Quantification of Bone Alkaline Phosphatase in Serum by Precipitation with Wheat-Germ Lectin: A Simplified Method and Its Clinical Plausibility

Werner Behr1 and Jürgen Bamert2

We report an easy, rapid method for quantifying bone isoenzyme of alkaline phosphatase (EC 3.1.3.1., ALP) in serum. The original method described by Rosalki and Ying Foo (Clin Chem 1984;30:1182–6) was somewhat simplified. In contrast to their results, we found that bone ALP is precipitated quantitatively by wheat-germ lectin. To check the clinical plausibility of the method, we used samples from several comparison groups (blood donors, children, pregnant women, patients with neoplasms but without skeletal involvement) and a large number of patients suffering from bone diseases and diseases of the liver and biliary tree. Measured activities of bone ALP nearly always correlated with the clinical diagnosis. Only patients with hepatitis often had pathological bone activities not in accord with the other findings. Possible reasons for this observation are discussed.

Additional Keyphrases: isoenzymes  variation, source of bonedisease  hepatitis

The great majority of requests for isoenzyme analysis of alkaline phosphatase [EC 3.1.3.1., orthophosphoric-monoester phosphohydrolase (alkaline optimum); ALP] arise from the need to distinguish between liver and bone as alternative or coexisting sources of an increased ALP activity in serum (1). At present there is no totally satisfactory method for quantifying these isoenzymes. The simple heat-inactivation test is susceptible to interference (2) and gives only a qualitative or semiquantitative estimate of bone ALP (1). The improved sequential heat-inactivation procedure (3) gives quantitative results, but is too tedious and impractical for use with large numbers of samples; moreover, scrupulous procedural control is essential for even moderate reproducibility (4). Electrophoresis is convenient only for qualitative examination of bone and liver phosphatases, because the overlap between the peaks makes difficult their quantification by densitometry, even though this may be suitable for measuring biliary and intestinal ALP activities (1, 4).

In 1984, Rosalki and Ying Foo described a new method for quantifying bone ALP (4). They used wheat-germ lectin to precipitate the bone isoenzyme. We re-examined the reliability of this easy and rapid method, investigated the extent of bone ALP precipitation, and simplified the test procedure.

Materials and Methods

Clinical Specimens

Serum samples were obtained from: (a) 40 male and 40 female healthy blood donors; (b) 20 healthy newborns (cord blood); (c) 20 children without hepatobiliary or bone diseases; (d) 10 healthy pregnant women in the third trimester; (e) 276 patients with different malignant tumors and 46 patients with bone pain or arthralgia (bone scans and radiographic bone surveys revealed no pathological findings or only showed degenerative changes or changes typical of rheumatism); and (f) 256 patients in whom a determination of bone ALP was requested for clarifying the cause of increased total ALP in serum or because a skeletal disease was suspected despite total ALP activity within normal limits.

The examinations were usually performed within 4 h of specimen collection; if not, the serum was stored at −18 °C. For calculating the between-day precision we prepared a pool of sera with increased bone ALP and stored aliquots at −18 °C. To check the linearity, we diluted with saline some serum from a patient having extensive bone metastases, but no clinical or chemical clinical sign of hepatobiliary disease.

Measurement of Total ALP Activity

We determined total ALP activity at 25 °C according to the recommendation of the German Society of Clinical Chemistry (5), using p-nitrophenyl phosphate substrate and diethanolamine buffer (Merck, Darmstadt, F.R.G.; nos. 14 000 and 14 001). The method was adapted to the Hitachi 705 analyzer (Boehringer, Mannheim, F.R.G.). In our laboratory the normal reference interval for adults is 40–190 U/L.

Precipitation and Calculation of Bone ALP Activity

The precipitation procedure was performed as described by Rosalki and Ying Foo (4), the only modification being a doubling of the quantity of serum and reagents for technical reasons (Hitachi 705 analyzer). All samples were pretreated by incubating 100 μL of serum for 30 min at 37 °C with 10 μL of a 20 g/L solution of Triton X-100 surfactant (Merck) in distilled water. After this pretreatement, we mixed the sample with 100 μL of an aqueous solution of wheat-germ lectin (5 g/L in distilled water) and incubated again for 30 min at 37 °C. Wheat-germ lectin was obtained as a lyophilized powder from Boehringer Mannheim Biochemica (cat. no. 1 660 061; we used reagent from lots 1026320-11, 10300520-12, and 1053820-16); after reconstitution it is stable for at least a month at 2–8 °C.

After the second incubation, the serum mixture was centrifuged (2000 × g) for 10 min. Without disturbing the precipitate, we removed the supernatant fluid, determined its ALP activity, and multiplied the measured value by 2.1 to correct for sample dilution. Bone ALP activity was calculated by subtracting this corrected value from the total ALP activity. For calculating the total analytical recovery of isoenzyme activities we resuspended the precipitate in 200 μL of isotonic saline and determined its ALP activity. Combining the activities of the precipitate and supernatant...
and adjusting for sample dilution gave a value for the total recovery of isoenzyme activity.

**Gel Filtration**

For gel-filtration studies we used Ultrogel AcA 34, a polyacrylamide–agarose gel (LKB Instrument GmbH, Gräfeling, F.R.G.), prepared and equilibrated in a 1.6 x 40 cm column with Tris HCl buffer (0.01 mol/L, pH 7.4) containing 1.0 mol of sodium chloride per liter. We applied 1.0 mL of serum and eluted with the Tris–saline buffer at a flow rate of 1 mL/min. The effluent was scanned at 280 nm with an Uvicord II absorbtiometer (LKB Instruments, Inc.) and collected in 1-mL fractions, all of which were measured for ALP activity. The effluent fractions corresponding to the peaks that contained enzymatic activity were pooled and concentrated by ultrafiltration (Amicon B-125 concentrator; Amicon, Oosterhout, The Netherlands).

**Statistical Methods**

We tested the significance of differences between the individual reference groups with the U-test of Wilcoxon, Mann, and Whitney or the Z-test (6). The significance level was set at p = 0.05.

**Results**

**Analytical Variables**

*Analytical recovery.* To calculate the analytical recovery of total ALP activity from precipitates and supernates combined, we examined 30 sera with a total ALP activity of 59 to 4428 U/L (mean 546, SD 101.8 U/L) and a bone ALP activity of 54 to 928 U/L (mean 222, SD 223.8 U/L). The mean figure for total recovery of isoenzyme activity was 99.5% (SD 4.73). The recovery rate was independent of the proportion of the bone or liver isoenzyme fraction.

*Between-day precision.* The between-day precision (n = 20) of the precipitation method was determined with use of a pooled serum specimen having a total ALP activity of 465 U/L and a mean bone ALP activity of 257 U/L (range 218–285). The CV was 6.3%.

*Linearity.* Checking linearity showed that for bone ALP activities > 1200 U/L, the lectin concentration had to be proportionately increased for complete precipitation of bone ALP. We do not recommend diluting sera containing high activities of bone ALP, because the reactivity of diluted sera to wheat-germ lectin is altered.

*Accuracy.* A direct check on accuracy presented problems, because the isoenzymes extracted from organs are not identical with the corresponding isoenzymes in serum (7, 8) and hence show a different precipitation behavior with wheat-germ lectin (8, 9). As an indirect check on accuracy, we mixed two sera having different bone ALP activities (serum I: total ALP/bone ALP = 442/30 U/L; serum II: 504/395 U/L) in various proportions (I:II = 1:9, 2:8, ..., 9:1), then measured bone ALP. The measured activities agreed with the calculated figures (Figure 1).

Cord blood usually contains just one ALP isoenzyme, which corresponds electrophoretically to the bone isoenzyme of adults (10). To investigate the extent of ALP precipitation by wheat-germ lectin, we examined cord blood from 20 neonates, with total ALP activities ranging from 113 to 382 U/L. We could precipitate 99.1% (SD 0.84%) of the ALP activity with lectin from any of three separate reagent batches. However, a fourth batch of lectin precipitated only 72.1% (SD 2.34%) of the ALP activity in cord blood (n = 20).

**Reference Interval**

**Blood donors.** The normal reference interval for bone ALP activity was established by use of data from 40 male and 40 female blood donors (Table 1). The Z-test showed no significant sex-related difference. The ALP values did not show a normal or log-normal distribution, so we determined the reference limits (2.5 and 97.5 percentiles) nonparametrically (11). The calculated reference interval for healthy adults was 35 to 95 U/L, which is comparable with other such published intervals (1, 12, 13).

**Patient groups.** To check the reliability of the method and the validity of the reference interval, we determined bone ALP in various groups of patients (Table 1). For children, we found the expected high bone ALP activities, which are also responsible for the high value for total ALP in children. Slightly increased values for total ALP, probably ascribable to placental ALP, were also found in gravidae. However, the mean bone ALP activities in pregnant women and female blood donors did not differ significantly (p = 0.05); placental ALP apparently does not interfere with the precipitation of bone isoenzyme.

In addition, we measured bone ALP activities in patients with malignant tumors (and in some instances additional fundamental diseases) and in patients with bone and joint pain, all with normal skeleton scintigrams and (where available) roentgenographic findings or with merely degenerative changes or changes typical of rheumatism. Again, the mean bone ALP activities in these patients did not differ significantly (p = 0.05) from those of the blood donors. However, the reference interval for this group of patients, also determined nonparametrically (11), was somewhat broader, 27–107 U/L, presumably ascribable to the slight increase in bone ALP in serum in persons of advanced age (13). Thus, for the subsequent investigations, we set the upper limit of the normal reference interval at 100 U/L.

**Fig. 1. Correlation of the measured and calculated bone ALP activities from serum mixtures having differing proportions of bone and liver isoenzymes**

Using this batch, we measured only relatively low bone ALP activities in sera from patients with osteoblastic bone metastases or Paget's disease, whereas with the other batches of wheat-germ lectin these bone ALP values were appropriately high.
Table 1. Total ALP and Bone ALP Activities in Various Groups of Subjects

<table>
<thead>
<tr>
<th></th>
<th>Healthy blood donors</th>
<th>Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>No.</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Age, yr Range</td>
<td>20–60</td>
<td>20–60</td>
</tr>
<tr>
<td>x</td>
<td>37</td>
<td>37</td>
</tr>
<tr>
<td>SD</td>
<td>11.5</td>
<td>14.3</td>
</tr>
<tr>
<td>Total ALP, U/L</td>
<td>Range</td>
<td>50–137</td>
</tr>
<tr>
<td>x</td>
<td>102</td>
<td>96</td>
</tr>
<tr>
<td>SD</td>
<td>17.4</td>
<td>29.6</td>
</tr>
<tr>
<td>x</td>
<td>61</td>
<td>56</td>
</tr>
<tr>
<td>SD</td>
<td>14.5</td>
<td>18.4</td>
</tr>
</tbody>
</table>

*Without hepatobiliary and osteoarthritis. * In third trimester. *The high total ALP activities in some of the patients are due to accompanying hepatobiliary diseases.

Bone ALP activities >100 U/L in adults were assessed as pathological. However, because of the measurement imprecision, which may be doubled on multiplying the measured value by the dilution factor 2.1, the range from 95 to at least 120 U/L should be regarded as a "borderline zone." At higher ALP values this "borderline zone" is probably even greater.

Checking the Plausibility of the Measured Bone ALP Activities with the Aid of Clinical Diagnoses

To check whether the bone ALP values we obtained via lectin precipitation are plausible with regard to the clinical picture and other findings, we evaluated the case histories of 258 patients in whom bone ALP activities were determined, either to elucidate increased total ALP or because of a suspected skeletal disease in the presence of normal values for total ALP. These patients were classified into the following groups:

Patients with increased bone ALP. Table 2 summarizes results for those 110 patients in whom increased bone ALP activities were caused by generalized or local bone disease. On average, the highest bone ALP activities are found in osteomalacia. In nine patients having cholestasis and clearly increased total ALP (375–1060 U/L), bone ALP values were pathologically increased without accompanying expression of symptoms in clinical examinations, radiological findings, or skeleton scintigraphy. Four of these patients had bone ALP activities in the borderline range (103, 105, 108, and 116 U/L, respectively). The laboratory values and diagnoses of the other five patients are listed in Table 3. The possible causes for these unclearly increased bone ALP activities are debated in the Discussion.

Patients with normal bone ALP and increased total ALP (>190 U/L). In all of these patients (n = 78), a hepatobiliary disease was the cause of the increased total ALP (Table 4). In four of the 10 patients having liver metastases and in two of the 68 patients with cholestasis or liver cell damage, additional skeletal metastases could be demonstrated radiologically and by skeleton scintigraphy. Apart from degenerative changes, no signs of local or generalized bone disease could be found clinically, radiologically, or—where performed—by skeleton scintigraphy in any of the other patients.

Patients with viral hepatitis, cholangiohepatitis, or other forms of hepatitis. In patients with hepatitis of differing origins, we found increased bone ALP activities in more than half of the cases, with no indication of a bone disease being given by clinical, radiological, or other laboratory measurements (Ca²⁺, PO₄³⁻, vitamin D, parathyrin). Therefore we pooled these patients into a separate group. Figure 2 summarizes the bone ALP values measured in the various forms of hepatitis. Immediately obvious is the fact that increased "bone ALP" values were found in all patients with cholangiohepatitis and in six of seven patients with hepatitis B, whereas all patients with Epstein–Barr virus–mediated hepatitis and the greater majority of patients with hepatitis A had bone ALP activities within the normal range.

For some patients, we monitored the increased "bone ALP" activities after a short interval; in all cases, the
Table 3. Diagnoses and Laboratory Values in Five Patients with “Unclear” Increase in Bone ALP

<table>
<thead>
<tr>
<th>Patient:</th>
<th>Sex, age (yr)</th>
<th>Diseases</th>
<th>Total ALP U/L</th>
<th>Bone ALP U/L</th>
<th>γ-GT U/L</th>
<th>LAP U/L</th>
<th>ASAT U/L</th>
<th>ALAT U/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>M.K. F, 74</td>
<td></td>
<td>Breast cancer, obstructive icterus due to calculi and tumor compression</td>
<td>1060</td>
<td>125</td>
<td>282</td>
<td>76</td>
<td>66</td>
<td>46</td>
</tr>
<tr>
<td>R.P. M, 55</td>
<td></td>
<td>Pancreatic carcinoma with liver metastases</td>
<td>653</td>
<td>141</td>
<td>348</td>
<td>—</td>
<td>43</td>
<td>32</td>
</tr>
<tr>
<td>B.L. F, 69</td>
<td></td>
<td>Intrahepatic cholestasis due to medication</td>
<td>940</td>
<td>129</td>
<td>328</td>
<td>108</td>
<td>21</td>
<td>44</td>
</tr>
<tr>
<td>E.S. M, 28</td>
<td></td>
<td>Testicular tumor with liver metastases</td>
<td>990</td>
<td>278</td>
<td>143</td>
<td>56</td>
<td>45</td>
<td>57</td>
</tr>
<tr>
<td>A.R. F, 74</td>
<td></td>
<td>Bile duct carcinoma with obstruction of choledochus</td>
<td>375</td>
<td>123</td>
<td>430</td>
<td>564</td>
<td>59</td>
<td>64</td>
</tr>
</tbody>
</table>

γ-GT, gamma-glutamyltransferase (EC 2.3.2.2); LAP, leucine aminopeptidase (EC 3.4.11); ASAT, aspartate aminotransferase (EC 2.6.1.1); ALAT, alanine aminotransferase (EC 2.6.1.2).

Table 4. Patients with Above-Normal Total ALP and Normal Bone ALP

<table>
<thead>
<tr>
<th>Clinical picture</th>
<th>No.</th>
<th>Total ALP, U/L, range (and X ± SD)</th>
<th>Bone ALP U/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver metastases</td>
<td>10</td>
<td>220–1500 (547 ± 438.8)</td>
<td>36–95 (69 ± 19.1)</td>
</tr>
<tr>
<td>Extrahepatic and intrahepatic cholestasis and liver cell damage from various causes*</td>
<td>68</td>
<td>209–1242 (343 ± 195.2)</td>
<td>19–96 (60 ± 22.0)</td>
</tr>
</tbody>
</table>

*Congestion of the biliary tract by calculi or tumors, drug-mediated intrahepatic cholestasis, nutritive-toxic liver damage, fatty liver, liver cirrhosis and perihepatic abscesses, but not cholangiohepatitis, viral, alcohol-mediated, autoimmune, or halothane-induced hepatitis.

previous values were confirmed. The possibility of mix-ups or technical errors as the cause of increased "bone ALP" values can therefore be ruled out.

Gel filtration. We fractionated by gel filtration the serum of a patient with hepatitis B and the serum of a patient with halothane-induced hepatitis. The total ALP/bone ALP activities were 235/180 and 253/163 U/L, respectively. The pathological values for bone ALP activities were not in accord with the clinical, radiological, or laboratory findings. In both cases, gel filtration showed a very small ALP activity peak excluded from Ultrogel AcA 34 (Figure 3), corresponding to the ALP fraction found in hepatic bile or in sera from patients with extrahepatic obstructions (14) and formed by complexing with membrane fragments (2, 15). The main activity was observed in the second peak, i.e., the 7S protein fraction (14). In both cases the pooled and concentrated mean peak contained a greater proportion of bone ALP than

![Graph showing Hepatitis type A, Hepatitis type B, Non A, Non B - hepatitis, Infectious mononucleosis, Cholangitis, Other types of hepatitis with ALP activity [U/L]](image)

**Fig. 2.** Bone ALP activities determined by lectin precipitation in patients with hepatitis (n = 61)

**Table 4.** Patients with Above-Normal Total ALP and Normal Bone ALP

- **Clinical picture**: Liver metastases, Extrahepatic and intrahepatic cholestasis and liver cell damage from various causes.
- **Bone ALP**: Normal levels.
- **Total ALP**: Above-normal levels.

**Table 3.** Diagnoses and Laboratory Values in Five Patients with “Unclear” Increase in Bone ALP

- **Patient**: M.K., R.P., B.L., E.S., A.R.
- **Diseases**: Breast cancer, obstructive icterus, Pancreatic carcinoma, Intrahepatic cholestasis, Testicular tumor, Bile duct carcinoma.
- **Laboratory Values**: Total ALP, Bone ALP, γ-GT, LAP, ASAT, ALAT.

**Graph**: ALP activity [U/L] vs Reference interval for Hepatitis type A, Hepatitis type B, Non A, Non B - hepatitis, Infectious mononucleosis, Cholangitis, Other types of hepatitis.

**Fig. 2**: Bone ALP activities determined by lectin precipitation in patients with hepatitis (n = 61).
did the unfractonated serum, i.e., 93.7 and 94.1% vs 76.7 and 64.6%, respectively.

We also fractionated by gel filtration the serum of a patient with extrahepatic obstruction, normal bone ALP, and high total ALP (1563 U/L); a main peak and a small 7S peak were excluded from the Ultrogel AcA 34. In this case, too, more than 90% of the ALP activity of both peaks was precipitated by wheat-germ lectin. A possible explanation for this phenomenon is discussed below.

**Discussion**

Lectins are proteins that react with certain carbohydrate structures; e.g., wheat-germ lectin reacts with N-acetylgalactosamine (16) and N-acetylneuraminic acid (5). ALP is a glycoprotein, with the bone and liver isoenzymes probably having the same structural gene (1). The differences between the two isoenzymes are apparently ascribable to posttranslational modifications of the carbohydrate side-chains (1). Furthermore, the isoenzymes circulating in serum differ from those extracted from the respective organs (7, 8), possibly because membrane-localized glycosyltransfereases might modify the enzyme molecule as it passes into the intravascular space (8). The isoenzyme isolated from liver and plasma and the ALP extracted from bone are bound by wheat-germ lectin (8, 9), whereas hepatobiliary and placental ALP in serum are obviously not precipitated. The method described by Rosalki and Ying Foo (4) for the quantification of bone ALP in serum is based on this differing behavior.

Our results agree with those of Rosalki and Ying Foo (4) with regard to the precision and linearity of the method. In contrast to these workers, however, we found that the recovery rate of ALP is independent of the proportion of the bone or liver isoenzyme fraction, and we observed no activation of ALP activity by wheat-germ lectin. Hence, after appropriate correction for dilution, we calculate the bone ALP from the difference between the total ALP activity and the activity in the supernate. This simplifies the determination, given the difficulty of complete removal of the supernate and resuspension of the precipitate. Incomplete resuspension is difficult to detect and yields values for bone ALP that are too low.

On the basis of the quantitative precipitation of the ALP activity in cord serum, we presume that bone ALP is completely precipitated by wheat-germ lectin. In contrast, Rosalki and Ying Foo (4) found that only about 80% of the bone ALP activity was precipitated. They obtained this value by comparing the lectin precipitation method with sequential heat-inactivation (3). This procedure is not correct, in our opinion, because sequential heat-inactivation is not an absolutely accurate method for the quantitative determination of bone ALP (2). An additional argument against only partial precipitation of bone ALP is the fact that in some sera from children more than 80% of the total ALP activity was precipitable by wheat-germ lectin. Multiplication of the precipitate activity by 1.25—as recommended by Rosalki and Ying Foo (4)—would therefore give a value for the bone ALP component clearly greater than that of the total ALP activity. In this regard our results agree with recently published observations (17).

It could also be that Rosalki and Ying Foo used a batch of lectin that did not completely precipitate bone ALP (17). To achieve reproducible results, we recommend that each batch of wheat-germ lectin be checked for complete precipitation of ALP activity (~98–99%) by using cord sera.

One of our objectives was to check the accuracy of the method. As mentioned above, defined enzymes extracted from various organs cannot be used for this. We therefore compared the measured bone ALP activity with the clinical diagnosis in a large number of patients. In almost all cases the bone ALP values agreed with the results of case history, physical examination, and additional technical investigations. The only exceptions were patients with hepatitis. In more than half of these cases, use of our method produced obviously falsely increased bone ALP activities. As shown by gel filtration, this "pseudo-bone ALP" does not consist of
high-\(M\), complexes of ALP and membrane fragments, but rather an ALP fraction having a molecular mass corresponding to that of liver or bone ALP, i.e., 225 and 180 kDa, respectively (18).

Perhaps pseudo-bone ALP is a result of the increased liver cell permeability and cytolysis during hepatitis, so that the ALP fraction from the hepatocytes increases (18). In the ALP isoenzyme forms that subsequently appear in serum, the carbohydrate component has not been modified by the above-mentioned membrane glycosyltransferases; they correspond to the hepatobiliary ALP that can be extracted from liver tissue and is precipitated by wheat-germ lectin. This theory is supported by the observation that four of the five patients with cholestasis and unclear increase of bone ALP had increased transaminases and de Ritis quotients \(>0.7\) (12), indicating enlarged necrosis of liver cells. As counter-evidence, however, the patients with hepatitis had transaminase activities and de Ritis quotients that did not correlate with the occurrence of "pseudo-bone ALP." Moreover, numerous patients with cirrhosis or other liver diseases and increased transaminase activities or de Ritis quotients did not have pathological values for bone ALP.

The numbers and intensities of ALP bands separated by electrophoresis depend on the differences in carbohydrate and acidic content (1, 2, 8, 9, 18) and vary considerably according to the methods used for extraction from tissues (2, 18). Obviously the carbohydrate side-chains responsible for lectin precipitation may be altered easily. For example, we noted that gel filtration with polyacrylamide-agarose gel clearly increases the proportion of ALP that can be precipitated by lectin.

Bile acids are cytotoxic to hepatocytes (20): they induce the synthesis of ALP in cultured hepatocytes (21), damage the cell membrane, and solubilize the ALP anchored in the membrane (18, 22, 23). Because various bile acids have significantly different effects on the liver plasma membrane (20, 23), the particular bile acids present may also be important. Thus, the action of special bile acids on the membrane-localized ALP could therefore play a decisive role in the formation of "pseudo-bone ALP." Further investigations in this point are necessary.

Mildly pathological bone ALP values, particularly those associated with high total ALP activities, can be ascribed to the imprecision of the method; in particular, a measuring or pipetting error may be doubled (via multiplication by the dilution factor 2.1). This could be the reason for the slightly increased bone ALP value in patient B.L. (Table 3).

The usefulness of bone ALP measurements in the search for skeletal metastases was demonstrated in our observation of 20 patients with breast cancer, bone metastases, and pathologically increased bone ALP but without any sign of liver disease: only nine had increased total ALP (unpublished data). Overall, however, bone ALP is not as sensitive as the skeleton scintigram, only 34 (43.6%) of 78 patients with various tumors and confirmed bone metastases showing pathological bone ALP values (unpublished data).

We conclude that the main clinical significance of the bone ALP determination is to:

- monitor the course and therapy of malignant diseases when the skeletal system is attacked, the isoenzyme determination being characterized by increased sensitivity and organ specificity (1)
- detect a pathological bone ALP activity when metabolic or endocrine osteopathy is suspected
- elucidate unclear increased total ALP activity, particularly when it is associated with only moderately increased gamma-glutamyltransferase and leucine arylamidase activities

As the isoenzyme analysis shows, increased total ALP is not rarely conditioned together by the bone and the hepatobiliary fraction; in 49 of the 110 patients with localized and generalized osteopathy (Table 2) we found an accompanying hepatobiliary disease.

The qualitative determination of bone ALP by precipitation with wheat-germ lectin is a rapid, technically simple method that can be performed in small, nonspecialized laboratories. Although the possible occurrence of falsely increased bone ALP values in acute hepatitis should be taken into account, this does not, in our opinion, substantially restrict the use of the lectin method. The reason for the increase of ALP in hepatitis is generally unequivocal and there should be no need to determine bone ALP.

We thank Dr. M. Tietze for the valuable discussions, Mr. J. Baijerlein for his excellent technical assistance, and Mrs. A. Fleck for her help in compiling the English manuscript.

References

Immunoradiometric Assay with Use of Magnetizable Particles: Measurement of Thyrotropin in Blood Spots, to Screen for Neonatal Hypothyroidism

Kay V. Walte, Glen F. Maberty, Gary Ma, and Creswell J. Eastman

We adapted a commercial immunoradiometric assay (IRMA) to measure thyrotropin in filter-paper blood spots. Two 3-mm blood spots are used for each standard and sample. These are incubated for 2 h with radiolabeled antibody and for 30 min with magnetic antibody, followed by a 10-min separation procedure. Assay sensitivity is 6 milli-int. units/L. Coefficients of variation (precision profile of the standard curve) ranged from 4.3 to 9.6%. The coefficient of correlation (r) between thyrotropin concentrations in the blood spots and in serum was 0.93. Pre-elution of the blood spots is necessary for short incubation time. Short incubation time, little need for specialized equipment, the high precision and sensitivity characteristic of IRMA, and ease of collection, transport, and storage of the blood-spot samples make this assay suitable for neonatal hypothyroidism screening.

The use of whole blood spotted on filter paper cards for estimating thyrotropin (TSH) and thyroxin concentrations in neonates is well established in screening for hypothyroidism (1-4). The method requires only a small blood specimen and thus is especially appropriate for use with neonates and where previous sampling is required (5). Dried blood spotted onto cards is also easy to transport and store. There are numerous in-house and commercially available RIAs for TSH in blood spots (2,6), but most of these require overnight incubation.

Immunoradiometric assays (IRMAs) are more precise and sensitive than RIAs for measuring TSH in blood spots (7). Recently, IRMAs with magnetizable particles have been described (8) and are being marketed for measurement of various compounds, including TSH in serum (9,10). The covalent binding of the magnetic particles to the antibody complex allows separation by use of a magnetic separation tray. This procedure requires little space and equipment and takes only 10 min per sample group.

To facilitate TSH screening, we have combined the ease of collection and storage of samples in the blood-spot methods with the simplicity of the magnetic separation method by modifying a commercially available solid-phase IRMA for TSH ("MAGIC"; Corning Medical and Scientific, Medfield, MA 02052).

Materials and Methods

Materials

Reagents: Blood-spot standards, 125I-labeled antibody to TSH, and goat antibody to human TSH, covalently bound to 0.4-1.5 μm (diameter) paramagnetic particles (silane-coated metal oxide), were obtained from Corning. Controls were made by adding dilutions of human TSH (Pituitary TSH; International Reference Preparation 68/38) to heparinized whole blood to produce a known range of TSH concentrations. Blood spotted onto filter papers (S & S 2992; Schleicher & Schuell, Keene, NH 03431) was dried for several hours at room temperature, then stored at -20 °C. Assay buffer contained, per liter, 25 mmol of phosphate (pH 7.4), 5.0 g of NaCl, and 1.0 g of sodium azide. Each assay tube contained 0.2 int. unit of human chorionic gonadotropin ("Primogonyl"; Schering AG, Berlin/Bergkamen, F.R.G.), to block antibody cross reaction with endogenous gonadotropin.

Apparatus: Incubations were carried out with shaking. A Magnetic Separation Unit, consisting of rows of parallel bar magnets embedded in a plastic base with a removable tube rack, was supplied by Corning. To count sample radioactivity we used a 1260 Multigamma gamma counter (LKB, Turku, Finland).

Procedures

TSH magnetic RIA. Punch two spots (3 mm in diameter) of standards, controls, or unknown samples from the filter

Endocrine Unit, Department of Medicine, Westmead Centre, Westmead, N.S.W. 2145, Australia.
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