PART I
REVIEW OF THE THYROID GLAND
Development of thyroid gland

The earliest stage of development of the thyroid gland is indicated as a median thickening of the entoderm in the pharyngeal floor between the level of 1st. and 2nd pharyngeal pouches. This area shows as a single median outgrowth in the furrow immediately caudal to the tuberculum impar by about four and a half weeks. The upper end of the duct is known as foramen caecum of the tongue in later life. It extends downwards as a tubular duct. By the early part of the 7th week it lies at the level of the laryngeal primordium. The lower level of this duct bifurcates and subsequently divides into series of double cellular plates. By the 8th week these cellular plates show a tubular arrangement and cell clusters become apparent. The bifurcated lower end of the duct ultimately gives rise to the isthmus and portions of the lateral lobes of the thyroid gland. By the 14th week primary thyroid follicles are filled with homogenous colloid. The thyroid starts functioning by this time since the gland at this stage can concentrate radiiodine. (Chapman, 1948). The pyramidal lobe is probably a secondary upward extension from the developing isthmus. On the grounds of comparative anatomy Davis and Davis (1962) failed to substantiate the contribution of the 4th pharyngeal pouch to the formation of lateral lobes of the thyroid gland.
The thyroid gland is a ductless, endocrine organ. It appears brownish red in colour and is highly vascular. It occupies the region between the 5th cervical to first thoracic vertebra. It weighs between 20-25 g. (Keele and Neil, 1961).

It consists of two lateral lobes connected medially by isthmus. Each lobe is conical and measures 5 cm. in length 3 cm. in width and 2 cm. in thickness. The isthmus measures 1.25 cm. in diameter. From the upper part of the isthmus or from the adjacent part of either lobe, another portion of the gland extends upwards towards the hyoid bone. This is conical in shape and is therefore called the pyramidal lobe.

Histology of thyroid gland

Microscopically the thyroid gland consists of groups of lobules. Each lobule is enclosed in a thin connective tissue capsule from which septa subdivide the lobule incompletely into groups of follicles. The follicles of the adult thyroid tissue are generally closed spherical sacs. They are lined by the basement membrane which is usually well defined in older individuals. Sommers and
Meissner (1954) described the basement membrane as "closely attached to the outer margin of the follicular epithelial cells. The membranes appeared as sharp, continuous, narrow, homogenous dark lines bounding each follicle". Stuart and Allan (1958) also demonstrated the normal basement membrane by silver stain and described this as a continuous unbroken lining adherent to the epithelial cells and this forms a complete barrier between the follicular epithelium and the capillary vessels. The thickness of the basement membrane usually varies between 0.1 \mu to 0.2 \mu. Thus, the follicular colloid is completely separated from the lymphoreticular system.

Each follicle measures 0.2 to 0.9 mm. in diameter (Maximow and Bloom, 1957). They are lined by a single layer of low cubical epithelium. Follicles are of small and large sizes. Nearly all have a lumen containing a homogeneous eosinophilic material called colloid. Normally in human beings small follicles are more numerous than the large follicles. Follicular lining cells usually have a pale eosinophilic cytoplasm and a large spherical basal or central vesicular nucleus containing one or more nucleoli. These cells are rich in enzymes. They remain in close relation to connective tissue and capillaries. The change in shape from low cubical to tall columnar epithelium
may take place during the functional activity of the gland. The inner margin of the epithelium sends cytoplasmic projections inside the colloid. Sometimes follicles may contain cells with deep eosinophilic cytoplasm and dark blue pyknotic nucleus. These are colloid cells of Langendüff (Maximow and Bloom, 1957).

The colloid is the homogeneous eosinophilic material, occupying the lumen, but may exhibit patchy basophilia (Finerty and Cowdry, 1960). The colloid is secreted into the lumen after being formed in the follicular epithelial cells, as a colourless unstainable material and subsequently modified to be stained with basic dyes. Later on, it acquires its affinity for acidic stains (Carleton & Short, 1957). The colloid contains both iodinated and non-iodinated proteins as determined from microchemical analysis, ultraviolet absorption studies and also from radioactive iodine study.

Finerty and Cowdry (1960) actually correlated the morphological changes of the colloid with the functional status of the gland. They observed that the density of the colloid varies inversely as the functional status and directly with the homogeneity of the colloid. Colloid is transported across the cells. This has been demonstrated
by Nadler, Sarkar and Leblond (1962) by electron microscopy. During the hyperactive state the colloid shows marginal scalloping characterised by round uniform vacuolated areas, distributed along the peripheral margins of the colloid. Follicular epithelium may become tall columnar at the site of the marginal scalloping. In the hypofunctional stage, the epithelium becomes flattened, colloid becomes eosinophilic without marginal scalloping. Vacuoles are probably the empty spaces which are formed as a result of pinocytosis by the follicular epithelial cell (Nadler, Sarkar and Leblond, 1962). Some colloid staining material may even appear intraepithelially during the functional stage.

Interstitial tissue:

This consists of the loose interfollicular connective tissue containing blood vessels and lymphatics. Histiocytes and lymphocytes are occasionally present. The connective tissue shows qualitative alteration with the functional status of the gland. It increases in hyperactive states and decreases during the hypoactive phase. Blood vessels and lymph capillaries are also influenced similarly and regress after the gland returns to the normal state.
Modell (1953) demonstrated arteriovenous communications and muscular cushions at places where arteries branch. He also attributed regulation of blood flow through the gland by the muscular cushions.

**Physiology of thyroid gland**

The thyroid gland is mainly concerned with intrathyroidal iodine metabolism. Thyroid follicular cells extract iodine from the circulation as iodide, concentrate it in the cells and secrete the final product into the colloid. Accumulation of inorganic iodide from the circulation and organification of the accumulated iodine from inorganic iodide to the proteins are the main steps in hormonogenesis (Pitt-Rivers and Trotter, 1953).

Synthesis of thyroglobulin takes place within the cells. Rankin (1941) demonstrated in the pig the formation of protein-bound-iodine in fetal thyroid cells prior to appearance of colloid. Nadler, Leblond and Carnerio (1960) demonstrated thyroglobulin synthesis intra-cellularly in the follicular epithelium with the help of autoradiographic technique. They suggested that thyroglobulin, after it is iodinated, is secreted into the follicle. Steps of hormonogenesis in the acinar cells could be postulated in the following way (Diagram 1). Thyroid draws iodine from
THE CYCLE OF IODIDE METABOLISM

Diag. 1
the plasma in the circulation. Iodide is concentrated within the follicular cells. It is then oxidised by the enzyme peroxidase. This enzyme peroxidase was first demonstrated histochemically by Dempsey (1944) and was subsequently confirmed by DeRobertis and Grasso (1946) in follicular cells. Alexander (1959) further demonstrated the existence of enzyme peroxidase by indirect means, since this enzyme could be blocked by thiocyanate group of drugs. Hydrogen peroxide is the substrate of iodide peroxidase. Hydrogen peroxide is derived in the wake of auto-oxidation of reduced flavin nucleotide. Flavin nucleotide is reduced by reduced triphosphopyridine nucleotide. The reduced triphosphopyridine nucleotide could be derived from within follicular cells, during glucose-6-phosphate dehydrogenase activity (Degroot and Davis, 1961; and Schussler et al., 1961). Iodide peroxidase oxidises iodide to a reactive 'higher valence state'. Their intermediate compounds would iodinate tyrosine either in its free or peptide-linked form. This is one of the pathways of iodination of tyrosine and is not enzyme dependant. Degroot and Davis (1962) recently isolated soluble iodide-peroxidase-tyrosine iodinase derived from mitochondria and microsomes of the follicular cells. This also helps organification of iodine to tyrosine to form
mono- and diiodotyrosine (MIT and DIT). The iodination of tyrosine is an orderly process. The ratio of mono-iodo and di-iodo forms is maintained constant by a regulating mechanism at the cellular level. After mono- and di-iodo tyrosines are formed, tri-iodothyronine appears within the cells after a short while in a measurable quantity (Degroot and Davis, 1961). That tri-iodothyronine is newly formed is evident from the fact that thyronine does not form a constituent of thyroid. Tri-iodothyronine is formed by 'coupling' of mono- and di-iodotyrosine. Similarly, two molecules of di-iodotyrosine couple into tetra-iodothyronine or thyroxine. Iodothyronine molecules are formed by joining the iodinated hydroxyphenyl group of one iodi tyrosine residue to the phenolic hydroxy group of another iodo tyrosine. This 'coupling reaction' is an extremely complicated reaction since this requires a transfer of large residue between the two peptide-linked amino acids.

Thyroglobulin acts as a ground substance within which iodo tyrosines are coupled to form iodothyronines. This thyroglobulin is an iodoprotein. Its molecular weight is approximately 650,000. It belongs to a class of glycoprotein, since it contains 40% of carbohydrate. It contains an excess of dicarboxylic amino acids. Thyroglobulin contains 10% arginine, 3% tyrosine, and 3% cysteine. During electrophoresis
in barbital buffer (pH 8.6) thyroglobulin is localised in interalpha fractions ahead of globulin towards cathode (Wynn, 1960). In starch gel electrophoresis several bands are obtained. These bands were thought to be due to impurities. But Spiro (1961) also observed several bands in starch gel electrophoresis with pure thyroglobulin. She suggested that thyroglobulin has the tendency to aggregate into multiples of a basic unit or to dissociate into dimers depending on the pH and ionic constituents in the solution of thyroglobulin (Spiro, 1961). Minor differences that are present are usually due to soluble iodinated and non-iodinated proteins which are distinct from thyroglobulin. One thyroglobulin molecule carries 110 tyrosyl residues (Robbins and Rall, 1960) and one thyroxine residue. Only a few tyrosyl residues are iodinated. Triiodothyronine is more scarce; it is not present in all thyroglobulin residues. Iodide deficiency alters di-iodotyrosine and mono-iodotyrosine ratio in thyroglobulin by altering the extent of iodination of tyrosyl groups. In iodide deficiency there is a relative increase in mono-iodotyrosine and tri-iodothyronine in thyroglobulin molecule. Larger amount of triiodothyronine is formed, perhaps to tide over the iodide deficiency. Reserve thyroxine in the normal gland can tide over the relative iodine deficiency of shorter duration. MIT and DIT ratio is also
increased in thyrotoxicosis and in multinodular goitre nodules (Pitt Rivers et al., 1957). The probable mechanism has not yet been elucidated. Thyroglobulin presents unique species specific antigenicity. Triiodothyronine is formed on the surface of thyroglobulin molecule where coupling of monoiodotyrosine and diiodotyrosine takes place. This triiodothyronine might also be formed by deiodination of thyroxine in the tissues (Keele and Neil, 1961). Thyroxine and Triiodothyronine after being formed on the surface of thyroglobulin molecule are stored in thyroglobulin in the follicle. Due to their linkage to thyroglobulin by hydrogen bond, they are not deiodinated by dehalogenase if present in the colloid. Under the influences of proteases (DeRobertis, 1941, McQuillan and Trikojus, 1953, Hadad and Rall, 1960) thyroglobulin containing the hormones are broken into larger fragments (McQuillan et al., 1961, and Litonjua, 1961). These larger fragments are hydrolysed by peptidases (Laver and Trikojus, 1956) into smaller fragments which are the transport forms.

Residual iodothyrosines released from thyroglobulin are deiodinated completely by an iodothyrosine deiodinase within the thyroid and is normally used again within the gland.
"S-L" protein constitutes 2-4% of the iodoprotein in normal gland. It is similar to albumin in electrophoretic mobility but is different immunologically from albumin. It contains iodothyronins and iodothyrosines. It passes rarely into the circulation and may appear in urine in peptide-linked forms.

A third iodoprotein is recognised which is distinct from previous iodoproteins. It has been demonstrated in vivo and in vitro and is clearly attached to mitochondria. It could be a precursor of thyroglobulin.

The thyroid hormones, thyroxine and triiodothyronine, in the smaller fragments are carried to their sites of action closely linked to plasma protein. These circulating thyroxine and triiodothyronine are simple amino acids as shown in two dimensional chromatography in Kieselguhr column (Gross & Pitt Rivers, 1953). Thyroxine and triiodothyronine circulate as components of peptide. Thyroxine is more intimately related to thyroglobulin than triiodothyronine. Triiodothyronine being loosely bound to plasma protein per se is more easily available to exert peripheral actions and is therefore 5 times more potent than thyroxine. During electrophoresis thyroxine moves with a specific protein migrating between alpha 1 and alpha 2.
globulin (Gordon et al., 1952) and is termed as thyroxine-binding globulin (TBG). Ingbar (1958) has demonstrated a second thyroxine-binding protein localised in prealbumin in both paper and starch-gel electrophoresis. The function of this thyroxine binding prealbumin (TBPA) is still disputed.

Thyroxine and Triiodothyronine bound to plasma protein could be precipitated out from plasma as protein bound iodine. The normal range is 4-8 micrograms per 100 ml. of plasma. This level is lowered to the range of 0.5 - 2.5 micrograms per 100 ml. of plasma in hypothyroidism and elevated to the extent of 14-30 micrograms per 100 ml. of plasma in hyperthyroidism.

Nature of hormone secreted by the thyroid:

Thyroxine and triiodothyronine are the hormones secreted by thyroid.

Thyroxine is usually bound to globulin and is located in the interalpha region in plasma protein electrophoresis in veronal buffer pH 8.6 (Gordon et al., 1952; Wynn, 1960). When this primary binding site is saturated, it is bound to prealbumin. Secondary association between thyroxine and other plasma proteins, including albumin, might also be present. A small fraction of thyroxine might
circulate in a free state in equilibrium with bound thyroxine so that when the concentration of bound thyroxine rises, free thyroxine concentration also rises. This free thyroxine has been suggested as a major regulating factor in the fractional turnover of thyroxine though such evidences are still lacking (Robbins and Boll, 1957; Ingbar and Fienkel, 1967).

Tri-iodothyronine has a low affinity for thyroxine binding globulin but no affinity for thyroxine binding prealbumin.

These protein hormone interactions influence the peripheral metabolism of thyroid hormone.

**Fate of thyroid hormones:**

Triiodo-thyroxine is completely used up in peripheral actions of the thyroid. When thyroxine binding globulin is saturated due to raised thyroxine level of the serum, thyroxine becomes loosely bound to albumin from which it is easily removed to be excreted in bile. Thus biliary excretion may help to regulate the concentration of thyroxine in plasma. Thyroxine is mostly destroyed in body and is excreted in bile either in free form or glucuronide form (Myant, 1958). Small amounts of glucuronide is excreted in the faeces.
Peripheral actions of the thyroid hormone:

The actions of the thyroid hormones are on the general metabolism and on the activity of different enzymes.

Action on General metabolism

The thyroid hormone increases oxygen consumption of heart, liver, muscle, kidney and blood cells. This hormone also has a calorigenic action. The hormone stimulates glycogenolysis, oxidation of sugar and reduces sugar tolerance. Enzymes of Kreb's cycle, though present in the thyroid, appear inactive due to hexose-monophosphate pathway (Dumont, 1960). Pentose phosphate pathway is stimulated by peripheral action of this hormone.

Thyroid hormone increases phospholipid and nucleic acid turnover in liver and controls excretion of cholesterol in bile. It also regulates the level of cholesterol in serum, induces negative nitrogen balance due to excretion of urea.

It exerts no effect on serum phosphorus level though the excretion of phosphorus is increased in urine and stool. It removes calcium from bones.
Thyroid helps conversion of carotene to Vitamin A. Brill & Truat (1947), Johnson & Bauman (1947), Kelley & Day (1948) demonstrated that administration of carotene to hypothyroid animals could not prevent iodine deficiency. On the other hand Concha et al. (1950) observed that administration of thyroid by oral route increased Vitamin A level and decreased carotene level in blood. They suggested that probable conversion of carotene to Vitamin A is helped by the thyroid hormone.

Thyroid hormone influences bone marrow activity as evidenced by presence of anemia only in hypothyroid individuals, which is corrected by thyroxine administration.

**Action on Enzymes**

The activities of hexokinase, TPNH, cytochrome C reductase, cytochrome oxidase and other respiratory enzymes are stimulated. Thyroxine inhibits DPN linked malic, lactic, glutamic and alcohol dehydrogenase. Due to inhibition of malic dehydrogenase, oxaloacetate does not accumulate in tissues.

Thyroxine breaks glutamic dehydrogenase into smaller units. Thyroid also reacts with the enzyme at structural positions involved in enzyme activation and enzyme metabolic activity.
Thyroxine causes selective inhibition of oxidative phosphorylation. Normally glucose oxidation is accompanied by conversion of adenosine diphosphate to triphosphate with storage of energy. With thyroxine this conversion to ATP from ADP is impaired, so usually greater amounts of substrate and oxygen require to be consumed to provide the same amount of energy.

Thyroxine stimulates adenosine triphosphatase which releases heat and dissipates high energy phosphate bonds. So more substrate and energy is needed to provide the same source of energy.

Thyroxine inhibits transhydrogenase reaction which inhibits the oxidation of reduced triphosphopyridine nucleotide and accordingly results in generation of high energy phosphate bonds.

Thyroxine induces mitochondrial swellings which result in loss of pyridine nucleotides causing decreased oxidative phosphorylation.