in the morning plasma and urine from 28 healthy men, mean ages 24.3 (SD 3.5) years, body weight 68.3 (SD 9.5) kg, and height 176 (SD 6.7) cm. Plastic syringes and tubes, free of contamination, were used. Blood collected in heparinized tubes was centrifuged to obtain plasma. All samples were stored at −20 °C for about two months until the day of the determination.

The sample preparation consisted only of diluting the samples sixfold: To 300 μL of plasma or urine, we added 1500 μL of a diluent containing HNO₃, 10 mL/L, and europium, 25 μg/L, used as an internal standard.

The spectrometer we used was a Nermag prototype (Argenteuil, France), consisting of a plasma source, tuned-line type, 56 MHz, from Durr (Aliberville, France), with a Fassel-type torch, a Meinhard nebulizer, and a Scott chamber obtained from Jobin-Yvon (Longjumeau, France). Argon gas flow rates, controlled by a Brooks Model 5878 mass flowmeter, were 12 L/min for outer gas and 0.75 L/min for nebulizer flow. The mass spectrometer, a specially modified Nermag Model R1010C, was equipped with a two-cone interface, three independent pumping stages, a quadrupole inlet ion optic, a 350-mm quadrupole mass analyzer, an analog mode of detection with a photonic converter, and an IBM AT data system. The inlet consisted of a Model 231 autosampler and Model Minipulse 3 peristaltic pump (Gilson, Villiers-Le-Bel, France) set at 1 mL/min uptake.

The selected ions from La, Ce, Gd, Tb, Yb, and Eu (internal standard) were measured at m/z = 139, 140, 156, 159, 174, and 153, respectively. The background signal, measured at m/z = 125.5, was automatically subtracted by the computer from each ion signal.

The detection limits (μg/L), with a confidence interval of 1 SD, were 0.006, 0.008, 0.020, 0.003, and 0.011 for La, Ce, Gd, Tb, and Yb, respectively, in agreement with the results recently published by Lichte et al. (3), who used a commercially available instrument and the same confidence interval.

The concentrations of La, Ce, Gd, Tb, and Yb in the plasma and urine of the 26 healthy subjects were all <0.3 μg/L, except one urine, which contained cerium at 1.5 μg/L.

The analytical recoveries obtained after addition of each of the rare earth elements, 5 μg/L, to six urines and plasmas (Table 1) demonstrate the ability of the method to accurately measure low concentrations.

In conclusion, we found that the concentrations of the rare earth elements measured—La, Ce, Gd, Tb, and Yb—in both plasma and urine of healthy subjects were <0.3 μg/L. Our results are in better agreement with the general data about the concentrations of rare trace elements in biological samples than those of Esposito et al. We suggest that the high concentrations they found came from a contamination.

Table 1. Analytical Recovery of Rare Earth Elements Added to Plasma and Urinea

<table>
<thead>
<tr>
<th></th>
<th>Mean (SD) measured, μg/L, in plasma</th>
<th>Mean (SD) measured, μg/L, in urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>La</td>
<td>5.04 (0.07)</td>
<td>5.43 (0.15)</td>
</tr>
<tr>
<td>Ce</td>
<td>5.08 (0.16)</td>
<td>5.60 (0.25)</td>
</tr>
<tr>
<td>Gd</td>
<td>4.83 (0.17)</td>
<td>5.07 (0.09)</td>
</tr>
<tr>
<td>Tb</td>
<td>4.80 (0.08)</td>
<td>5.22 (0.04)</td>
</tr>
<tr>
<td>Yb</td>
<td>4.90 (0.16)</td>
<td>5.20 (0.13)</td>
</tr>
</tbody>
</table>

a Recoveries were determined the same day after addition of 5 μg/L of rare earth to six different plasmas and urines.

References

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Variations in Measured Alkaline Phosphatase Activity: Influence of Isoenzymes and Buffer Systems

To the Editor:

Evaluating the Kodak Ektachem 700XR automated analyzer (Eastman Kodak Co., Rochester, NY), we noticed a peculiar phenomenon when measuring the activity of total alkaline phosphatase (ALP, EC 3.1.3.1). We compared the Kodak values obtained at 37 °C with those obtained at 25 °C with the Hitachi 705 analyzer (Boehringer Mannheim, Mannheim, F.R.G.) and the alkaline phosphatase kit from Baker Chemicals (Deventer, Holland). ALP activity measured with the Kodak analyzer (x) correlated with those measured with the Hitachi (y): y = 1.34x − 3 U/L. Results by both methods showed very good agreement (r = 0.99) for sera with a broad range of activities from 25 patients (selected without conscious bias). However, for other samples, we occasionally found differences >100 U/L between the two measurement techniques. Agarose electrophoresis with the substrate bromochlorindolyl phosphate (Isopal; Beckman, Analis, Belgium) (1) revealed high intestinal ALP activity in these latter samples. This finding agrees with the recently published results of Gorus and De Pree (2) regarding the effect of isoenzyme composition on Kodak Ektachem test results.

Because intestinal ALP is often present in sera from patients with terminal renal failure being treated with hemodialysis (3), we analyzed by both methods 40 samples from dialyzed patients. The overall coefficient of correlation between these results was low (r = 0.88); paired results for 11 samples (group 1) differed by >30 U/L (mean difference 70 U/L), whereas the mean difference between the two techniques for the remaining 29 samples (group 2) was only 9 U/L.

Table 1 summarizes the results of the ALP isoenzyme electrophoresis for group 1. Seven samples had a high intestinal ALP activity, but four had no considerable intestinal ALP activity (<20 U/L, measured with Baker reagents). However, two of the latter (patients 3 and 9) had a complex of ALP with an immunoglobulin (Ig). On the contrary, in the study by Gorus and De Pree (2), the presence of an Ig-ALP type of macro ALP in six samples did not appreciably influence the test results. Of the two other samples in group 1 that lacked intestinal ALP activity, one had a high-M fraction (patient 11), and one had a normal isoenzyme pattern (patient 10).

Only one of the 29 samples in group 2 had intestinal ALP >25 U/L (mean 9 U/L), none had an immunoglobulin-bound complex with ALP, and none had a high-M fraction >10 U/L.

Both systems use the same substrate, p-nitrophenyl phosphate (PNPP), but different buffers: The
Table 1. ALP Activities and ALP Isoenzyme Patterns in Sera of Dialyzed Patients with Discrepant ALP Measurements >30 U/L

<table>
<thead>
<tr>
<th>Patient</th>
<th>Kodak ALP acty, U/L</th>
<th>Baker ALP acty, U/L</th>
<th>Δ</th>
<th>IALP</th>
<th>High-M, ALP</th>
<th>Ig-ALP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>245</td>
<td>152</td>
<td>93</td>
<td>75</td>
<td>0</td>
<td>0</td>
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<tr>
<td>2</td>
<td>225</td>
<td>146</td>
<td>73</td>
<td>64</td>
<td>0</td>
<td>0</td>
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<tr>
<td>3</td>
<td>147</td>
<td>96</td>
<td>57</td>
<td>17</td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td>4</td>
<td>287</td>
<td>188</td>
<td>105</td>
<td>63</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>209</td>
<td>160</td>
<td>49</td>
<td>45</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>257</td>
<td>155</td>
<td>102</td>
<td>35</td>
<td>3</td>
<td>67</td>
</tr>
<tr>
<td>7</td>
<td>289</td>
<td>250</td>
<td>39</td>
<td>61</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>147</td>
<td>111</td>
<td>36</td>
<td>28</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>170</td>
<td>128</td>
<td>42</td>
<td>15</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>10</td>
<td>221</td>
<td>130</td>
<td>91</td>
<td>0</td>
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<tr>
<td>11</td>
<td>190</td>
<td>114</td>
<td>76</td>
<td>7</td>
<td>11</td>
<td>0</td>
</tr>
</tbody>
</table>

IALP, intestinal ALP; Ig-ALP, complex of ALP with an immunoglobulin.

Baker kit contains a diethanolamine (DEA) buffer; the Kodak slides, 2-amino-2-methyl-1-propanol (AMP). Gorus and De Pree (2) used reagents (Boehringer Mannheim) that contain PNPP as a substrate and a DEA buffer. DEAA activates ALP activity (4, 5), but apparently intestinal ALP is activated less than is bone and liver ALP. This was also found by Van Belle (4), who compared DEA with N-ethylaminoethanol buffer, and by Stinson et al. (5), who compared several phopsphoacceptors. Consequently, depending on the amount of intestinal ALP present, total ALP activity and, hence, all of its isoenzymes are more or less underestimated when DEA buffer is used as the phoshoacceptor.

We wanted to emphasize the clinical implications of these findings, especially in dialyzed patients and patients with liver cirrhosis, two conditions that have long been known to present a high incidence of intestinal bands (3, 6). We also found that the presence of ALP complexed with an immunoglobulin leads to higher ALP activities measured by the Kodak method. Finally, when intestinal ALP and high-M, ALP were within reference values (7), we observed no influence of the buffer on the determination of total ALP activity. For correct evaluation of the ALP isoenzyme activities in these patients, we recommend a method with an AMP buffer system.

References

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We received the following comments regarding this Letter:

To the Editor:

I have read the above comments by Van Hoof et al. with great interest. Their findings in hemodialysis patients are to a large extent in agreement with previous reports by us (1) and others (2), indicating that intestinal alkaline phosphatase activity (ALP; EC 3.1.3.1) is relatively more recognized by Ektachem slides (Eastman Kodak Co., Rochester, NY) than with certain conventional liquid reagents. The authors suggest that the different buffers used in the compared methods are responsible for the observed discrepancies. Wallinder et al. (2) have already raised this possibility previously and verified experimentally that this explanation is, to a large extent, correct in the presence of intestinal and placental ALP.

Van Hoof et al. also measured relatively higher ALP activities on Kodak Ektachem in six samples containing various amounts of macromolecular ALP forms. These results are at variance with our data on nine patients with high activities of circulating macro ALP (1). In five of six sera from the Antwerp group, low activities of macro ALP coincided with various amounts of intestinal ALP. The latter activities are likely to at least partially explain the observed differences. In the remaining sample the more important ALP-immunoglobulin (Ig) activity is probably better recognized by Ektachem slides than by Baker reagents. In view of the well-known molecular heterogeneity of enzyme-Ig complexes (1, 2, 3, 4), some of these entities might behave differently in various assays, as compared with the macro ALP-containing sera tested by us, one theoretical possibility being the occasional occurrence of intestinal ALP-Ig complexes. The impact of the different assay temperatures (25 °C vs 37 °C) and buffers—diethanolamide (DEA) for Baker and 2-amino-2-methyl-1-propanol (AMP) for Kodak—used in the comparison could also be investigated to reconcile divergent observations in the presence of ALP-Ig.

The available information indicates that Ektachem slides for ALP measure intestinal and placental forms with higher sensitivity than, and macro ALP with at least equal sensitivity as, a liquid reagents assay with a DEA buffer. Van Hoof et al. rightly emphasize the clinical relevance of these findings for patients treated with hemodialysis and in other conditions often accompanied by increased intestinal ALP activity. Generally, the frequent association of circulating intestinal, placental, and macro ALP with specific (patho)physiological conditions (5) is noted in my opinion, a practical advantage to AMP-buffered reagents (including dry-chemistry slides). Indeed, by more frequently yielding increased total ALP activities in samples with the above-mentioned ALP forms, these methodologies will more easily herald associated clinically relevant states. On the other hand, the majority of sera contain only