Evaluation of a solid phase radioimmunoassay (Austria-125) for the detection of hepatitis B antigen (Australia antigen) in two clinical materials

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SYNOPSIS A solid-phase radioimmunoassay (Austria-125) was compared to an immunoelectroosmophoresis method in detecting hepatitis-B antigen (HBAg). The radioimmunoassay detected HBAg in approximately 230 times higher dilutions than the immunoelectroosmophoresis method. In cases of acute hepatitis HBAg could be detected for about 70% longer after the onset of jaundice using radioimmunoassay compared with immunoelectroosmophoresis. Of 138 patients with acute hepatitis, who were HBAg-negative by immunoelectroosmophoresis, 23 (17%) were positive by radioimmunoassay. The specificity of the positive results with this test was investigated by neutralization tests with human antiserum to HBAg.

When testing blood for hepatitis B antigen (HBAg) (Australia antigen) it is desirable to use as sensitive and as rapid a method as possible. The method should also give a minimum of false positives. The methods currently used are Ouchterlony gel diffusion (Blumberg, Sutnick, and London, 1968; Prince, 1968), immunoelectroosmophoresis (Pesendorfer, Krassnitzky, and Wewalka, 1970; Gocke and Howe, 1970; Prince and Burke, 1970; Hansson and Johnsson, 1971), complement fixation (Shulman and Barker, 1970), immune adherence haemagglutination (Okochi, Mayumi, Hagino, and Saito, 1970; Mayumi, Okochi, and Nishioka, 1971; Hansson and Johnsson, 1973), passive haemagglutination inhibition (Vyas and Shulman, 1970), and certain variants of radioimmunoassay (Walsh, Yalow, and Berson, 1970; Aach, Grisham, and Parker, 1971; Hollinger, Vornad, and Dreeman, 1971; Coller, Millman, Halbherr, and Blumberg, 1971; Ling and Overby, 1972; Lewis and Coram, 1972; Hacker and Aach, 1973).

This comparative study includes immunoelectroosmophoresis, immune adherence and a solid phase radioimmunoassay developed by Abbott Laboratories (Austria-125), and is an extension of our previous evaluations of gel diffusion, immunoelectroosmophoresis, and immune adherence (Hansson and Johnsson, 1971 and 1973). The purpose of this investigation was to see how many more HBAg-positive patients could be detected in a clinical series of hepatitis cases using a radioimmunoassay compared with a good immunoelectroosmophoresis, and how long from the onset of jaundice HBAg could be detected by the two methods.

Materials and Methods

TEST SERA
Two-step dilutions of 10 sera containing HBAg of subtype ad and 11 sera of subtype ay were simultaneously tested by immunoelectroosmophoresis and radioimmunoassay. Six of the 10 subtype ad serum dilutions and seven out of the 11 subtype ay serum dilutions were also tested by immune adherence.

CLINICAL SAMPLES
Serum samples, consecutively drawn with an interval of about one week from 20 patients with HBAg-positive hepatitis, were tested. Sera from 138 cases, diagnosed clinically as acute hepatitis but HAg negative when tested with immunoelectroosmophoresis were also tested by immune adherence and radioimmunoassay. The serum samples were collected during the first two weeks after the onset of jaundice.
IMMUNOELECTROOSMOPHORESIS
This was performed as previously described (Hansson and Johnsson, 1971), using carefully selected agarose, and the test included staining of the electrophoresis plates with Coomassie brilliant blue. The antiserum was a goat antiserum made in our own laboratory. As a control for the method, reference hepatitis B antigen panel no. 2 was tested by our technique. This panel was provided by the Division of Biologics Standards, Bethesda, Maryland, and consisted of 61 serum samples. Thirty-one sera in the panel according to the Division of Biologics Standards should be positive for HBAg with immunoelectroosmophoresis or complement fixation and 41 with radioimmunoassay. The remaining 20 sera should be negative for HBAg. Our method detected the 31 sera expected to be positive with immunoelectroosmophoresis plus two expected to be positive only with radioimmunoassay.

IMMUNE ADHERENCE TECHNIQUE
These tests were done as described by Mayumi et al (1971) and the present authors (Hansson and Johnsson, 1973).

RADIOIMMUNOASSAY TECHNIQUE
In the Ausria-125 radioimmunoassay tests, 0-1 ml of the sera to be tested was added to polypropylene tubes coated with guinea pig hyperimmune HB serum, after which the tubes were incubated at room temperature overnight. Antigen, if present in the sera and which had not been bound to the tubes, was removed by rinsing with 5 \times 1 ml of 0-01 M Tris-HCl, pH 7.1, containing 0-02% sodium azide; 0-1 ml of 125I-labelled monospecific HB antibody (HBAb) from guinea pigs was added to each tube, which then were incubated at room temperature for a further 90 minutes. If HBAg was bound to the HBAb at the tube wall, this would mean that the radioactively labelled antibodies were bound to the antigen, thus forming an antibody-antigen-125I antibody "sandwich". Labelled antibodies, which had not bound, were removed by washing with 5 \times 2 ml of rinsing buffer and the radioactivity of the tubes was measured in a gamma counter (LKB-Wallac 80 000). One positive and at least 10 negative controls were included in each test series, and the mean and standard deviation of the negative controls were calculated. All tests giving count rates more than three standard deviation units (SD) above negative mean were repeated. If two consecutive tests of a serum had count rates greater than 5 SD above the negative mean, the result by radioimmunoassay for this serum was considered positive. As a specificity analysis, Ausria-positive sera were neutralized by a human HB antiserum: 0-1 ml of the antiserum was added to 0-1 ml of the serum to be tested. After incubation at +4°C for 24 hours the whole amount of serum mixture (0.2 ml) was tested with Ausria, together with a retest of the Ausria-positive serum. If the count rate of the neutralized serum had decreased to that of the negative controls, specificity for HBAg was considered to be confirmed.

RESULTS
COMPARATIVE ANTIGEN TITRATIONS
The mean antigen titre of the 10 sera with subtype ad was 240 times higher when tested with radioimmunoassay than with immunoelectroosmophoresis. When testing the 11 sera with subtype ay, radioimmunoassay gave 220 times higher titres than immunoelectroosmophoresis. The mean titre difference between the two methods was 230 and there was no significant difference between the ad and ay samples. Immune adherence gave, when six out of the 10 ad-sera and seven out of the 11 ay-sera were tested, 19 times higher antigen titres than the immunoelectroosmophoresis tests. The radioimmunoassay count rates of the highest dilutions positive with immunoelectroosmophoresis ranged from 64 to 109 SD above the mean of the negative controls.

CONSECUTIVELY DRAWN BLOOD SAMPLES FROM PATIENTS WITH HBAg-POSITIVE HEPATITIS
Serial bleedings from the 20 patients with acute, HBAg-positive hepatitis were tested with the immunoelectroosmophoresis and radioimmunoassay techniques. The time from the onset of jaundice, during which HBAg could be detected in 50% of the patients, was prolonged from 26 days with the former technique to 44 days with the latter, i.e., a prolongation of two-and-a-half weeks or 70% (figure). Each of the 20 patients turned negative with the radioimmunoassay test with count rates less than 3 SD above the mean of the negative controls. All sera drawn after the first negative one were also negative.

CASES OF ACUTE HEPATITIS
The results of the radioimmunoassay and immune adherence tests of the 138 cases of acute hepatitis, HBAg-negative with immunoelectroosmophoresis are shown in the table. Twenty-three sera (17%) were positive with radioimmunoassay, the count rates evenly distributed between 6 and 53 SD above the mean of the negative controls. Among sera drawn during the first and the second weeks after the onset of jaundice, the numbers positive by this test were 18 (18%) and five (13%) respectively. Of the 99 sera
Evaluation of a solid phase radioimmunoassay (Ausria-125)

Fig

Results in 20 cases of acute serum hepatitis.
Blood samples were serially drawn about once a week and tested with immunoelectro-osmophoresis and Ausria-125.

<table>
<thead>
<tr>
<th>Period after Onset of Jaundice</th>
<th>No. of Cases tested</th>
<th>Ausria-125</th>
<th>Immune Adherence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of Cases</td>
<td>%</td>
<td>No. Positive</td>
</tr>
<tr>
<td></td>
<td>&gt;5 SD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st week</td>
<td>99</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>2nd week</td>
<td>39</td>
<td>5</td>
<td>13</td>
</tr>
<tr>
<td>1st + 2nd weeks</td>
<td>138</td>
<td>23</td>
<td>17</td>
</tr>
</tbody>
</table>

Table Results in 138 cases of acute viral hepatitis negative with immunoelectroosmophoresis tested with Ausria-125 and immune adherence haemagglutination
The sera were drawn during the first and second weeks after the onset of jaundice

drawn the first week after the onset of jaundice, three (3%) were positive with immune adherence. In the radioimmunoassay tests these sera had count rates of 20 to 31 SD over the mean of the negative controls. Out of the 39 sera drawn during the second week after the onset of jaundice, no positive samples were found with immune adherence. In order to investigate the radioimmunoassay-positive samples for specificity to HBAg, neutralization tests with a human serum containing antibodies to HBAg were performed on 22 of the radioimmunoassay-positive sera. However, in one of the 23 cases no confirmatory test could be done, because the serum had been used up.

After neutralization and testing with radioimmunoassay, the count rates of the tests all were in the range of the mean of the negative controls ± 3 SD. This was considered to prove that the 22 radioimmunoassay-positive samples contained HBAg.

As a further control of the specificity analysis of the radioimmunoassay test in our study, the last positive sera by this test of the series of consecutively drawn blood samples, ranging from 5 to 38 SD above the mean of the negative controls, were neutralized by human HBAg-antiserum and tested with radioimmunoassay. After neutralization all samples were negative.

Discussion

Walsh et al (1970) introduced the first radioimmunoassay for HBAg. They used a system with 125I labelled antigen and a human antiserum. Separation of free labelled antigen from antibody-bound labelled antigen was done by paper chromatoelectro-
phoresis. Aach et al (1971) and Coller et al (1971) used the double-antibody radioimmunoassay, while Hollinger et al (1971) applied the double-antibody and solid-phase radioimmunoassay to the detection of HBAg. This report describes an evaluation study of Ausria-125, a solid-phase radioimmunoassay developed at Abbott Laboratories by Ling and Overby (1972). In our study, analysis of two-step dilutions of HBAg-positive sera showed that radioimmunoassay was almost 250 times more sensitive in recognizing HBAg than immunoelectroosmophoresis, which in our laboratory means about 5000 times GD (Hansson and Johnsson, 1971). The corresponding figures of Ling and Overby (1972) were 500 and 1000. In order to evaluate what these differences in titres would mean in terms of positives in clinical materials, two different studies were carried out. In the analysis of cases of acute hepatitis, the time from the onset of jaundice, during which HBAg could be detected, was prolonged approximately 70% when using radioimmunoassay compared with immunoelectroosmophoresis technique, ie, half of the patients were still HBAg-positive 44 days after the onset of jaundice. The corresponding prolongation with the immune-adherence method compared to immunoelectroosmophoresis was 14%, as reported in a previous investigation, where an equivalent material was tested (Hansson and Johnsson, 1973). Among the 138 cases of acute hepatitis negative by immune electroosmophoresis, 23 (17%) turned out to be HBAg positive with radioimmunoassay. This figure corresponds well with the study of Ling and Overby (1972) who in an examination of 245 hepatitis patients negative by immunoelectroosmophoresis found 41 (17%) to be Ausria-positive. However, no neutralization tests to verify the radioimmunoassay-positive results were done in their study. In our material, the 22 sera positive by this method which were tested for specificity to HBAg showed clear-cut neutralization of the positive radioimmunoassay results by antiserum against HBAg. When testing blood donor sera with Ausria, the incidence of false positives, ie, radioimmunoassay-positives which could not be neutralized by human HBAb, was about 1%. The count rates of these false positives ranged from 5 to 20 SD greater than the mean of the negative controls. Most of these false positives could, however, be neutralized by normal guinea-pig serum (Hansson and Johnsson, to be published). This could be due to antibodies against guinea-pig globulin in the sera of the donors. Prince, Brotman, Jass, and Ikram (1973) recently reported the same kind of false positives with Ausria. Thus, Ausria-positive results, especially low count rates, should be verified by neutralization tests with a human HBAg-antiserum. It is, however, quite clear that low values, eg, 5 to 7 SD greater than the negative control mean in the statistical evaluation applied in these studies, can be HBAg specific.

The difference in sensitivity between the immune adherence and immunoelectroosmophoresis techniques for the detection of HBAg was 20 times, while in a previous report (Hansson and Johnsson, 1973) the difference was found to be about 30 times. Three out of the 138 sera, from cases of acute hepatitis, negative with immunoelectroosmophoresis, were positive with immune adherence. In the previous report concerning immune adherence (Hansson and Johnsson, 1973) no positive sera were found among 240 cases of suspected immunoelectroosmophoresis-negative hepatitis. This material was, however, unselected and the diagnosis only preliminary.

As mentioned in the introduction, the purpose of this work was primarily to evaluate the sensitivity of a radioimmunoassay test compared with the immunoelectroosmophoresis technique. In a paper in preparation (Hansson, Johnsson, and Sundström) regarding testing of blood donors, the justification of using the more laborious and more expensive Ausria-125 will be discussed.

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