Differential staining of neuronal and glial nuclei

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The histological differentiation of neuronal from glial nuclei may be useful in the identification of tumours and in the study of developing nervous tissues. The following method does this by staining the glial nucleus red and the neuronal nucleus green. It also stains other structures in the neural parenchyma. Formol-fixed tissues taken down to water and embedded in paraffin are used.

1. The sections (10μ) are placed for 10 minutes in 1% brilliant crystal scarlet (Ponceau R or Xylo-dine) solution made up with 1% acetic acid diluted in distilled water.
2. Washed in distilled water
3. Five minutes in 0-5% phosphotungstic acid
4. Washed briefly in distilled water
5. Stained 30 seconds to one minute in 1% methyl green (made with distilled water)
6. Washed in distilled water
7. Dehydrate, clear, and mount in DPX

The glial nucleus stains red, neuronal nucleus green, the nucleolus red, myelin red, axons green, red blood cells red, and connective tissue red.

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An immunological method for the detection and estimation of fetal haemoglobin—continued


Improved sensitivity of the electrophoresis method by tannic acid for detection of Australia antigen

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For detection of Australia antigen (Au-Ag) by counter-immunoelectroosmophoresis (CIEOP) staining the agarose gel plates with certain dyes has been claimed to improve the sensitivity (Combridge and Shaw, 1971). In our experience, however, a simpler and less time-consuming procedure is that of layering the gel plates with 1% freshly made tannic for 10 minutes (Alpert, Munroe, and Schur, 1970) after the routine CIEOP procedure (Das, Hopkins, Cash, and Cumming, 1971). This has resulted in a significantly increased sensitivity by improving visualization of precipitin lines.

Serial dilutions of Au-Ag containing serum and anti-Au (human origin) were set up in the test system using a 'chessboard' design. After the electrophoresis 'run' the gel plates were observed at an angle under direct light over a dark background. The results were scored as + for sharp precipitin line, ± for weak precipitation, and — for no reaction. Table I shows that the titre of Au-Ag against the neat antiserum was 1/4, and no significant improvement was noticed when the same plate was reviewed after overnight incubation. Tannic acid was now added and the plate read after 10 minutes: the titre was now 1/16. This improvement reflects an increased sensitivity of the system as a whole; thus, before tannic acid treatment, the total number of

### Table 1 Results on serial dilutions of Au-Ag and Anti-Au tested in a 'chessboard' fashion before and after tannic acid treatment

<table>
<thead>
<tr>
<th>Au-Ag Dilutions</th>
<th>Counter Immunoelectrophoresis Routine</th>
<th>Overnight Incubation</th>
<th>After Tannic Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anti-Au Dilutions</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Neat</td>
<td>1/4</td>
<td>Neat</td>
</tr>
<tr>
<td>Neat</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>1/4</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>1/16</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Total positive</td>
<td>14</td>
<td>14</td>
<td>21</td>
</tr>
</tbody>
</table>

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Technical methods

Positives in the 'chessboard' was 14, and after tannic acid treatment they were 21. This was further confirmed over a period of two weeks during which 212 selected specimens from patients, including drug addicts, with clotting disorders and hepatitis, as well as blood donors, some of them already known to be carriers of Au-Ag, were subjected to the procedure described above. Results show (Table II) that the number of positive samples were eight before and 12 after tannic acid treatment; the additional positive results consisted of three from the patient group and one from the blood donors. In view of these results the above procedure was extended to the 'routine' laboratory where every unit of blood donated was screened for the presence of Au-Ag.

Table II  Number of positive specimens amongst 212 selected samples before and after tannic acid treatment of CIEOP plates

<table>
<thead>
<tr>
<th>No. of Samples Tested</th>
<th>Blood</th>
<th>Patient</th>
<th>Laboratory</th>
<th>Total Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Donor</td>
<td>Staff</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before tannic acid</td>
<td>3</td>
<td>5</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>After tannic acid</td>
<td>4</td>
<td>8</td>
<td>0</td>
<td>12</td>
</tr>
</tbody>
</table>

During a period of five months a total of 19,423 donors were tested, 10 were found to be positive by routine procedure and no additional positive appeared, however, after tannic acid treatment of the CIEOP plates.

The mechanism by which tannic acid increases the sensitivity of the system is not clear, but from a practical point of view there is no doubt that it is capable of bringing out Au-Ag-antibody precipitin lines, especially amongst the patient's sera. The present procedure in this laboratory is to score the results immediately after the routine CIEOP 'run', the plates are washed, then treated with tannic acid, re-read and photographed immediately. All positive samples are re-investigated for identity reaction, if necessary, after concentration.

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References


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R Hopkins and P C Das

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