Enhanced Detection of Australia Antigen in Serum Hepatitis Patients by Discontinuous Counter-Immunoelectrophoresis

CRAIG WALLIS AND JOSEPH L. MELNICK

Department of Virology and Epidemiology, Baylor College of Medicine, Houston, Texas 77025

Received for publication 14 January 1971

The use of discontinuous counter-immunoelectrophoresis enhanced the reaction between Au/SH antigen and its antibody in agarose. The ionic strength of the Veronal buffer used in the agarose was 0.015 μ, whereas 0.075 μ Veronal (both pH 8.6) was used for anode and cathode buffers. Electroendosmosis is increased under such conditions. Au/SH antigen and antibody reacted to give sharp lines within 30 to 45 min as compared with conventional counter-immunoelectrophoresis which required 1 to 3 hr or longer.

Counter-immunoelectrophoresis (CIE) has recently been applied to detection of Australia (Au) antigen in patients with serum hepatitis (SH) (1-3). The results of the CIE test can be read in 1 to 3 hr in contrast to the 1 to 3 days required for the immunodiffusion test, and lower concentrations of antigen can be detected. Prince and Burke (3) used a buffer for bridging to the agarose slide that had a lower ionic strength than the buffer in the agarose. Osmosis is decreased under these conditions, and acidic proteins move more slowly. They also recommended cooling the agarose slides to 23 C and maintaining this temperature while voltage is applied. In contrast, we found an advantage in using enough current to warm the agarose to between 30 and 35 C as proteins then move more rapidly.

This led us to investigate discontinuous CIE (DCIE), which was done by reducing the ionic strength of the agarose slides as compared to that of the buffers used for bridging the slides to the anode and cathode chambers. Under these conditions, gamma globulin and Au/SH antigen could be made to move more rapidly towards each other in the DCIE test.

MATERIALS AND METHODS

Electrophoresis equipment. All experiments were carried out with a Helena electrophoresis apparatus (model 6902, Helena Laboratories, Beaumont, Texas). Each of the two 800-ml chambers was filled with 600 ml of Veronal buffer, pH 8.6, ionic strength 0.075 (diethylbarbituric acid, 1.84 g; sodium diethylbarbiturate, 10.3 g; distilled water to make a 1-liter volume). A constant potential of 200 was applied (20 v/cm), which under these conditions gave about 3 ma/cm. Slides were bridged with a built-in sponge system.

Standardization of apparatus. If other equipment is used, especially if the volume of buffer differs from that described above, the system must be standardized so that the voltage will not excessively disfigure the agarose on the slide that has been made with 0.015 μ buffer. The slides were 8.3 by 10.2 cm (Kodak lantern slide cover glasses), and each was coated with 15 ml of 1% agarose (SeaKem). To ensure optimal results, slides made with 0.015 μ buffer were tested at various currents for 1 hr. Slides with disfigured agarose (excess endosmosis) received excess current. The level of current that was selected was about 50% of the minimal level that causes disfigurement.

RESULTS

Effect of discontinuous electrophoresis on serum proteins. Normal human serum was electrophoresed in 1% agarose for 15 min, at constant pH 8.6, in Veronal buffers with ionic strengths varying from 0.0125 to 0.075 μ. In all instances, the buffer used for bridging and in the chambers was 0.075 μ. Results are shown in Fig. 1. As the ionic strength of the agarose decreased, the acidic proteins moved more rapidly to the anode and the globulins to the cathode. At very low ionic strengths (0.0125 to 0.025 μ), the effect of electroendosmosis was manifest in that there was some distortion of the pattern of the acidic proteins. The distortion is probably due to the low solubility of certain proteins at this low ionic strength of buffer.

Effect of DCIE on Au/SH antigen. In a typical experiment (Table 1), a human serum containing Au/SH antigen was tested against an
Au/SH antiserum obtained from a hemophiliac. Four units of antigen (1:2 dilution of the Au/SH serum) were run against undiluted antiserum of low antibody titer in 2-mm wells, located 1 cm apart on agarose slides made with Veronal buffer of the ionic strength indicated. In each instance, antigen was diluted in the same ionic strength buffer as that used to dissolve the agarose. After different time intervals the slides were examined for precipitin lines.

From the results of a number of such experiments, it became evident that DCIE is significantly more rapid than the equi-ionic strength buffering systems of continuous electrophoresis. When a 0.0125 to 0.025 \( \mu \) buffer was used in the agarose, the antigen-antibody precipitin line could already be detected in 10 to 20 min, as compared to 60 min for the higher buffer level (0.075 \( \mu \)). The observed precipitin line with DCIE was sharp and clearly seen at 30 min; this degree of definition was not achieved by continuous CIE even after 90 min.

**Titrations of Au/SH antigen.** A typical comparative titration, with the same reagents, on slides made with 0.015 and 0.075 \( \mu \) buffers is shown in Table 2. A serum containing Au/SH antigen was serially diluted, and replicate amounts were placed in the wells on one side of the slide; the opposing wells (1 cm apart) were filled with undiluted antiserum. At the intervals indicated, the slides were examined for precipitin lines. The DCIE slide with 0.015 \( \mu \) buffer was a more rapid system for detecting Au/SH antigen than was the CIE slide with 0.075 \( \mu \) buffer. In the DCIE test, Au/SH antigen was first detected after 10 min at a dilution as high as 1:27, whereas in the

**Table 1. Effect of discontinuous counter-immunoelectrophoresis on the reaction of Au/SH antigen and antibody**

<table>
<thead>
<tr>
<th>Ionic strength of agarose, pH 8.6(^a)</th>
<th>Precipitin reaction after times indicated(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 min</td>
</tr>
<tr>
<td>0.075</td>
<td>0</td>
</tr>
<tr>
<td>0.050</td>
<td>0</td>
</tr>
<tr>
<td>0.0375</td>
<td>0</td>
</tr>
<tr>
<td>0.025</td>
<td>0</td>
</tr>
<tr>
<td>0.0125</td>
<td>(\pm)</td>
</tr>
<tr>
<td>0.00625(^c)</td>
<td>++</td>
</tr>
</tbody>
</table>

\(\pm\) Two-millimeter wells 1 cm apart in 1% agarose (3 to 3.5 ml per slide (25 by 75 mm)) containing Veronal buffer, pH 8.6, at the ionic strengths indicated were filled with Au/SH antigen (1:2 dilution of serum containing 4 antigen units, based on a 2-hr run with 0.075 \( \mu \) buffer) or with undiluted Au/SH antiserum. Each electrode chamber contained 600 ml, 0.075 \( \mu \) Veronal buffer, and the agarose slides were bridged with the same buffer (0.075 \( \mu \)). At the intervals indicated, the precipitin lines were scored. The slides were then returned to the chamber, and the electrophoresis was continued. Normal human serum samples served as negative control antigens.

\(\pm\) Scoring of precipitin lines: 0 = no precipitin, to ++++ = very discrete, readily apparent precipitin line (maximum reaction).

\(\pm\) Agarose was disfigured by electroendosmosis.
CIE test, antigen first became detectable after 40 min and then only with undiluted serum. The titers after 60 to 90 min were nine times higher in the DCIE test.

Similar experiments conducted with antisera prepared in baboons and guinea pigs gave essentially the same results.

A series of serum samples obtained from clinical cases of serum hepatitis were titrated by the CIE method with a 0.075 µ strength buffer through the entire system and simultaneously by the DCIE method described above. The results of over 50 titrations indicated that at 45 min full titers were reached with almost all sera in the DCIE test, whereas in the CIE test the titers at 90 min were lower and the lines were fainter than they were in the DCIE test at 45 min. Five samples were negative after 90 min in the CIE test, whereas all five samples were positive in the DCIE test.

**DISCUSSION**

Sensitivity and speed of detection of Au/SH antigen in blood donors are of prime importance in reducing the number of cases of serum hepatitis that result from transfusion with infectious serum. The introduction of the CIE test was a marked improvement over the immunodiffusion test (1-3). The DCIE test offers further advantages in speed and sensitivity. The Au/SH antigen chosen to illustrate the sensitivity of the procedure in Table 2 was selected because of its relatively low titer by the standard procedure. In addition, because of the increased electroendosmosis in the DCIE test, the precipitin lines between Au/SH antigen and antibody form at some distance from the wells and thus are not confused with nonspecific precipitin lines which may occur close to wells in the CIE test.

It should be emphasized that serial dilutions of antigen or antibody must be made in the low strength ionic buffer for maximum effectiveness of DCIE. In practice we routinely run DCIE tests with 0.015-µ agarose (pH 8.6), 0.075-µ bridging and chamber buffers. All antigen and antiserum samples are diluted in 0.015 µ Veronal buffer. Apparently, the maximum effectiveness occurs when the ionic strength of the buffer in the agarose slides is about one-fifth of the ionic strength of the chamber buffers.

**ACKNOWLEDGMENTS**

This study was supported by research contract NIH-70-2231 from the National Heart and Lung Institute.

The excellent technical assistance of Anne Shirley is gratefully acknowledged.

**LITERATURE CITED**

