INTRODUCTION AND REVIEW OF LITERATURE

This thesis attempts to study the metabolic alterations in thyroid disorders. The thyroid gland probably ranks first of all the endocrine glands of the body with respect to frequency of its dysfunction. Although the thyroid gland is not absolutely essential for the maintenance of life, its extreme or prolonged malfunction can change the affected individual into a creature unable to function or to celebrate.

In the early 17th century SANCTORIOUS began what could have been the first work on the thyroid in his attempt to measure the relationship between temperature, metabolism and food intake. Unfortunately, he was centuries ahead of his time and had no basis for adequate evaluation of his findings.

Important milestones in the evolution of our knowledge of the thyroid and its functions are :

1786 → Parry described an enlargement of the thyroid in connection with enlargement or palpitation of the heart.

1800 → Pinsuti proposed the therapeutic use of thyroid juice.

1802 → Flajani described exophthalmic goiter.

1806 → Meckel recognized enlargement of the thyroid conjunction with adolescence, menstruation, defloration and pregnancy.

1811 → The discovery of iodine by Courtois led shortly to its use in goiter by Coindet.

1835 → Grave's description of exophthalmic goiter appeared.

1856 → Schiff first understood experimental studies upon thyroid function wherein he observed that total ablation of the gland in dogs caused death.

1874 → Gull described hypothyroidism

1878 → ORD employed the term myxedema

1883 → Reverdin and Kocher surgically treated goiter and Kocher described cachexia strumipriva following total thyroidectomy.

1884 → Rehn suggested that Grave's disease was due to hyperthyroidism.

1886 → Horsley proved that myxedema was due to hypothyroidism.

1890 → Bettercourt and Servans produced temporary relief of myxedema by thyroid grafts.

1891 → 1. Nassale and Gley gave thyroid extracts to experimental animals.

2. Murray prevented myxedema by injections of thyroid extracts.

1892 → Von Müller related the clinical manifestations of hyperthyroidism to hypermetabolism.

Mackenzie, Howitiz and Fox proved the efficacy of orally administered thyroid gland in myxedema.

1893 → Greenfield correlated histologic hyperplasia and over activity of the thyroid as etiologic factors in Grave's disease.

1895 → Baumann proved that an organic combination of iodine is a constituent of normal thyroid gland.

1898 → Notthaft described a patient who after taking 1000 thyroid tablets in 5 weeks, developed Grave's disease, the symptoms of which gradually disappeared following withdrawal of the therapy.

1899 → Oswald isolated thyroglobulin from the thyroid.

1914 → Kendal and Kimball began the use of iodine for prevention of simple goiter.

1923 → Plummer and Boothley initiated the employment of Lugol's solution in hyperthyroidism.

Many hormones are small lipid soluble molecules that may act by dissociating from specific binding proteins in plasma and diffusing through cellular membranes to activate intracellular receptors. In most instances, the intracellular receptors for these hormones are transcription factors that become activated only after hormone binding.

Thyroid hormones are vital signaling molecules in both modern and primitive organisms. However, they were not discovered until around 1891 when George Murray noted improvement in certain myxedematous patients after administration of sheep thyroid extracts.

The fully developed thyroid gland in man is composed of two lobes connected by a thin band of tissue, the isthmus, which gives the gland the appearance of a butterfly. The gland is closely attached to the trachea in the anterior aspect of the neck. Each lobe measures ~ 2.0 – 2.5 cm in both thickness and width and 4.0 cm in length. The isthmus measures 2 cm in both length and width and 0.5 cm in thickness.

The secretory units of the gland are the follicles, which consist of an outer layer of epithelial cells that rest on a basement membrane and enclose an amorphous material called colloid. (fig-1)

Colloid is mainly composed of thyroglobulin, an iodinated glycoprotein, and small quantities of iodinated thyroalbumin. The follicles are embedded in stromal tissue, which contains blood vessels and autonomic nerve fibers. Increased activity of the gland is characterized by a decrease in the quantity of colloid, with the subsequent reduction of follicular volume; the lining cells become columnar and may even proliferate into the colloid.

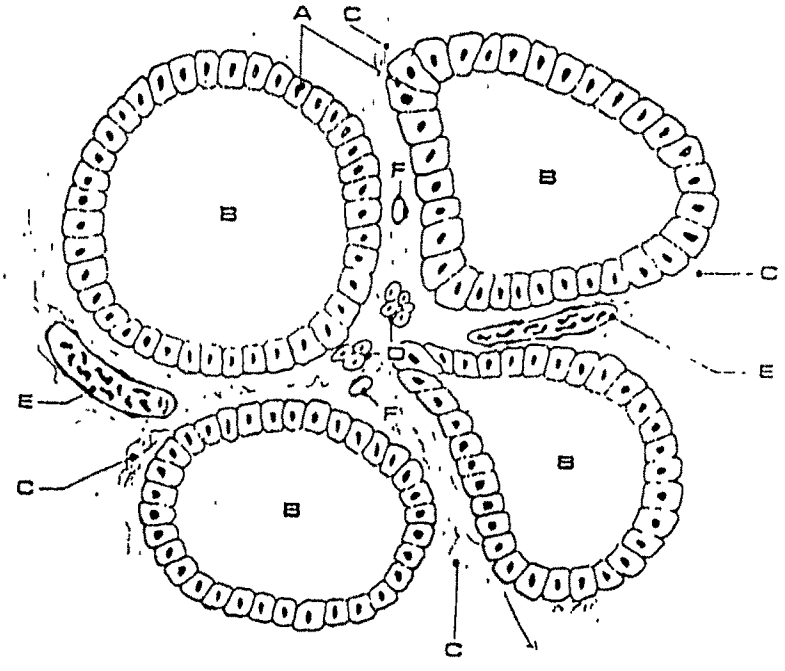


FIGURE-1 : SCHEMATIC DIAGRAM SHOWING THE THYROID FOLLICLE AND FOLLICULAR CELL

It consists of follicular cells (A), enclosing colloid (B) and Para follicular "C" cells (D), in the interstitium (C), (E), venule; F, capillary.

THYROID HORMONES

The thyroid gland secretes two hormones, thyroxine (3,5,3',5'-L-tetraiodo thyroxine) and triiodothyroxine (3,5,3'-L-triiodothyronine), which are commonly known as T_4 and T_3 respectively. In addition, the thyroid secretes minute amounts of biologically inactive 3,3',5'-L-triiodothyronine (reverse T_3 or rT_3). Approximately, 35% of the secreted T_4 is deiodinated by the liver and other peripheral tissues to yield T_3, and about 40% to yield rT_3. Therefore, with normal T_4 production of 90 μg/d, ~ 26 μg of T_3 and 30 μg of rT_3 would be produced by peripheral deiodinations. From the estimated daily production rates for T_3 (30 μg) and rT_3 (30 μg), it is evident that at least 80% of normal T_3 production and essentially all of rT_3 production can be accounted for by peripheral deiodination of T_4 rather than by direct secretion by the thyroid (Fig.2).

Until recently, T_4 was considered the principal biologically active hormone and T_3 on adjunct and relatively minor thyroid hormone. However, new, precise methods of measurement of T_3 in biological fluids and new knowledge of peripheral kinetics of T_3 and T_4 have shown that T_3 is biologically 4-5 times more potent than T_4. Some even consider T_4 primarily as a prohormone and T_3 is the biologically significant hormone (Tietz, 2nd Edition, page 1116).

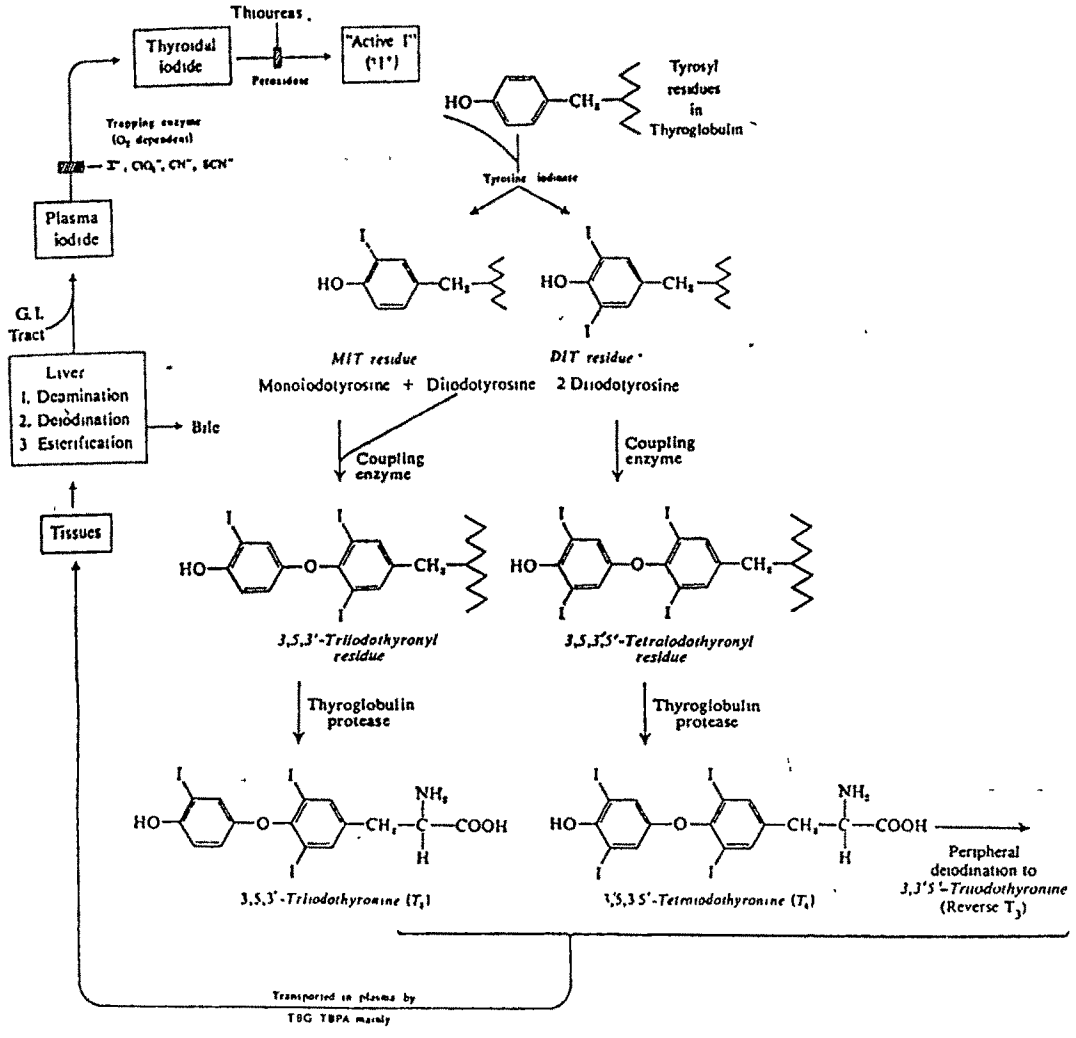


Fig.2 : The metabolism of iodine, emphasizing formation and secretion of the thyroid hormones. ▨ Indicates block in the pathway. Iodine transport is inhibited by monovalent anions such as thiocyanate (SCN^-), perchlorate (CLO_4), and pertechnetate (TcO_4^-). The oxidation and organic binding of iodide to thyroglobulin is blocked by thiourylenes, sulfonamides, and high concentrations of iodide.

Although thyroid hormones have many actions, the primary one is a calorigenic effect (increased oxygen consumption) on many tissues. However, some tissues, for example, the brain, retina, lungs, spleen and testes do not appear to be affected by this action of the hormones. Thyroid hormones are also indispensable for growth, development, and sexual maturation in mammals. Other actions include :

1. Stimulation of heart contractions
2. Maintenance of body weight
3. Stimulation of protein synthesis and carbohydrate metabolism
4. Increase in the synthesis and degradation of cholesterol and triglycerides
5. Increase in vitamin requirements, and
6. Enhancement of sensitivity of the β-receptor to catecholamines.

These effects are usually magnified in hyperthyroids and minimized in hypothyroids.

BIOSYNTHESIS, SECRETION AND METABOLISM

Thyroid hormones are synthesized in the thyroid gland. Although the precursor for both T_4 and T_3 is tyrosine, the synthesis does not occur on the free amino acid, but rather on the tyrosine residues of thyroglobulin. THYROGLOBULIN is a 6.60,000 dalton dimeric glycoprotein that is synthesized by the thyroid follicular cells. Thyroglobulin is stored inside the thyroid follicles as colloid

droplets that comprise ~ 75% of the protein. The formation of thyroid hormones starts with these follicles.

CONTROL OF THYROID HORMONE SECRETION

The thyroid gland is stimulated by the release of thyroid-stimulating hormone (TSH) from the pituitary TSH binds with high affinity receptor on the thyroid gland. The TSH receptor is a glycoprotein of ~ 100,000 daltons with an amino terminal domain that binds TSH and a carboxyl terminal domain containing seven membrane – spanning sequences typical to G-coupled receptors. TSH binding to the receptor stimulates adenylcyclase to produce cyclic adenosine monophosphate. At higher concentration, inositol phosphate pathways may be activated. TSH is under the control of both thyrotropin-releasing hormone (TRH) from the hypothalamus and feedback inhibition of thyroid hormones on the pituitary (Fig. 3).

The biosynthesis of thyroid hormones involves:

1. Thyroidal trapping of serum iodide (iodide transport)
2. Incorporation of iodine into tyrosine
3. Coupling of iodinated tyrosyl residues of thyroglobulin
4. Proteolytic cleavage of follicular thyroglobulin to free T_4 and T_3 and to MIT and DIT.

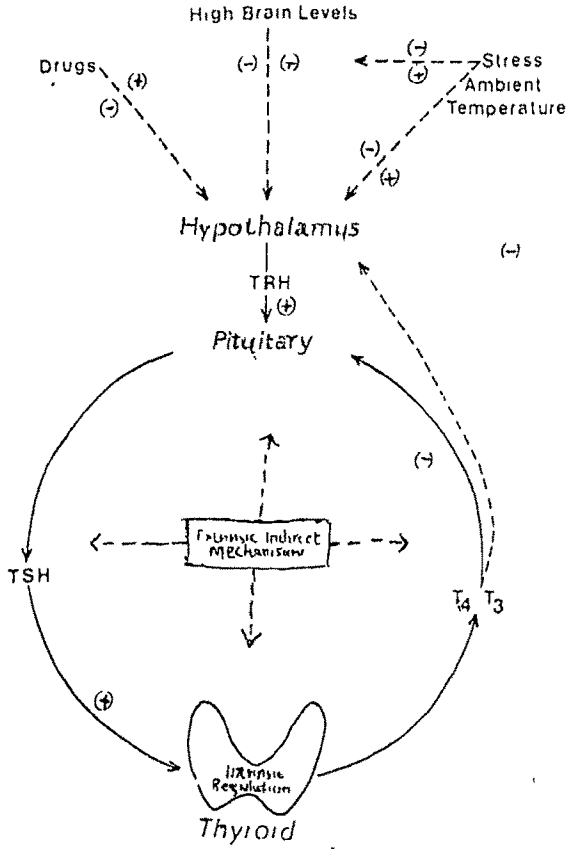


Figure-3 : Control of thyroid hormone secretion

Schematic outlines of iodine metabolism and formation and secretion of thyroid hormones are given in Fig.4.

The important element involved in the synthesis of thyroid hormone is iodine, which is normally ingested in the form of iodides. Iodide transport to the follicles is the first and rate limiting step in the synthetic process. The follicular cells concentrate iodide, by means of an energy-dependent pump mechanism, to some 30-40 times the normal plasma levels. Although the details are not complete, the iodine concentrating mechanism is linked to a TSH-sensitive Na^+, K^+-ATPase that is apparently unique to the thyroid. Drugs that block the ATPase, such as Ouabain, destroy the thyroids ability to concentrate iodine. It can accommodate a variety of large, inorganic anions, such as perchlorate and thiocyanate, that can inhibit iodide uptake. Thus patients with a diet high in rutabaga , which contains large amounts of thiocyanate, may become hypothyroid because of a lack of intrathyroidal iodine (Montgomery, 6th edition, page 588).

According to Ajjan R.A. (1998) iodide (I^-) uptake by the thyroid gland is an essential step for the formation of thyroid hormones and is mediate by sodium / iodide symporter (NIS). The NIS is localized to the basolateral membrane of thyroid follicular cells (TFC) transporting extrafollicular I^- using the sodium (Na) gradient generated by the Na^+ / K^+ ATPase as an energy source.

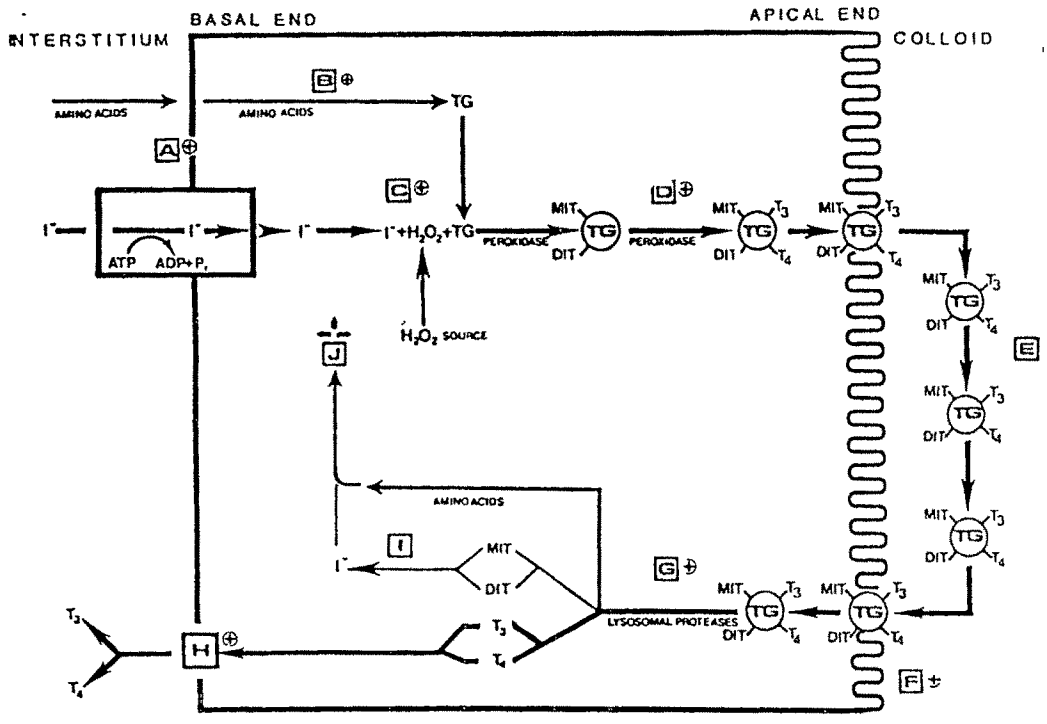


Fig.4 : Schematic diagram of the thyroid cell, depicting stages of thyroid hormonogenesis and intrathyroidal iodine metabolism.

A. Iodine transport
B. Thyroglobulin (TG) synthesis
C. Iodine oranification
D. Intrathyroglobulin coupling / condensation
E. Storage
F. Endocytosis
G. Hydrolysis
H. Hormone secretion
I. Intrathyroidal deiodination
J. Recycling

Steps influenced by TSH are indicated by (+)

The of membrane bound Na^+ / K^+ ATPase an integral membrane protein, is a member of P-type class of ATPases which includes the sarcoplasmic reticulum and plasma membrane Ca^{+2}-ATPases and the H^+, K^+-ATPase found in stomach and colon, in addition to several prokaryotic transport enzymes (Jerry B. Lingred, 1994).

The activity of Na / K ATPase has been proposed by M. Monti et al (1987), to be regulated by thyroid hormones and to account for a major proportion of the increased energy demand in thyroid hyper function.

Other anions are accumulated by the thyroid and act as competitive inhibitors of iodide transport. Two clinically useful anions are pertechnetate (TcO_4^-) and perchlorate (ClO_4). The ability of perchlorate to inhibit iodide transport allows its use in the perchlorate discharge test to detect defects in thyroid organic binding mechanism, it is also useful for thyroid imaging by radioscan.

The salivary gland, gastric mucosa, placenta, the ciliary body of the eye, the choroid plexus and the mammary gland also transport iodide against a concentration gradient, but their iodine uptake is not affected by TSH. The physiologic significance of extra-thyroidal iodide-concentrating mechanisms is obscure.

The next step in thyroid hormone synthesis involves the covalent attachment ("Organification") of the iodine to thyroglobulin. Electron microscopic evidence suggests that organification of iodine occurs in exocytic vesicles as they move towards the apical plasma membrane or within the storage follicle itself. Iodide is oxidized by H_2O_2 to form a positively charge iodine ion that reacts with specific tyrosine residue on thyroglobulin to produce MIT within the TG. This reaction is catalyzed by the enzyme thyroperoxidase. Formation of H_2O_2 is catalyzed by a special enzymatic complex on the apical membranes that uses reduced NADPH. In the presence of thyroperoxidase MIT undergo similar reaction to form DIT.

Coupling

$MIT + DIT \rightarrow T_3$

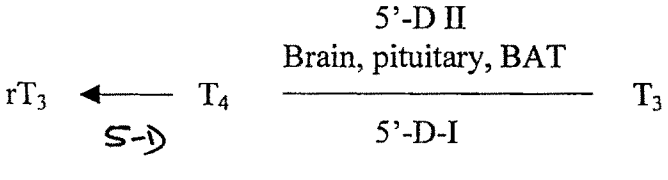
$DIT + DIT \rightarrow T_4 + Alanine$

$DIT + MIT \rightarrow rT_3$

Condensation reaction is an aerobic, energy requiring reaction, like oxidation and binding, it is catalyzed by thyroid peroxidase iodide thus bound to tyrosine residue can no longer be discharged by SCN^-, ClO_4^- or other inhibitors of iodide transport.

Each thyroglobulin dimer may contain upto 8 thyroxine molecules.Although there are 120 tyrosine residues in thyroglobulin, only a few residues located near the amino acid carboxyl termini are thought to be used for thyroid hormone synthesis. T_4 and T_3 are released from the thyroid after pinocytosis of the colloid by the follicular cells and proteolytic degradation of thyroglobulin in lysosomes. These cells salvage the iodine from monoiodotyrosines and diiodotyrosines for reuse. An inability to salvage iodine from iodotyrosine, although rare, leads to iodine deficiency and hypothyrodism.

Under normal conditions, T_4 makes up the majority of hormones produced by the thyroid gland; relatively little T_3 is synthesized. Instead most T_3 in plasma is found in extra-thyroidal tissues by 5'-deiodinase, a virtually ubiquitous enzyme that catalyzes the removal of an I^- from the outer ring of T_4.



Transport

Thyroid hormones in the blood are transported almost completely bound to specific binding proteins. (Table - 1, 2, & 3)

TABLE-1 : BINDING PROTEIN FOR THYROID

Protein	Half-life	Molecular weight	Hormone	Affinity M^{-1}	% Bound	Estrogen effect
TBG	5 days	54,000	T_4	10^{10}	80	Increased
TBPA	12 days	55,000	T_3	10^{9}	55	Increased
Albumin	15 days	69,000	T_4	10^{8}	15	None
			T_3	10^{8}	25	None

TABLE-2 : THYROID HORMONES IN SERUM

	T_4	T_3	rT_3
Serum concentration			
- Total	4000-11000 ng/dl	70-80 ng/dl	25-80 ng/dl
- Bound form	99.97%	99.8%	-
Half-life	6-7 days	1 day	-
- Free form	0.03%	0.2%	-
- Free amount	2 ng/dl	0.3 ng/dl	0.1 ng/dl
Total production rate	70-90 µg/day	~ 35 µg/dl	~ 35 µg/day
Fraction produced by thyroid	100%	20-25%	2%

TABLE-3 : ALTERATIONS IN CONCENTRATION OF THYROID HORMONE-BINDING PROTEINS

Decreased levels in
Treatment with anabolic steroids, androgens, diphenylhydantoin major illness or surgical stress
Nephrotic syndrome
Active acromegaly
Genetic (inherited) deficiency
Increased levels in
Treatment with estrogens, perphenezine
Pregnant or newborn state
Acute intermittent porphyrin
Infectious hepatitis
Genetic (inherited) increase in synthesis.

Degradative Metabolism : of T_3 and T_4 by oxidative deamination produces pyruvic acid analogs, which are converted to thyroacetates by subsequent decarboxylation. These analogs have some biologic activity, but there is no evidence that they are physiologically significant. In the liver, T_3 and T_4 are conjugated to form sulfates and glucuromides. These conjugates enter the bile and pass into the intestine.

The thyroid hormone conjugates are hydrolyzed and some are reabsorbed (enterohepatic circulation) or are excreted in the stool. The amounts of thyroidal substances in urine are very small and urinary assays have little clinical value.

SITES OF ACTION

Four different sites of action of TH'S within cell have been suggested. Binding proteins for TH'S have been found in (1) plasma membrane (2) cytoplasm (3) mitochondria and (4) nucleus (Fig.5).

In his basic Review on the thyroid hormone action at the nucleus level, Jack H. Oppenheimer (1985) suggested that the possibility of extra-nuclear effects of thyroid hormones continues to receive attention, there is general agreement that most of the characteristic biologic effects of thyroid hormone are mediated by an interaction of triiodothyronine with specific nuclear receptors. (Fig - 6)

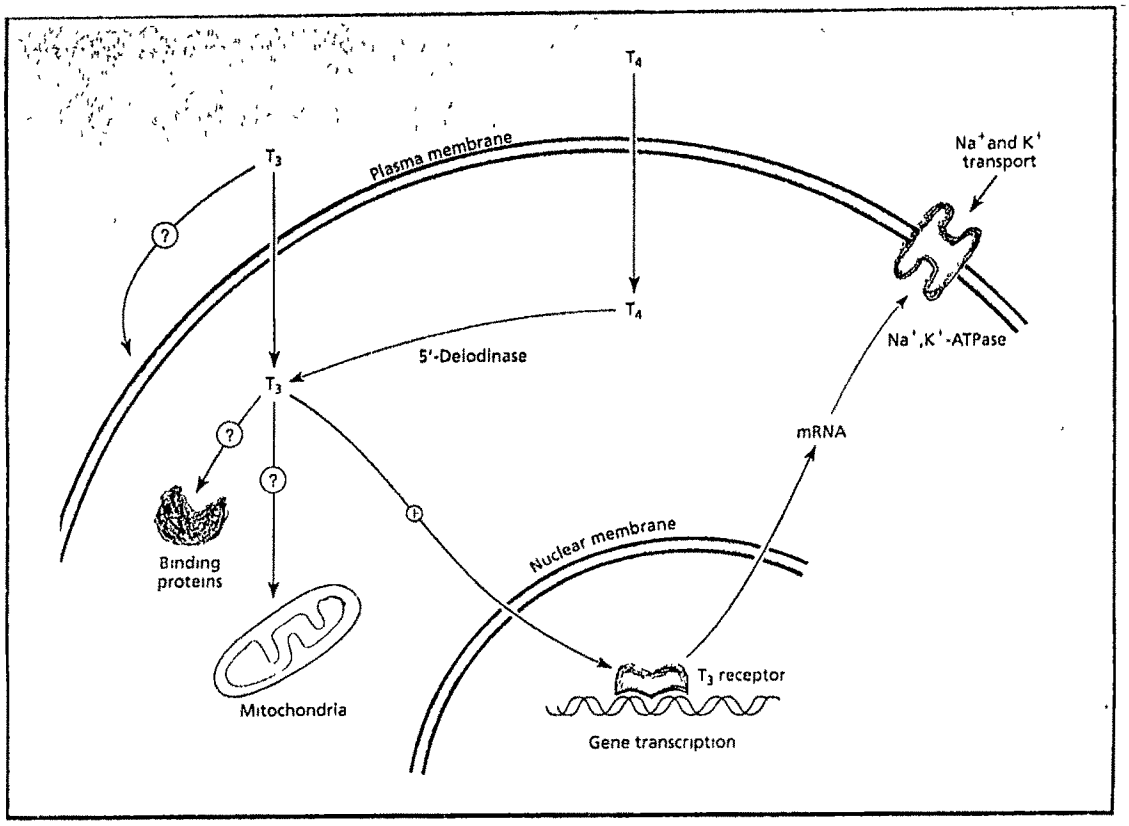


Fig.5 :Thyroid hormone action. Both T_3 and T_4 diffuse freely into cells where T_4 is converted to T_3 by 5'-deiodinase. The active thyroid hormone (T_3) diffuses into the nucleus and interacts with thyroid receptors, which bind to DNA at specific response elements (SRE) and induce gene transcription. There are additional binding proteins for T_3 in the mitochondria, cytoplasm and plasma membrane, but their function is unclear. One of the most important thyroid inducible gene products is Na^+, K^+ - ATPase, the main enzyme responsible for maintaining intracellular Na^+ and K^+ gradients throughout the body.

In brief, the nuclear hypothesis rests on the excellent rank correlation between the binding of T_3 analogs to nuclear receptors and their thyromimetric actions. Further, there is a clear relationship between nuclear occupation and nuclear response, when nuclear sites are fully saturated, the measured biological response is maximal. Nuclear receptors are found in all mammalian tissues responsive to T_3. Such receptors have identical physicochemical characteristics and relative analog binding spectra. These receptors in early vertebrates including the salmon, trout, and the lamprey, a species believed to be one of the first in which circulating TH'S have been identified.

Lastly, the rapidity with which certain mRNA sequences change in response to T_3 occupation of the nuclear sites further supports the concept of a casual relationship between these events.

Biologic Effects

Thyroid hormones are required for the survival of all mammalian cells. T_3 is the major mediator of hormone action. T_4 is predominantly prohormone that is converted to T_3 intracellularly. Thyroid hormones stimulate the metabolic rate in most animals. This is reflected as both an increase in thermogenesis (heat production) and an increase in the rate of metabolism of other substances, such as an increased clearance rate of various hormones and drugs. The increased rate of thermogenesis is thought to result largely from an increase Na^+, K^+-

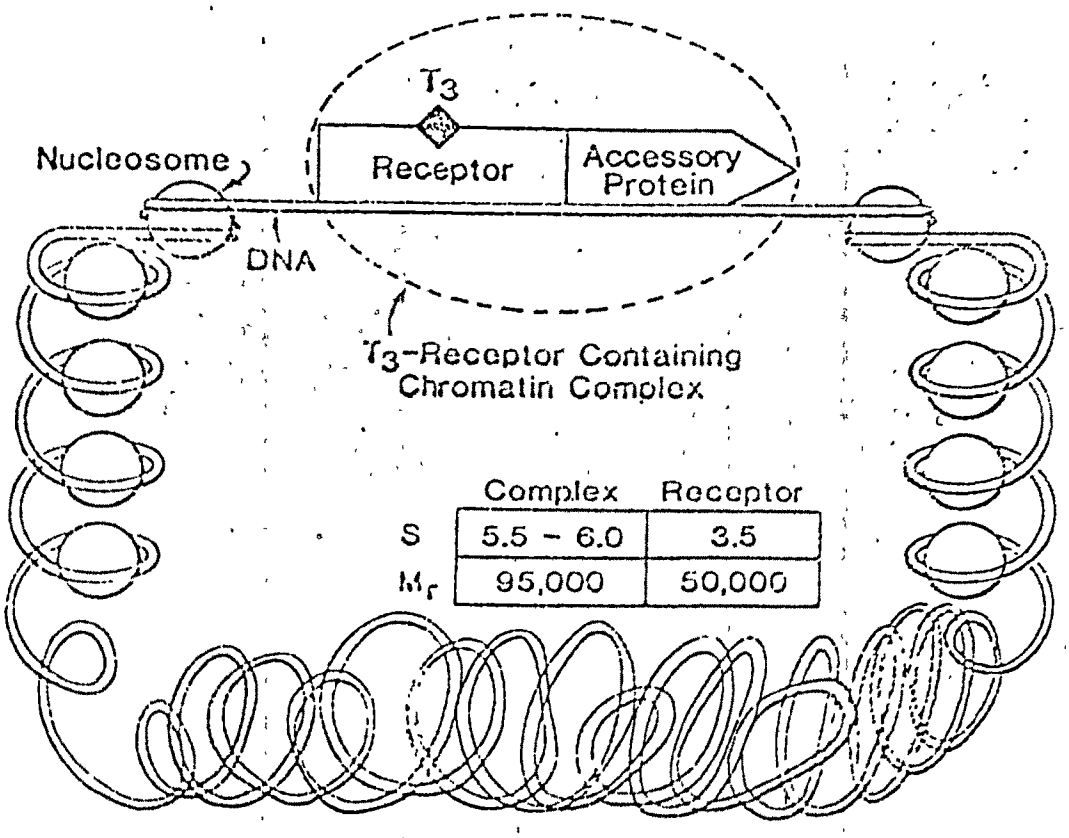


Fig.6 : Schematic representation of the structure of the receptor – containing complex, presumed to contain in addition to the 3.5 5-receptor of Mr (50,000) "accessory protein" and associated DNA sequences. The complex is located in relatively accessible or "open" DNA and is attached to the link region of DNA situated between two adjacent nucleosomes. These structures are represented in figure as spherical objects.

ATPase activity. Throughout the body, this enzyme is responsible for maintaining the normal gradient of high extra-cellular sodium and intra-cellular potassium. Inhibition of Na^+/K^+-ATPase activity with ouabain blocks most of the thyroid hormone-induced increase in oxygen consumption.

Effects of Fat / Lipid Metabolism

The lipid metabolism of patients with thyrotoxicosis changes towards an elevation of neutral fat in the blood and its decrease in the liver and lipid depots. This is explained by the greater transfer of lipid from the periphery to the glycogen-depleted liver and the more intensive burning up of fat in it.

Derangement of lipid metabolism in myxedema leads to obesity. This is explained, on the one hand, by the lower influx of fat from the periphery to the liver owing to the surplus storage of glycogen in it.

The course of vital processes in myxedema is marked by inhibition and determines the low level of metabolism in these patients. This is true of all kinds of metabolism, but against the background of their general inhibition, processes of assimilation prevail over processes of dissimilation.

Water metabolism is thyrotoxicosis is deranged the organism is dehydrated. Patients eliminate upto 10 litres of urine daily. The elimination of water, according to the Volhard and Fahr test, is much higher than usual : after 3 hours

all the ingested water is eliminated. Patients with thyrotoxicosis abundantly eliminate water not only through the kidneys but also through the skin, which is moist all the time. Alterations of the water exchange in thyrotoxicosis is mainly connected with reduced hydrophilia of the tissues and cells owing to an alteration of the other forms of metabolism in them. In this category, too, are greater proteolysis and change in the oncotic blood pressure, increased release of sodium chloride by the tissues and its excretion in urine.

The thyroid hormone influences the intermediary metabolism of fats as it plays a role in protein and carbohydrate metabolism. Epstein and Landi (1922) observed an increase of the blood cholesterol in myxedema and a decrease in hyperthyroidism. Hurxthal L. M. (1933, 1934) and his associates confirmed and extended these observations firmly establishing the important effect of thyroid hormone upon cholesterol levels in the blood. The cholesterol concentration was found to be an especially sensitive indicator of hypothyroidism.

Capillary permeability studies carried out by Magnus Levy in 1907, first suggested that myxedema might be characterized by an alteration in the permeability of cell membranes throughout the body. Associated with this derangement are the changes in water metabolism. Water metabolism is altered in myxedema. In addition to the obvious edema, the decrease in perspiration is too great to be accounted for on the basis of the lowered metabolism (Gilligan

D. R. et al, 1935). The capillary changes previously mentioned may be anatomic basis for this physiologic derangement.

Total body water is increased in myxedema (Aikawa J. K., 1956) with therapy transient further expansion of the extra-cellular volume is followed by a diuresis of water, sodium and chloride, along with a decrease in body weight.

An increase urinary excretion of hexosamine (Wiener R., 1955) and a transient increase in the level of serum mucoprotein suggest that salt and water, as well as proteins are stored in myxedema (Margitay, 1937). The extra fluid is presumably located in the intra-cellular fluid space, as well as in the extra-cellular compartment (Byrom F. B., 1934).

In addition to creatine phosphokinase (CPK), hyperthyroidism has been reported to result in the serum elevation of ribonuclease, aspartate transaminase (AST), lactic dehydrogenase (LDH) and malic dehydrogenase. In addition, the red cell cor tent of glucose-6-phosphatase dehydrogenase has been found to be quite considerably elevated in hyperthyroidism (Saunders, 7th edition).

Since muscle dysfunction is frequently associated with a hypothyroid state (Shimoda et al, 1980), many clinical reports have indicated that serum enzyme activities derived from the muscle such as CPK, LDH and AST are elevated.

The study carried out Afrasibi et al (1979) showed that patients with the nephrotic syndrome are clinically euthyroid and their thyroid function tests (TFT) were generally normal except for low serum T_3 levels and occasionally low serum TBG values impaired peripheral T_4 to T_3 conversion has been observed in many chronic diseases including renal failure (Nomira S. et al, 1975; Chopra I. J., 1976; Spector D. A. et al, 1976) and this may be responsible for the decreased serum T_3 levels in Afrasibi's study. However, a low serum TBG level may also contribute to reduction of serum T_3 level in nephrotic syndrome.

The mean serum TBG level in their patients was significantly lower than in the control subjects. This may be because of decreased synthesis, increased extra-vascular distribution, or increased losses and degradation. In the absence of malnutrition and liver disease, decreased synthesis is unlikely. Furthermore, protein synthesis is generally enhanced in the nephrotic syndrome. Edema and effusions in various serosal cavities, as they occur in the nephrotic syndrome, may lead to increased extra-vascular distribution of TBG. However, the most likely cause of the low serum TBG level is its renal loss. In their patients suffering from relatively mild proteinuria, urinary excretion seems to be insufficient to explain the observed low serum TBG levels. However, it should be noted that proteinuria may represent only a fraction of the total protein loss

in the kidney, because substantial portion of the filtered protein is catabolized by the renal tubular cells and thereby lost in the kidney without appearing in the urine (Oliver J. et al, 1958; Spector W. G., 1954).

A discrepancy between total T_4 and TBG binding capacity was also observed in patients in renal failure (Lim V. S., et al, 1977) where it was reversible upon renal transplantation. In their nephrotic patients, both urinary T_4 and T_3 were markedly increased. This could be correlated to some extent with the degree of proteinuria but not with urinary losses of TBG. Thyroxine-binding prealbumin (TBPA) is a smaller molecule than TBG, and TBPA-bound T_4 as well as free T_4 may be a significant source of urinary T_4 in nephrotic subjects in addition to the TBG-T_4 complex.

In normal persons, the urinary / serum ratio is higher for T_3 than T_4. This reflects differential binding to the plasma proteins. Because of the greater proportion of unbound T_3, a greater fraction of T_3 is filtered in the glomeruli, compared with T_4. In addition, it has been shown that the urinary excretion of T_3 involves glomerular filtration and tubular secretion, whereas, T_4 is subject to glomerular filtration and tubular reabsorption (Burke C. W. et al, 1976).

Finally, deiodination of iodothyroxines occurs in the kidney with the derived iodine preferentially excreted in the urine (Shimoda S. et al, 1972; Shimoda S.

et al, 1977). It seems plausible that some of the urinary T_3 is derived from partial deiodination of T_4 in the tubular cells.

In contrast to findings in the control subjects, the urinary T_4 level was increased considerably. Because T_3 does not bind to TBPA while a substantial part of T_4 is TBPA bound, the reversal of the usual urinary T_4 to T_3 ratio further suggests the possibility that TBPA-bound T_4 is a significant source of urinary T_4.

The lack of correlation of urinary T_4 and TBG was reported by Gravin and associates (1978). However, they found a correlation between daily total proteinuria and urinary T_4 in their patients.

The results obtained by Monti M. et al (1976; 1987) confirm an increased overall metabolic activity in erythrocytes from hyperthyroid patients. In their work published in 1987, showed that in microcalrometric measurements the activity of the Na / K-pump is reflected through its dependence on membrane bound Na/K-ATPase activity that can be inhibited by Ouabain. Their results indicate that the Na/K-pump energy expenditure is not different in the hyperthyroid compared to the euthyroid state. Thus, the Na/K-pump does not seem to account for any significant part of the increased energy expenditure in hyperthyroidism.

This conclusion is further supported by the relationship between changes in thyroid hormone levels and heat production rate during normalization of thyroid function. Significant correlation between these variables appeared only when the activity of Na/K-pump was blocked by ouabain. Furthermore, no relation was found between changes in serum T_3 levels and Na/K-pump energy expenditure during treatment.

Theoretically, differences between erythrocytes and nucleated cells might explain the disagreement between the results by Monti et al (1987) and the results obtained in animal studies (Ismail et al, 1970; Asanoy et al, 1976; Locs, 1976; Lin M. H., 1978) where Na/K-ATPase was found to be stimulated by thyroid hormones. However, in two investigations on the effects of thyroid hormones on thermogenesis in hepatocytes and skeletal muscle of rats, changes in metabolic activity were only to a minor degree mediated via the Na/K-pump (Lark D. G. et al, 1982; Biron R. et al, 1979).

There are only limited experimental data available on the contribution of the Na/K-pump to total energy expenditure. In the present study, it amounted to about 10% of total heat production rate before as well as after treatment for hyperthyroidism, which is similar to data from healthy subjects (13%) (Monti M. et al, 1985) and also in agreement with results from rat hepatocytes (Clark D. G. et al, 1982) and rat skeletal muscle (Biron R. et al, 1979) (about 10%).

Measurement by direct calorimetry of the Na/K-pump energy expenditure seems to reflect reliably the Na/K-pump function in view of the fact that a highly significant positive correlation was found between Na/K-pump power and ouabain sensitive Na^+ transport in the euthyroid state.

The significant decrease in erythrocyte sodium content in hyperthyroid patients during treatment observed in the study carried out by Monti et al (1985), is in agreement with earlier reports (Rubython E. J. et al, 1983; Smith E.K.M. et al, 1970; Golden A. W. G. et al, 1971; Kanagasabapathy A. S., 1973; Cole C. H., 1976).

The relative increase in erythrocyte sodium content after inhibition of Na/K-ATPase activity with ouabain was significantly lower before than after treatment for hyperthyroidism, thus suggesting that the Na/K-pump function is insufficient in the hyperthyroid condition. Other mechanisms may influence the erythrocyte sodium content in this disease, e. g., increased permeability of the cell membrane for electrolytes, suggested to occur in erythrocytes of hyperthyroid patients (Rubython E. J. et al, 1983).

The study by Deluise and Flier (1983) has shown that human hyperthyroidism is associated with a significant reduction in the number of Na/K-pump units on erythrocytes. It has also been demonstrated that the effect is reversible with

correction of hyperthyroidism, although at this stage there is insufficient information to enable determination of the time course of either the onset or the offset of the change in the sodium pump status. Hypothyroidism, on the other hand, may be associated with increase in both the number of pump units and their cation transport activity.

Deluise and Flier have in part elucidated the underlying biochemical mechanism showing that the changes seen represent alterations in the number of enzyme units, with a less marked reduction in the cation transport activity of these whole cells.

The factors responsible for the reduction in the number of erythrocyte Na/K-pump units are not known. It could be argued that the changes in the number of erythrocyte pump units represent changes in red cell populations since it is known that reticulocytes and younger cells have a higher complement of sodium pump units than older red cells. However, they are not aware of any data which suggest that hyperthyroidism is associated with either a subtle change in the differentiation of red cell precursors or an extension of the life span of red blood cells. It is clear that the reduction in red cell pump density is not due to a thyroid hormone induced reduction in the mean volume (and thus surface area) of erythrocytes, since the same differences are noted whether the

results are expressed in terms of cell numbers or in terms of total volume of cells in the assay and

An attractive but at this point unproven explanation for the paradoxical reduction in erythrocyte pump density in hyperthyroidism relates to a possible effect of thyroid hormone on the rate of disappearance of Na/K-ATPase from the red cell membrane.

It is known that thyroid hormone can accelerate the degradation of a number of cell proteins (Flaim K. E. et al, 1978).

CLASSIFICATION OF DISEASES OF THE THYROID

A perfect classification of disorders of the thyroid is not yet possible, owing to the lack of sufficient knowledge with respect to the etiology, physiology and pathology of most thyroid abnormalities. The term 'goiter' denotes enlargement of the thyroid gland whether or not the enlargement is diffuse or nodular, benign or malignant, or attended with symptoms and signs of increased or decreased function. A simple and useful clinical classification of thyroid disease should include only those quantifying terms that are justified by the clinical picture of a given thyroid disorder. The following classification has been used in our clinic and is in accord with that recommended by the American Association for the Study of Goiter.

1. Non-toxic diffuse goiter (iodine-deficiency goiter, endemic and sporadic)
2. Non-toxic nodular goiter
3. Toxic diffuse goiter (exophthalmic goiter, Grave's disease, thyrotoxicosis)
4. Toxic nodular goiter (toxic adenoma)
5. Thyroiditis (inflammatory disease of the thyroid)
    a) acute, suppurative and non-suppurative
    b) subacute or pseudotuberculous
    c) chronic
        1. lymphadenoid goiter (Hashimoto)
        2. Riedel's struma
        3. Tuberculosis
        4. syphilis
        5. actinomycosis
        6. echinococcus disease
        7. Chagas' disease
        8. Amyloid disease
6. Thyroid deficiency
    a) cretinism
        1. endemic
        2. sporadic
    b) myxedema
        1. primary
            i) idiopathic
            ii) following antithyroidal drugs, radioactive iodine therapy and thyroidectomy
        2. secondary to hypopituitarism
7. Neoplasms of the thyroid
    a) benign
        1. papilliferous adenoma or papillary cyst adenoma
        2. r on-papilliferous or simple adenoma
            i) the embryonal adenoma
            ii) the fetal adenoma
            iii) the simple adenoma
            iv) the colloid adenoma
            v) the Hürthle cell adenoma
    b) malignant
        1. low malignancy
        angioinvasive tumours
            i) adenoma
            ii) malignant papillary cystadenoma
        2. moderate malignancy

adenocarcinoma
   i) papillary
   ii) alveolar or solid
   iii) Hürthle cell
3. high malignancy
   i) carcinoma
      a) small cell (carcinoma simplex)
      b) giant cell
      c) epidermoid
   ii) Sarcoma
      a) fibrosarcoma
      b) lymphosarcoma

8. Thyroid anomalies
   a) Lingual goiter
   b) Lateral aberrant thyroid
   c) Thyroglossal cysts

THYROID DYSFUNCTION

Hypothyroidism and hyperthyroidism are the two primary pathological conditions that involve the thyroid glands.

HYPOTHYROIDISM is defined as a deficiency of thyroid activity. It is a common disorder that occurs in mild or severe forms in 2 to 15% of the population. Women are afflicted more often than men, and both sexes are affected more frequently with increasing age. Clinical symptoms may be obvious and easy to recognize or subtle and non-specific, escaping detection. *Myxedema* is a severe form of hypothyroidism in which there is accumulation of mucopolysaccharides in the skin and other tissues. *Cretinism* is the term used to describe severe hypothyroidism in the newborn period.

Many structural or functional abnormalities can lead to thyroid hormone deficiency (Table-4).

TABLE-4 : CAUSES OF HYPOTHYRODISM

PRIMARY (Goitrous) Hypothyroidism
Chronic lymphocytic (Hashimoto's) thyroiditis
Subacute thyroiditis
Post-partum thyroiditis
Endemic iodine deficiency
Defects in hormone biosynthesis and action
Drug-induced (lithium, iodine, anti-thyroid drugs)
PRIMARY (non-goitrous) Hypothyroidism
Spontaneous thyroid atrophy (atrophic myxedema)
Radioactive iodine therapy
Surgical ablation
External radiation
Sporadic athyreotic cretinism
SECONDARY Hypothyroidism
Primary disease
Hypothalamic disease
Peripheral Resistance to Thyroid Hormones

Hypothyroidism is commonly caused by diseases or treatments that destroy thyroid tissues or interfere with thyroid hormone biosynthesis (primary *hypothyroidism*) and less often by disorders of the pituitary or hypothalamus (*secondary hypothyroidism*).

PRIMARY HYPOTHYROIDISM

Primary goitrous hypothyroidism results when the synthesis of T_4 and T_3 is impaired, either because of some extrinsic factor or because of an intrinsic, inherited defect in thyroid hormone biosynthesis, compensatory thyroid

enlargement (goiter) is usually present. Primary non-goitrous hypothyroidism is characterized by loss or atrophy of thyroid tissue, resulting in decreased production of thyroid hormones despite maximum stimulation by TSH. Hashimoto's thyroiditis is the most frequent cause of primary hypothyroidism in developed countries where iodine intake is sufficient. Worldwide, iodine deficiency is the most common cause of goitrous hypothyroidism. The most common cause of non-goitrous hypothyroidism is surgical or radio-ablation of the thyroid in the treatment of Grave's disease. Primary hypothyroidism is frequently associated with circulating anti-thyroid antibodies and may co-exist with other diseases in which autoantibodies are found. In addition, primary hypothyroidism may be one manifestation of an autoimmune syndrome of polyglandular endocrine failure.

Biochemically, decreases in T_4 and T_3 concentrations lead to hyper-secretion of pituitary TSH and an amplified increase in serum TSH levels. The latter is a key laboratory findings, particularly in the early detection of thyroid failure. In mild or subclinical hypothyroidism, thyroid hormone levels may remain within the normal reference interval, but the TSH concentration is elevated. Determining the etiology of primary hypothyroidism is usually possible on the basis of history, physical examination, and tests for circulating thyroid autoantibodies.

Congenital hypothyroidism may be due to absence of the thyroid gland (athyreosis) or may occur secondarily to defects of thyroid hormone synthesis. This disorder occurs once in every 3500 to 4000 live births, and early treatment with thyroid hormone replacement is critical if irreversible neurological damage is to be prevented, screening program for congenital hypothyroidism have been established in almost all developed countries of the world. A majority of North American programs use a two-tiered laboratory approach in which an initial T_4 measurement is followed by a measurement of TSH in specimens with low T_4 values. In most programs, T_4 is measured in blood eluted from a filter paper spot collected from the newborn infant using a heel stick.

Primary hypothyroidism can be easily treated by daily administration of oral thyroxine. During initial treatment serum free T_4 levels adjust quickly, but TSH levels remain high. Because the pituitary is slow to register acute changes in thyroid hormone status ("pituitary lag"), 4 to 8 weeks may be needed for serum TSH values to reach a new steady state after dose changes. Periodic monitoring of serum TSH, one to three times a year, is recommended to help maintain clinical euthyroidism and a normal serum TSH concentration. Excessive oral T_4 should be avoided to minimize the risk of bone absorption or atrial fibrillation.

SECONDARY HYPOTHYROIDISM

Secondary hypothyroidism (central thyroid disease) results from pituitary or hypothalamic diseases that produce a deficiency of TSH, TRH, or both. Isolated TSH deficiency is rare, and most patients with secondary hypothyroidism also have other pituitary hormone deficiencies (parahypopituitarism). With secondary hypothyroidism, serum thyroid hormone concentrations are low, but TSH levels are either low or within the reference interval. When T_4 and TSH levels are both low, a TRH test may offer some benefit (Table-5).

TABLE-5 : TRH TEST : THYROTROPIN-RELEASING HORMONE STIMULATION OF THYROTROPIN RELEASE

Rationale : The pituitary cells that produce TSH are exquisitely sensitive to changes in thyroid hormone concentrations. When thyroid hormone levels rise above normal, TSH release from the pituitary gland is reduced or blocked when thyroid hormone levels fall below normal, TSH release in response to TRH is exaggerated.
Procedure : No patient preparation is necessary. A baseline specimen is collected for TSH determination (and for that of FT_4 if not already done). Five hundred micrograms of TRH is given intravenously and repeat specimens are drawn for TSH determination at 30 and 60 minutes after injection.
Interpretation : A typical response is a 5- to 10-fold increase of TSH level above baseline, with the peak value occuring at 30 minutes. Flat or blunted response are seen in hyperthyroidism (usually with a high T_4 or T_3 concentration) and in hypothyroidism secondary to hypopituitarism (usually with a low T_4 concentration). An exaggerated response (peak TSH level > 35 mU/ml) is seen in early primary hypothyroidism. Patients with hypothyroidism secondary to hypothalamic disorders may show a response of normal magnitude but also a delayed peak.

In patients who have destructive lesions of the pituitary gland that result in TSH deficiency, no TSH response is expected. In patients with hypothalamic abnormalities that affect TRH and TSH release, the peak TSH response to TRH may be normal but is generally delayed until 45 or 60 minutes after the TRH administration rather than after the usual time of 20 to 30 minutes.

HYPERTHYROIDISM

Hyperthyroidism is defined in Dorland's Medical Dictionary as a condition caused by excessive production of iodinated hormones. This disorder can be caused by a number of conditions resulting from excessive quantities of thyroid hormones (Table-6).

TABLE-6 : CAUSES OF THYROTOXICOSIS

Associated with thyroid hyperfunction
• Diffuse toxic hyperplasia (Grave's disease)
• Toxic multinodular goiter (Plummer's disease)
• Solitary toxic adenoma
• TSH-secreting pituitary tumor
• HCG-secreting trophoblastic tumor
• Iodine-induced hyperthyroidism
• Hypermesis gravidarum
Not associated with thyroid hyperfunction
• Subacute thyroiditis
• Silent lymphocytic thyroiditis
• Thyrotoxicosis facticia
• Drug-induced thyrotoxicosis
• Struma ovarii
• Hyperfunctioning metastatic thyroid carcinoma

Some clinicians prefer the general term thyrotoxicosis rather than hyperthyroidism to define the hypermetabolic syndrome associated with increased amounts of thyroid hormones. Causes of thyrotoxicosis can be subdivided into two types : those associated with hyperthyroidism and increased production and secretion of thyroid hormones from the gland, and those that are not. In North America, the most common cause of thyrotoxicosis due to hyperthyroidism is Grave's disease, an autoimmune disorder; its etiology an antibody against the thyroid TSH receptor that results in overproduction of T_4 and T_3 by the thyroid gland. Overproduction of thyroid hormones can also result from autonomous production by solitary or multiple thyroid nodules or from excessive TSH secretion by pituitary tumours (rare). A major cause of non-hyperthyroid thyrotoxicosis is increased leakage of stored hormone from the thyroid gland as a result of inflammatory changes secondary to various forms of thyroditis; new hormone synthesis is decreased due to suppression of TSH secretion by the hormone excess. A miscellaneous cause of non-hyperthyroid thyrotoxicosis is one in which the source of excess thyroid hormone is outside the gland, as in exogenous ingestion (thyrotoxicosis facticia), metastatic thyroid carcinoma, or stuma ovarii.

The prevalence of hyperthyroidism is fairly low in the general population (0.3-0.6%). Hyperthyroidism is often easier to diagnose on clinical grounds than is hypothyroidism (Table-7).

TABLE-7 : SYMPTOMS AND SIGNS OF HYPERTHYROIDISM AND HYPOTHYROIDISM

HYPERTHYROIDISM	
Symptoms	Signs
- Weight loss - Fatigue - Menstrual irregularities - Heat intolerance - Hyperdefecation - Nervousness - Restlessness	- Fine hair, thin skin - Onycholysis - Muscle weakness - Low cholesterol - Glucose intolerance - Tachycardia - Widened phase pressure - Tremor, rapid deep tendon reflexes - Stare, lid lag
HYPOTHYROIDISM	
Symptoms	Signs
- Weight gain - Easy fatigue - Lethargy - Cold intolerance - Hair loss - Constipation - Depression	- Growth retardation - Deep, hoarse voice - Dry, coarse skin - Myxedema - High cholesterol - Bradycardia - Slow reflex relaxation

In some patients with hyperthyroidism, particularly people older than 60 years, the diagnosis may not be self-evident and symptoms may be dismissed or attributed to stress or other causes. Biochemically, increases in T_4 and T_3 suppress circulating TSH to undetectable levels, except in rare cases in which hyperthyroidism is mediated by TSH itself (for example, TSH-secreting pituitary tumor or pituitary resistance to thyroid hormones). Patients with hyperthyroidism have serum TSH less than 0.1 mU/L and usually less than 0.05 mU/L. A serum TSH within the euthyroid reference internal almost always eliminates a diagnosis of hyperthyroidism.

When the TSH level is low, the serum FT_4 concentration should be measured and will be elevated in most cases of hyperthyroidism. Finding of low TSH level and an elevated FT_4 level is usually sufficient to establish the diagnosis of hyperthyroidism. If the TSH level is low but the free T_4 level is within the normal reference interval, a T_3 measurement should be performed, because serum T_3 concentration is often elevated to a greater degree than is T_4 concentration in the early phase of Grave's disease and in some patients with solitary or multinodular toxic goiters (so-called T_3 thyrotoxicosis). A persistently depressed serum TSH concentration associated with normal concentrations of serum T_3 and FT_4 may signify "subclinical hyperthyroidism", a defined biochemical entity with few or subtle clinical symptoms. Because

only the free fraction of T_3 is active, the estimation of free T_3 (e.g. by the use of a total T_3 measurement with a thyroid hormone binding ratio [THBR] and calculation of an FT_3 index) is helpful in adjusting the total T_3 for variations in binding proteins. If needed, FT_3 may be measured directly. Numerous medications and acute and chronic illnesses may cause a transient lowering of T_3 concentrations and a reduction in TSH level. In patients with non-thyroidal illnesses (NTIs), a definite diagnosis of early hyperthyroidism may not be possible, until the other illness has resolved.

Occasionally, increases in serum levels of T_4 and T_3 will occur owing to the ingestion of large quantities of exogenous thyroid hormones or to the release of thyroid hormones as a result of damage to the thyroid parenchyma associated with subacute thyroiditis or chronic lymphocytic thyroiditis. The increase in T_4 and T_3 levels may be associated with clinical findings that suggest true hyperthyroidism. This diagnostic dilemma, however, can be solved by finding a low radioactive iodine uptake (percent of orally administered radioactive iodine taken up by the gland at 6 or 24 hours) that accompanies these non-hyperthyroid varieties of thyrotoxicosis. In most cases of thyroiditis, the condition is self-limited and will resolve without residual thyroid function abnormality.

Until sensitive TSH assays became available, the most conclusive test for hyperthyroidism was the TRH stimulation test (Table-5) in which high levels of thyroid hormone block the release of TSH from the pituitary gland. In a patient with equivocal but suggestive symptoms of hyperthyroidism and T_4 or T_3 levels that are not clearly elevated, failure of the TSH level to rise after TRH administration establishes the diagnosis of hyperthyroidism. The TRH test is rarely needed for the diagnosis of hyperthyroidism now that sensitive TSH assays can directly measure suppressed TSH levels, provided the patient has intact hypothalamic pituitary function.

Treatments for hyperthyroidism – antithyroid drugs radioiodine ablation, or thyroidectomy are designed to decrease thyroid hormone production or inhibit peripheral conversion of T_4 and T_3. Measurements of serum FT_4 are recommended every few weeks until symptoms abate and serum values normalize; continuous monitoring for recurrence of hyperthyroidism is suggested two or three times a year after successful therapy. Because the pituitary gland has been suppressed for a long time, serum TSH is not an accurate measure of effective treatment and remains undetectable for months after the patient becomes clinically euthyroid. Ablation of thyroid tissue or over-treatment with an antithyroid drugs may lead to clinical hypothyroidism

and an increase in serum TSH' surveillance for hypothyroidism must continue for the life of the patient.

NON-THYROIDAL ILLNESS

Many disorders are characterized by thyroid hormone excess or deficiency in the absence of definable thyroid disease. These states of euthyroid hyperthyroxinemia or euthyroid hypothyroxinemia often result from alteration in the concentration of thyroid hormone-binding proteins, the actions of certain drugs, the effects of acute and chronic NTIs, or peripheral resistance to thyroid hormones.

A progressive spectrum of test abnormalities accompanies NTIs in euthyroid patients (Table-8) (the "euthyroid sick syndrome"). The earliest and most common changes that occur are a reduction in the serum concentrations of total and free T_3, sometimes to extremely low levels and an elevation in the serum level of rT_3 (the "low T_3 level"). These changes have been ascribed to a block in the conversion of T_4 and T_3 in peripheral tissues.

Acute and chronic nutritional problems, poorly controlled diabetes mellitus and drugs such as β-blockers can also inhibit this conversion.

TABLE-8 : EFFECT OF ACUTE AND CHRONIC ILLNESS ON THYROID FUNCTION

Reduced peripheral conversion of T_4 to T_3
Increased production of rT_3
Reduced production of thyroid hormone binding proteins
Circulating inhibitors of extra-thyroidal binding
Circulating inhibitors of thyroid hormone binding
Reduced cellular T_3 and T_4 receptors
Reduced cellular T_3 action
Mild elevation of serum TSH during recovery phase
Mild depression of serum TSH during acute phase.

Declining levels of total T_4 may also be seen in NTIs; however, FT_4 concentrations, determined using reference methods, usually remain within the normal reference interval or are mildly elevated. This disparity between falling total T_4 values and normal, or even elevated, free T_4 levels may be caused by decreases in serum concentrations of thyroid hormone-binding proteins, changes in bind ng properties induced by circulating inhibitors and drugs, or both. Unfortunately, some methods for estimating FT_4, such as the conventional FT_4 index and one step immunoassays, may provide artifactually low values in euthyroid patients with increasing severity of chronic illness.

Serum TSH concentrations are usually normal in euthyroid sick patients but may be mildly depressed during the acute phase of NTI or slightly elevated

during recovery from severe illness. Causes of these transient abnormal TSH levels are not fully understood but may relate to the effects of endogenous or exogenous hormones, such as glucocorticoids or dopamine, which independently suppress pituitary TSH secretion; altered nutrition; or altered biological activity of immunoreactive TSH.

As patients recover from NTIs, many of these test abnormalities revert to normal. Total T_4 levels will be corrected first followed by a rise in T_3. Serum TSH may also transiently rebound to high levels for several days or weeks before returning to normal.

HYPOTHYROIDISM VERSUS EUTHYROID SICK

The most common dilemma presented by the test abnormalities seen with the euthyroid sick syndrome occurs when hypothyroidism is suspected in an ill patient. Total and free T_3 levels are expected to be low in non-thyroidal illness and should not be measured for this purpose. If the total or free T_4 level is normal, hypothyroidism is most unlikely; however, a reduced total T_4 and a slightly subnormal free T_4 estimate may be seen in extremely ill euthyroid patients. Serum TSH is probably the best single test to address the differential between euthyroid sick and hypothyroidism (in absence of suspected pituitary or hypothalamic disease). A clear elevation of TSH level (> 30 mU/L) would indicate hypothyroidism. Lesser TSH elevations may be seen transiently in

euthyroid sick patients during recovery. Increasing the upper limit of the TSH reference limit to 8 to 12 mU/L in critical illness has been suggested to improve specificity for hypothyroidism with little loss of sensitivity. Dopamine or glucocorticoids also suppress pituitary TSH release and may result in a normal TSH level in a hypothyroid patient. If the question of hypothyroidism in acutely ill patients cannot be resolved with TSH and free T_4 testing, measurement of rT_3 may help (rT_3 being low in hypothyroidism [either primary or secondary] and normal or high in euthyroid subjects). Documentation of a normal serum cortisol may help distinguish euthyroid sick patients from those with hypothalamic or pituitary hypothyroidism.

HYPERTHYROID VERSUS EUTHYROID SICK

As many as 3% hospitalized patients on admission have subnormal TSH values often associated with the acute phase of illness or with glucocorticoid or dopamine therapy. With more sensitive TSH always, however, it is possible to separate hyperthyroid sick patients with profoundly low TSH values (less than 0.01 mU/L) from critically ill euthyroid patients with only mild suppressive of TSH in the 0.01 to 0.1 mU/L range.

EFFECT OF DRUGS

Medications may alter thyroid function and thyroid function tests. Few drugs are associated with the development of clinically significant thyroid disease, but

difficulty in interpretation of thyroid function tests often results. In general, drugs do not interfere chemically with assays for thyroid hormones or TSH. The medications most likely to affect TSH levels are glucocorticoids and dopamine (reduced TSH levels) and amidodarone (increased TSH levels). The most commonly encountered variations in thyroid hormone measurement induced by medication are reduced peripheral conversion of T_4 to T_3 or altered binding of T_4 and T_3 to carrier proteins. Some drugs that affect laboratory findings are shown in Table-9.

TABLE-9 : EFFECT OF SOME DRUGS OF TESTS OF THYROID FUNCTION

Cause	Drug	Effect
Inhibit TSH secretion	Dopamine L-dopa Glucocorticoids Somatostatin	$\downarrow T_4$; $\downarrow T_3$; $\downarrow$TSH
Inhibit thyroid hormono synthesis or release	Iodine Lithium	$\downarrow T_4$; $\downarrow T_3$; $\uparrow$TSH
Inhibit conversion of T_4 to T_3	Amidodarone Glucocorticoids Propranolol Propylthiouracil Radiographic contrast agent	$\downarrow T_3$; $\uparrow rT_3$; $\uparrow, \leftrightarrow$, $\uparrow T_4$ and FT_4; $\leftrightarrow$, $\uparrow$ TSH
Inhibit binding T_4/T_3 to serum proteins	Salicylates Phenytoin Carbamazepine Furosemide Non-steroidal anti-inflammatory agents Heparin (in vitro effect)	$\downarrow T_4$; $\downarrow T_3$ $\downarrow FT_4E$; $\leftrightarrow$, $\uparrow FT_4$; $\leftrightarrow$ TSH

Stimulate metabolism of iodothyronines	Phenobarbital Phenytoin Carbamazepine Rifampicin	$\downarrow T_4$; $\downarrow FT_4$; $\leftrightarrow$ TSH
Inhibit absorption of ingested T_4	Aluminium hydroxide Ferrous sulphate Cholesytramine Colestipol Iron sucralfate Soyabean preparations Kayexalate	$\downarrow T_4$; $\downarrow FT_4$; $\uparrow$TSH
Increase in concentration of T_4 binding proteins	Estrogen Clofibrate Opiates (heroin, metadone) 5-Fluouracil Perphenazine	$\uparrow T_4$; $\uparrow T_3$; $\leftrightarrow FT_4$; $\leftrightarrow$ TSH
Decrease in concentration of T_4 binding proteins	Androgens Glucocorticoids	$\downarrow T_4$; $\downarrow T_3$; $\leftrightarrow FT_4$; $\leftrightarrow$ TSH

DIAGNOSIS OF THYROID DYSFUNCTION

Laboratory tests most commonly used to evaluate patients for thyroid hormone dysfunction are listed in Table-10.

In the past, thyroid testing was performed stepwise, with the initial step being either a total serum T_4 measurement or a free thyroxine estimate (FT_4E) using, for example, the FT_4 index. Total T_4 measures both bound and free hormone and is a good reflection of thyroid hormone production; however, changes in the concentration or affinity of serum thyroid-hormone-binding proteins affect total T_4 levels without changing the free, active hormone. Total T_4 and FT_4E are not ideal indicators of thyroid status, in part because of the effects of variations

in serum binding protein levels, but also because T_3 is the primary active thyroid hormone and because the relationship between these hormones (T_4 and T_3) are not always predictable. In patients with hyperthyroidism, T_3 is usually elevated to a greater degree than T_4 because it is derived from two sources, increased thyroidal secretion of T_3 and increased peripheral conversion of T_4 to T_3. T_3 determination is sometimes a useful adjunctive test in patients suspected to have hyperthyroidism. However, because T_3 levels fluctuate rapidly in response to stress and other non-thyroidal factors, T_3 levels are low not only in hypothyroidism, but also in many other conditions. Thus, measurement of T_3 is also not a good general test of thyroid status.

The serum level of TSH reflects the integrative action of all thyroid hormones in one of its target tissues – the pituitary cells that secrete TSH. Measurement of TSH is more reliable in the diagnosis of thyroid hormone abnormalities than is measurement of thyroid hormone levels. Pituitary TSH secretion is very sensitive to circulating thyroid hormones; in fact, a two-fold change in free T_4 causes an approximate 100-fold change in the serum TSH concentration.

In the past, clinical use of TSH measurements was limited by the inability of most immunoassay to differentiate the lower limit of normal from abnormally low levels. Improved assay techniques that can distinguish low from normal levels are now available, which have altered the approach to thyroid function

testing. Instead of beginning with an FT_4E test, a sensitive TSH assay is now the accepted initial screening test of thyroid function (Fig.3 : May ID, 1988, EMCNA)

FIGURE-7

Proposed TSH based strategy for the laboratory investigations of thyroid function

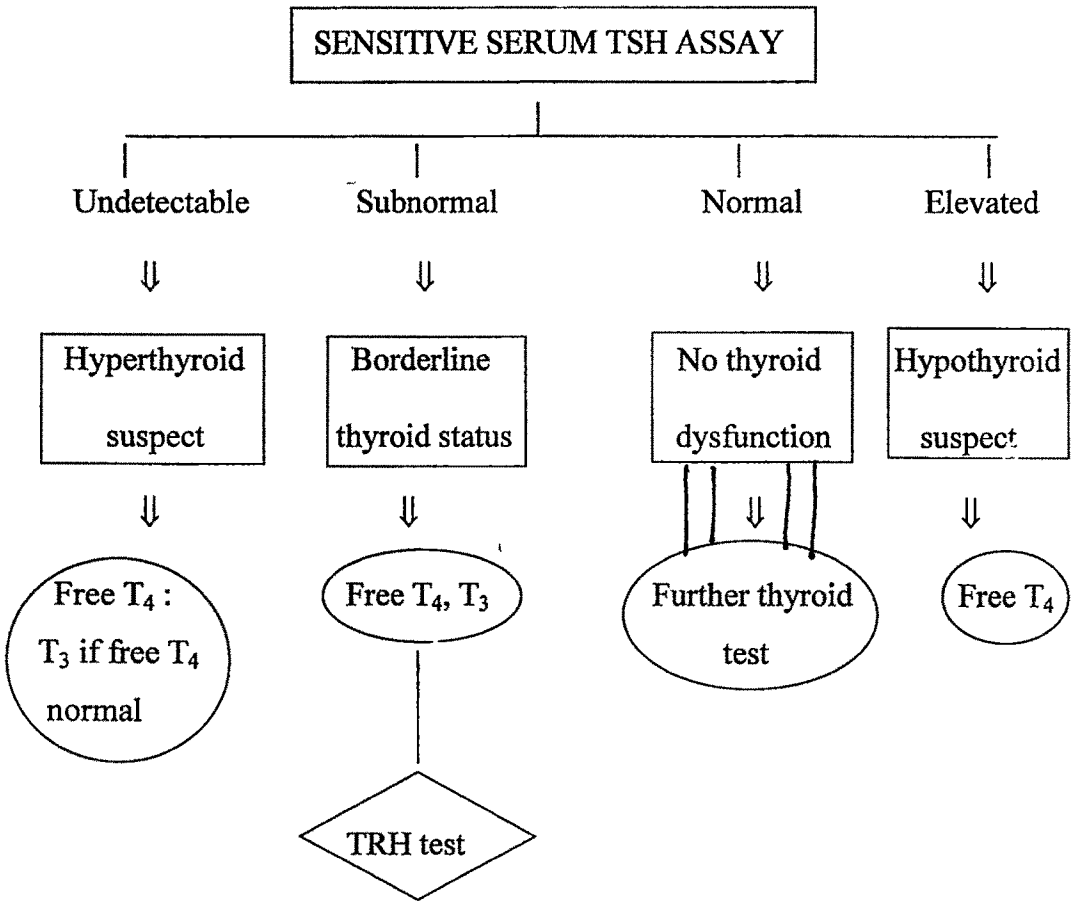


TABLE-10 : NOMENCLATURE – FOR THYROID TESTS

[I] Hormone Concentrations		
	1. T_4	Total serum thyroxine
	2. T_3	Total serum tri-iodothyronine
	3. FT_4D	Free thyroxine (dialysis method)
	4. FT_3D	Free tri-iodothyronine (dialysis method)
	5. rT_3	Reverse 3,3'5'-tri-iodothyronine
	6. TSH	Thyroid stimulating hormone, thyrotropin
[II] Indirect measure of binding sites		
	1. T_3U	T_3 resin uptake
[III] Indirect estimates of free hormone concentration		
	1. FT_4 index (FT_4I)	Free thyroxine index (T_4 X T_3U)
	2. FT_3 index (FT_3I)	Free tri-iodothyronine index (T_3 X T_3U)
[IV] Serum binding proteins		
	1. TBG	Thyroxine-binding globulins
	2. TBPA	Thyroxine-binding prealbumin
[V] Determination of Thyroglobulin (TG)		
[VI] Determination of human thyroid stimulating immunoglobulin (HTSI)		
[VII] Determination of antithyroglobin and antimicrosomal antibodies.		

Table-11 explains the physiological effects of thyroid hormone.

TABLE-11 : BASIC PHYSIOLOGICAL EFFECTS OF THYROID HORMONE AND THEIR RELATIONSHIP WITH SYNDROMES OF DYSFUNCTION

System	Thyroid hormone effects	Usual symptoms	
		Hyperthyrodism	Hypothyroidism
Metabolic	Increased calorigenesis and O_2 consumption Increased heat dissipation Increased protein catabolism Increased glucose absorption and production (gluconeogensis)	Heat intolerance Flushed skin Increased perspiration Increased appetite And food ingestion Muscle wasting and weakness Weight loss	Cold intolerance Dry and pale skin Decreased appetite and food ingestion Generalized weakness Weight gain
Cardio-vascular	Increased adrenergic activity and sensitivity Increased heart rate Increased cardiac output	Palpitations Fast heart rate (tachycardia) Increased BP mainly systolic Bouncy hyperdynamic arterial pulses Shortness of breath	Slow heart rate (Bradycardia) Heart failure Heart enlargement
Central nervous system	Increased adrenergic activity and sensitivity	Restlessness, hypermotility Nervousness Emotional lability Fatigue Exaggerated reflexes	Apathy, mental sluggishness Depressed reflexes Mental retardation
Gastro-intestinal (GI)	Increased motility	Hyperdefecation	Constipation

Determination of Thyroid Stimulating Hormone (TSH) in Blood

Measurement of TSH were originally based on bioassays, such as the stimulation of colloid droplet formation in the guinea pig thyroid gland and the release of labelled thyroidal iodide into mouse blood. These early in vivo bioassays, however, were of limited sensitivity and precision and were not applicable to the measurement of TSH in infractionated serum. Most TSH bioassays have involved the in vitro stimulation of thyroid cyclic adenosine monophosphate (cAMP) or adenylate cyclase activity. The rat FRTL-5 thyroid cell line is an example of a particularly convenient and precise assay system.

Principle

The first immunological assay for TSH was based on the cross-section of human and bovine TSH in a hemagglutination inhibition test, however, this method was too insensitive for clinical purposes. Immunoassay measurement of serum TSH did not become a routine test until the purified pituitary hormone became available for immunization and iodination.

Radioimmunoassay

The traditional radioimmuno assay (RIA) was based on competition between endogenous and radiolabelled hormone for binding sites on a limited amount of antibody. Separation of antibody-bound and free radio ligands was conveniently performed by double-antibody precipitation (enhanced by the

addition of polyethylene glycol) or by using a solid-phase second antibody procedure. The amount of labelled TSH bound to the antibody was inversely related to the amount of unlabelled TSH present in the serum specimen.

The determination of serum TSH by RIA proved very valuable in assessing the elevated TSH values in primary hypothyroidism. However, because they could only detect 1 mU of TSH per litre, most conventional RIAs could not distinguish normal values from subnormal values associated with hyperthyroidism.

Immunometric Assays

New immunometric techniques are now capable of measuring TSH at levels that routine clinical laboratories need to detect low levels of serum TSH. These new sensitive methods resulted from the application of the immunometric sandwich configuration, in which a serum TSH molecule forms a bridge between two or more distinct anti-TSH antibodies.

In first antibody (of monoclonal origin) is often directed at the specific β-subunit and is anchored to a solid-phase separation system. This antibody is present in excess and selectively immuno extracts the majority of TSH molecules from the serum specimen. Bound hormone is then quantitated using a second TSH antibody (of either monoclonal or polyclonal origin) that is

directed against a distinctly different antigenic site on the TSH molecule for example, the α-subunit). This detection antibody is labelled with a signal molecule, such as a radioisotope, enzyme, fluorophore, or luminescent molecule.

Immunometric assays for TSH are available commercially as manual kit procedures or for use on automated systems. For practical reasons, non-isotopic methods dominate the market and have replaced radioactive tracer methods in most routine laboratories. The majority of immunometric TSH assays label the detection antibody with peroxidase or alkaline phosphatase; sensitive photometric, fluorescent or chemiluminescent substrates are used to measure enzyme activity.

A "generational" classification based on functional sensitivity has been suggested to describe improvements in TSH assays. Each generation of assay represents one log improvement in its detection limit.

First generation RIAs typically had a functional detection limit of 1 to 2 mU/L. The subsequent immunometric assays had about a 10-fold improvement in their functional detection limit (0.1-0.2 mU/L) and are considered second-generation TSH assays, which can reliably distinguish normal from hyperthyroid (or hypothyroid) values in ambulatory patients; however, they have limited value

for distinguishing mildly subnormal values (0.01-0.1 mU/L) from the very low values (< 0.01 mU/L) that are typical thyrotoxicosis.

Third-generation immunometric assays have been developed for TSH determination that have enhanced precision at even lower detection limits. Most of these assays use chemiluminescent technologies and have a functional detection limit of less than 0.02 mU/L.

Research assays capable of fourth-generation functional detection limit in the 0.001 to 0.002 mU/L range also have been developed.

Reference Intervals

Secretion of TSH occurs in a circadian fashion : highest level prevail at night between 0200 to 0400 hr, and lowest levels occur between 1700 to 1800 hr. TSH surges immediately after birth, peaking at 30 minutes at 25 to 160 mU/L; values decline back to cord blood levels by 3rd and reach adult values in the first week of life.

Expected values using a third-generation immunochemiluminometric assay are as follows :

	μU/L
▯ Premature, 28-36 weeks (1st week of life)	0.7 – 27.0
▯ Cord blood (> 37 weeks)	2.3 – 13.2
▯ Children	
- Birth – 4 days	1.0 – 39.0
- 2-20 weeks	1.7 – 9.1
- 21 weeks – 20 years	0.7 – 64.0
▯ Adults	
- 21-54 years	0.4 – 4.2
- 55 – 87 years	0.5 – 8.9
▯ Pregnancy	
- First trimester	0.3 – 4.5
- Second trimester	0.5 – 4.6
- Third trimester	0.8 – 5.2

Determination of Thyroxine in Serum

Indirect Methods

Serum total T_4 concentrations were initially determined indirectly, using methods that measured the amount of iodine in a protein precipitate of serum (protein-bound iodine, PBI). In addition to hormonal iodine, the PBI tests also measured iodoproteins, iodotyrosines, inorganic iodine, and Tg. Nevertheless, these methods are relatively non-specific and are rarely used.

Competitive Protein Binding Assays

These methods were based on the competition between labelled and unlabelled T_4 for a limited number of protein-binding sites. The specific binding protein used in the majority of these assays was TBG, which was usually obtained from pooled human sera. Anion-exchange resins were often used to separate free and bound hormone fractions. Competitive protein binding methods, required a time-consuming step to extract T_4 from serum prior to assay. Although these methods were free from interference by non-hormonal iodine, they have subsequently been replaced by more sensitive and specific immunoassay methods.

Instrumental Methods

Isotope dilution-mass spectrometry is a proposed method for assay of T_4 in serum, in which, tritium-labelled T_4 is added to a fixed amount of serum. T_4 is then extracted from the serum, derivatized and subjected to combined gas chromatography – mass spectrometry. Electron capture gas chromatography and high performance liquid chromatography are also been used for measuring T_4 in human serum.

Immunoassays

At present, all clinical laboratory assays of total T_4 are competitive immunoassays. Many T_4 immunoassay use high affinity antibodies produced

against an albumin – T_4 conjugate. These polyclonal antisera are also highly specific and are able to distinguish among molecules differing by only one atom (e. g. T_3 and T_4). Tg is also used as an immunogen, because it contains T_4 (and T_3) as part of its structure. Monoclonal antibodies against T_4 have also been developed.

Immunoassays of total T_4 measure both free and protein bound thyroxine, which requires dissociation of T_4 from its serum transport proteins, because 99.97% of T_4 circulates tightly bound to TBG albumin, and TBPA. Binding of T_4 to albumin is usually not a concern, because the association constant of T_4 for antibody (usually 10^9 L/mol or greater) is several orders of magnitude higher than that of T_4 for albumin (~ 1.6 X 10^6 L/mol). However, binding constants for T_4 with TBG and TBPA are high (2 X 10^{10} L/mol and 2 X 10^8 L/mol, respectively). Binding of T_4 to TBPA is overcome by the use of barbital buffers, because barbital ions selectively inhibit this binding. Various blocking agents can be used to inhibit this binding of T_4 to TBG; 8-amino-1-naphthalene-sulfonic (ANS) acid appears to be the agent of choice.

Determination of Reverse Triiodothyronine using Radioimmunoassay

Numerous RIA's for rT_3 measurements have been published. Antisera for rT_3 are generally obtained by immunizing rabbits using rT_3 conjugates with HASA (Human serum Albumin) or BSA (Bovine Serum Albumin). The antisera are

usually devoid of significant cross reactivity with T_2 or T_3; cross-reaction with T_4 varies between 0.01 and 0.15%.

Like T_3 and T_4, rT_3 also binds with TBG, TBPA and albumin in serum. The binding of rT_3 to serum proteins has been overcome by the use of an ethanol extract of serum or by the addition of ANS to the serum in a manner similar to that described for T_3 and T_4. Separation of free and antibody-bound hormone is often achieved using a second-antibody (e. g. anti-rabbit γ-globulin) procedure accelerated with polyethylene glycol.

Reference Intervals

	ng/dl	mmol/L
Cord blood (> 37 weeks)	130-300	2.00-4.62
Children		
- 1 day	83-194	1.28-2.99
- 2 days	107-209	1.65-3.22
- 3 days	102-166	1.57-2.56
- 1 month to 20 years	10-35	0.15-0.54
Adult	10-28	0.15-0.43
Maternal serum (15-40 weeks)	11-33	0.17-0.51
Amniotic fluid (17-22 weeks)	163-599	2.51-9.22

Determination of Free Thyroid Hormones

Direct Reference Methods

Direct measurement of FT_4 and FT_3 in serum presents a considerable technical challenge. Free hormone concentrations are exceedingly low in normal serum (approximately 0.03% of the total serum T_4 and 0.3% of the total serum T_3 concentrations). Consequently, an assay must be able to measure subpicomole amounts. Theoretically, the most reliable methods for measuring FT_4 and FT_3 in serum are equilibrium dialysis and ultrafiltration methods that physically separate free hormone from protein-bound hormone before direct measurement of the free fraction with a sensitive T_4 or T_3 immunoassay. Only minimal dilution of serum specimens is allowed, because dilution alters the binding of drugs, free fatty acids, and other substances to serum proteins, thus disturbing the equilibrium between bound and free hormone.

Reference Intervals

Expected values for FT_4 using direct equilibrium dialysis are:

	ng/dl	mmol/L
Newborn (1-4 days)	2.2-5.3	28.4-68.4
Children (2 weeks-20 years)	0.8-2.0	10.3-25.8
Adults (21-87 years)	0.8-2.7	10.3-34.7
Pregnancy		
- First trimester	0.7-2.0	9.0-25.7
- Second and third trimester	0.5-1.6	6.4-20.6

Comments:

FT_4 assays based on direct equilibrium dialysis or ultrafiltration measure free hormone without knowledge of total hormone. These methods are unaffected by either variations in serum binding proteins or thyroid hormone autoantibodies.

Indirect Methods for Estimating Free Thyroid Hormones

Unlike direct reference assays, indirect index methods use two separate tests to estimate the free hormone concentration : a total serum T_4 (or T_3) measurement and an assessment of either the serum TBG level or the fraction of T_4 (or T_3) that is free in serum; the latter is traditionally derived using equilibrium tracer dialysis or "T uptake" methods. Results of these tests are then combined mathematically to give estimates of the free hormone concentrations.

Free Hormone Fraction by Indirect Equilibrium Tracer Dialysis

Principle

Test serum is enriched with a very small quantity of radioiodine-labelled T_4, which quickly distributes between free and bound T_4 to match the distribution of endogenous hormone. The fraction of labelled hormone that dialyzes through a semipermeable membrane is then measured.

Reference Intervals for % FT_4 range between 0.02% and 0.04% of total hormone concentration. Because T_3 is less firmly bound by TBG than is T_4, the dialyzable fraction of T_3 is appreciably greater (by almost 10 times) than that of T_4. Thus, the reference interval of % FT_3 is 0.2 to 0.4%.

Calculation of Free Hormone Estimates

Principle

The free hormone fraction, as measured by dialysis or ultrafiltration of diluted serum containing tracer T_4 or T_3, is multiplied by the respective total hormone concentration to obtain indirect estimates of FT_4 or FT_3.

Total T_4 X % FT_4 = FT_4E

Total T_3 X % FT_3 = FT_3E

Reference Intervals

The expected calculated values for FT_4 and FT_3 using equilibrium tracer dialysis are as follows:

	Ng/dl	Pmol/L
Cord	1.7-4.0	22-52
	2.6-6.3	34-81
	0.8-2.3	10-30

FT_3E

	Pg/dl	Pmol/L
Cord	15-391	0.2-6.0
Child and adult	210-440	3.2-6.8
Pregnancy	200-380	3.1-5.9

Free Hormone Fraction by T uptake Methods (Thyroid Hormone Binding Ratio)

The THBR is derived from a version of the T_3 or T_4 uptake test. Uptake tests are used to estimate the number of unoccupied (unsaturated) thyroid hormone binding sites on serum proteins. Measurement of THBR, in conjunction with a total hormone concentration, is a clinically useful indirect method for calculating the FT_4 (or FT_3) index.

Isotopic Methods

Many different radioactive tracer methods are available for estimating the unsaturated thyroid hormone binding capacity. The simplest is the T_3 uptake test. Traditionally, this test is performed by measuring the distribution of radiolabelled T_3 between serum binding proteins and a solid-phase binding material.

A diluted sample of patient serum is allowed to equilibrate with a trace amount of ^{125}I-T_3 and the secondary binder. A portion of the radioactive T_3 binds to empty binding sites in serum proteins, principally TBG, and the remainder attaches to the solid (the "uptake") is measured. The amount of T_3 tracer bound to the secondary binder varies inversely with the number of unoccupied TBG binding sites in the serum specimen and directly with the free fraction of T_4 and T_3.

Non-Isotopic Methods

Several T_4 and T_3 uptake assays that use non-isotopic tracers have been marketed. Many methods use enzymes to label T_4 and T_3; different photometric, fluorescent and luminescent substrates are available for monitoring the enzyme activity. Direct labelling of T_4 or T_3 antigens with fluorophors or chemiluminescent molecules is also popular. These non-isotopic methods are generically named "T uptake" assays, regardless of whether T_4 or T_3 is used as the labelled analogue. The competitive binder commonly used in many of these assays is a T_4 antibody bound to solid phase. Relatively little attention has focused on the analytical and clinical performance of these T uptake assays.

Reference Intervals

	THBR
Cord	0.75-1.05
Children	
- 1-3 days	0.90-1.40
- 1-2 weeks	0.85-1.15
- 1-4 months	0.75-1.05
- 1-15 years	0.88-1.12
Adult	
- > 50 years	0.83-1.11
- Male	0.87-1.11
- Female	0.80-1.04
Pregnancy (las. 5 months)	0.68-0.87

Comments

In hyperthyroidism, more binding sites and in hypothyroidism, fewer binding sites on TBG are occupied by T_4. As a result, the THBR is high in hyperthyroidism and low in hypothyroidism. In cases of decreased and increased TBG, the THBR is high and low, respectively. Deviations of THBR and total T_4 in the same direction from normal (i. e. a concordant variance) suggest an abnormality in thyroid hormone production; a discordant variance suggest primary change in the concentration or affinity of circulating TBG.

Diagnostic Utility of THBR and FT_4 Index

Clinical condition	Total T_4	THBR	FT_4I
Euthyroid	N	N	N
Hyperthyroid	↑	↑	↑
Hypothyroid	↓	↓	↓
Increased TBG	↑	↓	N
Decreased TBG	↓	↑	N

Calculation of Free T_4 and T_3 Indexes

The THBR assay is not designed to be used as an independent and isolated test for thyroid disease. It is useful only when combined with a measurement of total T_4 (or T_3) level to calculate on FT_4 or FT_3 index.

Principle

The calculation of a free T_4 index is based on the equilibrium relationship that exists between bound (TBP : T_4) and free T_4 :

$FT_4I = [T_4] \times THBR$

Reference Intervals

	FT_4 Index	
	µg/dl	nmol/L
Cord	6.0-13.2	77-170
Infants		
- 1-3 days	9.9-17.5	128-226
- 1 week	7.5-15.1	97-195
- 1-12 months	5.0-13.0	65-168
Children		
- 1-10 years	5.4-12.8	70-165
Pubertal child and adult	4.2-13.0	54-168

HDL ALTERATIONS

Continuing interest in the structure and function of human high density lipoproteins (HDL) derives in major part from their important role in lipid metabolism and from the epidemiologic observation of their inverse correlation with the risk of arterial disease. Early ultracentrifugal studies demonstrated heterogeneity in the density and size distributions of HDL class, and defined two major subclass HDL_2 (d 1.063 – 1.125) and HDL_3 (d 1.125 – 1.200). More recently, additional heterogeneity within each of the above two subclasses has been described. Reports of occurrence, to a greater or lesser extent, of two subpopulations within the HDL_2 subclass were published by Anderson D. W., Nichols A. V. et al (1977). These subpopulations, HDL_{2b} and HDL_{2a} were defined according to the density intervals d 1.063-1.100 and d 1.100-1.125, respectively, and were characterized according to particle size by gradient gel electrophoresis.

The metabolism of plasma high-density lipoprotein (HDL) appears to be closely related to that of the triglyceride-rich lipoproteins, chylomicrons and very low density lipoprotein (VLDL). A negative correlation between the VLDL triglyceride and HDL-cholesterol concentrations has been found in several communities (S. N. Rao, 1980). Changes in VLDL triglyceride concentrations in response to diet or drugs are occuring physiologically are commonly

accompanied by reciprocal changes in HDL-cholesterol concentration. The major apoproteins of HDL, apoprotein AI (Apo AI) and apoprotein AII (apo AII) have been shown to enter plasma partly as components of nascent chylomicrons.

Cholesteryl esters have been shown to transfer from HDL to VLDL in exchange for triglyceride in vitro. HDL's act as a reservoir for apolipoprotein C-II, the activator of lipoprotein lipase.

Furthermore, hydrolysis of VLDL triglyceride by lipoprotein lipase in the isolated perfused rat heart results in the transfer of surface components to the HDL density range (Chajeck T. Eisenberg S., 1978).

Alfred G. Scottolini et al (1980) studied alterations in the concentrations of high density lipoprotein cholesterol and of other lipid categories (total cholesterol, VLDL and TG) in serum in hypo- and hyperthyroid states. In untreated hypo- and hyperthyroid subjects, all of the lipid values differed significantly from those of the controls but promptly returned to normal values upon treatment. In hypothyroid patients, who are prone to develop coronary heart disease, the concentrations of high-density lipoprotein cholesterol were high, suggesting protection against heart disease. In our study, the HDL concentrations in the patients suffering from type III hyperlipoproteinemia had increased HDL

concentrations ($P < 0.001$) whereas those with hypercholesterolemia and type IIb hyperlipoproteinemia the change in the HDL concentration was not statistically significant. The changes in patients with hyperthyroidism and T_3 toxicosis were found to be statistically significant ($P < 0.001$), so also were the changes in HDL of the last two groups of patients secondary to liver disease and renal disease significantly ($P < 0.001$) decreased as compared to the normal.

According to Erik Muls et al (1985) severe hypothyroidism has a differential influence on the serum levels of the major HDL proteins apo AI and AII that might reflect changes in the HDL subfraction distribution and/or composition. In the hypothyroid state the major increase appeared in the lighter HDL_{2b} (d.1.063 to 1.100 gm/ml) fraction. The elevation of the HDL 3b + 3c (d. 1.150 to 1.210 g/ml) subclass was less pronounced and the intermediate HDL 2a + 3a fraction was unaffected by the disease. This redistribution of the HDL subfraction was associated with increased concentrations of cholesterol, phospholipid and Apo AI in HDL_{2b}, and of phospholipid and apo AI in HDL_{3b+3c}. Treatment of hypothyroidism, the concentrations of these fractions, $HDL_{2a + 3a}$ became the major HDL subclass in the euthyroid state. These data were in agreement with previous studies, documenting an increased mass of HDL_2 relative to HDL_3 in severe hypothyroidism.

The differential influence of severe hypothyroidism on apo AI and apo AII suggest that apo AI metabolism is more sensitive to thyroid regulation than that of apo AII. Moreover, changes in the surface apoprotein distribution of HDL particles might contribute to the observed accumulation of heavier $HDL_{3b + 3c}$ particles as the conversion of these particles into less dense $HDL_{2a + 3a}$ lipoproteins seems to be modulated by apoprotein transfer, specifically of apo AII. The major compositional difference between these two fractions in the presence of one apo AII molecule more in $HDL_{2a + 3a}$ than in $HDL_{3b + 3c}$. Valdemarsson et al (1983) have recently proposed that changes in the activity mainly of hepatic lipase, but also of lipoprotein lipase and LCAT, contribute to the alterations in HDL distribution in hypothyroidism. The cholesterol esterifying ability, as measured in an endogenous substrate assay of LCAT, is moderately lowered. The hepatic lipase activity is consistently decreased in hypothyroid patients. This might induce a deficient HDL_2 clearance from plasma and contribute to the accumulation of unusually light LDL particles. The low lipoprotein lipase activity observed in most, but not all, patients with severe hypothyroidism might counterbalance the hepatic lipase mediated effects on HDL_2 by decreasing the input of cholesterol into the HDL system.

Previous studies have suggested that hypothyroidism influences apo E metabolism in the following way: The VLDL apo E to apo C's ratio increases

in hypothyroid women (Pagnan A. et al, 1980) and apo E-rich, larger HDL particles accumulate in experimental hypothyroidism in rats (Dory L. et al, 1981).

The data obtained from hypothyroid women with marked increases in HDL_{2b} indicate that the apo E concentrations in HDL_{2b} increased to a lesser extent than the apo E content of VLDL. The major effect of hypothyroidism on apo E metabolism therefore seems to be an enrichment of VLDL with apo E, resulting in a significant increase of the plasma apo E level.

Hypothyroidism is associated with an increased risk for atherosclerosis (Erik Muls, 1985) despite the elevated plasma HDL cholesterol, apo AI and HDL_{2b} levels. This seems to be contrary to the commonly accepted protective effect of HDL against atheromatous disease. In this context, it should be emphasized however, that the major aspect of dyslipoproteinemia in hypothyroidism consists in a marked and consistent increase in cholesterol-rich lipoproteins, which play a causal part in development of coronary heart disease.

Current cross-cultural epidemiological and clinical data do not permit to interpret the relation between HDL and atherosclerosis in causal terms, ethnic and geographic differences in the incidence of coronary heart disease are not

match by reciprocal differences in HDL levels, and some of the genetic low HDL syndromes are apparently not associated with accelerated atherogenesis.

Moreover, the elevation of HDL cholesterol, apo AI and HDL_{2b} in hypothyroidism may not represent an enhanced mobilization of cholesterol from tissues but rather an impaired cholesterol elimination to the liver via the HDL system (Valdemarsson S. et al, 1983).

Liver Disease

Various abnormalities in thyroid hormone indices are seen, depending on the type and severity of hepatic disease. In chronic alcoholic liver disease, serum T_3 is decreased roughly in proportion to the degree of hepatocellular dysfunction. (The lowest levels of T_3 are found in end-stage cirrhosis). In many cases of cirrhosis, T_4 binding to TBG is diminished end, therefore, total T_4 levels are low. The mechanisms of low plasma binding of T_4 are complex. FIRST, the liver is the site of TBG synthesis, so this protein may participate in a general decline in the levels of plasma proteins that are made in the liver. SECOND, TBG is normally removed by the liver following removal of sialic acid residues. Hepatocellular disease causes a built up in the blood of partially desialylated TBG ("Slow" TBG), which binds T_4 less avidly than normal TBG (Marshall et al, 1977). THIRD, evidence has been presented indicating the existence of circulating inhibitors of T_4 binding to TBG in liver disease, as well as in other types of NTI. However, the quantitative importance and the nature of such inhibitors are still controversial.

An elevation in serum total T_4 due to increased serum TBG concentration is seen in some forms of liver disease, namely, chronic active hepatitis and primary biliary cirrhosis (nearly 50% of cases according to Schussler), less frequently in some cases of viral hepatitis in the acute phase, and in acute

intermittent porphyria (Hollander E, et al, 1967) it is not clear whether in these conditions the synthesis and release of TBG from the liver are increased or whether TBG removal is impaired. Free T_4, when measured, has been normal in these patients, unless they also were hypothyroid (Schussler et al, 1978).

Some cases of acute alcoholic hepatitis exhibit a transient elevation in both total and free T_4 (Gravin L. A. et al, 1979). Obviously, increased T_4 binding to TBG cannot account for this pattern. One can postulate an acute redistribution of T_4 out of the liver into the circulating pool, but there is no direct evidence for this mechanism in these patients. Nevertheless, the elevated free T_4 poses a diagnostic problem in excluding hyperthyroidism. A normal TSH (sensitive assay) would virtually rule out the latter diagnosis. A low serum T_3 concentration does not exclude this diagnosis, as such patients have been reported with co-existing hyperthyroidism and serious liver disease (Gravin et al, 1979).

Patients dying of severe thyrotoxicosis show a high incidence of pathological change in the liver, ranging from fatty infiltration through various stages of hepatitis and cirrhosis to necrosis and atrophy. These changes are not specific and are associated with the varied factors of undernutrition, emaciation, hypovitaminosis, and hypermetabolism which are characteristic of fatal hyperthyroidism.

Renal Disease

The kidneys have two major functions in relation to the thyroid : The *first* concerns iodine metabolism, since the kidneys represent a major pathway of iodine elimination; the *second* is the prevention of excessive losses of thyroxine-binding globulins in the urine. Kidney disease can result in either decreased iodine clearance and excretion, with secondary increases in blood and interstitial pools of iodine as seen in renal failure, or augmented loss of thyroxine-binding proteins as seen in the nephrotic syndrome. Finally, although less important than that of the liver, the kidney has the metabolic function of converting T_4 to T_3.

NEPHROTIC SYNDROME

Nephrotic syndrome affects thyroid function tests in several ways. When urinary losses of plasma proteins (including TBG) are extreme, the ability of the liver to compensate may become limiting and the serum TBG concentration falls. Consequently, total T_4 and T_3 levels decline. If this were the abnormality, free T_4 and free T_3 would remain normal. However, studies of nephrotic humans have shown that T_4 deiodination is also impaired, with the result that free as well as total T_3 concentration is low (Afrasibi et al, 1979). In a series of nephrotics excreting less than 20 g of total protein per day, Gavin et al found a

normal plasma TBG concentration but an abnormally low T_4 binding affinity of the TBG or the presence of circulating inhibitors of binding.

CHRONIC RENAL FAILURE

Multiple abnormalities are often found in patients with chronic renal failure, involving thyroid iodide uptake, circulating levels of thyroid hormone, the peripheral metabolism of T_4 and regulation of TSH secretion. In common with other forms of NTI, a low T_3 level is typical, the total T_3 concentration being more affected than the free T_3 because of diminished binding by TBG. In chronic renal failure, unlike other types of NTI, the rT_3 concentration is not increased, apparently because of a redistribution of rT_3 out of the vascular space into the cellular compartment (Kaptein E. M. et al, 1983).

The low T_3 state is not remedied by hemodialysis but is corrected by renal transplantation (Lim S. et al, 1977). Free T_4 is usually normal in uremia, but total T_4 is often low because of abnormally low binding activity of TBG of T_4. The latter may be due to decreased TBG concentration or to altered binding affinity (in turn caused by circulating inhibitors or a structural change in the protein). Heparin administration (in hemodialysis) can cause a falsely elevated free T_4.

Basal TSH levels are normal or slightly increased (Felicetta J., 1979). The TSH response to TRH is commonly blunted in patients with renal failure.

A number of abnormal results of thyroid function tests have been reported in patients with the nephrotic syndrome in the presence of clinical euthyroidism. These include low protein-bound iodine (PBI) (Peterman J. B., Man E. B., 1948), low thyroxine binding globulins (TBG) (Robbins J. et al, 1957), and increased urinary PBI and TBG levels (Recant L. et al, 1952; Rasmussen H. et al, 1956; Musa B. U. et al, 1967; Börner E. et al, 1970; Hermann J. et al, 1972). There is, therefore, the general assumption that standard thyroid function study results are abnormal in nephrotic patients. The present study was undertaken to examine thyroid function in the nephrotic syndrome with modern techniques.