The effect of bilirubin on the assay of gentamicin

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SYNOPSIS An experiment was set up to examine the effect of 'jaundiced' serum on the estimation of gentamicin levels in serum. Varying amounts of gentamicin were assayed in the presence of a range of bilirubin concentrations and compared with assayed levels in normal serum.

It has been reported by Stratford in a personal communication that 'jaundiced' serum inhibits gentamicin activity. When known amounts of gentamicin were added to serum from one jaundiced patient (bilirubin 23 mg/100 ml) there was a definite discrepancy between expected and observed values. The jaundiced serum clearly masked the gentamicin activity. This was shown to be due to inhibition, rather than inactivation, for sufficient dilution with normal serum permitted the gentamicin activity to be fully expressed. In contrast, serum from a patient with a serum bilirubin level of 8 mg/100 ml failed to antagonize any added gentamicin (Sabath, Casey, Ruch, Stumpf, and Finland, 1970). Secondly, agar-diffusion assay of gentamicin is not valid for serum from jaundiced patients. Extreme masking was observed and the idea was put forward that inhibition was due to formation of an inactive complex of gentamicin with bile acids (Stratford, 1970).

Materials

ASSAY ORGANISM

Spore suspension of *B. subtilis* ATCC 6633.

ASSAY MEDIUM

Baltimore Biological Laboratories streptomycin assay agar. The spore suspension was added to melted agar 50-55°C and a single seeded layer was poured into a large glass plate and permitted to harden. Antibiotic standards and bilirubin concentrations were added to punched-out wells in the agar.

POOLED SERA

Sterile, antibiotic-free pooled plasma with normal level of bilirubin was used as a diluent.

ANTIBIOTIC STANDARDS

The following concentrations of gentamicin were prepared: 0, 2, 10, 20, 100, and 200 µg/ml. These were used, diluted in equal quantities, with pooled sera, and with the range of bilirubin concentrations. Final concentrations were 0, 1, 5, 10, 50, and 100 µg/ml.

BILIRUBIN CONCENTRATIONS

These were made from highly purified bilirubin in a human albumin base (freeze dried). This was reconstituted with distilled water to give a range of concentrations 1, 2, 4, 8, 12, 14, 20, and 40 mg bilirubin/100 ml. When added to equal quantities of gentamicin standards the following range (comparable to blood levels) was obtained: 0.5, 1, 2, 4, 6, 8, 10, and 20 mg/100 ml.

Method

The range of gentamicin standards was added to equal quantities of pooled sera to give control zone sizes with a normal bilirubin level (0.2-0.8 mg/100 ml).

The range of gentamicin standards was also added to equal quantities of each bilirubin concentration to detect any inhibitions produced by 'jaundiced serum'.

The samples were all assayed in triplicate and incubated at 37°C for 18 hours. The resulting zone sizes were measured with a caliper to the nearest 0.5 mm. The results were tabulated and compared.

Results

No difference in zone size was noticed between gentamicin standards diluted in the varying bilirubin concentrations and those diluted in pooled sera of normal bilirubin level (see table).

Bilirubin Concentration in Normal Serum (mg/100 ml)	Average of Zone Sizes (mm)					
	Gentamicin Standards (μ g/ml)					
	0	1	5	10	50	100
0.5	Insignificant	18.5	21.0	22.0	23.5	24.5
1	Zone	18.0	20.5	22.0	24.0	24.0
2	Neither	18.5	21.5	22.0	24.0	24.0
4	bilirubin nor	18.5	20.5	22.0	23.5	24.5
6	pooled sera	18.5	20.5	22.0	23.5	24.5
1	had any	18.0	21.0	22.0	23.5	24.0
10	bactericidal	18.5	20.5	22.0	23.5	24.0
20	properties	18.5	20.5	22.0	23.5	24.5
	demonstrable					
Diluent of pooled sera control zone size		18.5	20.5	22.0	23.5	24.5

Table Zone sizes of standard gentamicin concentrations with added increasing concentrations of bilirubin compared with same standards with normal serum

The results show that bilirubin levels of up to 20 mg/100 ml do not interfere with biological assays by the agar diffusion technique, and levels above 20 mg/100 ml are so uncommon that they are not a major problem.

References

- Sabath, L. D., Casey, J. I., Ruch, P. A., Stumpf, L. L., and Finland, M. (1971). Rapid microassay for circulating nephrotoxic antibiotics. *Antimicrob. Agents and Chemother.*, **10** (1970), 83-90.
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