Effect of Various Sample-Handling Conditions on Radioassay Results

To the Editor:

Samples brought to the laboratory for routine radioassay often have been under suboptimal conditions. We compared the effect of some sample-handling conditions on the values for T₃, T₄, resin uptake (T₃RU), T₄, thyrotropin, cortisol, and prolactin in serum as determined by radioassays. Blood was collected in three separate plain red-top Vacutainer Tubes from each of six subjects. Serum was separated from one of the three tubes, divided into portions, and frozen within 1 h of collection. Another tube from each patient was allowed to sit at room temperature for 7 h and then the serum was separated, portioned, and frozen. The third tube from each patient was allowed to sit at room temperature for 7 h, then left overnight in the refrigerator and the serum separated and frozen after a further 17 h. All assays were done with commercially available kits: T₄, T₃RIA, and thyrotropin kits from Beckman Instruments Inc., Fullerton, CA 92634; T₃RU kits from E.R. Squibb and Sons Inc., Princeton, NJ 08540; cortisol kits from Clinical Assays, Cambridge, MA 02139; and prolactin kits from Abbott Laboratories, Diagnostics Division, North Chicago, IL 60064.

We could detect no significant detrimental effect of these various sample-handling conditions on the results of routine radioassays.

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Assay of UDP-Galactose 4-Epimerase

To the Editor:

Uridine diphosphate galactose (UDP-gal) 4-epimerase (UDPGlc 4-epimerase, EC 5.1.3.2) deficiency in humans was first described by Gitzelmann (1). This defect has been found in several families; however, the patients showed no significant clinical manifestations (2-4). All cases had either a complete or a partial deficiency of epimerase activity in erythrocytes, but normal activity in the liver (2-4). Recently, Holton et al. (5) reported the first case of deficiency of this epimerase with severe clinical symptoms of classical galactosemia. This case raises the question of whether there are heterogeneities in the epimerase deficiency. Therefore, we decided to determine the epimerase activity in all patients who were selected by screening for galactosemia. The epimerase assay we used previously is the two-step procedure of Gitzelmann and Steinmann (2); this spectrophotometric estimation of UDP-galactose is often not sensitive enough to determine the activity in cultured cells. The new method we describe here, a radioactive dilution technique, is very sensitive and also suitable for multiple analyses.

First we add 2-4 µL of erythrocytes to a reaction mixture consisting of UDP-gal, 1 mmol/L, NAD⁺, 5 mmol/L, and glycylglycine buffer, 100 mmol/L, pH 9.0, in a total volume of 100 µL. After a 10-min incubation at 37 °C, the reaction is terminated by heating at 95 °C for 4 min. To determine UDP-galactose, we let the mixture cool, centrifuge, and add 20 µL of the supernate to a second reaction mixture containing UDP-¹⁴C-glucose (0.05 µCi or 0.25 mmol; from Amersham Buchler, F.R.G.), pyrophosphate, 0.1 mmol/L, MgCl₂, 20 mmol/L, and Tris HCl buffer, 100 mmol/L, pH 8.0, in a total volume of 100 µL. To this we add 2 µL (0.1 U) of UDP-glucose pyrophosphorylase (EC 2.7.7.9; from Sigma Chemie GmbH, Munich, F.R.G.) to start the reaction. For the standard curve, we used 20 µL of 0, 25, 50, and 100 µmol/L UDP-galactose solutions. After incubation for 20 min at 37 °C, the reaction is terminated by heating at 95 °C for 3 min. We separate UDP-¹⁴C-glucose from °¹⁴C-glucose 1-phosphate as in the uridylyltransferase assay (6), by partly automated chromatography on a miniature DEAE-cellulose column.

For cultured cells and liver, the concentrations of each compound in the reaction mixture of the first step were two-thirds as great as those for the assay of erythrocyte epimerase. For cultured cells we used extracts containing 1 to 10 µg of protein; for liver, 0.1-1.0 µg of protein. Incubation was at 37 °C for 10-60 min according to the protein content. The formation of UDP-galactose was measured as described above.

Figure 1 shows the standard curve for UDP-galactose and a typical reaction of

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Table 1. Epimerase Activity in Erythrocytes, Tissues, and Cultured Cells

<table>
<thead>
<tr>
<th>Sample</th>
<th>Age of patient</th>
<th>Activity, µmol/h per mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocytes</td>
<td>1 wk</td>
<td>10.6, 11.3, 10.3</td>
</tr>
<tr>
<td>Erythrocytes</td>
<td>2–3 wk</td>
<td>13.4, 9.2</td>
</tr>
<tr>
<td>Erythrocytes</td>
<td>3–7 mo</td>
<td>9.1, 11.2, 10.0</td>
</tr>
<tr>
<td>Erythrocytes</td>
<td>23–30 yr</td>
<td>7.8, 9.0, 9.0</td>
</tr>
<tr>
<td>Liver</td>
<td>(autopsy)</td>
<td>41 yr</td>
</tr>
<tr>
<td></td>
<td>(biopsy)</td>
<td>10 mo</td>
</tr>
<tr>
<td></td>
<td>(biopsy)</td>
<td>4 yr</td>
</tr>
<tr>
<td></td>
<td>(fetus)</td>
<td>16 wk</td>
</tr>
<tr>
<td>Fibroblasts</td>
<td>(autopsy)</td>
<td>10.6, 9.8, 17.6</td>
</tr>
<tr>
<td>Amniotic fluid cells</td>
<td></td>
<td>7.1, 6.9, 10.8</td>
</tr>
<tr>
<td>Amniotic fluid cells</td>
<td></td>
<td>7.7, 6.4, 13.3</td>
</tr>
<tr>
<td>Leukocytes</td>
<td>2 yr</td>
<td>3.0</td>
</tr>
<tr>
<td>Leukocytes</td>
<td>25–30 yr</td>
<td>3.2, 3.1</td>
</tr>
</tbody>
</table>

* All liver samples were stored at −20 °C for two months and the fetal liver for 10 months at −20 °C.
* Cultured cells and leukocytes were grown to confluency and frozen as cell extracts for two to four days at −20 °C.
epimerase in fibroblasts and leukocytes. The increase in the radioactivity in the diphosphate fraction (6) was linearly related to concentration up to 20 μmol/L. The epimerase reaction in fibroblasts as well as in leukocytes, expressed as the dilution of UDP-glucose with time, was linear up to 60 min.

Table 1 summarizes the activity of epimerase in various human tissues. In each case, the activity in erythrocytes, as determined with the radioactive method, was similar to that determined spectrophotometrically. Interestingly, in contrast with the values for erythrocytes, the specific epimerase activity in fibroblasts and amniotic fluid cells considerably exceeds that of uridylyltransferase (EC 2.7.7.12) (7).

We previously described a sensitive radioactive method for determination of galactose 1-phosphate in various human tissues (8). Here, we first tried the same procedure for the UDP-glucose determination, using [14C]galactose 1-phosphate and uridylyltransferase. However, UDP-gal inhibited uridylyltransferase activity, resulting in high blank values. We then tried the UDP-glucose pyrophosphorylase reaction (UDP-glucose + pyrophosphate == galactose 1-phosphate + UTP), using UDP-[14C]glucose. UDP-gal does not inhibit the latter reaction, most probably because UDP-gal is an ineffective substrate for pyrophosphorylase, even in a concentration as high as 200 μmol/L.

In conclusion, we found it relatively simple to determine epimerase activity in erythrocytes as well as in cultured cells. This method should be helpful for the study of an aberration in galactose metabolism, the epimerase deficiency.

References

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Variant Creatine Kinase Isoenzyme Band Induced by Glycine

To the Editor:

A 72-year-old man was admitted to the Indiana University Hospital with symptoms of urinary retention and urethral obstruction. He had a history of insulin-dependent diabetes mellitus and hypertension. The urologic workup revealed an enlarged prostate with bladder dilatation and possible neurogenic bladder dysfunction.

During transurethral resection of the prostate gland an irrigation solution containing glycine (15 g/L) was used. The patient developed electrocardiographic changes and decreased blood pressure, indicative of severe cardiac problems. Blood was drawn for several tests, including isoenzymes of creatine kinase (CK) and lactate dehydrogenase (LD).

The LD pattern showed the usual isoenzymes, but the CK pattern had a large atypical band between isoenzymes MM and MB. CK isoenzymes were resolved on cellulose acetate (Helena fluorescent procedure). We suspected that glycine absorbed during the irrigation may have been the cause of this atypical band. To test this idea, we added glycine to serum that exhibited MB and MM bands and to another serum that had BB, MB, and MM bands. In both instances a band formed that corresponded to the atypical band in the patient's serum.

Irrigation with glycine solution is a standard procedure in transurethral resection, so one should consider the possibility of a high glycine concentration in the blood if an atypical CK isoenzyme band is seen between MB and MM.

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Siliconized vs Nonsiliconized Evacuated Blood-Collection Tubes for Free Thyroxin Measurements

To the Editor:

It has been reported recently (1) that serum samples prepared from blood collected in siliconized evacuated blood-collection tubes may give artefactually increased results for free thyroxin (FT4) when assayed by some solid-phase direct radioimmunoassay (RIA) methods, whereas FT4 results for sera prepared with blood collected into equivalent nonsiliconized collection tubes were not affected.

The suitability of both siliconized and nonsiliconized evacuated tubes for blood collection for serum FT4 measurements by the Amerlex TM FT4 RIA method was examined during the development of this product, as part of its routine challenge testing program for product-claims-support purposes. Paired blood samples were drawn from each of 21 euthyroid volunteers into both siliconized collection tubes (Corvac integrated serum separation tubes; Sherwood Medical, St. Louis, MO 63103) and nonsiliconized tubes (Venocent; Kimble, Elkton, MD 21921). Serum samples were prepared and were concurrently assayed in duplicate in two separate assays by the Amerlex FT4 RIA method (Amersham Corp., Arlington Heights, IL 60005).

The mean FT4 value for samples collected into nonsiliconized collection tubes was 17.3 (SD 2.8) pmol/L; the mean for sera prepared by using siliconized tubes was 16.9 (SD 2.6) pmol/L. The within-run coefficient of variation in FT4 values over the range of samples studied was 2.6% (n = 84). A within-assay comparison (paired t-test) of results for both types of blood-collection tubes revealed no statistically significant difference in FT4 values at the p = 0.05 level. To test if the FT4 results for both types of collection tube behaved in the same manner for all 21 patients, a split-plot analysis of variance was carried out. The interaction (siliconized vs nonsiliconized tubes × patients) was not statistically significant (F-test) at the p