With another batch of Phadebas tablets with BSA (CE 0311) the same phenomenon was observed. Again the enzymic activities determined in the presence of BSA were lower than in the absence of BSA, while the absorbance values were quite the same.

Evidently this difference in activity is due to the converting of absorbances into activities with help of the standard curve supplied by the manufacturer.

The reported normal upper limit of serum amylase is 300 U/liter. In the determination of amylase with BSA the upper limit will be 260 U/liter, as calculated from the results shown in Figure 1. It can be argued that this difference will not be clinically important, because in pathological conditions amylase activities increase far beyond normal.

I thank Gist-Brocedes Farmaca, Nederland, for gifts of Phadebas amylase tablets.

References

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Pitfalls of Use of Lipemic Serum with the Technicon SMAC and Du Pont aca

To the Editor:

In general, lipemia of serum is caused by increased concentrations of the very-low-density lipoproteins (VLDL) and chylomicrons.

In these cases photometric analysis can be disturbed or even impossible, especially measurements of enzyme activities at short wavelengths. For the diagnosis of, e.g., liver disease and myocardial infarct, often accompanied with hyperlipoproteinemia, the accuracy of the enzyme activities is important. Therefore we examined the effect of lipemia on the results of some clinical chemical tests performed with our Technicon SMAC and Du Pont aca.

We studied this effect by three different methods:
(a) VLDL were removed from lipemic serum samples by precipitation with polyanionic compounds (magnesium chloride/sodium heparin mixture) according to the method of Lambrecht and Seidel (1). The results are given in Table 1.

(b) “Intralipid” (a fat emulsion used in clinical practice for intravenous nutrition) was added in increasing concentrations to clear serum samples. The results are presented in Table 2.

(c) VLDL were removed by ultracentrifugation (Heraeus-Christ Omega; 45 000 rpm, 35 min, 100 000 × g at the top of the tube), washed twice in distilled water to remove traces of serum, and added in increasing concentrations to clear serum samples. The results are given in Table 3.

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Table 1. Results for 10 Clear and 15 Lipemic Sera Before and After Treatment with MgCl₂/Sodium Heparinate Mixture

<table>
<thead>
<tr>
<th>Component</th>
<th>Unit</th>
<th>Appearance of serum</th>
<th>X(B)</th>
<th>X(A)</th>
<th>X ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate</td>
<td>U/I</td>
<td>clear</td>
<td>229.9</td>
<td>233.7</td>
<td>0.98 ± 0.02</td>
</tr>
<tr>
<td>dehydrogenase</td>
<td></td>
<td>lipemic</td>
<td>202.1</td>
<td>237.9</td>
<td>0.85 ± 0.06</td>
</tr>
<tr>
<td>Aspartate</td>
<td></td>
<td>clear</td>
<td>73.8</td>
<td>76.8</td>
<td>0.92 ± 0.10</td>
</tr>
<tr>
<td>aminotransferase</td>
<td></td>
<td>lipemic</td>
<td>27.6</td>
<td>27.9</td>
<td>1.02 ± 0.11</td>
</tr>
<tr>
<td>Alanine</td>
<td></td>
<td>clear</td>
<td>70.3</td>
<td>71.0</td>
<td>0.99 ± 0.13</td>
</tr>
<tr>
<td>aminotransferase</td>
<td></td>
<td>lipemic</td>
<td>22.8</td>
<td>27.9</td>
<td>0.81 ± 0.11</td>
</tr>
<tr>
<td>Bilirubin, total</td>
<td>µmol/l</td>
<td>clear</td>
<td>15.9</td>
<td>14.0</td>
<td>1.16 ± 0.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>lipemic</td>
<td>8.7</td>
<td>9.1</td>
<td>1.00 ± 0.08</td>
</tr>
</tbody>
</table>

Results for urea, uric acid, alkaline phosphatase, calcium (after correction for magnesium interference), inorganic phosphate, total protein, and iron from the SMAC showed no clinically important influence of VLDL removal.

a aca. Others are SMAC determinations.

b Concs. too low for definitive conclusions.

Table 2. Results with and without Increasing Concentrations of Intralipid, Expressed as Percentages of the Concentrations or Activities Measured in Sera without Intralipid

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration of Intralipid</th>
<th>X(A)</th>
<th>X(B)</th>
<th>Greatest difference (\Delta X)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate</td>
<td></td>
<td>100</td>
<td>106</td>
<td>6</td>
</tr>
<tr>
<td>dehydrogenase</td>
<td></td>
<td>100</td>
<td>104</td>
<td>0</td>
</tr>
<tr>
<td>Aspartate</td>
<td></td>
<td>100</td>
<td>104</td>
<td>0</td>
</tr>
<tr>
<td>aminotransferase</td>
<td></td>
<td>100</td>
<td>98</td>
<td>2</td>
</tr>
<tr>
<td>Alanine</td>
<td></td>
<td>100</td>
<td>98</td>
<td>2</td>
</tr>
<tr>
<td>Bilirubin, total</td>
<td></td>
<td>100</td>
<td>98</td>
<td>2</td>
</tr>
<tr>
<td>Bilirubin, direct</td>
<td></td>
<td>100</td>
<td>98</td>
<td>2</td>
</tr>
<tr>
<td>Albumin</td>
<td></td>
<td>100</td>
<td>98</td>
<td>2</td>
</tr>
</tbody>
</table>

Results for sodium, potassium, chlorine, urea, creatinine, uric acid, alkaline phosphatase, calcium, inorganic phosphate, total protein, cholesterol, and iron from the SMAC showed no clinically important influence of added Intralipid.

\(\Delta X = X(B) - X(A)\)

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Table 3. Results with and without Added Lipoprotein

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration with or without lipoprotein (n = 5)</th>
<th>Greatest difference</th>
<th>Added lipoprotein</th>
<th>Value found, % of original</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate dehydrogenase</td>
<td>390.0 U/l</td>
<td>-138</td>
<td>100 90 83 78 59</td>
<td></td>
</tr>
<tr>
<td>Aspartate</td>
<td>344.0 U/l</td>
<td>-18</td>
<td>100 100 101 101 100</td>
<td></td>
</tr>
<tr>
<td>Alanine</td>
<td>60.7 U/l</td>
<td>+11</td>
<td>100 102 106 110 116</td>
<td></td>
</tr>
<tr>
<td>Alaminotransferase</td>
<td>61.5 μmol/l</td>
<td>-3</td>
<td>100 97 97 99 98</td>
<td></td>
</tr>
<tr>
<td>Bilirubin total</td>
<td>36.0 μmol/l</td>
<td>+7</td>
<td>100 99 102 111 112</td>
<td></td>
</tr>
<tr>
<td>Bilirubin direct</td>
<td>24.5 μmol/l</td>
<td>-2</td>
<td>100 100 100 100 99</td>
<td></td>
</tr>
<tr>
<td>Albumin</td>
<td>36.5 g/l</td>
<td>-2</td>
<td>100 102 102 102 101</td>
<td></td>
</tr>
</tbody>
</table>

Results for sodium, potassium, chloride, urea, creatinine, uric acid, alkaline phosphatase, calcium, inorganic phosphate, total protein, and iron from the SMAC showed no clinical important influence of added lipoprotein.

* Greatest difference between results with and without lipoprotein preparation.
* Appearance of serum samples, respectively: clear, slightly turbid, turbid, very turbid, and like milk.
* These series of values from aca; others from SMAC.

From the results we draw the following conclusions:

1. Lipemia in serum has no important effect on the results of several tests on the SMAC (Tables 1, 2, and 3).
2. Lactate dehydrogenase (EC 1.1.1.27) activities measured with the SMAC are strongly decreased by lipemia (deviation 15 to 100%; measured with the aca, the activities of lactate dehydrogenase are hardly decreased (deviation 0 to 3%)).
3. Albumin concentrations as measured with the SMAC can be strongly increased by lipemia (deviation 1 to 38%), only slightly decreased (deviation 0 to 6%) as measured with the aca.
4. Total and direct bilirubin concentrations as measured with the SMAC can be decreased (deviation to about 20%); as measured with the aca the bilirubin concentrations can be even more influenced by lipemia (increase to about 45%).
5. Aspartate aminotransferase (EC 2.6.1.1) and alanine aminotransferase (EC 2.6.1.2) activities as measured with the SMAC can be increased (deviation to about 35%). Recently Technicon advised that, because of this phenomenon, the user should substitute "clearing agent" for the "Pegesperse.

We think this information is useful to all SMAC users. Lipemic serum samples can easily be cleared by precipitation of VLDL with polyionic compounds (1). However, doing this precures measurements of sodium, potassium, chloride, calcium, creatinine, cholesterol, and triglycerides with SMAC, because of interference.

References


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Another Source of Contamination from Blood-Sample Containers

To the Editor:

The use of nitrogen–phosphorus detection (NPD) with gas–liquid chromatography (GLC) has eliminated interferences formerly encountered when body-fluid extracts are analyzed with flame ionization detectors. Pthalate plasticizers, and blood constituents such as methylated fatty acids and cholesterol are not significantly detected by NPD systems.

In contrast, contaminants containing nitrogen or phosphorus can exert considerable interference. A number of authors (1–3) using GLC-NPD systems have referred to interference from the plasticizer tributyoxyethyl phosphate, encountered in the use of Vacutainer Tubes (Becton-Dickinson).

We have recently encountered another contaminant, basic in character, and with a retention time identical to that of methadone on a 3% OV-17 column. On a 3% OV-1 column the compound had a retention time of 0.82 relative to that of methadone.

Gas chromatography–mass spectrometry (GC-MS) of the compound gave the following spectrum, recorded according to the method of Finkle et al. (4).

Base peak: 211, 40, 58, 67, 77, 91, 105.5, 119, 139, 154, 167, 183, 195, 211, 226. This spectrum was identical to that of N-isopropyl-N'-phenyl-p-phenylenediamine (I), a compound used commercially as an anti-oxidant. An authentic sample (gift from Dunlop N.Z. Ltd) proved to be identical by GC-MS with the contaminant.

\[ \text{\begin{align*}
\text{\begin{array}{c}
\text{N} \\
\text{H} \\
\text{C} \\
\text{H} \\
\end{array}}\end{align*}} \]

The source of contamination was traced to the natural rubber seals inserted in the aluminum caps of bottles used for the storage of blood samples taken for law-enforcement alcohol analyses. The anti-oxidant in this rubber leached into blood samples, and when we extracted various blood samples by our routine Celite method (5) we recovered concentrations of anti-oxidant ranging from 0.03 to 0.46 μg/ml.

Extraction of blood samples supplemented with the anti-oxidant (1.0 and 12.0 μg/ml) gave low recoveries of anti-oxidant (5 and 10%, respectively) initially, and nil recoveries after two months of storage in glass-stoppered flasks at 4 °C. At the same time, a considerable darkening of the blood was observed. This darkening had previously been observed for blood samples stored in the blood-alcohol bottles, and could be explained by reaction of the anti-oxidant with oxyhemoglobin, causing the anti-oxidant to be destroyed in the process.

References