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Measurement of Magnesium in Mononuclear Blood Cells

To the Editor:

Because magnesium is mainly an intracellular ion, measurements of its cellular concentration have been advocated to disclose deficiency. Magnesium in muscle biopsies may be well suited (1), but such specimens will hardly be taken routinely. Mononuclear cells are more easily available, but results obtained with these cells have been contradictory (2, 3). Measuring mononuclear cell Mg concentrations in patients with acute myocardial infarction, we experienced considerable analytical variation. At present, analytical imprecision appears to reduce the usefulness of the mononuclear cell Mg method, at least when the results are used to detect Mg deficiency in single patients.

We isolated the mononuclear cells (monocytes and lymphocytes) from 8 mL of venous blood as described by Bøyum (4), suspended the cells in 2 mL of a 135 mmol/L NaCl–1 mmol/L glucose–10 mmol/L phosphate pH 7.4 buffer, counted them within 30 min (Ortho ELT 800), and kept them at $-20^\circ$C. Before analysis they were thawed and disrupted ultrasonically (50 W, 60 s, on ice). Protein was measured (5) in a Cobas Fara centrifugal analyzer with reagents from Bio-Rad (Richmond, CA). Magnesium was measured by atomic absorption spectrophotometry after addition of NaOH to give a final concentration of 0.1 mol/L.

We used samples from 25 healthy individuals (mean age 36 years, age span 24–54 years) to estimate the normal variation of magnesium in mononuclear cells. The cells were usually prepared on separate days, but were analyzed in five batches.

We used four different controls to examine the day-to-day precision of different parts of the method (Table 1). Solutions of magnesium oxide and human albumin (controls 1 and 2) were used to examine the magnesium and the protein assays. A suspension of cells, prepared in one batch and frozen in subsamples (control 3), was used to examine the magnesium and the protein assays, as well as the magnesium per protein estimate. Cells drawn repeatedly from one donor during three weeks, each time preserved, thawed, and analyzed on the same day (control 4), were used to examine the sum of analytical and biological variation.

The results for the magnesium and protein controls (controls 1 and 2, Table 1) indicated the magnesium and protein assays to be acceptably precise. With the cell suspension (control 3) we obtained larger variations in the Mg and protein results than we did with the control solutions 1 and 2. This may suggest that measurements vary more when the component to be measured is present in cells than when it is in a homogeneous solution.

The precision of control 4 was approximately the same as the same as that of control 3 (Table 1). Control 4, by contrast to control 3, was newly prepared from a new blood sample each day of analysis. Therefore, the magnesium and protein measurements appeared to be mainly responsible for the variation of control 4, whereas biological variation and variance to the isolation contributed less.

The Mg per cell estimate was less precise than the Mg per protein estimate when we used all 12 observations to calculate the variation (Table 1). However, the two estimates were equally precise (CV = 12%) when variation was recalculated, including only the nine cellular preparations containing $>10^5$ cells per liter.

We estimated the concentration of Mg in mononuclear cells from 25 healthy individuals. The results obtained compare well with those reported by others (Table 2). The CV for the results (15%, Table 2) was not larger than the day-to-day variation of the cell control materials (Table 1). Theoretically, intra-individual, inter-
individual, and analytical variation all contribute to this variation, but the present results reveal that analytical variation is the main source. An improved method might therefore make the Mg/protein estimate in patients more reproducible and clinically more useful than it appears to be at present.

There is no reason to believe that the Mg/cell estimate is more reproducible than the Mg/protein estimate. On the contrary, the consistent finding that the Mg/cell range is even wider (Table 2) does suggest that, if there is any difference, the Mg/cell estimate is subject to more analytical variation than is the Mg/protein estimate.

References

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Microalbuminuria in Diabetes Mellitus: More on Urine Storage and Accuracy of Colorimetric Assays

To the Editor:
Diabetics have a high risk of hypertension and renal disease, so assay of urinary albumin excretion (UAE) has recently assumed a central role in the prevention and follow-up of diabetic nephropathy (1). Because screening for UAE has become part of routine diabetes care (1) and concerns a great number of patients, a suitable method should be used for measuring UAE. Because its concentration in normal urine is low, albumin is usually undetectable by standard laboratory methods (precipitation or "dipstick" techniques). Thus, more-sensitive immunological procedures, such as RIA, EIA, ELISA, immununoturbidimetric assay, fluorochrome, and immunonephelometric assay have been proposed for routine measurement of UAE (2). Moreover, because of the great number of samples that may have to be processed, urine specimens are often stored before assay.

Elving et al. (3) claimed that freezing of urine samples for determination of UAE by laser immunonephelometry may yield falsely low results, thus suggesting that urine should be stored at 4 °C and assayed within two weeks.

We previously found no decrease in albumin values measured by RIA in several (n = 4) freezing and thawing experiments performed during three weeks on 10 urine samples with various albumin concentrations (4).

Moreover, in Figure 1, we have reported the time courses of albumin values measured in three urine pools by two different RIA methods, both used according to the manufacturer's instructions, as reported (4, 5). A low-and an intermediate-level pool (pools 1 and 2, part B of Figure 1) were assayed by an RIA in which double antibody is used for bound/free separation (H-Albumin kit; Sclavo Diagnostic and Instruments Division, Cinisello Balsamo, Italy). Another intermediate-level pool (pool 3, part A of Figure 1) was assayed by an RIA in which a solid phase is used for bound/free separation (Albumin RIA 100 kit; Pharmac AB, Uppsala, Sweden). Each pool was divided into various 0.5-mL aliquots stored at −20 °C until assay. The assay CVs were 9.76%, 7.03%, and 5.2%, for pools 1, 2, and 3, respectively (Figure 1). A very slight, but significant decrease of albumin concentration was found only in the low-level pool (pool 1, part B of Figure 1); the values of the two intermediate-level pools (pools 2 and 3) did not decrease. The rate of decrement of albumin concentration for pool 1, as estimated from the linear-regression analysis, was only 3.5 μg/L per day (about 0.6 mg/L for six months).

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Table 1. Day-to-Day Precision [Mean, SD (CV%), n = 12] of Various Control Materials

<table>
<thead>
<tr>
<th>Measured</th>
<th>Calculated</th>
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<tbody>
<tr>
<td>Mg, μmol/L</td>
<td>Mg, μmol/L</td>
</tr>
<tr>
<td>Protein</td>
<td>protein</td>
</tr>
<tr>
<td>Mg, μmol/g</td>
<td>protein</td>
</tr>
</tbody>
</table>

| Control 1 | 8.39 ± 0.22 (2.8%) | |
| Control 2 | 130 ± 8 (5.5%) | |
| Control 3 | 5.36 ± 0.31 (5.3%) | 73 ± 12 (16%) |
| Control 4 | 65 ± 8 (12%) | 6.0 ± 1.9 (32%) |

Table 2. Concentration of Magnesium in Mononuclear Cells [Mean, SD (CV%)] of Healthy Individuals, as Reported by Different Authors

<table>
<thead>
<tr>
<th>Ref. no.</th>
<th>Mg, fmol/cell</th>
<th>Mg, μmol/g protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elin and Johnson</td>
<td>6</td>
<td>3.3 ± 0.9 (28%)</td>
</tr>
<tr>
<td>Elin and Hosseini</td>
<td>7</td>
<td>2.9 ± 0.6 (20%)</td>
</tr>
<tr>
<td>Ryzen et al.</td>
<td>8</td>
<td>2.8 ± 0.6 (19%)</td>
</tr>
<tr>
<td>Reinhart et al.</td>
<td>9</td>
<td>5.4 ± 1.9 (33%)</td>
</tr>
<tr>
<td>Sjøgren et al.</td>
<td>10</td>
<td>4.0 ± 0.9 (24%)</td>
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