this antiphagocytic action in guinea-pigs and rats is due to the large amounts of exopolysaccharide produced by these bacteria. Hence, the ability of mucoid bacteria to escape the normal alveolar macrophage-mediated clearance mechanisms of the lung appears to be due primarily to a 'barrier' effect of the slime. In addition, it has been shown that slime from mucoid type Ps. aeruginosa will inhibit phagocytic killing of some bacteria by rabbit polymorphonuclear leucocytes.

If it is possible to inhibit production of the exopolysaccharide or to destroy it after formation, normal clearance mechanisms may be able to operate. An alternative is to stimulate macrophage activity so that phagocytosis is able to take place in the presence of the slime. We think that these findings are important in the study of the mucoid form of Ps. aeruginosa in chronic respiratory disease. The results may partly explain the difficulty in clearing mucoid organisms from the lung and the increase in severity of cystic fibrosis associated with the presence of this organism.

While it has long been recognised that capsules can confer virulence on some bacteria, there are no reports of previous studies demonstrating this using alveolar macrophages and the mucoid form of Ps. aeruginosa. This may be explained by the difficulty that exists in working with the mucoid form of this organism, as spontaneous reversion to the non-mucoid form occurs in vitro. Selection of the colony from solid media is important, particularly when non-mucoid forms are present in the culture. Finally, when long-term in vitro exposure to macrophages is attempted, spontaneous reversion to non-mucoid forms allows phagocytosis to take place, giving the false impression that mucoid forms have been engulfed.

Correlation between two commercial streptococcal grouping kits

In recent years, a number of rapid streptococcal grouping kits have become commercially available. We have evaluated one, the Streptosec test (Organon Teknika), which has recently been marketed, by comparing it with another, the Streptec test (Wellcome Reagents), a method in current use in our laboratory that has been shown to be satisfactory and reliable.

Streptosec is a coagglutination method employing antibodies to the streptococci (groups A, B, C, and G) bound to Cowan type I streptococci. The four reagents are stained and dried on to wells on a white plastic tile.

Both tests were carried out according to the manufacturers' instructions as follows:

Streptec A heavy suspension of beta-haemolytic streptococci was made in 0-4 ml (400 μl) of extraction enzyme in a test tube. The suspension was incubated at 56°C for 1 hour and then centrifuged at 1200 g for 10 minutes. Using a Pasteur pipette, 1 drop of the clear supernatant was added to each of the six circles on the glass tile provided with the kit. One drop of each well-mixed streptococcal latex suspension (groups A, B, C, D, F, and G) was added to the appropriate circle on the tile and mixed with the bacterial extract with a wooden stick. The slide was rocked gently for up to 2 minutes and examined for agglutination.

References


Immunoperoxidase staining

Recently, my MLSOs have been anxious to develop immunoperoxidase methods, and I have discovered that they were ordering the reagent diaminobenzidine tetrahydrochloride, which was described by Mason et al. (J Clin Pathol 1980;33:609-16). This reagent, of course, has been mentioned in previous papers.

The formula of this reagent suggests that it is related to the carcinogenic hydrocarbons, some of which, particularly benzidine, are prohibited for laboratory use. There is no indication either in the papers or in the catalogues of reagents that these substances are carcinogenic.

In the most recent publication, Safety in Pathology Laboratories, benzidine is included among the carcinogens, and it is recommended that methods using these chemicals should be discontinued (HM
Letters to the Editors

(69) 57 and 74); it is also stated that when these reagents must be used the Chester Beattie Code of Practice should be rigidly followed.

I suggest that papers reporting studies in which these reagents are used should state that these are carcinogenic hydrocarbons. The chemical firms supplying these materials should indicate in their catalogues that these are carcinogenic hydrocarbons and that the Chester Beattie Code of Practice should be followed in their use.

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Reference


Historic laboratory apparatus

The photograph shows a paraffin lamp; the coil-like objects on top are hollow, cone-shaped receptacles of copper which fit closely into one another. This paraffin lamp was found in an old laboratory in Valetta, Malta, where, at the beginning of this century, much of the research work that led to the discovery of the Micrococcus melitensis (now called Brucella melitensis) in the blood of the goat took place under the aegis of the Commission for the Investigation of Mediterranean Fever.

Happily, this old laboratory has now been restored (25 June 1980) by the expert and devoted work of the eminent Maltese medical historian, Dr Paul Cassar, who would appreciate very much the views and suggestions of your readers as to what could possibly be the name and uses of this ancient DIY laboratory equipment.

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Stability of heparin in intravenous fluids

Administration of heparin by continuous intravenous infusions has been shown to be as effective as intermittent intravenous injection in the treatment of thromboembolism, but major bleeding is a more frequent complication with intermittent injection of heparin than with continuous infusion. Nevertheless, reports that heparin may be unstable in intravenous fluids continue to cast doubt on continuous infusion being the optimal method of administration. Jacobs et al. and Okuno and Nelson, using different assays of heparin, reported a loss of its potency in commonly used intravenous fluids, even within a few hours, and measurements with anti-Xa assay apparently showed erratic behaviour. These observations require confirmation since they carry significant implications regarding heparin administration in the management of venous thromboembolism.

The stability of heparin in intravenous fluids has been re-examined using four assays which measure most known aspects of heparin activity. The activated partial thromboplastin time method, the protamine sulphate titration technique, the metachromatic assay, and an anti-Xa assay were used, and details of these methods have been reported previously. Heparin (mucous) from Allen and Hanburys (Glaxo Australia) batch No. 251324 (5000 units/ml) was used in these studies; 5 ml of heparin was added to 1 litre glass bottles of saline, 5% dextrose (Abbott Laboratories), and Hartmann’s solution (Travenol Laboratories). It was found that the initial volume of fluid in these bottles ranged from 1001 to 1069 ml (± 1 ml). Thus the final concentration of heparin was 24 ± 1 units/ml. The bottles were stored at room temperature (22 ± 2°C), and samples were removed with a syringe as required.

As can be seen from the Table, no loss was detected in the potency of heparin in intravenous fluids for up to 24 hours by either the chemical or biological assays. Contrary to the suggestion of Okuno and Nelson, sensitivity of the method of assay was found to have no relation to the stability of heparin. In a separate series of experiments, the pH was monitored continuously with a strip-chart recorder connected to a Radiometer PHM61 pH meter

<table>
<thead>
<tr>
<th>Intravenous fluid</th>
<th>pH after heparin addition</th>
<th>Time after heparin addition (hours)</th>
<th>Anti-Xa assay</th>
<th>Protamine sulphate titration</th>
<th>Metachromatic assay</th>
<th>APTT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hartmann's solution</td>
<td>5.92</td>
<td>0</td>
<td>100</td>
<td>100</td>
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<tr>
<td></td>
<td>3</td>
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<td>100</td>
<td>104</td>
<td>102</td>
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<td></td>
<td>6</td>
<td>104</td>
<td>100</td>
<td>97</td>
<td>98</td>
<td></td>
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<tr>
<td></td>
<td>24</td>
<td>104</td>
<td>100</td>
<td>100</td>
<td>98</td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>5.40</td>
<td>0</td>
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<td>100</td>
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<td>102</td>
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<tr>
<td>5% Dextrose</td>
<td>5.95</td>
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<td>100</td>
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<td>100</td>
<td>100</td>
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</table>

Results are the mean of six measurements. The pH of the final mixture did not show a change with time.