We see this effect most commonly in specimens from intensive-care units, where syringes, needles, and cannulas often are heparinized before specimens are collected for blood-gas analysis.

The minimum volume of anticoagulant that would be left in a heparinized syringe is the dead space of the nozzle, about 100 μL. If this volume of heparin solution, containing 3 g of chlorocresol, were added to 5 mL of blood, the result for glucose would be about 1 mmol/L too high, a difference that probably is of clinical importance only in hypoglycemia or in serial sampling. Any more of the preservative, however, would produce a proportionately greater effect.

This is a recognized chemical interference (the instrument handbook states that the YSI model 23 AM is not suitable for use with samples containing thymol or other phenol preservatives; these are interfering substances for the model 23 AM and are contraindicated), but is easily overlooked because of an unsuspected technique of specimen collection. We advise users of this instrument to check the techniques and anticoagulants used by requesting clinicians.

D. Peel
Dept. of Clin. Biochem.
Queen Elizabeth Hosp.
Gateshead, NE3 6SX
U.K.

J. D. S. Kay
F. Taylor
Dept. of Clin. Biochem.
Hosp. for Sick Children
Great Ormond St.
London, U.K.

Insulin Immunoreactivity Stabilized in Serum and Plasma at Ambient Temperatures

To the Editor:

Finding that insulin immunoreactivity is unstable in plasma at 20 and 37 °C (1), we then attempted to find substances capable of slowing the rate of loss sufficiently to allow postal transport of specimens for insulin assay. In a report showing that insulin is similarly unstable in serum (2), Oliver presented evidence that sodium fluoride, 5 g/L, is an effective preservative. We could not confirm this, but have found buffered N-phenylmaleimide (NPM) to have a useful stabilizing effect.

NPM tubes contained 40 μL of a 50 mmol/L solution of NPM in ethanol and 40 μL of aqueous buffer [0.8 mol/L NH₄H₂PO₄, 0.2 mol/L (NH₄)₂HPO₄] that had been air-dried together. NaF tubes contained 5 mg of NaF powder. Serum and plasma (~1 mg of Na₂EDTA per milliliter) were collected from normal subjects about an hour after lunch (range: 70–330 pmol of insulin per liter) and 1-mL aliquots were added to capped polypropylene NaF, NPM, and control (no additives) tubes. After mixing and dissolving the additives we incubated the tubes at 30 or 37 °C. Comparison aliquots were stored at −20 °C. After five days all aliquots were frozen and stored at −20 °C until the immunoreactive insulin concentration was determined by the method of Albano et al. (3), with use of an antisera raised in this laboratory. For analysis of the results, insulin concentrations for each aliquot were expressed as a percentage of the concentration in the corresponding aliquot stored at −20 °C. The results tabulated above confirm that significant insulin immunoreactivity is lost from serum and plasma stored five days at 30 °C.

Losses are even greater at 37 °C. However, addition of sodium fluoride to give a final concentration of 5 g/L had no useful preservative effect at either temperature of storage, the losses at 30 °C being virtually identical to control samples, and at 37 °C over 60% of insulin immunoreactivity was lost with NaF present in both serum and plasma. In contrast, buffered N-phenylmaleimide, 2 mmol/L, largely preserved insulin immunoreactivity in both serum and plasma at both 30 and 37 °C. This effectiveness of NPM was not unexpected, because it is a good inhibitor of insulin-specific protease (4) and glutathione-insulin transhydrogenase (5), either or both of which might be responsible for the insulin-degrading activity in plasma and serum. Buffered NPM does not interfere in the insulin assay and is more effective than unbuffered NPM for preserving insulin immunoreactivity in plasma (Livesey, unpublished results). It may be that our results with fluoride differ from those of Oliver (2) because of the possibility of differing specificity of our antiserum.

In conclusion, we could not confirm the effectiveness of fluoride as an inhibitor of insulin degradation in serum but we find that, as expected from the properties of known insulin-degrading enzymes, a maleimide is an effective inhibitor of immunoreactive insulin loss in serum and plasma.

References


John Livesey
Richard A. Donald
Helen K. Roud
Dept. of Endocrinol.
Princess Margaret Hosp.
Cashmere Rd.,
Christchurch, 2,
New Zealand

Conclusions from a Pilot Immunoreactive Trypsin Newborn Screen for Cystic Fibrosis

To the Editor:

We have now completed a two-year pilot screening program in which immunoreactive trypsin (IRT) was measured in blood eluted from filter papers on which it was collected as part of a
universal phenylketonuria/hypothyroidism screen. The project was based on an hypothesis first outlined by Crossley et al. (1), viz., that an abnormally high blood IRT value for a newborn is suggestive of cystic fibrosis (CF). Details of the methods were previously described (2).

We tested 25,000 newborns and identified seven infants confirmed to have CF. One of the seven was not detected in the initial assay, but a repeat card, taken one week later because the infant was premature (birth weight, 1086 g), revealed an increased value for IRT. Values for all affected infants were within the top 1% of each assay run of 200 specimens.

During the project period, eight infants were diagnosed with CF. The one not detected by the IRT program did not have a filter paper card taken at birth.

From our experience, we conclude that the IRT test is a reliable indicator of infants with CF if the reagents used are of good quality and the specimens are fresh. We would use only reagents comparable to those outlined by Crossley et al. (3) and would assay the specimens within a few days of their arrival at the screening laboratory.

References


L. T. Kirby
A. G. F. Davidson
D. A. Applegarth
L. T. K. Wong
D. F. Hardwick

Depts. of Pathol. and Pediatrics
Univ. of British Columbia and Children’s Hosp.
Vancouver, B.C. V6T 1W5, Canada

Abnormally High Concentrations of $\beta_2$-Microglobulin in Acquired Immunodeficiency Syndrome (AIDS) Patients

To the Editor:

$\beta_2$-Microglobulin, a small peptide ($M_r$ 11,600), is normally present in trace amounts in both serum and urine (1). It may play an important role in the immune function of the body because of its association with histocompatibility antigens (2) and also because its amino acid sequence closely resembles that of the constant part of the immunoglobulin chain (3).

This peptide not only is present on the surface membrane of both B and T cells (4) but also in the membranes of all nucleated cells (5). Data on its concentration have been used as a reliable indicator of glomerular and tubular functions (6). Abnormally high concentrations of it have been reported in patients with impaired renal functions (6), rheumatoid arthritis and Sjögren’s syndrome (7), and malignant tumors (8), and more striking increases were shown in lymphoproliferative disorders (9). More recently, increased concentrations of $\beta_2$-microglobulin were shown in an immunodeficient homosexual man (10).

In the present preliminary study, we have determined $\beta_2$-microglobulin in the sera of patients with AIDS, as tabulated below. Note 29 of 31 patients with AIDS had supranormal $\beta_2$-microglobulin concentrations, whereas five of 11 normal homosexual men had increased values as compared with normal controls.

<table>
<thead>
<tr>
<th>No.</th>
<th>Range</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>1.6–2.4</td>
<td>1.9</td>
<td>0.24</td>
</tr>
<tr>
<td>31</td>
<td>2.0–16.0</td>
<td>6.2</td>
<td>3.50*</td>
</tr>
<tr>
<td>11</td>
<td>1.7–4.5</td>
<td>3.0</td>
<td>1.00*</td>
</tr>
</tbody>
</table>

*Different from normal homosexual men at $p < 0.01$.

*Different from normal controls at $p < 0.001$.

Serum $\beta_2$-microglobulin is thought to originate mainly from the surface of cells, and it increases with increased cellular turnover (10). Hence, the increased values in AIDS patients are indicative of an increased turnover of cells in such patients.

Thus far it has been demonstrated that reversal of ratios of helper to suppressor T-lymphocytes and lymphopenia are markers of AIDS (11, 12). We believe that increased $\beta_2$-microglobulin may provide yet another diagnostic marker for AIDS patients.

References


Ravi B. Bhalla
Bijan Safai
Roland Mertelsmann
Morton K. Schwartz

Memorial-Sloan Kettering Cancer Center
New York, NY 10021

Choice of a Satisfactory Evacuated Blood-Collection Tube for Magnesium and Zinc Assays

To the Editor:

Requests for magnesium and zinc assays in plasma and erythrocytes relate to research studies entail extra work for hospital nursing personnel as well as an additional blood sample from the patient. Moreover, the recent widespread and generally mandatory practice of using evacuated blood-collection tubes in our regional hospital has obliged us to abandon the use of polystyrene tubes and of polyethylene natural stoppers, which had proved quite acceptable (1), and to search for an evacuated blood-collection tube yielding the least possible Mg and Zn. In effect (though too often apparently ignored) conditions of taking and preserving blood samples on results for