulin are important in resistance to infection. The γ-globulin includes antibodies to many Gram-negative bacteria and viruses. γ-macroglobulin includes antibodies to Gram-positive bacteria and spirochaetes. γ-globulin can cross the placenta. γ-macroglobulin cannot cross the placenta. Concentration of γ-globulin falls between the birth and 2nd to 3rd month as the infant cannot effectively produce its own γ-globulin.

INBORN ERRORS OF METABOLISM IN THE NEW-BORN

Galactosaemia and phenylketonuria are the two examples which appear shortly after birth. These conditions are rare but when present their early detection is of great clinical importance.

Galactosaemia

In this condition there is a failure of utilisation of galactose. Lactose is the principal sugar in the milk. Lactose is converted into galactose by the enzyme lactate in the small intestine. The galactose is converted into

glucose in the river. The glucose is metabolised with the production of heat and energy. In galactosaemia galactose cannot be converted into glucose. Galactose accumulates in the blood producing diarrhoea and vomiting. Galactose appears in the urine and reduces Benedict's regent. If the child survives there will be liver damage, cataract and mental retardation. Galactose-free diet is essential for these babies. Milk should be excluded from the diet of the baby.

Phenylketonuria

In this condition phenylalanine cannot be converted to tyrosine (then to thyroxine), epinephrine and norepinephrine. Phenylalanine and phenylpyruvic acid accumulate in the blood producing toxic changes in the brain and causing mental deficiency. The phenylketone is found in the urine and giaes a bluish-green colour with ferric chloride. In such cases the diet of the baby must have a low and controlled level of phenylalanine.

HORMONES EXCRETED IN THE URINE

The following hormones are excreted in the urine::

I. **Sex hormones**

1. **Derivatives of testosterone**—*Androsterone,* 17-ketosteroids (17-KS) and *dehydroepiandrosterone* (pages 4-208, 4-211).

2. **Derivatiaes of oestrogens**—*Oestrone and oestriol* mainly as glucuronides or sulphates but slightly in *free uncombined from* (page 4-249). **During menstruation**—pages 4-249, 4-263. **During pregnancy**—pages 4-249, 4-283.

Both oestrogens and androgens are found in both male and female urines but none before the age of 6 years. Found even after castration—probably source adrenal cortex.

3. **Derivatives of progesterone**—*Pregnanediol*—as glucuronides (page 4-255). **During menstruation** (pages 4-255, 4-263). **During pregnancy** (pages 4-255, 4-283).

4. **Placental gonadotrophins** (pages 4-279, 4-280, 4-283).

II. **Adrenocorticoid hormones and Neutral 17-ketosteroids.** Neutral 17-ketosteroids are end products of sterol metabolism with a CO group in the 17 position. They are chiefly derivatives of androgens (androsterone) and similar substances derived both from the adrenal cortex and testes in males and only adrenal cortex in females. Hence in males, the excretion is higher than in females.

Excretion of 17-ketosteroids depends upon the degress of activity of adrenal cortex. In hyperactive conditions of adrenal cortex, viz., Cushing's disease and adrenal virilism —excretion rises. In hypoactive conditions, viz., Addison's disease, Simmonds' disease etc.,—it falls (pages 4-155—4-156).

III. **Pituitary hormones.** *(a) Antidiuretic hormone, (b) Somatotrophic hormone* and *(c) Thyrotrophic hormone* are excreted in the urine. The excretion of thyrotrophic hormones increases after thyroidectomy.

IV. **Thyroid hormones.** In hyperthyroidism with increased secretion of thyroid hormones, excess hormones are excreted through the kidney and also through monther's milk (page 4-75).

DEVELOPMENT OF BREAST AND LACTATION

Histology (Fig. 4.194). Breast is a cutaneous structure. It develops as an invagination of the surface epithelium into the underlying connective tissue as solid columns of cells. Later on, the cell columns become hollowed out into ducts. These ducts branch out into *terminal tubules* which end in *alveoli* that secrete milk. Around the ducts and to a lesser extent the alveoli remain modified muscle fibres, called **myoepithelium.** They are elongated and longitudinally striated cells. Their contraction helps to press out milk. The mammary gland on each side consists of 15-20 lobes separated by connective tissue septa. In the actively secreting gland the number and size of alveoli increase and the secreting cells remain filled up with minute fat droplets. The secretion is discharged

by rupture of the free ends of the cells, leaving the nucleus and basal cytoplasm intact. Regeneration of these cells quickly takes place. In the resting non-lactating gland, these features are not seen. At menopause, the breasts involute, the alveoli shrink, and the gland atrophies.

Therefore, the mammary gland in its functional state is a **compound tubulo-alveolar gland.** The larger ducts open at the nipple where a stratified squamous epithelium is continuous with two-layered columnar epithelium of the primary duct. *Larger ducts* are lined by an inner tall columnar layer and an outer cuboidal layer of epithelial cells. *Smaller*

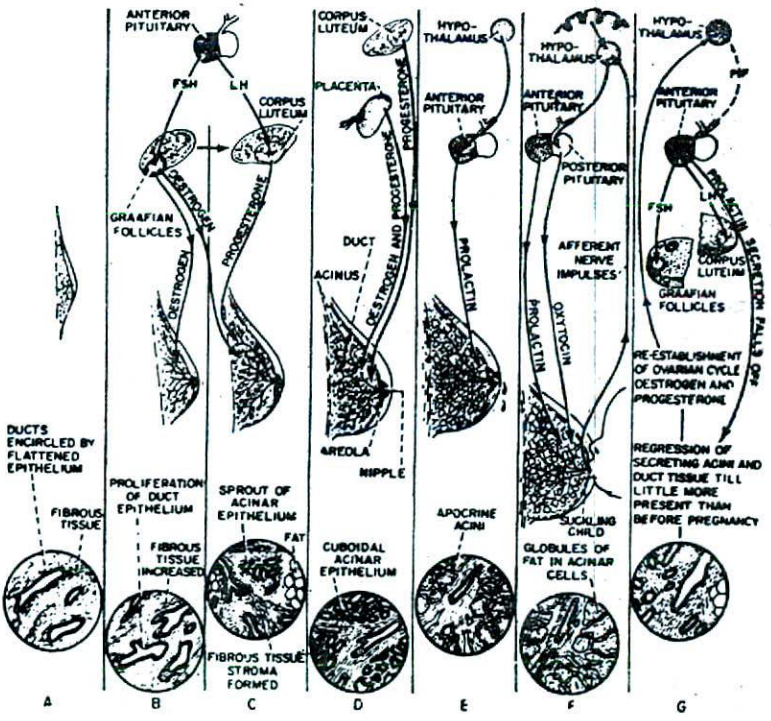


Fig. 4.194. Mammary glands at different phases of life. A = Small and undeveloped glands in childhood, B = In girls at puberty; C = In maturity when ovarian hormonal cycle established and ova being shed regularly; D = Breast in pregnancy; E = Breast gland after child-birth; F = Lactating breast; G = Post = lactation breast gland.

ducts have a single layer of columnar epithelial cells. The *smallest ducts* and *alveoli* contain irregularly depressed deep **myoepithelial cells (basket cells)** near the basement membrane. Height of the cells of the ducts and alveoli depends upon the functional state of gland and their relative distension. A clear basement membrane surrounds all the epithelial components. The basement membrane in turn is surrounded by a loose stroma, especially about the smallest intralobular ducts and alveoli. The intralobular stroma contrasts with the interlobular stroma. Stroma of the breast develops at the same time as the parenchyma

of the gland. *Fatty tobules* into which ducts project and in which lobules develop, contribute the looser intralobular stroma and more compact interlobular stroma. In some species, mammary epithelium will glow only in a fatty stroma.

Lactating mammary glands produce milk. This milk contains unique *proteins, casein, lactoglobulin, lactalbumin, lactose* and a high concentration of *fat*. Proteins, sugars and fats are all produced by the same cells. Fats first appear in the secretory cells as small droplets at about the level of nucleus. These fat droplets enlarge as they become more apically located. Fat droplets are always enveloped by smooth membranes and even retain the membrane as these are pinched off from the apex of cells. Sometimes, fat is found in epithelial cells of the smaller ducts and alveoli of non-lactating breasts. Proteins first appear in Golgi area and are located in smooth membrane vacuoles which may be derived from Golgi membranes. Aggregates of electron-dense elements accumulate in these vacuoles which move towards the free surface of cells and their membranes apparently establish contact with and then continuity with the surface membrane. Thus the membranes extrude their contents *(reversed pinocytosis)*. But where lactose and milk salts are elaborated and how they are extruded is not known. *Milk secretion is not an apocrine type of ecretion.*

Studies of fine structure have also revealed microvilli on the luminal surface of secretory cells and desmosomes. The cellular membranes are only slightly interdigitated and in close approximation with the basement membrane except where myoepithelial cells intervene. Cytoplasm contains rather large mitochondria that differ in size with changes of secretory activity, less compactly arranged endoplasmic reticulum but much increased during lactation, an apically-located Golgi body which is more conspicuous during lactation and lysosomes. Lysosomes may contain *cathepsin* and in some species *ferritin* as well. Mitochondrial functions of breast tissue increase 7- to 9-fold in succinic dehydrogenase, and cytochromic oxidase when gland is secreting.

Myoepithelial cells of the lactating brest contain alkaline phosphatase. Each cell possesses an irregular nucleus. Myofibrils have been observed and actual contraction is seen when the cells have been exposed to oxytocin.

Each brest covers a nearly circular space anterior to pectoralis muscles from the 2nd rib to the 6th rib and from the sternum into the axilla.

In infancy and childhood brests remain rudimentary. Development takes place at puberty and still more so during pregnancy. During pregnancy there is proliferation of the ducts and alveoli of the brests. In the early stage of pregnancy there is no secretion of milk by the cells lining the alveoli. During the later stage of pregnancy the cells lining the alveoli are swollen due to accumulation of secretion inside them. Gradually, milk is secreted by their cells inside the alveoli. Free lactation starts ony after delivery (Fig. 4.194).

The nipple is perforated at the tip by 15-20 minute openings of lactiferous ducts, each of which is an excretory duct from one of lobules to the surface. The skin of the nipple extends outwards upon the surface of the

brest for 1-2 cm to form a brown- and or pink-coloured *areola.* Areola contains numerous large sebaceous glands. These sebaceous glands secrete lipid material which protects and lubricates nipple during nursing of the infant.

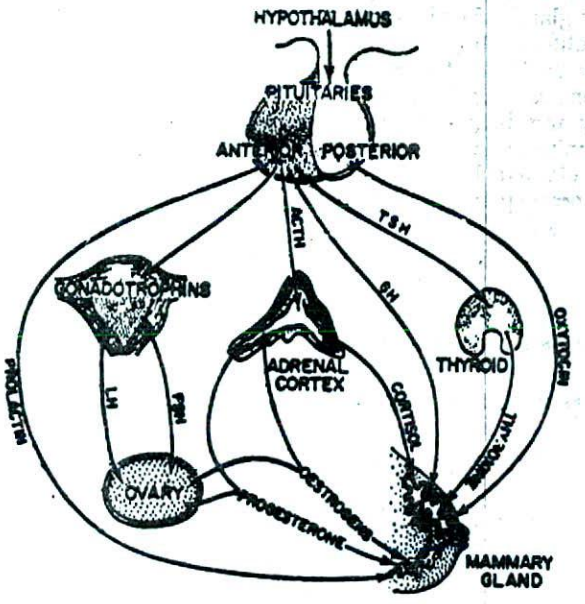


Fig. 4.195. The principal hormones that control the development and the function of the breast.

The mammary glands are well supplied with blood by the thoracic branches of the axillary, internal mammary, and intercostal arteries. The ventral and lateral cutaneous branches of 4th, 5th and 6th thoracic nerves innervate the glands.

Control of breast development and lactation

Development of mammary glands. In women, the mammary development requires direct concerted actions of oestrogens, progesterone, pituitary hormones, and the presence of basal levels of thyroxine, cortisol and insulin. Oestrogen causes the development of mammary ducts, and progesterone stimulates the formation of lobules and alveoli. Optimum mammary growth in animals also requires the presence of growth hormone and prolactin. In women, normal development is initiated by oestrogen, progesterone and growth hormone (Fg. 4.194).

With *the onset of puberty,* the growth of the mammary glands occurs due to liberation of *oestrogen.* This development is only due to proliferation of the duct. It has been observed that only oestrogen cannot initiate ductous proliferation and GH and adrenal corticoids are also required. Because in hypophysectomised animals if only oestrogen is administered then appreciable ductous growth does not occur.

During pregnancy breasts develop further chief due to the development of lobules and alveoli. This is caused by the simultaneous action of oestrogens, progesterone, GH, adrenal corticoids and prolactin (anterior prituitary). Injection of oestrogens alone or progesterone alone or even a course of oestrogens followed by a course of progesterone fails to cause such changes in the mammary glands. But if oestrogens and progesterone be given together, alveoli proliferate and the breasts show the same changes as in pregnancy. But prolactin, GH and adrenocorticoids along with oestrogen and progesterone are required for the development of mammary glands both at puberty and during pregnancy. This is proved by the fact that in rats after hypophysectomy all the above hormones must be present for the proper lobulo-alveolar development of the gland. During pregnancy, high levels of oestrogen and progesterone induce alveolar development

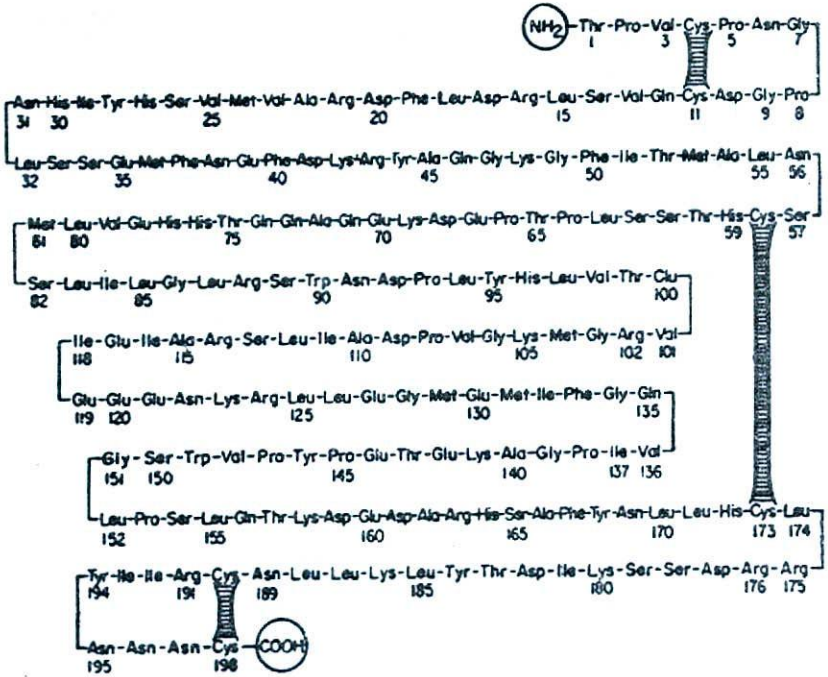


Fig. 4.196. Amino-acid sequence of prolactin.

in co-ordination with the stimulating effect of chorionic 'growth hormone prolactin' (CGP). Although CGP is lactogenic in other species, it does not cause milk secretion in the women during pregnancy. Practically the source of oestrogen secretion during pregnancy is mainly from the placenta, It has been observed that in pregnant mice if the ovaries are removed keeping the placenta *intact*, development of the breasts proceeds normally but in the same animals if the placenta is also removed there is rapid regression of the breasts.

Lactogenesis: Secretion of milk. Active lactation starts only after delivery and removal of placenta. This shows that placenta inhibits the action of prolactin. In all species other than the human female, prolactin is well-defined protein (Fig. 4.196) of the anterior pituitary and is normally kept under inhibitory control by the hypothalamus. Normally **prolactin levels are higher in females than in males** and are stimulated by oestrogen. But after delivery prolactin levels rise considerably and initiate lactation. It is possibly due to the action of large amounts of oestrogens and progesterone which have been experimentally found to inhibit milk section of lactating glands. When placenta is removed, prolactin acts unopposed due to withdrawal of oestrogen and progesterone and free lactation starts. Free flow of milk occurs after the birth of the child. There is diminution of oestrogen level in blood which in turn promotes the secretion of lactogenic hormone or prolactin from anterior pituitary. Adrenal cortex has a definite role in the initiation of lactation. Animals adrenalectomised during pregnancy have only a scanty milk and the litters cannot be raised. In hypophysectomised animals, prolactin in combination with adrenal cortical hormone can induce lactation.

Galactopoiesis (Maintenance of lactation). For the maintenance of lactation following parturition, continued secretions of prolactin along with ACTH, GH and TSH from the anterior pituitary are required. Besides these, suckling is required for the maintenance of lactation. Because by this process prolactin and oxytocin are both secreted. Prolactin helps in the process of lactation and oxytocin is required for milk ejection. If the litters are removed from the mother then mammary glands involute quickly and lactation thus ceases. Prolactin and gonadotrophin seem to have a reciprocal relationship. Ovulation tends to be depressed during the initial phases of lactation and when ovulation recurs, lactation often lessens.

Recently it has been found that prolactin has never been satisfactorily isolated from the human pituitary, except in the form of prolactin-rich growth-hormone preparation. However, various evidences indicate that prolactin is secreted by human pituitary under appropriate conditions. Bioassay studies have shown the presence of prolactin activity in urine during lactation. On the other hand, a sensitive plasma bioassay also depicts prolactin in the blood of lactating women, patients with acromegaly and inappropriate lactation, etc. Recent radio-immunoassay study has shown that plasma-prolactin rises during pregnancy progressively, falls after delivery, and rises again during breast-feeding.

During pregnancy and lactation, a specific acidophil cell type with characteristic staining and large granules is demonstrable in human pituitary. This type of cells is not present at other times and is termed as *prolactin-secreting cells of the hypophysis*. The appearance of this cell type is accompanied by a reduction in the number of somatotrophs in the hypophysis, consistent with the impairment of *maternal GH secretion* during pregnancy. Large amounts of secretion of CGP and oestrogens from the placenta may lead to supppression of GH secretion by the pituitary. High level of oestrogen may also stimulate the development of prolactin-secreting cells, probably from a common precursor cell of the acidophil type.

The importance of food and water intake in the maintenance of lactation is suggested by experimental hypophysectomised rat. In this animal

it has been possible to prepare mammary glands for lactation by administration of a combination of oestrogen, progesterone, growth hormone and prolactin. But when insulin has been given, only oestrogen and progesterone have been required. So pituitary hormones may have functioned by increasing food intake. But Lyons, after giving injection of prolactin into the mammary gland of a pseudopregnant rabbit, has found local stimulation of the injected segment of mammary gland only. Therefore it is complicated to believe that prolactin is not involved in the process of lactation under physiological condition.

Prolactin and insulin may possess very similar effects on the mammary gland. Because prolactin significantly accelerates the rate of glucose utilisation and secondarily stimulates various biosynthetic processes in the mammary gland, viz., lipogenesis from carbohydrate. The mammary gland cell can synthesise fat and protein for secretion in milk, and it appears to combine the metabolic characteristics of an adipose tissue cell which synthesises fat for storage and an exocrine pancreatic cell (for example) which synthesises large amounts of protein enzyme precursors.

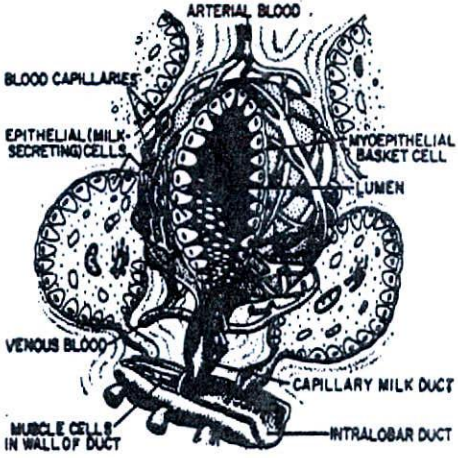


Fig. 4..197. It shows blood vessels and myoepithelial basket cells surrounding the alveoli of the mammary gland.

Ejection of milk

The milk that collects in the subareolar milk sinuses can be withdrawn by suckling. But the ejection of milk from the mammary acini into the duct system—a process commonly known as the *letting down*, in which the mother's subjective response and the mechanical stimulation of suckling both play a part. Suckling produces nerve impulses that are carried by way of the lateral funiculus of the spinal cord to the hypothalamus.

Effects of suckling. Suckling stimulates lactation as well as ejection of milk in two ways:

(a) **Mechanical.** It drains away the accumulated milk and makes room for fresh secretion.

(b) **Reflex.** Suckling reflexly stimulates anterior pituitary, which secretes more prolactin. The release of prolactin appears to be an intrinsic property of the anterior pituitary. The hypothalamus forms *prolactin-inhibitory factor* (PIF) that prevents secretion of prolactin. Oestrogen and chlorpromazine that stimulate lactation, lower the concentration of PIF in hypothalamus and probably permit thereby prolactin secretion. During suckling the afferent impulses from the nipple reach the hypothalamus and then through the hypothalamohypophyseal tract, neurohypophysis discharges oxytocin and little vasopressin in the blood.

The oxytocin circulates into the blood stream and causes contraction of the myoepithelial basket cells surrounding the alveoli of the gland and thus ejection of the accumulated milk (Fig. 4.137). So suckling produces both secretion and ejection of milk.

Inhibition. Emotional excitement like worry, fear, sadness, etc., during lactation may inhibit milk flow. Administration of epinephrine has similar effect. Emotional stress might inhibit the release of oxytocin acting through neurohypophysis. Effect of epinephrine,on the other hand, might be due to constriction of mammary blood vessels. Disturbances in milk ejection causes filling up of mammary gland and cessation of lactation.

Colostrum

Secretion of the mammary gland at the end of pregnancy and at the beginning of lactation is known as **colostrum.** Unlike milk, it is yellow in colour and is secreted from the breast only for a few days after the birth of the child. Colostrum contains little fat but numerous cell fragments, free-fat droplets, lymphocytes, monocytes, histiocytes, desquamated epithelial cells and colostrum corpuscles. It also contains a large amount of γ-globulins in most mammals and is formed due to imbalance between secretion and removal from the gland. Regarding composition and other details of milk, vide Volume I.

Hormone and Cancer

Carcinoma is the malignant growth of the tissue due to abnormal multiplications of cells at any part of the body. This abnormal multiplication is possibly due to absence of feedback controls that normally limit the cellular growth and differentiation. The aetiology of cancer is not yet known though investigations on various aspects have been undertaken all over the world. Considering the incidence of cancer in different tissues of the body in respect of male and female hormones have been indicated to be some of the causes of such disease. There is evidence that prolonged stimulation to cell division acts as one of the factors causing the tissue to become carcinomous. The oestrogen is not only stimulating the growth and differentiation of the accessory sex organs but also of other tissues of the body. The incidence of breast carcinoma in women of child-bearing ages are mostly *oestrogen-dependent.* This type of carcinoma is dependent upon the amount of oestrogen present in circulation. So this growth is highly stimulated during pregnancy and for any such condition when the oestrogen level of blood is heightened. This growth of the carcinoma can be inhibited for some time (months or years) but not are fully destroyed by bilateral ovariectomy. Because oestrogen secreted from the adrenal gland of this patient may maintain the growth of carcinoma. Bilateral adrenalectomy or administration of glucocorticoids which inhibit the ACTH secretion from anterior pituitary may produce another temporary remission of the carcinoma recurring after castration. When the disease recurs for the second time after adrenalectomy, it may be inhibited by hypophysectomy. There is also evidence that GH stimulates in breast carcinoma. There are some carcinomas of prostate which are *androgen-dependent* and regress temporarily after castration or hypophysectomy.

An understanding of lymphatic drainage of the breasts has great importance in relation to malignant condition of breasts. Malignant cells

may readily spread from the affected breast to other areas of the body though the lymphatic vessels and nodes that drain adjacent areas.

Rrecent studies reveal that prolactin can induce breast cancer, not oestrogen (*vide* page 4-258).

REGULATION OF GROWTH

Growth of the body (skeleton, muscles and viscera) does not proceed at a uniform rate. It shows the following phases: (1) During infancy, especially in the first year growth is very rapid. (2) Between 3 and 12 years, growth proceeds at a slower rate. (3) At puberty, growth again becomes very rapid. (4) After puberty, the rate slows down. (5) After 25 years, the epiphyseal cartilages ossify and growth in length ceases (but growth in thickness proceeds). (6) At old age degenerative changes occur in all the tissues of the body.

All tissues of the body do not show parallel growth at the same time. The growth rates of different tissues vary. The following types are seen: (1) *Skeletal type*—described above. (2) *Neural type*—The nerve tissues, viz., brain, spinal cord, visual and auditory apparatus, etc., show this type of growth. The growth rate is very rapid initially, reaching nearly the final maximum in the first few years. (3) *Lymphoid type*—The lymphoid tissues of the body, such as thymus, tonsils, adenoids, etc., have this type of growth. They reach their maximum at puberty and then decline. (4) *Reproductive type*—The sex organs have this type of growth. They show very little growth initially. Growth starts a little before puberty and continues throughout the sex life. At climacteric—sex organs degenerate.

It is obvious that there is an efficient mechanism for controlling the growth rate of the body and that, there seems to be a special mechanism for each type of growth mentioned above. The present state of knowledge is not sufficient to explain fully the different phases of skeletal growth. The following details are known:

Factors controlling growth

The following factors take part in growth regulation:

1. **Heredity.** The hereditary potentialities carried in the chromosomes (in the sperm and ovum) are the chief determining factors, Children of tall parents will usually grow tall stature. *Environment*—internal and external—plays a secondary role, either stimulating or retarding the growth potentialities.

2. **Diet.** Any sort of starvation depresses growth, whereas nutritious food always leads to better growth. Diet should have—(a) *enough calorie value*, (b) sufficient 'first class' proteins or proteins of 'high biological value', and (c) an adequate amount of minerals and vitamins. All vitamins help growth, especially A, D and C.

3. **Role of endocrines.** Disorders of endocrine glands leads to disturbances of growth and differentiation. (a) Growth hormone of the anterior pituitary, thyroid hormones and insulin help in the retention of nitrogen in the tissues and thereby help in the growth of tissues. (b) The gonadotrophic hormones of the anterior pituitary control the growth of gonads and secretion of sex hormones from the gonads. Sex hormones are required for the development of the secondary sex characters with the growth of the accessory sex organs in both male and female. During

pregnancy the growth of the uterus, vagina, breast, etc., are controlled by the different sex hormones, such as oestrogens, progesterone, placental growth hormone—prolactin. Besides these, GH, ACTH, thyroid hormones, corticoids and anterior pituitary prolactin play an important role in the special growth during pregnancy. (c) Thyrotrophic hormone and ACTH of the anterior pituitary regulate the growth and related functions of thyroid and addrenal cortex respectively. (c) The parathyroid glands control the growth of bones by regulating calcium and phosphorus metabolism.

Factors controlling sexual growth

Sex organs show the *reproductive type* of growth (*vide* above). They are rudimentary during infancy and childhood, mature at puberty and degenerate at climacteric. The following factors control sex:

I. **Endocrines.** (1) **Anterior pituitary**—(*a*) Follicle-stimulating hormone (FSH) (pages 4-31, 4-32), (*b*) Luteinising hormone (LH) (pages 4-31, 4-32), (*c*) Prolactin or Luteotrophic hormone (LTH) (page 4-33). (2) **Thyroid** (page 4-87). (3) **Adrenal cortex** (page 4-152). (4) **Testes** (page 4-208). (5) **Ovaries** (pages 4-233.). (6) **Thymus** (pages 4-188, 4-190).

II. **Role of hypothalamus.** Nervous reflexes play a considerable role in regulating anterior pituitary through hypothalamus (pages 4-32, 4-33, 4-35, 4-36, 4-60, 4-61, 4-62, 4-256). This organ synthesises different gonadotrophin-releasing factors, e.g., FSH-RF, LRF and prolactin-inhibiting factor (PIF) and thus controls the activity of primary and secondary sex organs through the pituitary.

III. **Vitamins.** Vitamin E is essential for reproduction. Other vitamins, especially A, C and some members of vitamin B complex are also important.

Endocrines on metabolism

1. **On BMR.** (*i*) Thyroid glands—Thyroid hormones. (*ii*) Anterior pituitary—Growth or somatotrophic, thyrotrophic and adrenocorticotrophic hormones. (*iii*) Adrenal medulla—Epinephrine. (*iv*) Adrenal cortex—Adrenal corticoids.

2. **On carbohydrate metabolism.** (*i*) Islets of Langerhans of the pancreas—Insulin and glucagon. (*ii*) Anterior pituitary—Growth or somatotrophic, thyrotrophic and adrenocorticotrophic hormones. (*iii*) Posterior pituitary—Antidiuretic hormone (vasopressin) and oxytocin produce hyperglycaemia. (*iv*) Adrenal cortex—Glucocorticoids. (*iv*) Adrenal medulla—Epinephrine (*vi*) Thyroid glands—Thyroid hormones.

3. **On fat metabolism.** (*i*) Anterior pituitary—Growth or somatotrophic hormone. (*ii*) Thyroid glands—Thyroid hormones. (*iii*) Islets of Langerhans of the pancreas—Insulin. (*iv*) Adrenal cortex—Glucocorticoids.

4. **On protein metabolism.** (*i*) Islets of Langerhans of the pancreas—Insulin prevents breakdown of proteins and gluconeogenesis. (*ii*) Anterior pituitary—Growth or somatotrophic hormone stimulates protein breakdown and its conversion into sugar. (*iii*) Adrenal cortex—Glucocorticoids (same as growth or somatotrophic hormone).

5. **On mineral metabolism.** (*i*) Thyroid glands—Thyroid hormones (T_3 and T_4) on iodine metabolism and thyrocalcitonin (on Ca and P

metabolism). (*ii*) Parathyroid—Parathormone (on Ca and P metabolism). (*iii*) Adrenal cortex—Mineralocorticoids (on Na, K, chloride, etc.).

6. **On water metabolism.** (*i*) Posterior pituitary—Antidiuretic hormone (vasopressin) (page 4-55). (*ii*) Adrenal cortex—Mineralocorticoids (page 4-150). (*iii*) Thyroid glands—Thyroid hormones cause diuresis by increasing the nitrogenous products, resulting from stimulated protein metabolism. (*iv*) Ovary, placenta and also adrenal cortex—Oestrogen and progesterone (pages 4-252, 4-253, 4-258).

Endocrines on bone formation

1. Anterior pituitary (growth hormone). 2. Thyroid. 3. Parathyroid. 4. Thymus, 5. Adrenal cortex. 6. Gonads.

Endocrines on kidney functions

1. Posterior pituitary (Antidiuretic hormone or ADH) (page 4-51). 2. Thyroid (page 4-78). 3. Adrenal cortex (pages 4-151, 4-152). 4. Adrenal medulla (Epinephrine—pages 4-170, 4-173).

CONTROLLED REPRODUCTION AND A PLANNED FAMILY

Today the world population is about 2·5 billion. The demographers predict that world population is likely to be doubled by 2000 A.D. and tripled by 2050 A.D. According to Malthus theory, the food production has to be doubled or tripled by 2050 A.D. to bring about a balance. But food production can be increased to some extent but certainly cannot be tripled. The only answer to this problem lies in the controlled reproduction and a planned family.

In order to check the population explosion, various sociomedical measures have been taken. Contraceptive measures to prevent pregnancy, are widely employed for the controlled reproduction and a planned family all over the world. Mass education is necessary for proper use of the contraceptiae measures in developing countries like India. Conception may be prevented in the following ways:

1. By **controlling the sexual intercourse** according to physiological principals, e.g., rhythm method and withdrawal method.

2. Use of some **barriers to the entry of sperms** into the cervix at the time of coitus.

(*a*) Use of rubber sheaths (condoms) over the penis during intercourse.

(*b*) Use of rubber diaphragm on the cervix by females. This is often combined with spermicidal jelly or foam.

3. Use of chemical **spermicidal agents** before or after intercourse.

(*a*) Creams, pashes, jellies, foams, suppositories, etc., used before coitus.

(*b*) Vaginal douches—used for irrigating the vagina to wash out the sperms after intercourse.

4. **Interruption of normal paths** of the sperms or ovum:

(*a*) Vasectomy in males and ligation of the Fallopian tubes (tubectomy) in females. Often these measures are permanent and are particularly useful for those who lack in strong motivation, intelligence and forethought before sexual intercourse.

5. If a foreign body is placed within the uterus the implantation of fertilised ovum in the uterus becomes difficult. This knowledge has been

utilised in the use of loop as an **intra-uterine contraceptive device** (IUCD).

6. Use of hormone or combination of hormones which prevent of conception, e.g., **oral contraceptive pills.**

1. *Withdrawal technique or coitus interruptus*—Withdrawal of penis just before orgasm (climax) is not at all dependable. Failure rate of this method is 17 per cent.

2. *Rhythm method*—In women with regular menstrual cycles ovulation occurs between the 9th and 19th days after the beginning of the menstrual bleed.

For birth-controlled purposes it is sufficient to know only the earliest possible and last possible dates on which the next period strts.

It is also important to remember that only one ovum normally is expelled per cycle and this ovum remains alive for only 24 hours. Spermatozoa after entering the uterus remain alive only for 48 hours. Thus there is a minimum period of 3 days in each cycle during which intercourse should not be allowed to avoid pregnancy. At the same time it is important to note that it is extremely difficult to precisely determine the exact day of ovulation.

To calculate the infertile days during the preovulatory phase, 18 is substracted from the shortest recorded cycles. Correspondingly in the luteal phase the infertile days are determined by substracting 11 from the longest cycle.

Thus, in the case of women whose cycles extend from 25 to 32 days, the 'safe period' would extend up to day 7 (25—18=7) and from day 21 (32—11=21) onwords.

3. *Intra-uterine contraceptive device* (IUCD) as a method for contraception has been widely tried in India. It is suggested that the presence of an IUCD makes the uterine environment hostile to the nidation of the fertilised ovum, or its presence interferes with the chain of events which leads to the attachment of the blastocyst to the uterine wall. Excessive bleeding, uterine cramp (pain), expulsion of the IUCD, infection and uterine perforation are some of the complications of IUCD. Failure rate of IUCD is about 5 per cent.

4. Sterilisation operations are being advocated more and more as a method of permanent contraceptive measure in devloping countries like India. Ligation and division of the Fallopian tubes are not associated with any known change in the female physiology. Ovulation and regular menstruation continue.

The unfertilised ovum is lost in the peritoneal cavity.

Following male sterilisation operation (vasectomy) spermatogenesis and hormone production continues normally. Macrophages phagocytose nearly all the sperms in the lumen of the epididymis and vas deferens.

5. *Oral contraceptives*—Of all the contraceptive measures, oral contraceptive pills are most effective. If properly taken, they are nearly 100 per cent. successful in preventing pregnancy. Oral contraceptive regimes are generally of three types: (*i*) classical pills regime, (*ii*) sequential pills regime and (*iii*) luteal supplementation pills regime. A 'classical pill' is a combination of an orally active progesterone-like substance (gestagen or progestational agent) and a small dose of oestrogen. In

'sequential pill regime' patient receives a high dose of oestrogen for part of the cycle followed by a mixture of oestrogen and gestagen. In 'luteal supplementation pill regime' the patient takes progestational agents in low dosage throughout the whole menstrual cycle.

Exact modes of action of various contraceptive pills are not definitely known. However, the classical pills inhibit ovulation by (*i*) suppressing of the midcycle peak in LH output which is brought about probably by modifying secretion of luteinising hormone release factor (LRF) by hypothalamus: (*ii*) modifying tubal transport of ovum; (*iii*) inducing endometrial changes which make nidation of the fertilised ovum unlikely; (*iv*) render the cervical mucus thicker and hostile to sperm penetration. The sequential pills inhibit ovulation due to its action on hypothalamus which in turn inhibits secretion of mainly follicule-stimulating hormone release factor (FSH-RF) and partly LRF. Sequential pills cause less alteration of the crevical mucus and endometrial changes. Luteal supplementation pills unlike other two preparations do not inhibit ovulation but there occur endometrial changes, changes of the cervical mucus and increases in the motility of the Fallopian tubes.

The hormonal checkmate produced by the pills leaves the ovary in a quiescent stage. The activity and size of the ovary are consequently reduced.

Side effects of the 'pills'

Use of oral contraceptive pills does not cause any major alteration of normal physiology. However, usages of oral contraceptives for a long time, have been shown to induce hypertension in susceptible women by causing a sodium loss which in turn increases renin release, thus stimulating aldosterone secretion. The chances of venous thrombo-embolism, cerebral thrombosis and jaundice are marginally increased after prolonged use of 'pills'.

6. *Progestin implant*—This method of contraception is still under experimental stages. Progestin capsules are implanted in the body and its action lasts for months or years. When pregnancy is desired the capsule may be taken out. Progestin in slowly released from the implant and prevents pregnancy by acting on ovulation. It does not stop normal cycles.

7. *Abortion*—Abortion is a method contraception when conception has already taken place. Adaptation of this method for controlling population explosion is perhaps the last resort.