Alkaline Phosphatase Activity in the Plasma of Children and Adolescents

Gerard A. Fleisher,¹ E. Stuart Eickelberg,² and Lila R. Elveback²

We determined plasma (serum) alkaline phosphatase activity in 854 healthy students of the Rochester, Minnesota, public schools. prepubertal girls had somewhat greater upper limits than did boys, and there was a low trend of increasing activity in both sexes. At the beginning of adolescence increasing activities were observed, which peaked at ages 11 to 12 years in girls and at ages 13 to 14 in boys. Adult values were not reached until six to eight years later. In 180 pairs of siblings, a significant intraclass correlation was noted. A possible role of alkaline phosphatase in the regulation of protein synthesis is suggested.

Additional Keyphrases: sex- and age-related effects, pediatric chemistry, enzyme activity, reference values, centrifugal analyzer, genetics, protein synthesis

During a comprehensive study of serum lipids in healthy students attending the Rochester, Minnesota, schools, the distribution of plasma alkaline phosphatase relative to age and sex also was investigated. We wish to report the results of this study.

Material and Methods

In the initial part of the study, done on grade school children between the ages of six and 12 years, heparinized plasma was used. In subsequent studies, plasma was replaced with serum after we determined that alkaline phosphatase activity in plasma did not differ significantly from that in serum (Figure 1). Health criteria for acceptance into the study were: negative findings at the last medical checkup, an absence of familial disease in parents and siblings, no history of major surgery, and no medication being received. The series involved 854 children (429 boys and 425 girls), about half of whom were of grade school age, one-fourth of junior high age, and one-fourth of senior high age. Blood samples were obtained at school in the early morning after an overnight fast, and the enzyme determinations were done on the same day.

Alkaline phosphatase activity was measured by the method of McComb and Bowers (1) with p-nitrophenyl phosphate as the substrate and diethanolamine buffer (DEA) as the phosphate acceptor. Final concentrations of the reactants were: DEA (Fisher Scientific, single lot), 1.00 mol/liter; MgCl₂, 0.5 mmol/liter; p-nitrophenyl phosphate (disodium, Sigma), 14 mmol/liter; and serum 1/27.7, and pH 10.15. A centrifugal analyzer (E.N.I.'s GEMSAEC or Union Carbide's CentrifChem), interfaced to a computer (PDP8I), was used for the phosphatase determinations with a working temperature of 30 °C. Delay time was 40 s. Five reading intervals at 15 s were used. Results were calculated in IUB units (U) per liter at 30 °C. All phosphatase activities were found to best fit a log-normal distribution, and statistical calculations by computer were made on that basis. To demonstrate activity–age relationships, we applied the method of moving geometric means, using groups of 80 children and an overlap of 50.

Results

The distribution of phosphatase activity with age, by sex, indicates many who have high activity, especially boys between the ages of 12 and 15 years (Figure 2). From Figure 3, which shows the moving geometric means by age for each sex, the following conclusions can be drawn. In children younger than 10 years, there is no significant phosphatase activity difference between sexes, and the mean is almost four fold that of adults. At the beginning of adolescence, the activity peaks, after which there is a gradual lowering toward adult values within six to eight years. The puberty peak activity occurs in girls between 11 and 12 years, in boys between 13 and 14 years, and is much more pronounced in boys, in whom the increase is nearly 50% over the phosphatase activity in young children.

For clinical use, lower and upper limits for the different age groups are desirable. The establishment of such limits was particularly difficult for two reasons: (a) although there were 854 children in the series, the greatest number in any single year–sex group was only

¹ Departments of Laboratory Medicine and Medical Statistics and Epidemiology, Mayo Clinic and Mayo Foundation, Rochester, Minn. 55901.
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Fig. 1. Parallel determinations of alkaline phosphatase activity in plasma and serum of 91 children.

Fig. 2. Scattergrams of alkaline phosphatase activity by age in children.
A, Males; B, females.

Fig. 3. Moving geometric means of plasma alkaline phosphatase activity relative to age.
The children were ordered on the basis of age. Each mean is based on data for 80 children. The next mean is based on data from the 30 oldest of these 80 plus the next 50 in order of age. Solid line, males; broken line, females.

45, and (b) the range of phosphatase activity in any one of the adolescent age groups was very large, for instance, from 180 to 1100 U/liter in the 15- to 16-year-old male group. Therefore nonparametric methods were used to arrive at the limits, which we chose at the 5th and 95th percentiles. These limits are empirical estimates based on a judgmental combination of nonparametric methods for subgroups on age and regression where it seemed appropriate (Table 1).

An inspection of Table 1 shows that, before puberty, the lower limit is constant. The upper limit increases by 50 U/liter per year, and in this respect, the girls are a year ahead of the boys. The upper limit for the girls reaches a maximum of 900 U/liter in the 12- and 13-year-olds and then declines sharply to 250 U/liter by the age of 15 years. At age 18, the upper limit is just 24 U/liter above the value for young women.

In boys, the upper limit increases sharply after the age of 11 years and remains high (1100 U/liter) until the age 15, while in this period the lower limit shows a moderate increase. After age 15, both limits decrease, and at the age of 18 years, the upper limit is still 111 U/liter greater than the value for adults.

The variability between individuals of the same age and sex (as reflected in the difference P95 - P5) is more than 400% of that in adults for girls of ages 10 through 13 years and for boys 12 through 15 years old. In girls, the range decreases to the adult value by age 15, while in boys it is still 150% of the adult value at age 18. Subgroups of the same sex and chronologic age are evidently far from homogeneous with respect to the determinants of alkaline phosphatase activity. In using the data in Table 1 the developmental age of a child as well as the chronologic age must be considered.

We found a significant (P < 0.01) intraclass correlation (r = 0.40) in serum alkaline phosphatase activities in 180 sets of siblings who were part of our population of normal children. The correlation was established in terms of age- and sex-specific relative deviates. There was no difference in correlation between brother-brother, sister-sister, or brother-sister pairings. The significant sibling-sibling correlation indicates that familial factors are at work in the regulation of alkaline phosphatase circulating in blood. These could be due to heredity or shared environment or both.

Discussion

Of earlier investigations on the age-sex distribution of plasma alkaline phosphatase activity in school-age children, the most comprehensive is that by Round (2), who studied 624 subjects between the ages of seven and 17 years, with results similar to our own. In particular, the youngest children showed no difference in phosphatase activity between sexes, whereas at a later age
both sexes showed a significant increase that seemed to parallel the adolescent growth spurt. In boys this increase occurred between the ages of 10 and 14 years; in girls it occurred about two years earlier. Adult values for serum phosphatase activity were reached in girls by the 15th year, whereas in boys, the activity was still increased by the end of the 18th year. The quantitative changes observed in Round’s study were not as great as in ours, and the ratio between the activity in the younger grade school children and that in adults was not as high as we found. The difference in analytical methods could explain this. Round used the King–Armstrong method for activity, in which the substrate was phenyl phosphate (rather than p-nitrophenyl phosphate), a phosphate-accepting buffer was not present, and the temperature was 37 °C, whereas we used 30 °C. In an earlier paper (3) on plasma alkaline phosphatase in boys, a puberty peak between the ages of 12 and 14 years also was reported. The extent of the increase was of the order of 40%, which is similar to our finding. Two earlier studies (4, 5) noted the lack of activity difference between sexes in young school children, as well as the marked peak in boys about 14 years of age. A corresponding peak in girls about 12 years of age was observed by Harrison’s group (4), but not by Clark and Beck (5).

Recently, Bennett et al. (6) studied the relationship in adolescents between serum alkaline phosphatase activity and sexual maturity rating. The study showed that maximal phosphatase activity occurred in boys at sexual maturity rating stage 3 and in girls at sexual maturity rating stage 2. However, the ranges of activity—particularly in sexual maturity rating stage 4 of the girls and stages 4 and 5 of the boys—were great, similar to those observed by us in the chronologic age groups of 12 to 14 years in girls and 15 to 17 years in boys. Thus, the growth spurt probably is not associated with one particular stage of sexual maturation, just as it is not associated with one chronologic age. Kantero et al. (7) investigated the changes in serum alkaline phosphatase activity, together with certain endocrine changes, in 146 healthy school girls and young nurses between the ages of seven and 20 years. Maximal activity of alkaline phosphatase occurred at a skeletal age of 11.5 years, which was about a year before the mean menarcheal age in their group and at an approximate sexual maturity rating of stage 2. This maximal activity was threefold the mean value for adult women. Somatotropin concentrations in serum did not peak until just after the menarche, that is, about a year after alkaline phosphatase activity became maximal. Additionally, they found no significant correlation between somatotropin and alkaline phosphatase activity. The same conclusion applied to urinary excretion of 17-hydroxycorticosteroids, which reached adult values immediately after the menarche, and to excretion of 17-ketosteroids, which showed a steady increase throughout adolescence.

Alkaline phosphatase activity in the blood of growing children presumably originates in the metabolically active chondroblasts and osteoblasts. The properties of alkaline phosphatase extracted from bone and of the enzyme in children’s blood are similar in electrophoretic pattern, urea and heat inactivation, and bile acid inhibition. Woodard and Kenney (8) found that growing bone contains at least 10 times as much alkaline phosphatase activity as does the corresponding adult bone. Conditions characterized by cessation of growth, such as cretinism, scurvy, and achondroplasia, are associated with abnormally low serum activity of the enzyme (9). Contrary to the older theory that the function of alkaline phosphatase lies only in the mineralization of the bone matrix, it is now thought that the enzyme also functions in the synthesis of collagen and perhaps of other proteins (10). Thus, the increased serum alkaline phosphatase activity in childhood not only reflects bone growth but also growth of connective tissue generally. Support for this comes from studies of fibroblastic proliferation during wound healing, which is associated with large increases of alkaline phosphatase production (10, 11). The recent discovery (12) that alkaline phosphatase at a more physiologic pH and low concentrations of Mg2+ has a strong affinity for inorganic pyrophosphate (PPi) suggests that one of its primary functions on a molecular level is to hydrolyze PPi. Among the various enzymatic reactions that produce PPi—particularly as they relate to the synthesis of DNA, RNA, and proteins—those that activate amino acids and produce amino acyl-tRNA seem to be the prime candidates for a regulatory function by alkaline phosphatase. Hydrolysis of PPi should greatly increase the available concentration of amino acyl-tRNA for protein synthesis, provide inorganic phosphate for the resynthesis of ATP as well as for bone mineralization, and abolish the known inhibitory effect of PPi on bone mineralization. The observation that in hypophosphatasemia the concentration of PPi in the blood increases (13) seems consistent with this role of alkaline phosphatase in amino acid activation.

Table 1. P5 and P95 Limits of Serum Alkaline Phosphatase According to Age

<table>
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<tr>
<th>Age, years</th>
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<th>Boys P95</th>
<th>Girls P5</th>
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References