Technical method

Modification of the urease method for gentamicin assays

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Established methods for the assay of aminoglycoside antibiotics in serum are imperfect for routine use in that either they are slow to yield results or they are time consuming in terms of laboratory labour. A recently introduced method which depends on inhibition of bacterial urease activity by the antibiotic goes some way towards overcoming these limitations (Noone, Pattison, and Samson, 1971), though in our hands it appears to be less reliable than some other methods (Phillips, Smith, and Warren, 1973). In the present communication we describe a modification of this assay method which needs development but which is potentially capable of automation.

Principle

The urease method, as originally described by Noone *et al* (1971), is based on inhibition by gentamicin of the urease activity of a strain of *Proteus mirabilis*. Measurements are made of the rise in pH caused by the liberation of ammonia from a urea-containing medium, diminution of the pH rise being indicative of the presence of the antibiotic. The overriding limitation of this method lies in the variable buffering powers of the sera under test which necessitate manyfold replication of the assay and yield data which are difficult to interpret accurately. In our modification, adapted from Delaunois' (1962) method for the determination of serum cholinesterase, the difficulty is overcome by continuous titration with HCl of the ammonia liberated using an automatic titrator. The effect of the serum buffering power is thus nullified.

The following account describes preliminary investigations with a mock-up apparatus designed to test the validity of the method.

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Received for publication 1 November 1973.

Procedure

The reaction mixture was made up as follows:

- 1 2% urea medium (Noone *et al*, 1971), 10 ml
- 2 Overnight culture of *Proteus mirabilis* (strain obtained from Dr P. Noone) in Todd-Hewitt broth, 0.3 ml
- 3 Serum, with or without added gentamicin, 0.5 ml.

The mixture was incubated at 37 °C, stirred with a rotating magnet and titrated continuously with 1.5N HCl at pH 7.8 using a Radiometer pH Stat automatic titrator. Addition of HCl against time was plotted automatically.

Results

The additions of HCl required to maintain pH in the presence of sera containing gentamicin 0, 5, 10, and 20 µg/ml are shown in figure 1. Over the 80-min incubation period the rate of addition increased progressively, but this increase was reduced by the presence of the antibiotic. Inhibition by gentamicin of additions of HCl in duplicate experiments, measured over a 16-min period (64 to 80 min after the start of incubation), was proportional to log concentration (fig 2).

Fig 1 Titrimetric addition of HCl to four incubation mixtures containing sera of known gentamicin content. Figures in parentheses indicate gentamicin concentrations (µg/ml). The graphs have been artificially superimposed for convenience.

Fig 2 Inhibition of urease activity by gentamicin at different concentrations (log scale). See text for details.

In these experiments the reaction mixtures were titrated sequentially in order of increasing concentration of gentamicin, the culture of *Proteus* being kept refrigerated at 4°C until required for last-minute addition to the mixture. Sequential titrations in the absence of gentamicin showed progressive increases in additions of HCl (about 50% in four hours), indicating that urease activity of the bacterial culture increased progressively in spite of refrigeration. Inhibition by gentamicin may therefore be greater than is suggested by the illustrated data.

Comment

The experiments have revealed a valid and simple method for the assay of gentamicin. The method, however, requires considerable development before it can be used routinely. Because of progressive changes in the bacterial culture and because of the need to obtain results quickly, such a development must include the ability to titrate a number of reaction mixtures, containing standard and unknown sera, simultaneously. With such provision, the method would be readily susceptible to automation, particularly with digital rather than graphic recordings of the additions of titrant. The method can also be made much more sensitive by the use of reaction mixtures of smaller volume and of a pH electrode of smaller size than was available for this investigation. This should enable the assay to be done with small volumes of sera, which would make it suitable for paediatric use.

We are grateful to Professor H. Harris, University College, for the generous loan of the Radiometer equipment, and to Nicholas Laboratories Limited for financial support.

References

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