Quantification of Monoclonal Human IgM

To the Editor:

Pontet et al. (1) recently argued that, in contrast to polyclonal IgM, accurate data on concentrations of monoclonal IgM in different patients is difficult to obtain because of the dependence of the results on the technique. This statement is at variance with previously published work of De Brujin and Klein (2, 3) who came to different conclusions. According to our findings, confirmed by Out et al. (4), the facts are as follows:

- Monoclonal as well as polyclonal human IgM from different individuals may both show large discrepancies in the results by radial immunodiffusion (RID), in the sense that the same quantity of different 19S IgM may give precipitation circles of widely different diameter.

- These discrepancies are caused by interaction with the diffusion medium (5) and therefore do not show up in nephelometric and turbidimetric methods, where polyethylene glycol does not seem to interfere (5).

- Reduction to 7S monomers (2) or the use of agarose (5) as diffusion medium abolishes the discrepancies and permits a quantification by RID of monoclonal was as well as of polyclonal IgM masses. Such figures compare well with those obtained by electrophoresis (monoclonal IgM only) and by nephelometry or turbidimetry without previous reduction.

- Determinations carried out in this way yield results (3, 6) well below those based on w/o estimates (7).

These properties of the RID technique are independent of the batch of antiserum. Their correctness has been proven by comparison with the results of quantifications by analytical ultracentrifugation or electrophoresis of sera containing monoclonal IgM (2, 3). Thus there can be no doubt that monoclonal IgM can be correctly determined in mass units by RID, electrophoresis, nephelometry, and turbidimetry.

Contrary to current expectations (e.g., 8) the discrepancies found when determining different monoclonal IgM by RID without previous reduction are not greater than when different polyclonal IgM are investigated. The significant differences between techniques found by Pontet et al. for monoclonal IgM may arise from comparison of results by electrophoresis with those by RID without reduction and partly also by use of different standards for each technique. Most commercial standards are directly or indirectly based on the data supplied by the WHO for their reference preparations (7). Such standards are therefore incorrect as far as mass units are concerned (3) and cannot be compared for use in RID without previous reduction. Correct primary standards may be prepared by adding known quantities of any purified human IgM preparation to sera previously freed of IgM by immunopreparation. The correctness of such standards has been demonstrated by recovery experiments (3, 5). Different standards must be used for high and low concentrations of IgM, because the RID results are affected by the ratio of IgM to other serum proteins (5). Secondary standards can then be derived from primary ones by RID with previous reduction or by nephelometry.

The nephelometric dose–response curves published by Pontet et al. (1) clearly show that use of that technique will produce comparable results if measurements are carried out in sufficient antibody excess, as most manufacturers prescribe. The shape of the dose–response curves around the equivalence points for various 19S IgM cannot be expected to be related to the diameter of precipitation circles of reduced IgM in RID. After reduction, antigenic or possibly other differences between the IgMs are no longer expressed in the latter technique. In addition, interference by native monomers is obviated because all IgM is quantified in the monomeric form.

Determination of monoclonal IgM should pose no problem if based on properly calibrated standards. If RID is involved, the IgM should be reduced first. Mass units or relative (international) units can be used, as desired.

References

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Urinary Ascorbate Measurement by Rate Analysis in a Centrifugal Analyzer

To the Editor:

As part of a study on oxalate precursors in urine we wished to measure ascorbate in urine, because this vitamin is a known major precursor of oxalate in man (1). Several methods for measuring ascorbate have been reviewed (2). We decided to use the colorimetric method of Roe (3) because it has been thoroughly investigated and found to be reliable and because it also measures dehydroascorbate, a metabolite of ascorbate and a precursor of oxalate (4). In this method, ascorbate is oxidized to dehydroascorbate, which is then condensed with 2,4-dinitrophenylhydrazine (DNPH) to form the colored osazone. This method, like many others, is labor-intensive and requires a 3-h incubation at 37 °C plus the addition of strong acids for the final color development (2, 3). In the development of this procedure we found that the rate of osazone formation in strong trichloroacetic acid correlated with the ascorbate concentration. Using this finding, we have modified the method so that 24 measurements of urinary ascorbate can be made in a Cobas Bio centrifugal analyzer in less
than 10 min, after a simple 60-min workup procedure.

Urines for analysis were collected over disodium EDTA/thimerosal (Na\(^1\), 50 mg/L urine) such that the final EDTA concentration exceeded 5 mmol/L. We found that this procedure inhibited the breakdown of ascorbate in the urine. The following solutions were used in the assay. The "ascorbate oxidizing solution," 50 g of acid-washed charcoal and 100 mmol of FeCl\(_3\) per liter of 200 g/L trichloroacetic acid solution, is indefinitely stable at room temperature. For the "color reagent," we dissolved 2.2 g of DNPH in 28 mL of concentrated H\(_2\)SO\(_4\) at 4°C and added this solution to 50 mL of water, keeping the temperature <10°C. After adding 0.25 g of thiourea and 0.03 g of copper sulfate in 10 mL of water to the DNPH solution, we stored this in a stoppered brown bottle at 4°C, where it was stable for six to eight months after preparation. Ascorbate standards of 5, 2.5, and 1.25 mmol/L in disodium EDTA (10 mmol/L) were stored frozen (−20°C).

Procedure: Mix urine and standards (0.2 mL) with the oxidizing solution (0.05 mL) and let stand at room temperature for about 50 min; longer incubation times (>80 min) result in deterioration of the ascorbate. Centrifuge urines and standards (2500 rpm for 5 min), then add 0.1 mL of the supernate to 0.05 mL of trichloroacetic acid (600 g/L) in analyzer cups.

Program the Cobas Bio as follows: Units, mmol/L; Calculation factor, 0; Standard 1, 1.25; Standard 2, 2.50; Standard 3, 5.0; Limit, 5.0; Temperature, 37°C; Type of analysis, 4; Wavelength, 520 nm; Sample volume, 80 \(\mu\)L; Diluent volume, 10 \(\mu\)L; Color reagent volume, 200 \(\mu\)L; Incubation time, 0 s; Start reagent volume, 0 \(\mu\)L; Time of first reading, 180 s; Time interval, 10 s; Number of readings, 6; Blanking mode, 1; Printout mode, 1.

The ascorbate concentration in urine is calculated by the analyzer and printed out in mmol/L.

We found that the standard curve was linear to 10 mmol of ascorbate per liter and satisfied the following equation:

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\text{absorbance change/min at 520 nm} = 0.028 \times \text{ascorbate, mmol/L}
\]

Using serial twofold dilutions, we found the smallest measurable concentration to be 0.15 mmol/L. The sensitivity of this assay can be increased by concentrating the urines. For example, lyophilized 2-mL aliquots of five different urines, when reconstituted with water to give a fourfold concentration, yielded results 94.4% (SD 6.2%) of those expected from the undiluted urines. Within-day precision for a urine containing ascorbate at the lower limit of detection (0.19 mmol/L) was ±0.03 mmol/L (CV 20%, n = 20). For the same urine supplemented with 2 mmol of ascorbate per liter, the precision was ±0.08 mmol/L (CV 3.9%, n = 20) and the analytical recovery 95.0% (SD 4.0%). Day-to-day precision for the above urines, analyzed 18 times during 29 days, was 0.19 (SD 0.04) mmol/L (CV 21%) for the unsupplemented urine, 2.08 (SD 0.12) mmol/L (CV 5.7%) for the supplemented sample; recovery was 94.6% (SD 5.2%).

Zero ascorbic acid results obtained for several urines after fivefold concentration (by lyophilization) confirmed the specificity. The following metabolites tested at 1–5 mmol/L did not interfere with the method: acetone, glyoxylate, \(\beta\)-hydroxybutyrate, pyruvate, lactate, acetylacetone, glucose, fructose, arabinose, maltose, trehalose, galactose, sucrose, lactulose, and raffinose.

The preliminary reference interval (0.19–2.2 mmol/day) for five men and six women (laboratory staff) not taking ascorbate preparations correlated well with that for a specific liquid-chromatographic method (5; range 0–3.4 mmol/day, n = 39) but was higher than that for two other end-point methods involving DNPH (6, 7; range 0.03 to 0.40 mmol/24 h). These differences may reflect different intakes of dietary ascorbate by the various groups (2, 5, 6).

The correlation between results for ascorbate in 10 urines estimated by the current method and an end-point method (9) was significant (r = 0.93, p < 0.01, range 0.41–2.26 mmol/L). The mean value by the present method was 1.20 (SD 0.85) mmol/L and by the end-point method 1.30 (SD 0.87) mmol/L.

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References

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