Diagnostic tests for thyroid antibodies: A comparison of the precipitin and latex-fixation (Hyland TA) tests


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SYNOPSIS  The clinical significance of thyroid antibodies is discussed and it is concluded that, of the antibody tests which have been extensively applied, the precipitin test is the most useful for diagnostic purposes. The recently introduced Hyland TA test is compared with the precipitin test. The TA test gives a rapid result and is slightly more sensitive, but it remains undecided whether a true positive result is always indicative of clinically important degrees of chronic thyroiditis. The TA test also gives occasional false positive results, not indicative of antibody to thyroglobulin. Furthermore, approximately 12% of precipitin-positive sera from patients with Hashimoto’s disease give negative TA tests, and since these also have low titres, or are negative, in the tanned red cell haemagglutination test, the precipitin test is the only simple method of demonstrating the antibody concerned. In spite of these disadvantages, the TA test is regarded as a useful procedure, and there are advantages in using both the precipitin and TA tests together for routine diagnostic purposes. A modification is described whereby the TA test reagent may be used to titrate antibody to thyroglobulin.

It is now well established that the presence of thyroid antibodies in a patient’s serum is strong evidence of chronic thyroiditis. There is also a general, although not perfect correlation between the amounts of antibodies and the severity and extent of the chronic thyroiditis. Thus very high concentrations of thyroid antibodies are commonly, but not invariably, present in Hashimoto’s disease which, by definition, is characterized by severe, extensive chronic thyroiditis. Lesser degrees of chronic thyroiditis, affecting microscopic foci of thyroid tissue, are of common occurrence and are, in general, associated with lesser concentrations of thyroid antibodies in the serum. These observations are discussed in the excellent review of Roitt and Doniach (1960).

For clinical purposes, it is important to make a distinction between mild (focal) and severe (extensive) chronic thyroiditis. It is not possible to make an absolute distinction, for all degrees of severity are encountered. Milder degrees of chronic thyroiditis, however, cause neither functional disturbance nor enlargement of the thyroid gland, and are of no clinical importance: they occur in association with various types of thyroid disorder, and also in individuals, particularly middle-aged and elderly women, without overt thyroid disease (Simmonds, 1923). Various degrees of chronic thyroiditis occur in most patients with thyrotoxicosis (Broders, 1936; see also Hertz, 1943). Usually the change is of mild degree, and is either not progressive or progresses very slowly (Spjut, Warren, and Ackerman, 1957). Severe chronic thyroiditis is important clinically, for it is a cause of thyroid enlargement and may be accompanied by features simulating thyrotoxicosis, including abnormalities in radioiodine tests, or by hypothyroidism, or it may be without obvious effect on thyroid function. Severe chronic thyroiditis is also the cause of some, if not all, cases of primary hypothyroidism without goitre, although by the time the patient seeks advice the inflammatory changes have largely subsided, leaving a shrunken fibrosed gland, and in most cases the serum levels of thyroid antibodies are low. Rarely, severe chronic thyroiditis accompanies true thyrotoxicosis or thyroid cancer.
From the above considerations it is clear that to be useful in clinical diagnosis a thyroid antibody test should be positive in the majority of patients with severe chronic thyroiditis (i.e., in Hashimoto's disease) and negative in patients with mild, clinically insignificant degrees of chronic thyroiditis. In the past few years, over a dozen thyroid antibody tests have been described, but only three of these can be evaluated from the available literature. These are the precipitin test, the tanned red cell test, both of which detect antibody to thyroglobulin, and the complement-fixation test for antibody to a constituent of thyroid epithelial 'microsomes'. The results obtained with these tests upon the serum of large numbers of patients with the commoner thyroid disorders were reported by Roitt and Doniach (1958) and by Fulthorpe, Roitt, Doniach, and Couchman (1961). It is apparent from these reports that the tanned red cell and complement-fixation tests are frequently positive in patients with mild degrees of thyroiditis, and that these tests, performed in titration, do not differentiate more accurately between mild and severe degrees of chronic thyroiditis than does the precipitin test upon undiluted serum. From these and our own observations (Anderson, Goudie, and Gray, 1959) we consider that a positive precipitin test is virtually always indicative of clinically important degrees of chronic thyroiditis, and because of its technical simplicity we regard it as the most suitable antibody test for diagnostic purposes.

A disadvantage of the precipitin test is that, with some sera, positive results take several days to appear, and as it is sometimes important to give a quick answer, we decided to compare it with the much more rapid 'TA' test introduced by Hyland Laboratories of Los Angeles.

**MATERIALS AND METHODS**

**SPECIMENS OF SERUM** These were obtained from 452 hospital patients, 223 of whom had thyroid diseases, and the remainder of whom had various surgical, gynaecological, and medical disorders but were not considered clinically to have any thyroid abnormality. Fifty-nine patients with rheumatoid arthritis were included in the latter group. This was done because the reagent for the TA test is a suspension of latex particles sensitized with thyroglobulin, and as preparations of thyroglobulin contain various other proteins, including γ globulin (Roitt and Doniach, 1958; Korngold, van Leeuwen, and Brener, 1959), we considered it possible that the presence of rheumatoid factors in the test serum might give rise to false positive TA tests. The numbers of patients with various thyroid disorders are given in Table I. The diagnostic criteria for Hashimoto's disease and simple non-toxic goitre were as stated elsewhere (Goudie, Anderson, Gray, Clark, Murray, and McNicol, 1957).

Thyrotoxicosis and primary hypothyroidism were diagnosed by the methods described by Crooks, Murray, and Wayne (1959) and Wayne (1960) respectively, and the diagnostic criteria for rheumatoid arthritis were those of Buchanan, Crooks, Alexander, Koutras, Wayne, and Gray (1961). The diagnosis of thyroid cancer was made histologically. Most of the specimens of serum were stored at −20°C, some for as long as 18 months, before testing.

**PRECIPITIN TEST** The precipitin test was performed by an Ouchterlony-Elek technique, details of which are appended on p. 467.

**TA TEST** The TA test detects antibody to thyroglobulin and is based on the method of Singer and Plotz (1956) in which a suspension of polystyrene latex particles sensitized by the addition of a solution of an antigen (in this case thyroglobulin) is aggregated by the corresponding antibody. The technique used was that recommended by the manufacturers of the test reagent. Briefly, the serum was inactivated by heating at 56°C for 30 minutes, and tested undiluted and diluted 1 in 20 with a glycine-saline buffer of pH 8.2. A drop each of the undiluted and of the diluted serum was placed on a glass slide, and to each was added a drop of the sensitized latex reagent. After mixing, the slide was slowly rocked and examined for three minutes. The formation of visible aggregates in either mixture was regarded as a positive result. The manufacturer's instructions do not state whether the slide should be examined by reflected or transmitted light. Experience proved this to be of some importance, for when viewed by transmitted light, fine, barely visible aggregates were observed with a significant number of serum specimens devoid of antibody to thyroglobulin. This difficulty was overcome by holding the slide over a dark background and examining it by oblique reflected light.

**INHIBITION TESTS** Selected specimens of serum giving a positive TA test were re-tested after treatment with an equal volume of a solution of thyroglobulin (0.5 mg. per ml.) prepared by the method of Derrien, Michel, and Roche (1948), and also after treatment with pooled human γ globulin (0.5 mg. per ml.). Inhibition of the TA reaction by thyroglobulin and not by γ globulin was regarded as evidence that the reaction was attributable to anti-thyroglobulin, whereas failure of inhibition by thyroglobulin suggested a false positive reaction.

**TA TITRATIONS** Although the manufacturers of the TA reagent do not advocate its use for antibody titrations, we considered it of interest to perform TA titrations on selected sera, in order to compare its sensitivity with that of other tests. In performing the titrations, in which sera were tested in fourfold dilutions, it was found that fine but visible aggregation of the latex particles occurred within three minutes of mixing the latex suspension with a drop of glycine-saline diluent, or with high dilutions of normal serum. This difficulty was obviated by adding to the glycine-saline diluent enough 30% bovine albumin
to Armour to give a concentration of 1%. This procedure did not significantly diminish the specific aggregation of the latex by serum containing antibody to thyroglobulin.

**TANNED RED CELL TEST** This test was performed by the technique described elsewhere (Anderson, Goudie, Gray, and Buchanan, 1961), with fourfold dilutions of serum, starting at 1 in 4. In our experience, positive results with this test are always inhibited by pre-treating the serum with thyroglobulin, and we regard them as indicative of antibody to thyroglobulin.

**RESULTS**

The results of the precipitin and TA tests upon the 452 specimens of serum examined are summarized in Table I. It should be explained that the specimens from patients with Hashimoto's disease, primary hypothyroidism, and thyrotoxicosis were selected purely for the purpose of comparing the two tests, and contain higher proportions of precipitin-positive specimens than those encountered in unselected groups of patients with these conditions. It is seen from Table I that of 70 precipitin-positive specimens of serum, 64 were positive with the TA test, and of 382 precipitin-negative specimens 362 were negative in the TA test. The six precipitin-positive, TA-negative specimens were from patients with Hashimoto's disease. Eleven of the 20 precipitin-negative, TA-positive specimens were from patients with thyroid diseases, and nine were from patients without clinical evidence of thyroid disease.

As there is little information available on the reliability of the TA test, we considered it important to investigate further the significance of the positive results with this test. This was done by two methods. First, by performing TA and tanned red cell titrations on 92 selected sera. The tanned red cell test, performed in titration, is regarded as a reliable, highly sensitive and semi-quantitative method of demonstrating antibody to thyroglobulin. Secondly, selected specimens of serum were examined by the TA test after treatment with thyroglobulin and with $\gamma$ globulin as described above. The results of these investigations are exemplified in Tables II and III.

In Table II, the results with sera nos 1-3 are typical of those obtained with most precipitin-positive, TA-positive specimens: the TA titre ranged from 64 to 4,000, and the tanned red cell titres from 4,000 to 256,000. There was a general, but not perfect correlation between the titres for the two tests, the tanned red cell titres being 16 to 1,000 times higher than the TA titres. Pre-treatment with thyroglobulin annulled the TA reactions of these sera. Pre-treatment with pooled $\gamma$ globulin did not annul the reactions of any of the TA-positive sera tested, and are not recorded in the tables. Occasional precipitin-positive, TA-positive specimens (e.g., no. 4) gave exceptional results in that their tanned red cell titres were disproportionately low, and some (e.g., no. 5) had lower TA and tanned red cell titres than is usual for precipitin-positive specimens. Sera 6 to 11 are the six precipitin-positive, TA-negative specimens from patients with Hashimoto's disease. Only nos. 6 and 7 gave tanned red cell reactions and their titres were very low for precipitin-positive specimens. It was notable that these six specimens all gave an unusual type of reaction in the precipitin test: all gave a line of precipitate which developed only after several days, and was shorter, wider, and more fuzzy than in the typical reaction. This type of precipitin reaction is illustrated and briefly discussed in the technical appendix to this

**TABLE I**

**RESULTS OF PRECIPITIN AND LATEX-FIXATION TESTS ON SERA OF 452 HOSPITAL PATIENTS**

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of Cases</th>
<th>Precipitin +ve</th>
<th></th>
<th>Precipitin -ve</th>
<th></th>
<th>Totals</th>
<th></th>
<th></th>
<th></th>
<th></th>
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<tbody>
<tr>
<td><strong>Thyroid Diseases</strong></td>
<td></td>
<td></td>
<td>---</td>
<td>----------------</td>
<td>---</td>
<td>--------</td>
<td>---</td>
<td>---</td>
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<tr>
<td>Hashimoto's disease</td>
<td>63</td>
<td>41</td>
<td>6</td>
<td>1</td>
<td>15</td>
<td>47</td>
<td>42</td>
<td>42</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary hypothyroidism</td>
<td>35</td>
<td>16</td>
<td>0</td>
<td>2</td>
<td>17</td>
<td>16</td>
<td>18</td>
<td>18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thyrotoxicosis</td>
<td>73</td>
<td>5</td>
<td>0</td>
<td>6</td>
<td>62</td>
<td>5</td>
<td>11</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Simple non-toxic goitre</td>
<td>40</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>40</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thyroid cancer</td>
<td>12</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>9</td>
<td>1</td>
<td>3</td>
<td>3</td>
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<td><strong>Controls</strong></td>
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<td>--------</td>
<td>---</td>
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<td>---</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>59</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>54</td>
<td>0</td>
<td>5</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other diseases</td>
<td>170</td>
<td>1</td>
<td>0</td>
<td>4</td>
<td>165</td>
<td>1</td>
<td>5</td>
<td>5</td>
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</table>

**TABLE II**

**LATEX-FIXATION AND TANNED RED CELL AGGLUTINATION TITRES OF PRECIPITIN-POSITIVE SERA**

<table>
<thead>
<tr>
<th>Serum No.</th>
<th>TA Titre</th>
<th>Tanned Red Cell Titre</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>4,000</td>
<td>256,000</td>
</tr>
<tr>
<td>2</td>
<td>64</td>
<td>16,000</td>
</tr>
<tr>
<td>3</td>
<td>256</td>
<td>4,000</td>
</tr>
<tr>
<td>4</td>
<td>64</td>
<td>64</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>1,000</td>
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<tr>
<td>6</td>
<td>0</td>
<td>256</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>4</td>
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<td>8</td>
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<td>9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td>0</td>
<td>0</td>
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</tbody>
</table>
Diagnostic tests for thyroid antibodies

TABLE III
LATEX-FIXATION AND TANNED RED CELL AGGLUTINATION TITRES OF 20 PRECIPITIN-NEGATIVE SERA

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Serum No.</th>
<th>TA Titre</th>
<th>Inhibited by Thyroglobulin</th>
<th>Tanned Red Cell Agglutination Titre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hashimoto's disease or primary hypothyroidism</td>
<td>12-14</td>
<td>4-16</td>
<td>Yes</td>
<td>256-1,000</td>
</tr>
<tr>
<td>Thyrotoxicosis</td>
<td>15-20</td>
<td>1-4</td>
<td>Yes</td>
<td>64-1,000</td>
</tr>
<tr>
<td>Thyroid cancer</td>
<td>21</td>
<td>4</td>
<td>Yes</td>
<td>1,000</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>16 (prozone)</td>
<td>No</td>
<td>1,000</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>23</td>
<td>64</td>
<td>Yes</td>
<td>4,000</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>4</td>
<td>Yes</td>
<td>4,000</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>16 (prozone)</td>
<td>No</td>
<td>1,000</td>
</tr>
<tr>
<td></td>
<td>26</td>
<td>16 (prozone)</td>
<td>No</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>27</td>
<td>16 (prozone)</td>
<td>No</td>
<td>0</td>
</tr>
<tr>
<td>Other diseases</td>
<td>28</td>
<td>16</td>
<td>Yes</td>
<td>4,000</td>
</tr>
<tr>
<td></td>
<td>29</td>
<td>16</td>
<td>No</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>16 (prozone)</td>
<td>—</td>
<td>4,000</td>
</tr>
<tr>
<td></td>
<td>31</td>
<td>16 (prozone)</td>
<td>No</td>
<td>0</td>
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</tbody>
</table>

paper, and evidence is presented that it indicates a true antigen-antibody reaction not demonstrable by the tanned red cell or TA tests. Since it was observed only in Hashimoto's disease, it is of diagnostic value.

Table III gives the results of investigations on the 20 precipitin-negative, TA-positive specimens of serum. Ten of the 11 specimens (nos. 12 to 21) from patients with thyroid diseases had tanned red cell titres 16 to 236 times higher than their TA titres, and both the tanned red cell titres (64 to 1,000) and the TA titres (1 to 16) were below the levels usually associated with a positive precipitin test. Their TA reactions were inhibited by thyroglobulin, and it is concluded that they contain antibody to thyroglobulin, but not enough to give a positive precipitin test. Subtotal thyroidectomy was subsequently performed in three of the six patients with thyrotoxicosis, and histological examination revealed in all three a degree of chronic thyroiditis unusually severe for patients with thyrotoxicosis. The biopsy from the patient with thyroid cancer (serum no. 21) contained only tumour tissue, and therefore the presence or absence of chronic thyroiditis could not be determined. The five specimens from patients with rheumatoid arthritis gave three patterns of results. With sera 23 and 24 the findings were similar to those just described for the thyroid disease specimens, and we conclude that the positive TA tests are indicative of antibody to thyroglobulin. Sera 26 and 27 gave a prozone effect in the TA titrations, being positive at a dilution of 1 in 16, but negative undiluted and weak or equivocal at 1 in 4. Their tanned red cell titres were 16 and nil respectively and their TA reactions were not annulled by thyroglobulin. We conclude that these two specimens give false positive TA reactions, not attributable to antithyroglobulin. Rheumatoid serum no. 25 appears, from the tanned red cell test, to contain anti-thyroglobulin, and yet the TA reaction was not annulled by thyroglobulin and is therefore of doubtful significance; the same applies to the case of thyroid cancer whose serum (no. 22) gave identical results. Of the four TA-positive sera from patients without thyroid or rheumatoid disease, one (no. 28) contained antithyroglobulin, and two (nos. 29 and 31) gave false positive TA tests. Thyroglobulin-inhibition could not be tested on serum 30 owing to lack of serum, and we can draw no conclusion concerning the positive TA test in this patient. Serum 31 was from a patient who subsequently died, and histological examination showed an insignificant degree of chronic thyroiditis.

DISCUSSION

The usefulness of tests for thyroid antibodies in clinical diagnosis is limited by the imperfection of the correlation between the amounts of antibodies in the serum and the degree of chronic thyroiditis. With the possible exception of the cytotoxic factor (Pulvertaft, Doniach, Roitt, and Hudson, 1959; Irvine, 1960) none of the antibodies so far described is invariably present in patients with severe chronic thyroiditis. It is true that one or other of the antibodies can always be demonstrated, at least in small amounts, by the use of multiple and elaborate tests (Fulthorpe, Roitt, Doniach, and Couchman, 1961), but this observation is of little diagnostic value since small amounts of thyroid antibodies are readily demonstrable in the sera of many individuals without severe chronic thyroiditis. The precipitin test has the advantages of simplicity and reliability, and when positive is virtually always indicative of severe chronic thyroiditis. The present investigation has shown the TA test to be more sensitive than the precipitin test, for most
precipitin-positive sera were found to have TA titres of 64 or more. It is thus not surprising that the TA test was positive in occasional patients with Hashimoto’s disease or primary hypothyroidism having a negative precipitin test. The greater sensitivity of the TA test is evident also in the results with the 73 patients with thyrotoxicosis, five of whom had positive precipitin and TA tests, while in six the TA test alone was positive. As already stated, the incidence of positive precipitin tests in this series is abnormally high, and in a larger, unselected series of thyrotoxic patients, the incidence was 2% (Anderson, Goudie, and Gray, 1959). The presence of severe chronic thyroiditis in patients with thyrotoxicosis has been shown to be associated with an increased incidence of hypothyroidism following treatment by subtotal thyroidectomy (Whitesell and Black, 1949; Levitt, 1951; Bartels, 1953; Greene, 1953), and a positive precipitin test should therefore be taken into consideration when deciding what form of therapy is to be adopted (Buchanan, Alexander, Crooks, Koutras, Wayne, Anderson, and Goudie, 1961). It is probable that, in thyrotoxic patients with a negative precipitin test, a positive TA test is of some importance, for our three patients who had these findings, and who were treated by subtotal thyroidectomy, all had degrees of chronic thyroiditis which, according to the above workers, are associated with a high incidence of hypothyroidism following operation. These observations are in agreement with the findings of Fulthorpe, Roitt, Doniach, and Couchman (1961), who reported a positive precipitin test in approximately 1% of patients with thyrotoxicosis, whereas the tanned red cell and complement-fixation titres reached Hashimoto levels in 10% and were associated with an increased tendency to develop postoperative hypothyroidism.

Of the 229 ‘control’ patients, one had positive precipitin and TA tests, and in three others the TA test was positive and was regarded as indicating antibody to thyroglobulin. In our experience a positive precipitin test is encountered in occasional patients not suspected of having thyroid disease, but more intensive investigation has always provided evidence of severe chronic thyroiditis. It remains undecided whether a true positive TA test alone in ‘control’ patients indicates progressive or clinically important degrees of chronic thyroiditis. The finding of two true positive TA tests in 59 patients with rheumatoid arthritis is in keeping with the increased incidence of Hashimoto’s disease, of complement-fixing antibody (Buchanan, Crooks, Alexander, Koutras, Wayne, and Gray, 1961), and of positive tanned red cell tests (Anderson, Goudie, Gray, and Buchanan, 1961) reported in this condition.

The differences in results of precipitin and TA tests are not all accounted for by the greater sensitivity of the latter test, for six precipitin-positive sera were TA-negative. The precipitin reactions of these sera were slow to develop and unusual in appearance, and the tanned red cell tests were of low titre or negative. For reasons stated below (see appendix) we regard these atypical precipitin reactions as indicative of large amounts of thyroid antibody, although it is not established that it is anti-thyroglobulin. The six patients concerned had Hashimoto’s disease, and since sera were not selected for their atypical precipitin reactions it is likely that this represents a true incidence, i.e., that the TA test will prove to be negative in approximately 12% of precipitin-positive sera from patients with Hashimoto’s disease.

The conclusion that false positive reactions, not indicative of thyroid antibody, are occasionally encountered with the TA test, is based on the results with our four specimens of serum which gave positive TA reactions, were not inhibited by thyroglobulin, and were negative or had a very low titre in the tanned red cell test. Histological examination of the thyroid of one of the patients showed an insignificant degree of chronic thyroiditis. Although two of the four false positive reactions occurred in patients with rheumatoid arthritis, they were not inhibited by pooled γ globulin, and there is no evidence that they are attributable to rheumatoid factors in the serum. For practical purposes, the false positive reactions are not a big disadvantage, for they occurred with only four of the 452 sera tested. All except one of the four showed a prozone effect, the TA test being negative with undiluted serum, and positive with serum diluted 1 in 16 (or 1 in 20 as in the standard technique). This prozone effect is not confined to false positive reactions, but was observed also with occasional precipitin-positive sera in Hashimoto’s disease, and is regarded by the manufacturers of the TA reagent as indicating a true positive result. It is noticeable (Table III) that for most of our precipitin-negative, TA-positive sera, including three of the four false positives, the TA test was positive with either undiluted serum, or with the 1 in 20 dilution, but not with both. By regarding as positive only those sera which reacted at both dilutions in the TA test, the results would be in closer agreement with those of the precipitin test. This modification in the reading of the TA test would, however, give a negative TA result with four precipitin-positive sera which aggregated the latex at one dilution only.

One of the more important purposes for which tests for thyroid antibodies are being used is to help in the differential diagnosis between thyroid cancer and Hashimoto’s disease. A negative precipitin test is of little value, since it is encountered in approxi-
mately 30% of patients with Hashimoto's disease. A positive precipitin test is helpful, but should not be regarded as excluding the possibility of thyroid cancer. In our series, the patient with thyroid cancer who had a positive precipitin test died recently, and at post-mortem examination the remaining thyroid showed severe extensive chronic thyroiditis, the appearances being closely similar to those in primary hypothyroidism. The association of chronic thyroiditis with thyroid cancer is well known, and positive precipitin tests have been described in such cases (Stuart and Allan, 1958), but are probably uncommon, for Fulthorpe, Roitt, Doniach, and Couchman (1961) encountered only one precipitin-positive serum among 64 patients with thyroid malignancy. We agree with the latter authors that a positive precipitin test should not dictate the diagnosis, but should be considered together with the clinical findings and results of other laboratory investigations. This warning applies also to the results of TA tests, which were positive in three of our 12 patients with thyroid cancer.

In conclusion, it is clear that the choice of thyroid antibody tests depends upon the purpose for which they are performed. In a unit with a special interest in thyroid diseases, and dealing with a large number of sera, there are advantages in performing several of the available antibody tests. Where the number of sera examined is small, and the main purpose is to assist in the diagnosis of primary hypothyroidism, and in the differentiation of Hashimoto's disease from other causes of thyroid enlargement, we consider the precipitin test to be the best single procedure available. The TA test is far more rapid, but in our hands proved less reliable: its greater sensitivity may, however, prove to be valuable in detecting clinically important degrees of chronic thyroiditis in some patients with thyrotoxicosis.

TECHNICAL APPENDIX

We have used the precipitin test for five years, and have encountered over 250 positive results. Below we describe the technique we have found most convenient, and illustrate the various appearances presented by positive specimens of serum.

METHOD

Agar plates, 9 cm. diameter, are prepared, containing 20 ml. of 1.7% Difco Bacto agar dissolved in 0.9% saline solution and containing 0.1% of sodium azide to inhibit bacterial growth. The azide is added as a 2% solution when the melted agar has cooled to 60°C, immediately before pouring. When the agar has set, circular cuts are made in it with cork-borers, using a template drawn on paper beneath the Petri dish to position the cuts correctly. It is convenient to use a piece of glass tubing, clamped vertically in a burette stand, as a guide to the cork borer, which is slid down inside the tubing to make the cuts (Fig. 1). The discs of agar are removed with a suction pipette. A well of 7 mm. diam. (No. 3 cork borer) is made for the antigen, and a well of 10 mm. diam. (No. 6 cork borer) for each test serum. The distance between the centres of the antigen and serum wells is 20 mm. Two antigen wells are made on each plate, and two specimens of serum are tested on opposite sides of each antigen well (Fig. 2). It is not advisable to place more than two serum wells around each antigen well, for when serum wells are close together a strongly-reacting serum may obscure a weak reaction of an adjacent serum.

The antigen and serum wells are filled, the latter with undiluted serum, and the plate is left at room temperature and examined at first daily, and then at two-day intervals, for a total of 14 days. The time taken for precipitation to occur may be reduced by leaving the lids off the plates for a few hours to allow most of the contents of the wells to pass into the agar: if the lids are left off too long (e.g., overnight), excessive drying of the agar interferes with the tests.

ANTIGEN This consists of a crude saline extract of normal post-mortem thyroid tissue, or of surgically removed thyroid tissue from patients with simple non-toxic goitre or thyrotoxicosis. Preparation of a more highly purified
antigen which is much too concentrated fails to detect weakly reacting serum, whereas use of a very dilute antigen solution results in the precipitate forming close to the antigen well where it may not be easily seen. A suitable dilution of antigen can readily be determined by testing doubling dilutions of the stock solution with strongly and weakly reacting (or diluted) specimens of serum. The plates are best examined against a dark background with bright, obliquely transmitted illumination.

Modification of the above technique by placing the antigen and antibody wells closer together resulted in the more rapid appearance of precipitate with most positive sera, but with this modification the antigen concentration was found to be a highly critical factor, and the modified test failed to detect antibody in approximately 25% of the serum specimens which gave a positive result with the method described above. Precipitin tests performed in cellulose acetate (Oxoid) film also gave more rapid results, but failed to detect antibody in a significant proportion of positive sera.

**THE APPEARANCES OF POSITIVE RESULTS**

**THE TYPICAL REACTION** Most positive serum specimens produce visible precipitation within one to three days of setting up the test. Typically the precipitate takes the form of a sharp white line, concave towards the antigen well, and gradually increasing in density and length (Fig. 2). This appearance is influenced by the relative concentrations of precipitin and thyroglobulin in the serum and antigen solution respectively. With serum containing a very high concentration of precipitin, the line of precipitate, originally narrow and sharply defined, becomes much wider by extending towards the antigen well, giving a 'fringe' effect (Fig. 3). With a weak serum, the line of precipitate gradually widens, and many actually change position, moving towards the serum well (Fig. 4):

![FIG. 2. The large wells contain four test sera, the small wells thyroid extract. The serum in the upper left well shows the typical appearance of a positive reaction. The serum in the upper right well shows a short, broad, fuzzy line of precipitate (× 1).](image)

![FIG. 3. The serum in the bottom well contains a strongly reacting serum, and the precipitate shows a fringe extending towards the antigen well (× 1).](image)

![FIG. 4. A weakly reacting serum (top well). The precipitate has been visible for several days, and has widened and moved towards the serum well (× 1).](image)
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FIG. 5. Two parallel lines of precipitate (× 1½). This and the following figures are photographs of agar plates which were set up to illustrate the various appearances described in the text. The sizes of the wells, and their arrangement in the agar are not necessarily those used in actual tests.

FIG. 6 A ‘clear line’ reaction produced by the serum in the bottom well (× 1¼).

FIG. 7. The lines of precipitate produced by the sera in the top and bottom wells are bent towards the antigen well by the serum in the right well, although this last serum does not give a precipitate (× 1½).

This last effect is seen particularly when the antigen solution contains a high concentration of thyroglobulin, and is attributable to solution of the precipitate in the gradually expanding zone of antigen excess.

MULTIPLE LINES OF PRECIPITATE The development of two parallel lines of precipitate (Fig. 5) is fairly common. This effect is influenced by the relative proportions of antigen and antibody, and probably does not indicate the presence of two antigen-antibody systems. Korngold and van Leeuwen (1959) have described a similar appearance in the reaction of antibody with highly purified protein antigen.

SHORT, FUZZY LINE Some specimens of serum react to give a short, broad, fuzzy zone of precipitation (Fig. 2) which usually takes five days or longer to appear. Changing the concentration of the antigen solution affects the position in the agar of this type of precipitate, but does not alter its short, broad, fuzzy appearance. Immunoelectrophoresis of serum giving this type of precipitate shows the serum factor to be a γ globulin. The same appearance is given when thyroglobulin solution, prepared by the method of Derrien, Michel, and Roche (1948), is used as the antigen in place of crude extract of thyroid tissue: extracts of tissue other than thyroid fail to react. We regard this type of reaction as indicative of a thyroid antibody, and quantitative precipitation tests in fluid medium indicated that it is present in large amounts in sera giving this type of precipitate. By performing such tests with an extract of the thyroid from a patient who had received a therapeutic dose of radioiodine shortly before death it was shown that the antigen precipitated by serum of this type is an iodine-rich protein. As stated above, such sera give a negative TA test, and are negative or have low titres in the tanned red cell agglutination test.
"CLEAR LINE" Very occasional specimens of serum give, in place of a precipitate, a line visible as a clearing of the slight turbidity of the agar (Fig. 6). In a previous report (Goudie, Anderson, and Gray, 1959) we have provided evidence that this is indicative of an antibody-thyroglobulin complex, and thus it is of diagnostic value.

COMBINATIONS Occasionally combinations of the above types of reaction are seen, e.g., a clear line together with a line of precipitation.

COMPLEX INTERACTIONS As already stated, the placing of serum wells close together in the agar may result in a strongly positive serum obscuring the reaction of a weakly positive serum in an adjacent cup. In other cases, and using a suitable arrangement of wells, we have observed bending of a line of precipitate in the region of an adjacent well containing serum which itself gave no precipitate (Fig. 7). In such cases, the second serum must be regarded as containing antibody to thyroglobulin in quite large amounts, but not enough to give a positive precipitin test.

Other interactions, of greater complexity, have been observed when sera are placed in closely adjacent wells. Some specimens of serum interact to give enhanced precipitation, and Fig. 8 shows enhancement of precipitation produced by the combined effect of two sera, one of which gives a 'clear line' the other a precipitate. Enhancement of this type has been described by Goudie, Anderson, and Gray (1959) and by Moore (1961). Even more complex interactions may be observed when sera giving the more unusual precipitin patterns are placed in wells in close juxtaposition (Fig. 9).

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