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 131I and 99mTc 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 introduction of fully automated techniques for measurement of serum PBI concentrations and of partially automated methods for thyroxine (T₄) 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 T₄ 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 genetic 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, these 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 T3) concentration and a serum uptake test, the two results in conjunction being taken to reflect the free T4 concentration. 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 these 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 T3) and second, a measure of the 'unoccupied' hormone-binding sites in serum proteins (serum uptake test).

The recently introduced effective thyroxine ratio (ETR) test derives a similar index from a single test (Thorson, Minsey, McIntosh, and Morrison, 1972), but not all workers have found good reproducibility. The test 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 131I-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.
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 \( T_3-\)

\(^1\)Radiochemical Centre, Amersham.

<table>
<thead>
<tr>
<th>Author</th>
<th>Dialyzable Fraction (% of total ( T_4 ))</th>
<th>Free ( T_4 ) (pmol/l)</th>
<th>Mean ± SD (no.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anderson (1968)</td>
<td>0.019 ± 0.002</td>
<td>34.8 ± 4.9 (42)</td>
<td></td>
</tr>
<tr>
<td>Arango et al (1968)</td>
<td>0.035 ± 0.001</td>
<td>49.0 ± 1.2 (316)</td>
<td></td>
</tr>
<tr>
<td>Fang et al (1970)</td>
<td>0.038 ± 0.004</td>
<td>49.0 ± 1.1 (20)</td>
<td></td>
</tr>
<tr>
<td>Ingbar et al (1965)</td>
<td>0.050 ± 0.009</td>
<td>79.4 ± 2.14 (105)</td>
<td></td>
</tr>
<tr>
<td>Liewendahl et al (1969)</td>
<td>0.054 ± 0.008</td>
<td>112.0 ± 9.5 (22)</td>
<td></td>
</tr>
<tr>
<td>Oppenheimer et al (1963)</td>
<td>—</td>
<td>46.3 ± 5.7 (22)</td>
<td></td>
</tr>
<tr>
<td>Sterling et al (1966)</td>
<td>0.046 ± 0.005</td>
<td>83.1 ± 14.4 (18)</td>
<td></td>
</tr>
<tr>
<td>Chan et al (1971)</td>
<td>Range calculated from urine ( T_4 ) excretion level</td>
<td>39.0 — 118.0 (35)</td>
<td></td>
</tr>
</tbody>
</table>

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)
The laboratory assessment of thyroid function

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 T₃ 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 T₄ concentrations and serum uptake tests in the hypothyroid range. This is often explained by thyroidal secretion of hormone with a high T₃/T₄ ratio, so that serum T₃ 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 T₃, T₄, and TSH should soon be widely available, and simple, reliable tests on serum and urine might then supersede the more complex studies described here.

References

Clark, F., and Horn, D. B. (1965). Assessment of thyroid function by the combined use of the serum protein-bound iodine and...
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