8. Endocrine System

8.1 Basic Concepts

The endocrine system is one of the two coordinating and integrating systems of the body. It acts through chemical messengers or *hormones* carried in the circulation. In contrast to the nervous system, the action of the endocrine system is slower in onset, more prolonged and generally more widespread. The two systems are linked, however, through the *hypothalamus* which controls the secretion of many of the endocrine glands. Consequently influences acting on or through higher centres of the brain, i.e., emotion, can affect endocrine secretion. Thus the brain is a *neuroendocrine organ.*

Hormones assist in

(i) maintaining the constancy of the internal environment (homeostasis);

(ii) controlling the storage and utilization of energy substrates;

(iii) regulating growth, development and reproduction;

(iv) responding to environmental stimuli.

Endocrine cells usually exist as discrete glands in the body. In contrast to exocrine glands they are ductless and empty their secretions directly into the bloodstream. Evidence that an organ functions as an endocrine gland can be obtained by studying the specific effects of its removal from the body, by transplantation of the gland back into the body and by injections of extracts of the gland. The first experiment in endocrinology is attributed to Berthold, who in 1849 showed that transplantation of a testis into the abdomen of a castrated cockerel restored its secondary sex characteristics. The principal endocrine glands in mammals are the *hypothalamus, pituitary, thyroid, parathyroids, adrenals, pancreas, gonads* and *placenta.* Other hormones and hormone-like substances are also produced in the kidney, liver, thymus, pineal gland and certain cells of the gastrointestinal tract.

Hormones

A hormone can be defined as a chemical substance that is transported by the circulation and at very low concentrations elicits a specific response in other tissues. Not all hormone-like substances meet these criteria. For example, some cells in the CNS and GIT release substances which diffuse into surrounding regions and act locally. These are referred to as *paracrine* secretions. Sometimes cells secrete enzymes that act on plasma proteins to produce hormones, i.e., the renin-angiotensin system.

Hormones may be classified according to their chemical structure into

(i) *peptide hormones* (proteins, glycoproteins and polypeptides), i.e., growth hormone, insulin and ADH;
(ii) *steroid hormones,* i.e., aldosterone, oestrogen and testosterone;
(iii) *tyrosine derivatives,* i.e., thyroxine and adrenaline.

The concentrations of hormones in the blood are extremely low, i.e., peptide hormones may range from $10x^{-10}$ to 10^{-12} mol/L and steroid hormones from 10^{-6} to 10^{-9} mol/L. Because of the low concentration of hormones in the blood, their presence is difficult, if not impossible, to detect by chemical analytic techniques and so bioassays, radioimmunoassays and radioreceptor assays have been devised for measuring hormone levels. The development of the radioimmunoassay technique, which is highly sensitive and usually less cumbersome to perform than the bioassay, has led to rapid advances in endocrinology.

Hormone Synthesis and Secretion

Peptide hormones are synthesized on the ribosomes of the rough (granular) endoplasmic reticulum as part of larger precursor proteins called *preprohormones,* which are subsequently modified by deletion of peptide sequences. The 'pre' sequence appears to be a signal peptide involved in ribosomal attachment and transfer of the newly synthesized protein across the membrane of the endoplasmic reticulum. From the rough endoplasmic reticulum the prohormone is transported to the Golgi apparatus where it is packaged into vesicles. Processing of the prohormone to the hormone occurs during these stages. With appropriate stimulation Ca^{2+} enters the cell, causing vesicles to fuse with the membrane and to extrude their contents into the extracellular fluid - a process known as *exocytosis.*

Steroid hormones are synthesized from cholesterol in steps which take place in the mitochondria, smooth endoplasmic reticulum and cytoplasm and require acetate, O_2, NADPH and other cofactors. Steroid hormones are not stored in vesicles and, being lipophilic, they readily cross the plasma membrane of the cell. Therefore their rate of release is directly related to their rate of synthesis.

Hormone Transport and Inactivation

Once released in the bloodstream, water-insoluble hormones, i.e., thyroid and steroid hormones, are bound to various plasma proteins. The free form is usually only a small fraction of the total hormone in the blood and exists in equilibrium with the bound form. Only the free hormone can affect its target cells. The concentration of the 'active' hormone in the blood is therefore determined by the dynamic relationship between its rate of secretion, its rate of inactivation and the degree to which it is bound to plasma proteins.

Hormones have a half-life in the body of minutes to hours. Inactivation may occur in the blood, in the liver or kidney, or in some cases in the target tissues. Hormones may be inactivated by degradation, oxidation, reduction, methylation or conjugation to glucuronic acid, and excreted in the urine or bile.

Hormone Actions

Hormones affect the growth, development, metabolic activity and function of tissues. The responses are often the result of the actions of several hormones. Actions may be *stimulatory* or *inhibitory* and *additive* or *synergistic.* A hormone, which has no effect *per se* but is necessary for the full expression of the effects of other hormones, is said to have a *'permissive'* action. Hormones may alter

(i) membrane permeability;

(ii) activity of rate-limiting enzymes in reaction pathways;

(iii) protein synthesis (blocked by puromycin or cycloheximide); or

(iv) gene activation leading to the transcription of new messenger RNA species (blocked by actinomycin D).

These actions are not mutually exclusive and hormones may act in one or more of these ways.

The first step in the action of a hormone is its binding to a specific cell receptor. Peptide hormones, which do not penetrate cells readily, act by binding to specific receptors in the plasma membrane; so too does adrenaline. Recently it has been shown that some of the peptide hormone-receptor complexes may be internalized, i.e., taken up into the cell by endocytosis. The reason for this is not clear but the process appears to be concerned with the regulation of the number of receptors per cell.

Steroid hormones readily cross the plasma membrane and bind to specific *cytoplasmic* receptors in target tissues. The receptor is a dimer that binds two molecules of steroid hormone. The steroid hormone-receptor complex is then translocated to the nucleus, where it induces gene activation leading to the transcription of new messenger RNA species and, consequently, to new protein synthesis. Thyroid hormones likewise cross the plasma membrane but bind directly to receptors in the nuclei of their target tissues.

The effects of many hormones - adrenaline (acting on beta receptors), ACTH, glucagon, LH, MSH, PH and TSH - are mediated by adenosine 3',5'-monophosphate (*cyclic AMP*). Hormone-receptor interaction at the membrane surface, via a *GTP-regulatory protein,* stimulates the enzyme *adenylate cyclase,* which catalyses the synthesis of cyclic AMP from ATP. The increased level of cyclic AMP within the cell in turn stimulates a *protein kinase* which by a series of reactions may bring about specific changes in enzyme activity, membrane permeability, protein synthesis, or gene activation depending on the tissue involved. The initial hormonal signal is amplified many times. Eventually the concentration of cyclic AMP is restored to its basal level by degradation to AMP, this being catalysed by the enzyme *cyclic nucleotide phosphodiesterase.* Sutherland called cyclic AMP the *'second messenger'.* Some hormones, i.e., insulin, growth hormone and adrenaline (acting on alpha receptors) that do not increase cyclic AMP concentrations in their target tissues. A possible second messenger in the case of adrenaline here is *calcium* acting through a calcium-binding protein called *calmodulin.* A more recent view, however, proposes that receptor activation leads to the hydrolysis of *polyphosphoinositide* resulting in the formation of *inositol triphosphate* and *diacylglycerol.* These molecules in turn are thought to act independently as second messengers, inositol triphosphate bringing about an increase in calcium ions in the cytoplasm. Other possible second messengers are cyclic GMP and prostaglandins.

Control of Hormone Secretion

The immediate stimulus for the secretion of a hormone may be neural, hormonal, or the level of some metabolite or electrolyte in the blood. In the long term, secretion rates are usually maintained at fairly constant levels by *negative feedback mechanisms,* whereby increased levels of hormone in the blood lead to the inhibition of further hormone secretion. Positive feedback control is much less common, but an example is to be found in the hormonal control of ovulation during the female reproductive cycle.

8.2 The Pituitary Gland

The *pituitary* or *hypophysis* is a small gland, approximately 0.5 g in man, situated at the base of the skull and connected to the brain by the hypophyseal stalk. It consists of an *anterior lobe,* and a *posterior lobe.* During the embryonic development the *neurohypophysis,* which includes the *pars posterior* is derived from a downward evagination of the brain. The *adenohypophysis,* which includes the *pars anterior* and the *pars intermedia,* comes from an outgrowth of the roof of the mouth known as Rathke's pouch. In the adult human the pars intermedia is only a remnant and the pars anterior and pars posterior may be equated with the terms anterior and posterior pituitary respectively.

The pituitary secretes at least nine hormones. Four of these regulate other endocrine glands and are referred to as *'trophic'.*

Anterior Pituitary

The anterior pituitary synthesizes and secretes at least six hormones, namely, *growth hormone (somatotrophin, GH). prolactin,* and the trophic hormones - *thyroid-stimulating hormone (TSH, thyrotrophin), adrenocorticotrophic hormone (ACTH, corticotrophin), follicle-stimulating hormone (FSH)* and *luteinizing hormone (LH).* The hormones are stored in granules and released in response to stimulation by their corresponding hypothalamic neurohormones. Many of these hormones are synthesized as larger molecules or *prohormones.* The prohormone may contain the sequences of a number of hormones, i.e., the prohormone for ACTH also contains the sequences of melanocyte-stimulating hormones, of lipotrophins, and of endorphins.

The trophic hormones influence the secretion of their target glands and their size and development. Hypophysectomy therefore causes:

(i) inability to grow due to lack of GH.

(ii) atrophy of the thyroid gland and hypothyroidism due to lack of TSH.

(iii) atrophy of the adrenal cortex and hypoadrenocorticalism due to lack of ACTH.

(iv) failure of the gonads to mature due to lack of FSH and LH.

Removal of the pituitary is not incompatible with life, although hypophysectomized animals have a low tolerance to cold and stress. In hypophysectomized animals the posterior pituitary hormones continue to be secreted because the neurones that synthesize

these hormone have their cell bodies in the hypothalamus.

Hypothalamic Neurohormones

Release of the anterior pituitary hormones is regulated by *neurohormones* which are elaborated in the hypothalamus. These neurohormones are released at the level of the median eminence. They diffuse into a primary plexus of capillaries and are transported down large portal vessels in the pituitary stalk to a secondary set of capillaries or sinusoids in the anterior pituitary - the s-called *hypophyseal portal system.* They comprise both releasing and release-inhibiting hormones and some of the anterior pituitary hormones may be subject to dual control. The following releasing hormones have been identified - *growth hormone-releasing hormone (GHRH), thyrotropin-releasing hormone (TRH), corticotrophin-releasing hormone (CRH),* and *luteinizing hormone-releasing hormone (LHRH).* Purified and synthetic preparations of LHRH can release both LH and FSH from the anterior pituitary and an alternative name for LHRH is *gondatotrophin-releasing hormone.* The release-inhibiting hormones secreted by the hypothalamus are *growth hormone-inhibiting hormone (GHIH, somatostatin)* and a *prolactin-inhibiting factor.* Thus secretion of GH is subject to dual control by the hypothalamus. TRH is a tripeptide (pyroglutamyl-histidyl-proline amide). The other neurohormones also appear to be small peptides, except prolactin-inhibiting factor which is almost certainly dopamine.

Control of Anterior Pituitary Hormone Secretion

The secretion of releasing and inhibiting hormones by the hypothalamus may be influenced by emotional and environmental factors acting through the CNS. In the long term, however, regulation of the secretion of the hypothalamic neurohormones and anterior pituitary hormones is usually achieved through negative feedback mechanisms triggered by the blood level of the anterior pituitary hormones (short feedback loop) and the target gland hormones (long feedback loop).

Growth Hormone

Thee conditions of *dwarfism* and *gigantism* are caused by disturbances in the function of the pituitary gland. GH was first isolated from bovine anterior pituitaries in the 1940s. It is a protein of MW 22000, there being some differences in the amino acid sequence between species. It has close structural similarities to prolactin and human placental lactogen, which suggests their evolution from a common progenitor molecule.

Growth and hormones. The major function of GH is to stimulate the growth of bones and other tissues. The growth process is not simple and in addition to an adequate food supply and to genetic endowment, a number of hormones are involved, *GH, sex hormones, thyroxine* and *insulin.* There are two periods of accelerated growth. The first occurs in the first two years of life and the second at the time of puberty. The period of accelerated growth at puberty is associated with increased levels of sex hormones, including androgens of adrenal origin. Paradoxically the cessation of growth around 18 to 20 years of age is also due to the sex hormones, which cause fusion of the growing ends of bones (*epiphyseal closure*). Thus the effect of excess GH secretion depends on whether it occurs before or after closure of the epiphyses.

Humans respond only to GH of human (or other primate) origin. This is obtained from the cadavers and hence is available only in limited amount. It is now possible to produce GH using genetic engineering techniques in bacteria.

Actions of GH. GH affects the metabolic activity of most of the tissues of the body. Its growth-promoting effect is due to its ability to stimulate the uptake of amino acids and their incorporation into proteins in muscle and bone. It is currently thought that the growth-promoting actions of GH are mediated by a group of polypeptides called *somatomedins* which are produced mainly in the liver. GH also has effects on carbohydrate and fat metabolism which are in general antagonistic to those of insulin, i.e., they are 'diabetogenic'. GH increases blood glucose (after an initial decrease) by stimulating hepatic gluconeogenesis and by inhibiting uptake by muscle. Large doses of GH raise free fatty acid levels by increasing fat mobilization in adipose tissue. The half-life of GH in blood is about 30 min.

Control of GH secretion. The level of GH is same in an adult as in a child and it fluctuates continuously. GHRH and GHIH (somatostatin) control the release of GH. Factors that stimulate growth hormone secretion are low blood glucose levels, high blood amino acid concentrations and stress. Bursts of hormone secretion also occur during certain periods of sleep.

Also found in the anterior pituitary are the opioid peptides.

Opioid Peptides

Endogenous peptides which interact with opiate receptors were first discovered in the brain and gut and later in the adrenal medulla. They were found to be pentapeptides and were named *met-enkephalin* (Tyr-Gly-Gly-Phe-Met) and *leu-enkephalin* (Tyr-Gly-Gly-Phe-Leu). Subsequently larger opioid peptides were discovered in the pituitary and named *endorphins* and *dynorphins.* The former are found in the pars anterior and pars intermedia and the latter in the pars posterior. These opioids are also present in brain.

The family of opioid peptids has grown to at last nine members, all of which contain either the met-enkephalin or leu-enkephalin sequence at their N-terminals. Complementary DNA-gene cloning techniques have shown that the family has three branches which stem from (i) *proopiomelanocortin,* the precursor of beta-endorphin and related peptides, (ii) *proenkephalin,* the precursor for met- and leu-enkephalin and (iii) *prodynorphin,* the precursor for dynorphins and neoendorphins. The proopiomelanocortin precursor contains the sequences of several active peptides including adrenocorticotrophic hormone, beta-lipotrophin, beta-endorphin, and alpha- and beta-melanocyte-stimulating hormones. This precursor molecule is processed differently depending on its location. In the pars anterior of the pituitary ACTH and beta-lipotrophin are produced (some of the beta-lipotrophin is split to produce beta-endorphin). In the pars intermedia ACTH is further cleaved to alpha-MSH and beta-lipotrophin is cleaved to produce beta-endorphin.

Opioid peptides are powerful analgesics when injected into the ventricles of the brain. There are at least three classes of opioid receptors: (i) the delta-receptor which binds enkephalins, (ii) the kappa-receptor which binds dynorphins and (iii) the mi-receptor which binds beta-endorphin and the enkephalins and is correlated with analgesia.

Melanocyte-Stimulating Hormone

The pars intermedia secretes *melanocyte-stimulating hormone (MSH)* which in amphibia and fish causes skin to darken by dispersing the melanin granules within the melanophores and thus enables these animals to blend their skin colour with the environment. In the human adult the pars intermedia occupies only 1% of the pituitary and its role is not known. There are two types of MSH in animals, alpha-MSH and beta-MSH, both of which are polypeptides containing structural sequences in common with ACTH. Excess production of MSH (or ACTH) in humans can cause an increase in melanin synthesis and hyperpigmentation. MSH is under dual hypothalamic control but inhibiting hormone plays the dominant role.

Posterior Pituitary

The posterior lobe of the pituitary secretes two peptide hormones, *antidiuretic hormone (ADH)* and *oxytocin.* These hormones are synthesized in discrete groups of neurones in the hypothalamus called the supraoptic and paraventricular nuclei. Each hormone is synthesized as a prohormone which also contains *neurophysin* (neurophysin I for oxytocin and neurophysin II for ADH). The prohormone is packaged into granules in the Golgi apparatus. Neurophysin is then cleaved from the hormone to which it binds non-covalently. This protects the hormone from degradation and also prevents it from leaking out of the storage granules. The granules are transported down the axons to their terminals in the posterior pituitary, where they are stored prior to their release into the circulation. Release of each hormone together with its corresponding neurophysin is triggered by nerve impulases originating in the hypothalamus. This process is known as *neurosecretion.*

Antidiuretic Hormone

ADH is an octapeptide (MW 1102). Its main role is to control the reabsorption of water by the kidneys but at higher concentrations it also constricts arterioles and so has a pressor effect. Hence an alternative name for ADH is *vasopressin.* In mammals there are two types of ADH which differ by a single amino acid - arginine (man) or lysine. ADH has a half-life of about 5 min in the plasma and is metabolized by the liver and kidneys.

Action of ADH. ADH increases the reabsorption of water by the kidneys and so reduces the excretion of water from the body. It acts on the distal portions of mammalian nephrons, increasing their permeability to water. Water moves passively out of the nephrons along an osmotic gradient and so urine volume is decreased. The action of ADH appears to be mediated by cyclic AMP.

Control of ADH secretion. Several factors influence its release:

(i) *Osmotic changes.* Osmoreceptors in the hypothalamus respond to an increase in osmolality of the extracellular fluid leading to the release of ADH. Subsequent renal conservation of water thereby restores the normal osmolality of the body fluids. Conversely, the ingestion of a large amount of water reduces the osmolality of the extracellular fluid leading to a reduction in ADH release and an increase in the renal excretion of water.

(ii) *Blood volume changes.* Haemorrhage promotes ADH release in response to decreased stimulation of stretch receptors in the atria and pulmonary veins. This mechanism acts to offset loss of circulatory volume. A change in body position from a supine to a sitting position has a similar transient effect on ADH release.

(iii) *Other stimuli.* Pain, exercise, stress, sleep and drugs such as morphine induce ADH secretion while alcohol strongly inhibits secretion. The well-known diuretic effect of alcohol beverages results not simply from increased fluid intake but also from a direct suppression of ADH release.

Damage to the ADH-producing neurones in the hypothalamus may result in the condition known as *diabetes insipidus*, which is characterized by the voiding of large volumes (polyuria) of dilute urine and excessive thirst (polydipsia). This condition can be treated satisfactorily with synthetic ADH administered as a nasal spray.

Oxytocin

Oxytocin is an octapeptide (MW 1025). In structure oxytocin differs from ADH in two amino acid residues. Its synthesis and metabolism are similar in many respects to those of ADH.

Actions of oxytocin. Oxytocin stimulates the myoepithelial cells of the mammary gland causing milk let-down. It also causes uterine contraction in the oestrogen-stimulated uterus during parturition and is used clinically for the induction of labour. It has no known function in males.

Control of oxytocin secretion. Milk let-down is a reflex action in response to suckling at the breast. A neuroendocrine reflex pathway is involved, in which impulses initiated by suckling are relayed to the hypothalamo-pituitary axis causing the release of oxytocin. This is carried by the circulation to the mammary glands. Pain, embarrassment and anxiety can cause inhibition of oxytocin release. There is a marked elevation of plasma oxytocin levels during parturition.

8.3 Thyroid Gland

Thyroid hormone deficiency in childhood produces the condition of *cretinism*, which is characterized by a failure to grow and severe mental retardation. A less severe form of thyroid hormone deficiency may give rise to *goitre* which is a gross enlargement of the thyroid gland. Both of these conditions are often associated with an inadequate intake of *iodine* in the diet and occur in areas where the soil is deficient in iodine.

The main hormones secreted by the thyroid gland are *thyroxine (T_3)* and *triiodothyronine (T_3)*, collectively known as the thyroid hormones. The thyroid gland also secretes the hormone, *calcitonin*, which lowers plasma calcium concentrations. Sufficient T_3 and T_4 are stored in the gland to last 2-3 months. The only structural difference is that T_4 contains four iodine atoms whereas T_3 has three. There is approximately fifty times more T_4 than T_3 in the plasma but T_3 is about five times more potent that T_4.

Synthesis and Secretion of Thyroid Hormones

The thyroid gland actively concentrates iodide to a level normally some 25 times that in the plasma. The iodide is oxidized by a peroxidase in the follicle cells to atomic iodine which immediately iodinates tyrosine residues contained in *thyroglobulin.* Thyroglobulin is a large protein (MW 670000) which is synthesized in the follicle cells and secreted into the follicular cavity. The iodinated tyrosine residues in thyroglobulin undergo coupling to form T_4 and T_3. Iodination and coupling are thought to take place at the cell surface bordering the follicular cavity and the thyroid hormones are stored in the cavity conjugated to thyroglobulin.

The synthesis of the thyroid hormones can be summarized as follows:

1. Iodide uptake and concentration (blocked by thiocyanate and perchlorate).

2. Oxidation of iodide to iodine (blocked by propylthiouracil and carbimazole).

3. Iodination of tyrosine molecules in thyroglobulin by atomic iodine (also blocked by propylthiouracil and carbimazole).

4. Coupling of either two diiodotyrosine residues to form T_4 or of a diiodotyrosine and a monoiodotyrosine residue to form T_3.

Too much iodide also inhibits the biosynthesis of the thyroid hormones.

All the steps are stimulated by TSH acting through cyclic AMP. TSH also stimulates their secretion. The first step in secretion is the uptake of small globules of colloid into the follicle cells by pinocytosis. The globules then fuse with lysosomes and their contents are digested, thus liberating the thyroid hormones which diffuse out of the follicle cells into the blood.

Transport and Inactivation of Thyroid Hormones

Most of the circulating T_4 is bound to plasma proteins, mainly to thyroxine-binding globulin and to a lesser extent to prealbumin and albumin. T_3 does not appear to bind as tightly to plasma proteins as does T_3. As 95% of the protein-bound iodine (PBI) in plasma is associated with thyroid hormones, the PBI was used as a diagnostic test for thyroid function. Now the free T_3 and T_4 levels can be estimated and more reliance is placed on these tests because only the free form of the hormones is active.

The thyroid hormones are broken down in several tissues, particularly the liver and skeletal muscle. T_4 has a half-life of 7 days, T_3 about 1 day. Much of the iodide that is released is reclaimed but about 150 microg of iodide is lost in the urine and faeces daily and must be replaced in the diet.

Actions of Thyroid Hormones

(i) *Calorigenic actions.* One of the principal effects of the thyroid hormones is to stimulate oxidative metabolism and thereby increase the production of heat in warm-

blooded animals. They increase oxidative metabolism in all tissues of the body except the brain, lungs, spleen and sex organs. The increase in basal metabolic rate produced by a single injection of T_4 begins after a latency of several hours and lasts 9 days or more. The basal metabolic rate may increase by as much as 100% while after thyroidectomy it may fall to 50% of normal. T_3 and T_4 may cause a slight increase in body temperature but their actions are not of direct importance in acute responses to cold. It has been suggested that their action on oxidative metabolism is due to at least in part to stimulation of sodium pump activity.

(ii) *Effects on growth and development.* Thyroid hormones are essential for normal growth in childhood. They stimulate growth by a direct effect on tissues but they also have a permissive action on GH secretion.

(iii) *Effects on the nervous system.* The thyroid hormones are essential for normal myelination and development of the nervous system in childhood. In the adult, a deficiency of thyroid hormones may lead to listlessness and blunting of intelect; an excess of listlessness and hyperexcitability.

(iv) *Effects on reproduction.* An adequate secretion of thyroid hormones is necessary for the development of the gonads, for normal menstrual cycles and for lactation.

(v) *Other effects.* Often associated with excess production of thyroid hormones are an increased cardiac output and tachycardia. These symptoms are partly a direct response to the thyroid hormones, which increase sensitivity to catecholamines, and are partly secondary responses to increased demands for oxygen associated with their calorigenic action. The thyroid hormones have less well-defined effects on carbohydrate and lipid metabolism. They lower cholesterol concentration. The metabolism of protein is also affected by thyroid hormones, and hyperthyroidism may lead to wasting of skeletal muscle and a negative nitrogen balance. The thyroid hormones influence calcium metabolism and demineralization of the skeleton is common in severe hyperthyroidism.

They bind to specific receptors in the cell nuclei of target tissues and appear to act by controlling gene expression. It is not clear how the resulting increase in new protein synthesis leads to the characteristic actions of T_3 and T_4.

Control of Thyroid Hormone Secretion

For normal thyroid hormone secretion there must be adequate intake of iodide in the diet. The immediate stimulus for the release of thyroid hormone is TSH secreted by the anterior pituitary. TSH appears to stimulate every step in the production and secretion of thyroid hormones. In addition it controls the size and vascularity of the gland. If the pituitary is removed the thyroid atrophies.

Secretion of TSH is stimulated by TRH which is secreted by neurones in the hypothalamus and transported to the anterior pituitary in the hypophyseal portal vessels.

Disorders of Thyroid Gland Function

Hypothyroidism may result from disease of the pituitary or thyroid gland, or from insufficient iodine in the diet. Severe hypothyroidism in the adult is called *myxoedema* because of the puffiness of the hands and face due to an abnormal accumulation of mucoproteins in the subcutaneous layers. Other symptoms are low metabolic rate, bradycardia, cold intolerance, mental and physical lethargy and slow hoarse speech. Severe hypothyroidism in children results in the condition of cretinism.

Hyperthyroidism or *thyrotoxicosis* results from the overproduction of thyroid hormones and is characterized by a high metabolic rate, tachycardia, heat intolerance, hyperexcitability, restlessness and weight loss. A common form of hyperthyroidism is *Graves' disease* which is also characterized by protruding eyeballs (exophthalmia) and goitre formation. Graves' disease is caused by abnormal thyroid stimulators in the blood. Long-acting thyroid stimulator (LATS) was the first of these to be discovered and it is now clear that it is one of a group of autoantibodies called *thyroid stimulating antibodies* that are responsible for this condition.

Goitre formation is often associated with hyperthyroidism. However, it may also be a manifestation of hypothyroidism, in which there is a compensatory increase in TSH secretion, as occurs with iodine deficiency (endemic goitre) or with a high intake of naturally occurring goitrogens found in vegetables of the *Brassica* genus.

In the diagnosis of disorders of thyroid function the physician is guided by estimates of plasma levels of free hormones and TSH, by responses to test doses of TRH, and by the pattern of iodine uptake by the thyroid after administration of radioactive iodine.

8.4 The Parathyroid Glands and Calcium Metabolism

In mammals, endocrine tissue important for controlling calcium balance is located in the region of the thyroid gland. Four parathyroid glands are usually embedded in the dorsal surface of the lobes of the thyroid gland. They secrete *parathyroid hormone,* which raises plasma calcium and lowers plasma phosphate concentration. In addition, the parafollicular ŽCŽ cells of the thyroid gland secrete *calcitonin,* which lowers the plasma calcium concentration. In non-mammalian vertebrates the cells producing calcitonin form distinct glands (ultimobranchial bodies) but in mammals they form part of the thyroid gland. Another important regulator of calcium metabolism is an active metabolite of *vitamin D.*

Calcium Metabolism

Calcium has a number of essential physiological functions in the body including:

(i) maintenance of normal permeability of cellular membranes;

(ii) maintenance of normal excitability of nerve and muscle;

(iii) release of neurotransmitters, many hormones and exocrine secretions;

(iv) muscular contraction;

(v) formation of bone and teeth;

(vi) coagulation of blood;

(vii) production of milk; and

(viii) activity of many enzymes.

More than 98% of body calcium is found in bone. The concentration of calcium in plasma is approximately 2.5 mmol/L of which 1.5 mmol/L is ionized. The remainder is bound to plasma proteins and to anions such as citrate. Calcium concentration in the interstitial fluid is about 1.5 mmol/L reflecting the relative impermeability of capillary walls to the bound complexes. On a typical diet about 25 mmol (1 g) of calcium is ingested daily. Balance is maintained by excreting a comparable amount, mostly in the faeces. Both faecal and urinary losses are under hormonal control.

Phosphorus metabolism is regulated conjointly with calcium and so needs to be considered. About 80% of the 16 mmol (500g) of body phosphorus is contained in bone. Phosphorus occurs in a number of organic constituents of blood including lipids and nucleotids. It is also present in inorganic phosphate at a concentration of about 1 mmol/L of plasma. The solubility product of calcium and phosphate is such that the product of the concentrations of the free ions remains constant.

Intracellular Calcium

In cells, free cytosolic calcium is regulated at around 10^{-7} to 10^{-8} mmol/L. Cellular membranes generally have very low calcium permeabilities. Calcium diffusing into the cell from the interstitial fluid down its electrochemical gradient may be expelled either by active transport of by Na-Ca counter-transport. Most calcium in cells is compartmentalized or is bound to cellular constituents, such as membranes and binding proteins. One particular calcium-binding protein, *calmodulin,* plays an important role in the regulation of a variety of enzyme activities. Calcium may also be stored in the nucleus on binding proteins, or be exchanged across the endoplasmic reticulum or mitochondrial inner membrane. If free ionized cytosolic calcium is increased, both endoplasmic reticulum and mitochondria will rapidly accumulate the ion. Mitochondria may accumulate calcium at the expense of ATP formation causing calcium phosphate to precipitate. As a consequence the mitochondria swell and their function may be irreversibly damaged. This may be of major importance in determining the extent to which ischaemic tissue will recover if perfusion can be restored.

Bone

Bone is composed of an organic matrix of collagen in a 'ground substance' consisting largely of mucopolysaccharides and non-collagen proteins, on to which crystals of a complex salt of calcium and phosphate are deposited. Bone also contains Na, Mg, S, K, Cl, F, carbonate and citrate, often in non-exchangeable forms. Three types of cells appear to function in the formation and resorption of bone, namely osteoblasts, osteoclasts,

and osteocytes. The *osteoblasts* synthesize and secrete collagen fibres and promote the deposition of calcium phosphate crystals, while *osteoclasts* cause resorption of bone. Bone resorption depends on the destruction of collagen by lysosomal enzymes and phagocytosis, and on the dissolution of bone mineral by an increase in lactate and citrate production. *Osteocytes* are the most numerous cells in mature bone and are formed from osteoblasts. They appear to play an essential role in both bone maintenance and bone resorption depending on the plasma concentration of parathyroid hormone. Osteocytes are probably responsible for the early phases of bone resorption following an increase in parathyroid hormone while osteoclasts contribute to the delayed response. Only 1% of the calcium and phosphate of bone is in equilibrium with the extracellular fluid - the so-called 'exchangeable' pool - which acts to buffer small, short-term changes in blood calcium and phosphate. The remaining 99% of bone is not in equilibrium with the extracellular fluid and is referred to as 'non-exchangeable' bone. However, the 'non-exchangeable' bone is constantly being broken down and remodelled by the action of the bone cells which are regulated by parathyroid hormone and calcitonin as well as by several other hormones, especially during growth.

Vitamin D

Vitamin D is essential for proper bone development and a deficiency of this vitamin in children causes *rickets*, a disorder characterized by stunted growth and bowing of the limbs. In adults vitamin D deficiency can cause a failure of ossification (*osteomalacia*). Vitamin D is a steroid found in a limited number of foodstuffs, i.e., cod-liver oil, and is also synthesized in the skin by the action of ultraviolet light on a cholesterol derivative. The vitamin occurring naturally in animals is vitamin D_3 (cholecalciferol).

Cholecalciferol is now regarded as a *prohormone* because it is converted to active metabolites which act on the *gut* and *bone* to *increase* the concentrations of extracellular calcium and phosphate. It is first converted to a 25-hydroxy derivative in the liver and then, in the kidney, to 1,25-dihydroxycholecalciferol (1.25-DHCC), if extracellular concentrations of calcium or phosphate are low. 1,25-DHCC acts on the small intestine to promote the absorption of calcium and phosphate and, with parathyroid hormone, it also causes release of these ions from bone. However, in vitamin D deficiency insufficient calcium is absorbed in the gut and bone calcium is depleted. If extracellular concentrations of calcium and phosphate are normal, most of the vitamin is transformed in the kidney to the 24,25-dihydroxy and 1.24.25-trihydroxy derivatives which are probably intermediates in degradative pathways.

Parathyroid Hormone

In addition to the parathyroid glands situated adjacent to the dorsal surface of the thyroid gland, accessory parathyroid tissue is not uncommon in other parts of the neck. The *'chief' cells* of the parathyroid tissue secrete parathyroid hormone, a polypeptide of MW 9500. Removal of the parathyroid glands (and accessory tissue) causes plasma calcium o fall as much as 50% resulting in *hypocalcaemic tetany* which is characterized by extensive spasms of skeletal muscle. This can lead to asphyxiation due to laryngeal spasm.

Actions of Parathyroid Hormone

Parathyroid hormone *increases* ionized plasma calcium and *lowers* plasma phosphate concentration. It acts of the *bone*, the *kidney* and, indirectly, the *gastrointestinal tract.* Its actions on bone and kidney appear to be mediated by cyclic AMP. Parathyroid hormone increases the rate of bone resorption by stimulating the activity of osteocytes and osteoclasts. This effect is important in long-term regulation of plasma calcium but the actions of parathyroid hormone on the kidney appear to be more important in compensating for short-term changes. In the kidney parathyroid hormone increases the tubular reabsorption of calcium and decreases that of phosphate. It also stimulates the formation of 1,25-DHCC in the kidney. Thus the absorption of calcium in the gastrointestinal tract is increased as a consequence of an increase in 1,25-DHCC. In the long term increased secretion of parathyroid hormone may result in a net loss of calcium from the body through the kidney. Under these conditions the increased ionized plasma calcium increases the filtered load to an extent greater than that by which the reabsorption of calcium has been stimulated.

Control of Parathyroid Hormone Secretion

Parathyroid hormone secretion is regulated solely by the level of *plasma calcium* acting on the parathyroid glands and varies inversely with plasma calcium levels. A decrease in plasma calcium concentration causes an increase in secretion of parathyroid hormone and vice versa. Since parathyroid hormone increases extracellular calcium, further release is inhibited - a typical negative feedback mechanism.

Calcitonin

Calcitonin is a polypeptide (MW 3500) secreted by the *parafollicular 'C' cells* of the thyroid gland. It *decreases* plasma calcium by decreasing the rate of resorption of bone and by inhibiting the re-uptake of calcium in the kidney. The release of calcitonin is stimulated by an increase in plasma calcium. It has been suggested that calcitonin protects animals against hypercalcaemia. However, in mammals it takes large doses of calcitonin to lower plasma calcium and the physiological significance of this hormone is unclear.

Disorders of Calcium Metabolism

Hypocalcaemia causes excessive neuromuscular irritability. A rapid decrease of ionized plasma calcium to below 1 mmol/L results in spontaneous firing of peripheral nerves. On the afferent side this causes unusual sensations such as tingling (paraesthesia). On the efferent side muscular twitching, spasm and cramps can develop, these motor manifestations being called *manifest tetany.* If ionized calcium decreases more slowly or to a lesser extent, stimuli such as localized ischaemia, hyperventilation or pressure over a nerve my be required to elicit the motor events. This is termed *latent tetany.* A low ionized plasma calcium may be consequent upon decreased levels of parathyroid hormone or vitamin D in the body. It may also result from increased plasma pH, i.e., in respiratory alkalosis, due to the release of hydrogen ions from plasma proteins making additional negatively-charged binding sites available for calcium. Tetany may also occur when plasma-ionized magnesium concentration is reduced or when plasma potassium concentration is raised abruptly in potassium-depleted patients.

Hypercalcaemia is seen most frequently in hyperparathyroidism. Increased calcium mobilization from bone leads to painful softening and bending of bones, and the increased calcium and phosphate excretion in urine may result in nephrocalcinosis and renal stones. Additionally, increased calcium may cause headaches and decreased tone (hypotonia) in skeletal and intestinal muscle.

8.5 The Adrenal Glands

There are two adrenal glands, situated one on top of each kidney. Each adrenal gland comprises two endocrine organs - the *adrenal medulla* and the *adrenal cortex.* The two parts of the adrenal gland have different embryonic origins and are anatomically quite distinct. The adrenal medulla secretes *catecholamines* while the adrenal cortex secretes *corticosteroids.*

Adrenal Medulla

The medulla is a modified nervous tissue derived from the neural crest and can be regarded as a collection of postganglionic sympathetic neurones in which the axons have not developed. The catecholamines are produced in chromaffin cells which are of two types, one secreting *adrenaline* and the other *noradrenaline.* In man, adrenaline constitutes about 80% of the catecholamines produced by the medulla. Small collections of chromaffin cells are also located outside the adrenal medulla, usually adjacent to the chain of sympathetic ganglia.

In addition to catecholamines the adrenal medulla also contains enkephalins, dynorphin, neurotensin, somatostatin and substance P. The role of these adrenal peptides has not yet been elucidated although it is known that met- and leu-enkephalins are located within the adrenaline-containing chromaffin cells and are co-secreted with catecholamines.

The synthesis of catecholamines from tyrosine has been dealt with previously. The amines are stored in membrane-bound granules and their secretion is initiated by acetylcholine released from *preganglionic fibres* in the splanchnic nerves. Acetylcholine depolarizes the chromaffine cells causing calcium to enter and trigger the release of the granular contents by exocytosis.

Once released into the bloodstream the catecholamines have only a short half-life (minutes). They are rapidly taken up into extraneural tissues and degraded by catechol-O-methyltransferase (COMT), or into nerve terminals and degraded by monoamine oxidase (MAO), the degradation products eventually appearing in the urine.

Actions of Catecholamines

The actions of adrenaline and noradrenaline are complex and depend on their effects on the various subclasses of alpha and beta receptors which in their distribution differ from tissue to tissue. Noradrenaline causes widespread vasoconstriction and a marked increase in peripheral resistance while adrenaline causes vasoconstriction in skin and viscera but vasodilatation in skeletal muscles. Both catecholamines increase heart rate and contractility directly but in the intact animal the increase in peripheral resistance and

mean arterial pressure caused by noradrenaline administration leads to reflex bradycardia. Adrenaline has a more pronounced effect on metabolic processes and increases the basal metabolic rate, stimulates glycogenolysis and mobilizes free fatty acids. Catecholamines also cause bronchodilatation and relaxation of the gastrointestinal tract.

Control of Catecholamine Secretion

The secretion of catecholamines is initiated by sympathetic activity controlled by the hypothalamus and occurs in response to such stimuli as pain, excitement, anxiety, hypoglycaemia, cold and haemorrhage. Increased secretion is part of the 'fight or flight' reaction described by Cannon. In an emergency, catecholamines released by the adrenal medulla are disseminated in the bloodstream while noradrenaline released from sympathetic nerve terminals acts at discrete points in the body. In frightened or stressed animals there is a general increase in sympathetic activity in which the sympathetic nerves appear to play the dominant role because the removal of the adrenal medulla does not seriously impair an animal's ability to cope with stress.

Catecholamine-secreting tumours of the adrenal medulla, one type of which is known as *phaemochromocytoma,* can result in severe hypertension.

Adrenal Cortex

The adrenal cortex secretes *corticosteroids* which can be classified as follows:

1. *Glucocorticoids,* i.e., *cortisol* and *corticosterone,* which affect the metabolism of carbohydrates, fats and proteins.

2. *Mineralocorticoids,* mainly *aldosterone,* which are essential for the maintenance of sodium balance and extracellular fluid volume.

3. *Sex hormones,* mainly *androgens,* which may play a minor role in reproductive function, particularly as a source of androgens in the female, and are involved in growth at puberty.

The cortex is organized into three zones - the outer *zona glomerulosa,* which secretes aldosterone, the middle *zona fasciculata,* which secretes mainly glucocorticoids, and the inner *zona reticularis,* which secretes mainly androgens. Removal of the pituitary causes the fasciculata and reticularis zones to atrophy but has little effect on the zona glomerulosa.

The gluco- and mineralocorticoids contain 21 carbon atoms and the androgens 19. They are synthesized from cholesterol and acetate in reactions that take place in the mitochondria and the cytoplasm. The adrenal cortex contains the highest concentration of ascorbic acid of any tissue in the body but its role in steroidogenesis is unknown. There is an appreciable storage of corticosteroids in the adrenal cortex and the rate of release corresponds to the rate of synthesis. In man approximately 20 mg of cortisol, 3 mg of corticosterone and 0.2 mg of aldosterone are secreted per day. The adrenal cortex also secretes significant amounts of androgens, particularly dehydroepiandrosterone (but no testosterone).

The corticosteroids are transported in the circulation mostly bound to plasma proteins (90% of glucocorticoids, 60% of aldosterone). The bound form acts as a reserve and protects the steroids from degradation which takes place mainly in the liver. Circulating glucocorticoids have a half-life of approximately 1 h whereas aldosterone, of which less exists in the bound form, has a half-life of approximately 20 min.

Actions of Glucocorticoids

They play an important role in the control of the *intermediary metabolism* of carbohydrate, fat, protein and purines throughout the body. Their modes of action are poorly understood but in many instances they appear to have a 'permissive' action on the effects of other hormones.

(i) *Effects on intermediary metabolism.* They promote glycogen storage in the liver by stimulating both *glycogenesis* and *gluconeogenesis.* The main substrates for gluconeogenesis are amino acids derived from protein breakdown in skeletal muscle. In addition to stimulating gluconeogenesis, glucocorticoids also stimulate *protein catabolism* in skeletal muscle and excess production of glucocorticoids causes severe muscle wasting. The glucocorticoids are also *diabetogenic* in that they raise blood glucose, effectively by inhibiting glucose uptake in muscle and adipose tissue. They also enhance *fatty acid mobilization* from adipose tissue either by direct action or indirectly by potentiating the lipolytic effects of catecholamines and growth hormone.

(ii) *Maintenance of normal circulatory function.* Glucocorticoids are essential for the maintenance of normal myocardial contractility and vascular resistance. Their action on the vasculature is a permissive one in that they potentiate the vasoconstrictor effects of catecholamines.

(iii) *Adaptation to stress.* The way in which the body adapts to stress is not well understood but cortisol appears to play an important role. It is known that the release of cortisol increases during stress and that, in patients with adrenocortical insufficiency, stress factors such as heat, cold, infection or trauma, can cause hypotension and death.

In addition they have some mineralocorticoid activity and this may be of significance because of their comparatively high secretion rate. In large doses the glucocorticoids suppress the *immune response.* They decrease the number of circulating lymphocytes and eosinophils, cause involution of the thymus and lymph nodes, and depress the antibody response. Therefore synthetic corticosteroids are used therapeutically to suppress rejection of transplanted organs and to treat allergies. They also have *anti-inflammatory* properties and are used in the treatment of rheumatoid arthritis and related diseases.

Control of Glucocorticoid Secretion

The release of cortisol and corticosterone (and androgens) is controlled by ACTH which is released in response to CRH. A negative feedback mechanism operates in which cortisol inhibits the release of ACTH by actions on the hypothalamus or the pituitary. The

level of plasma cortisol follows a diurnal pattern, peak levels occurring in the morning just before waking.

In addition to stimulating the release of glucocorticoids which enhance fat mobilization, ACTH increases fat mobilization by a direct action on adipose tissue.

Actions of Mineralocorticoids

Aldosterone is the main mineralocorticoid produced by the adrenal cortex. It acts chiefly on the distal tubules of the *kidney* to promote the *reabsorption of Na* in exchange for K and H ions which are excreted. Excess production of aldosterone with retention of Na leads to expansion of ECF volume and hypertension. Adrenalectomy leads to a fall in extracellular Na, hypotension and eventually death.

Control of Mineralocorticoid Secretion

ACTH is not the major regulator of aldosterone secretion, in contrast to the other corticosteroids, but it does play a supportive role. The primary regulator of aldosterone secretion appears to be angiotensin II produced by the renin-angiotensin system. An increase in plasma K or a fall in Na concentration also stimulate the release of aldosterone by the adrenal cortex.

Disorders of Adrenocortical Function

Addison's disease is due to a generalized adrenocortical insufficiency, the cause of which is often obscure. This disease usually develops slowly and is characterized by lethargy, weakness, weight loss and hypotension. A sudden stress can precipitate a crisis requiring emergency medical treatment. A common feature of this disease is hyperpigmentation of the skin due to excessive secretion of ACTH leading to stimulation of melanocyte activity in the skin. The elevation in ACTH levels is brought about by removal of the negative feedback provided by cortisol. Patients with Addison's disease require replacement therapy with both a glucocorticoid and a mineralocorticoid.

Cushing's syndrome is a condition associated with excess secretion of glucocorticoids resulting from excess ACTH production, tumours of the adrenal cortex or over-administration of glucocorticoids in the course of therapy. This disease is characterized by redistribution of body fat ('moon-face'), severe muscle wasting, a predisposition to diabetes and hypertension. A diagnosis of Cushing's syndrome is indicated if plasma cortisol is elevated throughout the day and if ACTH secretion is not suppressed by low-dose administration of the potent glucocorticoid drug, dexamethasone.

Conn's syndrome, or primary aldosteronism, is due to excess mineralocorticoid secretion caused by a tumour of the adrenal cortex. This leads to K depletion and Na and water retention, resulting in hypertension, muscle weakness, tetany and hypokalaemic alkalosis.

Adrenogenital syndrome is associated with excessive androgen secretion, which may cause masculinization in the female and precocious puberty in the male. This may be due to

an androgen-secreting tumour or it may be congenital. The latter is known as *congenital hyperplasia* in which one of the enzymes involved in cortisol synthesis is deficient. This leads to increased ACTH secretion by the pituitary and hence excess production of adrenal androgens. Treatment with glucocorticoids corrects the deficiency and also suppresses excess ACTH secretion.

8.6 The Endocrine Pancreas

The pancreas is both an endocrine and an exocrine gland. The endocrine portion of the pancreas is localized in the *islets of Langerhans* which constitute only 2% of the mass of the pancreas. *Insulin* is produced in the B (beta) cells and *glucagon* in the A (alpha) cells of the islets. Recently it has been discovered that a third hormone, *somatostatin,* is also produced in the islets from D (delta) cells. Insulin was successfully extracted by Banting and Best in 1921 from the dog pancreas after they had first depleted if of proteolytic enzymes by ligating its exocrine ducts. The disease known as *diabetes mellitus* is due to a deficiency of insulin or to insulin resistance.

Insulin

Insulin is a small protein (M 6000) consisting of two peptide chains, called A and B, which are linked by two disulphide bonds. The A chain contains 21 amino acid residues and the B chain 30 residues. Beef and pig insulin differ from human insulin in only a few residues and both are used in the treatment of diabetes mellitus. Insulin is synthesized as a larger single polypeptide pre-proinsulin, which is cleaved soon after synthesis to form *proinsulin.* Proinsulin is packaged into vesicles and converted to insulin by cleavage of a connecting peptide to form two peptide chains. Insulin is released from thee cell by exocytosis in response to an increase in blood glucose. Once released into the bloodstream insulin has a half-life of only a few minutes as it is rapidly metabolized in the liver and kidneys.

Actions of Insulin

Insulin *lowers blood glucose levels* by facilitating the uptake of glucose into muscle and adipose tissue. It has powerful anabolic effects and *stimulates* the synthesis of *glycogen, fat* and *protein.* Although insulin is known to act by increasing the uptake of glucose and amino acids into cells, this simple explanation is not sufficient to explain all the effects of insulin. Some of its effects such as an increase in glycogen synthesis, a decrease in glycogenolysis and a decrease in fat mobilization may be due to a reduction in cyclic AMP. Insulin also increases K uptake into cells and consequently *lowers plasma K.*

Control of Insulin Secretion

An increase in plasma *glucose concentration* provides the major stimulus for insulin secretion. There is an initial rapid phase of secretion followed by a second slower phase of sustained secretion. Some *amino acids,* such as arginine, are also potent stimulators of insulin release. After feeding, the level of insulin may rise even before that of blood glucose because gastrointestinal hormone can also stimulate insulin release. Other potent stimuli for insulin release are *glucagon, growth hormone* and thee *sulphonyl urea drugs,* such as tolbutamide,

which are used in the treatment of mild cases of diabetes. *Adrenaline* inhibits insulin release and so too does *somatostatin.* Somatostatin produced in the islets of Langerhans probably plays an important role in the local control of insulin secretion. Neural control is also important; parasympathetic activity enhances insulin release while sympathetic activity inhibits it.

Glucagon

Glucagon is a polypeptide (M 3000) which is secreted by the pancreas in response to low blood glucose. In contrast to insulin, it *increases blood glucose* levels. Glucagon acts on the liver to stimulate glycogenolysis and gluconeogenesis and its action is mediated by cyclic AMP. Glucagon also has a pronounced lipolytic effect in adipose tissue and has a positive inotropic effect on the heart.

Control of Energy Utilization and Storage

The brain uses glucose almost exclusively as an energy source and it is necessary to maintain an adequate blood glucose concentration at all times or convulsions and coma will ensue. Blood glucose is maintained at fairly constant levels of 4-6 mmol/L by the interactions of several hormones, namely *insulin, glucagon, growth hormone, adrenaline* and *cortisol.* Only insulin lowers blood glucose levels, while the actions of other hormones tend to oppose the actions of insulin and are said to be 'diabetogenic'. During the *absorptive state* following a meal, there are adequate glucose supplies and this causes the secretion of insulin which enhances the utilization of glucose and the storage of energy as glycogen and fat. During thee *post-absorptive or fasting state* blood glucose falls and insulin secretion decreases in relation to that of the diabetogenic hormones. The ratio of insulin to glucagon is probably the most important factor in controlling the shift from the absorptive to the post-absorptive state. This ensures that during fasting adequate glucose levels are maintained for the brain by gluconeogenesis in the liver and by glucose-sparing reactions in other tissues.

Effects of Insulin Deficiency

Diabetes mellitus is a major health problem which affects about 2% of the population. A predisposition to diabetes is inherited but the genetic factors are complex. Two types of diabetes are recognized clinically - juvenile-onset (Type I) and maturity-onset (Type II). Type I patients (10-12% of diabetics) have low plasma insulin and require injections of insulin. Type II patients may have normal or elevated levels of insulin but show decreased sensitivity to insulin, often correlating with a reduction in insulin receptor concentration. Type II patients are often obese and generally show improvement with weight reduction. Diabetes can be simulated in experimental animals by treatment with alloxan which destroys the B cells of thee islets of Langerhans.

By 'diabetes mellitus' is meant the passing of sweet urine. The capacity of the renal tubules to reabsorb glucose actively is exceeded and glucose spills over into the urine. The loss of so much solute causes an *osmotic diuresis* resulting in polyuria and polydipsia. Large amounts of salts are consequently lost and this can lead to dehydration and hypotension. The blood concentration of free fatty acids is raised and their metabolism produces ketones which

cause *metabolic acidosis.* If left untreated the patient may become unconscious (*diabetic coma*). The administration of too much insulin to relieve this condition can also lead to coma (*insulin coma*) because of the sudden lowering of blood glucose and the brain's dependence on glucose as an energy source.

There are also long-term effects of the disease; in particular atherosclerosis in the heart, retina, brain and kidney is common. Diabetics are slower to heal and more prone to develop gangrene.

In the diagnosis of diabetes the physician is guided by the fasting blood glucose concentration and the presence of glucose and ketones in the urine. A *glucose tolerance test,* in which changes in the blood glucose are measured in response to oral administration of glucose, provides a more definite indication. In diabetics the blood glucose rises higher in response to a glucose load and returns to baseline more slowly than in normal subjects.

8.7 Appendix

Hormone Assay

There are three methods for assaying hormone concentrations in serum or urine - bioassay, radioimmunoassay and radioreceptor assay - although some hormones, i.e., catecholamines and steroid hormones, may be estimated by chemical analysis.

Bioassay

This involves quantifying the responses of tissues to various concentrations of a hormone (standard or unknown) either *in vitro* or *in vivo.* For example human chorionic gonadotrophin was formerly assayed by its ability to induce ovulation in rabbits. A standard curve is plotted and the concentration of the unknown is extrapolated from it. Such assays are often cumbersome to perform and variable in their response. However they do measure the biological activity of the hormone.

Radioimmunoassay

This assay, now widely used, depends on the availability of radioactively-labelled hormone and on antibodies that react with it. Peptide hormones can be labelled with ^{125}I to a high degree of specific activity and steroid hormones with ^{3}H or ^{14}C. Antibodies to the larger peptide hormones are prepared by immunizing experimental animals against these molecules. Antibodies to smaller polypeptides, steroid hormones (and even to cyclic AMP) can also be produced if those molecules are first made antigenic by attaching them to proteins.

A competition assay is set up in which the standard or unknown hormone competes with the labelled hormone for a sit on the antibody. The more unlabelled hormone present the less labelled hormone is bound to the antibody. The hormone-bound complex is then separated from the free hormone by various physico-chemical means (i.e., filtration, precipitation, charcoal exclusion) and the radioactivity of the hormone-bound complex

determined. A standard curve is prepared and the concentration of the unknown is extrapolated from this.

One of the advantages of the radioimmunoassay is that it is extremely sensitive. However it does not necessarily measure the biological activity of a hormone and it can detect precursors and degradation products of a hormone, thus leading to overestimation of hormone concentration.

Radioreceptor Assay

This is similar to the radioimmunoassay but instead of an antibody it uses a receptor of the hormone for binding. Theoretically such assays are ideal because they simulate the first step in the action of a hormone but in practice they are not as sensitive as radioimmunoassays.