The present role of *in-vitro* tests of thyroid function

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In recent years a swing has taken place away from tests *in vivo* of thyroid function, which require the administration of radioactive isotopes, towards tests performed on patients' sera *in vitro*. This swing has followed the introduction of new, simple, and rapid assay methods, a fresh approach to their interpretation and application, and a simultaneous awareness of the limited discriminatory power of *in vivo* isotopic methods.

Advantages of Methods *in vitro*

The advantages of methods *in vitro* include the following:

1 The patient need not attend the hospital. While I hesitate to suggest that examination of a patient's serum is a proper substitute for examination of the patient, there are situations in which such a mechanistic approach might be justified. For instance, it is generally agreed that all hyperthyroid patients treated by surgery, radioactive iodine, or long-term administration of antithyroid drugs should be followed up at regular intervals for the rest of their lives. The snowball effect of this accumulation of patients at a thyroid clinic can only lead to less thorough methods of examination, longer waiting times, errors and confusion, and, finally, the patient's disillusionment and non-attendance. It would surely be simpler and less traumatic to ask each patient to attend her general practitioner at, say, annual intervals for clinical assessment and for removal of a blood sample which would be sent to the laboratory for routine thyroid function tests. If either clinical or laboratory assessment suggested thyroid dysfunction the patient would be asked to attend the thyroid clinic.

2 The administration of radioactive isotopes is avoided. The use of short-lived isotopes such as ^{132}I and $^{99\text{m}}\text{Tc}$ has made this consideration less cogent, but it is probably undesirable to administer even these to pregnant women, nursing mothers, or infants.

3 Large numbers of samples can be processed. The in-

roduction of fully automated techniques for measurement of serum PBI concentrations and of partially automated methods for thyroxine (T_4) permits the processing of a hundred or more samples per day in a single laboratory. No isotopic test *in vivo* could compete in terms of throughput. Commercially available kits for T_4 assay and for serum uptake tests require relatively little technical skill or training.

4 Diagnostic discrimination is improved. In general, tests *in vitro* offer better discrimination than tests *in vivo*, an advantage which applies most clearly to the assessment of hyperthyroid patients after treatment or hypothyroid subjects on maintenance thyroxine replacement.

Disadvantages of Methods *in vitro*

The disadvantages of methods *in vitro* include the following:

1 For proper interpretation in the majority of cases a minimum of two tests has been necessary, ie, a measurement of thyroxine level and of unsaturated binding sites which are combined to give a free thyroxine index (FTI). However a single-stage FTI such as the ETR (effective thyroxine ratio) provides similar information from a single test and will be further discussed below.

2 Lack of specificity of the PBI assay: many laboratories still favour PBI measurement on the grounds of the availability of fully automated methods and of cheapness. It is, however, most susceptible to the influence of iodine contamination and false high values are frequently misleading. This can be overcome by measuring thyroxine.

3 Delay in producing results: although individual tests may take only a few hours to perform, tests *in vitro* are usually done in batches, once or twice a week in most laboratories, so that a delay of several days is customary; with measurement of 'early' thyroid uptake *in vivo* definitive results can be given within half an hour of referral of the patient.

4 Tests *in vitro* are not 'dynamic'. Whereas tests

in vivo may give an indication of the degree and rate of thyroid function, tests *in vitro* merely express the concentration of hormone in the serum at a particular moment. This disadvantage is probably trivial.

5 Lack of accuracy: with the exception of the PBI assay (by automated techniques) tests *in vitro* are poorly replicable and coefficients of variation may exceed 20% in the lower physiological ranges.

Interpretation of Tests *in vitro* and the Determination of 'Free' T₄ in Serum

The major problem with tests *in vitro* lies in their interpretation, basically because of the influence of the plasma proteins which bind T₄ and T₃ (fig 1). An increase in the concentration of serum thyroxine-binding globulin (TBG) with a resultant increase in T₄ concentration may be of genetical origin but is most commonly due to the effect of oestrogens secreted in pregnancy or contained in oral contraceptive agents; an increase has also been noted in acute intermittent porphyria and in infective hepatitis. A reduced concentration of TBG with low T₄ values may also reflect a genetic variation or result from the administration of androgens or anabolic agents, urinary loss of protein in the nephrotic syndrome, or an unexplained effect of

acute surgical stress or major illness, especially in the elderly; certain drugs (diphenylhydantoin or salicylates) displace T₄ from TBG thereby simulating a low TBG state.

Where circulating concentrations of T₄ (and T₃) are above or below the physiological range because of abnormal concentrations of serum TBG, the subjects are clinically euthyroid and have normal T₄ (and T₃) secretion rates. This reflects the normal concentration of 'free' (non-protein-bound) hormone which correlates more precisely with the metabolic status of the individual. The serum total T₄ is an index of the free T₄ concentration only if the binding proteins are present in physiological amounts.

A fundamental requirement, therefore, for proper evaluation of a subject's thyroid status is measurement of the circulating free T₄ concentration, which normally comprises only about 0.02 to 0.05 % of the total T₄ or 40-100 pmol/l serum (2.5 ng/100 ml). Direct methods, using equilibrium dialysis or gel filtration, are not suitable for routine use and most laboratories offer as a substitute a 'free thyroxine index' (FTI) based on an original formulation by Clark and Horn (1965). This makes use of measurement of the circulating PBI (or T₄) concentration and a serum uptake test, the two results in conjunction being taken to reflect the free T₄ concentration¹. Howorth and MacLagen (1969) introduced a variant of the serum uptake test using T₄ instead of T₃ and Goolden, Gartside, and Sanderson (1967) recognized the non-linear nature of the relationship between the two tests which they attempted to overcome by calculating a free thyroxine factor. Interpretation of these indices is not always straightforward, due primarily to the effect of other plasma proteins that bind T₄ (fig 1) but their validity in the diagnosis of the majority of cases is unquestioned. It should be stressed that this approach to the assessment of free T₄ requires two tests: first, a measure of total circulating hormone in the serum (PBI or T₄) and second, a measure of the 'unoccupied' hormone-binding sites in serum proteins (serum uptake test). The recently introduced effective thyroxine ratio² (ETR) test derives a similar index from a single test (Thorson, Mincey, McIntosh, and Morrison, 1972); not all workers have found good reproducibility, the cost is not significantly less than that of the two standard tests combined, and somewhat greater technical precision is required; good diagnostic discrimination, however, is achieved. A full evaluation of the ETR test cannot yet be made but it does not appear to provide the final answer to the assay of free T₄.

Fig 1 Distribution of ¹²⁵I-T₄ in serum protein peaks as demonstrated by two-dimensional crossed immunoelectrophoresis, with subsequent autoradiography. Hormonal radioactivity is noted on thyroxine-binding globulin (TBG), thyroxine-binding prealbumin (TBPA), albumin, α₁ and β₁-lipoproteins and to a minor degree on other proteins.

¹See appendix to contribution on Nomenclature by G. K. McGowan

²Based on the Mallinckrodt kit.

The laboratory assessment of thyroid function

Chan and Landon (1972) have recently presented a novel and apparently useful approach to this measurement. Arguing that T_4 bound to plasma proteins should not be filtered by the kidney, they set out to extract and measure T_4 in the urine. In a 24-hour sample they found amounts of T_4 which, by calculation, appeared to reflect the serum free T_4 (see table). Their results are in agreement with most other published series and excellent diagnostic discrimination has been reported. Closer examination of the excreted iodinated compounds (Burke, 1972; Black, Griffiths, Hoffenberg, and Leatherdale, 1973) raises doubts about the precise interpretation of their urinary findings, but the overall value of the test is highly promising.

A review of *in-vitro* tests of thyroid function would be incomplete if it failed to discuss the major contributions of serum TSH and T_3 measurements, but these items are the subjects of separate papers (for recent reviews see Hall (1972) on TSH and Hoffenberg (1973) on T_3). Urinary T_3 has been measured by Chan, Besser, Landon, and Ekins (1972) in precisely the same way as T_4 and seems to provide equally good if not better diagnostic discrimination. Finally, a word should be said about the use of erythrocyte sodium assay (Goolden, Bateman, and Torr, 1971). This test suffers from the defect that it cannot be performed accurately on stored blood, but it distinguishes hyperthyroidism readily and is one of the few tests that directly measures an end-organ response to thyroid hormones. For this reason alone, it deserves a place in the *in-vitro* thyroid testing armamentarium.

A 'Routine' Approach to Tests of Thyroid Function

From knowledge of the availability and value of

individual thyroid function tests, can a worthwhile 'routine' approach be provided to help to resolve a clinical problem? Or, to look at it from a different angle, what tests should be performed on a serum sample sent to the laboratory for the exclusion of thyroid disease? Figure 2 illustrates an approach developed by Miss H. E. A. Farran (Northwick Park Hospital, Harrow, Middlesex) and myself to cope with the increasingly heavy demands of a busy routine laboratory. As basic tests on all samples we measure the T_4 concentration (by a modified competitive protein-binding technique) and serum uptake (initially using resin but latterly a Thyopac-3¹ kit). Consequential tests are based on the results of these two. Where both tests conform with the clinical assessment no problems arise and nothing further needs to be done; in a proportion of cases calculation of a free thyroxine index (or factor) will be needed to quantify the effect of a disturbance of plasma hormone-binding proteins. In a few cases there will be some conflict between the test results and clinical assessment and it is this group that I shall now consider. (One assumes for the sake of discussion that technical or labelling errors have been excluded; in practice, mismatching clinical and laboratory assessments should lead one immediately to reconsider the patient's clinical picture and to repeat the tests.)

It is apparent that four types of conflict may arise, and the steps taken to resolve them are discussed below.

1 THE PATIENT IS CLINICALLY HYPERTHYROID BUT THE TESTS ARE 'EUTHYROID'

Recent work would suggest a diagnosis of T_3 -

¹Radiochemical Centre, Amersham.

Author	Dialyzable Fraction (% of total T_4)	Free T_4 (pmol/l) (ng/100 ml) Mean $\pm$ SD (no.)
Anderson (1968)	0.019 $\pm$ 0.002	34.8 $\pm$ 4.9 (42) (1.77 $\pm$ 0.25)
Arango <i>et al</i> (1968)	0.035 $\pm$ 0.001	49.0 $\pm$ 1.2 (316) (2.49 $\pm$ 0.06)
Fang <i>et al</i> (1970)	0.038 $\pm$ 0.004	49.0 $\pm$ 7.7 (20) (2.49 $\pm$ 0.39)
Ingbar <i>et al</i> (1965)	0.050 $\pm$ 0.009	79.4 $\pm$ 2.14 (105) (4.03 $\pm$ 1.08)
Liewendahl <i>et al</i> (1969)	0.054 $\pm$ 0.008	112.0 $\pm$ 9.5 (22) (5.67 $\pm$ 0.47)
Oppenheimer <i>et al</i> (1963)	—	46.3 $\pm$ 5.7 (22) (2.35 $\pm$ 0.29)
Sterling <i>et al</i> (1966)	0.046 $\pm$ 0.005	83.1 $\pm$ 15.4 (18) (4.22 $\pm$ 0.77)
Chan <i>et al</i> (1971)	Range calculated from urine T_4 excretion level	39.0 — 118.0 (35) (2.0 — 6.0)

Table Variability of free T_4 measurements by different authors using different techniques and overall comparability of figures derived from the urine method of Chan *et al* (1972)

Fig 2 An approach to thyroid function testing, based on the routine measurement of (a) circulating thyroxine concentration, and (b) the availability of binding sites on thyroxine-binding proteins to eradicate problems arising from abnormal concentrations of these proteins. Further investigation (or reconsideration of the diagnosis) follows when tests and clinical assessment fail to conform.

toxicosis (Sterling, Refetoff, and Selenkow, 1970; Hollander, Mitsuma, Nihei, Shenkman, Burday, and Blum, 1972) and estimation of serum (or urine) T_3 concentration should be undertaken. Thyroidal uptake studies *in vivo* may help to rule out hyperthyroidism, as uptakes are usually high in T_3 toxicosis as well as in the commoner form of the disease. A TRH (thyrotrophin-releasing hormone) test (Hall, Amos, Garry, and Buxton, 1970) may also help to rule out hyperthyroidism since an increase of serum TSH in response to TRH injection is incompatible with this diagnosis. The T_3 suppression test (Werner and Spooner, 1955)—a valuable standby for almost 20 years—appears now to have been superseded. If doubt still exists, or if the more sophisticated tests are not available, the clinician may decide to watch the patient for a time or, if the picture warrants it, to give a therapeutic trial of an antithyroid drug.

2 THE PATIENT IS CLINICALLY EUTHYROID, THE TESTS 'TOXIC'

Clearly the same battery of tests can be applied in this situation, but there is no real need to treat unless the clinical picture changes. A clinical trial of antithyroid drug treatment would be misplaced, as one would be treating the tests and not the patient! Further investigation is probably unnecessary and one would simply watch the patient.

3 THE PATIENT IS CLINICALLY HYPOTHYROID, THE TESTS 'EUTHYROID'

The most useful test in this instance would be an assay of serum TSH as primary (thyroidal origin)

hypothyroidism is probably always associated with a raised serum concentration of this trophic hormone; a low or normal TSH result is not really compatible with the diagnosis. In the same way a normal response to TRH is considered to rule out a diagnosis of hypothyroidism, in which an exaggerated and prolonged rise of serum TSH is anticipated. The response of the thyroid gland to TSH stimulation may expose the syndrome of 'low thyroid reserve' but, like the T_3 suppression test, this dynamic test is falling out of favour and is unlikely to remain in use for much longer. Simple 'old-fashioned' tests like measurement of the serum cholesterol, an electrocardiograph, or tendon jerk reflex time are of limited diagnostic value and are most useful in the serial assessment of response to treatment. A therapeutic trial of thyroxine may be helpful in difficult cases.

4 THE PATIENT IS CLINICALLY EUTHYROID, THE TESTS 'HYPOTHYROID'

Especially after treatment of hyperthyroidism (by surgery, radioactive iodine, or antithyroid drug) some apparently euthyroid patients have been observed to show low circulating PBI or T_4 concentrations and serum uptake tests in the hypothyroid range. This is often explained by thyroidal secretion of hormone with a high T_3/T_4 ratio, so that serum T_3 concentration is normal or high, the patient thus being maintained in a euthyroid state (Sterling, Brenner, Newman, Odell, and Bellabarba, 1971; Hoffenberg, 1973). Measurement of serum TSH and, perhaps, its response to TRH might throw further light on the problem, but as long as the patient remains well, fuller investigation or treatment would generally be unnecessary.

In presenting this review and an 'approach' to the use and interpretation of *in-vitro* thyroid function tests, I feel I should stress that I do so in a rapidly changing world. What seems reasonable in 1972 to 1973 is unlikely to remain so for very long. Radioimmunoassays for T_3 , T_4 , and TSH should soon be widely available, and simple, reliable tests on serum and urine might then supersede the more complex studies described here.

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