Significance of ‘High’ Acid Phosphatase Activity in the Serum of Normal Children

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Serum acid phosphatase activity in normal children (newborn to 18 years) is several fold that in normal adults. Activity is age-related but not sex-related. The isoenzyme pattern in children is similar to that in adults and contains no prostatic fraction. Quantitatively, most of the enzyme activity in the serum of children is tartrate-resistant and correlates well with heat-labile fractions of alkaline phosphatase activity in serum, suggesting that the source of the higher acid phosphatase activity in children is bone. Significant tartrate-resistant acid phosphatase activity was demonstrated in the giant cells in three patients with giant-cell tumors, but not in the “osteoblasts” in six patients with osteogenic sarcomas and many other normal or abnormal tissues. This work suggests that the higher enzyme activity in the serum of children represents a normal physiological phenomenon resulting from their greater osteoclastic activity.

Additional Keyphrases: age-related effects · pediatric chemistry · alkaline phosphatase compared · normal values · changes seen in cancer patients’ sera · isoenzymes

The clinical significance of serum acid phosphatase (EC 3.1.3.2) in prostatic diseases has been established since the classic studies of Gutman et al. (1, 2). Assessment of serum acid phosphatase activity has subsequently been extended to nonprostatic diseases (3). In normal children, serum enzyme activity is higher than in normal adults (4, 5). The cause of this difference is unclear, but this “elevated” enzyme activity may lead to erroneous clinical interpretation, and, consequently, unwarranted extensive diagnostic studies.

Recently, we had the opportunity to study serum acid phosphatase activity in a 10-year-old boy hospitalized for urinary tract obstruction and infection. In the course of the clinical study, his serum acid phosphatase activity was found to be several fold the established normal adult value. This “high” serum enzyme activity led to further extensive studies, but to no avail. His serum acid phosphatase isoenzyme pattern was normal, although activity of isoenzyme 5 was markedly increased. No definite prostatic isoenzyme (isoenzyme 2) was detected. Because this sort of experience has been reported (6) and may be repeatedly encountered in the future, we undertook a study to investigate further the origin of serum acid phosphatase activity in normal children in order to avoid repetition of this experience.

Materials and Methods

Subjects and Samples

We studied 78 persons under 18 years of age: 49 children 12 years old or younger and 29 adolescents 13 to 18 years of age. Informed consents were obtained to draw blood samples for study. The samples were allowed to clot at 10 °C. The serum was separated and, if not used immediately, was stored at ~70 °C.

Fresh prostatic and nonprostatic tissues obtained at surgery were homogenized in the presence of a surfactant, Triton X-100 (50 mL/L) in a tissue homogenizer. Acid phosphatases were extracted from these tissues by six freeze–thaw cycles followed by centrifugation (7). The supernatant fluid was used for study.

Control subjects included 51 healthy blood donors over 18 years of age. In addition, sera from five patients with metastatic prostatic cancer with increased serum AcP activity, prostate tissue from five patients with either benign hyperplasy or cancer, tumor tissue from six patients with osteogenic sarcoma, and three patients with giant-cell tumor of the bone were studied. Total acid phosphatase (AcP) and tartrate-resistant acid phosphatase (TAcP) activities as well as isoenzyme and histochemical studies were performed for comparison.

Enzyme Assay

For fluorometry of AcP activity in the serum and in tissue extracts, we used a Turner fluorometer according to the method of Babson et al. (8), with minor modifications. α-Naphthyl phosphate in citrate buffer (70 mM/L, pH 5.2) was used as the substrate. TAcP was measured in an identical manner except that tartrate was added to the incubation solution to make a final concentration of 50 mM/L.

Total and heat-labile alkaline phosphatase (EC 3.1.3.1) activities were assayed according to the method of Johnson (9) with naphthol AS-MX phosphate as substrate.

Isoenzyme Studies

Isoenzyme activity in sera and tissue extracts was examined by disc polyacrylamide-gel electrophoresis (75 g/L gel, pH 4.3, 90 min) (10). After the electrophoresis was completed, the gel columns were washed with acetate buffer (0.1 mol/L, pH 5.2) and stained with α-naphthyl phosphate–Fast Garnet GBC

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**Table 1. Serum Enzyme Activities (U/L) of Males and Females in Three Age Groups (Mean ± 1 SD)**

<table>
<thead>
<tr>
<th>Enzyme Type</th>
<th>Group</th>
<th>Mean ± SD</th>
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<tbody>
<tr>
<td></td>
<td>Newborn-12 yr. (48)</td>
<td>13-18 yr. (28)</td>
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<tr>
<td>Acid phosphatase</td>
<td>6.1 ± 1.5</td>
<td>2.9 ± 0.7</td>
</tr>
<tr>
<td>Tartrate-resistant acid phosphatase</td>
<td>5.6 ± 1.5</td>
<td>2.3 ± 0.8</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>34.2 ± 11.1</td>
<td>23.7 ± 12.3</td>
</tr>
<tr>
<td>Heat-labile alkaline phosphatase</td>
<td>31.7 ± 10.2</td>
<td>21.7 ± 11.4</td>
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*Numbers within parentheses are number of subjects.

in the presence and absence of 50 mmol/L tartrate. The staining reaction was terminated by immersing the gel column in a 75 mL/L acetic acid solution. The relative intensity of each isoenzyme in the gel was assessed densitometrically and correlated with the enzyme activity measured biochemically.

**Enzyme Histochemistry**

Histochemical demonstration of AcP and TAcP in cryostat-sectioned tissues was done according to the method of Li et al. (11), with naphthol AS-BI phosphoric acid as the enzyme substrate and hexazotized pararosaniline as the coupler.

**Results**

For apparently normal adults (Table 1, Figure 1), our mean serum AcP activity was 1.6 ± 0.51 international (IUB) units (range, 0.9 to 3 U/L) and appeared not to be correlated with age. About two-thirds of the activity of serum enzyme was tartrate-resistant, one-third tartrate-sensitive. In normal children, the total serum AcP activity was 4.6 ± 2.35 (range 0.9 to 10.2) U/L and the TAcP activity was 4.14 ± 2.3 (range, 0.8 to 9.6) U/L. In children ranging in age from newborns to 12 years old the total AcP activity in serum was 6.0 ± 1.6 (range, 2.7 to 10.2) U/L and the TAcP activity was 5.5 ± 1.6 (range, 2.5 to 9.6) U/L, whereas in adolescents these values were 2.24 ± 1.3 (range, 0.9 to 7.7) U/L and 1.8 ± 1.3 (range 0.8 to 7.7) U/L, respectively. The enzyme activity in serum appeared to correlate with age: it was highest in infants, gradually declined thereafter, but remained two- to fourfold above the normal adult value until the age of 14, at which time it declined sharply to normal adult values. There was no significant sex-related difference in enzyme activity in either the children or the adult groups (Table 1).

Isoenzyme studies (Figure 1) indicated that adult sera contained isoenzymes 3 and 5, the former being tartrate-sensitive and having an electromobility identical to that of platelets, isoenzyme 5 being tartrate-resistant. The isoenzyme pattern in normal children was comparable to that in adults, except that isoenzyme 5 activity was markedly greater. No abnormal protein bands with AcP activity were seen in the children's sera.

Extracts of prostatic tissue and sera from patients with metastatic cancer of the prostate contained isoenzymes 2 and 4 in our gel-electrophoretic system. These two isoenzymes, like isoenzyme 3, are tartrate-sensitive, but are not present in sera from either normal children or normal adults. When serum samples from normal children were mixed with prostate ex-
tract or sera from patients with prostatic carcinoma, isoenzymes 2, 3, 4, and 5 were noted. In six patients with osteogenic sarcoma, the neoplastic osteoblasts invariably contained strong alkaline phosphatase activity and weak acid phosphatase activity, whereas in three patients with giant-cell tumor of bone, intense TAcP activity was demonstrated in the giant cells or the osteoclasts. The enzyme in the giant cells has electromobility identical to that of isoenzyme 5 of the sera of both children and adults (Figure 2).

When the activity of AcP in serum was compared with that of alkaline phosphatase, with and without heat inactivation (Figure 3), the TAcP activity generally correlated with that of the heat-sensitive fraction of alkaline phosphatase.

**Discussion**

This study indicates that serum AcP activity in normal children exceeds that in adults. The fraction contributing to this greater value in children is the tartrate-resistant isoenzyme 5. The serum enzyme activity in children is age-dependent; it is highest in infants, declines slowly with age, and declines sharply to normal adult values after the age of 14. This "elevated" serum enzyme activity in children is not sex-related, and is nonprostatic in origin.

Our previous studies have indicated that TAcP is present in adrenal adenoma, granulomatous lesions of Hodgkin's disease, and in hairy cells of leukemic reticuloendotheliosis (12, 13) but not in 23 human organs and tissues (14). Bone tissue, however, has not been adequately investigated in these studies.

In many human bone diseases, serum acid phosphatase activity is often above normal (3). At least in Paget's disease and in breast cancer with bone metastasis, much of the increased serum enzyme activity was tartrate-resistant (3, 7). In animal experiments, intense TAcP activity has been shown in osteoclasts, whereas significant activity of alkaline phosphatase is demonstrated in the osteoblasts (15-19). This finding suggests that the heat-sensitive fraction of alkaline phosphatase in human serum may originate from osteoblasts and the TAcP activity from osteoclasts. Attempts to study the TAcP activity in adult bone-marrow biopsy samples have met with technical difficulties inherent in the small size of the biopsy specimens. Nevertheless, the fact that the serum TAcP activity is greater in young children and drops sharply at pre-puberty suggests that it may be related to body maturation or bone growth. The close correlation between activity of serum heat-sensitive (bone) alkaline phosphatase and TAcP, and the absence of significant TAcP in various normal and abnormal human tissues other than bone support the interpretation that the TAcP in the serum of children may originate from bone. The presence of intense TAcP activity in giant cells or osteoclasts in the giant-cell tumors would reinforce this possibility.

The clinical implications of elevated serum TAcP activity

![Fig. 3. Relationship between age and activities of acid and alkaline phosphatases](image)

Note that acid phosphatase activities fluctuate with age in a similar fashion as alkaline phosphatase.
in diseases with bone involvement remain to be elucidated. Nevertheless, it is useful to know that serum AcP activity in children many times greater than in the normal adult may not be pathologic, but merely represents a normal physiologic phenomenon.

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