Alleged Effect of Bile Constituents on Gentamicin Assays

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It has been claimed that gentamicin assays on the serum of jaundiced patients give falsely low levels and suggested that the antibiotic in such sera is bound to bile acids and thus is partly inactivated. No evidence of such inactivation has been obtained (i) in the serum of jaundiced patients treated with gentamicin, (ii) in sera with high bilirubin contents to which gentamicin was added, or (iii) when bile or bile acids were added with gentamicin to normal serum.

Sabath et al. (1) in a paper on the assay of nephrotoxic antibiotics reported an observation by B. C. Stratford that the agar diffusion method was not valid for serum from jaundiced patients. He assayed a serum that gave no zone of inhibition by the paper disk method, although he demonstrated the exceptionally high level of 80 \( \mu \)g of gentamicin per ml by the broth dilution method. No further details are given. Sabath et al. (1) reported that "assays of sera from a number of jaundiced patients fell within the broad range seen with serum from non-jaundiced patients." However, when gentamicin was added to one serum with a bilirubin of 23 mg/100 ml, there was a definite discrepancy between the expected and observed values. No such discrepancy was seen when gentamicin was added to one serum with a bilirubin of 8 mg/100 ml. Sabath et al. suggest that the loss of activity is due to the formation of an inactive complex of gentamicin with bile acids and that this interferes with the assay of the drug by diffusion, although this binding is reversible by dilution. They also suggest that in severely jaundiced patients, aminoglycoside antibiotics may be circulating mainly as inactive complexes and that their use in such patients should be reevaluated.

The paper by Sabath et al. was seen by one of us (P.M.W.) while a small investigation on gentamicin serum levels in neonates was in progress in this hospital. As a considerable proportion of these sera were visibly jaundiced and some of the levels had been rather low, we questioned the validity of the assays. The next nine sera from such infants were therefore assayed by the vertical diffusion method, both undiluted and diluted 1:10 by adding 1 drop to 9 drops of pooled human serum. In no instance was there any discrepancy between the results. Another explanation for the low levels was discovered later, namely the incomplete emptying of syringes containing the small volumes being given to neonates. After this, assays were done on two jaundiced adults receiving gentamicin, and again there was no discrepancy between the results with the serum diluted. The visit of D.R.M. to this department made a more thorough investigation of this problem possible.

MATERIALS AND METHODS

Blood was collected from 17 patients whose serum bilirubin ranged from 2.4 to 24 mg/100 ml; 13 were >5.5, and 6 were >10 mg/100 ml, including one each of 17, 20, and 24 mg/ml. Causes of jaundice included obstruction, cirrhosis, infectious hepatitis, chronic active hepatitis, and drug-induced and hemolytic jaundice. Blood was also obtained from two patients under treatment with gentamicin and from five who had received a single intramuscular injection of 80 mg 1 h before the blood was collected. All specimens were protected from the light, and after separation the serum was kept frozen until tested.

Assays were done by the agar cup method. Oxoid diagnostic sensitivity test agar, with a pH of 7.4, was preseeded with Klebsiella edwardsii var. atlantae NCTC 10896, and 9-ml volumes were poured into 85-mm plastic petri dishes. Holes were cut with a metal cutter with a diameter of 7 mm. Gentamicin standards of 10, 2, and 0.4 \( \mu \)g/ml in pooled human serum were used for assays of serum from patients receiving the antibiotic, and the patient’s serum was diluted 1:5 in pooled serum. Standards containing 100 and 10 \( \mu \)g/ml in pooled serum were used when the antibiotic was added to jaundiced serum in vitro. The same pooled serum with the pH corrected to 7.3 was used throughout the investigation. All assays were done in triplicate and incubated overnight at 37 C. Zone diameters were measured to 0.1 mm by using calipers with a vernier scale.

Broth dilution assays were done in 1-ml volumes of Oxoid nutrient broth no. 2 with 20% horse serum added, preincubated to contain about 10\(^7\) organisms...
per ml. Two sets of doubled dilutions were made, one starting from 1:4 and one from 1:6 with the serum and from 2 and 1.5 μg of gentamicin per ml for the standards. Tubes were incubated overnight at 37 C, and the end point was taken as the last tube showing visible growth.

RESULTS

Addition of gentamicin to jaundiced serum.
Gentamicin was added to serum from 17 patients having serum bilirubins ranging from 2.4 to 24 mg/100 ml (the majority being between 6 and 11 mg/100 ml) to give 100 and 10 μg/ml. These were then placed in cups cut in assay plates seeded with Klebsiella, and the resulting inhibition zones were compared with those produced by the same concentrations of gentamicin in pooled human serum. The sera were then incubated overnight and assayed again. The results are summarized in Table 1; there was a difference of >1 mm in zone diameter in only eight of sixty-six assays, and in five of these the jaundiced serum gave the larger zones.

The same amounts of gentamicin were also added to seven samples of serum from patients who had received the drug, and in every case the resulting zones were larger than those given by the control sera.

Assays on serum from patients receiving gentamicin.
Sera from two patients receiving treatment with gentamicin and from five who had received a single injection of 80 mg 1 h before the blood was collected were assayed both undiluted and diluted 1:5 in normal human serum by the plate diffusion method.

The results are given in Table 2 and show no discrepancy between diluted and undiluted sera. Five of the specimens were also assayed by the broth dilution method, using the Oxford Staphylococcus aureus in one and the Klebsiella in the remainder. The results are given in Table 2, and we do not consider the differences between these and those obtained by the diffusion method to be significant.

Addition of bile salts to serum and medium.
An attempt was made to demonstrate a reaction between gentamicin and bile salts. The antibiotic was added to reconstituted dehydrated ox bile and fresh human bile to give 100 and 10 μg/ml. Similar concentrations were also added to both normal human serum and water containing the sodium salt of taurodeoxycholic acid (100 mg/100 ml). Several dilutions, ranging from 1 to 50% of both biles, were tested. The solutions were placed in cups in assay plates, and the resulting zones were compared with those produced by control solutions in water or serum. There was always <1 mm difference in the diameter of the zones. The addition of

### Table 1. Differences between the diameter of zones produced by gentamicin when added to jaundiced serum and normal human serum*

<table>
<thead>
<tr>
<th>Jaundiced serum compared to normal human serum</th>
<th>Difference (mm) between the diameter of zones</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100 μg/ml</td>
</tr>
<tr>
<td>Maximum increase</td>
<td>1.5</td>
</tr>
<tr>
<td>Maximum decrease</td>
<td>1.1</td>
</tr>
<tr>
<td>Mean increase</td>
<td>0.51 (18 assays)</td>
</tr>
<tr>
<td>Mean decrease</td>
<td>0.37 (12 assays)</td>
</tr>
<tr>
<td>(3 identical)</td>
<td></td>
</tr>
<tr>
<td>Mean diameter of standards</td>
<td>25.1</td>
</tr>
</tbody>
</table>

* Seventeen sera were assayed immediately after the drug was added and 16 of them again after overnight incubation; there was no significant difference between these results.

* Quantity of gentamicin added.
gentamicin often produced a visible deposit; this disappeared if the pH was raised to 7.3 and did not affect the assay.

It has been suggested that false results obtained with the diffusion tests in Stratford's patient may be caused by the bile products interfering with the diffusion of gentamicin. This clearly has not happened with the jaundiced sera tested here. One percent ox bile was also added to assay medium, and the zones produced on this and on control plates were compared. There was no significant difference when the test organism was Escherichia coli or Klebsiella, but with both S. aureus and B. subtilis, zones were appreciably larger on plates containing bile, presumably because this slowed the rate of growth of these organisms.

DISCUSSION

In the present work, no evidence has been found to suggest that jaundiced serum inactivates gentamicin. It must be accepted that the addition of the drug in vitro does not necessarily correspond to conditions in the body, and it is of interest to note that the one instance in which Sabath et al. (1) saw any masking activity was when gentamicin was added to the serum of a patient receiving the drug. Nevertheless, it may be asked why this patient should have the expected serum level with apparently no masking of activity in the first instance, but a considerable loss when further gentamicin was added. No explanation is offered for this, nor for the fact that when 5 µg of gentamicin per ml was added to this serum, already containing 3.4 µg/ml, the assay showed 6.7 µg/ml, but when 25 µg/ml was added it was only 5.2 µg/ml. Because the results of adding 50 and 100 µg/ml to this serum to another with a bilirubin of 8 mg/100 ml and to the normal control were all given as >10 µg/ml, it is not possible to tell what loss of activity there was with even higher levels. These authors found this inactivation to be reversible by dilution (as did Stratford in the original observation) and suggest that it is due to the formation of an inactive complex of gentamicin with bile acids, although no evidence is given to support this suggestion.

The present work shows no evidence that gentamicin is inactivated by jaundiced serum, either in vivo or when added to such serum in vitro. Likewise it was not inactivated when bile acids were added to normal serum. Although the tube dilution assays usually gave higher results than the diffusion method, the differences are such as can be accounted for by the recognized inaccuracy of the dilution method. There was clearly something unusual about the two sera referred to by Sabath et al. (1), but there is no evidence that the apparent inactivation of gentamicin was due to bile acids; even assuming that it was, it is clear from the present work that this cannot be a common phenomena, and indeed it was only observed in one of a number of jaundiced patients referred to by Sabath et al. (1). A more likely explanation for "some poor results noted in the use of gentamicin for infections of the biliary tree" referred to by these authors might be the failure of this drug to pass into the bile in adequate concentrations.

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LITERATURE CITED