A RAPID METHOD OF ASSAYING GENTAMICIN AND
KANAMYCIN CONCENTRATIONS IN SERUM

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The aminoglycoside group of antibiotics, notably kanamycin and gentamicin, are of considerable therapeutic value in the treatment of severe infections due to Gram-negative bacilli. However, they are all highly toxic, particularly to the eighth cranial nerve and to the kidney; it is therefore necessary to monitor the patient's serum for both peak and residual levels of antibiotic. This is particularly important in patients with impaired renal function since, because of poor excretion, the antibiotic may accumulate in the blood.

The assay methods most commonly used, i.e., tube dilution (Garrod and O'Grady, 1971) and diffusion (Mitchison and Spicer, 1949; Bennett et al., 1966), require overnight incubation. Even if optimal cultural conditions and heavy inocula are used, the results are available only after 5-8 hours (Dutton and Elmes, 1959). All the methods in current use suffer from the disadvantage that small inocula of sensitive bacteria must give rise to visible growth before reliable results can be obtained. When toxic blood levels are suspected, therefore, it is often necessary to reduce the dosage or stop therapy altogether for 12 hours, until estimates of the amount of drug in the blood are available. A more rapid method of estimating this is needed so that accurate adjustment of antibiotic dosage can be made. Because aminoglycosides are given every 8-12 hours, such a method should yield a result in 2-4 hours, well before the next dose is due.

Faine and Knight (1968) observed that, under suitable conditions, the addition of varying concentrations of an aminoglycoside affected the rate of hydrolysis of a monosaccharide, such as glucose, by an induced organism, i.e., one that has previously been grown on a medium in which the monosaccharide is the main source of energy. Although they showed that the rate of hydrolysis was inversely proportional to the concentration of antibiotic, it appeared from some of their results that the minimum concentration of drug detectable by the method was too high for it to be of clinical value. In addition, it is not possible to draw any firm conclusions from their work because of varying conditions used in their experiments. This study was a re-investigation of the principle described by Faine and Knight (1968) with a view to finding a rapid method of assaying gentamicin and kanamycin at concentrations likely to be found in the sera of patients receiving these drugs.

MATERIALS AND METHODS

Bacterial strains. Many clinical isolates were examined for their sensitivity to kanamycin and gentamicin and, from these, three organisms were selected. These were Staphylococcus

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aureus (no. 938), Enterobacter aerogenes (strain EAB) and Klebsiella aerogenes (strain Kl). A type-culture stain of Escherichia coli (NCTC no. 10418) was also included. The selected strains were maintained on nutrient-agar slopes stored at room temperature. The minimum inhibitory concentration (MIC) of gentamicin, kanamycin, tetracycline and ampicillin was determined for these organisms.

Titration based on growth inhibition

Antibiotic assay. The method used was a two-row doubling dilution technique in which the first row started with a serum dilution of 1 in 2 and the second row started at a dilution of 1 in 3. Dilutions of an antibiotic standard (20 µg per ml) were prepared in pooled human serum (PHS) that had previously been tested to exclude antibacterial activity. Thus, the first standard row started at 10.0 µg per ml and the second at 6.6 µg per ml. Subsequent dilutions were in nutrient broth (Southern Group Laboratories).

Determination of MIC. Serial dilutions in broth were made of a 20-µg-per-ml standard made up in nutrient broth. An inoculum of 10³–10⁴ viable organisms per tube (1 ml of medium per tube) was used for both antibiotic assays and MIC determinations. Inoculation of a blood-agar plate was included to check the purity of the culture and confirm the inoculum size. Titrations were incubated overnight in a 37°C water bath and the end-point was taken as the lowest drug concentration completely preventing growth.

Assays based on enzyme inhibition

Inducing medium. The monosaccharide-hydrolysing enzyme systems of the selected organisms were induced by overnight growth on 1% peptone water agar containing 1% glucose (GPA). This medium was prepared by adding 1 g New Zealand agar to 100 ml of 1% peptone water (Southern Group Laboratories). After autoclaving at 115°C for 10 min., the medium was cooled, and 5 ml of 25% glucose in 1% peptone water (Seitz filtered) was added aseptically before the plates were poured. The inducing medium was flooded with a log-phase peptone-water culture of the test organism and after overnight incubation the resulting growth was harvested and suspended in 2.5 ml of 1% glucose in peptone water (GPW). The suspension was refrigerated at 4°C before being used within 6 hours.

Test medium. GPW was dispensed aseptically in 25 ml volumes into sterile round-bottomed glass tubes (16 mm x 110 mm) and steamed for 30 min. After incubation at 37°C overnight to confirm sterility the tubes were stored at 4°C.

Method. On the basis of the results of preliminary work, described below, the following procedure was adopted to determine aminoglycoside concentrations in test sera.

1. Kanamycin or gentamicin controls were prepared in PHS at concentrations of 1, 2, 3, 4 and 6 µg per ml. These are stable at -20°C.
2. Patient's serum was diluted 1 in 2, 1 in 5 and 1 in 10 with PHS.
3. To duplicate tubes of GPW were added 0.3 ml of the five standard and three test dilutions. All the tubes were heated in a waterbath at 95°C for 5 min., cooled to 40°C, and 0.2 ml of the bacterial suspension was added to each.
4. All tubes were incubated in a water bath at 37°C for 3 hours with vigorous shaking.
5. The pH of each mixture was measured in a Pye 290 pH meter (or similar model). The values of pH against aminoglycoside concentration in controls were plotted on linear graph paper to give a standard curve.
6. The concentration of antibiotic in the test serum was calculated from this graph.

RESULTS

The MICs of the aminoglycosides and of ampicillin and tetracycline for the test organisms are shown in the table. These organisms, induced with glucose, were tested in triplicate in tubes of GPW which contained a final volume of 3.0 ml after antibiotic-free PHS and bacterial suspension had been added. The
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fall in pH over a 3-hour period was recorded, and the results are shown in fig. 1. It can be seen that all four strains produced a considerable drop in pH, and that the effect of *Staphylococcus aureus* was greater than that of the other organisms. Replicate results did not differ by more than 0.01 pH units.

**TABLE**

Minimum inhibitory concentrations of four antibiotics for the four bacterial strains tested

<table>
<thead>
<tr>
<th>Organism</th>
<th>Minimum inhibitory concentration (µg per ml) of</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>kanamycin</td>
</tr>
<tr>
<td><em>Enterobacter aerogenes</em></td>
<td>1.25</td>
</tr>
<tr>
<td>strain EAB</td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>1.25</td>
</tr>
<tr>
<td>strain 938</td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em> NCTC</td>
<td>2.50</td>
</tr>
<tr>
<td>no. 10418</td>
<td></td>
</tr>
<tr>
<td><em>Klebsiella aerogenes</em></td>
<td>2.50</td>
</tr>
<tr>
<td>strain K1</td>
<td></td>
</tr>
</tbody>
</table>

The effect of aminoglycosides in the serum

Early results showed that if organisms sensitive to kanamycin, *e.g., S. aureus* strain 938 with an MIC of 1.25 µg per ml, were used the initial pH of the medium was unchanged for 4 hours in the presence of all serum concentrations of the drug above 8 µg per ml. Therefore, samples of PHS containing 1, 2, 4, 6, and 8 µg per ml of kanamycin were prepared, and were tested by the technique described above, together with a control serum containing no antibiotic. Inhibition of fall in pH proportional to kanamycin concentration was observed (fig. 2). Similar results were obtained with gentamicin at concentrations of 2, 4, 6, and 8 µg per ml of PHS and the same indicator organism (fig. 3). Results obtained with other sensitive organisms showed that the sensitivity of the test
(i.e., pH drop per μg aminoglycoside per ml serum) was directly proportional to the degree of sensitivity of the test organism to the aminoglycoside.

On the basis of these results the procedure for a 3-hour assay of aminoglycoside in serum, as set out in the Materials and Methods section, was adopted. Each set of assays requires the construction of a reference graph relating pH to drug concentration, and fig. 4 shows typical examples of such curves for *S. aureus* with gentamicin and kanamycin.

**Comparison of results obtained by the two assay methods**

Samples of patients' sera were assayed by the growth-inhibition and the glucose-hydrolysis methods. Whenever possible both assays were carried out simultaneously, but if this was not convenient the sera were stored at −20°C until tested. Twenty sera containing either gentamicin or kanamycin were examined by both methods. Twelve of these showed an agreement of ±0.5 μg aminoglycoside per ml of serum and seven had an agreement of ±1.0 μg per ml serum. The results with the remaining specimen showed a difference of 1.1 μg per ml. Neither method of assay gave results consistently higher than the other.

**DISCUSSION**

Although many techniques have been developed for the assay of antimicrobial drugs in body fluids, the majority of them suffer from the disadvantage that they require sufficient incubation to allow bacterial growth, or its inhibition, to be determined macroscopically. With such techniques it is impossible to reduce the time taken for an antibiotic assay to less than 5–8 hours and most require overnight incubation.
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However, by the use of parameters of microbial activity other than visible growth, various methods of antibiotic assay may be developed that will provide results within 1–5 hours, and the technique described is an example. Other possibilities include measurement of substrate utilisation, inhibition of specific enzyme production as shown by Noone, Pattison and Samson (1971), and production of specific detectable metabolites.

The apparent discrepancies between the MIC results in the table and the enzyme-inhibitory concentrations of antibiotic in serum are due to the very heavy inoculum used in the test compared with the small inoculum used to determine the MIC. Moreover, the test shows the extent of enzyme inhibition after only 3 hours’ incubation and may therefore reflect only a decreased growth rate in the presence of antibiotic. It was noted that any further increase in inoculum size for the test produced a more rapid fall in pH but was accompanied by a loss of sensitivity.

It is common experience that patients given gentamicin or kanamycin are also receiving ampicillin, carbenicillin or cephaloridine. In the monosaccharide-hydrolysis assay method this should not constitute a major problem because the heating process will virtually ensure the inactivation of these labile drugs. The selection of aminoglycoside-sensitive indicator strains that are resistant to the second chemotherapeutic agent would provide an alternative method of assaying mixtures if the organism chosen is able to hydrolyse the monosaccharide employed.

The method described was also found to be suitable for streptomycin assay although no clinical samples were tested.

**Summary**

A simple method is described for the assay of gentamicin and kanamycin in human serum. It is a modification of the monosaccharide hydrolysis method of Faine and Knight (1968) and will estimate low concentrations of antibiotic accurately within 3 hours. The method gives results similar to those obtained by a double-row tube-dilution growth inhibition technique, is unaffected by the presence of penicillins or cephalosporins, and requires only standard laboratory equipment. Because the method gives readily reproducible results and is easy to perform, it should commend itself to all laboratories dealing with patients receiving aminoglycoside therapy.

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**REFERENCES**

