Thyroid Hormones Assay in Diagnosis of Thyroid Disorders

Pages with reference to book, From 124 To 127

Farida Agha (PMRC Research Centre, Jinnah Postgraduate Medical Centre, Karachi-35.)

The study of the hormones of the thyroid gland has developed in stages over the last 80 years, when it was recognised that the thyroid gland contained a considerable amount of iodine. The two main iodine containing hormones are thyroxine (T4) and tri-iodo-thyro-nine (T3).

The role of the thyroid gland is to synthesize, store and secrete the thyroid hormones thyroxine (T4) and tri-iodothyronine (T3). This function is controlled by pituitary thyroid stimulating hormone (TSH, thyrotrophin). The TSH secretion is controlled by the levels of T3 and T4 in conjunction with the action of the hypothalamic hormone (TRH). Iodine is an essential part of the thyroid hormones and an adequate dietary intake is important. Iodine ingested in foodstuffs or in drinking water is absorbed as iodide from the gut into the plasma. The iodide which is circulating in the plasma is trapped by the thyroid follicular cells, and appears to be changed into some "activated" form of iodine probably by an oxidative process as a result of the thyroid peroxidase enzymes. This activated form of iodine is then incorporated into tyrosine residues on previously synthesized non-iodinated polypeptide chains of thyroglo-bulin, contained in the intra-follicular colloid. Iodination takes place at or near the apical (Luminal) surface of the follicle cell during and following aggregation and leads to the formation of mono and di-iodotryosine (MIT and DIT) in approximately equal quantities. The relative proportions of MIT and DIT formed are dependent on the availability of iodine; when iodine is in short supply, the MIT/DIT ratio increases.

The subsequent step in synthesis consist of the joining together of two iodotyrosine residues: two DIT residues result in the formation of T4 (DIT and DIT), whereas one MIT and one DIT residue result in the formation of T3 (MIT+DIT). The reaction appears to occur most readily between molecules which are in close physical proximity on the thyroglobulin frame work.

When the thyroid hormones are formed they normally remain in the gland for an appreciable period of time and act as a store of hormones. When required the hormones are released by the intervention of some proteolytic mechanism.

Within the plasma the hormones are strongly bound to specific proteins so that only a minute fraction exist in a free or unbound state. The portion of the hormones may be important in controlling their metabolic effects despite the fact that approximately 99% of the total hormone is carried bound to plasma proteins. The major binding proteins are thyroxine binding globulin (TBG), thyroxine binding prealbumin (TBPA) and albumin which respectively carry 60%, 30% and 10% approximately of total circulating T4. Free T4 is about 0.05% of the total and free T3 0.5% of the total T3. In a state of equilibrium, the level of free T4(FT4) is dependent upon the concentration of total T4 bound to the transport proteins (TBPT4) and the unsaturated or residual binding capacity of these proteins (UTBP) in the following manner:

$$1/KFT4 = \frac{TBPT4}{UTBP}$$

where 'K' is the dissociation constant of the reaction. Thus if, UTBP rises because of increased production of binding protein then FT4 will fall unless there is a compensatory rise in TBPT4 due to an appropriate increase in T4 production as in pregnancy. The role of binding proteins appears to act as a buffer in the extra-thyroidal metabolism of the thyroid hormones, modulating their transfer to intracellular sites and preventing their loss in urine. The roles for TBG and TBPA would appear to differ; TBG is the main binding protein for normal purposes and TBPA is the protein which can exhibit a more flexible response than TBG to sudden changes in requirements for the thyroid hormones. The

two hormones T3 and T4 have different binding affinities: T3 is much less firmly bound than T4. T3 has greater biological activity than T4 (3-4 times) and has a more rapid metabolic effect and turnover largely because of decreased protein binding. It has been calculated that T3 accounts for two-thirds of the total metabolic contribution of the thyroid hormones. Only a proportion of the total amount of T3 produced daily is actually secreted direct from the thyroid gland, the remainder results from peripheral mono-deicdination cf T4, one sixty of which is converted (Braveman et al., 1970). Following the breakdown, it is known that deiodination takes place and most of the iodine is recycled through the thyroid. A small amount of thyroxine is excreted as a glucuronide conjugate in the bile, but most of the iodine is reabsorbed further down the intestinal tract. A small quantity of thyroxine is excreted in the urine, and some T3 in a sulphated form is also excreted in the bile and in the urine. The production of thyroid hormone is controlled through the hypothalmus and anterior pituitary by secretion of thyrotrophin releasing hormone (TRH) and thyroid stimulating hormone (TSH, thyrotrophin).

TSH is a glycoprotein and formed by specific thyrotroph cells in the anterior pituitary under the influence of TRH. TSH is largely responsible for the maintenance of normal thyroid hormone synthesis and secretion, TSH increases the size and vascularity of the gland and height and activity of the follicular epithelium. In addition, TSH stimulates many other metabolic processes in the thyroid gland such as cyclic AMP generation, glucose oxidation, 02-consumption and phospholipid and protein synthesis. The pituitary secretion of TSH is influenced by the free circulating levels of T4 and T3. High levels of thyroid hormones reduce TSH output whereas low levels of thyroid hormone increase its release.

Abnormal levels of T4 are generally an indication of thyroid malfunction. Changes in T4 levels are generally paralleled by similar changes in T3, but in some circumstances the changes are independent and the measurement of both levels is therefore, an important part of the investigation of the dysfunction of the hypothal-mic/pituitary/thyroid axis.

Many techniques have been developed for the measurement of serum T4, the competitive protein binding analysis (Murphy and Pettee, 1964) and radioimmunoassay techniques for T4 (Mitsuma et al., 1972; Chopra, 1972; Dunn and Foster, 1973) have permitted more specific measurements of this hormone. Although reliable methods for "assaying" T4 have been available for some time, the radio-immunoassay for T4 could supplant competitive protein binding analysis by virtue of its greater simplicity and sensitivity in the lower range.

In contrast, the minute concentration of T3 normally present in serum made quantitation by routine chemical means a formidable task. The development of competitive protein binding analysis (Nauman et al., 1967; Sterling et al., 1969) and gas liquid chromatography (Hollander, 1968) for T3 were useful in providing pioneering studies on T3 but these methods were cumbersome and generally over estimated T3-concentra-tion. The more recent development of radioimmunoassay techniques for measuring T3 (Lieblich and Utger, 1972; Mitsuma et al., 1971; Larsen, 1972; Huefner and Hesch, 1973; Chopra et al., 1971; Hesch and Evered, 1973) has allowed for a greater understanding of the contribution of T3 to thyroid economy. Moreover, the advent of a T3-RIA affords, for the first time, and simple reproducible and accurate assay suitable for routine clinical use.

Tri-iodothyronine (T3) and Thyroxin (T4):

Measurement of serum T3 and T4 levels are every useful in the diagnosis of thyroid disorders. There is in general, a good correlation between T3 and T4 levels over a wide range of concentrations. In overt hypothyroidism both T3 and T4 levels are depressed, and in overt hyperthyroidism both levels are raised. In some clinical situations, however, the T3-value can be disproportionately high with respect to the T4 levels as conventional hyperthyroidism regardless of cause (i.e., whether the thyrotoxicity result from toxic diffuse goitre, toxic nodular goitre, or autonomously functioning adenoma), T3 levels are invariably elevated.

Most interest in the clinical use of total serum T3 measurement has been in respect of the condition which is known as T3-toxicosis, i.e., thyrotoxicosis caused by excessive secretion of T3 rather than T4

(Hollander et al., 1972). In this condition the total serum T4 value is normal and the sole biochemical abnormality is an elevated T3-level (Larsen, 1971; Sterling et al., 1970; Wahner and Gorman, 1971; Bella-barba, 1971). Patients with T3-toxicosis, reported to have normal total and free-T4 levels, normal thyroid binding proteins, and a normal or elevated thyroidal binding proteins, and a normal or elevated thyroidal uptake of radio-iodine that could not be suppressed with exogenous thyroid hormone. All patients found to have elevated total T3-levels, ranged from 228 to 2000 ng/ 100 ml, and high free T3 level as well (Jeereddi et al., 1979).

In a series of 64 patients with usual forms of thyrotoxicosis, it was found that T3-varied from 232 to 1700 ng/100 ml with a mean of 495 ng/100 ml (Jeereddi et al., 1979). This contrasts with the findings in normal and hypothyroid subjects. Mitsuma et al. (1971) have reported T3-levels in 82 normal subjects, ranged from 96 to 172 ng/100 ml with a mean of 138 ng/ml while in 45 patients with primary hypothyroidism the mean T3 was found to be 62 ng/ 100 ml and in patients with hypothyroidism secondary to pituitary disease, mean T3 level was 57 ng/100 ml.

In general, patients with hypothyroidism were found to have T3-levels which were approximately one half those found in normals.

The discrepancy between the T3 and T4 values occur particularly after therapy with radioiodine or surgery for thyrotoxicosis. The elevated TJ-levels in subjects with conventional hyperthyroidism fell to normal with the induction Of euthyroid state by surgery, radio-iodine or antithyroid drugs (Mitsuma et al., 1971). However, it has been observed that several patients with conventional hyperthyroidism, remained early in the course of antithyroid drug therapy clinically toxic, despite a fall in theirs T4 levels to normal (Hollander et al., 1972b). Similar observations have been made by Bellabarba et al. (1972).

Sterling et al. (1970) and Bellabarba et al. (1972) have observed the maintenance of normal clinical status in patients who have normal or elevated T3, but low serum T4-conccntration, after iodine-I131 therapy for thyrotoxicosis.

More recently Marsden and McKerron (1973) have critically examined the diagnostic value of T3-in hyper thyroidism and confirmed the finding that T3-estimation in serum is the best single thyroid function test in hyper-thyroidism.

Considerable attention has also been focused on the measurement of serum reverse T3 (inactive metabolite) 3,3,5, tri-iodo thyronine (T3). Reverse T3 is formed during hormone metabolism and is a noncalorigenic and biologically inactive congener of T3. Measurement of T3 may have clinical usefulness in the evaluation of thyroid function in the fetus. Recent studies indicate that serum T3 is increased in several conditions, e.g., starvation (Vegenakis et al., 1975), hepatic cirrhosis, chronic renal failure, acute febrile illness, protein calorie malnutrition (Chopra et al., 1975b), after surgical operations (Burr et al., 1975), and in amniotic fluid (Chopra and Crandall, 1975).

In hypothyroidism, both T4 and T3 levels are generally depressed, while in subjects with mild thyroid failure T3 levels may remai nnormal.

The diagnosis of overt hypothyroidism is readily confirmed by the finding of a low corrected T4-value. However, the recognition of mild cases of thyroid failure may be more difficult, as the corrected T4 value is frequently within the normal range, although it may tend towards the lower end of normal. A raised TSH level in these cases is confirmation of mild primary hypothyrodin (Evered et al., 1973; Hall et al., 1971).

Thyroid Stimulating Hormone (TSH):

The most important application of the TSH assay has been to exclude primary thyroid failure. A low or normal serum TSH level in cases of hypothyroidism excludes primary thyroid failure and suggests dysfunction of the pituitary or hypothalamus.

The pituitary reserve of TSH may be tested by monitoring serum TSH levels after the intravenous administration of TRH. In normal subjects, serum TSH levels rise in"mediately after injection of TRH and reach a maximum 20 to 30 minutes later. The TSH response to TRH in hypothyroid patients is

exaggerated and prolonged, and this may be useful in diagnosing cases of mild hypothyroidism where the basal scrum TSH level is onlv slightly raised (Ormston, 1972).

Thyroid failure:

Evered etal. (1973) proposed a classification of thyroid failure into 3 grades: (1) Overt hypothyroidism in which the classical features of thyroid failure are present together with evidence of low circulating thyroxine (T4) levels and a raised TSH; (2) mild hypo-thyroidism in which the clinical features are non-specific yet improve with thyroxine medication. Routine thyroid function tests may be equivocal or normal but serum TSH is elevated; (3) Sub-clinical hypothyroidism in which serum TSH is elevated in an asymptomatic subjects with normal routine thyroid function tests.

Mild hypo-thyroidism justifies a therapeutic trial of thyroxine, when patients with hypothyroidism are treated with thyroxine their TSH levels return to normal on adequate replacement and this may be a useful guide to therapy. The finding of a raised TSH level in patients taking larger doses of thyroxine may imply that the patient is not taking the prescribed medication. Patients with subclinical hypothyroidism progress to more severe degree of thyroid failure and such patients should not be given thyroxine, unless they develop symptoms or signs, consistent with hypothyroidism.

Congenital hypothyroidism is the most frequent known endocrine or metabolic cause of mental deficiency in the new born. Its incidence in most series has varied from one in 3000 to 1 in 10,000. Prevention of mental deficiency due to congenital hypothyroidism requires early detection and treatment of hypothyroidism before the age of 3 months (Kleinetal., 1972; Raiti and Newns, 1981). Unfortunately, prompt clinical diaenosis of congenital hypothvroidism is often difficult (Klein et al., 1974, 1975).

The measurement of TSH levels in the newborns has been shown to be useful in the early detection of congenital hypothyroidism. Serum TSH levels are usually elevated immediately after birth and fall to normal adult levels after about 3 days (Fisher and Odell, 1969). Measurement of neonatal TSH have been done on serum from cord blood (Klein et al., 1974) and the recent development of more sensitive and specific measures of thyroid hormones and TSH by filter paper methods (Larsen et al., 1976; Dussault et al., 1973-1975, 1976b) has prompted a number of workers to undertake large scale screening of infants to detect congenital hypothyroidism at birth. Some observations suggest that 10-15% of cases would be missed if screening is conducted with TSH alone (Dtssault et al., 1976). For this reason, screening for congenital hypothyroidism using the filter paper T4 method with filter paper TSH testing of suspicious samples, is advised (Fisher et al., 1976) to increase the frequency of case detection.

References

1. Bellabarba, D. (1972) Further observation on T3-Thyro-toxicosis. Clin. Res., 20:421.
2. Bellabarba, D., Bernard, B. and Langlois, M. (1972) Serum pattern of T4, T3 and TSH after treatment of thyrotoxicosis. Clin. Res., 20:421.
3. Braveman, L.E., Ingbar, S.H. and Sterling, K. (1970) Conversion of thyroxine (T4) to tri-iodo-thvronine (T3) J. Clin. Invest., 49:855.
4. Burr, W.A., Griffith, R.S., Black, E.G., Hoffenberg, R., Meinhold, II. and Weznel, K.W. (1975) Serum T3 concentration after surgical operation. Lancet, 2:1277. Chopra, I.J., Chopra, U., Smith, S.R., Reza, M. and Solomon, D.H. (1975) Reciprocal changes in serum concentration of 3, 3, 5-tri-iodothyronine (rcverse-T3) and 3, 3, 5 tri-iodothyronine (T3) in systemic illnesses. J. Clin. Endocrinol. Metab., 41:1043.
5. Chopra, I.J. and Crandall,B.E". (1975) Thyroid hormones and thyrotrophin in amniotic fluid. New Eng. J. Med., 293:740. Chopra, I.J. (1972) A radioimmunoassay for measurement of Thyroxine in unextracted serum. J. Clin. Endocrinol. Metab., 35:938.

6. Chopra, I.J., Solomon and Berk, A.N. (1971) Radioimmunoassay for measurement of tri-iodothyronine in human scrum. J. Clin. Invest., 50:2033.
7. Dunn, R.T. and Foster, L.B. (1973) Radioimmunoassay of thyroxine in unextracted serum by a single antibody technique. Clin. Chem., 19:1063.
8. Dussault, J.H. and Laberge, C. (1973) Thyroxine (T4) determination in dried blood by radioimmunoassay: Screening method for neonatal hypothyroidism. Union Med. Can., 102:2062.
9. Dussault, J.H., Coulombe, P., Laberge, C, Letarte, J., Guyda, H. and Khoury, K. (1975) Preliminary report on a mass screening program for neonatal hypothyroidism. J. Paeditr., 86:670.
10. Dussault, J.H., Letarte, J., Guyda, II. and Laberge, C. (1976-a) Thyroid Function in neonatal hypothyroidism. J. Pediatr., 89:541.
11. Dussault, J.H., Parlow, A. and Laberge, C. (1976-b) TSH measurements on blood spots on filter paper: A confirmatory screening test for neonatal hypothyroidism, J. Pediatr., 89:550.
12. Evered, D.C., Ormston, B.J., Smith, P.A., Hall, R. and Bird, T. (1973) Grades of Hypothyroidism. British. Med. J., 1:657.
13. Fisher, D.A., Burrow, G.N., Dussault, J.H., Hollingsworth, D.R., Larsen, P.R., Man, E.B. and Walfish, P.G. (1976) Recommendations for screening programmes for congenital hypothyroidism. J. Pacdiatr., 89:692.
14. Fisher, D.A., "and Odell, W.D. (1969) Acute release of Thyrotrophin in the new-born. J. Clin. Invest., 48:1670.
15. Hall, R., Amos, J. and Ormston, B.J. (1971) Radio-immuno-assay of human serum Thyrotrophin. Br. Med. J., 1:582.
16. Hesch, R.D. and Evered, D.C. (1973) Radioimmunoassay of T3 in unextracted human serum. British Med. J., 1:645.
17. Hollander, C.S. (1968) On the nature of circulating thyroid hormone: Clinical studies of tri-iodothyronine and thyroxine in serum using gas chromatographic methods. Trans. Assoc. American Physicians, 81:76.
18. Hollander, C.S., Mitsuma, T., Nihci, N., Shenkman, L., Burday, S.Z. and Blum, M. (1972a) Clinical and laboratory observations in 40 cases of T3-Toxicosis confirmed by radioimmunoassay. Lancet, 1:609.
19. Hollander, C.S., Shenkman, L., Mitsuma, T. and Asper, S.P. (1972-b). Tri-iodothyronine Toxicosis developing during antithyroid drug therapy for hyper-thyroidism. Johns Hopkins Med. J., 131:184.
20. Huefner, M. and Hesch, R.D. (1973) Radio-immunoassay for tri-iodothyronine in human serum. Acta Endocrinol., 72:464.
21. Jeereddi, A., Parasad and Charles, S., Hollander (1979) Thyroxine and Tri-iodothyronine: Methods of Hormone radio immunoassay. Second. Ed. Acad. Press, P. 375.
22. Klein, A.H., Augustin, A.V., Hopwood, N.J., Pericill, A., Johnson, L. and Foley, T.P. Jr. (1975) Thyrotrophin Screening for congenital hypothyroidism. Pediater. Res., 9:291.
23. Klein, A.H., Augustin, A.V. and Foley, T.P. (1974) Successful Laboratory screening for cogenital hypothyroidism. Lancet, 2:77.
24. Klein, A.H., Meltzer, S. and Kenny, F.M. (1972) Improved prognosis in congenital hypothyroidism treated before the age of 3 months. J. Pacdiatr., 81:912.
25. Larsen, P.R. (1972) Direct immunoassay of tri-iodothyronine in human serum. J. Clin. Invest., 51:1939.
26. Larsen, P.R. (1971) Technical aspects of the estimation of T3 in human serum: Evidence of conversion of thryoxine to tri-iodothyronine during assay. Metab. Clin. Exp., 20:609.
27. Larsen, P.R., Merker, A. and Parlow, A.F. (1976) Immunoassay of human TSH using dried blood samples. J. Clin. Endocrinol. Metab., 42:987.
28. Lieblich, J. and Utger, R.D. (1972) Tri-iodothyronine radioimmunoassay. J. Clin. Invest., 51:157.
29. Marsdcn, P. and McKerron, C.G. (1973) Serum T3-concentration in the diagnosis of hyper-

thyroidism. Clin. Clin. Endocrinol., 4:183.
30. Mitsuma, T., Nihci, N., Gershengorn, M.C. and Hollander, C.S. (1971) Serum tri-iodothyronine. Measurements in human serum by radio-immunoassay with collaboration by gas liquid chromatography. J. Clin. Invest., 50:2679.
31. Mitsuma, T., Colucci, J., Shenkman, L. and Hollander, C.S. (1972,a) Rapid simultaneous radioimmunoassay for Tri-iodothyronine and Thyroxine in unextracted serum. Biochem. Biophy. Res. Commun., 46:2107,
32. Murphy, B.E.P. and Pettee, C.J. (1964) Determination of thyroxine utilizing the property of protein binding. J. Clin. Endocrinol. Metab., 24:187.
33. Nauman, J.A., Nauman, A. and Warner, S.C. (1967) Total and free tri-iodothvroninc in human serum. J. Clin. Invest., 46:1346.
34. Ormston, B.J. (1972) Clinical affects of TRH on TSH release after, i.v. and oral administration in normal volunteers and patients with thyroid disease. Frootiers of Hormone Research, Vol. I. P.-45.
35. Raiti, S. and Newns, G.H. (1971) Creatinism early diagnosis and its relation to mental prognosis. Arch. Dis. Child., 46:492.
36. Sterling, K., Bellabarba, D., Neuman, E.S. and Brener, M.A. (1969) Determination of tri-iodothyronine concentration in human scrum. J. Clin. Invest., 48:1150.
37. Sterling, K., Refetoff, S.O. and Selenvow, H.A. (1970) T3 thyrotoxicosis due to elevated serum T3-lcvels. J. Am. Med. Assoc., 213:571.
38. Vegenakis, A.G., Burger, A., Portnay, G.I., Rudolph, M., O'Brien, J.J., Azizi, F., Arky, R.A., Nicod, P., Ingbar, S.H. and Braveman, L.E. (1975) Diversion of peripheral thyroxine metabolism from activating to inactivating pathways during complete Fasting. J. Clin. Endocrinol. Metab., 41:191.
39. Warmer, W.H. and Gorman, C.A. (1971) Interpretation of serum T3-levels measured by Sterling Technique. New Engl. J. Med., 284:225.