Counter immunoelectrophoresis as a rapid screening test for amoebic liver abscess

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SUMMARY Counter immunoelectrophoresis using cellulose acetate as the supporting medium was used as a rapid screening test for amoebic abscess. All the sera from 40 cases gave positive results. No false positives were obtained, but the results in intestinal amoebiasis were less reliable. An attempt was made to account for discrepancies in previous reports.

Although the use of counter immunoelectrophoresis (CIEP) in the diagnosis of amoebiasis has been well documented, reports of the reliability of the test have not been unanimous. However, it appears to offer the prospect of being a useful screening test for amoebic abscess, for which there is a need. Studies so far have been carried out using agar or agarose as the supporting medium, though Stamm, Phillips, and Warhurst (unpublished observations) carried out promising preliminary tests with cellulose acetate membrane. From the point of view of a rapid screening test, cellulose acetate provides a more rapid diffusion medium than gel; it is also more standardised and easier to handle.

The purpose of this paper is to evaluate the cellulose acetate CIEP test and to seek the causes of discrepancies in previously reported results.

Material and methods

SERA

Sera were available from 40 patients with confirmed amoebic liver abscess and from three with amoeboma of the colon. About half the patients were in the Hospital for Tropical Diseases, while other sera were referred to the department from outside sources. A systematic study of amoebic dysentery was not possible, but 13 patients with intestinal symptoms and a positive immunofluorescent test were included. As controls, sera were tested from 50 patients attending the hospital for tropical investigations who were thought not to have amoebiasis, and from 50 patients attending an antenatal clinic. The sera were not inactivated but stored at −20°C for a maximum period of six months.

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ANTIGEN

A lyophilised preparation of Entamoeba histolytica strain HK9 amoeba grown in TPS−1,9 was kindly supplied to the Amoebiasis Unit by Dr E M Proctor. The powder was dissolved in distilled water at a concentration of 13·2 mg/ml (10⁷ amoebae/ml) and freeze-thawed three times. The turbid solution was lyophilised in 0·1 ml aliquots for storage at −20°C. Each ampoule was reconstituted with 0·1 ml distilled water. The antigen was subsequently diluted for use, as described below. It could be stored in diluted form at −20°C for up to 12 days. CIEP has also been carried out with antigen prepared similarly from E. histolytica strain NIH200, which appears to give identical results.

COUNTER IMMUNOELECTROPHORESIS

CIEP was carried out on cellulose acetate membrane (Shandon CELAGRAM strip) using Tris-barbital-sodium-barbital buffer (pH 8·8) ionic strength 0·06 in a Gelman chamber. The paper was floated on the buffer and gently blotted. Using a Perspex template and rounded metal rod, two parallel rows of equally spaced indentations, 3 mm in diameter, were made on the paper strip, 80 mm and 93 mm from one end. A strip measuring 78 × 150 mm accommodated three indentations in each row. Each strip was supported by the two bridges in the tank and held in place with the aid of four magnets. The wells were nearer the cathode end. Equilibration was carried out at a constant voltage of 200 V, giving a current of 2 mA per strip, for 2 minutes. Then 4 µl of each test serum was placed in the anodal wells. It was found by experiment that a 1/10 dilution of the antigen in PBS (pH 7·6) was optimal. The diluted antigen was placed in each of the wells nearer the cathode. The current was then reconnected, and after 35 minutes...
at room temperature the strips were removed from the chamber and washed by shaking in a saline bath for 10 to 15 minutes. The strips were stained for 5 minutes in Nigrosin\textsuperscript{10} and washed for 1 minute in tap water. A positive result was indicated by the presence of one or more precipitin lines between antigen and serum wells when the wet strip was held against a bright light source.

The amoebiasis sera were all tested for comparison by immunofluorescence (IFAT) and cellulose acetate precipitation (CAP)\textsuperscript{10}

### Results

The results are summarised in Table 1. The specificity of CIEP was found to be high, as no false-positive results were obtained in the 100 sera taken from patients without evidence of amoebiasis. The sensitivity of the test in detecting liver abscess and amoeboma was also 100\%. However, the CIEP was found to be considerably less reliable in cases of invasive intestinal amoebiasis, in which only 61\% of cases which had given positive results by IFAT were positive by CIEP.

<table>
<thead>
<tr>
<th>Reference No.</th>
<th>Positivity</th>
<th>False positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abcess</td>
<td>Intestinal</td>
<td></td>
</tr>
<tr>
<td>1 (quoted by 5)</td>
<td>98</td>
<td>5.8</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>0.0</td>
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<tr>
<td>3</td>
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<td>0.0</td>
</tr>
<tr>
<td>6</td>
<td>89</td>
<td>1.7</td>
</tr>
<tr>
<td>7</td>
<td>88</td>
<td>3.1</td>
</tr>
<tr>
<td>8</td>
<td>84</td>
<td>3.6</td>
</tr>
</tbody>
</table>

CIEP was found to be less sensitive than IFAT and more sensitive than CAP. IFAT remains the first choice as a general screening test, partly for its sensitivity and partly because it is well suited to large-scale operations. But IFAT positivity needs to be checked by CAP. None of these tests has given reliable results with invasive intestinal amoebiasis, and CIEP is not recommended in this situation.

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### References

\textsuperscript{1}Sepulveda B, Aubanel M, Landa L, Velasques G. Avances en la técnica de contra-immunoelectroforesis para el estudio serológico de la amebiasis. Arch Invest Med (Mex) 1972; 3: Suppl. 2; 363-370.
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