Table 1. Precision Data for Phenylalanine Determinations

<table>
<thead>
<tr>
<th>Serum pools</th>
<th>I</th>
<th>II</th>
<th>III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within-run (n = 10)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean, mg/L</td>
<td>22</td>
<td>41</td>
<td>73</td>
</tr>
<tr>
<td>SD, mg/L</td>
<td>1.5</td>
<td>1.0</td>
<td>1.5</td>
</tr>
<tr>
<td>CV, %</td>
<td>6.8</td>
<td>2.4</td>
<td>2.1</td>
</tr>
<tr>
<td>Between-run (n = 5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean, mg/L</td>
<td>22</td>
<td>42</td>
<td>69</td>
</tr>
<tr>
<td>SD, mg/L</td>
<td>2.3</td>
<td>3.2</td>
<td>5.4</td>
</tr>
<tr>
<td>CV, %</td>
<td>10.5</td>
<td>7.6</td>
<td>7.8</td>
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</table>

We compared the results for phenylalanine obtained by our enzymic procedure (γ) with those by the spectrofluorometric method (χ) of McCaman and Robins (5). Linear regression analysis of 25 serum samples from phenylketonuric patients gave the following: γ = 0.971x - 2.35; r = 0.990; ȳ = 74 mg/L, x̄ = 79 mg/L. Phenylalanine concentrations ranged from 26 to 200 mg/L for the fluorometric procedure and from 19 to 191 mg/L for our PAL-catalyzed assay.

Our adaptation of an enzymic determination of serum phenylalanine for use on a centrifugal analyzer provides a simple, rapid means for monitoring phenylalanine concentrations in phenylketonuric patients and for screening newborns for this disorder.

References

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Heterogeneity of Calcitonin in Patients with Neoplasia

To the Editor:
Circulating calcitonin (CT) consists of as many as four or five immunoreactive forms larger than the 32-amino acid CT monomer (CT M) (1). Some of these species could be formed by noncovalent binding to plasma proteins; others probably result from disulfide bridge formation between CT monomers. The origin of this heterogeneity might be the secretion of multiple CT species that appear to be a feature of normal thyroid tissue (2). Increased concentrations of immunoreactive CT (iCT) have been found in the serum of patients with medullary thyroid carcinoma and with various nonthyroid tumors. However, the validity of serum iCT as a tumor marker is controversial (3–5).

In a recent study to evaluate the CT content of nonthyroid human tumors, we found a high content of iCT in hepatic metastases from a patient with bronchial carcinoid (5). Further to analyze the nature of this iCT material, we fractionated the tissue extract by reversed-flow chromatography on a 2.5 × 65 cm column of Ultragel AcA-202 (Pharmacia, Uppeala, Sweden), eluting phosphate buffer, pH 7.5. We measured iCT in the effluent by RIA (5). The goat antiserum we used, 03-37-77 (European PTH Study Group), recognized some sequence between amino acid residues 11 and 32, as shown in studies with synthetic fragments of CT (kindly provided by Dr. B. Argéni, Groupe Hospitalier de la Timone, Marseille, France). Material with immunoreactivity was eluted in a broad elution zone, from the void volume to beyond the position of the CT M (Figure 1). Nevertheless, RIA of the immunoreactive material in the tumor extract gave a dilution curve that was superimposable on the standard curve for CT M.

The immunochromatographic heterogeneity in this tumor extract differs from that found for sera from three patients with medullary thyroid carcinoma, in which CT exceeded 20 ng/mL (normal: <125 pg/mL). The distribution of the immunoreactivity in these sera after gel filtration, as described previously, showed essentially three peaks. One peak appeared at the void volume of the column (I), another peak at the position of the CT monomer (II), and the third peak corresponded to the CT M (III). The distribution of this profile was analogous in two cases: about 30, 20, and 45% of total immunoreactivity eluted in peaks I, II, and III, respectively. However, in the third case studied, the distribution was about 30, 40, and 30%, respectively.

Radioimmunoassayable CT in peaks I and III, but not in peak II, was superimposable on the standard curve for CT M. Thus, we found the same chromatographic profile but a heterogeneous distribution of serum iCT species among individual patients with medullary thyroid carcinoma.

These results and those of others (6–8) suggest that at least some of the divergent findings among different groups who measured CT in tumor patients may be explained by the heterogeneity of iCT among different neoplasia and also among patients with the same kind of neoplasm.

References

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Differentiation of Mislabeled Samples by LDH Isoenzyme Assay

To the Editor:
The clinical laboratory is often faced with the problem of mislabeled speci-
Electroimmunoassay of Apolipoprotein B in Triacylglycerol-Rich Serum

To the Editor:

Apolipoprotein B (apo B) is distributed in low- and very-low-density lipoproteins (LDL and VLDL), which represent a large and heterogeneous family. Apo B in serum is usually measured by electroimmunoassay (EI) (I) with use of anti-LDL antibodies.

For triacylglycerol-rich serum (type IIa, or IV) the apo B value obtained by EI is abnormally high. As compared with the apo B protein concentration (2) in lipoproteins (density <1.063 kg/L) isolated from the same serum, the amount of apo B was overestimated in EI. This difference was related only to VLDL-apo B; the quantification of apo B in LDL was in good accord in both methods.

As already suggested by other authors (3–5), a lipase-catalyzed hydrolysis of VLDL could adequately correct EI values for triacylglycerol-rich serum. However, unlike the previous data (4), we point out that albumin in excess in the reaction medium is absolutely necessary, to bind the free fatty acids released by the triacylglycerol hydrolysis. The required quantity of albumin depends on the triglyceride concentration; 100 g of BSA (Pentex; bovine serum albumin, Cohn Fraction V, fatty acid poor) can bind the fatty acids released by 12 mmol of triglycerides. We found the optimum final concentration of BSA in the sample to be 65 g/L.

Thus we propose the following modifications for determination of apo B by EI: hydrolyze with 10 μL of lipase (EC 3.1.1.3, from Rhizopus arrhizus; Boehringer Mannheim GmbH) 0.1 mL of serum or standard mixed with 0.2 mL of a 100 g/L solution of BSA. After a 30-min incubation at 37 °C, the mixture is treated as in the usual procedure (I) for EI.

Table 1 shows apo B values we obtained for several normal and hypertriglyceridemic samples by EI and after lipase hydrolysis, with or without addition of BSA, compared with the concentration of apo B protein as determined by chemical analysis. These treatments caused no changes for sera having normal triacylglycerol content. In contrast, for triacylglycerol-rich sera these values were lowered after lipase hydrolysis in the presence of the BSA solution and were identical to the concentration of apo B protein.

Guided by these results, we recommend this procedure for correct determination of apo B by EI in hypertriglyceridemia, for sera with triglyceride content between 1.5 and 12 mmol/L.

References


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A Simple Immunoenzymometric Assay for Choriongonadotropin in Serum

To the Editor:

We adapted the Beta Quik V4 kit, an immunoenzymometric (IEM) assay developed by Pacific Biotech, Inc., San Diego, CA 92121, to accurately estimate human choriongonadotropin (HCG) in serum, and compared results with those by the RIA procedure of Leeco Diagnostics, Inc., Southfield, MI.

Table 1. Influence of BSA on Apo B Concentrations as Determined by EI in Triacylglycerol-Rich Serum Treated with Lipase

<table>
<thead>
<tr>
<th>Apo B, g/L, in serum</th>
<th>BSA, mg/L</th>
<th>Un- treated</th>
<th>Treated with lipase</th>
<th>Treated with lipase and BSA</th>
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<tr>
<td>0.65</td>
<td>80</td>
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<tr>
<td>1.20</td>
<td>80</td>
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<tr>
<td>1.50</td>
<td>90</td>
<td>0.85</td>
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<tr>
<td>2.20</td>
<td>110</td>
<td>1.10</td>
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<tr>
<td>3.75</td>
<td>1.20</td>
<td>1.60</td>
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<tr>
<td>5.30</td>
<td>1.40</td>
<td>1.70</td>
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<td>1.70</td>
<td>1.90</td>
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<td>2.30</td>
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<tr>
<td>11.50</td>
<td>2.00</td>
<td>2.60</td>
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Tg, triglycerides. *ml of BSA, 100 g/L. *By chemical analysis.