RECEPTOR SITE ASSAY OF STREPTOMYCIN AND DIHYDROSTREPTOMYCIN

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Streptomycin (SM) and dihydrostreptomycin (DHS) are estimated in dilute aqueous solution by the conventional agar diffusion method with *Bacillus subtilis* (ATCC 6633) as test organism[1]. The lower limit is about 0.25 μg/ml, depending on the presence of interfering substances. In milk or serum the standard deviation is more than 25 %.

We have previously investigated the competition between SM/DHS for the sites on their natural receptor, the 70S ribosomes from a sensitive bacteria. Typical isotope dilution curves suitable for an assay system were obtained, but the sensitivity was low due to the low specific activity of the tracer[2]. With a new tracer (Amersham, Bucks. England) the sensitivity of the receptor site assay became more than 50-times higher than that of the agar diffusion method. Fig. 1 shows that as little as 2.5 ng/ml can be estimated with a standard deviation of 0.5 ng. The detection limit, *i.e.* the lowest concentration that can be distinguished from zero with 95 % confidence, is 0.50 ng/ml. Fig. 1 further shows that the presence of biological fluids, milk and serum has no detectable influence on the results. This means that this assay is useful in the estimation of residual amounts of antibiotic after human or veterinary therapy.

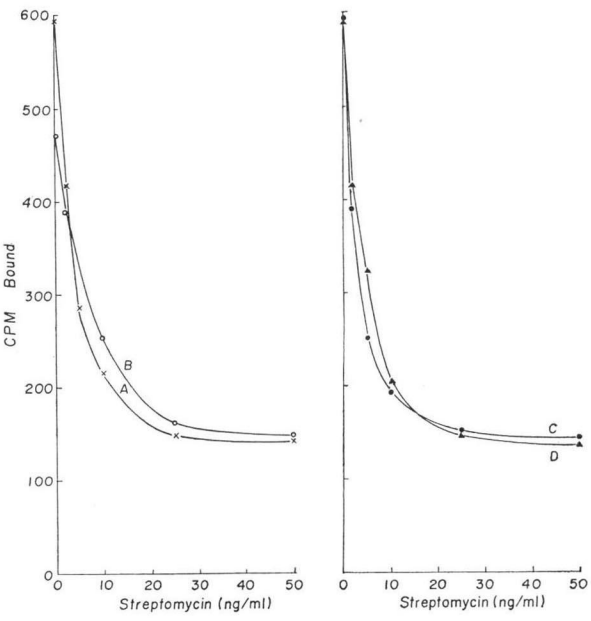
It should be pointed out that the assay is very specific. Other classes of antibiotics, penicillins, tetracyclines, bacitracins *etc.* do not interfere. Interference of other aminoglycosidic antibiotics with the assay described above cannot be excluded and is currently under investigation.

The receptor site assay system described here has other advantages than high sensitivity and specificity: Ribosomes can be prepared in quantity, and maintain their binding capacity for years when kept at −20°C or lyophilized. A 100-liter fermentation yields ribosomes to perform approximately 20,000 estimates. The results can be obtained in 2~3 hours as compared to 16~20 hours for the agar diffusion method.

We therefore feel that this approach to the analytical estimation of many drugs deserves much attention when the relevant receptor can be isolated, and when a tracer

Fig. 1. Isotop dilution curves.
A: In buffer solution with frozen ribosomes, B: In buffer solution with freeze-dried ribosomes, C: In milk with frozen ribosomes, and D: In human serum with frozen ribosomes.

This assay was performed in the following way: 500 μl test sample was mixed with 5 μl T-DHS (5 μg/ml), specific activity 3 Ci/mmol. The mixture was added to 25 μl ribosomes (0.92 A_{260} units) prepared and suspended in a buffer pH 7.8 as described by NIRENBERG[3]. The reaction mixture was incubated at 37°C for 30 minutes. The ribosome-T-DHS complex was isolated by Millipore filtration (0.45 μ), and the filters washed three times with 2 ml of the buffer. The bound radioactivity was measured by liquid scintillation in a standard toluene scintillation liquid. The DHS/SM containing milk samples were added 1/3 volume chloroform, shaken and centrifuged (30 minutes at 4,000 rpm) before estimation of antibiotic in the aqueous phase. Recovery was more than 95 %.



of sufficiently high specific activity can be prepared.

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