Clinical Tests of THYROID FUNCTION

John A. Thomson

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John A Thomson
MD, FRCP (Glasgow), MRCP (London)

Senior Lecturer in Medicine
University Department of Medicine
Royal Infirmary, Glasgow

Consultant Endocrinologist
Glasgow Royal Infirmary and Royal Samaritan
Hospital for Women, Glasgow

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Foreword

Even amidst the current plethora of publications on the thyroid gland and its disorders there is still room for a text which provides straightforward and clear-cut advice to the clinician on the selection, from the many that are available, of the tests of thyroid function that are most likely to illuminate his particular problem and help him with their interpretation. In my view, Dr Thomson's book admirably achieves these aims. His own experience is essentially based in clinical practice leavened by the special insights that research in the laboratory provides. His approach is practical. He accepts the truism that common disorders occur most commonly and fully appreciates that relatively few clinicians have access at the present time to the full range of the most recent and more sophisticated tests, no matter how great their theoretical attractions.

Physicians with special interests other than thyroid disease will particularly benefit from this practical approach, but many thyroidologists will also find in the text sound advice which will help them in the management of their patients.

E M McGirr
Muirhead Professor of Medicine
University Department of Medicine
Royal Infirmary, Glasgow

Erratum

Page 60, Estimation of the serum triiodothyronine (T_3)

***For* 'Normal ranges have been quoted as 138 ± 23 μg/100 ml for radioimmunoassay' *please read* '. . . quoted as 138 ± 23 ng/100 ml . . .**

Preface

The objective of this book is to provide the interested clinician with a background knowledge of the principles behind the thyroid function tests which are used, to give an explanation of how the tests are performed and to explain where clinically useful data can be derived from the tests. It is not intended as a detailed bench manual and technical details of the tests are not given, but reference to appropriate original sources is made.

The opinions expressed in the book are the result of thirteen years' experience in the busy thyroid clinic at Glasgow Royal Infirmary. The author over this period has been grateful for the continuing support of Professor E. M. McGirr, Muirhead Professor of Medicine, Royal Infirmary, who has also been kind enough to write a foreword. Over the years thyroid problems have been discussed with many colleagues, especially Dr W. R. Greig and Dr H. W. Gray, and continuing contact with these and other colleagues has helped the author to formulate his opinions.

The author is, of course, entirely responsible for the opinions expressed in this book.

The able assistance of Mrs June Buntain in typing the manuscript is gratefully acknowledged. The figures are based on artwork prepared by Mrs Brenda Burn, medical artist in the University Department of Medicine.

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1 *Background anatomical and physiological data*

Anatomy of the thyroid gland

The thyroid gland develops in the human embryo from the primitive foregut. This site of origin is seen in the adult as the foramen caecum in the floor of the mouth at the junction of the anterior two-thirds and posterior one-third of the tongue. During early embryonic life the primitive thyroid descends in the mid-line of the neck to occupy the normal adult position in front of the thyroid cartilage. There the thyroid gland derives from the fourth pharyngeal pouch other elements which go to form part of the lateral lobes of the thyroid gland. The thyroid gland is recognisable in the human embryo in its final site from about the seventh week of intra-uterine life and it develops its characteristic follicular structure round about the twelfth to fourteenth week of foetal life. Co-incidentally with the changes in structure, biochemical studies have shown that the thyroid develops its capacity to secrete thyroid hormone. It is interesting that the functional capacity of the thyroid appears in a stepwise fashion, the ability to accumulate iodine being followed by the development of the other stages of thyroid hormone synthesis outlined below.

In the adult the thyroid is about 20 grams in weight and consists of two fairly symmetrical lateral lobes each about $5 \times 2 \times 2$ cm. As a normal variant the right lobe is often larger than the left lobe. The two lateral lobes are joined by a thin portion of thyroid tissue called the isthmus. From the isthmus of the thyroid a variable sized lobe extends upwards in the neck. This is referred to as the pyramidal lobe and it is clinically obvious in certain thyroid diseases, particularly thyrotoxicosis and autoimmunising thyroiditis.

The thyroid is situated in front of the thyroid cartilage and the trachea to which it is closely attached by the pretracheal fascia. This explains the well-known clinical sign that the thyroid moves upwards on swallowing. It is partially covered by the strap muscles of the neck and by the edge of

the sterno-mastoid muscles. The parathyroid glands which are also derived from the pharyngeal pouches are closely related to the posterior aspect of the thyroid gland.

The thyroid gland derives its extremely rich blood supply from paired superior and inferior thyroidal arteries, with a less important supply directly from the aorta or the innominate artery via the thyroidea ima artery. The venous drainage of the thyroid is to the internal jugular and innominate veins and the lymphatic drainage of the gland is to the lateral lymph nodes of the neck, in the first instance; there is, however, some drainage down into the anterior mediastinal lymph nodes.

When the thyroid is examined microscopically it is found to consist of follicles lined in the resting state by a low cuboidal epithelium. The height of this epithelium is dependent on the degree of stimulation of the thyroid. Under appropriate stimulation by thyroid stimulating hormone (TSH) the low cuboidal epithelium can be converted into a tall columnar epithelium. The thyroid follicles are variable in size and their diameters are of the order of 50–500μ. They are filled with a protein material which consists largely of thyroglobulin which, in fact, constitutes 75 per cent or more of the soluble proteins of the normal thyroid gland and is the main storage form of the thyroid hormones. The thyroid is unique in this way amongst the endocrine organs in having a large store of pre-formed hormone in the gland. The follicles are surrounded by a very vascular stroma which also contains the lymphatic channels.

The thyroid epithelial cells themselves are of interesting structure. The border which impinges on the central colloid of the follicle has a regular formation of microvilli. The base of the cell is separated from the interfollicular stroma by a well-formed basement membrane. The cell nucleus of the epithelial cell is usually situated in the basal part rather than the apex and the cell is well supplied with the usual subcellular bodies concerned in protein and carbohydrate synthesis, namely rough endoplasmic reticulum and Golgi apparatus. In addition, within the cell cytoplasmic vesicles can be seen and many of these at least are absorption vesicles, the colloid being absorbed from the follicles by a process of engulfment by the thyroid cell processes. This mechanism is known as pinocytosis.

Amongst the thyroid epithelial cells there are in addition certain cells which can be stained by special techniques and shown to be different in their staining characteristics from the thyroid epithelial cells. These cells have been recognised in recent years to be the cells responsible for production of the calcium-lowering hormone calcitonin and are usually

referred to as the 'C' cells or light cells. The hormone does not seem to be of great physiological importance in normal man but it is produced in excess in certain types of thyroid tumours (medullary carcinoma) and in this condition assay of the serum calcitonin can yield valuable diagnostic information (Chapter 4).

Control of thyroid function

The thyroid gland is under the control of the anterior pituitary which produces from certain of its basophil cells (thyrotrophs) a glycopeptide hormone, thyroid stimulating hormone (TSH). This hormone acts on the thyroid gland to increase many of the biosynthetic processes which take

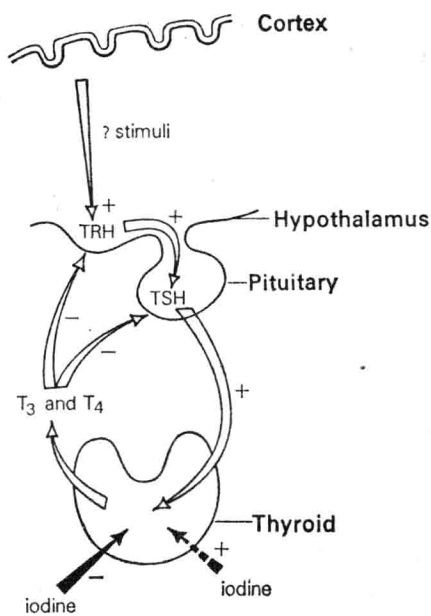

Fig. 1. Regulation of thyroid function

place there (Fig. 1). A great deal of work has been carried out on the various possible effects of thyroid stimulating hormone but there is no agreement at present on what is the prime effect. The mode of action of TSH is not completely settled. As with many other hormone actions it is thought that the prime effect may be through the cyclic 3′, 5′ AMP

mechanism. Certainly the present evidence is highly suggestive that this is the likely mode of action.

The TSH production from the anterior pituitary is under a negative feedback control mechanism whereby increasing blood levels of the thyroid hormones diminish the production or release of TSH from the pituitary gland. The fact that the thyroid influences the pituitary has been known for many years from the histological changes produced in the appropriate pituitary cells (thyrotrophs) following thyroidectomy, when it can be shown that there is a decrease in the stores of secretory granules in the pituitary cells. Another indicator of the effect of the thyroid on the pituitary is the well recognised enlargement of the pituitary in long-standing untreated cretinism. The pituitary gland itself is under the control of the hypothalamus by means of thyrotrophin releasing hormone (TRH) which stimulates the TSH production from the anterior pituitary. There is also evidence that TRH itself is under a negative feedback control via the thyroid hormones triiodothyronine (T_3) and thyroxine (T_4) from the thyroid gland. It must further be remembered that the hypothalamus itself is not the ultimate centre but is, in fact, influenced by the higher centres in the cortex, thus explaining the response of the thyroid gland to circumstances of stress.

It is often forgotten, with the current interest in effects of TSH and abnormal thyroid stimulators such as long acting thyroid stimulator (LATS) and, more recently, with the work being done on TRH, that the thyroid gland itself has definite autoregulatory functions. It has, for instance, been shown that the hypophysectomised experimental animal is capable of increasing the uptake of iodine into its thyroid when subjected to the stress of a low iodine diet. This would therefore imply that increased trapping ability is not necessarily mediated by the TSH mechanism.

Perhaps the best known example of the intrathyroidal control of thyroid hormone synthesis is that known as the Wolff-Chaikoff effect. These workers showed that when the experimental animal is exposed to a high inorganic iodine load such that the thyroidal inorganic iodine rises above a critical level, the thyroid stops hormone synthesis so that excess thyroxine and triiodothyronine are not formed. Normal hormone production is resumed when the level of intrathyroidal inorganic iodine is allowed to reach low enough levels. This mechanism has obvious biological advantages in enabling the animal to deal with wide fluctuations in iodine intake without going through periods of thyroid over- and under-activity.

Biosynthesis of the thyroid hormones

In the normal functioning adult thyroid the mechanism of production of the thyroid hormones is as outlined in Figs 2–4. The thyroid gland is the body's main iodine-containing organ. This has been recognised for some time, but only early in this century when the structure of thyroxine was elucidated was the importance of iodine in metabolism recognised. The

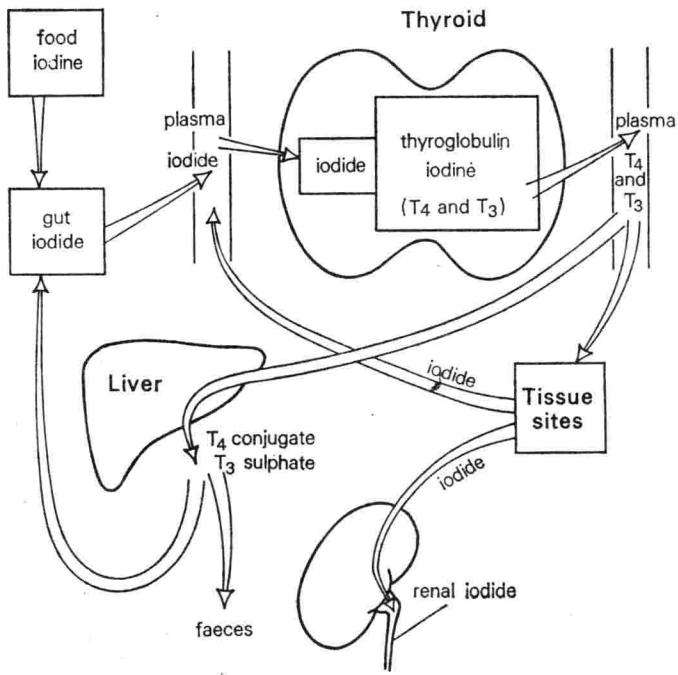

Fig. 2. The iodine cycle

normal adult takes in approximately 100–150 μg of iodine per day in food (Fig. 2). This iodine is broken down in the gastro-intestinal tract and is usually absorbed as iodide, which passes into the blood stream where it circulates in the systemic circulation and is actively accumulated by the thyroid from the blood passing through it.

The first step, therefore, in the synthesis of the thyroid hormones is that the thyroid gland has to accumulate inorganic iodide; this is done by an

active process which is usually referred to as *trapping* (Fig. 3). The resting thyroid gland has been shown to accumulate iodine in the ratio of approximately 20:1 from the plasma. This can be increased markedly under the appropriate conditions of stimulation. This ability to trap iodine is not unique to the thyroid gland but is shared by the other derivatives of the embryonic foregut such as the salivary glands, the gastric mucosa and the

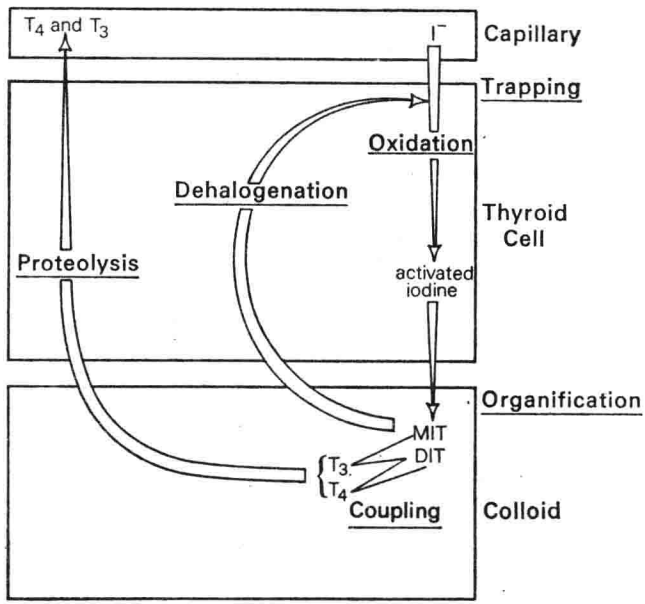

Fig. 3. Details of thyroid hormone synthesis and degradation

lactating breast. The process can be demonstrated readily both *in vivo* and *in vitro* and is stimulated by TSH and also by abnormal thyroid stimulators such as long acting thyroid stimulator (LATS). It can be shown *in vitro* to be inhibited by processes interfering with enzyme function, such as boiling of the tissue. The process is also inhibited by certain substances such as perchlorate and thiocyanate. These may act by having a similar molecular size to iodide and they do not appear to act as competitive inhibitors for the enzymatic process.

Once the iodine has been accumulated in the thyroid gland it undergoes a process of *oxidation*. A peroxidase enzyme system has been shown to be present in abundance in the thyroid gland and it is assumed that this

is involved in the oxidation process by the local manufacture of tiny amounts of hydrogen peroxide within the cell. The exact mechanism of this oxidation process has not been fully worked out, nor has the main product of this reaction. There are those who feel that free iodine is momentarily produced in the thyroid gland, but others consider that another unstable iodine ion is produced. The identification of this intermediate form of iodine is subject to great technical problems because of its very evanescent nature and the minute quantities produced.

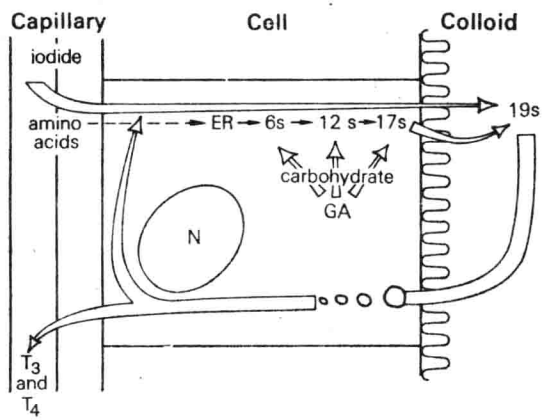

Fig. 4. Stages of thyroglobulin formation and degradation (ER = endoplasmic reticulum; GA = Golgi apparatus; N = nucleus)

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

In recent years a great deal of attention has been paid to the processes of thyroglobulin synthesis (Fig. 4). Thyroglobulin is an iodinated glycopeptide of molecular weight approximately 660,000. On analysis in the ultracentrifuge it has an 'S' value of 19 and is recognised as the main protein present in the thyroid. Studies of the synthesis and degradation of thyroglobulin have been consistent with the view that this large protein is composed of smaller polypeptide sub-units which coalesce to form 6S and 12S sub-units. The 12S sub-units combine to form larger units, such as 17S, which is the largest non-iodinated part of the thyroglobulin

molecule recognised. The polypeptide part of this glycoprotein is synthesised by the rough endoplasmic reticulum of the thyroid cell. The carbohydrate moiety is added probably mainly in the Golgi apparatus, which results in the formation of a fully synthesised carbohydrate-containing protein of 17S size. This protein is then iodinated.

It is well recognised that the thyroid cell passes iodine rapidly through itself into the colloid. There is a certain amount of controversy as to whether iodination takes place at the apex of the thyroid epithelial cell or whether it takes place within the colloid. The weight of evidence is that in normal circumstances iodination takes place in the colloid at the edge of the follicle, but this is not to say that cellular iodination is impossible and does not occur under certain abnormal circumstances.

The processes of synthesis of the basic thyroglobulin molecule and the processes of iodination are quite separate both in time relationship and in anatomical site within the cell. It has been shown, for instance, that whereas iodine enters the colloid within seconds in the experimental animal, several hours are required for amino acids to reach the colloid. Furthermore, it can be shown that substances such as thiouracil which interfere with iodination do not interfere with amino acid incorporation in the short term and, conversely, substances such as actinomycin which affect amino acid incorporation do not interfere with short term iodination experiments.

The addition of one atom of iodine per tyrosine residue results in the formation of monoiodotyrosine (MIT) and the addition of a further atom of iodine per tyrosine residue results in the formation of diiodotyrosine (DIT). As mentioned above, these processes are interfered with by the commonly used anti-thyroid drugs such as propylthiouracil and carbimazole. This is also a site of action of many of the drugs which have an anti-thyroid effect such as sulphonamides and phenylbutazone.

The next stage in the pathway is the formation of the thyroid hormones which are thyroxine (tetraiodothyronine, T_4) and triiodothyronine (T_3). There is considerable evidence that the formation of these depends on the iodinated tyrosine residues containing mono- and diiodotyrosine being present in the correct number and in the correct geometrical relationship on the thyroglobulin molecules. This process has been called *coupling*. There is no evidence that this particular step is enzymatically controlled.

The thyroid gland has therefore, at this stage in the formation of hormone, a store of the active hormones T_4 and T_3 bound to the protein part of thyroglobulin which is present as the intrafollicular colloid. As the

hormone is required it is broken down by a series of proteolytic enzymes which are present in the thyroid epithelial cells, and the T_4 and T_3 are released into the blood stream. This process of breakdown is accomplished by the thyroid epithelial cell engulfing small parts of the colloid which are seen as intracellular vesicles.

The thyroglobulin not only acts as a store of formed hormone but also has usually an excess of MIT and DIT present, and in this sense also acts as a store of iodine. During protein breakdown this excess of MIT and DIT is released. The body conserves this additional iodine by means of a *deiodinase* or *dehalogenase* enzyme system. The iodine is then available to be reutilised by the thyroid gland. There is some evidence, however, that the iodine produced from the deiodination of MIT and DIT is handled differently by the thyroid from iodine newly entering the thyroid from the plasma. This iodine derived by dehalogenation is often referred to as the second iodide pool because it does not seem to be immediately exchangeable with inorganic iodine pools of the plasma and the thyroid.

Transport of the thyroid hormones

The thyroid hormones T_3 and T_4 are transported in the plasma in the main bound to the plasma proteins. Less than 0·1 per cent is in the form of free thyroxine or triiodothyronine. The main specific thyroxine binding protein is an interalphaglobulin referred to as thyroxine binding globulin (TBG). This protein is present in quite low concentration but its binding affinity is very high. It is interesting that cortisol binding globulin also is a similar interalphaglobulin. Apart from TBG several other binding proteins are recognised. The one which has given rise to most controversy is a binding protein which runs in front of albumin on electrophoresis of serum in certain buffers. This protein is referred to as thyroxine binding prealbumin (TBPA) and is present in larger concentration than TBG. It was first isolated from the cerebrospinal fluid but has been regularly found in the plasma provided an appropriate buffer system is used. It is not seen if barbitone buffer is used, as this seems to inhibit thyroxine binding to TBPA. The binding affinity of this protein for thyroxine is less than TBG but its total binding capacity is much greater.

Over the last decade there has been controversy over the role of TBPA in thyroid hormone homeostasis. The consensus of opinion is that this protein does play a role in normal thyroid control by being probably a