Letters to the Editor

Increased erythrocyte rigidity in chlorate poisoning

Methaemoglobin formation is a prominent feature of chlorate poisoning; available evidence suggests that this condition is neither relieved by methylene blue treatment nor does it determine the often fatal outcome of this intoxication. We recently observed a case of severe sodium chlorate poisoning with methaemoglobinemia unresponsive to methylene blue and ascorbic acid. We could also demonstrate that the red cell defect induced by methaemoglobin forming agents varied greatly in its responsiveness to methylene blue. After a short in vitro incubation with chlorate, the red cell defect was no longer reversed by the addition of methylene blue.

We have demonstrated that the incubation of red cells with chlorate induces not only the oxidation of haemoglobin, but also an increase in the membrane rigidity. Human red cells were incubated in chlorate buffer (110 mmol/l NaCl, 30 mmol/l NaClO3, 10 mmol/l glucose, 10 mmol/l Tris, pH 7-4) at 37°C for two hours, and the passage time through a polycarbonate membrane with 5 μm pores measured, using a modification of the technique described by Reid et al.

Filtration times for 0-5 ml of a 15% suspension of erythrocytes rose from 2-5 ± 0-26 s before incubation (mean ± SD) to approximately 100 s after two hours’ incubation.

This dramatic increase in rigidity will lead to impairment of microrcirculation and to the destruction of the red cells in the spleen. Both factors will contribute to the life-threatening sequelae of chlorate poisoning—that is, disseminated intravascular coagulation and renal failure. As these complications cannot be prevented by methylene blue treatment, an exchange transfusion should be performed very early in all cases of chlorate poisoning.

Is guinea-pig inoculation ever justified for the diagnosis of tuberculosis?

Tests involving animals are costly and undesirable. Fortunately improved culture methods for Mycobacterium tuberculosis as well as the eradication of Brucella abortus from cattle have very greatly reduced the need for guinea-pig inoculation in medical laboratory practice in the United Kingdom. Routine guinea-pig tests are no longer recommended in the British Isles but published data show that the same advice would be inappropriate in the Federal Republic of Germany and, by implication, in a number of other countries in the world, usually because less satisfactory culture methods are used.

To see if guinea-pig inoculation continues to play any useful role we have made a sample study of the records of two laboratories, one in a rural area (Shropshire) and the other in an urban area (South Yorkshire). In the rural laboratory the number of guinea-pig inoculations (GPI) has declined from 408 in 1971 to only 29 in 1982 and a comparable decline has been experienced in the urban laboratory. To identify which specimens remain most appropriately examined by animal inoculation as well as culture, the results have been recorded in Table 1 and further details of the materials giving positive results are shown in Table 2. In this series 56 (4%) of 1258 of samples showed evidence of tuberculosis. Approximately one in five of the positive samples was identified by GPI alone. A similar proportion were found positive by culture alone. There seems little doubt that in some instances splitting of the sample may have reduced the inoculum to a level too small to produce a positive result by one test or the other, but this can not be presumed to be the sole explanation especially as the guinea-pig test is well established as one of very high sensitivity.

Animal inoculation is very seldom justified, particularly so when multiple specimens can readily be obtained, as for

Table 1  Comparison of culture with guinea-pig inoculation

<table>
<thead>
<tr>
<th>Test result</th>
<th>Positive tests</th>
<th>Total tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture:</td>
<td>N + N + N +</td>
<td>N + N +</td>
</tr>
<tr>
<td>Rural (1972–1982)</td>
<td>993 22 1 5 3 30 1023</td>
<td></td>
</tr>
<tr>
<td>Urban (1976–1979)</td>
<td>210 13 6 7 26 236</td>
<td></td>
</tr>
<tr>
<td>1203 35 11 10 56 1259</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

N = negative result
+ = test for M tuberculosis positive

Table 2  Type of specimen giving positive culture or animal test result

<table>
<thead>
<tr>
<th>Origin of specimen</th>
<th>Culture + Animal +</th>
<th>Culture + Animal N/V</th>
<th>Culture N/V Animal +</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulmonary</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Urine</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pus/abscess</td>
<td>5</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Lymph node</td>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Pleural fluid</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Synovial fluid</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pericardial effusion</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cerebrospinal fluid</td>
<td>5</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Spinal/vertebral aspirate</td>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Tissue</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Peritoneal biopsy</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Liver biopsy</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Endometrium</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Mycobacterial culture</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Other/unknown</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
<td>10</td>
<td>11</td>
</tr>
</tbody>
</table>

+ = positive for M tuberculosis;
N/V = negative or void for M tuberculosis;
V = void (cultures contaminated or premature death of guinea-pig before completion of test).

References

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example urine or sputum. However when an invasive procedure is used on a patient and only scanty material obtained, such as spinal aspirate or endometrium, the inclusion of an animal test may be a valuable additional diagnostic aid. In particular, GPI of cerebrospinal fluid can be especially helpful when acid fast bacilli are not found on direct microscopy but the clinical observations, as well as the biochemical and cytological findings on the fluid, favour a diagnosis of tuberculous meningitis. A positive test in these circumstances confirms the diagnosis but when culture and animal tests are both negative this may allow the diagnosis to be reconsidered after at worst six weeks of chemotherapy.

The advantage of GPI in these few patients has, we believe, usually depended more on the scanty nature of the sample than on inadequate culture technique. Experience with numerous other samples examined by microscopy and culture alone (mainly sputum or urine) has shown close correlation between clinical and laboratory findings; after decontamination material has been cultured on Löwenstein-Jensen medium, pyruvate egg medium and into Kirscher enrichment medium and strains of M tuberculosis of both human and bovine type have been isolated satisfactorily by this means. In addition, with very small samples it is also worthwhile adding Kirscher enrichment medium to an apparently empty or almost empty specimen container or washing through the aspiration needle with this medium to permit growth of very scanty bacilli adherent to the container or needle surface.

By use of the tuberculin test, knowledge of the BCG immunisation history, and by selection of "at risk" patients (as for example those with a history of contact, or with a past history of pneumonia or pleurisy around the menarche) many inessential animal inoculations can be avoided. Culture of samples from more than one site—for example, urine and endometrium, may also assist in establishing the diagnosis without need to resort to guinea-pig inoculation.

The answer to the question, "Is guinea-pig inoculation ever justified for the diagnosis of tuberculosis?" must be, for the United Kingdom, that it is rarely justified but is nevertheless valuable for the selected "problem" situation. The decision to perform a guinea-pig test can usually be determined by discussion between microbiologist and clinician. For this purpose few animal houses are now needed and those that remain will provide facilities for other tests (especially virus, toxicity, and some pathogenicity tests) for which animal inoculations still remain essential.

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References

In vitro stability of cyclosporin A

We would like to report some new data on the stability of cyclosporin A (CyA) in human serum which is relevant to our article on this subject in the January edition of the Journal.1 Investigation at the Sandoz Research Laboratories indicated that when tritiated CyA was mixed with normal blood the percentage of whole blood CyA present in the plasma was dependent on the temperature at which the blood was stored prior to separation. When the blood was stored at 21°C before separation the plasma concentration of CyA was 50–70% of the plasma concentration when the blood was stored at 37°C before separation. The decrease in plasma CyA concentration on storage at room temperature (21°C) occurred within one hour of collection and was probably due to temperature dependent alterations in the cellular binding of CyA (Sandoz, unpublished data).

We have repeated in vitro temperature studies using the blood of patients treated with CyA. Measurements were by radioimmunoassay.2 Figures 1 and 2 show our results which confirm the Sandoz findings, using both plasma and serum samples. Our published work on in vitro stability of CyA in human serum was carried out on blood samples stored at room temperature for 2–3 hours before separation.1 The "toxic" and "therapeutic" ranges for serum CyA in our patients were based on samples handled in the same way. Using this method we have found a useful correlation between trough serum CyA concentrations and renal toxicity.3

The data shown in Figs. 1 & 2 suggest that plasma and serum concentrations of CyA in vivo are consistently higher than our measurements indicate. However, at present we still recommend that trough serum concentrations of CyA should be used to monitor patients but that the blood

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**Fig. 1** Decrease of plasma concentrations of cyclosporin A after storage of whole blood at room temperature (20°C) and 37°C. The initial plasma cyclosporin A concentration was 445 ng/ml.
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