Evaluation of discordant laboratory data in patients with thyroid disorders

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SYNOPSIS The usefulness of the various tests of thyroid function has been evaluated over a three-year period. It is concluded that an uptake test and estimation of the serum protein-bound radioactive iodine (PB$^{131}$I), supplemented as required by the protein-bound iodine (PBI), remain the best routine tests of thyroid function. Forty-five patients who gave discordant results on these tests over this period have been studied in detail and the value of other tests of thyroid function has been explored.

With the advent of radioisotopes a variety of tests has become available to aid in the diagnosis of thyroid disorders. It must, however, be kept in mind that in the majority of patients the diagnosis of thyrotoxicosis or hypothyroidism can and should be made on clinical grounds. For example, in one series (Wayne, 1960) it was estimated that 85% of untreated thyrotoxic patients and 81% of hypothyroid patients could be diagnosed on clinical grounds. In such patients, while confirmatory tests may provide a valuable baseline for future reference, especially in hypothyroidism where lifelong therapy is needed, they are not essential for the initial diagnosis. Unfortunately most publications dealing with the various thyroid tests have concentrated on establishing the feasibility of the test in question and defining the expected ranges in thyrotoxicosis, euthyroidism, and hypothyroidism. The present communication deals with a series of 45 patients seen over a three-year period who presented to us a thyroid diagnostic problem of varying complexity.

METHODS

Routine $^{131}$I tests consisted of a 24-hour thyroidal uptake and a 48-hour serum protein-bound $^{131}$I (PB$^{131}$I). Normal ranges in our laboratory are for a 24-hour uptake between 20 and 50% dose, and a 48-hour PB$^{131}$I $< 0.3$% dose/litre plasma.

When indicated, the percentage of the PB$^{131}$I which was extractable in butanol (BE$^{131}$I) was estimated by counting the radioactivity of an aliquot of serum and acidifying with concentrated HCl to pH 2. The serum was then shaken with an equal volume of butanol/thiosulphate for five minutes and then centrifuged at 3,000 rpm for five minutes and the supernatant decanted. The precipitate was then counted again. This procedure was performed as a minimum on three occasions but was continued as long as the count rate was falling. The normal range is $> 80$% extractable.

The protein-bound iodine was estimated by the method of Farrell and Richmond (1961).

The degree of saturation of the thyroxine-binding globulin (TBG) was estimated by the electrophoretic method of Osorio, Jackson, Gartside, and Goolden (1961) and latterly indirectly by a labelled triiodothyronine resin uptake test (T$_3$ resin uptake test$^3$).

The ranges found in our laboratory in normal subjects and in unequivocally thyrotoxic or hypothyroid subjects using the above three tests is shown in Table I.

The thyroxine suppression test was performed by repeating the $^{131}$I tests (with appropriate corrections for background activity) after the administration of sodium L-thyroxine 0-1 mg t.i.d. for 10 days.

The thyroid precipitin test was estimated by the method of Anderson, Buchanan, Goudie, and Gray (1962).

It should be noted that not all tests were performed in every patient. In part, this is because the T$_3$ resin test was not available at the beginning of the period of study and in other cases because the diagnosis became clear after clinical re-evaluation of the patient, especially with regard to eliciting a history of drug administration or performing a single further test such as finding the presence of serum antibodies to thyroglobulin.

CASE MATERIAL

The patients studied attended the thyroid clinic at the Royal Infirmary, Glasgow, or had been referred

$^3$Trisorb test, Abbott Laboratories Ltd.
to the Radioisotope Unit at the Royal Infirmary. In this unit approximately 3,000 131I tests are performed annually. The period of study is the years 1963-65 inclusive. The series of 45 patients who caused diagnostic difficulty is therefore a highly selected one. Table II indicates the type of patient in whom difficulty arose. These will now be considered in greater detail.

### TABLE II
SUMMARY OF PATIENTS STUDIED WITH CLINICAL STATUS OUT OF KEEPING WITH 131I STUDIES OR PBI

<table>
<thead>
<tr>
<th>Case</th>
<th>Clinical Status</th>
<th>Toxic</th>
<th>Euthyroid</th>
<th>Hypothyroid</th>
<th>Eventual Clinical Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-14</td>
<td>10</td>
<td>4</td>
<td>Hashimoto's thyroiditis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15-21</td>
<td>7</td>
<td>1</td>
<td>Thyrotoxicosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15-26</td>
<td>2</td>
<td>1</td>
<td>Treated thyrotoxicosis (131I)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>27-29</td>
<td>3</td>
<td>1</td>
<td>Treated thyrotoxicosis (thyroidectomy)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30-31</td>
<td>2</td>
<td>1</td>
<td>Treated thyrotoxicosis (carbimazole)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>32-33</td>
<td>3</td>
<td>1</td>
<td>Single thyroid nodule</td>
<td></td>
<td></td>
</tr>
<tr>
<td>36-38</td>
<td>3</td>
<td>1</td>
<td>Iodine administration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>39-41</td>
<td>3</td>
<td>1</td>
<td>Other drugs (excluding iodine and antithyroid drugs)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>42-44</td>
<td>3</td>
<td>1</td>
<td>Hypopituitarism</td>
<td></td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>27</td>
<td>9</td>
<td>Dyshormonogenesis</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Total 9

### CASES 1-14
This, the largest single group (Table III), consisted of patients in whom eventually the diagnosis of Hashimoto's disease appeared probable. This does not, of course, include the patients in whom the diagnosis of Hashimoto's disease had already been considered by the referring physician, usually because of the consistency of the thyroid gland or associated hypothyroidism. The group under consideration were usually patients in whom 131I studies had been performed as part of the investigation of a non-toxic goitre and in whom, usually as a result of a raised PBI131I out of proportion to the uptake and in the presence of euthyroidism or mild hypothyroidism, the possibility of Hashimoto's disease was raised. The results are summarized in Table III. It is noteworthy that all were women and, with four exceptions, were aged over 40 years. In four cases there was mild hypothyroidism; the remainder were euthyroid. In the nine cases in which the PBI131I was high enough to allow a BE131I to be estimated, the percentage extractable was less than normal in five. In nine of the 14 cases, the thyroid precipitin test was positive. The PBI was < 3.3 μg in seven cases.

The only certain way to diagnose Hashimoto's disease is by histological examination of the thyroid tissue. However, there is a good correlation between the results of antibody tests and the thyroid histology. We have included several patients in this group because of the overall clinical picture despite the absence of a positive precipitin test and the lack of thyroid histology. One of these patients is described in detail, since she illustrates well the type of problem which we encountered.

Case 5 was a 28-year-old housewife who was referred for evaluation of slight weight loss and a small goitre which had appeared about two years after having had a mitral valvotomy for rheumatic mitral stenosis. Despite a persistent sinus tachycardia, her cardiac status was excellent. She had no other symptoms or physical findings suggestive of thyrotoxicosis. The 131I studies showed a 24-hour uptake of 88% of the dose and a PBI131I of 0.40% of the dose/litre plasma. This was > 90% butanol extractable. The PBI was 10.1 μg on three occasions and the T3 resin uptake test was 29.4%. Because of the absence of clinical features of thyrotoxicosis a thyroxine suppression test was performed which showed normal suppression. The thyroid precipitin test and tanned red cell agglutination test was negative on two occasions. Because of the small
size of the thyroid gland and relative lack of symptoms, it was not thought justifiable to proceed to thyroid biopsy. A two-year follow up on this patient has shown no change in the clinical picture or protein-bound iodine. Obviously in this patient the diagnosis of Hashimoto’s disease can only be provisional.

**CASES 15-21** This group consisted of seven clinically thyrotoxic patients in whom discordant results were obtained. The results of the laboratory tests are detailed in Table IV. In three patients (cases 15-17) the pattern of results was a negligible PBI\(^{131}I\) with raised \(^{131}I\) uptake. The PBI was raised in all three cases. In none could the \(^{131}I\) uptake be suppressed by thyroxine administration. In three other patients the \(^{131}I\) studies were in the thyrotoxic range but the PBI was in the euthyroid range at 6-8, 5-4, and 3-4 \(\mu g/100\) ml respectively. In only the last of these three was the cause known. In this patient the anomalous result was due to the fact that the patient was receiving phenytoin as treatment for major convulsive seizures. In this patient the \(T_3\) resin uptake test was in the normal range at 31-4. In two other patients (cases 18 and 19) it is unfortunate that no estimations of the TBG capacity were made.

The remaining patient in this group (case 21) is of some interest. This patient was clinically thyrotoxic and had \(^{131}I\) studies in the thyrotoxic range. The PBI, however, was in the hypothyroid range at 2-4 \(\mu g/100\) ml (2-5 \(\mu g/100\) ml on repeat estimation). There was no obvious lead from the history of why this should be so. She was questioned closely for any history of drug administration, but with the exception of sedative doses of barbiturates (sodium amytal 4 gr bd) she was receiving no medication. The estimation of the TBG capacity by electrophoresis was low at 18-8 \(\mu g/100\) ml and the \(T_3\) resin uptake test was in the normal range at 28-8%. Following restoration of euthyroidism by \(^{131}I\) therapy the PBI has paradoxically risen and is now at the upper limit of normal at 7-4 \(\mu g/100\) ml. She has continued to take sodium amytal in the same dosage throughout.

**CASES 22-31** These were all patients who had been previously treated for thyrotoxicosis (Table V). In five cases the therapy had been with \(^{131}I\). In patients 22 and 23, although they appeared overall euthyroid, the possibility of residual thyrotoxicosis could not be excluded. The PBI\(^{131}I\) was much higher than is usual in the previously treated patients, in whom in our experience it is usually less than 1-0%...
dose/litre plasma. The $^{131}I$ uptake was not suppressible with thyroxine. The PBI in both was normal. On follow up they remained euthyroid. In cases 24 and 25, although the patients were still clinically thyrotoxic and the $^{131}I$ studies were consistent with this, the PBI levels were in the euthyroid range although towards the upper limit of normal. Unfortunately no estimations of TBG capacity were performed in these patients. Both were treated again with $^{131}I$ and the resulting clinical improvement confirmed our diagnosis of thyrotoxicosis. In the remaining case treated with $^{131}I$, although the patient was obviously hypothyroid and the $^{131}I$ studies were consistent with this impression, the PBI was elevated to 8.6 μg/100 ml. In view of this, the patient was again questioned with regard to drug administration and eventually admitted to having recently taken a few doses of an iodide-containing cough mixture.

In cases 27 and 28, who had been treated by thyroidectomy, the $^{131}I$ studies were repeated because of the suspicion of thyrotoxicosis. In both cases the $^{131}I$ studies gave results at the upper limit of normal. The PBI in both cases was in the euthyroid range and subsequent clinical follow up has confirmed this impression. Conversely, in case 29 although the patient appeared euthyroid the $^{131}I$ uptake was just below our normal range. The PBI was normal.

In both cases 30 and 31 where carbimazole had been recently discontinued (two months and three days respectively) and where the referring clinician was anxious about the question of possible active thyrotoxicosis the $^{131}I$ studies were at or just above normal in both, with the PBI levels in the euthyroid range. The overall clinical impression of euthyroidism was confirmed on prolonged clinical surveillance and in case 31 by repeating the $^{131}I$ studies after an interval of two months when the 24-hour uptake was 63% of the dose and the 48-hour PB$^{131}I$ was negligible.

**CASES 32-35** In these four cases (Table VI) with a single nodule, the PB$^{131}I$ was elevated in three, with a normal BE$^{131}I$. In two cases (32 and 33) scanning of the gland showed the presence of a 'hot' thyroid nodule. In the other two cases (34 and 35) the uptake was just below our normal range although associated with a very high PB$^{131}I$ level (1.81% of the dose/litre plasma in case 34). It was of interest that in these two patients the uptake which was present was not over the thyroid nodule but was over the residual thyroid tissue. In all four cases the PBI was in keeping with the clinical impression of euthyroidism.

**CASES 36-38** These three patients were referred for evaluation of a small non-toxic goitre. Difficulty...
arose because of failure to elicit in the initial history the fact that the patient had taken some iodide-containing preparation. In two of these patients this was quickly realized from the combination of a low $^{131}$I uptake (3% and 8% respectively) combined with a grossly raised PBI (18-6 µg and 10-6 µg/100 ml plasma respectively). In the other patient the situation was more difficult as the $^{131}$I studies were in the euthyroid range (24-hour uptake 40% of the dose and the 48-hour PB$^{131}$I negligible). In this case the PBI was raised to 8-0 µg/100 ml. This patient would not admit to any iodide ingestion but the real clue came from the fact that she had chronic bronchitis. After repeated questioning she did admit to taking a few doses of cough mixture about three months before the test was performed.

**CASES 39-41** In these three patients other drugs (excluding antithyroid drugs and iodine) interfered with interpretation of the results. In two of these, this was because of administration of oestrogens as part of an oral contraceptive preparation. Both had normal $^{131}$I uptake (50% and 31% of the dose respectively) with a negligible PB$^{131}$I and raised PBI (7-6 µg and 8-8 µg/100 ml). In one, the $T_3$ resin uptake test was normal (30-2%) and in the other it was in the hypothyroid range at 22-5%. The TBG capacity was raised in both cases at 37-0 and 40-0 µg/100 ml respectively.

The third case in this group presented a more difficult problem of evaluation. This was a mentally retarded girl of 18 years (case 41) who was referred to the clinic as being possibly hypothyroid. She was of small stature, was moderately obese, and severely mentally retarded. The $^{131}$I studies were in the euthyroid range (uptake 50% of the dose and PB$^{131}$I negligible). However, the PBI was low at 2-7 µg/100 ml. The $T_3$ resin test gave a result at the upper limit of normal (34-3%). The explanation was that she was receiving phenytoin therapy to control major convulsive seizures.

**CASES 42-44** These three patients with hypopituitarism are of interest. One was a man aged 33 years who had been operated on some years previously for a craniopharyngioma; another was a man of 35 years who had had a chromophobe adenoma removed; and the third case in this group was a boy of 13 years with idiopathic hypopituitarism. All three had clinical secondary hypothyroidism. The $^{131}$I uptakes were normal (40%, 49%, and 33%) with negligible PB$^{131}$I, but the low PBI levels (2-3, 1-6, and 3-2 µg/100 ml) were in keeping with the patients' clinical status.

**CASE 45** This last patient was a girl of 11 years who was referred to the clinic because of a large goitre. There was no family history of thyroid enlargement. Although she was clinically euthyroid or mildly hypothyroid the $^{131}$I study was in the thyrotoxic range (uptake 81% of the dose, PB$^{131}$I 0-75% of the dose/litre plasma). The BE$^{131}$I was 78% of the PB$^{131}$I. The PBI was 2-1 µg/100 ml and the TBG capacity was in the hypothyroid range at 30-0 µg/100 ml, as was the $T_3$ resin uptake test at 20%. Biochemical studies on the resected gland showed that she had a dys hormonogenetic goitre of the iodoprotein type (McGirr, Hutchison, Clement, Kennedy, and Currie, 1960).

**DISCUSSION**

The patients reported in this paper do not fall into any homogenous group. The largest single group was those patients who were thought to have Hashimoto's disease. This is a condition in which anomalous $^{131}$I results have been recognized for some time (Murray and McGirr, 1960). In the cases under discussion, however, it should be emphasized that these were cases in which the diagnosis was not entertained by the referring clinician. Once the possibility had been raised (usually by the elevated PB$^{131}$I often combined with a lowered PBI) the diagnosis could be simply made by estimation of the serum antibodies. This would seem to be the investigation of choice in the middle-aged woman with a raised PB$^{131}$I without obvious cause. It must be admitted, however, that there were some cases, for example case 5, in which there is still doubt about the final diagnosis.

The seven cases of thyrotoxicosis are of interest. In the three cases with the negligible PB$^{131}$I the only possibly explanation is that the intrathyroidal turnover of iodine is unusually slow. We have seen this pattern of results in three subsequent patients after the period under consideration. In the group of four with the low or normal PBI the explanation was only clear in one (case 20) who was receiving phenytoin, which is recognized as depressing the PBI by occupying the binding sites on the TBG molecule (Oppenheimer and Tavernetti, 1962). A similar false low PBI in a euthyroid patient is illustrated by case 41. In one other patient (case 21) the low PBI when thyrotoxic and the return to normal levels when euthyroid is inexplicable. Cases of thyrotoxicosis in which the PBI has been low despite clinical thyrotoxicosis have been described by Cavalieri (1961). This has been attributed to congenitally low levels of circulating TBG. This explanation would seem unlikely in our patient in view of the response of the PBI to the
control of the thyrotoxic state. Our patient had no close relatives available for examination. It is worthwhile emphasizing that in all of this group the diagnosis of thyrotoxicosis was finally confirmed by observing the clinical response to antithyroid therapy.

The difficulty in interpreting 131I studies when there is only a small rapidly turning over thyroidal iodine pool is well recognized. This occurs after treatment of thyrotoxicosis with 131I, thyroidectomy, or antithyroid drugs. In this latter group a temporary state of iodine deficiency is induced (Alexander, Harnden, Koutras, and Wayne, 1965). In all of this group of previously treated thyrotoxic patients most help was obtained from estimation of the protein-bound iodine. In two cases, however, the opposite problem was present of patients treated with 131I who were still clinically thyrotoxic (and who responded clinically to anti-thyroid therapy) who had 131I studies consistent with thyrotoxicosis but PBI in the euthyroid range. In these two cases, however, we lack the data to make any valid comment on the TBG levels.

Cases of non-toxic goitre with a single 'hot' nodule may also have a small intrathyroidal iodine pool and this occurred in patients 32 and 33. In cases 34 and 35 the uptakes were rather low although associated, in case 34, with a greatly elevated PB131I. In both these cases the scan showed no uptake over the nodule. The most reasonable explanation would seem to be that there was only a small rim of normally functioning thyroid tissue around the nodule and this was giving the pattern of results more usually associated with a 'hot' nodule.

The three patients to whom iodine had been administered illustrate the extreme importance of getting a good history of the drugs which the patient is taking or has taken in the recent past. This is sometimes not as simple in practice as it theoretically should be. During the period of study we, of course, saw a much larger number of patients in whom such a history of iodine administration was obtained and we either postponed the tests for a suitable interval or the T3 resin uptake test was used, this being the most suitable test as it is not affected by iodine administration. In case 38 difficulty arose because there was apparently only enough residual iodine to affect the PBI without influencing the 131I uptake.

Similarly, in cases 39 and 40 the history of administration of oral contraceptive drugs was overlooked. The administration of an oestrogen-containing drug was suspected on noting the combination of a normal uptake and an elevated PBI with elevated TBG capacity. In one of these cases the T3 resin uptake test was as expected falsely low but in the other it was normal. It is worthwhile noting that in one series of T3 resin tests in pregnancy (Clark and Horn, 1965), where a similar alteration of TBG capacity exists, the T3 resin test was in the normal range in 8.7% of cases. With the increasing use of oral contraceptive agents this group is becoming more important as a fallacy of the protein-bound iodine test.

The three patients with hypopituitarism are interesting. Again it must be emphasized that during the three-year period of study we encountered several other hypopituitary patients who gave the more usual results of a low 131I uptake and protein-bound iodine. The explanation of the anomalous 131I uptakes in our patients who were clinically hypothyroid is either that the thyroid had retained some autonomous function, which is recognized as occurring in animals, which, despite having had hypophysectomy performed, can still respond to iodine deficiency by increased thyroid function (Means, De Groot, and Stanbury, 1963), or more probably that a minimal amount of TSH was being secreted which enabled the thyroid to trap a normal proportion of the test dose but which was not enough consistently to produce sufficient thyroxine secretion to maintain clinical euthyroidism. The discrepancy of a normal 131I uptake with a low PBI in hypopituitarism has been previously noted (Christy, 1960) but is not apparently widely recognized.

In the final patient, case 45, the absence of a family history of goitre and the absence of significant hypothyroidism, although not exclusive, are points against the suspected diagnosis of dysmamorogenesis. The pattern of results, however, made this diagnosis clear and this was confirmed by studies in vitro upon the resected thyroid tissue, which showed that she was producing an abnormal iodoprotein (McGirr et al, 1960).

One point which is well brought out by these studies is that there is no single ideal thyroid test at present in current use. In practice the tests used are a compromise between a wide battery of testing for all patients, covering many aspects of thyroid function, and a selective procedure designed to achieve a correct diagnosis in the majority of patients with minimal investigation. Nowadays all centres dealing with large numbers of thyroid cases should have access to an isotope laboratory containing at least an uptake counter and a well-typed scintillation counter. It is probable that some form of uptake measurement together with an estimation of the PB131I at 48 hours will remain the best routine tests of thyroid function. Indeed it was interesting in the present series (for example cases 20, 21, and 39-41) where the peripheral transport of the thyroid hormone was altered that the
131I studies gave a truer reflection of the clinical status than did the other tests.

The recently suggested combination of an uptake measurement and a T3 resin uptake test (Carter, 1966) would seem to us unsatisfactory in that having given the patient a dose of radioactivity one might as well proceed to estimate the PBl31I.

If tests other than 131I studies are to be used the PBI is the most useful, especially in our experience in patients previously treated for thyrotoxicosis. The T3 resin uptake test is an alternative but in the form of the Triosorb test it is rather expensive and the test kit has to be used within a short time of receipt. It is, however, of definite value in the investigation of a patient known to have taken iodides in whom doubt exists as to the clinical thyroid status and in whom an urgent answer is necessary. It is also of value in conditions of abnormal thyroxine binding. In this latter context the 'free' thyroxine index (Clark and Horn, 1965) has been used. This is an arbitrary product of the PBI and T3 resin uptake. The value of the test is that in conditions such as pregnancy where the PBI and TBG capacity alter, they are changed in opposite directions thereby cancelling out in the euthyroid subject. We have insufficient data of our own to comment on the usefulness of this test, especially in states of altered TBG capacity in the presence of clinically altered thyroid function, but it is of interest to note that in neither of our two thyrotoxic patients with altered TBG capacity (cases 20 and 21) would the correct clinical diagnosis have been made on the basis of these two tests alone.

A direct measurement of the free thyroxine (Oppenheimer, Squef, Surks, and Hauer, 1963) may well prove to be an extremely important test but is at present too cumbersome for routine use.

These results seem to us worth reporting to illustrate the type of clinical problem encountered at a thyroid clinic and to illustrate how the various laboratory tests may help in achieving the correct diagnosis where clinical doubt exists.

REFERENCES