of cefazolin immediately post-infusion were 250 mg/L after a 2-g infusion, 100 mg/L after a 1-g infusion. The increase in apparent plasma creatinine declined more slowly with time than did cefoxitin. This is likely due to the higher concentrations of creatinine in plasma and to the additional interference by deacetylcephalothin. Cefoxitin is essentially unmetabolized.

Two 24-h urine specimens, one collected during steady state and the other at least five biological half-lives after the drug was discontinued, were collected from each of two patients receiving cefoxitin and two receiving cefazolin. Urinary creatinine concentrations and their differences were calculated, as well as the creatinine clearances and their differences (Table 1). We calculated creatinine clearances when the patients were on cephalosporin, using plasma creatinine values obtained when cephalosporin concentrations were lowest.

In three patients, there were increases in apparent urinary creatinine, and the average increase in clearance was 18 mL/min. Because cefazolin and cefoxitin are eliminated primarily by renal tubular secretion, whereas creatinine is lost mainly by glomerular filtration, the status of a patient's glomerular function relative to tubular function may affect the degree of interference with respect to urine. This may explain the failure of one patient to demonstrate such interference. Other factors could be an incomplete 24-h urine collection or a change in renal function on the collection day.

Overall, creatinine clearance is not a clinically reliable index of glomerular filtration for patients being treated with cephalosporins, because the values for apparent plasma creatinine vary during a dosing interval as cephalosporin concentrations fluctuate. If the clearance is calculated with the plasma creatinine value obtained when cephalosporin values are minimal, false increases in creatinine clearance are observed. This confirms the results of Durham et al. with cefoxitin (4), but contradicts those of Rankin et al. with cefalothin (3). The latter authors may have minimized increases in the creatinine clearance by fortuitously using plasma creatinine values at the highest cephalosporin concentrations.

In summary, cephalosporins falsely increase apparent creatinine in plasma and in urine, both in vitro and in vivo when a direct Jaffé reaction is used. The significance of the in vivo interference depends on the clinician's interpretation of results; therefore, to promote awareness and management of potential interferences, we developed the following guidelines for interpretation of plasma creatinine and creatinine clearance results:

1. In patients receiving cefazolin and cefoxitin, use caution in interpreting creatinine values from patients on maximal doses or patients with renal dysfunction.
2. Draw blood for the measurement of creatinine values when cephalosporin concentrations are low, to minimize interference.
3. Verify creatinine clearance calculations with an estimate from a nomogram such as that of Siersback-Nelson. The plasma creatinine concentration measured when cephalosporin is lowest should be used.
4. When doses of drugs are calculated on the basis of a creatinine clearance value obtained during concomitant cephalosporin administration, monitor the serum drug concentrations where possible.

References
then some of the peroxide produced in the enzyme reactor would be lost, and the amount of color formed in the indicator reaction would decrease. Longer enzyme loops contain proportionally more catalase, which could account for the plateau in the plot of assay sensitivity vs IME reactor length.

To test this hypothesis, we added sodium azide, a known inhibitor of catalase (6), to the buffer, and repeated the study of assay sensitivity vs reactor length. In addition, we did several other experiments designed to determine the effect of azide on the system. The results are given below.

Glucose oxidase (GO) was immobilized on nylon tubing by a method adapted from procedures described by Morris et al. (7) and Sundaram et al. (8). Four 25-cm enzyme coils with almost identical activities were prepared and used, alone or in tandem, for this study. Sodium azide solutions were prepared in a pH 6.85 phosphate buffer and were stable for several weeks at room temperature. A miniature continuous-flow system, developed in this laboratory, was used in all the experiments.

Addition of azide improved the linearity of the curves dramatically (Figure 1, curves B and C). To prove that the effect of azide was to inhibit catalase, we did additional experiments.

First, the immobilized GO coil was removed from the continuous-flow manifold, and standard peroxide solutions were assayed. The instrumental response was reduced by only 3% at an azide concentration of 100 mmol/L. Thus, the indicator reaction was not significantly inhibited by azide. Next, a 25-cm GO-IME reactor (assumed to contain co-immobilized catalase) was added to the manifold. The peroxide standards now gave a much smaller instrumental response than before, presumably owing to reaction with co-immobilized catalase. As azide was added in increasing amounts to the buffer, the loss of peroxide decreased as shown in Figure 2, curve A. At a concentration of 100 mmol/L azide, the instrumental response was 98% of that with no catalase present (the GO coil removed). Thus, the presence of co-immobilized catalase in the IME reactor was confirmed.

Curve B of Figure 2 shows the combined effect of azide on glucose oxidase and catalase. Glucose replaced peroxide as the sample in these experiments. The decrease in response when the azide concentration exceeded ~5 mmol/L might be due to inhibition of glucose oxidase, but we did not investigate the nature of this inhibition.

Three other facts deserve mention. First, the effect of azide on glucose oxidase and catalase was completely reversible. Enhanced sensitivity was observed in less than 5 min after azide was added, while the sensitivity returned to "normal" after the azide was removed. Second, we assayed control sera, using a dialyzer and a 25-cm GO-IME reactor. The results showed that there were no significant differences between the values obtained with and without azide, except that the assay sensitivity was increased by a factor of 1.7 with added azide. Third, the effect of azide would be less apparent if the analytical and indicator reactions were not separated, because peroxidase could compete favorably with catalase for the peroxide produced in the GO-catalyzed reaction.

Thus we recommended the addition of azide to the buffer reagent in continuous-flow determinations of glucose by use of immobilized glucose oxidase-colorimetric methods. The optimum azide concentration actually in contact with the immobilized glucose oxidase (considering dilution in the manifold) is 1.67 mmol/L. With the addition of azide and the use of a 75-cm GO coil, the assay sensitivity was increased by a factor of 4.8 over that observed with no azide and a 25-cm coil.

References

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Quantitation of Serum IgM by Graded Hemaggulination Inhibition

To the Editor:

Graded hemaggulination inhibition (GHI), hitherto used for determination of IgG and IgA (1, 2), has now been elaborated for the determination of IgM in the serum. GHI is a quantitative hemaggulination method in which the inner ring diameter of sedimented erythrocytes is inversely related to the concentration of antigen in question (Figure 1).

For determination of IgM in human sera, the only reagents needed are: sheep erythrocytes, coated with purified IgM with the aid of gluteraldehyde, according to Avrameas et al. (3), and anti-IgM, monospecific for the M chain. Serum with known concentration of IgM (obtained from WHO as "the international reference preparation and British standard for human serum immunoglobulins G, A, and M") is used as the standard. The reaction is arranged to take place in polystyrene spherical bowls 23 mm wide and 18 mm high, all made.

Fig. 1. Assay sensitivity vs enzyme coil length. Curves at various azide concentrations. Curve A, 0.0 mmol/L azide; curve B, 1.0 mmol/L azide; curve C, 10.0 mmol/L azide.

Fig. 2. Relative sensitivity vs log azide concentration with hydrogen peroxide (curve A) and with glucose (curve B) as the sample.