What Is the Cause of Benign Transient Hyperphosphatemia? A Study of 35 Cases

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In a study of 35 children with benign transient hyperphosphatemia, I found a marked seasonal clustering of cases after the summer months. Furthermore, plasma 25-hydroxyvitamin D concentrations were almost twice those of controls matched for age and time of year. Many children had evidence of weight loss and one had idiopathic hypercalcemia of infancy. Activities both of liver and bone isoenzymes of alkaline phosphatase (EC 3.1.3.1) in plasma were increased. The liver and (to a lesser extent) bone isoenzymes had enhanced electrophoretic mobility, and both showed increased binding to wheat-germ lectin by affinity electrophoresis. For the liver (and probably also the bone) isoenzyme, these changes were due to an increased content of sialic acid. A possible etiology for the condition is proposed involving (a) increased synthesis of alkaline phosphatase, mediated by vitamin D metabolites, and (b) decreased hepatic clearance caused by the high sialic acid content and exacerbated in some cases by the effects of some drugs on the liver.

Additional Keyphrases: vitamin D · alkaline phosphatase isoenzymes · binding to wheat-germ lectin · seasonal differences · neuraminic acid · half-life of enzyme · diagnosis

Benign transient hyperphosphatemia (TH) was first described by Posen et al. (1). Since that report there have been many individual case reports and a few longer series, recently reviewed by Stein et al. (2). TH usually occurs in children under five years of age, and it is characterized by a sudden transient increase in alkaline phosphatase (ALP, EC 3.1.3.1) activity in plasma, often to spectacular amounts. Activities then return to normal, usually within four months. The clinical presentation is variable, and the condition has been found in healthy children. Extensive physical and biochemical investigation reveals no evidence of liver or bone disease to account for the ALP increase. The etiology of TH remains a mystery. I report here an investigation conducted on 35 children with TH, the largest series to date, with the aim of discovering a possible cause for the condition.

Materials and Methods

Electrophoresis and measurement of total ALP and its isoenzymes. I measured total ALP activity in plasma, using p-nitrophenyl phosphate as substrate in diethanolamine buffer at 37 °C (3). The upper limits of the adult and pediatric reference intervals were 250 and 850 U/L, respectively. For electrophoresis of ALP isoenzymes, I used agarose film, “Tris-Bicine” (tris(hydroxymethyl)aminomethane-N,N,N-tris(2-hydroxyethyl)glycine) buffer, and 4-methylumbelliferyl phosphate as substrate. All these were from Corning Medical and Scientific, Halstead, U.K., and were used according to the manufacturer's instructions. Fluorometric scanning of the gels allowed exact measurement of the mobilities of the isoenzyme bands. A reference plasma (from an adult with a slight increase in liver ALP activity) was included in each gel, and the mobilities (R) of all isoenzymes were calculated relative to that of the liver isoenzyme in this reference plasma. I alternated TH and control samples to avoid bias. Because the liver isoenzyme was not always resolved from the predominant bone isoenzyme in age-matched control samples, the data for the liver isoenzyme were fewer than for the bone isoenzyme. However, any bias so introduced into the comparison with TH samples was in the conservative direction; i.e., it would tend to underestimate rather than overestimate differences in isoenzyme mobility between control and TH samples (see Results).

Quantification of ALP isoenzymes, and assessment of relative degrees of binding to wheat-germ lectin (from Triticum vulgaries; Sigma Chemical Co., Poole, U.K.), was carried out by affinity electrophoresis in “lectin–agarose,” agarose gel impregnated with the lectin (4). I calculated an indirect “lectin binding index” for each isoenzyme by subtracting its relative mobility in lectin-agarose from its relative mobility in unmodified agarose.

General properties of ALP isoenzymes. I measured inhibition by L-homocysteine and L-phenylalanine, thermal inactivation at 56 °C, apparent relative molecular mass, and quantitative binding to wheat-germ lectin–agarose, 5-hydroxytryptamine–agarose, and concanavalin A–agarose (all from Sigma Chemical Co.) by methods previously described (5).

Removal of sugars from ALP isoenzymes. To remove N-acetylgalactosamine, I treated samples with an equal volume of a 5 kU/L solution of N-acetylβ-glucosaminidase (from jack beans, EC 3.2.1.30; Sigma Chemical Co.) for 24 h at 37 °C. Sialic acid (neuraminic acid) residues were removed in either of two ways. For complete desialylation I incubated samples with an equal volume of a 1 kU/L solution of sialidase (EC 3.2.1.18, from Clostridium perfringens; Sigma Chemical Co.) in 0.1 mol/L acetate buffer, pH 5.5, containing 0.15 mol of sodium chloride and 10 mmol of magnesium chloride per liter, for 4 h at 37 °C. For partial removal of the more accessible sialic acid residues, I used a short incubation with 1 kU/L sialidase solution in a sample:sialidase volume ratio of 5:1 for exactly 15 min at 37 °C, after which I rapidly cooled the samples in ice and, without delay, subjected them to electrophoresis.

25-Hydroxyvitamin D (25-OHVitD) measurement. A competitive protein binding assay was used, based on that of Preece et al. (6).

Statistical tests. I used parametric statistical tests unless F-tests demonstrated unequal variances in the groups being compared. All statistical tests were two-tailed.

Results

Clinical Features of Benign TH

Over a period of four years, I have identified 35 patients (all white Caucasians) with benign TH. Within the post-
neonatal pediatric age group, this represents a detection rate of approximately 1 in each 300 samples for which ALP analysis was requested. I discovered one case accidentally while carrying out biochemical investigations on a small group of "control" children for whom ALP assay was not requested. Especially in view of its transient nature, benign TH must therefore be relatively common. Eighteen of the cases were males and 17 females. Excluding one case, the mean age of presentation was 24.8 months (range 7–47 months). The exception was a boy of 8.5 years with rhabdomyosarcoma in remission; he had finished chemotherapy over 2 years previously, had no evidence of recurrence, and satisfied all the criteria of benign TH.

The children presented with a wide variety of clinical conditions, ranging from the relatively trivial to the serious, in all cases with no evidence of liver or bone involvement. Twelve children had evidence of infection, 11 had seizure disorders (of various etiologies), and nine had gastrointestinal symptoms (usually diarrhea). Of the serious disorders, one child had severe encephalitis, one was in complete remission after removal of a Stage I orchidoblastoma, one (see above) had rhabdomyosarcoma in remission, and two were receiving chemotherapy for acute lymphoblastic leukaemia. One child died of causes unrelated to the TH: a girl with a ventricular septal defect and pulmonary hypertension who died during surgery. One child was on a low calcium diet for idiopathic hypercalcaemia of infancy (Williams' syndrome) at the time of the TH. Her plasma calcium concentration was towards the upper end of the normal reference interval. A roentgenogram of her wrist showed no radiological evidence of rickets, and the ALP value reverted to normal within eight weeks, with no change in therapy.

Growth was recorded in 27 cases. Seventeen children had recent or long-standing weight loss (for a variety of clinical reasons), and four of these had evidence of recent catch-up growth. Radiological examination in nine cases showed no evidence of rickets. Eighteen children were receiving drug therapy: sodium valproate (six), trimethoprim-sulfamethoxazole (three), and a wide variety of other drugs, including simple analgesics. The remaining 17 children were taking no drugs at all.

There was a marked seasonal clustering of cases (Figure 1). A disproportionate number of cases presented during the second half of the calendar year (binomial test, $P < 0.01$).

Biochemical Features of Benign TH

**Total ALP and Isoenzymes.** The median peak activity of ALP in plasma was 4415 U/L (range 1255–16 300 U/L). Isoenzymes were quantified in 17 cases of TH and in 18 age-matched hospitalized controls without the condition (Table 1). In nearly every TH case, the liver and bone isoenzymes were both increased, maintaining similar relative proportions to those found in controls. I also measured isoenzymes in six cases of TH after total ALP had returned to normal. In every case the liver and bone ALP activities had returned to the range seen in the controls.

**Dynamic behavior of ALP.** In five TH patients the ALP activity increased still further after initial detection. The mean rate of increase in these patients was 590 U/L per day (range 385–700). In 13 of the TH patients, it was possible to approximate the half-life of the enzyme in plasma as its activity decreased from the peak. For 11 of these patients, the mean half-life was 7.6 days (SD 2.2 days). The remaining two patients had acute lymphoblastic leukaemia and were receiving maintenance chemotherapy. In these two the ALP half-lives were much longer, 24 and 25 days, respectively. The time taken to revert to the highest ALP activity identified (which may not necessarily have coincided with the peak) to normal values varied according to the extent of the ALP increase, but was always less than 12 weeks. The probable total duration of the ALP increase was two to four months.

In four cases of TH, I monitored isoenzymes serially during the period of increased total ALP. The activities of the two isoenzymes peaked at approximately the same time, but the liver isoenzyme formed a progressively larger proportion of the total from pre-peak to peak to post-peak samples, suggesting that either its synthesis or its clearance (or both) lagged behind that of the bone isoenzyme.

**General properties of ALP isoenzymes.** The liver and bone ALP in TH were indistinguishable from normal liver and bone ALP in age-matched controls and adults, in terms of inhibition by L-homoarginine, resistance to inhibition by L-phenylalanine, half-life at 56 °C (6.6 min and 2.5 min for the liver and bone isoenzymes, respectively), and apparent relative molecular mass. Binding to 5-hydroxytryptamine–agarose and concanavalin A–agarose (19% and 34%, respectively) was also close to that of control samples.

**Electrophoretic mobility.** The electrophoretic mobilities of the liver isoenzyme were significantly higher in TH samples

![Fig. 1. Seasonal presentation of 35 TH cases over four complete calendar years](chart)

The dashed line represents the expected monthly incidence based on an even incidence throughout the year. By the chi-square test, comparing the number of cases presenting in bimonthly periods through the year, $P < 0.01$. By the binomial test, comparing the number of cases presenting in the first half of the year with the number presenting in the second half, $P < 0.01$.

### Table 1. ALP Isoenzymes in Selected Samples from Cases of TH and Age-Matched Controls

<table>
<thead>
<tr>
<th>Isoenzyme</th>
<th>TH (n = 17)</th>
<th>Controls (n = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total ALP (U/L)</td>
<td>Median 4225*</td>
<td>347</td>
</tr>
<tr>
<td>Liver ALP (U/L)</td>
<td>Median 628*</td>
<td>97</td>
</tr>
<tr>
<td>Bone ALP (U/L)</td>
<td>Median 2993*</td>
<td>251</td>
</tr>
<tr>
<td>Liver ALP, in % of total ALP</td>
<td>Median 21.1</td>
<td>26.5</td>
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*Significantly different from controls: $P < 0.001$ (Mann–Whitney U test).
than in age-matched controls (P <0.001, Student's unpaired t-test), with only one TH sample being in the control range (Figure 2). The bone isoenzyme in TH samples showed a slight increase in mobility, which did not reach statistical significance (possibly owing to the large interindividual variation among TH patients and controls) but was correlated with the mobility of the liver isoenzyme (r = 0.627, P <0.01). Once the ALP values had returned to normal in six TH patients, the liver isoenzyme could no longer be resolved from the bone isoenzyme, suggesting that it no longer had enhanced mobility. In these post-TH samples, there was also a slight, but significant (P <0.05, Student's paired t-test) decrease in the mobility of the bone isoenzyme (mean 0.88) compared with samples from the same patients taken during TH (mean 0.92). Within individuals, therefore, TH was associated with an increased mobility of the bone isoenzyme, which decreased after total ALP activity had returned to normal.

Three patients with biliary hypoplasia (ages 10–30 months) and large non-transient increases in liver ALP isoenzyme activity in plasma had no enhancement of liver isoenzyme mobility, suggesting that the phenomenon was not associated with high enzyme activities per se.

**Binding to wheat-germ lectin.** The lectin binding index for liver ALP was greater (P <0.005, Student's unpaired t-test) for TH samples (mean +0.06, SD 0.07) than that for control samples (−0.04 ±0.04). It was also greater (P <0.01, Student's unpaired t-test) for bone ALP in TH samples (+0.24 ±0.08) compared with control samples (+0.17 ±0.06). In six post-TH samples, the lectin binding index of bone ALP had returned to control values (+0.19 ±0.04). Among TH samples, there was a strong correlation between the lectin-binding indices for liver and bone ALP (r = 0.825, P <0.001); no such correlation was found for control samples. In both TH and control groups, the lectin binding indices of both isoenzymes were correlated with their mobility in unmodified agarose gel, the correlation being strongest for the liver isoenzyme in the TH group (r = 0.909, P <0.001).

There was no overall correlation between the activity of liver ALP in plasma and its electrophoretic mobility or lectin binding index. Serial samples from four TH patients also showed an inconsistent relationship between these variables. On the other hand, although the bone isoenzyme showed little change in its electrophoretic mobility over time in these patients, it did show parallel behavior between the lectin binding index and activity during the rising and falling phases of the disorder.

Table 2 confirms that a TH liver isoenzyme with a high lectin binding index by affinity electrophoresis also showed enhanced quantitative binding to wheat-germ lectin–agarose.

**Removal of sugar residues.** Treatment with N-acetyl-β-glucosaminidase resulted in a considerable reduction in the mobility of liver and bone ALP from both TH cases and controls (P <0.001, Student's paired t-test), but did not abolish the enhanced mobility of the TH liver isoenzyme (Table 3). In addition, quantitative binding to wheat-germ lectin–agarose was unchanged by the treatment.

Complete desialylation decreased the electrophoretic mobility of liver and bone ALP to nearly zero in all samples, and this was associated with a diminished quantitative binding to wheat-germ lectin–agarose, to 60% to 70% bound. Incubation with sialidase for only 15 min had only a small effect on the mobility of liver ALP in control samples, but decreased its mobility in TH samples to a value indistinguishable from that for controls (Table 3). The mobility of the bone isoenzyme was decreased to the same extent in TH samples and controls.

**Relationship with 25-OHvitD.** Following the discovery of a marked seasonal incidence of TH (see above), I examined plasma ALP activities in a group of 88 hospitalized control children, matched for age and race, who were not on anticonvulsant therapy and who had no evidence of nutritional, liver, or bone disease (data extracted from a previous study, 7). Although there was a marked seasonal variation

![Fig. 2. Electrophoretic mobility (Rf) of ALP isoenzymes from TH patients and age-matched controls.](image)

Table 2. Lectin Binding Index by Affinity Electrophoresis Compared with Quantitative Binding to Wheat-Germ Lectin–A garose

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Liver ALP</th>
<th>Bone ALP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>Binding to lectin–agarose, %</td>
<td>LBI*</td>
</tr>
<tr>
<td>Case 1</td>
<td>−0.06</td>
<td>73</td>
</tr>
<tr>
<td>Case 2</td>
<td>−0.02</td>
<td>74</td>
</tr>
<tr>
<td>TH</td>
<td>Case 1</td>
<td>−0.04</td>
</tr>
<tr>
<td>Case 2</td>
<td>+0.18</td>
<td>88</td>
</tr>
</tbody>
</table>

* LBI, lectin binding index: relative mobility in lectin–agarose subtracted from the relative mobility in unmodified agarose.

* The liver isoenzyme in TH Case 1 had a relatively low Rf value (1.07) and low lectin binding index within the control range, whereas the liver isoenzyme in TH Case 2 had a relatively high Rf value (1.15) and high lectin binding index.

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of 25-OHVitD (7), I found no seasonal fluctuation of ALP and no overall correlation of plasma ALP activities with 25-OHVitD concentrations. Three children in this control group had ALP activities close to the upper limit of the pediatric reference interval; all of them had 25-OHVitD concentrations close to the mean values for the time of year.

I then compared the plasma 25-OHVitD concentrations in 13 TH patients who presented between October and January with the concentrations in 32 of the controls who presented during those months (control data from ref. 7). The mean 25-OHVitD concentration in the TH patients was 55.4 (SD 24.6) nmol/L, compared with a mean of 30.9 (SD 15.9) nmol/L in the control group (P < 0.001, Student's unpaired t-test). As far as could be ascertained, none of the children were taking vitamin D supplements at the time these measurements were made. Owing to small sample volumes and ethical considerations which precluded obtaining additional samples from the children, I was unable to measure 1,25-dihydroxy-vitamin D.

**Discussion**

The enhanced electrophoretic mobility of the liver isoenzyme in TH has been described qualitatively by previous authors (8-11). In the present investigation, the quantitative comparison with age-matched controls provides a sound basis for concluding that TH can be reliably diagnosed from a single plasma sample, providing that the mobility of the liver isoenzyme is increased. Immediate diagnosis of TH offers an advantage over retrospective diagnosis (made after plasma ALP activities have returned to normal), especially in patients with malignant disease, because considerable anxiety and unnecessary further investigation can then be avoided. Other authors have found no enhancement of mobility for the bone isoenzyme beyond the rather wide control range during TH. In the present study, some of the patients did show a slight increase in the mobility of bone ALP, which declined after total ALP had returned to normal.

Both liver and bone ALP in TH samples were bound more readily by wheat-germ lectin during affinity electrophoresis compared with age-matched controls. This binding was correlated with electrophoretic mobility. Wheat-germ lectin specifically binds glucose derivatives with a 2-acetoamido group and a free 3-hydroxyl group (12), a description that applies equally to N-acetylglucosamine and sialic acid (N-acetyneuraminic acid). The enhanced mobility could therefore have been due to an increased content of either of these sugars. Experiments showed that it was ascribable to sialic acid. Unlike the sialic acid attached to the normal liver isoenzyme, the excess sialic acid present in TH liver ALP was readily removable by very brief incubation with sialidase. Rosalki and Foo also described, in qualitative terms, the retardation of liver ALP by sialidase in a patient with TH (10).

Normal bone ALP also contains readily removable sialic acid (13) and binds strongly to wheat-germ lectin (14). Perhaps this high-background "masking" effect was the reason that I was unable to demonstrate directly any increased content of sialic acid (Table 3) or quantitative binding to wheat-germ lectin-agarose (Table 2) in bone ALP from TH patients. However, the greater binding of TH bone ALP to lectin during affinity electrophoresis and the increase and decrease of the lectin binding index in the increasing and decreasing phases of the disorder suggest that either (a) there was indeed an overall increase in the number of sialic acid residues or (b) they were more accessible for rapid binding to lectin during the 20-min course of electrophoresis. The correlation, both in electrophoretic mobility and in lectin binding index, between liver and bone ALP in TH patients suggests that the same process was affecting both isoenzymes, resulting in an increased sialic acid content. The alternative possibility remains that the "liver" isoenzyme in TH patients did not originate in liver at all, but was an excessively sialylated bone isoenzyme. This explanation is rendered less likely by the observation that the rate of heat denaturation of the isoenzyme resembled that of the liver form rather than the bone form. Schoenau et al. (15) found unusual ALP elution profiles by high-performance liquid chromatography in two cases of TH, which they attributed to the presence of ALP isoenzyme fragments. I have found no evidence for the presence of fragments with low molecular mass by gradient polyacrylamide gel electrophoresis. Rather, it seems likely that the retention times of the unusual peaks were prolonged owing to increased sialylation.

The clinical disorders with which the children with TH presented probably largely reflect the spectrum of conditions in which an ALP assay may be requested as part of the biochemical investigation in children of this age. TH in malignant disease, including acute lymphoblastic leukemia, has been reported before (16). The presence of several children with malignant disease in this series probably merely reflects the fact that ALP, among other assays, is regularly monitored in these children. Such regular moni-
toring may also be the reason why TH was found in a case of idiopathic hypercalcemia of infancy on treatment; alternatively, this case may shed light on the fundamental mechanism of TH. The precise etiology of idiopathic hypercalcemia of infancy is obscure but, like TH, it tends to be transient, albeit of longer duration than TH. The condition is associated with abnormally high rates of calcium uptake by the intestine, possibly as a result of hypersensitivity to vitamin D. There is some evidence of increased conversion of vitamin D to 25-OHvitD and reduced conversion of the latter to 1,25-dihydroxyvitamin D (17). TH has also been described in a case of vitamin D-dependent rickets being treated with 1α-hydroxyvitamin D (18); in this disorder the basic defect also results in decreased conversion of 25-OHvitD to 1,25-dihydroxyvitamin D. In both idiopathic hypercalcemia and in vitamin D-dependent rickets there may therefore be accumulation of 25-OHvitD and decreased concentrations of 1,25-dihydroxy-vitamin D. Treatment by diet may conceivably reverse this pattern. In my investigation of TH, the seasonal clustering of cases after summer exposure to sunshine (which has not previously been described) and the observation that 25-OHvitD concentrations were almost twice those of control children matched for age and time of year suggest that 25-OHvitD may be involved in the etiology of TH. In most previous studies this metabolite was not measured, although an increased concentration of 25-OHvitD in plasma was reported in one case of TH (19).

In more than half of the cases where growth indices were recorded, I discovered evidence of recent or long-standing weight loss (for a variety of clinical reasons), in some cases with evidence of recent catch-up growth. Previous investigators who have looked for evidence of unusual growth spurts have found none (16). Records have not generally been systematically examined for evidence of weight loss, but a recent study (2) recorded failure to thrive and poor growth to be the main clinical presentation in six of 21 cases of TH. Weight loss in adults has been reported to be associated with decreases in concentrations of 1,25-dihydroxy-vitamin D, and weight gain with increases in concentrations (20), although I am not aware of any such study on children. 1,25-Dihydroxy-vitamin D itself stimulates the synthesis of ALP in bone (21), and possibly also in liver, because both forms of ALP are almost certainly coded for by a single gene. One could speculate that a period of weight loss (for any reason) during the summer, when 25-OHvitD levels are relatively high, might block conversion to 1,25-dihydroxy-vitamin D, leading to further accumulation of 25-OHvitD. Then, as catch-up growth occurs, the block may be lifted and the accumulated 25-OHvitD converted to 1,25-dihydroxy-vitamin D, causing a surge in this metabolite that stimulates synthesis of ALP. The seasonal clustering of cases may be particularly striking in more northern latitudes where exposure to sunlight follows a marked seasonal cycle. This suggested mechanism may be particularly sensitive in children under five years of age, accounting for the age preference of TH, but TH has also been found in an eight-year-old boy (this series) and in a 21-year-old man (22).

Previous discussions of the etiology of TH have centered around the theory of impaired clearance of ALP from the circulation. This has been attributed to a postulated viral infection (2, 15), the hypothesis being based on impaired clearance of certain enzymes by the reticuloendothelial system in mice infected with Riley virus (23). However, the clearance of ALP itself in mice is unaffected by viral infection (23), rendering this hypothesis less likely. Like other glycoproteins (24), normal liver ALP appears to be cleared from the circulation by hepatocyte uptake, not via the reticuloendothelial system, and is protected from rapid removal by its sialic acid residues (25). In fact, removal of these sialic acid residues is probably required before substantial clearance can occur. Bone ALP has not been studied but probably shares a similar mechanism of clearance. It is therefore likely that the increased sialylation of the liver (and probably also the bone) isoenzyme in TH leads to some impairment of clearance. The only two children in this series who were on cytotoxic chemotherapy for leukemia showed a much longer half-life for ALP in the circulation than did the children not on chemotherapy. Two similar patients in another series also appeared to show a prolonged course (16). This suggests that adverse effects of drugs on liver parenchymal cells may prolong the course of TH by further impairment of clearance. The cause of the increased sialylation of ALP in TH remains uncertain. Sugar residues are attached to the protein moiety by a complex process of post-translational modification that is ill-understood, as is their removal during catabolism. Increased amounts of sialic acid may remain attached to the enzyme simply because the excessive rate of production of the enzyme and its sialylated form exceeds the capacity of any desialylation mechanisms.

In summary, the available evidence suggests that the etiology of TH is linked with increased synthesis of ALP, mediated by vitamin D metabolites, and is exacerbated by decreased clearance that is caused by increased sialylation of ALP and, sometimes, drug therapy.

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References


