testing hypercholesterolemia must have the best possible analytical performance.

We suggest that, in screening a population for hypercholesterolemia, serum or plasma samples are preferable to blood spots on filter paper. Instruments such as the Cobas-Bio centrifugal analyzer require less than 50 μL of sample and the typical overall CV is 4% for assays of cholesterol in plasma.

References

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Oxalate Oxidase from Banana Peel for Determination of Urinary Oxalate

To the Editor:

The many procedures proposed for determination of oxalate in urine reflect the continuing need for a simpler, more sensitive, and more rapid method. Zerwekh et al. (1) recently compared six different techniques for determining oxalate in urine, including colorimetry, liquid chromatography, gas chromatography, enzymic analysis, and ion chromatography. They noted that the enzymic method is sensitive, but the high expense of the necessary enzymes and the presence of interfering factors in the urine limit the usefulness of the procedure. Using oxalate oxidase derived from beet stem, Obzansky and Richardson (2) recently reported an automated method for determination of urinary oxalate.

We outline below a simple method for determination of urinary oxalate by use of oxalate oxidase (oxalate:oxygen oxidoreductase, EC 1.2.3.4) from banana peel. The possible occurrence of oxalate oxidase in banana peel was first suggested by Richardson (personal communication). The banana peel enzyme is the least expensive to prepare, and its isolation is simple and rapid. In other enzymic methods, substances in the urine, such as oxalate oxidase activity, are removed by treatment with charcoal or ferric chloride and then by Dowex AG 50 W × 8, or Chelex (Bio-Rad Labs.), or both. To simplify these steps, we suggest using a single resin for the selective absorption and elution of urinary oxalate.

The enzyme is isolated as follows: Add cold acetone to a 100 g/L homogenate of peels from over-ripened and darkened banana fruit (Musa paradisiaca; the French Plantain or common green variety, Giant Cavendish, can be used). Prepare a series of precipitates (which are removed by centrifugation) by successively adding the acetone to produce 300, 500, 640, 950, 1100, 1400, 1660, 2000, and 2400 mL/L (10 000 × g), and 600 mL/L (15 000 × g) concentrations. Suspend the last precipitate, which shows the greatest oxalate oxidase activity (2 kUg of protein, 11% yield), in the minimum necessary amount of phosphate buffer (0.1 mol/L, pH 5.2) and use this as enzyme source. One unit (U) is defined as the amount of oxidase required to convert 1 μmol of substrate to product in 1 min. Oxalate in urine is determined as follows: To a tube or vial containing oxalate-extracting absorbent (obtained from Sigma Chemical Co., cat. no. 590–4), add 1.0 mL of urine sample (24-h urine, acidified with HCl to a pH of 3) and mix for 6 min; after allowing the absorbent to settle, aspirate the liquid and discard it. To the absorbent, add 2.0 mL of water, shake, and aspirate the water and discard. Add 1.0 mL of 0.2 mol/L sodium hydroxide to the absorbent, mix for 6 min, and aspirate and save the clear extract, free of absorbent. To 0.1 mL of this extract, add 1.9 mL of phosphate buffer (0.1 mol/L, pH 5.0) containing 0.2 U of banana oxalate oxidase, 0.2 U of peroxidase, 0.21 μmol of 3-methyl-2-benzothiazolinone hydrazone (MBTH), and 3.2 μmol of 3-(dimethylamino)benzoic acid (DMAB). (This is the reaction used in Sigma kit 590 for oxalate.) Maintain the pH of the final assay mixture at 5.2. After incubating the reaction mixture for 30 min at 37 °C, measure the absorbance of the sample at 590 nm.

urinary oxalate excretion rate for 20 normal persons to be 18.2 to 37.4 mg/24 h (mean 26, SD 5.1). Analytical recovery of oxalate (25 and 50 mg) added to 24-h urine specimens ranged from 94 to 102%. The results obtained by this method correlated well with those of deoxytreated samples (range 4.7–36.3 mg/24 h, mean 20.5), Castello et al. (4) (range 14.0–37.6, mean 25.9), and Obzansky and Richardson (2) (range 22.9–46.1, mean 33.8).

References

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Li+ and Sicking of Homozygous HbS Cells

To the Editor:

Recently, Diorio (1) reported that Li+ might be effective in therapy for the prevention of the sickling phenomenon in sickle cell anemia, because he had observed that homozygous S erythrocytes did not sickle when mixed with a 20 g/L solution of sodium metabisulfite if the cells were treated with LiBr. Also, radial immunodiffusion of hemolysates of sickle cell hemoglobin (HbS) was reported to demonstrate an "isothermally induced melting of HbS" (1). Here, we present data and studies of others that refute the idea that Li+ salts may be useful in treatment of sickle cell anemia.

First, we have repeated the metabisulfite experiment and find that LiBr at 5, 10, 15, and 20 mmol/L (final concentration) has no effect in decreasing sickling, even after 24 h of incubation at 4 °C. Low-temperature incubation increases uptake of Li by erythrocytes (2). We repeated this same experiment, using a 7 g/L solution of sodium dithionate, a more suitable deoxygenating agent. Table 1 summarizes our findings for Li+-incubated cells and controls, treated with the above deoxygenating agents. Clearly, Li+ has little or no effect in reversing the sickling phenomena.

We also measured the effect of Li+ on the filterability of homozygous S cells pushed through a 5-μm pore-size filter, at a constant flow rate, under anaerobic conditions (Table 1). There was actually a small increase in pres-
Table 1. Effect of LiBr on Homozygous HbS Erythrocytes

<table>
<thead>
<tr>
<th>Incubation conditions</th>
<th>LiBr treatment, mmol/L</th>
<th>% of cells that sickled*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>3 h, RT, dithionate</td>
<td>95</td>
<td>93</td>
</tr>
<tr>
<td>24 h, 4 °C, dithionate</td>
<td>99</td>
<td>97</td>
</tr>
<tr>
<td>3 h, RT, metabisulfite</td>
<td>98</td>
<td>94</td>
</tr>
<tr>
<td>24 h, 4 °C, metabisulfite</td>
<td>86</td>
<td>82</td>
</tr>
</tbody>
</table>

Deoxygenated cells

<table>
<thead>
<tr>
<th>LiBr treatment, mmol/L</th>
<th>Oxygenated cells (control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3</td>
</tr>
<tr>
<td>10</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td></td>
</tr>
<tr>
<td>2 h, RT</td>
<td>55</td>
</tr>
<tr>
<td>68</td>
<td></td>
</tr>
<tr>
<td>24 h, 4 °C</td>
<td>68</td>
</tr>
</tbody>
</table>

*At the end of the specified time, the cells were treated with deoxygenating agent (Na dithionite, 7 g/L, or Na metabisulfite, 20 g/L) for 1 h, then fixed with glutaraldehyde before normal vs sickle-deformed cells were counted under a microscope. RT, room temperature.

Moreover, adverse effects are observed if Li⁺ concentrations in plasma exceed 1.5 mmol/L, and serious toxicity is common above 2.0 mmol/L. This indicates a very small therapeutic index.

REFERENCES

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Reference Interval for Thyroxin as Measured by Fluorescence Polarization Immunoassay

To the Editor:

Total thyroxin (T₄), as measured by fluorescence polarization immunoassay (FPIA), is an accurate and precise index to thyroid homeostasis. Initial evaluations in our laboratory (Clin Chem 30: 1035, 1984, abstract 492) showed the euthyroid range we found (45 to 105 μg/L) to be considerably lower than that recommended by the manufacturer (Abbott Diagnostics): 45 to 120 μg/L. Patients’ samples run by FPIA and RIA showed FPIA to have a method bias of −8 μg/L, and the t-test result was 4.3. The sensitivity of the FPIA method is 6 μg/L.

We used a population of ostensibly normal, ambulatory “well” volunteers in statistically determining a suitable normal reference interval: 52 men and 48 women ranging in age from 17 to 67 years. Individuals receiving any exogenous hormone therapy were excluded.

The mean value by FPIA was 66.2 (SD 11.7), range 43 to 97 μg/L, and the data were normally distributed (Figure 1). The 95% confidence interval thus was 42.7 to 89.7 μg/L.

Total T₄, determined by RIA was measured with Tetra Tab reagents (Nuclear Medical Laboratories, Dallas, TX); their recommended euthyroid range is 45 to 115 μg/L. We measured thyrotropin (TSH) with Hybritech Tandem-R TSH reagents; their suggested upper limit for euthyroid values is 7 μg/L. Total triiodothyronine (T₃) was measured with Corning reagents; their recommended euthyroid range is 60 to 200 μg/L.

We determined the inter- and intra-assay precision of the FPIA assay by running, in replicates of five on five different days, three control sera with different thyroxin concentrations:

<table>
<thead>
<tr>
<th>T₄ concn, μg/L</th>
<th>Within run SD, μg/L</th>
<th>Between run SD, μg/L</th>
<th>CV, %</th>
<th>CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>48.7</td>
<td>2.6</td>
<td>5.69</td>
<td>3.4</td>
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</tr>
<tr>
<td>80.9</td>
<td>2.2</td>
<td>7.22</td>
<td>3.1</td>
<td>3.81</td>
</tr>
<tr>
<td>156.0</td>
<td>5.3</td>
<td>3.37</td>
<td>5.2</td>
<td>3.37</td>
</tr>
</tbody>
</table>

n = 25 each.

Patients with confirmed clinical hypothyroidism had T₄ values ranging from 11 to 48 μg/L and TSH results from 17 to >100 μg/L T₄ measured by RIA on the same samples ranged from 4 to 55 μg/L. For hyperthyroid patients T₄ values by FPIA ranged from 101 to 219 μg/L. Hyperthyroidism was confirmed by increases in T₃ values or T₄ asayed by RIA.

In our laboratory, a value for total T₄ by FPIA outside the limits of 43 and 97