Time-Dependent Changes in Bone, Placental, Intestinal, and Hepatic Alkaline Phosphatase Activities in Serum during Human Pregnancy

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To measure changes in bone alkaline phosphatase (EC 3.1.3.1) activity in serum as a function of duration of pregnancy, we adapted our existing alkaline phosphatase (ALP) isoenzyme assay (which has been used to measure bone, hepatic, and intestinal ALP activities in serum, in the absence of placental ALP) to allow quantification of individual ALP isoenzyme activities in the presence of placental ALP. The resulting CV for repeat measurements of bone ALP activity in artificial isoenzyme mixtures ranged from 23% for samples in which the bone isoenzyme represented 7% of total ALP activity to 11% for samples in which bone ALP accounted for 48% of total ALP activity. Values for repeat determinations of bone ALP activity in human serum samples (i.e., including samples obtained from pregnant women and from non-pregnant controls) varied by an average of 18%. We find, in initial applications of this method, that (a) the amount of bone ALP activity in serum is increased during pregnancy (P < .001), and remains increased at six weeks postpartum, in non-lactating women (P < .001), and (b) bone ALP activity at term was not significantly different in pregnant women with pre-eclampsia, diabetes, premature rupture of membranes, or premature labor, compared with normal pregnancies at term. Our data support the hypothesis that maternal bone formation may be increased during pregnancy.

Additional Keyphrases: pregnancy, alkaline phosphatase, and bone formation • isoenzymes

Indirect evidence indicates that pregnancy and lactation can represent a physiological challenge to maternal homeostasis and skeletal metabolism. By the end of pregnancy, the human fetus has accumulated 30 g of calcium, most of it during the last nine weeks before delivery (1). To meet this demand, maternal calcium fluxes are altered such that both intestinal calcium absorption and bone resorption are increased, as respectively evidenced by increases in the concentration of 1,25-dihydroxy-vitamin D3 in serum (2, 3), and the ratio of calcium to creatinine in the urine of the fasting subject (4). Calcium tracer kinetics have similarly shown increases in both intestinal calcium absorption and the rate of bone resorption during normal pregnancy (5). Surprisingly, those studies also revealed a pregnancy-dependent increase in skeletal calcium accretion (i.e., bone formation). Presumably, this increase in maternal bone formation is compensatory with respect to prior bone resorption (to protect against a nonessential decrease in bone volume) and depends upon a supply of dietary calcium sufficient to more than meet the fetal demand.

To test the hypothesis that maternal bone formation may be increased during pregnancy, and to quantify the increase, we measured alkaline phosphatase (ALP) skeletal isoenzyme activity in serum as an index of the rate of bone formation. We had three reasons for making this choice of analyte. First, previous studies had shown a close association between skeletal ALP isoenzyme activity in serum and the rate of bone formation, both in vitro (6) and in vivo (7–9). Second, previous studies also indicated that the pregnancy-dependent increase in total ALP activity in serum might reflect increases in both the placental (10, 11) and skeletal ALP isoenzymes (12, see reference 13 for a general review). And finally, we had already established an assay that would allow us to quantify individual ALP isoenzyme activities in the circulating mixture of bone, hepatic, and intestinal ALPs of the non-pregnant subject (14).

We had two objectives in the current studies:

(a) to modify our existing ALP isoenzyme assay so that we could accurately measure bone ALP activity in a four-component isoenzyme mixture (i.e., bone, hepatic, intestinal, and placental ALPs); and

(b) to apply the modified assay to determine time-dependent changes in serum ALP isoenzyme activities during normal pregnancy, and to compare ALP isoenzyme distributions, at term, in normal and abnormal pregnancies.

Materials and Methods

Chemicals and Supplies

Purified human placental ALP, NaHCO3, Na2CO3, and p-nitrophenyl phosphate were from Sigma Chemical Co., St. Louis, MO. Pooled human serum (mycoplasma-free) was from Flow Laboratories, McLean, VA. For colorimetry of ALP activity we used an automated motorized micro-well plate reader (Dynatech, Richmond, VA; Model MR-600).

Description of the Study Population

We first studied 19 non-pregnant women, to evaluate the possible significance of circadian rhythms and fasting on ALP isoenzyme activities in serum. Serum samples were obtained from volunteers (a) at 1-min intervals, (b) at different times of the day, (c) during fasting, and (d) after a meal. No variations were found.

For our pregnancy study, we recruited 22 women, 26 (SD 3) years old, who had received prenatal care at the Loma Linda University Hospital. No distinctions were made with respect to previous pregnancies and or deliveries. After informed consent was obtained, blood specimens were taken at one or more of the following four times during pregnancy: 10–12 weeks (i.e., near the end of the first trimester), 18–20 weeks (near the midpoint of the second trimester), 32–34 weeks (near the end of the third trimester), and six weeks postpartum (non-lactating women). We originally intended...
to obtain longitudinal data, but had to revert to a cross-sectional design because of (e.g.) poor compliance with our sampling schedule, change in subject's residence, etc. Of the 22 women enrolled: two were sampled only at 10–12 weeks; two were sampled only at 18–20 weeks; three were sampled only at 32–34 weeks; one was sampled only at 10–12 and 18–20 weeks; two were sampled only at 10–12 and 32–34 weeks; four were sampled only at 32–34 weeks and six weeks postpartum; one was sampled only at 10–12, 18–20, and 32–34 weeks; one was sampled only at 10–12 and 18–20 weeks, and six weeks postpartum; two were sampled only at 10–12 and 32–34 weeks, and six weeks postpartum; and four were sampled at all four times.

Blood was also sampled at term from (a) pregnant women with a diagnosis of pre-eclampsia (based on two separate measurements of blood pressure 140/90 mmHg and a minimum value of +1 for albumin in the urine); (b) pregnant women with class B diabetes mellitus; (c) normal pregnant women, admitted in active labor, with and without spontaneous rupture of membranes; and (d) age-matched, non-pregnant, volunteer controls.

ALP Isoenzyme Assay

To evaluate the time-dependent changes in ALP isoenzyme activities in serum during pregnancy, we first needed to adapt our assay methodology (14), which was designed for ALP isoenzyme analysis of a three-component circulating isoenzyme mixture (i.e., bone, hepatic, and intestinal ALPs), to allow for the analysis of four-component mixtures (i.e., including the placental isoenzyme).

In preliminary studies we assessed the sensitivities of the bone, hepatic, intestinal, and placental ALP isoenzymes to chemical and thermal inhibitions. As described below in Results, our data were consistent with previous reports (13), indicating that a quantitative analysis of a four-component ALP isoenzyme mixture could be based on (a) the sensitivity of intestinal and placental ALP activities to chemical inhibition by L-phenylalanine, (b) the differential sensitivities of bone and hepatic ALP activities to thermal inactivation, and (c) the thermal stability of placental ALP activity. As in our previous studies (14) the results of our isoenzyme assays were normalized by including isoenzyme standards in each assay. Serum isoenzyme standards were used for bone ALP (i.e., serum from subjects with active Paget's disease, for whom we assume that total serum ALP activity = bone ALP activity >800 U/L) and hepatic ALP (i.e., serum from subjects with obstructive jaundice and cholestasis, for whom we assume that total serum ALP activity = hepatic ALP activity >500 U/L). Human intestinal ALP activity was extracted (and partly purified) from sections of duodenum, obtained at autopsy. A highly purified preparation of human placental ALP was obtained commercially.

For the current studies, we also adapted our assay to an automatic microtiter plate spectrophotometer/printer. (The automated plate reader can measure and record the absorbancies of 96 separate samples in less than 2 min.) To adapt the assay to this system, total reaction volumes were decreased from 1.5 mL to 0.3 mL, but the composition of the ALP reaction mixture was unchanged: per liter, 0.15 mol of carbonate buffer (pH 10.3), 1 mmol of MgCl₂, 10 mmol of p-nitrophenyl phosphate, and the enzyme. Based on our preliminary studies of chemical and thermal stability, we used the following protocol to measure ALP isoenzyme activities.

(a) A 75-μL aliquot of each serum sample to be assayed was diluted with 75 μL of 25 mmol/L carbonate buffer (pH 8.0, containing sodium azide, 500 mg/L), and 150 μL of de-ionized water, in a 12 mm × 75 mm test tube (i.e., a fourfold dilution).

(b) Duplicate 25-μL aliquots of the diluted serum samples were transferred to a 96-well immunotiter plate and 30 μL of carbonate buffer (pH 10.3) was added to each well, for subsequent measurements of total serum ALP activity.

(c) Additional duplicate 25-μL aliquots were transferred to a second microtiter plate along with 30 μL of a 100 mmol/L solution of L-phenylalanine in carbonate buffer (pH 10.3) for subsequent measurement of phenylalanine-sensitive ALP activity.

(d) The test tubes containing the remaining 200 μL of diluted sera were incubated in a heated water bath (53 °C) for 11 min, then cooled in an iced-water bath. Duplicate 25-μL aliquots were then transferred to a third microtiter plate, and 30-μL of carbonate buffer (pH 10.3) was added to each well for subsequent measurements of heat-resistant ALP activity.

(e) Finally, the test tubes were returned to the 53 °C water bath for an additional 90 min, then duplicate 25-μL aliquots were transferred to a fourth microtiter plate, and 30 μL of carbonate buffer (pH 10.3) was added to each well for subsequent measurements of heat-stable ALP activity.

At this point, the diluted serum samples were distributed over four microtiter plates such that duplicate aliquots of untreated sera were in one plate, sera with phenylalanine in the second plate, 11-min-heated sera in the third plate, and 101-min-heated sera (i.e., 11 min + 90 min) in the fourth plate. The total volume in each well was 55 μL. ALP activities were then determined by adding 245 μL of our standard ALP reaction mixture to each well. Controls without added enzyme were included on each plate to correct for nonspecific hydrolysis of p-nitrophenyl phosphate. As in previous studies (14), isoenzyme standards (diluted in carbonate buffer, water, and heat-inactivated human serum, as appropriate) were also included on each plate, to correct for interassay variations.

The reactions were initiated by adding p-nitrophenyl phosphate (in the reaction mixture) and monitored by the time-dependent increase in absorbance at 404 nm (i.e., reflecting the production of the p-nitrophenolate anion in the alkaline assay environment), at ambient room temperature (22 °C). The standard assay ran for 40 min, but both shorter and longer incubations were used to measure high and low activities, respectively. As outlined in Results, ALP isoenzyme activities were then calculated by the simultaneous solution of four equations containing four unknowns.

Equations: (a) Total (untreated) ALP activity = B + H + I + P; (b) phenylalanine-resistant ALP activity = aB + bH + cl + dP; (c) heat-resistant ALP activity = eB + fh + gI + hP; (d) heat-stable ALP activity = iB + jH + kI + lP.

Unknowns: B = bone ALP activity; H = hepatic ALP activity; I = intestinal ALP activity; P = placental ALP activity.

Isoenzyme activity factors (a, b, c, etc.) were determined from the isoenzyme standards in each assay (e.g., if 11 min at 53 °C decreased the activity of the bone ALP standard by 65%, the factor "e" would be equal to 0.35 for that assay).

All ALP activities are reported in U/L (of serum)—the number of micromoles of product formed per minute in our standard assay (i.e., at 22 °C, with 10 mmol of p-nitrophenyl phosphate per liter of carbonate buffer, pH 10.3). Unrelated studies have established that total ALP activities in serum
measured under these conditions must be multiplied by a factor of 2.7 for comparison with values obtained from the Pettis Veterans' Hospital clinical laboratory, which uses 2-amino-2-methyl-1-propanol buffer at 30 °C.

Using this method, we could assay as many as 40 serum samples on each set of (four) microtiter plates, allowing space for controls and isoenzyme standards on each plate. Because the aliquots of the diluted sera could also be stored frozen (−20 °C) in the microtiter plates without apparent loss of activity, multiple sets of plates could be included in an assay, so that 80 or 120 serum samples could be analyzed together.

Sample Storage and Stability

The serum samples used in these investigations were collected over a period of 15 months and stored at −20 °C until the assays were performed. Because previous studies (14) had shown that the variation between ALP isoenzyme assays exceeded the variation within assays, we planned to assay the samples all together, or, at least, in three large groups (i.e., including preliminary samples from the non-pregnant, volunteer controls, time-dependent pregnancy samples, and term samples from women with different metabolic status, respectively). To ensure that ALP isoenzyme activities were not lost or altered during frozen storage, we removed aliquots from some samples (including both controls and pregnant subjects) before they were frozen, to allow a comparison of ALP isoenzyme activities before and after eight months of frozen storage.

Statistical Analyses

As mentioned, we were only able to obtain a complete series of samples (i.e., at 10–12, 18–20, and 32–34 weeks, and six weeks postpartum) from a total of four subjects. Therefore, we have treated the data as cross-sectional. For statistical comparisons we used analysis of variance and Student's two-tailed t-test (paired or unpaired, as appropriate) and linear regression analysis.

Results

ALP Isoenzyme Assay

(a) Chemical and thermal stabilities of ALP isoenzymes. ALP isoenzyme standards, diluted in appropriate mixtures of carbonate buffer (25 mmol/L, pH 8.0), water, and heat-inactivated human serum, were used to determine the inhibitory actions of 10 mmol/L L-phenylalanine and incubation at 53 °C. (Heat-inactivated human serum was prepared by incubating serum at 53 °C, until ALP activity could no longer be detected in a 25-μL aliquot.) As summarized in Table 1, we found that both the intestinal and placental ALP isoenzymes were significantly inhibited by phenylalanine, all four isoenzymes were differentially inactivated by an 11-min incubation at 53 °C, and only placental ALP activity was retained after a 101-min incubation at 53 °C. These data, which are consistent with previous reports (see reference 13 for a review), allowed us to define the four equations (i.e., for total ALP activity, phenylalanine-resistant ALP activity, heat-resistant ALP activity, and heat-stable ALP activity—see Methods) required to measure the four unknown ALP isoenzyme activities (i.e., bone, hepatic, intestinal, and placental).

(b) Design and characterization of the assay. The 110-min incubation at 53 °C allowed for quantification of placental ALP activity, so we defined heat-stable ALP activity as placental ALP. Serum samples that contained significant amounts of placental ALP (i.e., >0.1 U/L) were analyzed by the simultaneous solution of four equations containing the four unknown ALP isoenzyme activities. Serum samples that did not contain placental ALP (i.e., samples from the non-pregnant controls and some first-trimester and postpartum samples) were analyzed by the simultaneous solution of three equations (i.e., omitting the value for heat-stable ALP activity) containing the remaining three unknowns. Table 2 gives the CVs for multiple determinations of ALP isoenzyme activities in artificial mixtures, simulating pregnant and non-pregnant sera (i.e., with and without placental ALP).

(c) Sample storage and stability. Consistent with previous studies (see reference 13, pp 301–302, for a review), we found that eight (SD 1.7) months of storage at −20 °C had no effect on ALP activity (Table 3). Neither total ALP activity nor any of the isoenzymes measured showed evidence of time-dependent changes in activity during frozen storage (data for hepatic and intestinal ALP activities not shown). Because our method estimates total ALP activity and placental ALP activity directly (i.e., by measurements of total and heat-stable ALP activities), it is not surprising that the variations for repeat measurements of total and placental ALP activities were significantly lower than variations for repeat determinations of bone ALP activity. As a further test for time-dependent changes in ALP activity with storage we also subjected the eight-month repeat isoenzyme

<table>
<thead>
<tr>
<th>Isoenzyme distribution</th>
<th>CV, %a</th>
</tr>
</thead>
<tbody>
<tr>
<td>13% 77% 10% 0</td>
<td>37.0% 5.0% 28.0% 0</td>
</tr>
<tr>
<td>42% 48% 10% 0</td>
<td>9.2% 4.8% 27.8% 0</td>
</tr>
<tr>
<td>65% 25% 10% 0</td>
<td>4.0% 10.9% 26.8% 0</td>
</tr>
<tr>
<td>7% 53% 6% 34%</td>
<td>23.1% 10.2% 49.7% 2.6%</td>
</tr>
<tr>
<td>24% 36% 6% 34%</td>
<td>16.3% 4.1% 38.9% 5.1%</td>
</tr>
<tr>
<td>48% 12% 6% 34%</td>
<td>11.1% 17.4% 39.9% 4.2%</td>
</tr>
</tbody>
</table>

* ALP isoenzyme composition of artificial mixtures, shown as a percentage of total ALP activity, for six isoenzyme combinations prepared by mixing Paget's disease sera, cholestatic sera, purified human placental ALP, and partly purified human intestinal ALP. All combinations were diluted in an appropriate mixture of heat-inactivated, ALP-negative human serum, carbonate buffer, and water, as described in Methods.

*CVs for calculated ALP isoenzyme distributions, as determined for each artificial sample in a total of four assays. The last three samples (i.e., with placental ALP) were assessed by simultaneous solution of three equations with three unknowns. The last three samples (i.e., with placental ALP) required four equations for the four unknowns.

Table 1. Differential Inhibitions of ALP Isoenzymes

<table>
<thead>
<tr>
<th>Isoenzyme * L-Phenylalanine</th>
<th>11 min at 53 °C</th>
<th>101 min at 53 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone 83–86% (85%) 28–33% (31%) 0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Hepatic 84–90% (85%) 59–71% (70%) 0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Intestinal 35–43% (41%) 24–29% (27%) 0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Placental 11–13% (12%) 96–103% (99%) 96–106% (101%)</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

* ALP isoenzymes (i.e., Paget's disease serum, cholestatic serum, purified placental ALP, and partly-purified intestinal ALP) were diluted in ALP-negative (i.e., heat-inactivated) human serum, NaHCO3 buffer, and water (see Methods) for triplicate determinations of isoenzyme sensitivity to chemical and thermal inhibitions.

Percent remaining ALP activity (i.e., percent of untreated triplicate controls, for each isoenzyme) for ALP isoenzymes assayed in the presence of L-phenylalanine (10 mmol/L), after heating for 11 min at 53 °C; or for 101 min at 53 °C. Range of triplicate values and mean (in parentheses) are shown.

Table 2. Quantification of ALP Isoenzyme Activities in Artificial Mixtures

CLINICAL CHEMISTRY, Vol. 33, No. 10, 1987 1803
Table 3. ALP Isoenzyme Activities during Pregnancy: Stability of Frozen Samples

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Total serum ALP</th>
<th>Bone ALP</th>
<th>Placental ALP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range, first assay</td>
<td>8.3–44.2</td>
<td>2.6–16.9</td>
<td>0.0–38.1</td>
</tr>
<tr>
<td>Range, second assay</td>
<td>8.5–42.2</td>
<td>3.2–16.8</td>
<td>0.0–40.3</td>
</tr>
<tr>
<td>Correlation coefficient, r</td>
<td>0.81*</td>
<td>0.68*</td>
<td>0.84*</td>
</tr>
<tr>
<td>Correlation, slope</td>
<td>0.93</td>
<td>0.89</td>
<td>0.98</td>
</tr>
<tr>
<td>Mean variation between assays</td>
<td>7.6%</td>
<td>18.3%</td>
<td>8.2%</td>
</tr>
<tr>
<td>Range of variation between assays</td>
<td>0.2–21%</td>
<td>0.6–39%</td>
<td>0.0–24%</td>
</tr>
</tbody>
</table>

*To detect time-dependent changes in ALP activities during storage we compared the first and second assays with respect to: (a) range—indicates the range of ALP activity (U/L) observed in each assay; (b) correlation—indicates the correlation coefficient and the slope for a linear regression analysis of first vs second assay values for each subject (* indicates a significant correlation, P < 0.001); none of the slopes differed significantly from 1.0; (c) variation between assays—indicates the mean value and the range for the variation between ALP activities determined in the first and second assays, for each subject.

A total of 24 serum samples from non-pregnant controls (n = 6), women during pregnancy (n = 13), and women at term (n = 5) were assayed for ALP isoenzyme distribution (first assay) and then reassayed eight (SD 1.7) months later (second assay). The samples were stored at −20 °C between the two assays.

Data to a paired analysis and found no significant differences.

ALP Isoenzymes during Pregnancy

(a) Cross-sectional analysis of data. The data summarized in Table 4 indicate the time-dependent changes in serum ALP isoenzyme activities during normal pregnancy. ALP isoenzyme activities in the serum of age-matched non-pregnant controls are also shown in Table 4 (i.e., in the legend). Table 5 summarizes the results of our one-way analysis of variance of the time-dependent changes shown in Table 4. Both placental and bone ALP activities were significantly increased during pregnancy, and remained increased six weeks postpartum.

(b) Serial samples from four subjects. For each of the four women from whom we obtained a complete series of serum samples during pregnancy, the time-dependent changes in serum ALP activities during pregnancy (data not shown) followed the same pattern as our cross-sectional data.

(c) Effects of metabolic status on serum ALP isoenzymes at delivery. The data summarized in Table 6 demonstrate that both total and placental ALP activities at delivery were significantly lower for subjects with ruptured membranes and for subjects in pre-term labor as compared with normal pregnancy, but values for bone ALP were not significantly different.

Discussion

Our results demonstrate that bone ALP activity can be quantified in human serum, even in the presence of placental ALP. Our isoenzyme assay, adapted from previous methods (14–17), recognizes placental ALP activity by its unique stability to inactivation by heat (13), intestinal ALP activity by its sensitivity to inhibition by L-phenylalanine (13, 14, 18), and bone and hepatic ALP activities by their different sensitivities to heat inactivation (6, 13, 14, 19, 20). In characterizing this assay we found that: the CV for repeat determinations of bone ALP activity in four-component, artificial isoenzyme mixtures ranged from 11% to 23% for mixtures containing high and low percentages of bone ALP, respectively. Neither total ALP activity nor any of the isoenzymes measured showed evidence of time-dependent

Table 4. ALP Isoenzyme Activities during Pregnancy: Time-Dependent Changes

<table>
<thead>
<tr>
<th>Isoenzyme(s)</th>
<th>10–12 weeks (n = 11)</th>
<th>18–20 weeks (n = 11)</th>
<th>32–34 weeks (n = 16)</th>
<th>6 weeks post. (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total ALP in serum</td>
<td>7.6 (1.0)</td>
<td>11.1 (3.8)*</td>
<td>20.4 (4.8)**</td>
<td>13.7 (2.8)**</td>
</tr>
<tr>
<td>Bone ALP</td>
<td>3.7 (1.2)*</td>
<td>6.1 (2.2)*</td>
<td>7.6 (1.6)**</td>
<td>6.9 (1.4)**</td>
</tr>
<tr>
<td>Hepatic ALP</td>
<td>3.1 (1.1)</td>
<td>2.1 (2.6)</td>
<td>1.8 (2.6)</td>
<td>4.0 (1.9)</td>
</tr>
<tr>
<td>Intestinal ALP</td>
<td>0.8 (0.5)</td>
<td>0.9 (0.4)</td>
<td>0.5 (0.5)</td>
<td>2.9 (2.9)</td>
</tr>
<tr>
<td>Placental ALP</td>
<td>0.1 (0.1)</td>
<td>2.0 (2.3)**</td>
<td>10.4 (4.8)**</td>
<td>0.2 (0.2)</td>
</tr>
</tbody>
</table>

*ALP isoenzyme activities (U/L) during pregnancy, measured at 10–12, 18–20, and 32–34 weeks, and six weeks postpartum, are shown as group mean values (SD in parentheses). Postpartum samples were obtained only from non-lactating women. As indicated in the Table, a total of 11–16 of the 22 volunteers were sampled at each time point, most women at least twice, but only four at all four indicated times.

* Indicates a significant difference from age-matched non-pregnant female controls, P < .01; ** indicates P < .001. ALP isoenzyme values in the non-pregnant controls (n = 12) were as follows: total ALP, 9.3 (SD 2.2); bone ALP, 2.3 (SD 1.2); hepatic ALP, 3.8 (SD 1.7); intestinal ALP, 3.3 (SD 2.4); placental ALP, 0 (SD 0).

Table 5. ALP Isoenzyme Activities in Serum during Pregnancy: Analysis of Variance of Time-Dependent Changes

<table>
<thead>
<tr>
<th>ALP isoenzyme</th>
<th>Pregnancy samples only</th>
<th>Pregnancy samples + controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total serum ALP</td>
<td>F = 11.49, P &lt; .0001</td>
<td>F = 15.08, P &lt; .0001</td>
</tr>
<tr>
<td>Bone ALP</td>
<td>F = 11.84, P &lt; .0001</td>
<td>F = 14.50, P &lt; .0001</td>
</tr>
<tr>
<td>Hepatic ALP</td>
<td>F = 2.36, N.S.</td>
<td>F = 2.85, N.S.</td>
</tr>
<tr>
<td>Intestinal ALP</td>
<td>F = 6.03, P &lt; .0001</td>
<td>F = 5.90, P &lt; .001</td>
</tr>
<tr>
<td>Placental ALP</td>
<td>F = 48.60, P &lt; .0001</td>
<td>F = 25.80, P &lt; .0001</td>
</tr>
</tbody>
</table>

*One-way ANOVA for: (a) pregnancy samples only (data shown in Table 4), reflecting ALP activities at 10–12, 18–20, and 32–34 weeks, and six weeks postpartum, allowing for 3 and 45 degrees of freedom in the ANOVA; and (b) pregnancy samples with additional data from age-matched non-pregnant female controls (control data given in the legend to Table 4), allowing for a total of 6 and 62 degrees of freedom in the ANOVA. N.S.: no significant difference between groups.
changes during eight (SD 1.7) months of frozen storage.

Initial applications of this method have revealed that: (a) placental ALP activity in serum is increased during pregnancy (from a value of zero in the non-pregnant woman), then disappears from the circulation; (b) bone ALP activity is also increased during pregnancy, and remains so six weeks postpartum, in non-lactating women; and (c) total and placental, but not bone, ALP activities were lower, at delivery, in women with premature rupture of membranes and those in pre-term labor than in normal-term controls.

The first of these three observations—that placental ALP activity is increased during pregnancy but is virtually non-detectable six weeks postpartum—is consistent with previous reports of (a) time-dependent changes in total serum ALP activity during human pregnancy (10–13), and (b) a biological half-life in serum of approximately seven days for the placental isoenzyme (21). Similarly, our observations that total and placental ALP activities were lower, at delivery, in subjects with premature rupture of membranes and those in pre-term labor (i.e., compared with normal-term controls) are also consistent with previous data, although our observation of unchanged placental ALP in pre-eclamptic subjects is not (22, 23).

Our observations of increased bone ALP activity in serum during pregnancy both confirm and extend a previous report of increased heat-labile (presumably, bone) ALP activity in serum of pregnant women (12). Previous studies indicate that bone ALP activity can provide a useful index of the rate of bone formation, both in vitro (6) and in vivo (7–9, 14, 24). Thus our data support the hypothesis that maternal bone formation may be increased during pregnancy (5). Presumably, this increase in maternal bone formation is compensatory, allowing for replacement of bone lost to resorption when calcium is mobilized in order to meet the fetal demand (2, 3). Although the mechanisms of calcium mobilization during pregnancy are incompletely understood, our data are also consistent with a previous report (5) that both bone formation and bone resorption are increased in advance of the fetal demand for skeletal calcium (i.e., before the last nine weeks of pregnancy). Because we also found that bone ALP activity remained increased six weeks postpartum, our data further indicate that maternal bone formation may remain increased in non-lactating women after delivery.

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References
20. Posen S, Neale FC, Clubb JS. Heat inactivation in the study of


