Chapter 66

Hypophosphatasia: nature's window on alkaline phosphatase function in humans

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Chapter outline

Introduction

Alkaline phosphatase (ALP) was discovered by Robert Robison, PhD, in 1923 (Robison, 1923). During the next decade, he and his coworkers would advance his hypothesis that this phosphomonoester phosphohydrolase functioned importantly in skeletal calcification, emphasizing the liberation of inorganic phosphate (Pi), perhaps from hexosephosphoric ester substrate. Liberation of Pi to bind with calcium (Ca^{++}) would allow for hydroxyapatite (HA) crystal formation and growth. However, by 1932 Robison had concluded that some additional unknown factor conditioned this process (Robison, 1932). As I will review, this proved to be the ALP natural substrate and inhibitor of biomineralization, inorganic pyrophosphate (PPi).

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In the 1930s, physicians began to appreciate the important clinical insight that derives from quantitation of ALP activity in serum. Elevated levels (*hyper*phosphatasemia) usually denoted skeletal or hepatobiliary disease. In contrast, *hypo*-phosphatasemia usually went ignored (Wolf, 1978). Quantitation of serum ALP activity soon became the most frequently performed enzyme assay (McComb et al., 1979; Siller and Whyte, 2018).

In 1948, implication of the ubiquitously expressed "tissue nonspecific" (bone/liver/kidney) isoenzyme of ALP (TNSALP) as essential for skeletal mineralization began with the discovery by John C. Rathbun, MD, of hypophosphatasia (HPP) (Rathbun, 1948). In 1988, HPP was confirmed to be an inborn error of metabolism, as it was caused by loss-of-function mutation of the *ALPL* gene that encodes TNSALP (OMIM, 171760). The defective skeletal mineralization of HPP had been shown in the 1960s to involve deficient hydrolysis of PPi by TNSALP (Weiss et al., 1988a; Whyte, 1994). We now understand that ALPs are ubiquitous in nature (McComb et al., 1979; Harris, 1990; Moss, 1992; Whyte, 1994), yet there is uncertainty about the purpose of the three additional ALP isoenzymes in humans (Millán, 2006; Millán and Whyte, 2016).

In this chapter, I provide a brief history of the discovery of ALP, discuss the proposed function(s) of ALP in humans, and review the molecular and biological chemistry of the ALPs. Subsequently, HPP is described in some detail, and I summarize the most significant revelations about ALP from this "experiment-of-nature." Advances and refinements concerning the role of TNSALP revealed by mouse models of HPP are discussed (Millán, 2006; Millán and Whyte, 2016). Finally, the multinational approval in 2015 of asfotase alfa, a TNSALP-replacement therapy that restores "hard tissue" mineralization in HPP, returns us "full circle" concerning ALP.

History and proposed physiological roles of alkaline phosphatase

A 1923 publication by Robert Robison, PhD (Fig. 66.1**),** reported abundant phosphatase activity in bone and cartilage extracts from rats and rabbits, especially those with rickets. Thus, he hypothesized that the new enzyme acted in skeletal mineralization, perhaps by hydrolyzing a hexosephosphoric ester to increase the concentration of Pi available locally to bind with calcium (Ca^{++}) for HA crystal formation and growth (Robison, 1923). In 1924, he and Katherine Soames, PhD, reported that this phosphatase precipitated mineral into rachitic rat bone when monophosphate esters were the only source of Pi. In their laboratory, the enzyme became detectable using a distinctly alkaline pH optimum (Robison and Soames, 1924). However, Robison knew this pH was not physiological and called the enzyme "bone phosphatase" (Robison, 1932). The term "alkaline phosphatase" was introduced in the 1930s by others to distinguish it from a recently identified "acid phosphatase" that was becoming implicated in certain metastatic bone diseases (Siller and Whyte, 2018). However, concerns were emerging that challenged Robison's hypothesis (McComb et al., 1979). ALP activity was abundant not only in the skeleton but also in tissues that normally did not calcify (e.g., liver, intestine, and placenta). Furthermore, Robison had not identified its physiological substrate(s) (Neuman and Neuman, 1957).

FIGURE 66.1 Robert Robison, PhD, DSc, FRS (1883−1941), the discoverer of alkaline phosphatase in 1923. *Reproduced with permission from the Godfrey Argent Studio, as published in Obituary Notices of Fellows of The Royal Society, Vol. 3, 1941, p. 929.*

Then in the 1960s, electron microscopy rejuvenated Robison's hypothesis when the earliest site of HA crystal deposition in the skeleton was discovered by H. Clark Anderson, MD, to be within unique extracellular structures called "matrix vesicles" (MVs) (Anderson, 1969). MVs seem to be buds of the plasma membrane of chondrocytes and osteoblasts. They contain many enzymes including pyrophosphatase (PPi-ase) and ATPase (Anderson, 1992) and are especially rich in ALP (Ali, 1986). During the initial ("primary") phase of skeletal mineralization, HA crystals appear and then grow within MVs. When they rupture the MV, "secondary" mineralization features HA crystal growth for their deposition into the organic matrix of the skeleton (Ornoy et al., 1985). In 1975, nucleoside phosphate released from dying cells was proposed as the ALP substrate for Pi necessary to fulfill Robison's hypothesis (Majeska and Wuthier, 1975).

Further evidence that ALP functions in skeletal mineralization came from reports that used stereospecific inhibitors of ALP activity, such as L-tetramisole, which blocked calcification in vitro (Fallon et al., 1980). However, it was later shown that stereoisomers that did not block ALP activity would also impair mineralization (Whyte, 1994). In the clinic, it had been known for decades that circulating ALP activity correlated with the severity of disorders that involve accelerated bone formation (McComb et al., 1979), such as Paget bone disease (Kanis, 2002).

By the 1990s, many biological roles had been proposed for ALP (McComb et al., 1979; Harris, 1990; Moss, 1992; Whyte, 1989, 1994) and included provision of the nonphosphate moiety, transferase action in the synthesis of phosphate esters, regulation of Pi metabolism, maintenance of phosphoryl metabolite levels, and action as a phosphoprotein phosphatase (McComb et al., 1979; Alpers et al., 1990; Harris, 1990; Muller et al., 1991; Simko, 1991; Moss, 1992; Whyte, 1994; Millán and Whyte, 2016). Cell membrane ALP was hypothesized to condition not only the active transport of Pi but also Ca^{++}, fat, protein, carbohydrate, and Na^{+}/K^{+} (Muller et al., 1991; Simko, 1991). As the four isoenzymes of ALP in humans became recognized (see later), sequence analyses suggested they coupled to other proteins including collagen (Wu et al., 1992). In the placenta, ALP bound the Fc receptor of IgG and perhaps transcytosed this immunoglobulin (Makiya et al., 1992). During embryogenesis, ALP seemed to act intracellularly (Narisawa et al., 1992), although we now know that ALP functions importantly when bound to cell surfaces (see later).

Additional proposals also emerged for TNSALP action specifically in skeletal mineralization (Table 66.1) (Whyte, 1989, 1994). Perhaps TNSALP was a plasma membrane transporter for Pi (Wuthier and Register, 1985), an extracellular Ca^{++}-binding protein that stimulates Ca^{++}-Pi precipitation and orients mineral deposition into osteoid (DeBarnard et al., 1986), a Ca^{++}/Mg^{++}-ATPase (Birge and Gilbert, 1974), or a phosphoprotein phosphatase that conditions skeletal matrix for ossification (Lau et al., 1985; Tsonis et al., 1988). Certain structural domains of ALP suggested it could bind to types I, II, and X collagen in cartilage and bone (Tsonis et al., 1988; Wu et al., 1992). Nevertheless, a theory that captured Robison's missing "factor" (Robison, 1932) emerged and gained preeminence in the 1960s; i.e., TNSALP hydrolyzes an inhibitor of calcification (Neuman and Neuman, 1957; Caswell et al., 1991; Moss, 1992; Whyte, 1994; Heinonen, 2001; Millán, 2006; Millán and Whyte, 2016), with the principal candidate being PPi. High concentrations of extracellular PPi bind to HA and thereby impair HA crystal growth. TNSALP was shown to hydrolyze PPi (Moss et al., 1967). In fact (see later), plasma and urine levels of PPi became recognized as increased in HPP (Russell, 1965; Russell et al., 1971) consistent with PPi being a natural substrate of TNSALP.

Ironically, however, approaching a century after its discovery, methods for assaying ALP activity still do not deal with Robison's quandary of the alkaline pH optimum (McComb et al., 1979; Harris, 1990; Moss, 1992; Whyte, 1994; Coburn et al., 1998). In both clinical and research laboratories, ALP continues to be measured using nonphysiological alkalinity (e.g., pH 9.2 to 10.5). Furthermore, the assays involve high concentrations (millimolar) of artificial substrates whose

TABLE 66.1 Suggested roles for alkaline phosphatase in skeletal mineralization.

Locally increase Pi levels
Destruction of inhibitors of HA crystal growth
Transport of Pi
Ca^{++}-binding protein (Ca^{++} uptake by cells)
Ca^{++}/Mg^{++}-ATPase
Tyrosine-specific phosphoprotein phosphatase

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hydrolysis products can be followed colorimetrically (e.g., p-nitrophenylphosphate) (McComb et al., 1979). Also, biological specimens for ALP assay are diluted into buffers without Pi, although Pi competitively inhibits TNSALP (Coburn et al., 1998). Such assays were devised especially for their clinical utility (Wolf, 1978), yet it had been known for decades that the pH optimum for ALP is considerably less alkaline for lower concentrations of physiological substrates, although hydrolytic rates are reduced (McComb et al., 1979; Moss, 1992). Until the discovery of the natural substrates for TNSALP in studies of HPP, the significance of this was unknown (McComb et al., 1979; Harris, 1990; Moss, 1992; Whyte, 1994).

To understand what HPP teaches us about ALP, it is helpful to review the genomic structure, protein chemistry, and enzymology of ALP.

Genomic structure, protein chemistry, and enzymology of alkaline phosphatase

ALP (orthophosphoric-monoester phosphohydrolase, alkaline optimum, EC 3.1.3.1) is found throughout nature in plants and animals (McComb et al., 1979). In humans, four ALP isoenzymes are encoded by four separate genes (Millán, 1988, 2006; Harris, 1990; Moss, 1992). Three of the isoenzymes have essentially tissue-specific expression and are designated intestinal, placental, and germ-cell (placental-like) ALP. The fourth ALP isoenzyme is expressed ubiquitously and therefore is designated TNSALP (Stigbrand and Fishman, 1984; Harris, 1990; Moss, 1992). Skeletal, hepatic, and renal tissue are especially rich in TNSALP. The distinctive physicochemical properties (heat stability, electrophoretic mobility, etc.) among ALPs purified from human bone, liver, and kidney are lost upon exposure to glycosidases (Moss and Whitaker, 1985), and TNSALP is therefore a family of "secondary" isoenzymes (I will call them "isoforms"). They are encoded by the same gene, have the identical polypeptide sequence, and differ only by posttranslational modifications involving carbohydrates (Harris, 1980).

The gene mapping symbol for TNSALP is *ALPL* ("ALP-liver"), although the function of the liver isoform of TNSALP is not known (see later). *ALPL* is located near the tip of the short arm of chromosome 1 (1p36.1−p34), whereas the genes for the intestinal, placental, and germ-cell ALPs are at the tip of the long arm of chromosome 2 (2q34−q37) (Harris, 1990; Millán, 2006). *ALPL* seems to represent the ancestral gene, whereas the tissue-specific ALPs were likely formed by gene duplication (Harris, 1990). *ALPL* is somewhat larger than 50 kb and contains 12 exons, 11 of which are translated to form the mature enzyme consisting of 507 amino acid residues (Weiss et al., 1988b). TATA and Sp1 sequences may be regulatory elements, but basal expression seems to reflect "housekeeping" promoter effects, whereas differential expression in various tissues may be mediated by a posttranscriptional mechanism (Kiledjian and Kadesch, 1990). *ALPL* has two promoters and two corresponding 5′-noncoding exons, 1a and 1b. Their expression results in two different mRNAs with differing 5′-untranslated regions (Nosjean et al., 1997). Transcription occurs preferentially from the upstream promoter (1a) in osteoblasts and from the downstream promoter (1b) in the liver and kidney (Millán, 2006).

The tissue-specific ALP genes are smaller than *ALPL*, primarily owing to shorter introns. Amino acid sequences deduced from their cDNAs suggest 87% positional identity between placental and intestinal ALP but only 50%−60% identity between the tissue-specific ALPs and TNSALP (Harris, 1990).

The amino acid residue sequence of TNSALP indicates five potential N-linked glycosylation sites (Weiss et al., 1988). N-glycosylation is necessary for catalytic activity. O-glycosylation characterizes the bone, but not the liver, isoform (Nosjean et al., 1997).

In 2000, the crystal structure for human placental ALP was delineated at 1.8-Å resolution (Le Due et al., 2000). The active site of TNSALP would derive from a nucleotide sequence conserved in ALPs throughout nature (Henthorn and Whyte, 1992), reflect six exons, and comprise 15 amino acid residues (Zurutuza et al., 1999).

ALPs are Zn^{++}-metalloenzymes (McComb et al., 1979). Catalytic activity requires a multimeric configuration of identical subunits with each monomer having one active site and two Zn^{++} atoms that stabilize the tertiary structure (Kim and Wycoff, 1991).

ALPs are generally considered homodimeric in the circulation (McComb et al., 1979). TNSALP, in its symmetrical dimeric form, has αβ topology for each subunit including a ten-stranded β-sheet at its center (Hoylaerts and Millán, 1991). However, in tissues, ALPs are tethered (see later) to cell surfaces, perhaps as homotetramers (Fedde et al., 1988).

In vitro, ALPs have broad substrate specificities and pH optima that depend on the type and concentration of phosphocompound undergoing catalysis (McComb et al., 1979). Catalytic activity requires Mg^{++} as a cofactor (McComb et al., 1979). PPi as well as phosphoesters can be hydrolyzed (Xu et al., 1991). The reaction involves phosphorylation−dephosphorylation of a serine residue. Dissociation of the covalently linked Pi seems to be the rate-limiting step. In fact, Pi is a potent competitive inhibitor of ALP (McComb et al., 1979; Kim and Wyckoff, 1991; Coburn et al., 1998). However, it may also be that Pi stabilizes the enzyme (Farley, 1991).

Uncertainties persist about the biosynthesis of ALP in higher organisms. The gene sequences of the human ALP isoenzymes indicate that the nascent polypeptides have a short signal sequence of 17−21 amino acid residues (Harris, 1990) and a hydrophobic domain at their carboxyl terminus (Weiss et al., 1988b). Intracellular degradation of ALPs can involve proteasomes (Cai et al., 1998). Nevertheless, these ALPs link to the external surface of plasma membranes tethered to the polar head group of a phosphatidylinositol−glycan moiety (Whyte et al., 1988; Whyte, 1994) and can be released by phosphatidylinositol-specific phospholipase (Fedde et al., 1988). However, their precise interaction with phosphatidylinositol may differ among ALP isoenzymes (Seetharam et al., 1987).

Lipid-free ALP is the moiety normally found in the circulation. Yet, the mechanisms for ALP release from cell surfaces are not known. The process could involve a phosphatidase of the C or D type, detergent action, proteolysis, membrane fractionation, or lipolysis (Alpers et al., 1990).

In healthy men and women, nearly all ALP activity in serum or plasma derives from approximately equal amounts of the bone and liver isoforms of TNSALP (Millán et al., 1980). Infants and children, particularly newborns and adolescents, have higher bloodstream levels of the bone isoform (McComb et al., 1979). Some individuals with B and O blood types who are "secretors" increase the small amount of intestinal ALP in their circulation after ingesting a fatty meal (Langman et al., 1966; McComb et al., 1979). Typically, however, intestinal ALP contributes just a few percent to serum total ALP activity (maximum 20%) (McComb et al., 1979; Mulivor et al., 1985). Placental ALP is usually expressed and circulates only during the last trimester of pregnancy (Birkett et al., 1966). Various cancers, however, release placental or germ-cell ("placental-like") ALP (Millán, 1988) into the bloodstream. Clearance of circulating ALP, as for many other glycoproteins, probably involves uptake and degradation by the liver (Young et al., 1984).

Hypophosphatasia

Subnormal extracellular levels of Ca^{++} and Pi, or Pi alone, cause nearly all types of rickets and osteomalacia (Whyte, 2002; Drezner and Whyte, 2018). In fact, some believe that hypophosphatemia is common to all such patients (Tiosano and Hochberg, 2009). HPP is, however, a distinctive and remarkably instructive exception. This heritable form of rickets and osteomalacia has hypophosphatasemia as its biochemical hallmark. In HPP, circulating levels of Ca^{++} and Pi are typically normal but often elevated, and the skeleton does not mineralize properly (Whyte, 1994, 2001). Thus, HPP has been "nature's window" for understanding the important physiological role of TNSALP in humans. With the discovery beginning in 1988 (Weiss et al., 1988a) of loss-of-function mutations in TNSALP in HPP, Robison's hypothesis that ALP acts in biomineralization became confirmed. TNSALP was necessary not only for skeletal mineralization but also for mineralization of the teeth (Whyte, 1994). However, undisturbed function of other organs/tissues in HPP, notably the liver and kidney, questioned the biological significance for TNSALP elsewhere (Whyte, 1994, 2001).

History

John C. Rathbun, MD (Fig. 66.2), a Canadian pediatrician, coined the term "hypophosphatasia" in 1948 when he reported an infant boy who died of seemingly acquired rickets and epilepsy whose ALP activity in serum, bone, and other tissues

FIGURE 66.2 John C. Rathbun, MD (1915−1972), who identified and characterized hypophosphatasia in 1948.

obtained at autopsy was paradoxically subnormal (Rathbun, 1948). Hundreds of case reports of HPP are now in the medical literature (Whyte, 1994, 2001), and we now know the disorder's etiology and its key clinical, radiographic, biochemical, and skeletal histopathological features, and in addition understand well (but not completely) its pathogenesis (Whyte, 2018). A PubMed (http://www.ncbi.nlm.nih.gov/sites/entrez/) search from 1948 onward shows 687 articles with "hypophosphatasia" in the title, and 1072 where it appears in the text. Reviewed following, our understanding of both the metabolic basis for HPP and the physiological function of TNSALP was advanced importantly by the discoveries of elevated endogenous levels of three phosphocompounds (i.e., TNSALP natural substrates) in affected individuals. In 1955, in the era of paper chromatography to diagnose inborn errors of metabolism, identification of increased amounts of phosphoethanolamine (PEA) in urine provided a second biochemical marker for HPP other than hypophosphatasemia (Fraser et al., 1955; McCance et al., 1955). In 1965 and 1971, the discovery of elevated levels of PPi in the urine (Russell, 1965) and plasma (Russell et al., 1971), respectively, of HPP patients would explain the disorder's defective skeletal mineralization, because PPi was now recognized as inhibiting this process (Heinonen, 2001). In 1985, the discovery of often markedly elevated plasma concentrations of pyridoxal 5′-phosphate (PLP), the major circulating form of vitamin B_6 in HPP, coupled with an understanding of vitamin B_6 metabolism, revealed that TNSALP functions as a cell-surface enzyme. This explained the extracellular accumulation of these three phosphocompounds in this "inborn-error-of-metabolism" (Whyte et al., 1985).

Clinical features

Hypophosphatasia (OMIM #146300, #241500, and #241510) seems to occur in all ethnicities (Whyte et al., 2006). However, it is especially prevalent in Mennonites in Manitoba, Canada, where about 1 in 25 individuals carries a "founder" *ALPL* missense mutation (Greenberg et al., 1993), and 1:2500 newborns manifests the severe autosomal recessive disease (Leung et al.). Canadian Hutterites too have a relatively high prevalence of HPP. In Toronto, Canada, the incidence for what would be the severest forms of HPP was estimated in 1957 to be 1 per 100,000 live births (Fraser, 1957). Inexplicably, HPP seems to be particularly rare in people of black ancestry (Whyte et al., 2006).

Despite the high expression of TNSALP in bone, cartilage, liver, kidney, and adrenal tissues (and at least some ubiquitous TNSALP) in healthy individuals (McComb et al., 1979), HPP seems to disrupt only the skeleton and dentition directly (Whyte, 2001). Discussed later, vitamin B_6–dependent seizures are a metabolic consequence of HPP when it is most severe (Baumgartner-Sigl et al., 2007). Muscle weakness is often an important finding, but its pathogenesis is not understood.

Nevertheless, a remarkable feature of HPP is its extraordinarily wide-ranging expressivity, spanning from death in utero with an essentially unmineralized skeleton to early shedding of deciduous "baby" teeth without skeletal disease (Fraser, 1957; Whyte, 2001). In fact, many individuals with relatively mild biochemical characteristics of HPP and harboring one defective *ALPL* allele seem, at least early in adult life, to have escaped the disorder's complications and can be considered "carriers" (Whyte et al., 1982a). I consider HPP to manifest the most broad-ranging severity of all skeletal diseases. This partly reflects the polymeric nature of the active enzyme, the many ($>$ 360) different deactivating predominantly missense mutations found throughout *ALPL*, and the two patterns of autosomal inheritance (Weiss et al., 1988; Henthorn et al., 1992; Mornet et al., 1998; Whyte, 2000; Mumm et al., 2002). Some *ALPL* mutations have dominant/negative effects and cause autosomal dominant disease. However, it is increasingly apparent that other unknown genetic or nongenetic factors can significantly condition HPP expressivity. This is obvious from the discordant HPP severity that sometimes occurs among siblings sharing identical *ALPL* defects (Henthorn et al., 1992; Whyte et al., 2006; Mumm et al., 2006) **(**Fig. 66.3**)**. Accordingly, the prevailing nosology for patients with HPP (Whyte, 2001) remains a clinical one based primarily on consideration of age-of-onset as suggested by Fraser beginning in 1957 (Fraser, 1957). Understandably, an alternative *ALPL* mutation-based nosology might add too little for prognostication (Whyte, 2001).

Six principal forms of HPP are now discussed. The age at which the disorder presents and is diagnosed distinguished early on the perinatal, infantile, childhood, and adult forms (Fraser, 1957; Whyte, 2001). Those affected individuals who do not have skeletal disease but instead manifest dental features only are said to have "odontohypophosphatasia" (odonto-HPP) (Whyte, 2001). With the approval in 2015 of asfotase alfa as a TNSALP-replacement therapy for HPP, it became important to recognize "mild" versus "severe" forms of childhood HPP (see later). Nevertheless, this nosology of six forms of HPP does not unambiguously classify all patients, and it is important to appreciate that the disorder features a continuum of expressivity. An extremely rare form of HPP called "pseudohypophosphatasia" recapitulates infantile HPP, except that serum ALP activity is not subnormal but instead within or above the age-appropriate reference range in the clinical laboratory, where the assay reflects highly nonphysiological conditions (see later) (Scriver and Cameron, 1969; Whyte, 2001). Importantly, a not uncommon "benign perinatal" form of HPP, characterized from fetal sonography, revealed that

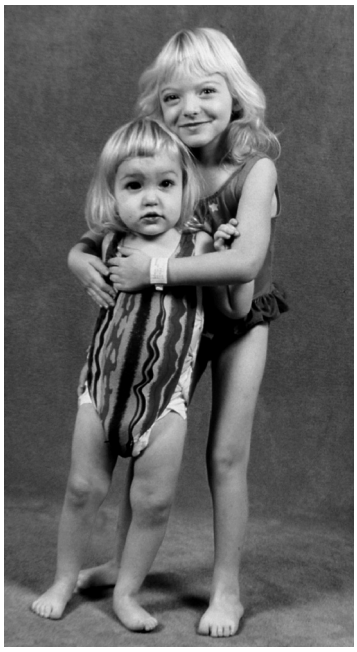

FIGURE 66.3 Variable clinical expressivity of hypophosphatasia is exemplified by these sisters who are compound heterozygotes sharing the same two *ALPL* missense mutations. The proposita (right) at age 5−3/12 years survived infantile HPP featuring poor weight gain, hypercalcemia with nephrocalcinosis, and severe rickets (Barcia et al., 1997) that was followed by short stature, premature loss of teeth, and craniosynostosis. Her younger sister (left) at age 2−2/12 years appears well and has mild rickets despite very similar hypophosphatasemia and endogenous elevations of the TNSALP substrates.

skeletal deformity in utero owing to HPP does not predict a lethal outcome (Moore et al., 1999; Pauli et al., 1999). This form of HPP manifests skeletal disease at birth but with spontaneous postnatal ex utero improvement, although with a broad-ranging clinical outcome (Wenkert et al., 2011).

The prognoses for the six major forms of HPP are determined by the severity of the skeletal disease. Typically, the earlier in life that skeletal signs and symptoms present, the worse the outcome (Fraser, 1957; Whyte, 2001). The benign prenatal form of HPP is, however, an important exception.

Perinatal hypophosphatasia

This most severe form of HPP (OMIM #241500), featuring profound generalized skeletal disease obvious in a neonate, reflects nearly a complete lack of mineralization of endochondral and membranous bone and typically causes death before or soon after birth. Remarkable skeletal softening can result in caput membranaceum and limbs that are short and deformed. Some affected newborns survive a few days or weeks but succumb to respiratory compromise owing to rachitic disease of the chest. In some, the lungs appear hypoplastic (Silver et al., 1988). There may be vitamin B_6−dependent seizures (Baumgartner-Sigl et al., 2007). Myelophthisic anemia can occur, perhaps from encroachment of excessive osteoid on the marrow space (Terheggen and Wischermann, 1984). Long-term survival is very rare (Whyte et al 2016a, 2019a).

Skeletal radiographs (Fig. 66.4) taken at birth readily distinguish perinatal HPP from even the most severe types of osteogenesis imperfecta or congenital dwarfism; the findings are diagnostic. Nevertheless, there is patient-to-patient variability (Shohat et al., 1991). In some stillborns, the bones appear nearly devoid of mineral (see Fig. 66.4A). In others, severe rachitic changes are apparent (see Fig. 66.4B). Occasionally, individual or sequential vertebrae appear completely or partly missing (Shohat et al., 1991). In the skull, the membranous bones may calcify only at their centers, giving the illusion that cranial sutures are widely separated ("open"), although they may be functionally closed (see Fig. 66.4C). Other unusual radiographic features (Whyte, 1988) include bony protrusions (Bowdler spurs) extending from the midshafts of the ulnas and fibulas (see Fig. 66.4D).

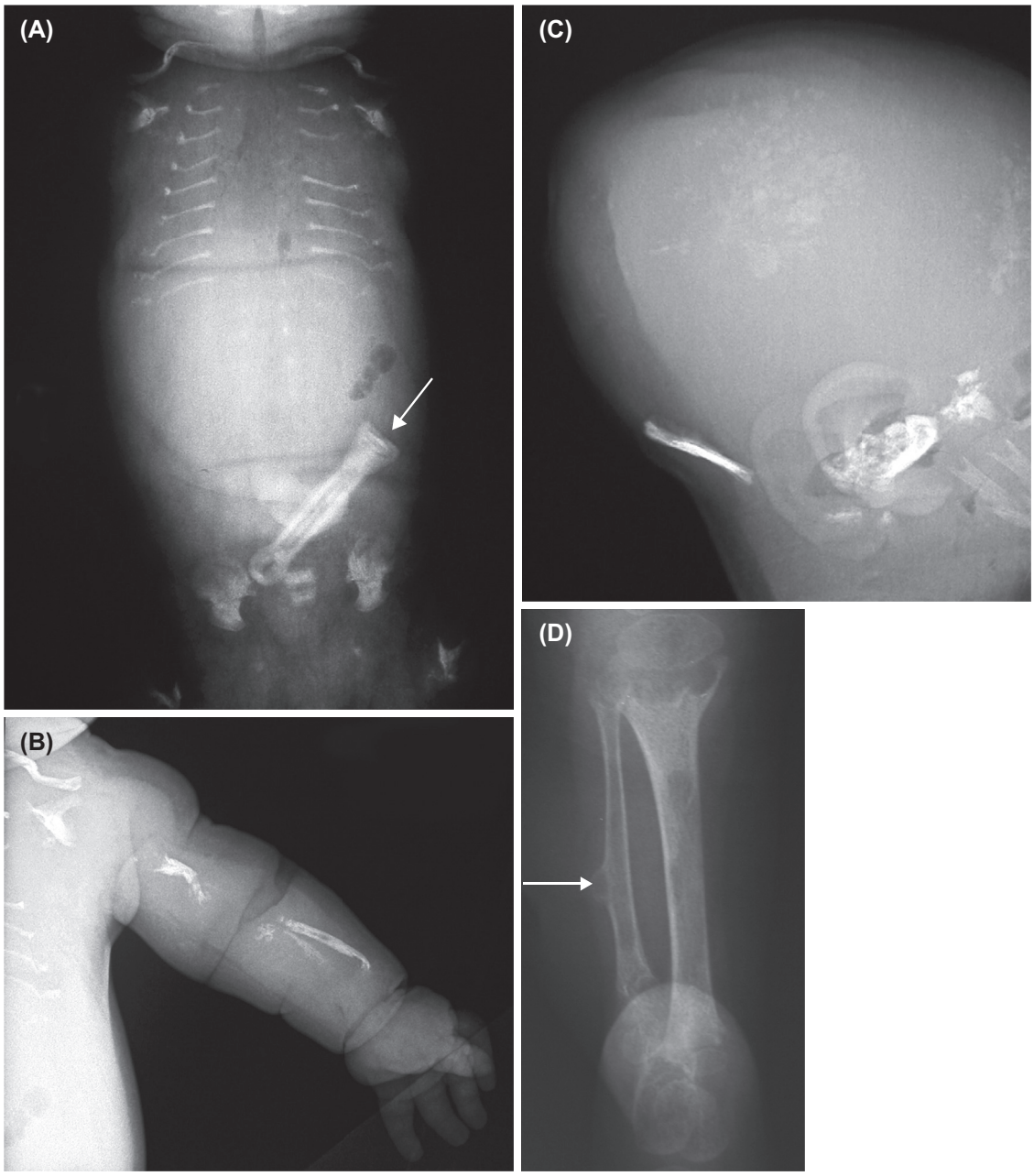

FIGURE 66.4 Perinatal hypophosphatasia. (A) Profound skeletal hypomineralization is obvious at birth (arrow points to umbilical cord clip). (B) The ends of the upper extremity long bones at 1 day of age show characteristic, extreme, rachitic changes. (C) Severe hypomineralization of the calvarium is present at 1 day of age. (D) A Bowdler spur (arrow) is found in some patients.

Infantile hypophosphatasia

This is the form of HPP (OMIM #241500) that was encountered by Rathbun (Rathbun, 1948). Signs and symptoms manifest after birth but before 6 months of age (Fraser, 1957; Whyte, 2001). Development may seem normal until there is hypotonia, poor feeding, and inadequate weight gain. At this time, the clinical and radiographic manifestations of rickets appear. Rarely, vitamin B_6–dependent epilepsy precedes the skeletal changes, and this complication predicts a lethal outcome (Baumgartner-Sigl et al., 2007). A flail chest from rib fractures, rachitic deformity, etc. often leads to pneumonia. Hypercalcemia and hypercalciuria are common and may explain episodes of recurrent vomiting as well as acquired nephrocalcinosis and renal compromise (Fraser, 1957; Whyte et al., 1982b, 2012).

Although the striking radiographic features of the skeletal disease of infantile HPP are diagnostic (see Fig. 66.5A), they are less severe than in perinatal HPP. Radiographs may suggest that the cranial sutures are wide open, but this can be an illusion from hypomineralization of the calvarium, and instead "functional" craniosynostosis can be present. Later, bony fusion of the sutures can occur if the patient survives infancy, causing the symptoms and complications of craniosynostosis (see Fig. 66.5B). In some babies, an abrupt transition from relatively normal-appearing diaphyses to poorly mineralized metaphyses (see Fig. 66.5C) suggests that metabolic deterioration occurred suddenly (Fraser, 1957). This observation is supported by the hypercalciuria and hypercalcemia that can develop in this form of HPP. Serial radiographs may reveal not only impaired skeletal mineralization (i.e., rickets) but also gradual, generalized demineralization of all osseous tissue (Whyte et al., 1982b) indicating a lethal outcome (Whyte et al., 2003; Cahill et al., 2007) (see Fig. 66.5D). However, spontaneous but unexplained improvement sometimes occurs (Ish-Shalom et al., 1986).

Childhood hypophosphatasia

This form of HPP (OMIM #241510) has especially wide-ranging expressivity (Fallon et al., 1984; Whyte, 2001). When asfotase alfa TNSALP-replacement therapy became available multinationally in 2015, characterization of mild versus

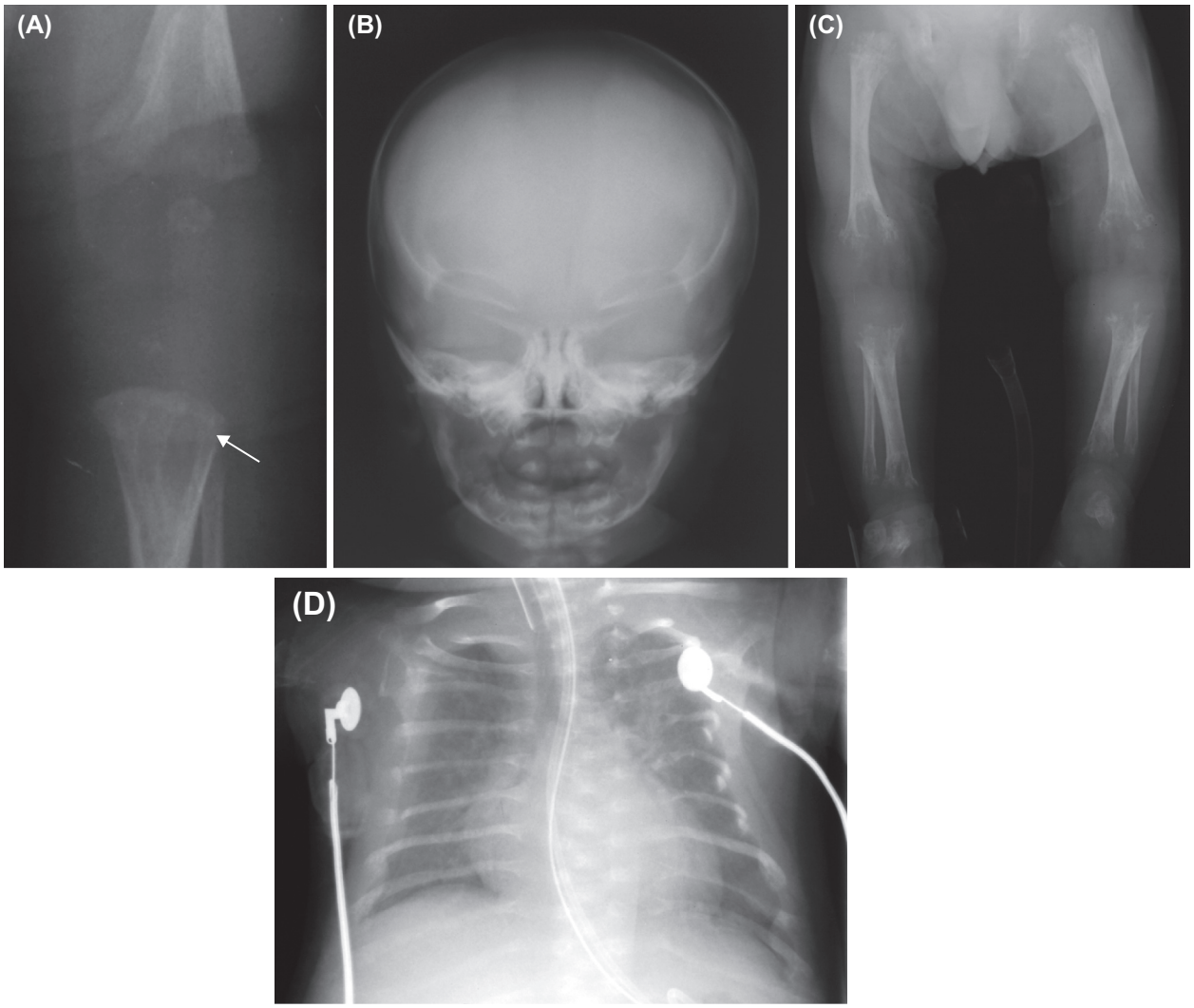

FIGURE 66.5 Infantile hypophosphatasia. (A) Characteristic tongues of radiolucency (arrow) extend from the growth plate into the metaphysis. (B) Cranial sutures appear widened in this hypomineralized skull at 1 month of age. (C) An abrupt transition seems to have occurred from well-mineralized diaphyses to poorly mineralized metaphyses by the age of 3 months. (D) The "bell-shaped" configuration of the chest from a soft thorax together with rib fractures will predispose to respiratory complications at 23 months of age.

severe childhood HPP helped in appreciating the clinical trial assessments of treatment (Whyte et al, 2015, 2016b). Childhood HPP is diagnosed when the presentation is after 6 months of age but before skeletal maturity. In 1953, premature loss of deciduous teeth was found to be a major clinical feature (Sobel et al., 1953). Early shedding of "baby" teeth (i.e., at less than 5 years of age) results from hypomineralization of aplastic or hypoplastic dental cementum (Van den Bos et al., 2005). Consequently, tooth roots are not bound sufficiently to the periodontal ligament (Lundgren et al., 1991), and teeth are shed painlessly, bloodlessly, and without trauma (see Fig. 66.6A). The mandibular incisors are typically lost first, and occasionally nearly all the primary dentition exfoliates prematurely. We have encountered premature loss of at least one deciduous tooth in 98% of our pediatric patients with HPP (Whyte et al., 2015). Delayed walking with a characteristic

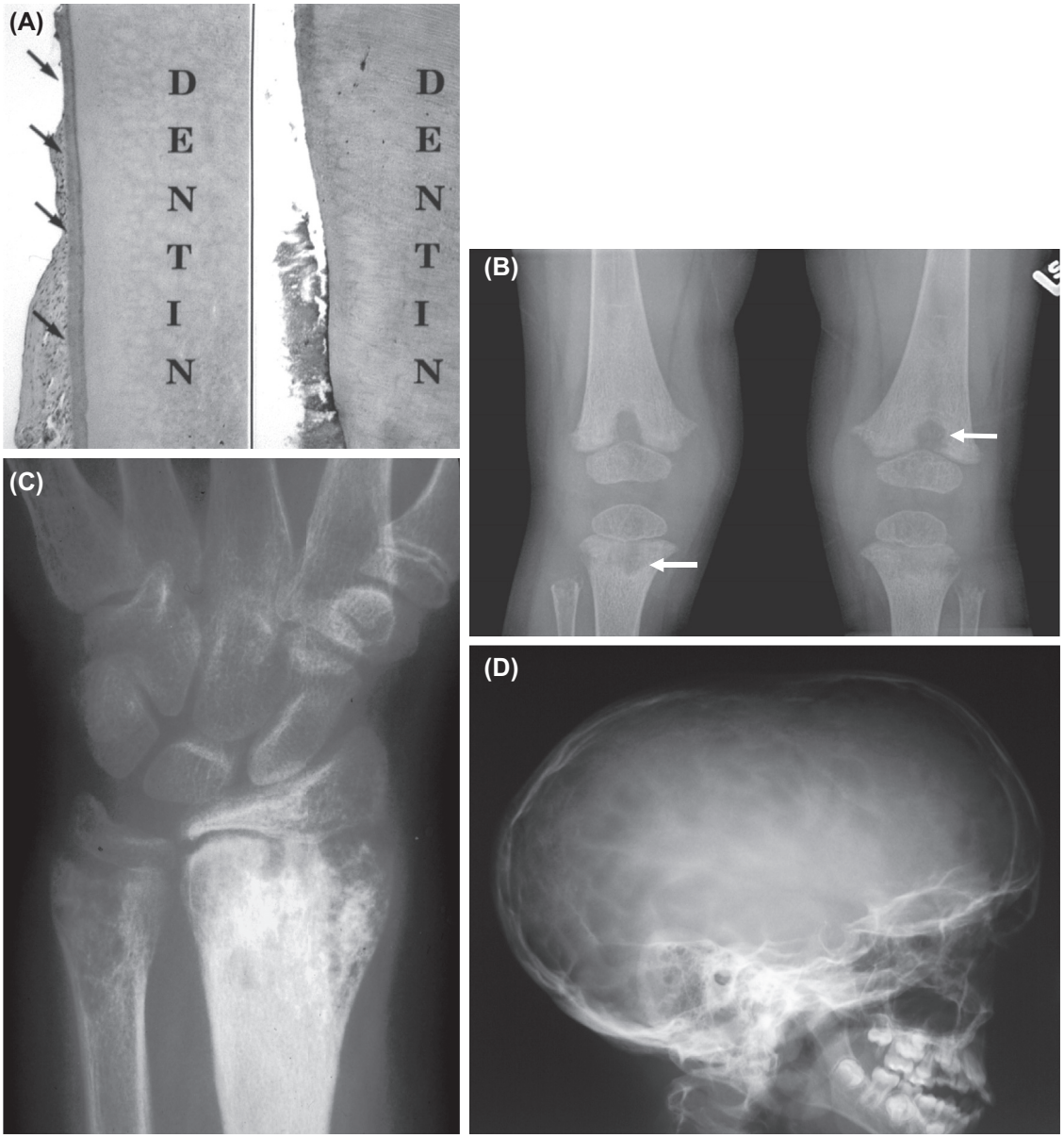

FIGURE 66.6 Childhood hypophosphatasia. (A) Dental findings. (Left) Decalcified section of part of the root of a maxillary incisor from a child with X-linked hypophosphatemia is essentially normal and shows primary cementum (delineated by arrows) at its surface (original magnification, ×150). (Right) In hypophosphatasia, cementum is absent (original magnification, ×150). (B) Characteristic tongues of radiolucency (arrows) project from growth plates into metaphyses at 16 months of age. (C) Idiopathic, patchy, metaphyseal osteosclerosis is a common finding. (D) A "beaten copper" appearance in the calvarium, here at 11 years of age, signifies premature closure of cranial sutures (craniosynostosis) that can lead to raised intracranial pressure. *(A) Reproduced with permission from Whyte, M.P., 2001. Hypophosphatasia. In: Scriver, C.R., Beaudet, A.L., Sly, W.S., Valle, D., (Eds.) The Metabolic and Molecular Bases of Inherited Disease, eighth ed., pp. 5313–5329. McGraw-Hill, New York.*

waddling gait is typical of severe childhood HPP. Patients may have appendicular muscle weakness (especially in the thighs) consistent with a nonprogressive myopathy (Seshia et al., 1990) and complain of stiffness and pain. There may also be delayed speech and language acquisition (unpublished observations).

Radiographs usually show characteristic focal "tongues" of radiolucency that project from rachitic growth plates into adjacent metaphyses (see Fig. 66.6B). Patchy metaphyseal osteosclerosis can occur (see Fig. 66.6C). True premature bony fusion of cranial sutures may raise intracranial pressure (see Fig. 66.6D). Dental radiographs sometimes show enlarged pulp chambers and root canals that characterize the "shell teeth" of various types of rickets.

Adult hypophosphatasia

This form of HPP (OMIM #146300) typically presents in middle age or later (Whyte, 2001; Whyte et al., 1982a). Not infrequently, however, such patients recall being told of early loss of their deciduous teeth followed by rickets or weakness in childhood. Subsequently, there is good health during young adult life (Weinstein and Whyte, 1981). Others among these patients may have been considered "carriers" of HPP until they manifested the characteristic complications of the disease (Sutton et al., 2012).

In adult HPP, osteomalacia often presents as recurrent metatarsal stress fractures (see Fig. 66.7A) (Whyte et al., 2007; Camacho et al., 2016). With more advanced disease, persistent aching or tenderness in the thighs or hips may be explained by femoral pseudofractures (see Fig. 66.7B) that will not heal spontaneously unless they progress to complete fractures (Coe et al., 1986; Khandwala et al., 2006; Whyte, 2009). Early loss or extraction of the secondary dentition is not uncommon, although the pathogenesis is not well understood (Whyte et al., 1982a). Calcium pyrophosphate dihydrate (CPPD) deposition disease troubles some patients, and occasionally overt attacks of pseudogout also reflect the increased endogenous levels of PPi (see later) (O'Duffy, 1970; Whyte et al., 1982a; McKiernan et al., 2014). Affected adults may suffer degeneration of articular cartilage from "pyrophosphate arthropathy" (Whyte et al., 1982a). Radiographs often reveal chondrocalcinosis and osteopenia (Lassere and Jones, 1990; Whyte et al., 1982a). In certain families manifesting hypophosphatasemia, Ca^{++}-Pi deposition manifests as "calcific periarthritis" (Guañabens et al., 2014) or ossification of ligaments (syndesmophytes) resembling spinal hyperostosis (Forestier's disease) (Lassere and Jones, 1990).

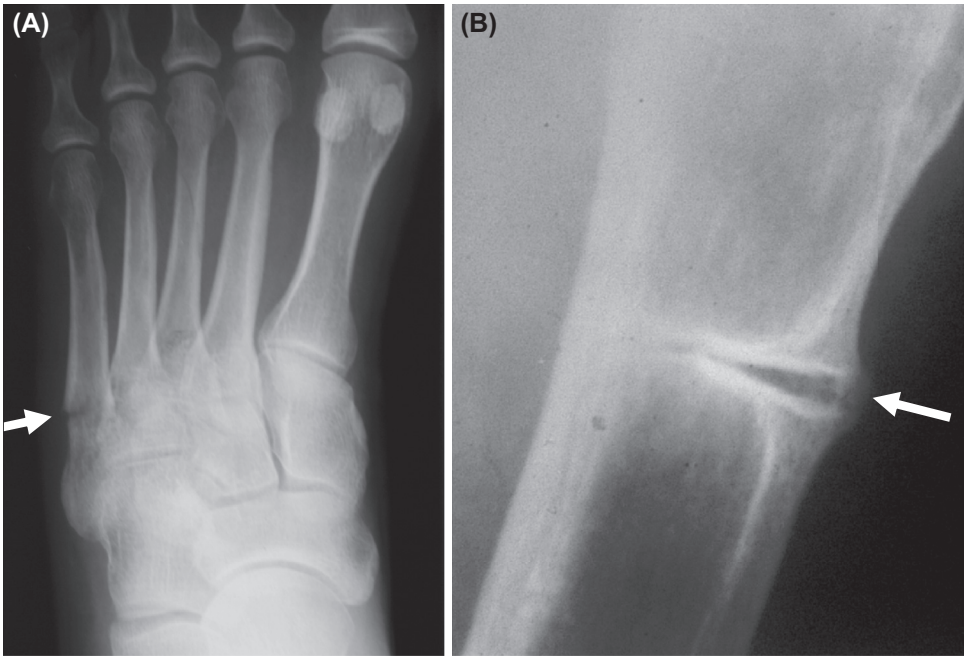

FIGURE 66.7 Adult hypophosphatasia. (A) Recurrent, poorly healing metatarsal stress fractures (arrow) may be the most common skeletal manifestation of the adult form of hypophosphatasia. (B) Femoral pseudofractures (arrow) are often painful and characteristically occur on the lateral aspect proximally in adult hypophosphatasia. They do not heal spontaneously unless a through-and-through break occurs.

Odontohypophosphatasia

This form of HPP is diagnosed when there are characteristic dental manifestations but no radiographic or histological evidence of rickets or osteomalacia.

Pseudohypophosphatasia

This extremely rare variant of HPP has been documented convincingly in two infants (Scriver and Cameron, 1969; Whyte, 2001). The clinical, radiographic, and biochemical findings are those of infantile HPP with one key exception—serum total ALP activity is not low but instead normal or increased (Whyte, 1994, 2001). The enzymatic explanation for pseudo-HPP involves defective TNSALP with diminished hydrolysis for PPi, PLP, and PEA endogenously, but normal or increased catalysis for artificial substrates in the nonphysiological conditions of the ALP assays used in clinical laboratories (see later) (Fedde et al., 1990).

Benign prenatal hypophosphatasia

Sonography may reveal a fetus with HPP showing bowing of major long bones that does not represent a lethal form of the disease. Spontaneous improvement of the skeletal deformities may occur later in the pregnancy as well as ex utero and then the severity range from infantile HPP to odonto-HPP (Moore et al., 1999; Pauli et al., 1999; Wenkert et al., 2005).

Laboratory diagnosis

Biochemical findings

HPP can be diagnosed with confidence from a consistent medical history and physical findings together with typical skeletal radiographic changes and serum ALP activity that is subnormal for the patient's age (Whyte, 2001, 2017b, Whyte et al., 2018). Even individuals with odonto-HPP are expected to have low serum ALP activity for their age, although their values can approach the lower end of carefully established reference ranges (see Fig. 66.8). Although HPP severity correlates inversely with age-appropriate serum ALP activity (Whyte et al., 2018), this correlation is not sufficiently strong to offer help with prognostication. In perinatal and infantile HPP, hypophosphatasemia is detectable at birth in umbilical cord blood (Whyte, 2001), while circulating ALP levels in carrier or affected mothers normalize or become elevated from placental ALP (Whyte et al., 1995).

Hypophosphatasemia can occur from other conditions (starvation, hypothyroidism, scurvy, severe anemia, Wilson's disease, celiac disease, multiple myeloma, hypomagnesemia, Zn^{++} deficiency, etc.), certain drugs (glucocorticoids, clofibrate, chemotherapy, vitamin D intoxication, milk-alkali syndrome, etc.), and exposure to radioactive heavy metals or a massive transfusion of blood or plasma (Macfarlane et al., 1992; McKiernan et al., 2014). These clinical situations should, however, be readily recognized and diagnosed. Especially rare cases of extremely severe osteogenesis imperfecta (Royce et al., 1988) and cleidocranial dysplasia in some infants (Unger et al., 2002; Wycoff et al., 2005) can also manifest hypophosphatasemia (apparently from the paucity of skeletal mass together with impaired cellular processing of bone ALP, or from osteoblast hypofunction, respectively). The skeletal changes of cleidocranial dysplasia might pose the greater confusion (Unger et al., 2002; Wycoff et al., 2005).

Transient increments in circulating bone ALP activity have been postulated for individuals with HPP after orthopedic surgery or significant fracturing (Whyte et al., 2013). In theory, circumstances that increase serum levels of any type of ALP (e.g., pregnancy, liver disease) could obscure the enzymatic and biochemical diagnosis of HPP. Thus, documenting hypophosphatasemia on more than one occasion during clinical stability, particularly from the earliest medical record, seems advisable for the exceptional, confusing patient. Quantitation of circulating ALP isoenzymes (Mulivor et al., 1985), isoforms of TNSALP (Whyte et al., 1996), or leukocyte ALP (Iqbal et al., 2000) in clinical laboratories may also be helpful.

Mineral homeostasis

Neither circulating Ca^{++} nor Pi levels are subnormal in HPP. Serum levels of 25(OH)D, $1,25(OH)_2D$, and parathyroid hormone (PTH) are typically normal (Whyte and Seino, 1982) unless altered physiologically by hypercalcemia or renal failure (Fallon et al., 1984). In fact, in infantile HPP there may be hypercalcemia, secondary hypoparathyroidism, and hyperphosphatemia together with hypercalciuria (Fraser, 1957; Shohat et al., 1991; Whyte et al., 1982b, 2012). The

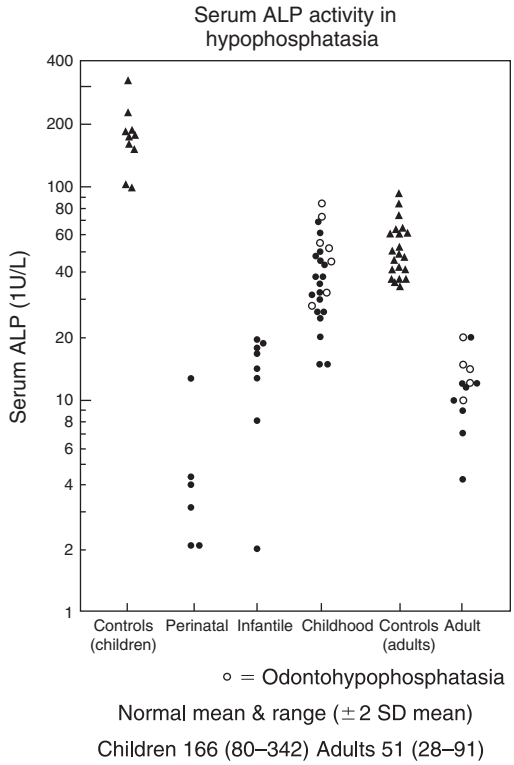

FIGURE 66.8 Serum alkaline phosphatase activity in hypophosphatasia. Serum total ALP activity in healthy children and adults and in 52 patients from 47 families with the various clinical forms of hypophosphatasia (note the logarithmic scale). All assays were performed at the Center for Metabolic Bone Disease and Molecular Research, Shriners Hospitals for Children-St. Louis, St. Louis, MO, USA. *Reproduced with permission from Whyte, M.P., 2001. Hypophosphatasia. In: Scriver, C.R., Beaudet, A.L., Sly, W.S., Valle, D., (Eds.) The Metabolic and Molecular Bases of Inherited Disease, eighth ed., pp. 5313–5329. McGraw-Hill, New York.*

disturbed mineral homeostasis seems largely due to impaired Ca^{++} and Pi uptake by a poorly mineralizing and growing skeleton, sometimes with progressive skeletal demineralization perhaps reflecting patient immobility. Low circulating levels of PTH, reflecting increased ionized Ca^{++} levels, are common in severely affected pediatric patients. In childhood HPP, affected individuals may manifest hypercalciuria but usually not with hypercalcemia. In the past, several HPP patients were reported to have elevated serum PTH levels, but impaired kidney function may have been the explanation in some (Whyte, 1994). Rarely, affected adults with HPP do have primary hyperparathyroidism (Faas et al., 1974; Whyte, 2001).

Of interest, individuals with the childhood or adult forms of HPP have serum Pi levels above average values for age-matched controls (Whyte, 2001). Indeed, >50% of these patients are distinctly hyperphosphatemic. Enhanced renal reclamation of Pi (increased TmP/GFR) underlies this finding (Whyte and Rettinger, 1987). However, in only some instances is suppressed circulating PTH contributory. The pathogenesis seems complex, perhaps involving low levels of TNSALP activity in the kidney tubules, subnormal or inappropriately normal circulating levels of phosphatonins, and/or elevated levels of urinary PPi (Whyte et al., 2019). In contrast, hypophosphatasemia with hypophosphatemia from renal Pi wasting was reported in 1981 (Juan and Lambert, 1981).

Phosphoethanolamine

Elevated urine levels of PEA support a diagnosis of HPP (Rasmussen, 1968) but are not pathognomonic. Licata et al. (1978) demonstrated that phosphoethanolaminuria occurs in other conditions including several metabolic bone diseases. Reference ranges for urine PEA vary according to patient age and somewhat by diet and follow a circadian rhythm. PEA values can be unremarkable in mildly affected HPP patients. Age-adjusted normal ranges (expressed as micromoles of PEA per gram of creatinine) are for less than age 15 years, 83 to 222; for 15–30 years, 42 to 146; for 31–41 years, 38 to 155; and for more than 45 years, 48 to 93 (Licata et al., 1978).

Pyridoxal 5′-phosphate

Increased plasma PLP ("vitamin B_6") is a sensitive and reliable marker for HPP (Coburn and Whyte, 1988; Whyte et al., 2018) including pseudo-HPP (Cole et al., 1986). Even patients with odonto-HPP manifest this biochemical finding (Whyte et al., 1985, 2018). However, the earliest studies showed that PLP values overlap between the different clinical forms of HPP (see Fig. 66.9). Now testing is available from commercial laboratories. In order to avoid false-positive results, vitamin supplements containing pyridoxine should not be taken, if possible, for 1 week before blood is obtained for the assay. HPP disease severity correlates positively but not precisely with the elevation in plasma PLP concentration (Whyte, 2001; Whyte et al., 2018). Quantitation of plasma PLP after challenge with pyridoxine given orally once each day for 6 days seems to distinguish HPP patients especially well (Whyte, 1994). The procedure has been used to identify Canadian Mennonite carriers of severe HPP (Chodirker et al., 1990).

Inorganic pyrophosphate

Assay of PPi in plasma or urine is not commercially available. Urine PPi levels are increased in most HPP patients but can be unremarkable in mildly affected individuals (Caswell et al., 1991). Nevertheless, this test may help with carrier detection (Whyte, 2001). Assays of both circulating PPi and PLP were used as efficacy and safety parameters during the clinical trials of asfotase alfa treatment for HPP (Whyte et al., 2016b, 2019b; Kishnani et al., 2019).

Radiographic findings

Radiographic features are illustrated elsewhere for the principal clinical forms of HPP (see Figs. 66.4 to 66.7).

Histopathological findings

Histopathological disturbances in HPP that are a direct consequence of TNSALP deficiency seem to be hard tissue hypomineralization and perhaps the seemingly paradoxical ectopic mineralization of calcific periarthritis and enthesopathy.

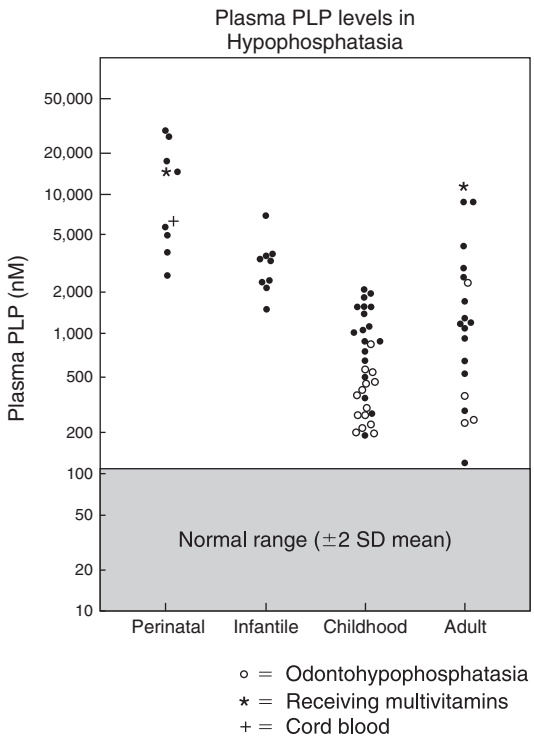

FIGURE 66.9 Plasma pyridoxal 5′-phosphate (PLP) levels in hypophosphatasia. PLP concentrations in plasma in various clinical forms of hypophosphatasia (hatched area is the normal range for children and adults). Note the logarithmic scale with some overlap between clinical forms (assays performed courtesy of Dr. Stephen P. Coburn, Fort Wayne State Developmental Center, Fort Wayne, IN). *Reproduced with permission from Whyte, M.P., 2001. Hypophosphatasia. In: Scriver, C.R., Beaudet, A.L., Sly, W.S., Valle, D., (Eds.) The Metabolic and Molecular Bases of Inherited Disease, eighth ed., pp. 5313–5329. McGraw-Hill, New York.*

Pulmonary hypoplasia (Silver et al., 1988) seems secondary to a small thorax. Myopathic changes are not observed despite muscle weakness.

Skeleton

In all but the mildest cases of HPP (i.e., odonto-HPP), nondecalcified sections of the skeleton reveal defective mineralization (Fallon et al., 1984; Ornoy et al., 1985). The degree of rickets or osteomalacia generally reflects the clinical severity overall (Fallon et al., 1984). Features of secondary hyperparathyroidism (present in rickets or osteomalacia associated with hypocalcemia) are typically absent (Fallon et al., 1984; Anderson et al., 1997). In severe cases, extramedullary hematopoiesis (Fallon et al., 1984; Ornoy et al., 1985) may reflect marrow space crowding owing to the osteomalacia.

In growth plates and osseous tissue, cellular sources of the bone isoform of TNSALP (chondrocytes and osteoblasts) are present (Anderson et al., 1997) but their TNSALP activity is deficient (Fallon et al., 1984; Ornoy et al., 1985). Other forms of rickets or osteomalacia cannot be excluded by their histopathological features unless ALP activity is assessed. In HPP, ALP activity in bone inversely reflects the degree of osteoid accumulation and therefore the severity of the skeletal disease (Fallon et al., 1984).

Electron microscopy of skeletal tissue obtained at autopsy from perinatal HPP has shown normal distribution of MVs, proteoglycan granules, and collagen fibers in the extracellular space of cartilage (Fallon et al., 1984; Ali, 1986), although the MVs lacked ALP activity. Early reports described only isolated or tiny groups of HA crystals (calcospherites) that frequently were not associated with vesicular structures (Ali, 1986; Shohat et al., 1991). Actually, HPP MVs do contain HA, but the crystals fail to enlarge due to the superabundance of extracellular PPi after these structures rupture (Anderson et al., 1997). Thus, "secondary," but not "primary," mineralization is compromised in HPP.

Dentition

Premature exfoliation of primary teeth occurs in a few diseases other than HPP, such as cyclical neutropenia and Papillon–Lefèvre syndrome (Van den Bos et al., 2005). In HPP, a paucity of mineralized cementum (despite the presence of cells that look like cementoblasts) seems to explain this complication (see Fig. 66.6A) (el-Labban et al., 1991; Lundgren et al., 1991). Desiccated teeth remain useful for histopathological examination. What cementum is present appears afibrillar (el-Labban et al., 1991). The severity of this defect varies from tooth to tooth but typically reflects the degree of skeletal disease (Whyte et al., 2015). Incisors are usually affected most and are the first to be shed.

In addition to defects in cementum, dentinogenesis seems to be impaired as shown by big pulp chambers. Dentin tubules may be enlarged although reduced in number. The excessive width of predentin, increased amounts of interglobular dentin, and impaired calcification of cementum are analogous to the osteoidosis found in bone. Reportedly, the enamel is not impacted directly (Lundgren et al., 1991). The histopathological changes of the permanent teeth are similar to those in deciduous teeth (el-Labban et al., 1991), but their prognosis early on is better (Lepe et al., 1997).

Biochemical and genetic defects

HPP is caused by mono- or biallelic loss-of-function mutation(s) in *ALPL*.

Tissue-nonspecific alkaline phosphatase deficiency

Early on, postmortem studies of perinatal and infantile HPP revealed selective deficiency of TNSALP isoenzyme activity and thereby identified HPP as an inborn error of metabolism while suggesting its genetic basis. Profound deficiency of ALP activity was discovered in bone, liver, and kidney tissue but not in intestine or placenta (fetal trophoblast) (Vanneuville and Leroy, 1981). This observation matched emerging amino acid sequence analyses from proteolytic peptide digests of ALPs purified from healthy human tissues (Stigbrand and Fishman, 1984) and indicated selective deficiency of the various isoforms within the TNSALP isoenzyme family. Leukocyte ALP activity can be subnormal in any form of HPP except perhaps pseudo-HPP, and therefore likely represents a type of TNSALP (Fallon et al., 1984).

Postmortem studies of children or adults with HPP have not been reported, but globally diminished TNSALP activity is indicated by deficiency in serum, bone, circulating granulocytes (Fallon et al., 1984), and skin fibroblasts (Vanneuville and Leroy, 1981; Whyte and Vrabel, 1985).

Hypophosphatasemia in HPP does not seem to involve accelerated clearance of TNSALP from the circulation (Gorodischer et al., 1976). Purified placental ALP (Whyte et al., 1992) as well as the bone isoform of TNSALP contained in the

plasma of patients with Paget bone disease (Whyte et al., 1982b) showed normal circulating half-lives of several days when infused intravenously into infants with life-threatening HPP during attempted ALP replacement therapy (see later).

Furthermore, coincubation experiments with mixtures of serum and coculture studies with fibroblasts provided evidence against the presence of an inhibitor or the absence of an activator of TNSALP in HPP (Fraser, 1957; O'Duffy, 1970; Whyte and Vrabel, 1985). Then, in 1985, skin fibroblast heterokaryon studies indicated a defect at a single gene locus causing severe HPP (Whyte and Vrabel, 1985).

ALP immunoreactivity has been studied in tissues obtained at autopsy as well as in skin fibroblasts from patients with severe HPP (Fallon et al., 1989; Fedde et al., 1996). In a preliminary report, a polyclonal antibody to the liver isoform of TNSALP indicated normal amounts of immunoreactive TNSALP in bone, liver, and kidney tissues from five patients (Fallon et al., 1989). Others, using isoelectric focusing and enzyme inhibition studies, suggested that the low ALP activity in one HPP patient reflected intestinal ALP (Mueller et al., 1983). In a fibroblast study, ALP had somewhat different physicochemical and immunological properties compared with ALP from healthy controls but seemed to be a type of TNSALP (Fedde et al., 1996). In studies of ALP in the circulation, monoclonal antibody-based immunoradiometric assays for polymeric TNSALP demonstrated low levels of the bone and liver isoforms in sera from patients with all clinical types of HPP except pseudo-HPP (Whyte et al., 1996). Upon release from cell surfaces into blood, TNSALP in HPP seemed altered in such a way that immunoreactivity was diminished and/or clearance was accelerated (Whyte et al., 1996). Fedde et al. (1996) concluded that the precise impact of the underlying *ALPL* mutation(s) (see later) must be understood to fully appreciate its effect(s). Now, these early findings are explained by the identification of >360 typically missense loss-of-function mutations of *ALPL* in HPP, including their effects on cellular processing of the enzyme as well as its structure (see later).

Inheritance

Mutational analysis of *ALPL* established both autosomal dominant and autosomal recessive patterns of inheritance for HPP. Perinatal and infantile HPP represent autosomal recessive disease (Weiss et al., 1988; Henthorn et al., 1992; Mumm et al., 2001; Whyte et al., 2003, 2006, 2012, 2015), whereas all milder forms of HPP can reflect either autosomal recessive (Henthorn et al., 1992) or autosomal dominant transmission of *ALPL* defects (Mumm et al., 2006; Whyte et al., 2007). Certain *ALPL* mutations have dominant-negative effects and account for multigenerational HPP (Mumm et al., 2006) (see later). Pseudo-HPP too involves *ALPL* defects (Madson et al., 2015). The utility of quantitating the biochemical parameters of HPP to identify carriers has been discussed (Sorensen et al., 1978; Chodiker et al., 1990; Whyte et al., 2017b).

ALPL gene defects

In 1988, proof that HPP can be caused by loss-of-function alteration of *ALPL* came with the discovery by Weiss and coworkers of a homozygous missense defect within *ALPL* in a consanguineous boy who died from perinatal HPP (Weiss et al., 1988). Transfection studies showed that his single-base transition in *ALPL* compromised the enzyme's activity, perhaps by impairing the binding of a metal ligand to an important arginine residue at the catalytic pocket (Weiss et al., 1988). In 1992, Henthorn et al. reported eight further missense mutations in four additional unrelated patients with perinatal or infantile HPP (Henthorn et al., 1992) and found two siblings with childhood HPP and one unrelated woman with adult HPP who were compound heterozygotes for the identical *ALPL* defects. In 1993, Greenberg and coworkers demonstrated that homozygosity for a "founder" tenth *ALPL* missense mutation accounts for the high prevalence of HPP in Mennonites living in Manitoba, Canada (Greenberg et al., 1993). Now, all clinical forms of HPP have been shown to involve loss-of-function mutation(s) in *ALPL* (Whyte, 2000, 2016, 2019), and all patients are expected, depending on sufficient rigor of the search, to carry one or two defective *ALPL* alleles (Whyte, 2017a; 2017b, 2018). There has not been genetic heterogeneity for HPP. A web site organized by Etienne Mornet, PhD, summarizes the *ALPL* mutations identified in HPP patients worldwide (http://www.sesep.uvsq.fr/Database.html). Currently, >360 different *TNSALP* mutations are recorded, of which the considerable majority are missense. Some seem to have increased regional or national prevalence (Mumm et al., 2006).

ALPL structural defects

Many *ALPL* mutations causing HPP (Taillander et al., 2001; Mumm et al., 2002) alter the amino acid residue in all mammals (Henthorn and Whyte, 1992; Mornet et al., 1998) and sometimes conserved in the ALPs of bacteria (Henthorn et al., 1992). Characterization of the three-dimensional structure of *Escherichia coli* ALP (Kim and Wyckoff, 1991) and human placental ALP (Le Due et al., 2000) by X-ray crystallography accelerated our understanding of the enzymatic basis for HPP (Henthorn et al., 1992; Kim and Wyckoff, 1991; Millán, 2006). Missense mutations in *ALPL* could now be

examined for their impact on the catalytically active dimeric TNSALP molecule (Chemscape Chime version 1.02 by MDL Information Systems, Inc., San Leandro, CA, at http://www.mdli.com and RasWin Molecular Graphics, Windows version 2.6 by Roger Sayle, Glaxo Wellcome Research and Development, Stevenage, Hertfordshire, UK, at http://www.umass.edu/microbio/rasmol/index.html). Some *ALPL* mutations would disturb the enzyme's catalytic pocket or structurally important metal ligand-binding sites; others possibly compromised the formation of TNSALP dimers (Mornet et al., 1998; Millán, 2006). Nevertheless, why all *ALPL* loss-of-function (Brunt-Heath et al., 2005) mutations associated with HPP are deleterious is not understood (Whyte, 2000; Millan and Whyte, 2016). For example, site-directed mutagenesis with the Ala161-Thr substitution first discovered to cause HPP (Weiss et al., 1988) did not compromise the catalytic activity of *E. coli* ALP (Chaidaroglou and Kantrowitz, 1993). In fact, some *ALPL* mutations alter the intracellular processing of TNSALP (Mornet et al., 1998; Shibata et al., 1998; Fukushi et al., 1998). Clinical, radiological, and perhaps histopathological studies of elderly individuals harboring the wide range of *ALPL* defects may prove especially helpful for understanding whether they ever cause HPP complications (Whyte, 2000, 2017a, b).

Prognosis

Untreated perinatal HPP is almost always rapidly fatal (Whyte et al., 2019). With intensive life support, these neonates may live for a short time, but long-term survival is rare. Now, however, asfotase alfa treatment can rescue these patients and lead to enjoyable health (Whyte et al., 2012; 2019b) (see later).

Infantile HPP, when first diagnosed, has a less certain outcome. Often there is clinical and radiographic deterioration, with historically ~50% of affected babies said to die from pneumonia and respiratory compromise owing to worsening skeletal disease particularly affecting the chest (Fraser, 1957; Whyte et al., 2003; Cahill et al., 2007). Sometimes instead, considerable spontaneous improvement occurs. The prognosis seems better if there is survival past infancy. In fact, a preliminary report in 1986 from Canada (Ish-Shalom et al., 1986) suggested that the adult stature of survivors of infantile HPP may be normal. Nevertheless, I am aware of less favorable outcomes (Whyte, 2001). Childhood HPP may also spontaneously improve, typically after growth plate fusion in young adult life. During childhood, however, the various pediatric forms of HPP usually do not alter their expressivity (Whyte et al., 2016c). Unfortunately, recurrence of skeletal symptoms and complications later in adulthood can occur (Fraser, 1957; Weinstein and Whyte, 1981; Khandwala et al., 2006; Whyte et al., 2007), but not predictably. Adult HPP often presents with recurrent metatarsal stress fractures and then further orthopedic difficulties become chronic (Whyte et al., 1982a). Worsening osteomalacia associated with osteopenia and fractures was not prevented in two affected women by estrogen replacement given at their menopause (personal observation).

Treatment

When the previous 2008 version of this chapter was published, clinical trials of HA-targeted asfotase alfa (Strensiq) for HPP had just begun. The results were transformative, especially for affected neonates, infants, and children, and this biologic was approved multinationally in 2015, typically for pediatric-onset HPP (Whyte, 2017b). The evolution of treatment trials for HPP is instructive concerning the physiological role of TNSALP.

Supportive

Symptoms from CPPD crystal deposition or periarticular HA precipitation (calcific periarthritis) may respond to nonsteroidal antiinflammatory agents.

Intramedullary rodding of femoral fractures or pseudofractures has been the mainstay of orthopedic management (Coe et al., 1986; Sutton et al., 2012). Recurrent metatarsal stress fractures have benefitted from wearing a boot.

Medical

Before asfotase alfa, there was no established medical treatment for HPP. Several informative approaches had been attempted (Fraser et al., 1955) including trials of nontargeted ALP replacement therapy (Whyte et al., 1982b, 1984, 1992), marrow cell transplantation (Whyte et al., 2003; Cahill et al., 2007), and teriparatide administration (Whyte et al., 2007). Cortisone given to a few pediatric patients with severe HPP reportedly coincided with periods of normalization of serum ALP activity and radiographic improvement (Fraser and Laidlaw, 1956) but was an inconsistent finding. Brief supplementation with Mg^{++} or Zn^{++} had been unsuccessful (Fraser, 1957). Because patients with HPP are often hyperphosphatemic (Whyte, 2001; Whyte et al., 2019), restriction and/or pharmacological binding of dietary Pi seemed

potentially useful for HPP (Wenkert et al., 2002). The superabundance of extracellular Pi might be competitively inhibiting TNSALP (McComb et al., 1979; Coburn et al., 1998) or suppressing *ALPL* gene expression (Goseki-Sone et al., 1999). Nevertheless, full clinical studies did not follow.

Hypercalcemia in infantile HPP can be treated by restriction of dietary Ca^{++} that hopefully does not exacerbate any progressive skeletal demineralization reflecting patient immobility (Whyte et al., 1982b, 1986). If necessary, glucocorticoids may be helpful for elevated blood Ca^{++} levels (Whyte et al., 1982b) but could pose an additional risk. Hypothetically, an antiresorptive treatment like subcutaneous injections of salmon calcitonin might address some of the hypercalcemia while blocking skeletal demineralization in HPP (Barcia et al., 1997), but experience has not been positive, likely because the skeletal disease involves osteoidosis covering bone surfaces. In fact, antiresorptive bisphosphonates have not had success and are derivatives of PPi that theoretically could exacerbate the rickets or osteomalacia (Whyte, 2001; Sutton et al., 2012). Some drugs that stimulate TNSALP biosynthesis in the skeleton, such as teriparatide, may benefit HPP, especially if there is only one defective *ALPL* allele (Whyte et al., 2007; Camacho et al., 2016).

Assessing therapy for infantile HPP can seem uncertain because some patients demonstrate spontaneous, and sometimes quite significant, improvement (Ish-Shalom et al., 1986; Whyte et al., 1986), whereas others show progressive skeletal demineralization leading to a fatal outcome (Whyte et al., 1982b). Now, we understand that the latter is more common (Lueng et al., 2013; Whyte et al., 2016a, 2019a).

Enzyme replacement therapy for HPP has been attempted by intravenous infusion of several types of ALP, but generally the results have been disappointing. In 1972, serum from a patient with Paget bone disease given to an affected infant was said to precede radiographic improvement (Macpherson et al., 1972). In 1982, weekly infusion of fresh plasma from healthy subjects was followed by clinical and radiographic advances in a child (Albeggiani and Cataldo, 1982). However, subsequent infusions of Paget plasma were without significant clinical or radiographic benefit for four patients with infantile HPP (Whyte et al., 1982b, 1984). One boy with infantile HPP showed remarkable but transient correction of hypophosphatasemia and substantial clinical, radiographic, and histological improvement after a brief trial of prednisone, bovine PTH 1−34, and then pooled plasma infusions from healthy individuals, but succumbed soon after to pneumonia (Whyte et al., 1986). He was later confirmed to have a homozygous *ALPL* missense mutation (Whyte et al., 2006). In follow-up to a brief report in 1989 that suggested infusions of ALP purified from liver improved the histological appearance of bone and decreased urinary PEA levels (Weninger et al., 1989), purified placental ALP was given to a severely affected infant, but despite repeated doses that led to transient hyperphosphatasemia there were only modest decrements of plasma PLP and urinary PEA concentrations, no change in urinary PPi levels, and no clinical or radiographic improvement (Whyte et al., 1992). Placental ALP was given because elevated concentrations of PLP in plasma and increased levels of PEA and PPi in urine diminished as endogenous natural production of this ALP isoenzyme corrected the hypophosphatasemia of pregnant women who were carriers of HPP (Whyte et al., 1995). Perhaps pregnancy with large amounts of placental ALP present physiologically in situ represents an "endogenous" form of enzyme replacement in HPP. However, the symptoms, radiology, skeletal histology, etc. of women who are carriers or affected by HPP have not been assessed across a pregnancy. Extreme skeletal disease in perinatal HPP despite placental ALP circulating in the mother shows that the in utero environment may not be protective for the fetus (Whyte, 2001).

These cumulative discouraging observations, showing that circulating ALP activity could be corrected in infantile HPP yet have no clinical benefit, suggested that ALP must be within the skeleton itself for physiological activity and therapeutic efficacy (Whyte et al., 1995). In fact, two subsequent reports of patient rescue and clinical and radiographic improvement in infantile HPP following mesenchymal cell transplantation suggested sufficient numbers of TNSALP-replete osteoblasts had formed (Whyte et al., 2003; Cahill et al., 2007). These improvements occurred without significant biochemical alterations of mineral metabolism, consistent with a beneficial effect limited to the skeleton.

In 2007, injections of teriparatide (recombinant PTH 1−34) were associated with clinical, biochemical, and radiographic improvement in a woman with adult HPP (Whyte et al., 2007), possibly from increased expression of her normal *ALPL* allele in her osteoblasts and/or PTH-induced phosphaturia documented by increased serum ALP and decreased Pi, respectively.

Later, I discuss the multinational availability of asfotase alfa to treat HPP.

Prenatal diagnosis

A number of reports have concluded that lethal perinatal HPP has been diagnosed in utero during the second trimester using ultrasonography with particular attention to the shape and mineralization of the skull and major long bones (van Dongen et al., 1990). However, relatively mild HPP, inherited either as an autosomal dominant or autosomal recessive trait, can bow major bones in utero and suggest the presence of this lethal form of HPP, yet the deformities correct postnatally,

reflecting instead the benign prenatal form of HPP (Moore et al., 1999; Pauli et al., 1999; Wenkert et al., 2005, 2011; Stevenson et al., 2008). The >360 specific defects possible in *ALPL* show that for mutation analysis it is necessary to examine *ALPL* exons 2−12 and their splice sites for new patients with HPP (Mumm et al., 2002). Microarray study for *ALPL* deletion/duplication is occasionally necessary. *ALPL* mutation analysis is possible to detect HPP prenatally (Henthorn and Whyte, 1995), but prognostication from the results and early fetal sonography can be problematic (Wenkert et al., 2011).

Physiological role of alkaline phosphatase explored in hypophosphatasia

As reviewed previously, several roles for TNSALP in skeletal formation have been proposed (Table 66.1). Hence, investigation of HPP has been a "window of opportunity" to explore a number of them directly in humans.

The discovery by Weiss and coworkers more than 3 decades ago that homozygosity for a loss-of-function missense mutation within *ALPL* caused severe HPP (Weiss et al., 1988) confirmed Robison's hypothesis; i.e., the enzyme that came to be called ALP functions crucially in skeletal mineralization (Robison, 1923). Subsequent characterization of the dental manifestations of HPP revealed that ALP is important also for the formation of teeth; i.e., "hard tissues." Now, it appears that all bona fide cases of HPP are explained by mono- or biallelic loss-of-function mutation(s) in *ALPL* (Whyte, 2016; 2017b, 2018, 2019).

Electron microscopy of bone and cartilage obtained at autopsy from patients representing the most severe forms of HPP demonstrated that deficient activity of TNSALP is associated with a fundamental disturbance in the process of "secondary" skeletal mineralization. Extravesicular mineralization is impeded in HPP (Anderson et al., 1997). HA crystal growth appears to fail after MVs rupture.

Mineralization defects in the cementum and dentin of HPP teeth have been considered as analogous to those in the skeleton (Lundgren et al., 1991). A prominent role for TNSALP during two critical phases of dental mineralization, initiation and completion, was proposed (Hotton et al., 1999). In 2005, Van den Bos and coworkers reported that both cellular and acellular cementum formation was impaired, but not mineralization of the dentin (Van den Bos et al., 2005).

Although healthy liver, kidneys, and adrenal glands are rich in TNSALP activity (McComb et al., 1979), they seem to function normally in HPP (see later) with the exception that reclamation of filtered Pi by the kidney is enhanced and hyperphosphatemia is common. This can be explained by suppression of circulating PTH levels in the often hypercalcemic perinatal and infantile forms of HPP (Whyte and Rettinger, 1987; Whyte et al., 2012), but otherwise seems to have a complex pathogenesis also involving a role for TNSALP in kidney tubules; phosphatonin deficiency or inappropriately normal levels despite hyperphosphatemia; and/or effects on TmP/GFR by the elevated levels of urinary PPi (Whyte et al., 2019).

It has been suggested that the TNSALP deficiency of severe HPP might impair the biosynthesis of phospholipids and thereby explain occurrences of pulmonary atelectasis (Silver et al., 1988). However, the respiratory problems of severely affected patients are attributable to rib cage fractures, thoracic deformity, and chest muscle weakness, perhaps also accounting for hypoplastic lungs.

In the 1980s, it became clear from investigation of HPP patients that TNSALP is important not only for hard tissue mineralization but also for dephosphorylation of extracellular PLP and therefore vitamin B_6 metabolism (Whyte, 1994). In severe pediatric HPP, insufficient hydrolysis of PLP to pyridoxal (PL) for entry into neurons impairs the biosynthesis of the neurotransmitter γ-aminobutyric acid (Baumgartner-Sigl et al., 2007). In fact, epilepsy is an important characteristic of the murine model of infantile HPP and is associated with low levels of γ-aminobutyric acid in the central nervous system (Waymire et al., 1995). Pyridoxine supplementation briefly extends the lives of these patients (Baumgartner-Sigl et al., 2007) and mice (Fedde et al., 1999) with HPP.

Tissue-nonspecific alkaline phosphatase substrates

The discoveries that PEA, PPi, and PLP accumulate endogenously in HPP were essential for elucidating the physiological role of TNSALP in humans. Each was thereby correctly inferred to be a natural substrate for TNSALP. However, a preliminary study using 31P-magnetic resonance spectroscopy of urine from patients with HPP suggested several additional phosphorylated TNSALP substrates (Whyte et al., 2000).

Phosphoethanolamine

The reports in 1955 by McCance and colleagues (1955) and Fraser and coworkers (1955) that PEA levels are elevated in the urine and plasma of HPP patients provided a second biochemical marker for the disorder and identified the first natural

substrate for TNSALP. In 1968, Rasmussen showed that this phosphocompound appears in the urine when plasma levels are scarcely detectable; i.e., there is essentially no renal threshold for PEA excretion (Rasmussen, 1968).

Although the metabolic origin of PEA is uncertain, the principal source of circulating PEA in HPP is considered to be the liver, which normally metabolizes PEA to ammonia, acetaldehyde, and Pi in a reaction catalyzed by O-phosphorylethanolamine phospholyase (Gron, 1978). In one family with adult HPP, urine levels of PEA correlated inversely with circulating activity of the liver but not the bone isoform of TNSALP (Millán et al., 1980). PEA is a component of the phosphatidylinositol-glycan linkage apparatus for cell surface proteins (Low and Zilversmit, 1980). Hence, PEA could be a degradation product of this link, but is not considered a degradation product of phosphatidylethanolamine; i.e., not from plasma membrane phospholipid breakdown.

Pyridoxal 5′-phosphate

The discovery in 1985 that circulating levels of PLP are elevated in HPP was key to understanding the physiological role of TNSALP (Whyte et al., 1985).

Reviewed later (Fig. 66.10), the forms of dietary vitamin B_6 (pyridoxine, pyridoxal, pyridoxamine, and their phosphorylated derivatives) are converted to PLP in the liver (Dolphin et al., 1986). Organ ablation studies of mammals identified hepatic tissue as the principal source of circulating PLP. PLP is released from hepatocytes into the bloodstream where more than 95% couples to albumin (Dolphin et al., 1986). Some PLP also binds in the circulation to various enzymes, but a small amount circulates freely.

Like many phosphorylated compounds, PLP cannot cross plasma membranes but must first be dephosphorylated to PL before it can enter tissues. Inside cells, PL is rephosphorylated to PLP or converted to pyridoxamine 5′-phosphate that then act as cofactors for many and varied enzymatic reactions. Ultimately, vitamin B_6 is degraded to 4-pyridoxic acid (4-PA), primarily in the liver, and then excreted into the urine (Dolphin et al., 1986; Coburn and Whyte, 1988).

In disorders that elevate circulating bone and liver TNSALP, plasma PLP concentrations are decreased (Anderson et al., 1980). Thus, discovery of elevated plasma PLP levels in HPP extended this reciprocal relationship. However, the wide-ranging elevated levels in circulating PLP are probably not of pathophysiological consequence in HPP because cell surface TNSALP controls extracellular but not intracellular dephosphorylation of PLP. Elevated plasma PLP levels in HPP do not reflect enhanced PLP synthesis but instead extracellular accumulation from diminished hydrolysis of PLP (Whyte

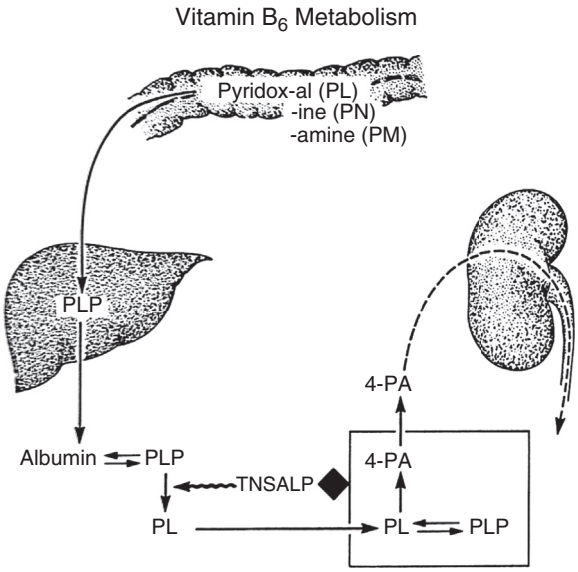

FIGURE 66.10 Role of tissue-nonspecific alkaline phosphatase (TNSALP) in vitamin B_6 metabolism. The various forms of vitamin B_6 are plentiful in the diet and absorbed into the hepatic portal circulation (phosphorylated forms are first dephosphorylated in the gut). In the liver, each is converted to PLP, which is then secreted bound to albumin into the plasma. In order to enter tissues, plasma PLP must be dephosphorylated to PL that can traverse membranes. 4-Pyridoxic acid (4-PA), the major degradation product of vitamin B_6, is excreted in the urine. High plasma levels of PLP in hypophosphatasia, yet normal plasma concentrations of PL, are consistent with a cell surface role for TNSALP in the extracellular dephosphorylation of PLP to PL. *Reproduced with permission from Whyte, M.P., 2001. Hypophosphatasia. In: Scriver, C.R., Beaudet, A.L., Sly, W.S., Valle, D., (Eds.) The Metabolic and Molecular Bases of Inherited Disease, eighth ed., pp. 5313–5329. McGraw-Hill, New York.*

et al., 1985). Clinical investigation of HPP led to this overview of vitamin B_6 metabolism because patients with HPP do not have symptoms of vitamin B_6 toxicity such as peripheral neuropathy (Dolphin et al., 1986) (see later). Similarly, in all types of HPP except the most severe perinatal and infantile forms where vitamin B_6–dependent epilepsy can occur, there are no signs or symptoms of chronic vitamin B_6 deficiency such as stomatitis, dermatitis, peripheral neuritis, anemia, or depression (Dolphin et al., 1986). Furthermore, a variety of biochemical findings indicated that intracellular levels of vitamin B_6 are unremarkable in HPP. First, urine levels of 4-pyridoxic acid are normal in patients with childhood HPP (Whyte et al., 1988). Second, these children respond normally to L-tryptophan loading—a test for vitamin B_6 deficiency (Whyte and Coburn, unpublished observation). Third, in homogenates of severely TNSALP-deficient HPP fibroblasts in culture, levels of PLP and the various other forms of vitamin B_6 are normal (Whyte et al., 1988). Finally, tissues obtained at autopsy from patients with perinatal HPP (plasma PLP concentrations elevated 50 to 900 times normal) contain essentially normal levels of PLP, PL, and total vitamin B_6 (Whyte et al., 1988). Accordingly, TNSALP seemed to function as a cell surface enzyme (Whyte et al., 1985; Fedde et al., 1988), and soon after how ALP and other proteins anchor to the plasma membrane was identified (Low and Saltiel, 1988).

Because TNSALP seemed responsible for the extracellular dephosphorylation of PLP to PL, plasma levels of PL could be low in HPP. However, only patients with the severest forms of HPP have low plasma PL concentrations, helping to explain the vitamin B_6–dependent seizures; the others show normal or sometimes elevated circulating PL levels (Whyte et al., 1985).

Vitamin B_6 deficiency is associated with renal stone disease and epilepsy. However, nephrocalcinosis in infants with HPP likely reflects hypercalciuria, although oxalate excess (a consequence of vitamin B_6 deficiency) has not been searched for in HPP (Dolphin et al., 1986). Epilepsy in severe HPP has the aforementioned metabolic explanation but also occurs in patients who may have cranial deformity, intracranial hemorrhage, periodic apnea, etc. Of interest, PEA reportedly caused seizures when given intravenously to an infant severely affected by HPP during a study of PEA metabolism (Takahashi et al., 1984). In two patients with perinatal HPP and epilepsy, both of whom had plasma PL levels below assay sensitivity, administration of vitamin B_6 as pyridoxine did not correct the seizure disorder (personal observation), perhaps because the pyridoxine was converted to more PLP rather than PL. Vitamin B_6–dependent seizures can be the first manifestation of infantile HPP. All reported cases of HPP with such seizures proved fatal (Baumgartner-Sigl et al., 2007). *Alpl* knockout mice manifest this type of epilepsy (see later), and require pyridoxine administration to extend their lives (Waymire et al., 1995; Fedde et al., 1999). Treatment of these mice, an excellent model for infantile HPP (Fedde et al., 1999), with asfotase alfa prevented their skeletal and dental disease and vitamin B_6-dependent epilepsy (Millán et al., 2008).

The clinical and biochemical observations concerning vitamin B_6 metabolism in HPP have indicated an extracellular role for TNSALP (Whyte et al., 1985). In 1988, Fedde and coworkers exposed cultivated human osteosarcoma cells (Fedde et al., 1988), and then dermal fibroblasts from patients with infantile HPP (Fedde et al., 1990), to PLP and PEA in the medium and confirmed that TNSALP is a plasma membrane-associated enzyme (see later). In 1980, characterization of porcine kidney ALP as membrane-bound by phosphatidylinositol (Low and Zilversmit, 1980) indicated this attachment apparatus for TNSALP (Low and Saltiel, 1988).

Inorganic pyrophosphate

Discovery in 1965 and 1971, respectively, that PPi levels are increased in HPP patient urine (Russell, 1965) and plasma (Russell et al., 1971) provided a plausible explanation for defective skeletal mineralization in HPP (Caswell et al., 1991) (Fig. 66.11). At that time, PPi was becoming recognized as a potent inhibitor of mineralization (Heinonen, 2001). Although low concentrations of PPi could enhance Ca^{++} and Pi precipitation from solution to form amorphous calcium phosphate, at higher concentrations PPi prevented the growth and dissolution of HA crystals (Caswell et al., 1991). Caswell and co-workers demonstrated that nucleoside triphosphate pyrophosphatase (NTP-PPi-ase, PC-1) activity is unremarkable in TNSALP-deficient fibroblasts from perinatal and infantile HPP patients. Extracellular generation of PPi from ATP by these cells was not hindered (Caswell et al., 1986). Therefore, NTP-PPi-ase seemed distinct from TNSALP. In 2000, this conclusion was supported by studies of osteoblasts from *Alpl* knockout mice (see later) (Johnson et al., 2000). From understanding PLP metabolism in HPP, it became clear how the clearance of 32PPi administered in the 1960s into the circulation of two adults with HPP was markedly delayed (R. G. G. Russell, personal communication). Endogenous accumulation of PPi in HPP reflects diminished extracellular hydrolysis (Caswell et al., 1991).

Consonant with the in vitro effects of PPi, several other abnormalities of mineralization in HPP may reflect the local concentration of PPi. Perhaps relatively minor excesses of extracellular PPi explain the precipitation of amorphous calcium phosphate and the calcific periarthritis observed in some adults with HPP (Lassere and Jones, 1990). Furthermore, ALP can dissolve CPPD crystals in vitro (Xu et al., 1991), and this PPi-ase activity seems additional to its capacity to hydrolyze

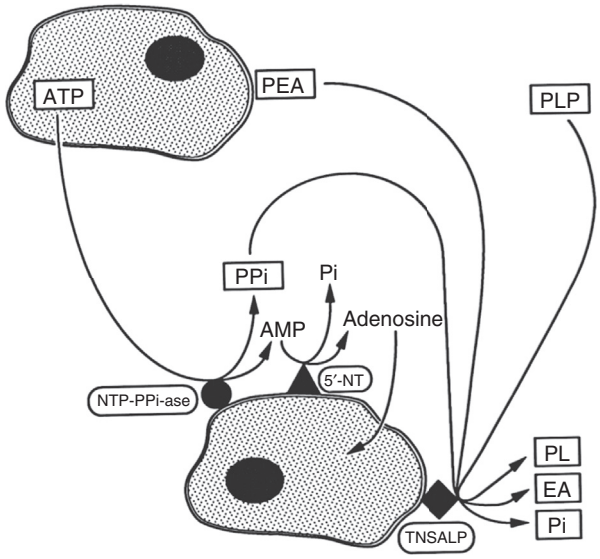

FIGURE 66.11 Metabolic basis for hypophosphatasia. Extracellular generation of PPi, presumably by the action of nucleoside triphosphate pyrophosphatase (NTP-PPi-ase), is normal. PPi is also pumped extracellularly from within cells via an ANK channel (not shown) (Millán, 2006). Extracellular degradation of PEA, PPi, and PLP is diminished because of deficient cell-surface TNSALP activity. Accumulation of PPi extracellularly accounts for CPPD precipitation and sometimes associated calcium phosphate crystal deposition. The inhibitory effect of PPi on extravesicular HA crystal growth accounts for the rickets/osteomalacia. *Reproduced with permission from Whyte, M.P., 2001. Hypophosphatasia. In: Scriver, C.R., Beaudet, A.L., Sly, W.S., Valle, D., (Eds.) The Metabolic and Molecular Bases of Inherited Disease, eighth ed., pp. 5313–5329. McGraw-Hill, New York.*

phosphoesters. Thus, CPPD crystal deposition leading to chondrocalcinosis, pseudogout, and pyrophosphate arthropathy could be explained by the failure of TNSALP to hydrolyze not only PPi but also CPPD crystals. The especially high extracellular concentrations of PPi in HPP would inhibit HA crystal formation and growth; hence, rickets or osteomalacia becomes readily explained. As discussed, electron microscopy of skeletal tissue from lethal HPP and in Tnsalp knockout mice (see later), showed that the precise pathogenesis involves secondary mineralization surrounding MVs (Anderson et al., 1997).

Circulating tissue-nonspecific alkaline phosphatase

Several observations suggest that circulating ALPs are physiologically inactive. Infants with severe HPP who received intravenous infusions of plasma from patients with Paget bone disease or purified placental ALP demonstrated no significant clinical or radiographic improvement despite transient correction in circulating ALP activity, sometimes to elevated levels. Such therapy failed to normalize urinary PEA or PPi levels or plasma PLP concentrations (Whyte et al., 1982b). The deficiency of TNSALP activity within the skeleton itself seemed to account for the rickets and osteomalacia of HPP. In fact, in studies reminiscent of Robison's work (Robison, 1932), Fraser and Yendt reported in 1955 that rachitic rat cartilage would calcify in serum obtained from an infant with HPP, yet slices of HPP costochondral junction would not mineralize in synthetic calcifying medium or in pooled serum from healthy children (Fraser and Yendt, 1955). Subsequently, transfection studies using ALP cDNA showed both catalysis against Pi esters and mineralization in a calcification system (Yoon et al., 1989; Farley et al., 1991). Nevertheless, there has been skepticism that such experiments reflect true biomineralization, because increased Pi in metastable solutions is expected to simply precipitate as calcium phosphate (Khouja et al., 1990). As discussed, it seemed necessary to augment ALP activity in the skeleton tissue itself to treat HPP. In fact, the aforementioned experience with marrow cell transplantation for infantile HPP suggested that even small increases in ALP activity within the skeleton can improve its mineralization in HPP (Whyte et al., 2003). Transient transfection studies of various *ALPL* mutations causing HPP also indicated that small differences in the magnitude of the deficiency of ALP activity within the skeleton can account for lethal versus nonlethal outcomes (Mornet et al., 1998).

Hypophosphatasia fibroblast studies

Healthy dermal fibroblasts in culture (Fedde et al., 1990; Whyte and Vrabel, 1985; Whyte et al., 1986) show some TNSALP-like activity that peaks at confluency (Whyte and Vrabel, 1987). Fibroblasts from severely affected HPP patients

are profoundly deficient in this ALP activity (less than 5% control) (Whyte et al., 1983), but the residual activity seems to be a type of TNSALP (Whyte et al., 1987). Using this model, preliminary studies indicated that the phospholipid composition and rates of 32Pi accumulation by these TNSALP-deficient cells were normal (Tsutsumi et al., 1986; Whyte and Vrabel, 1983). In 1990, Fedde et al. demonstrated that TNSALP is present primarily on the surface of these cells (Fedde et al., 1990) and hydrolyzed extracellular PEA and PLP under physiological conditions. Although some reports suggested that ALP conditions cell growth and differentiation by influencing the phosphorylation of nucleotide pools, HPP fibroblasts proliferated at normal rates in culture (Whyte et al., 1983), and TNSALP did not seem to be a phosphoprotein phosphatase acting at the plasma membrane (Fedde et al., 1993).

Alpl knockout animals

Since 1995, studies of *Alpl* knockout mice and then the creation of other murine models of ALP function have complimented and expanded the insights gained from investigation of HPP patients (Millan and Whyte, 2016). In 1995, Waymire and coworkers developed an *Alpl*-null mouse that manifested the deranged vitamin B_6 metabolism of HPP, including lethal seizures from deficient γ-aminobutyric acid in the brain (Waymire et al., 1995). With pyridoxine treatment, the epilepsy was controlled temporarily, and the animals survived long enough to develop dental disease. In 1997, Narisawa and colleagues created a different *Alpl*-null mouse with vitamin B_6–dependent epilepsy and dental defects that also manifested skeletal disease (Narisawa et al., 1997; Millán, 2006). In 1999, Fedde and coworkers demonstrated that both models recapitulated the infantile form of HPP remarkably well, including the acquired skeletal and dental disease and epilepsy together with endogenous accumulation of PPi, PLP, and PEA (Fedde et al., 1999). In 2000, the skeletal mineralization defect of HPP was reproduced in vitro using osteoblasts in culture from these mice (Wennberg et al., 2000). This disturbance did not seem to be related to the aberrations in vitamin B_6 metabolism (Narisawa et al., 2001). Defective secondary (extravesicular) skeletal mineralization was then confirmed by electron microscopy (Anderson et al., 2004). Subsequently, double-knockout mouse studies showed, as might be expected, that skeletal formation is essentially normal in mice that lack both Tnsalp and the PPi-generating enzyme PC-1 (NTP-PPi-ase) (Hessle et al., 2002). Furthermore, Tnsalp expressed under control of the APO-E promoter in the liver of *Alpl* knockout mice prevented the skeletal disease of HPP. It is uncertain, however, whether Tnsalp in the liver itself or in high amounts in the circulation of these mice explained the beneficial skeletal effects. In 2007, Hough and coworkers characterized a much milder, acquired, semi-dominant form of HPP in mice generated from N-ethyl-N-nitrosourea exposure. The adult mice manifested late-onset skeletal disease and arthropathy (Hough et al., 2007). Later, *Alpl*-null mice became the key preclinical model to test asfotase alfa therapy for HPP (Millán et al., 2008). Now, further mouse models of relatively mild HPP are undergoing investigation (Millán and Whyte, 2016; Foster et al., 2015). Recently, a large animal model (sheep) for HPP that includes dental findings has begun characterization (Williams et al., 2018). In 2019, infantile HPP was documented in a breed of dogs (Kyöstila et al., 2019).

Asfotase alfa treatment for hypophosphatasia

HPP was the last type of rickets or osteomalacia to have a medical treatment (Whyte, 2017a). In 2015, asfotase alfa was approved multinationally for HPP, typically if there was pediatric-onset disease. It is an expensive, HA-targeted, recombinant, human TNSALP-replacement therapy administered by subcutaneous injection with dosing based on the patient's weight (Whyte et al., 2012, 2016a; 2019b). The clinical trials encompassed patient ages ranging from newborn to adult life (Whyte et al., 2012, 2016a; Kishnani et al., 2019). Radiographic assessments using validated scales revealed relatively rapid and then sustained improvement in rickets—7 years for 1- to 3-year-old patients with perinatal or infantile HPP (Whyte et al., 2019b), and 5 years for 6- to 12-year-old survivors of infantile HPP or with severe childhood HPP (Whyte et al., 2016b). Osteomalacia seemed improved in both affected children and adults (Whyte et al., 2016b; Kishnani et al., 2019). Circulating ALP activity becomes markedly elevated with this treatment, but the clinical trials were reassuring against ectopic mineralization from excessive lowering of endogenous PPi levels. With treatment, circulating PPi and PLP levels were generally lower and often in the normal range. Rapid and marked improvements were demonstrated, especially by the most severely affected pediatric patients, but bony craniosynostosis was not prevented. Muscle weakness seemed to improve first. Thus, this experience has brought us "full circle," showing that today Rathbun's original patient (Rathbun, 1948) could overcome the pathogenesis of his TNSALP deficiency and enjoy good health. In the future, we must now aim to cure this highly informative inborn error of metabolism.

Summary and conclusions

HPP is the rare but remarkably instructive inborn error of metabolism that demonstrates a critical role for the tissue-nonspecific isoenzyme of ALP in hard tissue mineralization. Subnormal serum ALP activity (hypophosphatasemia), the biochemical hallmark of HPP, reflects a generalized deficiency of TNSALP activity. The three tissue-specific ALP isoenzymes (intestinal, placental, and germ-cell ALP) are not compromised in HPP, but the physiological function of these ALP isoenzymes is not certain.

HPP features impaired skeletal mineralization that manifests as rickets in newborns, infants, children, and adolescents and as osteomalacia in adults. Involvement of the dentition as well shows that all hard tissues are compromised. Clinical expressivity of HPP is, however, extremely broad-ranging and largely but not completely explained by autosomal dominant and autosomal recessive patterns of inheritance with a multitude of underlying loss-of-function mutations, primarily missense defects, in *ALPL*-encoding TNSALP. Perinatal HPP, apparent in utero, causes neonatal death from skeletal hypomineralization that is so severe that no calcification may be seen radiographically. Infantile HPP presents postnatally by the age of 6 months. Sometimes, there is nephrocalcinosis from hypercalcemia and hypercalciuria and functional craniosynostosis as well. Untreated, about 50% of these babies succumb to worsening rachitic disease that compromises pulmonary function. Vitamin B_6–dependent epilepsy indicates particularly severe TNSALP deficiency and a lethal outcome. Childhood HPP features premature loss of deciduous teeth, often preceding rickets and muscle weakness. Adult HPP causes recurrent metatarsal stress fractures, especially femoral pseudofractures, and often arthritis from CPPD crystal deposition, rarely calcific periarthritis from precipitation of amorphous calcium phosphate, and PPi arthropathy or pseudogout. Odonto-HPP refers to premature tooth loss from defective mineralization of dental cementum but no skeletal manifestations.

Perinatal and infantile HPP are transmitted as autosomal recessive traits involving >360 different loss-of-function, often missense, mutations scattered throughout *ALPL*. They compromise the catalytic site and/or structure of this cell surface homodimeric or homotetrameric phosphomonoester phosphohydrolase and sometimes its intracellular processing. All of the more mild forms of HPP can be inherited as an autosomal dominant trait owing to dominant-negative *ALPL* mutations. However, individuals with even the mildest forms of HPP can reflect autosomal recessive inheritance.

Prenatal diagnosis of HPP now involves *ALPL* mutation analysis. During the second trimester, fetal ultrasonography may identify skeletal disease; however, the considerable number and variety of *ALPL* mutations as well as the influence of other factors on the HPP phenotype preclude precise prognostication.

Discovery in HPP of increased endogenous levels of PEA, PPi, and then PLP demonstrated that TNSALP is a cell-surface phosphomonoester phosphohydrolase catalytically active toward a variety of natural substrates, acting also as a PPi-ase. However, additional substrates for TNSALP seem likely. Because these substrates are typically at nanomolar or micromolar concentrations, TNSALP acts physiologically at much lower pH and concentrations than those of the artificial substrates used in clinical and research assays of ALP activity. Clearly, "alkaline phosphatase" is a misnomer.

Clinical investigation of vitamin B_6 metabolism in HPP, supported by HPP fibroblast studies, confirmed that TNSALP acts as a cell-surface enzyme. Typically normal levels of PL in plasma, despite the superabundance of plasma membrane-impermeable PLP, explain the absence of signs or symptoms of vitamin B_6 deficiency (or toxicity) in all but the most severely affected HPP patients.

Attempts to treat HPP by intravenous infusions of ALPs purified from various human tissues was disappointing and suggested that circulating ALP is physiologically inactive. Marrow cell transplantation could be associated with clinical and radiographic improvement but without significant biochemical changes in blood or urine. Teriparatide stimulated bone ALP levels and appeared to heal fractures in several adults with HPP. Following successful treatment of *Alpl*-null mice with recombinant HA-targeted human TNSALP (asfotase alfa), clinical trials proved similarly beneficial, especially for the most severely affected pediatric patients.

Circulating PPi may be formed from extracellular ATP by cell surface NTP-PPi-ase and pumped there from cells by a channel protein called ANK. PLP appears to be from the liver. PEA seems to come from degradation of the phosphatidylinositol-glycan moiety that anchors many proteins to cells surfaces. Hyperphosphatemia owing to increased renal reclamation of Pi in HPP suggests that TNSALP plays a direct role in renal Pi excretion, although additional factors seem likely.

In HPP, PPi excess frequently results in chondrocalcinosis and sometimes in pseudogout or PPi arthropathy. Rickets and osteomalacia in HPP reflect the effect of high extracellular concentrations of PPi to inhibit HA crystal growth at sites of skeletal mineralization. Success using HA-targeted TNSALP-replacement therapy (asfotase alfa) for HPP was consistent with TNSALP acting physiologically within the skeleton but not in the circulation.

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