Chapter 1

REVIEW OF LITERATURE

THYROID GLAND & ITS REGULATIONS

Anatomy and histology of the thyroid gland

The thyroid, from the Greek ‘thyreoeides’, meaning shield shaped, is made up of two lobes and a bridging ‘isthmus’. The thyroid gland is a highly vascularised organ located anteriorly in the neck, deep to the platysma, sternothyroid and sternohyoid muscles, and extending from the 5th cervical (C5) to the 1st thoracic (T1) vertebrae. The gland consists of two lobes (left and right) connected by a thin, median isthmus overlying the 2nd to 4th tracheal rings, typically forming an "H" or "U" shape. Embryologically, the thyroid gland develops as a thickening in the pharyngeal floor that elongates inferiorly as the thyroglossal duct, dividing into two lobes as it descends through the neck (Kim et al., 2013). The capsule sends projections into the thyroid forming septae and dividing it into lobes and lobules. Dense connective tissue attachments secure the capsule of the thyroid to both the cricoids cartilage and the superior tracheal rings (Zoeller, 2007).

The thyroid gland is a hormone secreting organ, it is highly vascularised. It receives its blood supply from the superior and inferior thyroid arteries (Xiao et al. 2002). The inferior thyroid artery is a branch of the thyrocervical trunk that arises from the subclavian arteries. It ascends behind the carotid sheath to enter the thyroid posteriorly. The superior thyroid artery is usually the first branch of the external carotid artery.

The fundamental functional unit of the thyroid gland is the follicle cells and their diameter is in the range of 100-300 μm. The gland is organized into spherical follicles whose walls are composed of epithelial cells that surround a lumen filled with colloid (Whittow, 2000). Each follicle is lined by a single layer of cells of endodermal origin. The shape of these cells, together with the size and shape of the follicles, depends upon the activity of the thyroid gland (Hodges, 1974). Follicle cells in the thyroid gland create a lumen, and there exists a protein named thyroglobulin that they synthesize in the colloid in this lumen. The apical part of these follicle cells makes contact with colloidal lumen and its basal part with blood circulation through rich capillaries. Thus, thyroid hormones easily pass into circulation and can reach target tissues (Di Lauro and Defelice, 2004; Dillmann, 2004).

On electron microscopy, the thyroid follicular epithelium has many features in common with other secretory cells and some peculiar to the thyroid. From the apex of the
follicular cell, numerous microvilli extend into the colloid. It is at or near this surface of the cell that iodination, exocytosis and the initial phase of hormone secretion, namely colloid resorption occur (Ericson, 1990). The nucleus has no distinctive features and the cytoplasm contains an extensive endoplasmic reticulum laden with microsomes. The endoplasmic reticulum is composed of a network of wide irregular tubules that contain the precursor of Tg. The carbohydrate component of Tg is added to this precursor in the Golgi apparatus which is located apically. Lysosomes and mitochondria are scattered throughout the cytoplasm. Stimulation by TSH results in enlargement of the Golgi apparatus, formation of pseudopodia at the apical surface and the appearance in the apical portion of the cell of many droplets that contain colloid taken up from the follicular lumen.

THYROID HORMONE SYNTHESIS AND SECRETION

Overview

The function of the thyroid is to generate the quality of the thyroid hormone necessary to meet the demands of the peripheral tissues. This requires the daily thyroidal uptake of sufficient iodide and its oxidation by thyroid peroxidase (TPO) to allow the synthesis of approximately 110 nmoles (85 µg) of T₄, which is 65% iodine by weight. This requires the synthesis of a 660-kd glycoprotein homodimer, Tg. Tg contains specific tyrosine residues that are then iodinated at the apical portion of the thyroid cell to form mono- and diiodotyrosine (MIT and DIT). TPO-catalyzed coupling of two molecules of DIT, or one of DIT and one of MIT, leads to formation of T₄ and T₃, respectively, which are then stored as colloid, still as part of the Tg molecule. Pinocytosis of stored colloid leads to the formation of phagolysosomes, the colloid droplets in which Tg is digested, releasing T₄, T₃, DIT and MIT as the droplet is translocated toward the basal portion of the cell. Thyroxine and T₃ exit the cell into the capillaries, and DIT and MIT are deiodinated by an iodotyrosine deiodinase to allow recycling of the iodide to iodinate newly synthesized Tg. The synthesis of thyroid hormones requires the expression of a number of thyroid cell–specific proteins. In addition to Tg and TPO, the TSH receptor is also required to transduce the effects of extracellular TSH for efficient hormone synthesis. Several thyroid cell–specific proteins—thyroid transcription factors 1 and 2 (TTF-1 and TTF-2) and PAX-8 stimulate transcription of the Tg and TPO genes. One or more of these proteins may also influence expression of the TSH receptor (Missero et al., 1998; Damante et al., 2000).
Iodine availability and transport

Formation of normal quantities of thyroid hormone requires the availability of adequate quantities of exogenous iodine to allow thyroidal uptake of about 60 µg daily, taking into account the fecal losses of about 10 to 20 µg iodine of iodothyronines as glucuronides and about 100 to 150 µg as urinary iodine in iodine sufficient populations. Plasma iodide (I⁻), the form of the element in biologic solutions, is completely filterable with about 60% to 70% of the filtered load reabsorbed passively. At least 100 µg of iodine per day is required to eliminate all signs of iodine deficiency. Milk, meat, vitamin preparations, medicines, radio contrast material, and skin antiseptics are important sources of iodine. Even in a single area, iodine intake varies among different individuals and in the same individual from day to day (Larsen et al., 1998).

Uptake of iodine by the thyroid

The concentration of iodide in plasma is extremely low; a mechanism is required for the thyroid cell to concentrate the required amounts of this element. This process, called iodide trapping, is accomplished by a membrane protein, the sodium-iodide symporter (NIS) (Kosugi et al., 1998; De La et al., 2000). Human NIS is a 643 amino acid protein with 13 membrane-spanning domains. NIS-mediated I⁻ accumulation is a Na⁺-dependent active
transport process that couples the energy released by the inward translocation of Na\(^+\) down to its electrochemical gradient to the simultaneous inward translocation of I\(^-\) against its electrochemical gradient. The maintenance of the Na\(^+\) gradient acting as the driving force is insured by (Na\(^+\)-K\(^+\))-ATPase. NIS belongs to the sodium/glucose cotransport family as the SLC5A5 member. Iodide transport is energy-dependent and requires O\(_2\). Ouabain, digitoxin, thiocyanate and other cardiac glycosides block transport \textit{in vitro} (Wolff, 1960; Tyler \textit{et al.}, 1968). Iodide uptake by thyroid cells is dependent on membrane ATPase. During gland hyperplasia, iodide transport usually varies concordantly with plasma membrane Na\(^+\)-K\(^+\)-activated, ouabain-sensitive ATPase activity (Brunberg and Halmi, 1966). Transcription of the NIS gene is increased by TSH. The mechanism for this has not been completely elucidated, but studies of the rat NIS promoter suggest that there is an NIS upstream enhancer, which confers a cyclic adenosine monophosphate (cAMP) response but also contains binding sites for the thyroid specific transcription factors PAX-8 and TTF-1, as well as a degenerate cAMP response element sequence (De La \textit{et al.}, 2000).

Following active transport across the basolateral membrane, iodide is translocated across the apical membrane by pendrin, the PDS gene product, which is a chloride/iodide transporter (Bidart \textit{et al.}, 2000; Everett \textit{et al.}, 1997). Pendrin, encoded by the PDS gene (Everett \textit{et al.}, 1997) and composed of 780 amino acids, is expressed in different organs including kidney, inner ear and thyroid. In the thyroid, pendrin is a 110kDA membrane glycoprotein (Porra \textit{et al.}, 2002), selectively located at the apical plasma membrane (Bidart \textit{et al.}, 2000). Its activity as transporter of anions including iodide has been demonstrated in different experimental systems (Yoshida \textit{et al.}, 2002; Gillam \textit{et al.}, 2004). Pendrin belongs to the SLC family under the reference SLC26A4. However, the implication of pendrin in thyroid iodide transport remains uncertain for several reasons.

Iodide that enters the thyroid remains in the free state only briefly before it is further metabolized and bound to tyrosyl residues in Tg. A major fraction of the intrathyroidal free iodide pool comes from deiodination of MIT and DIT; this iodide is either recycled within the thyroid or leaked into the circulation. Some data suggest that iodide entering the gland by active transport segregates from that generated by deiodination of Tg within the gland (Hildebrandt \textit{et al.}, 1979; Rosenberg \textit{et al.}, 1961). In contrast, NIS activity is sensitive to both iodine availability and TSH stimulation, and transport rather than intrathyroidal binding is the controlling factor in making iodide available for hormonogenesis.
The thyroid is not the only organ to concentrate iodine; the others endowed with this capacity are salivary glands, gastric mucosa, mammary glands, and choroid plexus. Ductal cells of the salivary glands express NIS (Jhiang et al., 1998).

**Iodide Oxidation and Organification of Iodide**

**Role of thyroperoxidase (TPO)**

After concentrating iodide in thyroid, iodide participates in a series of reactions that lead to the synthesis of the active thyroid hormones. The first of these involves oxidation of iodide and incorporation of the resulting intermediate into the hormonally inactive iodotyrosines MIT and DIT, a process termed organification. The process requires the presence of iodide, a peroxidase (TPO), a supply of H$_2$O$_2$, and an iodine acceptor protein (Tg). The iodinations that lead to formation of iodotyrosines occur within Tg rather than on the free amino acids. Oxidation of thyroidal iodide is mediated by the heme-containing protein TPO. The complementary DNA (cDNA) for human TPO encodes 933 amino acids with a molecular size of 103 kd, 10% of which is due to carbohydrate. The protein contains a membrane spanning region near the COOH terminus, and it is oriented in the apical membrane of the thyroid cell with residues 1 to 844 in the follicular lumen (Yokoyama and Taurog, 1988).

In vitro, TPO, in the presence of H$_2$O$_2$, iodinates Tg as well as other proteins. The reaction catalyzed by peroxidase in vitro has many properties of the iodination reaction in vivo, including inhibition by PTU and MMI and by high concentrations of iodide (Wolff-Chaikoff effect) (Wolff and Chaikoff, 1948). The evanescent product of the peroxidation of iodide (i.e. the active iodinating form) may be free hypoiodous acid, iodine, or iodinium (I$^+$). The H$_2$O$_2$ that serves as the oxidant of iodide is generated through the auto-oxidation of flavin enzymes acting as NADH - and particularly NADPH - oxidases. In this way, generation of H$_2$O$_2$ is linked to electron transfers due to substrate oxidations within the thyroid. Radioautographic and histochemical evidence suggests that the iodination reactions occur at the cell colloid interface (Izumi and Larsen, 1977). Thus mitochondrial systems provide a source of H$_2$O$_2$, cell membranes contain TPO, and the cytoplasmic fraction the regulatory inhibitors of organic iodinations.
Fig 2. Schematic representation of the membrane topology of Thyroperoxidase, TPO (A) and NADPH thyroid oxidase, ThOX (Duox) (B) at the apical plasma membrane of thyrocytes. (C) hypothetical reaction scheme for TPO. \( \text{H}_2\text{O}_2 \) is presumed to oxidize the free enzyme with a loss of two electrons leading to the formation of complex I. Iodide binds to complex I, is oxidized and form complex II, which then reacts with a tyrosyl residue of Tg, Tyr-Tg. The newly-formed \( \text{I}^0 \) and Tyr\(^\text{0}\)-Tg free radicals interact to form MIT-Tg and the enzyme returns to its free state. I\(_2\) may be generated from two oxidized iodine atoms (Izumi and Larsen, 1977).

**\( \text{H}_2\text{O}_2 \) GENERATING SYSTEM**

Thyroid peroxidase requires \( \text{H}_2\text{O}_2 \) for its oxidative function. It was already suggested in 1971 that \( \text{H}_2\text{O}_2 \) would be produced at the apical plasma membrane of the thyrocyte by an enzyme that requires calcium and NADPH originating from the stimulation of the pentose phosphate pathway (Bjorkman et al., 1984). Further biochemical studies showed that the enzymatic complex producing \( \text{H}_2\text{O}_2 \) for TPO is a membrane-bound NADPH-dependent flavoprotein (Deme et al., 1985; Dupuy et al., 1989).

The molecule for \( \text{H}_2\text{O}_2 \) production in the thyroid gland has been known as dual oxidase 2 (DUOX2). Duox proteins are localized at the apical plasma membrane of the thyrocyte as fully glycosylated forms (~190kDa) and in the endoplasmic reticulum as high
mannose glycosylated forms (~180kDa). Both DUOXs contain intracellular EF-hands and respond to increase in intracellular calcium by a marked activation. The DUOX molecule contains peroxidase-like and NADPH oxidase-like domains. Human thyroid gland also contains DUOX1 that shares 83% similarity with the DUOX2 gene. However, thyroid DUOX1 protein appears to play a minor role in \( \text{H}_2\text{O}_2 \) production. DUOX proteins require DUOX maturation or activation factors (DUOXA1 or 2) for proper translocation of DUOX from the endoplasmic reticulum to the apical plasma membrane, where \( \text{H}_2\text{O}_2 \) production takes place (Ohye and Sugawara, 2010).

Recently, NADPH oxidase 4 (NOX4), a homolog of the NOX family, was added as a new intracellular source of reactive oxygen species (ROS) in the human thyroid gland. These enzymes produce \( \text{H}_2\text{O}_2 \) or the \( \text{O}_2^\cdot \) superoxide, which is rapidly converted to \( \text{H}_2\text{O}_2 \) by superoxide dismutases. The role of the various NOXs has been recently reviewed (Dumont et al., 2005; Geiszt, 2006; Quinn et al., 2006). Intracellular \( \text{H}_2\text{O}_2 \) is also generated by intracellular metabolism, for instance by mitochondria and peroxisomes presumably as a by product. TSH stimulates NADPH oxidase activity and \( \text{H}_2\text{O}_2 \) production and this effect is mediated through cAMP. \( \text{H}_2\text{O}_2 \) generation in \textit{in vitro} system appears to be regulated by iodine. At low concentrations and short-term incubation, iodide increases \( \text{H}_2\text{O}_2 \) production whereas at high iodide concentrations \( \text{H}_2\text{O}_2 \) generation is inhibited (Deleu et al., 2000).

**THYROGLOBULIN (Tg)**

Thyroglobulin is the most abundant protein in the thyroid gland; its concentration within the follicular lumen can reach 200-300 g/L. Its main function is to provide the polypeptide backbone for synthesis and storage of thyroid hormones (Dunn and Dunn, 2000). It also offers a convenient depot for iodine storage and retrieval when external iodine availability is scarce or uneven. Newly synthesised Tg polypeptide chains entering the lumen of the rough endoplasmic reticulum (RER) are subjected to core glycosylation, dimerise and are transferred to the Golgi where they undergo terminal glycosylation. Iodination and hormone formation of Tg occur at the apical plasma membrane-lumen boundary and the mature hormone-containing molecules are stored in the follicular lumen, where they make up the bulk of the thyroid follicle colloid content.

**Thyroglobulin iodination and hormone synthesis**

The step preliminary to thyroid hormone formation is the attachment of iodine to tyrosyl residues in Tg to produce MIT and DIT. This process occurs at the apical plasma
membrane-follicle lumen boundary and involves H$_2$O$_2$, iodide, TPO, and glycosylated Tg. All rendezvous at the apical membrane to achieve Tg iodination.

First, iodide must be oxidized to an iodinating form. An extensive literature has sought to identify the iodinating species, but the issue is still not resolved for a detailed review. One scheme proposes that oxidation produces free radicals of iodine and tyrosine, while both are bound to TPO to form MIT which then separates from the enzyme. Further reaction between free radicals of iodine and MIT gives DIT. Experimental studies by Taurog, (2000) and others suggest that the TPO reduction occurs directly in a two electron reaction. A second proposal, based on studies of rapid spectral absorption changes (Ohtaki et al., 1982a; 1982b), is that TPO$^{-}\Gamma^+$ is the iodination intermediate and that the preferred route is oxidation of TPO by H$_2$O$_2$ followed by two electron oxidation of $\Gamma$ to $\Gamma^+$, which then reacts within a tyrosine. As a third possibility, Taurog, (2000) proposed a reaction between oxidized TPO and $\Gamma$ to produce hypoiodite (OI$^-$), which also involves a two electron reaction. Whatever the precise nature of the iodinating species, it is clear that iodide is oxidized by H$_2$O$_2$ and TPO, and transferred to the tyrosyl groups of Tg. All tyrosine residues of Tg are not equally accessible to iodination. The molecule has about 132 tyrosyl residues among its two identical chains; at most, only about 1/3 of the tyrosyls are iodinated.

The final step in hormone synthesis is the coupling of two neighbouring iodotyrosyl residues to form iodothyronine. Two DIT form T$_4$; one DIT and one MIT form T$_3$. Coupling takes place while both acceptor and donor iodotyrosyl are in peptide linkage within the Tg molecule. The reaction is catalyzed by TPO, requires H$_2$O$_2$ (Cahnmann et al., 1977; Deme et al., 1978; Virion et al., 1981; Virion et al., 1985) and is stringently dependent on Tg structure (Lamas and Taurog, 1977). The generation of the iodothyronine residue involves the formation of an ether bond between the iodophenol part of a donor tyrosyl and the hydroxyl group of the acceptor tyrosyl. After the cleavage reaction that gives the iodophenol, the alanine side chain of the donor tyrosyl remains in the Tg polypeptide chain as dehydroalanine (Gavaret et al., 1980; Gavaret et al., 1981). Observations both in vivo and in vitro show an appreciable delay in coupling after initial formation of iodotyrosines. A typical distribution for a Tg containing 0.5% iodine (a normal amount for iodine-sufficient individuals) is 5 residues MIT, 5 of DIT, 2.5 of T$_4$ and 0.7 of T$_3$ (Dunn and Dunn, 2000). More iodine increases the ratios of DIT/MIT and T$_4$/T$_3$, while iodine deficiency decreases them.
**Hormone storage**

Tg molecules vectorially delivered to the follicular lumen by exocytosis accumulates to reach uncommon concentrations i.e. 0.3-0.5 mM. The mechanism operating such a “packaging” is unknown. Water and ion extraction from the follicle lumen might represent an active process leading to Tg concentration. As the follicle lumen is a site of Ca\(^{++}\) accumulation (Haeberli *et al.*, 1978; Fonlupt *et al.*, 1997), the high degree of compaction of luminal Tg might depend on electrostatic interactions between Ca\(^{++}\) and anionic residues of Tg, which is an acidic protein. Stored Tg molecules undergo iodination and hormone formation reactions at the apical plasma membrane-lumen boundary (Haeberli *et al.*, 1978; Ekholm, 1981; Wollman and Ekholm, 1981), where TPO and H\(_2\)O\(_2\) generating system reside. The mature Tg molecules, now containing MIT, DIT, T\(_4\) and T\(_3\), remains extracellular in the lumen of thyroid follicles. Turnover of intra-follicular material or so-called colloid varies greatly with gland activity. When the turnover increases, less Tg is stored, and with extreme hyperplasia, none is evident and the entire organic iodine content may be renewed daily (De Groot, 1966). In this situation, secretion of Tg and resorption of Tg probably occur at similar rates and only tiny amounts of intra-follicular material are present at any time.

Thyroglobulin as usually isolated from the thyroid is chiefly the 19S 660kDa dimer that has been glycosylated and iodinated. Iodination and hormone formation of Tg is more complex than generally thought because of the slow diffusion of molecules that are in a colloidal state in the follicle lumen. It has been reported that TSH alters the hydrodynamic properties of intra-follicular Tg molecules (Ofverholm and Ericson, 1984; Gerber *et al.*, 1985). There is evidence for the presence of insoluble Tg in the form of globules of 20-120 microns, at a protein concentration of almost 600 mg/mL, in the lumen of thyroid follicles of different animal species (Herzog, 1983). Insoluble Tg has many internal crosslinks through disulfide bonds, dityrosine and glutamyl-lysine bonds, the latter generated by transglutaminase (Saber-Lichtenberg *et al.*, 2000). The formation of Tg multimers that probably results from oxidative processes might be limited by the presence of molecular chaperones such as the protein disulfide isomerase (PDI) and BiP in the follicle lumen (Delom *et al.*, 2001).

**Thyroglobulin endocytosis**

To be useful, thyroid hormones must be released from Tg and delivered to the circulation for action at their distant target tissues. Depending on numerous factors including the supply of iodide as substrate, the activity of enzymes catalyzing hormone formation, the
concentration and physico-chemical state of Tg - the hormone content of lumenal Tg molecules varies to a rather large extent. Tg molecules newly arrived in the follicle lumen with no or a low hormone content would co-exist with “older” Tg exhibiting up to 6-8 hormone residues. The downstream processes responsible for the production of free thyroid hormones from these prohormonal molecules must therefore adequately manage the use of these lumenal heterogeneous Tg stores to provide appropriate amounts of hormones for peripheral utilization.

The way the thyroid follicle proceeds to generate free hormones from stored hormone containing Tg molecules has been known for a long time. Tg molecules are first taken up by polarized thyrocytes and then conveyed to lysosomal compartments for proteolytic cleavage that release T₄ and T₃ from their peptide linkages. The first step represents the limiting point in the thyroid hormone secretory pathway. Over the last decade, there has been substantial improvement in the knowledge of the cellular and molecular mechanisms governing the internalization or endocytosis and intracellular transport of the prohormone, Tg. The evolution has first been to consider that it could proceed via a mechanism different from phagocytosis, also named macropinocytosis, evidenced in rats under acute TSH stimulation. Results obtained in rats and dogs have been for a long time extrapolated to the different animal species including human. There is now a number of experimental data indicating that in the thyroid of different species under physiological circumstances, basal internalization of Tg, mainly if not exclusively, occurs via vesicle-mediated endocytosis or micropinocytosis (Marino and McCluskey, 2000), while macropinocytosis results from acute stimulation (Rocmans et al., 1978; Unger et al., 1978).

The internalization process starts with the organization of microdomains at the apical plasma membrane of thyrocytes; these microdomains or pits, resulting from the recruitment and assembly of proteins (clathrin, adaptins etc.) on the cytoplasmic side of the membrane, invaginate to finally generate coated vesicles after membrane fission. Lumenal Tg molecules, either free or associated to membrane proteins acting as Tg receptors, enter the pits and are then sequestrated into the newly-formed vesicles (Pierce et al., 1985; Bernier-Valentin et al., 1990; Bernier-Valentin et al., 1991). Tg internalization via vesicle-mediated endocytosis is regulated by TSH (Bernier-Valentin et al., 1991). The vesicles lose their coat and, through a complex fusion process, deliver their content into a first type of endocytic compartments, the early apical endosomes (Kostrouch et al., 1993). In these compartments, Tg molecules probably undergo sorting on the basis of recognition of different physico-chemical parameters either linked or independent such as the hormone content, exposed carbohydrates,
conformation of peptide domains; a step of sorting appears as a prerequisite for subsequent differential cellular handling of Tg molecules.

It has been shown that internalized Tg molecules can follow different intracellular pathways. Part of Tg molecules are conveyed via a vesicle transport system to the second type of endocytic compartments, late endosomes or prelysosomes. This route ending to lysosomes corresponds to the Tg degradation pathway for the generation of free thyroid hormones. It is reasonable to think that Tg molecules following this route are the more mature molecules (with a high hormone content) but, this has not been firmly demonstrated. The other Tg molecules with no or a low hormone content, present in early apical endosomes, enter either of the two following routes; they are recycled back into the follicle lumen through a direct vesicular transport towards the apical plasma membrane (Kostrouch et al., 1993) or via a two-step vesicular transport to the Golgi apparatus and then to the apical plasma membrane (Miquelis et al., 1993). Alternately, Tg molecules are transported and released at the basolateral membrane domain of thyrocytes via transcytotic vesicles (Herzog, 1983; Romagnoli and Herzog, 1991); a process accounting for the presence of Tg in plasma. The orientation of Tg molecules towards one or the other of these three routes requires the presence of receptors.

Following endocytosis, endocytotic vesicles fuse with lysosomes, and proteolysis is catalyzed by cathepsin L- and D-like thiol proteases, all of which are active at the acidic pH of the lysosome. The iodotyrosines released from Tg are rapidly deiodinated by an NADPH-dependent iodotyrosine deiodinase and the released iodine is recycled. The T4 is released from Tg, but it is not clear how its transfer into the plasma is regulated. Release is acutely stimulated by TSH, as may be the 5’-monodeiodination of small amounts of T4 to T3 by the types 1 and 2 iodothyronine deiodinases (D1 and D2), which are both expressed in human thyroid (Laurberg et al., 1984; Salvatore et al., 1996; Gereben et al., 2001).

Tg proteolysis and T4 release are inhibited by several agents, the most important of which is iodide. Inhibition of hormone release is responsible for the rapid improvement that iodide induces in hyperthyroid patients. Increasing iodination of Tg also increases its resistance to hydrolysis by acid proteases in the lysosomes. Lithium also inhibits thyroid hormone release in circulation (Wu et al., 1982).
THYROID HORMONES IN PERIPHERAL TISSUES

Plasma transport

The metabolic transformations of thyroid hormones in peripheral tissues determine their biologic potency and regulate their biologic effects. Consequently, an understanding of thyroid physiopathology requires knowledge of the pathways of thyroid hormone metabolism. A wide variety of iodothyronines and their metabolic derivatives exist in plasma. Of these, T₄ is highest in concentration and the only one that arises solely from direct secretion by the thyroid gland. In normal people, T₃ is also released from the thyroid but about 80% is derived from the peripheral tissues by the enzymatic removal of a single 5’-iodine atom (outer ring or 5’-monodeiodination) from T₄ (Bianco et al., 2002). The remaining iodothyronines and their derivatives are generated in the peripheral tissues from T₄ and T₃. Principal among them are 3, 3’, 5’-triiodothyronine (rT₃) and 3, 3’-diido-L-thyronine (3, 3’-T₂). Trace concentrations of other diiodothyronines, monoiodothyronines and conjugates thereof with glucuronic or sulfuric acid are also present (Curran and De Groot, 1991; Findlay et al., 2000). Deaminated derivatives of T₄ and T₃ that bear an acetic acid rather than an alanine side chain (tetra and tria) are also present in low concentrations.

The major iodothyronines are poorly soluble in water and thus bind reversibly to plasma proteins. The plasma proteins with which T₄ is mainly associated are TBG and transthyretin (TTR), formerly termed T₄-binding prealbumin (TBPA) and albumin. About 75% to 80% of T₃ is bound by TBG, with the remainder bound by TTR and albumin.

Thyroxine-Binding Globulin

TBG is a glycoprotein with a molecular mass of about 54 kDa, about 20% of which is carbohydrate. The gene that encodes the protein is on the X chromosome (Schussler, 2000). The protein sequence of TBG resembles that of the SERPIN family of serine antiproteases (Grasberger et al., 1999; Buettner et al., 1999). The TBG molecule has a single iodothyronine-binding site with affinity slightly higher for T₄ than T₃ (Hocman, 1981). The molecule caries T₄ in a surface pocket held by a series of hydrophobic interactions with underlying residues and hydrogen bonding of the aminopropionate of T₄ with adjacent residues. Optimal binding activity requires the presence of the L-alanine side chain, an unsubstituted 4’- hydroxyl group, a diphenyl either bridge and halogen (I or Br) constituents at the 3, 5, 3’ and 5’ positions (Cody, 1980).
**Transthyretin (TTR)**

TTR exists in part as a complex with retinol (vitamin A) binding protein, hence its name. It consists of four identical polypeptide chains, with a total molecular mass of approximately 55 kDa and is not glycosylated. TTR binds 1 mole of T₄ with high affinity and a second T₄ molecule is bound with lower affinity at high concentrations of T₄ (Bartalena, 1990). Binding of T₄ by TTR is independent of the association with retinol-binding protein. Its half-life in plasma is normally about 2 days, but this decreases during illness (Surks and Oppenheimer, 1964). TTR circulates in blood as a stable tetramer of identical subunits, each containing 127 amino acids (Tsuzuki *et al*., 1985). Each TTR subunit has 8 β-strands four of which form the inner sheet and four the outer sheet. The four subunits form a symmetrical β-barrel structure with a double trumpeted hydrophobic channel that traverses the molecule forming the two iodothyronine binding sites. Despite the apparent identity of the two iodothyronine binding sites, TTR usually binds only one T₄ molecule because the binding affinity of the second site is greatly reduced through a negative cooperative effect (Irace and Edelhoch, 1978). Despite the 20-fold higher concentration of TTR in serum relative to that of TBG, it plays a lesser role in iodothyronine transport. In the presence of normal levels of TBG, wide fluctuations in TTR concentration or its removal from serum by specific antibodies has little influence on the concentration of free T₄ (Woeber and Ingbar, 1968).

**Lipoproteins**

Lipoproteins bind T₄ and to some extent T₃ (Freeman and Pearson, 1969; Benvenga and Robbins, 1993). The affinity for T₄-binding is similar to that of TTR. These proteins are estimated to transport roughly 3% of the total T₄ and perhaps as much as 6% of the total T₃ in serum. The binding site of apolipoprotein A1 is a region of the molecule that is distinct from that portion which binds to the cellular lipoprotein receptors and the physiological role of such binding is still unclear.

**Albumin**

The affinity of albumin for T₄ and T₃ binding is much lower than that of either TBG or TTR, but the high concentration of this protein results in the binding of 10% of the plasma thyroid hormones. Changes in albumin concentration per se have little influence on the total hormone levels unless they are accompanied by alterations in TBG and TTR, all three of which are synthesized in the liver. Hepatic failure or nephrotic syndrome leads to a decreased
plasma concentration of all three and the albumin concentration serves as a surrogate for estimating TBG concentrations (Sunthornthepvarakul et al., 1998).

**Free Thyroid Hormones**

Because most of the circulating $T_4$ and $T_3$ are bound to TBG, its concentration and degree of saturation are the major determinants of the free fraction of $T_4$. Binding of the thyroid hormones to the plasma proteins alters their metabolism. The negligible urinary excretion of $T_3$ and $T_4$ is due to the limited filterability of the hormone-binding protein complexes at the glomerulus. The volume of distribution and rate of turnover of the hormones are also affected by their protein associations. In vitro, the interaction between the thyroid hormones and their binding proteins conforms to a reversible binding equilibrium that can be expressed by conventional equilibrium equations (Izumi and Larsen, 1977).

It is the free hormone that is available to the tissues for intracellular transport and feedback regulation that induces its metabolic effects and that undergoes degradation. The bound hormone acts merely as a reservoir. It follows that the concentration of the free hormone is the determinant of the metabolic state and it is this concentration that is defended by homeostatic mechanisms. If an increase in the overall net binding affinity for $T_4$ occurs, the free $T_4$ concentration can be maintained at normal levels only if the bound $T_4$ increases. The plasma concentration of $T_4$ is determined by its rate of entry into and exit from, the plasma. The metabolic clearance rate relates the quantity of $T_4$ removed from the plasma per unit time to the quantity available for removal that is, its plasma concentration (Woeber and Ingbar, 1968).

**Thyroid Hormone Activation and Inactivation by the Selenodeiodinases**

The most important pathway for $T_4$ metabolism is its outer ring (5’) monodeiodination to the active thyroid hormone, $T_3$. This reaction is catalyzed by type 1 and type 2 deiodinases (D1 and D2). Inner ring deiodination, catalyzed primarily by type 3 deiodinase (D3), inactivates $T_4$ and $T_3$ (St. Germain and Galton, 1997; Bianco et al., 2002). These reactions can be considered physiologically activating and inactivating pathways that control $T_3$ concentrations in peripheral tissues. The structures of the three human deiodinases are similar to one another and are conserved from tadpoles to humans (Bianco et al., 2002). All three contain selenocysteine in the active catalytic centre and hence are termed selenodeiodinases. Selenocysteine has nucleophilic properties that make it ideal for catalysis of oxidoreductive reactions such as iodothyronine deiodination and the reduction of $H_2O_2$ by another family of...
selenoenzymes, the glutathione peroxidases (Berry and Larsen, 1992; Toyoda et al., 1997). Selenium acts as the iodine acceptor during deiodination reactions.

Figure 3. Pathways for thyroid hormone activation and inactivation catalyzed by human Iodothyronine selenodeiodinases. Numbers refer to the iodine positions in the iodothyronine nucleus. The iodothyronine deiodinases are abbreviated D1, D2, and D3 for types 1, 2, and 3 deiodinases, respectively. Arrows refer to monodeiodination of the outer or inner ring of the iodothyronine nucleus, termed 5’ or 5 by convention (Williams Textbook of Endocrinology, 2008).

**Type I deiodinase (D1)**

Type I deiodinase (D1) is expressed mainly in liver, kidney and thyroid. Among the nonsulfated conjugates, rT3 is by far the preferred substrate, the ORD of which is orders of magnitude faster than the deiodination of any other iodothyronine (Hennemann and Visser, 1997). Therefore, it is not surprising that D1 is probably the primary site for the clearance of plasma rT3. Although it catalyzes the conversion of T4 to T3 much less effectively, D1 is supposed to be the major source of circulating T3 (Visser, 1988; Kohrle et al., 1991; Larsen and Berry, 1995). The conjugated compounds T4 sulfate and T3 sulfate are preferred substrates for IRD (St. Germain and Galton, 1997). Dithiothreitol (DTT) is the in vitro cofactor and D1- catalyzed deiodination is sensitive to inhibition by PTU (Oppenheimer et al., 1972). Thyroid hormone-induced stimulation of D1 activity is exerted at the
transcriptional level (Berry et al., 1990; Berry et al., 1991), which in the human Dio1 gene can be attributed to the presence of 2 thyroid hormone response elements (TREs) in the 5’ flanking region (FR) of the gene (Toyoda et al., 1995; Zhang et al., 1998).

Type II deiodinase (D2)

Type II deiodinase (D2) is an obligate ORD and the preferred substrate of D2 is T₄. Unlike D2, the role of D2 is in local T₃ production. Hypothyroidism increases and hyperthyroidism decreases D2 activity. Regulation is predominantly posttranslational through substrate-induced enzyme inactivation, involving ubiquitination and degradation in the proteasome. D2 activity is expressed in pituitary, brain, and brown adipose tissue. D2 is particularly important in the brain, where it produces more than 75% of the nuclear T₃ in the cerebral cortex in the rat (Crantz et al., 1982). D2 activity in skeletal muscle could serve as a source of extrathyroidally generated plasma T₃ (Salvatore et al., 1996a). Furthermore, thyroidal D2 activity in patients with Graves’ disease and follicular adenomas may give rise to the relative increase in thyroidal T₃ production seen in these cases as well as in iodine deficiency (Salvatore et al., 1996a). While D2 mRNA was detected in human heart, no D2 activity was found (Salvatore et al., 1996b). However, in a recent paper D2 mRNA as well as D2 activity was reported to be present in mouse and rat heart with an increase in D2 mRNA expression and/or activity in hypothyroidism (Wagner et al., 2003).

Type III deiodinase (D3)

Type III deiodinase (D3) is an obligate IRD with T₃ as the preferred substrate. It is the major T₃ and T₄ inactivating deiodinase, catalyzing their conversion to 3, 3’-T₂ and to rT₃, respectively. D3 is expressed in placenta, pregnant uterus, brain, human embryonic liver and infantile hepatic and cutaneous human hemangiomas and adult vascular tumor (Santini et al., 1999; Galton et al., 1999; Bernal, 2002). D3 activity in the latter two was so high, that the inactivation rate of thyroid hormone by D3 in the tumor exceeded the secretory capacity even of the TSH-stimulated normal thyroid gland, resulting in a hypothyroidism. This clinical picture was referred to as consumptive hypothyroidism after its nature of origin. Its main function in thyroid hormone homeostasis is to protect tissues from an excess of active hormone. Regulation of D3 is less obvious; whereas T₃ positively regulates D3 activity at the transcriptional level in brain, no regulation could be observed in placenta. This indicates that this gene is differently responsive to T₃ in different tissues (Mori et al., 1995; Roti et al., 1982).
Mechanism of Thyroid Hormone Action

Thyroid hormone acts by binding to a specific nuclear DNA-bound thyroid hormone receptor (TR), usually as a hetero-dimer with retinoid X receptor (RXR) at specific sequences thyroid hormone response elements (TREs) dictated by the DNA binding-site preferences of the RXR-TR complex.

Nuclear receptor binding sites

In humans, two TR genes (α and β) are found on different chromosomes (TRα on chromosome 17, TRα on chromosome 3). The active proteins are TRα-1 and TRs β1, β2 and β3 (Williams, 2000). The structure of the TRs conforms to a protein with three major functional domains, one binding DNA, one binding ligand and two major transcriptional activation domains. These activation domains of the TRα and β are similar to but not identical with, the major differences in the amino-terminal portion of the molecule. In general, TRβ, particularly TRβ-2, is thought to be important in the hypothalamus and pituitary gland, where regulation of thyroid function occurs (Abel et al., 1999). In addition to differences in the amino-terminus between TRβ-1 and β2, the two proteins are under the regulation of different promoters that can function in tissue-specific patterns. TRβ-2 is down-regulated by T₃, whereas TRβ-1 mRNA expression is not affected (Forrest et al., 1996). TRβ-2 is also expressed in the cochlea. TRβ-1 is expressed in all tissues, although its mRNA is especially highly expressed in the kidney, liver, brain and heart. TRα-1 mRNA is also expressed in the brain and at lower levels in skeletal muscle, lungs and heart. TRβ-3 mRNA is expressed at very low levels but is more abundant in the liver or kidneys and lungs in comparison with other tissues.

Receptor specifically binds T₃. Under physiological conditions, 90% of receptors bound hormone is T₃; the affinity of nuclear receptor for T₃ is 10 fold more than that of T₄ (Samuels and Tsai, 1973). These receptors bind to the specific TRE sites on DNA in the absence of T₃ unlike the case with steroid hormone receptors. The TREs are located near, generally upstream with respect to the start of transcription, to the promoter where transcription of specific thyroid hormone responsive genes is initiated. T₃ binding to the receptors result in stimulation or in case inhibition of the transcription of these genes with consequent changes in the levels of mRNA transcribed from them. The changes in mRNA levels alter the level of the protein product of these genes. These proteins then mediate the thyroid hormone response (Greenspan, 1994).
**Extra nuclear binding sites**

In addition to the nuclear receptor mediated actions, there are several well characterized non genomic actions of thyroid hormones, including these occurring at the level of the plasma membrane (Davis and Davis, 1996) and on the cellular cytoarchitecture. There are well characterized thyroid hormone binding sites on the mitochondria (Sterling, 1989). In several of those processes T\(_4\) is the hormone that produces the response. The overall contribution of the extra nuclear sites to cellular regulation by thyroid hormone is likely to be minor.

![Diagram of thyroid hormone activation and inactivation](image)

Figure 4. Schematic diagram of thyroid hormone activation and inactivation in a cell expressing D2 and D3, such as an astroglial cell or a neuron. The triiodothyronine (T\(_3\)) that enters the cell can either be deiodinated to 3, 3’ T\(_2\) (diodothyronine) or can enter the nucleus and bind to the thyroid hormone receptor. An additional source of T\(_3\) is that generated by outer ring deiodination of thyroxine (T\(_4\)) within the cell. The interaction of T\(_3\) with the thyroid hormone receptor (TR) bound as a heterodimer with a retinoid X receptor (RXR) to the thyroid hormone response element (TRE), causes either an increase or a decrease in the transcription of that gene (Williams Textbook of Endocrinology, 2008).

**REGULATION OF THYROID FUNCTION**

**Hypothalamic-Pituitary-Thyroid Axis**

The thyroid gland participates with the hypothalamus and pituitary gland in a classic feedback control loop. The levels of thyroid hormones in the blood are regulated by a negative feedback mechanism involving the hypothalamic-pituitary-thyroid (HPT) axis. The
hypothalamus releases thyrotropin releasing hormone (TRH), which stimulates the anterior pituitary to produce thyroid stimulating hormone (TSH). TSH then prompts the thyroid to produce thyroid hormones. Cells in both the hypothalamus and pituitary respond to levels of circulating thyroid hormones. When these are high, the output of both TRH and TSH declines. When levels of thyroid hormones are low, the outputs of TRH and TSH are raised, prompting the thyroid to increase the output of $T_4$ and $T_3$. The negative feedback loop helps the body to respond to varying demands for thyroid hormone and to maintain hormone homeostasis. Interactions from several types of growth factors are also involved in stimulation of the follicular cells (NCM, 2002; Zoeller, 2007).

**Thyrotropin-Releasing Hormone Synthesis and Secretion**

TRH, a modified tripeptide (pyroglutamyl-histidyl-prolineamide), is derived from a large pre-pro-TRH molecule that contains five progenitor sequences. The TRH peptides are released from the pre-pro molecule by a peptidase that acts at flanking lysine/arginine residues. TRH is expressed in the hypothalamus, the brain, the C cells of the thyroid gland, the beta cells of the pancreas, the myocardium, the reproductive organs (including prostate and testis) and the spinal cord. The parvocellular region of the paraventricular nuclei of the hypothalamus is the source of the TRH that regulates TSH secretion. The 5’-flanking region of the gene encoding TRH has sequences for mediating responses to glucocorticoids and cAMP. In addition, at least two elements in this region of the gene can confer negative regulation of thyroid hormone receptor complexes (Hollenberg et al., 1995).

$T_3$ suppresses the levels of pre-pro-TRH mRNA by $T_3$ in the hypothalamus (Segerson et al., 1987), but normal feedback regulation of prepro-TRH mRNA synthesis by thyroid hormone requires a combination of $T_3$ and $T_4$ in the circulation, with the latter giving rise to $T_3$ via $T_4$ 5’-deiodination in the CNS (Kakucska et al., 1992). In rats, there is a dense expression of D2 in the specialized ependymal cells (tanycyes) in the inferior portion of the third ventricle (Riskind et al., 1987; Tu et al., 1997). These cells have processes extending into the median eminence and arcuate nucleus, where active conversion of $T_4$ to $T_3$ releases $T_3$ in the region of the hypothalamic-pituitary portal system. Thus, part of the negative feedback induced by $T_4$ may be generated both indirectly at the level of the paraventricular nucleus by suppressing TRH and at the median eminence and arcuate nucleus at a point where neuropeptides and $T_3$ enter the pituitary portal system.
**Thyrotropin Synthesis and Secretion**

TSH is the major regulator of the morphologic and functional states of the thyroid gland. It is a glycoprotein secreted by thyrotrophs in the anteromedial portion of the adenohypophysis composed of an α subunit of 14 kd (92 amino acids) that is common to LH, follicle-stimulating hormone (FSH) and hCG as well as a specific β subunit a 112 – amino acid protein synthesized in thyrotrphs. The peptide sequence cysteine-alanine-glycine-tyrosine-cysteine (CAGYC) is highly conserved in the β subunits of TSH, FSH, LH and hCG and is required for heterodimerization with the α sub unit (Hayashizaki et al., 1989). An autosomal recessive form of hypothyroidism is associated with a glycine to arginine mutation in this sequence of the TSH β subunit which blocks its capacity to heterodimerize and renders it non-functional (Hayashizaki et al., 1990). In normal thyrotrophs and in thyrotroph tumors synthesis of the α subunit is in excess, indicating that the quantity of the β subunit is rate-limiting for TSH secretion. TRH increases and thyroid hormone suppresses the transcription of both subunits; these are the most important influences on TSH synthesis. The physiologic glycosylation of TSH involves addition of preformed asparagine-linked oligosaccharides in the rough endoplasmic reticulum modifications in proximal and distal Golgi apparatus and the appearance of the intact, folded hormone in the secretory granules (Magner, 1990).

![Diagram of thyroid hormone regulation](image)

**Figure 5.** Role of thyroxine and triiodothyronine (T₄ and T₃) in the feedback regulation of thyrotropin-releasing hormone (TRH) and thyrotropin (TSH) secretion. Secreted T₄ must be converted to T₃ to produce its effects. This conversion may take place in tissues such as the liver (L) and kidney (K) and thyroid (T) catalyzed by D1 or D2 in the human thyroid gland (T), skeletal muscle (SM), and, possibly, cardiac muscle (CM). SRIH, somatostatin (Williams Textbook of Endocrinology, 2008).
FACTORS REGULATING THYROID GLAND FUNCTION

A large number of agents in the environment, both naturally occurring and anthropogenic, as well as some medications, are known to interfere with thyroid gland morphology and function posing the danger of thyroid disease. Agents that cause thyroid enlargement are known as environmental goitrogens which may cause the goitrous condition by acting directly on the thyroid gland but also indirectly by altering the regulatory mechanisms of the thyroid gland and the peripheral metabolism and excretion of thyroid hormones (Gaitan, 1988, 1990). However, the mechanism that induces the trophic changes leading to goitre formation is not well understood, because besides thyrotropin other humoral, paracrine and autocrine growth factors may be involved in the process (Gaitan, 1990).

Environmental agents acting through the foodstuffs or water exposure along with bacterial contamination, malnutrition with chemicals and drugs responsible for the thyroid disorders are summarized.

ANTI-THYROID DRUGS

According to their principle mode of action on thyroidal iodine metabolism, anti thyroid drugs are divided into two categories:

1) The monovalent anions which inhibit iodide transport into the thyroid gland viz. thiocyanate, perchlorate and nitrate ions (SCN⁻, ClO₄⁻ and NO₃⁻) inhibit transport of iodide into the thyroid gland and thereby depress iodide uptake and hormone formation (Ermans and Goossens, 1961; Chow et al., 1969, Chandra et al., 2008). Thiocyanate stimulates efflux of iodide from the thyroid and also inhibits iodide binding and probably coupling (Scranton et al., 1969). These ions have a molecular volume and charge similar to those of iodide and may compete with iodide for transport.

2) A large number of compounds that act through inhibition of thyroidal iodide binding and iodotyrosine coupling. The most important representatives of this category of compounds are the group of thionamides. They may be competetives substrates for thyroid iodide peroxide, preventing the peroxidation of iodide by this enzyme (Yamazaki et al., 1960).

3) A number of other drugs, including the amionoheterocyclic and substituted phenols, act as goitrogens principally by impairing Tg iodination. They are in general far less potent in their goitrogenic effect than the thionamides (Milne and Greer, 1962).
The effect of the drugs in the first category is counteracted by exposure to excess iodine, whereas iodine has no effects and at times even potentiates the action of drugs in the second category. Other drugs inhibit thyroid hormone secretion or act through yet unknown mechanism.

**Mechanism of action of anti thyroid drugs**

![Diagram of thyroid gland and anti-thyroid drugs](https://www.google.co.in/search)

Fig 6. Schematic representation of the mechanism of action of anti thyroid drugs on thyroid follicular cell (https://www.google.co.in/search).

**PROTEIN CALORIE MALNUTRITION (PCM)**

Protein intake might be determining factors in the malnourished individual and in experimental animals while on a poor protein diet. Apart from iodine intake, other factors are known to interfere with adequate iodine nutrition, these includes protein-energy malnutrition (PEM) (Gaitan *et al.*, 1983). The effect of both iodine deficiency and PEM may be pronounced among the school age children as they seem to be the most exposed to the fundamental risk factors; poor food consumption patterns, poor sanitation, infections, illnesses (Amigo *et al.*, 2000). PCM is associated with a low serum T₃ concentration and increased rT3 levels, probably due to similar changes in iodothyronine monoiodination (Matisonn and Pimstone, 1973). A low protein diet in rats impairs the thyroidal transport of iodine, decreases iodine concentration in the thyroid and is accompanied by an enlargement of the thyroid. Serum T₄ and T₃ levels were found to be both elevated (Tulp *et al.*, 1979). Under these circumstances the goitrogenic effect of anti-thyroid substances are enhanced.
The administration of protein reverses these alterations and decreases the action of such goitrogenic agents (Gaitan et al., 1986).

**MINERALS**

**Iodine**

Iodine is an essential element for thyroid hormone synthesis. The thyroid gland has the capacity and holds the machinery to handle the iodine efficiently when the availability of iodine becomes scarce, as well as when iodine is available in excessive quantities. The latter situation is handled by the thyroid by acutely inhibiting the organification of iodine, the so-called acute Wolff-Chaikoff effect. It is proposed that iodopeptide(s) are formed that temporarily inhibit thyroid peroxidase (TPO) mRNA and protein synthesis and, therefore, thyroglobulin iodinations. The Wolff-Chaikoff effect is an effective means of rejecting the large quantities of iodide and therefore preventing the thyroid from synthesizing large quantities of thyroid hormones. This is achieved by decreasing the intrathyroidal inorganic iodine concentration by down regulation of the sodium iodine symporter (NIS) and therefore permits the TPO-H₂O₂ system to resume normal activity. The mechanism through which iodide acts via desensitization of the thyroid gland to TSH (Robinson et al., 1998). Iodine also antagonizes TSH stimulated thyrocyte proliferation (Uyttersprot et al., 1997). The most intriguing effects of iodine are the involution of hyperplasia and the decrease in vascularity that occur when the ion is administered to patients with diffuse toxic goitre. Iodine may be able to induce apoptosis in thyroid cells (Burikhanov and Matsuzaki, 2000). Under different circumstances, iodide may intensify the hyperplasia and produce goitre (Fisher, 1996). Excess iodine can be responsible for the development of goitre, hypothyroidism and thyrotoxicosis.

Iodine deficiency used to be the leading cause of goitre in the world and still remains so in certain regions. When severe, it can cause hypothyroidism and cretinism (Gaitan, 1990).

**Other Antithyroid Agents**

**Thionamides**

The major agents for treating thyrotoxicosis are drugs of the thionamide class, most commonly propylthiouracil, methimazole and carbimazole (Cooper, 1998). These agents inhibit the oxidation and organic binding of thyroid iodide and, therefore, produce intrathyroidal iodine deficiency that further increases the ratio of T₃ to T₄ in the thyroid secretion, as reflected in the high T₃/T₄ ratio in the serum. In addition large doses of
propylthiouracil, but not methimazole, impair the conversion of T$_4$ to T$_3$ by deiodinase type 1 in the peripheral tissues. Because of this additional action, large doses of propylthiouracil may provide rapid alleviation of severe thyrotoxicosis (Cooper, 1998; Abuid and Larsen, 1974; Laurberg and Weeke, 1981). The propylthiouracil concentration in serum correlates with the extent of blockade of organic binding of iodine within the thyroid gland (Cooper, 1998). Thionamide drugs may also directly influence the immune response in patients with autoimmune thyroid disease (Weetman, 1994). This action occurs within the thyroid gland, where the drugs are concentrated.

**Lithium**

Lithium carbonate also inhibits thyroid hormone secretion, but, unlike iodine, it does not interfere with the accumulation of radioiodine. Lithium, 300 to 450 mg every 8 hours, is employed only to provide temporary control of thyrotoxicosis in patients who are allergic to both thionamide and iodide (Wu *et al.*, 1982). This is because the blocking effect is often lost with time. The goal is to maintain a serum concentration of 1 mEq/L.

**Dexamethasone**

Dexamethasone, 2 mg every 6 hours, inhibits the glandular secretion of hormone inhibits the peripheral conversion of T$_4$ to T$_3$ and has immunosuppressive effects (Williams *et al.*, 1975). The inhibitory effect of dexamethasone on the conversion of T$_4$ to T$_3$ is additive to that of propylthiouracil, suggesting a different mechanism of action.

**Beta-Blocking Agents**

Agents that block the response to catecholamines at the receptor site (e.g., propranolol) ameliorate some of the manifestations of thyrotoxicosis and are often used as adjuncts in management. Tremulousness, palpitations, excessive sweating, eyelid retraction, and heart rate decrease; effects are rapidly manifested and appear to be mediated largely through the adrenergic nervous system, although propranolol may also impair the conversion of T$_4$ to T$_3$.

**Hardness of water**

In 1931 slott suggested that goitre in India was directly related to the high calcium content in drinking water (Slott, 1931). Murray et al. concluded on the basis of their studies that even where the iodine levels were similar, there was a greater incidence of visible thyroid
glands in people in areas with hard water as in England, than in areas with soft water, as in Scotland (Murray et al., 1961). However, it has also been mentioned that goitre could take place with soft water. High mineral content, particularly of magnesium and calcium salt have implicated as goitrogenic factor in water (Koutras, 1980. Chandra et al., 2014).

**WATER-BORNE GOITROGENS**

Water-borne goitrogens may be the most important factor underlying the goitre endemia of regions. Coal is a source of large variety of anti-thyroid and goitrogenic compounds viz. phenols, dihydroxyphenols (resorcinol), substituted dihydroxybenzenes, phthalic acid, pyridines, halogenated and polycyclic aromatic hydrocarbons (PAH) (Gaitan, 1988). Most of these compounds have been identified in drinking water from the iodine-sufficient goitrous areas of Kentucky and Columbia (Gaitan, 1986).

These xenobiotics may impact the binding of TH to serum proteins, and consequently affect the TH transport. Polychlorinated biphenyls (PCBs) are persistent industrial chemicals, used earlier as fire retardant and cooling fluids e.g. transformers. Even though their production stopped in the late 1970’ies, they are still leaching out into the environment where they bioaccumulate (Brucker and Davis, 1998). Numerous animal studies have shown thyroid effects after exposure to several PCB congeners (Gray et al., 1993; Ness et al., 1993; Goldey et al., Barter and Klaassen, 1994; 1995a; Morse et al., 1996) and the main mode of action for the coplanar PCBs is most likely induction of UDPGT metabolism, thereby increasing metabolism and excretion of T₄. There are however, in vitro studies showing that hydroxylated PCBs can also act by displacing T₄ from TTR (Lans et al., 1993; Chauhan et al., 2000). A group of chemicals that also seem to affect TTR are the brominated flame retardants. These chemicals are widely used in computers, television and other electronic devices (Kashiwagi et al., 2009) and the group includes both polybrominated diphenyl ethers (PBDEs) and tetrabromobisphenol A (TBBPA). Both compounds have been shown to reduce T₄ levels in animal studies (Hallgren et al., 2001; Zhou et al., 2002; van der Ven et al., 2008) and mechanistic studies indicate that PBDEs may act by down-regulating the transport protein TTR (Richardson et al., 2008), while TBBPA has been documented to interfere with binding to TTR (Meerts et al., 2000).

**Other organochlorides**

DDT (2, 2 bis-[p-chlorophenyl]-1, 1- trichloroethane) is polychlorinated and non-degradable. It is insoluble in water and resistant to destruction by light and oxidation. Its
stability has created difficulties in residue removal from water, soil and foodstuffs (Gaitan, 1990). The dominant degradative reaction of DDT is dehydrochlorination to form DDE. Another breakdown product of DDT is DDD. All these compounds (DDT, DDE and DDD) induce microsomal enzyme activity that may affect thyroid hormone metabolism in way to that of polyhalogenated biphenyls and polycyclic aromatic hydrocarbons. (PAH) (Barsano, 1989).

**GOITROGENS FROM FOODSTUFFS**

Plants are known to produce more than 200,000 different bio-active natural products (also denoted secondary metabolites, specialty compounds) including cyanogens and polyphenols (Bak et al., 2006; Zagrobelny et al., 2008). Regular consumption of food containing cyanogenic glucosides, glucosinolates, and thiocyanate affect thyroid physiology and may lead to the development of endemic goitre, especially in iodine deficient environments (Delange et al., 1982).

Most of the plant foods contain thyroid disrupting factors like cyanogenic glucosides, glucosinolates, thiocyanate and flavonoids, more than one of which may occur in the same plant. The goitrogen content in the same plant shows extreme differences from region to region or in the same region because of genetical and ecological factors (Delange, 1989). Besides these, the actual concentration of goitrogens in foodstuff do not always represent their true goitrogenic potentiality and even their absence do not negate the possible antithyroid effect because after ingestion, inactive precursor are converted to active goitrogens in animal body by widespread glucosidases and sulphur transferase enzymes (Lang and Die et al., 1933; Montgomari, 1969).

There are plant foods which are generally consumed by the people of third world countries including India are rich in cyanogenic constituents and polyphenols. Most interestingly endemic goitre is prevalent from mild to moderate degree in those regions during post-salt iodization phase. Therefore in the present investigation certain plant foods containing both cyanogenic constituents and polyphenols have been selected and attempt has been made to evaluate their goitrogenic /anti-thyroid potential as the studies in this aspect found unavailable.