Improved Method for Quantitative Determination in Serum of Alkaline Phosphatase of Skeletal Origin


In this quantitative method for detection of skeletal alkaline phosphatase (EC 3.1.3.1) activity in human serum, intestinal and placental alkaline phosphatase activities are recognized by their susceptibility to inhibition by L-phenylalanine, and skeletal and hepatic alkaline phosphatases are distinguished by their different sensitivities to inactivation by heat. Alkaline phosphatase isoenzymes prepared from organ sources may behave differently from the corresponding isoenzymes in serum. Our procedure allows us to include organ-derived internal standards of skeletal, intestinal, and biliary alkaline phosphatase to minimize between-assay variation. In preliminary applications, we have found that (a) total serum alkaline phosphatase activity is extremely variable in post-menopausal osteoporotic subjects and is not a reliable index of skeletal alkaline phosphatase activity; (b) seven osteoporotic patients responding to therapy with sodium fluoride with increased bone formation showed increased skeletal alkaline phosphatase activity in their serum as compared with age-matched controls (p <0.005); and (c) 10 post-menopausal osteoporotic patients responding to therapy with stanozolol with increased total body calcium showed an increase in circulating skeletal alkaline phosphatase activity (p <0.001).

Additional Keyphrases: osteoporosis · rate of bone formation · Paget's disease · organ-specific isoenzymes: activity in organ extracts vs activity in serum · intra-individual variation · reference interval

The AIP activity in normal human serum consists of a mixture of organ-specific isoenzymes derived from intestine, bone, liver, and (sometimes) placenta. Experimental evidence suggests that the amount of skeletal AIP activity in serum may reflect the rate of bone formation (1, 2). However, we cannot establish, or even study, this relationship until we can quantitatively determine the amount of skeletal AIP in serum, in the presence of the other isoenzymes.

The difficulty lies in distinguishing skeletal from hepatic AIP activity. We can easily quantitate the amounts of intestinal and placental AIP because they are uniquely sensitive to inhibition by L-phenylalanine (3), but the skeletal and hepatic isoenzymes are affected by the same inhibitors (e.g., homocarnosine, levamisole) to similar extents (4, 5). However, the latter two isoenzymes can be distinguished by their differential sensitivities to inactivation by heat or denaturation by urea (6, 7).

In adapting the heat-inactivation technique (6) to our studies of skeletal AIP and its role in bone formation, we were confronted with two interrelated problems that affected reproducibility: (a) the crucial heat inactivation was extremely sensitive to changes in the time and temperature of incubation (8, 9), and (b) the inclusion of internal standards to normalize these variations was made less valid by the fact that AIP isoenzymes prepared from organ sources did not always behave the same as their counterparts in serum (6, 10). We have therefore sought to determine the basis for these differences between AIP isoenzymes in organ extracts and the same isoenzymes in serum, with the immediate goal of finding suitable internal standards for a quantitative isoenzyme assay, and the ultimate goal of determining the usefulness of skeletal AIP activity in serum as an index of the rate of bone formation.

Materials and Methods

Chemicals and Supplies

p-Nitrophenyl phosphate (PNPP), levamisole, Tris, L-phenylalanine, NaHCO3, and Na2CO3 were purchased from the Sigma Chemical Co., St. Louis, MO 63178. Cellex-D (DEAE-cellulose) and Bio-Gel A-0.5 were obtained from Bio-Rad Labs., Richmond, CA 94804.

Procedure

Purification of AIP isoenzymes. Skeletal AIP was partly purified from human bone (adult femoral heads obtained at time of total hip replacement) as previously described (11). The bones were frozen in liquid nitrogen, powdered, thawed, and repeatedly rinsed in carbonate buffer (25 mmol/L carbonate buffer, pH 8.0, prepared by titration of NaHCO3 with NaOH) to remove contaminating marrow, and extracted with butanol, 200 mL/L. The butanol extract was further purified by ammonium sulfate fractionation, column chromatography on DEAE-cellulose, and gel filtration on Bio-Gel A-0.5. The overall purification was about 70-fold.

Intestinal and hepatic AIP isoenzymes were partly purified from human tissues obtained at autopsy (two different specimens of each tissue were compared). The tissues were rinsed repeatedly in the carbonate buffer and minced; the mince was repeatedly rinsed in the buffer (to remove contaminating serum and bacteria) and then extracted with the butanol solution. The butanol extracts were further purified by ammonium sulfate fractionation. The overall purifications were about 30-fold and 17-fold for liver and intestinal AIP activities, respectively.

Biliary AIP was partly purified (about 30-fold) from three different specimens of human bile, obtained as T-tube drainage. Extensive dialysis and DEAE-cellulose column chromatography removed contaminating bilirubin, and ammonium sulfate fractionation provided further purification.

Assay for AIP activity. The standard assay was conducted at 22 °C in carbonate buffer (150 mmol/L, pH 10.3, containing 1 mmol of Mg2+ and 20 mmol of PNPP per liter) in a total volume of 1.5 mL. Adding the enzyme initiated the reaction. Incubations were usually for 15–20 min, but more prolonged when necessary. The linear range of activity vs enzyme concentration was determined for each AIP isoenzyme, and all assays were performed within these limits. Controls without added enzyme were included with each experiment to correct
for nonspecific hydrolysis of PNPP. We determined product
(p-nitrophenol, PNP) concentration from the increase in
absorbance at 410 nm, using a molar absorptivity value of
18.75 × 10³. Sample absorbance was determined by sequential
readings (e.g., once at 0 min and again at 20-min intervals, as
required) with a Model DU spectrophotometer (Beckman
Instruments, Fullerton, CA 92634) modified to include an
automatic-recording cuvette chamber (Gilford Instrument
Labs., Oberlin, OH 44074). Quartz cuvettes with a 1-cm
lightpath were uniformly rinsed and dried between readings.

Heat inactivation. Aliquots of AIP isoenzymes (or sera)
were heated in a circulating water bath incubator in buffer/
sample mixtures as described below. The total volumes heated
were usually less than 0.2 mL, although the volumes had no
effect on the inactivation rates over the range we tested (0.1
to 0.5 mL).

Statistical analysis of data. All data are reported as mean
values ± SD, and groups are compared by using Student’s
two-tailed t-test, except where otherwise indicated.

Subjects in Clinical Applications of the Isoenzyme
Method

(a) Serum samples from a group of 43 untreated post-
menopausal osteoporotic subjects (selected by criteria in-
cluding presence of vertebral crush fractures without evidence
of osteomalacia or secondary osteoporosis) were analyzed for
AIP isoenzyme distribution.

(b) Serum samples from seven additional osteoporotic
patients (original selection criteria as above) who were re-
spending to therapy with NaF (22 mg four times daily for
two to eight years) were also analyzed for AIP isoenzyme
distribution, as were serum samples from additional groups
of eight age-matched, non-osteoporotic controls, and eight
volunteer controls (not age-matched).

(c) Serum samples collected from another group of 10
postmenopausal osteoporotic patients both before and after
treatment with the anabolic agent stanozolol (6 mg/day for
an average of six to eight weeks) were also analyzed for AIP
isoenzyme distribution.

Results

Preparation of AIP Organ Standards

Table 1 shows results of inhibition studies with AIP iso-
enzymes prepared from human organs. Liver AIP2 is
apparently less heat-stable than the biliary isoenzyme. Previous studies
have shown that the AIP isoenzyme that predominates in the
serum of patients with obstructive jaundice (and other hepatic
diseases) behaves like the biliary isoenzyme and not like the
activity obtained in liver extracts (9, 12), suggesting that the
biliary isoenzyme is the more appropriate hepatic standard.
We have therefore used it in these studies. The rationale for
this selection and the question of why the AIP activity in liver
tissue extracts is not the same as in biliary extracts are ad-
dressed in the Discussion.

Standardization of the Quantitative Assay for
Skeletal AIP in Serum

Normal human serum was heated at 60 °C until AIP activity
was no longer detectable, then added in aliquots to AIP organ
standards for the heat inactivation. The results of these
studies are typified by Figure 1. Adding serum to a sample of
liver AIP in carbonate buffer increased the rate of heat inac-
tivation. When the same sera were dialyzed overnight the ef-
fect was no longer observable (Figure 2). Similar effects were
also observed with the skeletal and intestinal isoenzymes: serum
enhanced their inactivation by heat but dialyzed serum did not.
This effect of serum was not caused by changes in pH be-
cause the rate of heat inactivation of liver AIP was not af-


\[ \text{Table 1. Differential Sensitivities of AIP Isoenzymes} \]

<table>
<thead>
<tr>
<th>Isoenzyme</th>
<th>Treatment</th>
<th>Remaining Acty, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skeletal</td>
<td>Assay with L-Phe</td>
<td>95–102</td>
</tr>
<tr>
<td>Skeletal</td>
<td>Heat, then assay</td>
<td>11–15</td>
</tr>
<tr>
<td>Intestinal</td>
<td>Assay with L-Phe</td>
<td>15–21</td>
</tr>
<tr>
<td>Intestinal</td>
<td>Heat, then assay</td>
<td>2–7</td>
</tr>
<tr>
<td>Hepatic</td>
<td>Assay with L-Phe</td>
<td>85–94</td>
</tr>
<tr>
<td>Hepatic</td>
<td>Heat, then assay</td>
<td>28–35</td>
</tr>
<tr>
<td>Biliary</td>
<td>Assay with L-Phe</td>
<td>97–103</td>
</tr>
<tr>
<td>Biliary</td>
<td>Heat, then assay</td>
<td>48–54</td>
</tr>
</tbody>
</table>

* Prepared from human tissues as described in Methods. * L-Phe, L-phenyl-
alanine, 10 mmol/L. Heat inactivation: 10 min at 96 °C in carbonate buffer (pH
8.0, 4.25 mmol/L) with AIP-negative (heat-inactivated) human serum added
(one-fourth of total volume). * Range of three determinations.

We will refer to the AIP activities in extracts of liver and bile as
"liver" and "biliary," respectively, and the circulating isoenzyme that
originates in liver and (or) bile as "hepatic" AIP.

Fig. 1. Percentage of remaining activity (see footnote a, Table 2) of liver AIP (standard assay conditions) vs dilution factor for liver AIP diluted in serum and not heated (a), or diluted in water (W) or serum (B) and heated for 16 min at 55 °C

A fixed amount of liver AIP was diluted as indicated, heated, and then assayed
under the standard reaction conditions (e.g., 1.5 mL total reaction volume with
20 mmol/L PNPP in 0.15 mol/L carbonate buffer, pH 10.3)

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Fig. 2. Percentage of activity (standard assay conditions) of liver AIP remaining after heating at 55 °C for 16 min in an equal volume of dialyzed (+) or non-dialyzed (−) human serum from subjects A, B, C. Controls, either unheated or heated without additions, are also shown.

Fig. 3. K_inact (inactivation rate) vs temperature for skeletal (Δ) and biliary (■) AIP activities. K_inact was determined from plots of ln (% remaining activity) vs time at the indicated temperatures for samples heated in 6.25 mmol/L carbonate buffer, pH 8.0, in a total volume of 0.2 mL containing 50 μL of AIP-negative (heat-inactivated) human serum. Remaining activity was determined with the standard assay.

Inhibits the intestinal isoenzyme by 75–85% and the biliary and skeletal isoenzymes by 0–5%. (Typically, we found that intestinal AIP accounts for 0–15% of the circulating total. Fasting reduces this proportion to 0–5%, so that omitting the L-phenylalanine control from sera from fasted patients would have little effect.)

3. The activity of heat- and L-phenylalanine-insensitive AIP is measured. An aliquot of serum is heated in carbonate buffer (final concentration, 6.25 mmol/L) for 10 min at 56 °C, and the remaining AIP activity is measured as above, in the presence of L-phenylalanine, 10 mmol/L. Typically, we heat a total volume of 0.2 mL containing 0.05 mL of serum, 0.05 mL of carbonate buffer (25 mmol/L, pH 8.0), and 0.1 mL of water. Under these conditions, we see 11–15% of the original activity remaining for skeletal AIP, 48–54% of the activity remaining for biliary AIP, and 2–7% of the activity remaining for intestinal AIP.

Internal standards are included along with each set of patients' sera, and are heated under identical conditions. Normal, AIP-negative serum and carbonate buffer are added to the isoenzyme standards to give the same final concentrations as used for the patients' sera. The actual reaction conditions (buffer concentration, serum volume, temperature and time of incubation, etc.) may be varied so long as the samples and standards are treated the same. For instance, when serum AIP activity is low, we can increase the volume of serum assayed to as much as 250 μL, so long as the controls are treated identically. The data are then analyzed by the simultaneous solution of the following three equations, where S, H, and I represent skeletal, hepatic, and intestinal AIP activities, respectively,

(a) Total AIP activity = S + H + I
(b) L-phenylalanine-insensitive activity = x(S) + y(H) + z(I)
(c) Heat- and L-phenylalanine-insensitive activity = q(S) + r(H) + s(I)

and the factors x, y, z, q, r, and s are determined from the standards every time the assay is performed.

A sample calculation may be useful. AIP activity (U/L) is equal to [(ΔA410 × 1.5)/(reaction time × 18.75)] × volume

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Table 2. Ionic Effectors of AIP Inactivation

<table>
<thead>
<tr>
<th>Ionic strength</th>
<th>Remaining act., %</th>
<th>Unheated</th>
<th>Heated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (no additions)</td>
<td>—</td>
<td>100</td>
<td>71</td>
</tr>
<tr>
<td>+ AIP-negative serum</td>
<td>—</td>
<td>102</td>
<td>18</td>
</tr>
<tr>
<td>+ NaCl, 0.1 mol/L</td>
<td>0.1</td>
<td>98</td>
<td>71</td>
</tr>
<tr>
<td>+ NaCl, 0.4 mol/L</td>
<td>0.4</td>
<td>104</td>
<td>48</td>
</tr>
<tr>
<td>+ lactose, 0.4 mol/L</td>
<td>—</td>
<td>101</td>
<td>71</td>
</tr>
<tr>
<td>+ carbonate, 0.1 mol/L</td>
<td>0.15</td>
<td>97</td>
<td>6</td>
</tr>
<tr>
<td>+ Tris, 0.1 mol/L</td>
<td>0.025</td>
<td>105</td>
<td>54</td>
</tr>
<tr>
<td>+ MgCl₂, 0.1 mol/L</td>
<td>0.30</td>
<td>102</td>
<td>26</td>
</tr>
<tr>
<td>+ Pi, 0.1 mol/L</td>
<td>0.11</td>
<td>64</td>
<td>17</td>
</tr>
</tbody>
</table>

* Skeletal AIP prepared from human bone was incubated with the indicated compounds with or without heating (10 min at 50 °C) in 6.25 mmol/L carbonate buffer, pH 8.0, total reaction volume 0.2 mL, before remaining activity was determined with standard reaction conditions. Average of two determinations.


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factor, where the absorbance change at 410 nm (corrected for nonspecific hydrolysis of PNPP) is divided by the millimolar absorptivity of PNP (18.75) to give micromoles of product per milliliter. This is multiplied by the reaction volume (1.5 mL) and divided by the reaction time (in minutes) to give a value for the micromoles of product per minute (units) produced by a given volume of serum. The volume factor (e.g., 2 x 10⁴ for a 50-μL volume) adjusts the result to units per liter of serum.

If a given sample shows total AlP = 54 U/L, 1-phenylalanine-insensitive activity = 49 U/L; and heat- and 1-phenylalanine-insensitive activity = 13 U/L (we will assume the values of x, y, z, q, r, and s are 1, 0.98, 0.2, 0.15, 0.5, and 0.05, respectively), we use equations a, b, and c as follows:

(a) 54 U/L = S + H + I; I = (54 U/L - S - H)
(b) 49 U/L = 1.0(S) + 0.98(H) + 0.2(I)
(c) 13 U/L = 0.15(S) + 0.5(H) + 0.05(I)

We then substitute for I in b and c and solve these equations simultaneously to determine S or H, then whichever unknown remains. In this case, S = 32.5 U/L, H = 15.6 U/L, and I = 5.9 U/L.

The skeletal and hepatic activities behave independently with respect to heat inactivation, as shown in Figure 4, and a nomogram such as Figure 4 is prepared, by determining the 100% skeletal and hepatic standards every time the assay is performed. The advantage of this method is that we can normalize the large and often unavoidable variations between separate assays. Using this technique, we can calculate the amount of skeletal AlP activity in predetermined mixtures of AlP organ standards with a precision of 3–4% and an accuracy of 6–13% for a range of 75–25% skeletal AlP (Table 3).

The following observations support the validity of this method: (a) the increase in serum AlP activity in five patients with active Paget’s disease was due to an increase in the skeletal isoenzyme, from a normal value of about 40% (13) to 82–98% of the circulating total; and (b) the increase in serum AlP activity in four patients with liver disease was caused, predominantly, by an increase in the biliary isoenzyme, from a normal value of about 40% (13) to 65–92% of the circulating total.

Applications of the Method: Skeletal AlP as a Possible Index of Bone Formation

In the following applications of this method, we used frozen human serum and AlP organ standards prepared from human tissues as described in Materials and Methods.

(a) Serum samples from the 43 untreated, postmenopausal osteoporotic subjects were analyzed for skeletal AlP activity. The degree of variability was surprising. Total serum AlP activity ranged from 48 to 3.1 U/L (mean = 17.4 U/L, SD 9.2), and the skeletal AlP activity ranged from 29.7 to <0.2 U/L (mean = 10.4 U/L, SD 7.24), with no apparent correlation. For example, total AlP activity was 15.2 U/L and skeletal AlP activity was 6.3 U/L for serum from one patient; total AlP activity was 16.2 U/L and skeletal AlP activity <0.2 U/L (the minimum detectable activity) for another. In our age-matched control group, total serum AlP activity ranged from 3.1 to 12.6 U/L (Table 4). Previous investigators have reported a similar range of normal values, when adjustments are made for differences in AlP units (13, 14).

(b) The group of seven osteoporotic patients responding to therapy with NaF showed an increase in serum AlP activity as compared with values for age-matched controls, corresponding to the previously noted increased bone density (by roentgenogram) and visibly coarsened trabeculae, indicating increased bone formation (15, 16). The increase in total serum AlP was due almost entirely to an increase in skeletal AlP activity (Table 4).

(c) Sera from the group of 10 postmenopausal osteoporotic patients treated with stanozolol were analyzed for AlP isoenzyme composition and compared with baseline (pretreatment) samples from the same patients. Although there was no significant change in total AlP activity, every patient showed an increase in the skeletal component of her serum total (Table 5). These patients also showed an increase in total body calcium (17).

Discussion

The data in Table 3 indicate that skeletal AlP can be assayed quantitatively in human serum with reasonable precision. The method we have developed is similar to methods previously described by Green et al. (6) and by Whitaker et al. (18), but has the advantage of including internal standards to normalize variations between assays. These internal standards could be included only because we could account for the

Table 3. Determinations of Skeletal AlP Activity In In Vivo Mixtures

<table>
<thead>
<tr>
<th>Actual proportion of bone isoenzyme</th>
<th>25%</th>
<th>50%</th>
<th>75%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean of calculated percentage</td>
<td>24.48</td>
<td>48.5</td>
<td>72.7</td>
</tr>
<tr>
<td>Overall SD of calculated percentage (precision)</td>
<td>3.36</td>
<td>3.74</td>
<td>4.33</td>
</tr>
<tr>
<td>SD from true values (e.g., assumed mean percentage)</td>
<td>3.4</td>
<td>4.66</td>
<td>4.90</td>
</tr>
<tr>
<td>CV, %</td>
<td>13.6</td>
<td>9.3</td>
<td>6.7</td>
</tr>
</tbody>
</table>

* Mixtures of AlP, semi-purified from organs and used as standards, were made containing various amounts of bone and biliary AlP. These were analyzed as described in Results. 100% bone and 100% biliary standards were used to normalize the assays. These values were calculated from six separate determinations, each performed in duplicate.
Table 4. Activity of Skeletal AIP in Serum Response to Therapy with NaF

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Age, yr</th>
<th>Total AIP, U/L</th>
<th>Skeletal AIP, U/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaF responders</td>
<td>7</td>
<td>57 ± 13.4</td>
<td>24.6 ± 4.5</td>
<td>14.5 ± 3.96</td>
</tr>
<tr>
<td>Age-matched</td>
<td>8</td>
<td>58.5 ± 3.82</td>
<td>16.0 ± 6.7</td>
<td>7.05 ± 3.63</td>
</tr>
<tr>
<td>Volunteer controls</td>
<td>8</td>
<td>—</td>
<td>13.3 ± 4.4</td>
<td>4.68 ± 3.89</td>
</tr>
</tbody>
</table>

* Described in text. † Significantly different from patients, p < 0.005. ‡ Significantly different from patients, p < 0.001.

differences between the behaviors of AIP isoenzymes prepared from organ sources and the same isoenzymes in human serum. Previous investigators avoided this problem by using serum standards in place of organ-derived standards (6, 7). This practice is justifiable in the case of skeletal AIP, because patients with active Paget's disease may have 20-fold increases in total serum AIP so that the skeletal isoenzyme comprises more than 95% of the circulating total activity. But with liver disease the serum AIP activity is seldom increased to this extent (except in extreme cases of obstructive jaundice), and there may still be significant contributions from the skeletal and intestinal isoenzymes, particularly since bone disease is an expected complication of liver dysfunction. For this reason, and because human organs are more readily available than the necessary serum, we have used organ-derived standards of AIP activity, rather than patients' sera, to normalize our results.

Our selection of internal standards depends on the following assumptions: (a) that serum AIP activity in humans is almost entirely composed of isoenzymes derived from bone, intestine, liver, and placenta; and (b) that the AIP activity found in bile is the proper hepatic isoenzyme standard.

Regarding the first assumption, we know that AIP activity is far more abundant (per milligram of protein) in bone, intestine, kidney, placenta, and liver than in other human tissues, so these organs are the most likely sources of AIP activity in serum (19). AIP activity of renal origin has been shown to appear in urine and not in serum (20), so its contribution is minimal. We also know that changes in the metabolic states of the liver, placenta, intestine, and skeleton can change serum AIP activity, and that no other organ has such an effect. Although these arguments are circumstantial, all of the available evidence is consistent with our assumption. There is also clinical evidence to support the presence of another AIP type, the Regan isoenzyme (21), in the serum of 4.6% of tumor patients; however, this activity is indistinguishable from placental AIP in heat-inactivation or inhibition studies. We have ignored this isoenzyme in our calculations because it would appear as placental or intestinal AIP.

With regard to the second assumption—that the AIP activity found in bile is the proper hepatic standard—the increase in serum AIP activity associated with liver disease is attributable to an increase in the biliary isoenzyme, as has been repeatedly confirmed (6, 12, 22). Furthermore, the data of Fitzgerald et al. (8) indicate no difference from the biliary isoenzyme in the heat-inactivation properties of the predominant form of AIP in the sera of patients with various forms of liver disease. Intrahepatic disease was not distinguishable from extrahepatic obstruction, suggesting that the AIP activity endogenous to liver (and, presumably, released into the circulation) is not different from that found in bile. What, then, could account for the difference in heat stabilities of liver and biliary AIP activities? One possible explanation for these apparently discordant results is that liver tissue extracts may contain not only endogenous hepatic AIP but also other isoenzymes (e.g., skeletal and intestinal) that have been cleared from the circulation. Although the existence of such a clearance process has not been established, 125I-labeled canine AIP, re injected into dogs, was rapidly cleared by the liver and did not reappear in bile or in the general circulation (23). Data obtained by Klein et al. (24) are also consistent with the possibility that AIP isoenzymes are cleared from the circulation by human liver. Specifically, they showed that the increase in serum intestinal AIP activity associated with dietary fat loading lasts for three to six days in cirrhotic persons, as compared with 8 h in normal individuals, which suggests that normal clearance is impaired in cirrhosis.

The final bit of evidence to support our second assumption is circumstantial. We had originally prepared a liver extract to use as an internal standard, but several serum samples, and particularly those from patients with liver disease, were more heat stable than this standard. According to our nomogram (Figure 4), that would indicate >100% liver AIP in the serum total. It was this inconsistency that led us to question the homogeneity of AIP derived from liver extracts. Since we began using the biliary standard, we have found no such inconsistencies.

Once we had selected organ standards, the anticipated problem of forcing them to behave like their counterparts in serum was easily overcome. The factors that ordinarily affect the stability of enzymes at high temperatures have to do with the molecular environment—concentrations of substrates, products, and effector ions; pH; and the buffer type. Moss et al. (25) had previously reported that the stability of AIP isoenzymes to heat and to urea was affected by factors such as pH, protein concentration, and endogenous urea. They also suggested that other factors, including intrinsic differences in the heat stability of individual patients' AIP isoenzymes, could not be ruled out. While we cannot disagree with the latter observation, we can argue from our own data that slight variations in pH and protein concentration among sera are not important in our assay, and that variations owing to differences in the concentrations of effector ions in serum (see Table 2) are either insignificant or advantageously masked by our addition of carbonate buffer. According to Cornish et al. (26), AIP isoenzymes in serum are inactivated less rapidly by heat when the samples are diluted with anything but AIP-negative serum. This is consistent with our observations.

Using our protocol (as described in Results) and including
internal standards, we were able to determine the amount of skeletal AlP activity in artificial mixtures (with serum) to within 14% in the low-normal range and 6% in the upper-normal range (Table 3). This means that we should be able to detect a 10 to 20% change in the circulating skeletal AlP of individual patients. In Paget’s disease, a 10% change in serum AlP (which is almost entirely the skeletal isoenzyme) reflects a 10% change in the bone formation rate (I). If this correlation holds within the normal range, a 10% change in the bone formation rate would translate to about 20 mg of calcium per day. Because this is about the degree of the negative calcium balance in osteoporosis, our method could be sufficiently sensitive to be applied to studies of osteoporosis. The data in Tables 4 and 5 show that osteoporotic patients who were responding to therapy with increased bone formation also had increased skeletal AlP in their serum. If the activity of this isoenzyme in serum does indeed reflect the rate of bone formation, determinations of skeletal AlP activity could be a useful adjunct to measurements of bone density by roentgenograms or photon absorption.

In summary, these studies show that (a) skeletal AlP activity in human serum can be measured with precision; (b) total AlP activity in serum is not a reliable index of skeletal AlP activity; and (c) changes in the activity of skeletal AlP in serum may reflect changes in bone formation.

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References