B$_6$-responsive disorders: A model of vitamin dependency

Peter T. Clayton

Summary Pyridoxal phosphate is the cofactor for over 100 enzyme-catalysed reactions in the body, including many involved in the synthesis or catabolism of neurotransmitters. Inadequate levels of pyridoxal phosphate in the brain cause neurological dysfunction, particularly epilepsy. There are several different mechanisms that lead to an increased requirement for pyridoxine and/or pyridoxal phosphate. These include: (i) inborn errors affecting the pathways of B$_6$ vitamer metabolism; (ii) inborn errors that lead to accumulation of small molecules that react with pyridoxal phosphate and inactivate it; (iii) drugs that react with pyridoxal phosphate; (iv) coeliac disease, which is thought to lead to malabsorption of B$_6$ vitamers; (v) renal dialysis, which leads to increased losses of B$_6$ vitamers from the circulation; (vi) drugs that affect the metabolism of B$_6$ vitamers; and (vii) inborn errors affecting specific pyridoxal phosphate-dependent enzymes. The last show a very variable degree of pyridoxine responsiveness, from 90% in X-linked sideroblastic anaemia (δ-aminolevulinate synthase deficiency) through 50% in homocystinuria (cystathionine β-synthase deficiency) to 5% in ornithinaemia with gyrate atrophy (ornithine δ-aminotransferase deficiency). The possible role of pyridoxal phosphate as a chaperone during folding of nascent enzymes is discussed. High-dose pyridoxine or pyridoxal phosphate may have deleterious side-effects (particularly peripheral neuropathy with pyridoxine) and this must be considered in treatment regimes. None the less, in some patients, particularly infants with intractable epilepsy, treatment with pyridoxine or pyridoxal phosphate can be life-saving, and in other infants with inborn errors of metabolism B$_6$ treatment can be extremely beneficial.

P. T. Clayton (✉)
Biochemistry, Endocrinology and Metabolism, Institute of Child Health, 30 Guilford St, London WC1N 1EH, UK
e-mail: p.clayton@ich.ucl.ac.uk

Chemical structure of B$_6$ vitamers

Vitamin B$_6$ is present in the human body as six vitamers, which all share a 2-methyl-3-hydroxypyridine structure but differ in the nature of the C4 and C5 substituents (Fig. 1) (Sauberlich 1999). The C4 carbon bears a hydroxymethyl group ($-CH_2OH$) in pyridoxine, an aldehyde group ($-CHO$) in pyridoxal, and an aminomethyl group ($-CH_2NH_2$) in pyridoxamine. All three of these C4 variants can exist with the C5 substituent as a hydroxymethyl group or with this group esterified to phosphate (e.g. pyridoxal 5′-phosphate). In plants the C5 hydroxymethyl group of pyridoxine can be esterified to glucose, forming pyridoxine-5′-β-D-glucoside.

Sources of vitamin B$_6$

Vitamin B$_6$ in human breast milk is present as pyridoxal phosphate plus pyridoxal (Morrison and Driskell 1985). In animal-derived foods it is also present largely as pyridoxal phosphate and in greatest amounts in meat (including poultry and fish) because, in animals, the largest store of pyridoxal phosphate is present in muscle, associated with glycogen phosphorylase. Smaller amounts of pyridoxamine phosphate are also present in animal-derived foods. In plant-derived foods, B$_6$ is present mainly as pyridoxine, pyridoxine phosphate and pyridoxine glucoside (which appears to have reduced bioavailability because of the need for hydrolysis by
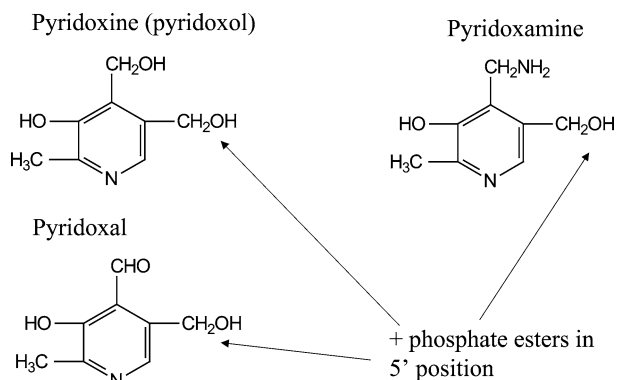
Fig. 1 B_6 vitamers

an intestinal glucosidase) (Mackey et al 2003). When foods are fortified with B_6 (e.g. soya meat substitutes), the form that is used is usually pyridoxine (hydrochloride). The two B_6 vitamers that have been used therapeutically are pyridoxine (hydrochloride) and pyridoxal 5′-phosphate (PLP); this review will discuss disorders responsive to pyridoxine and/or PLP.

Reactivity of pyridoxal 5′-phosphate

Pyridoxal 5′-phosphate is a highly reactive chemical. The aldehyde group can react with:

1. Amino groups, by condensation reactions to form a covalent Schiff base, e.g. the ε-amino groups of lysine residues at the active sites of PLP-dependent enzymes, the α-amino groups of amino acids during PLP-catalysed reactions, or the ε-amino group of lysine-130 in albumin
2. Hydrazines, by condensation reactions e.g. hydralazine, isoniazid, carbidopa
3. Substituted hydroxylamines, by condensation reactions e.g. D-cycloserine
4. Sulphydryl compounds, e.g. L-penicillamine

The ability of small molecules to react with PLP and inactivate it is an important factor in pyridoxine dependency, both in patients taking drugs such as isoniazid and in certain inborn errors of metabolism; the latter will be discussed in more detail below. The reactions of PLP with lysine residues of proteins and with the α-amino groups of amino acids are essential to the numerous reactions involving amino acids that are catalysed by PLP-dependent enzymes.

Physiological roles

Our requirement for B_6 arises mainly from the fact that PLP is the cofactor for over 100 enzyme-catalysed reactions that occur in humans. The majority of these reactions involve amino acids. When PLP reacts with the α-amino group of an amino acid substrate, it stabilizes a negative charge at C_α. This facilitates reactions at the α, β and γ positions (Eliot and Kirsch 2004). Some of the important pyridoxal-phosphate-dependent enzymes in relation to B_6 deficiency, known or suspected inborn errors of metabolism and/or seizures are listed in Table 1. It can be seen that the metabolism of the sollowing neurotransmitters could be affected by PLP deficiency: dopamine, serotonin (5-hydroxytryptamine), glycine, D-serine, glutamate, γ-aminobutyrate (GABA), and histamine. Furthermore, the following might be indicators of deficient flux through a PLP-deficient pathway: raised CSF 3-methoxytyrosine (3-O-methyl-dopa); raised urinary vanillyllactate; low CSF homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA); raised threonine and/or glycine and/or serine and/or histidine in CSF and perhaps in plasma; raised plasma total homocysteine, and plasma and urine homocystine; raised urinary cystathionine; increased urinary xanthurenic acid (particularly following a tryptophan load); and microcytic, hypochromic anaemia.

PLP also acts as the cofactor for glycogen phosphorylase, although the mechanism is different from that seen in reactions involving amino acids (Takagi et al 1982). Other physiological roles of B_6 include modulation of steroid receptor interaction (Tully et al 1994) and regulation of immune function. PLP binds tightly to the D1 domain of the CD4 molecule (Salhany and Schopfer 1993) and it has been suggested that this may interfere with the reaction of CD4 with MHC II (Namazi 2003)

There is some evidence that in some situations B_6 vitamers can function as antioxidants, quenching singlet oxygen at a rate comparable to vitamins C and E (Ehrenshaft et al 1999; Bilski et al 2000).

Dietary deficiency of vitamin B_6

Isolated dietary deficiency of vitamin B_6 is rare. The first reports were of an ill-defined syndrome characterized by weakness, irritability, nervous disorder, insomnia and difficulty with walking. In1954, Coursin observed that when infants were fed on a formula containing only 60 µg/L of B_6 they developed irritability and seizures. These changes were reversed when their B_6 intake was restored to 8 µg/kg per day. A dietary intake of B_6 of less than 7 µg/kg per day also causes EEG changes in adults (Kretsch et al 1991). The changes can be reversed by restoring vitamin B_6 intake to 8 µg/kg per day. Biochemical changes observed in vitamin B_6 deficiency include increased urinary excretion of xanthurenic acid (Fouts and Lepkovsky 1942), raised plasma concentrations of threonine, glycine and serine (Park and Linkswiler 1971).

Hyperhomocystinaemia has also been seen in elderly subjects who are receiving insufficient B_6. A daily supplement

Table 1 Pyridoxal 5′-phosphate-requiring enzymes

Known to be involved in neurotransmitter metabolism	
Aromatic amino acid decarboxylase	Formation of dopamine and 5-hydroxytryptamine
Branched chain amino acid 2-oxoglutarate aminotransferase	Synthesis of glutamate
Glutamate decarboxylase	Conversion of glutamate to GABA
GABA transaminase	Breakdown of GABA, regeneration of glutamate
Glycine cleavage enzyme	Catabolism of neurotransmitter, glycine
L-Serine racemase	Formation of neurotransmitter, D-serine
Histidine decarboxylase	Synthesis of histamine
Providing markers of B$_6$-deficiency/dependency	
Cystathionine β-synthase	Raised plasma total homocysteine
Cystathionine γ-lyase	Increased urinary cystathionine
Kynureninase (?+ kynurenine aminotransferase)	Increased urinary xanthurenic acid
Threonine dehydratase	Raised plasma/CSF threonine
Serine dehydratase	Raised plasma serine
Aminolevulinate synthase	Microcytic, hypochromic, (sideroblastic) anaemia

of 30 μg/kg per day is sufficient to correct the hyperhomocystinaemia in those who are folate and riboflavin replete (McKinley et al 2001).

Decreased absorption of B$_6$ vitamers

There is evidence to suggest that absorption of B$_6$ vitamers is impaired in coeliac disease (Kowlessar et al 1964). Surprisingly, PLP concentrations in plasma are still low in some patients who have been on a gluten-free diet for 10 years (Hallert et al 2002).

Metabolic pathways, including interconversion of B$_6$ vitamers

The phosphorylated B$_6$ vitamers in the diet are thought to be hydrolysed to pyridoxal, pyridoxamine and pyridoxine by intestinal phosphatases prior to absorption (Fig. 2). Pyridoxine-glucoside is hydrolysed to pyridoxine by a glucosidase. The absorbed pyridoxal, pyridoxamine and pyridoxine are rapidly cleared, principally by uptake into the liver, where they are phosphorylated by pyridoxal kinase. Pyridoxine phosphate and pyridoxamine phosphate are then converted to pyridoxal phosphate by pyridox(am)ine-5′-phosphate oxidase (PNPO). Pyridoxal phosphate re-enters the circulation bound to the lysine-190 residue of albumin. Delivery of active cofactor to the tissues requires hydrolysis of circulating pyridoxal phosphate to pyridoxal by the ecto-enzyme tissue nonspecific alkaline phosphatase. Pyridoxal is able to cross the blood–brain barrier (and enter other tissues) but then needs to be re-phosphorylated by pyridoxal kinase to produce active cofactor.

In the next sections we will discuss the effect of deficiency of PNPO and tissue nonspecific alkaline phosphatase (inborn errors affecting the interconversion of B$_6$ vitamers).
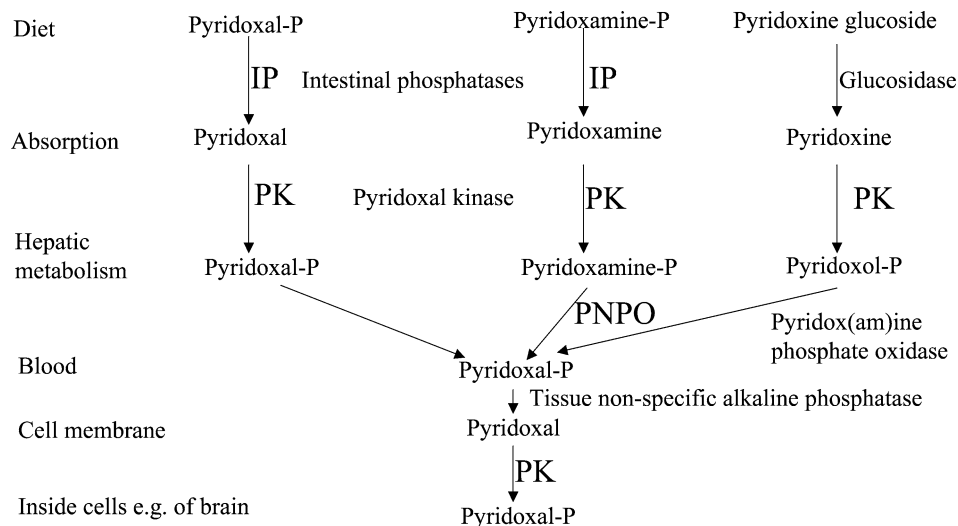
Inborn errors affecting interconversion of B$_6$ vitamers

Pyridox(am)ine phosphate oxidase deficiency

Autosomal recessive loss-of-function mutations in the *PNPO* gene encoding pyridox(am)ine-5′-phosphate oxidase have been described in 5 infants with neonatal epileptic encephalopathy from three families (Mills et al 2005). In all of these newborns, the severe seizure disorder did not respond to treatment with pyridoxine; in the one infant in whom pyridoxal phosphate was tried there was a dramatic cessation of seizures. These 5 infants will be described below. Much larger numbers of infants have been described in whom severe epilepsy has been better controlled with the use of PLP than with the use of pyridoxine (Wang et al 2005). It is not yet known whether any of these infants have mutations or polymorphisms in the *PNPO* gene.

All 5 infants with homozygous PNPO deficiency were born prematurely and all but one had low Apgar scores and/or required intubation. Early acidosis was also common. Seizures commenced on the first day of life and were associated with an EEG showing a burst suppression pattern. The convulsions, which included myoclonic jerks and severe tonic-clonic seizures, were resistant to conventional anticonvulsant drugs. In two patients pyridoxine treatment led to a partial improvement in clonic contractions and lip-smacking automatisms.

Fig. 2 Conversion of dietary vitamin B_6 to intracellular pyridoxal 5′-phosphate cofactor

Diet	Pyridoxal-P	Pyridoxamine-P	Pyridoxine glucoside
	IP Intestinal phosphatases	IP	Glucosidase
Absorption	Pyridoxal	Pyridoxamine	Pyridoxine
	PK Pyridoxal kinase	PK	PK
Hepatic metabolism	Pyridoxal-P	Pyridoxamine-P	Pyridoxol-P
		PNPO	Pyridox(am)ine phosphate oxidase
Blood		Pyridoxal-P	
		↓ Tissue non-specific alkaline phosphatase	
Cell membrane		Pyridoxal	
		PK	
Inside cells e.g. of brain		Pyridoxal-P	

CSF concentrations of the dopamine metabolite HVA and of the serotonin metabolite 5HIAA were low. The CSF concentration of the L-dopa metabolite 3-*O*-methyl-dopa (3-methoxytyrosine) was very high. The urinary excretion of another L-dopa metabolite, vanillyllactic acid, was increased. These changes indicated reduced activity of the PLP-dependent enzyme aromatic L-amino-acid decarboxylase. Raised CSF concentrations of glycine and threonine (4/5) could be explained by reduced activity of the PLP-dependent glycine cleavage enzyme and threonine dehydratase, respectively. CSF concentrations of histidine and taurine (4/5) were also increased. Plasma and CSF concentrations of arginine were low. CSF concentrations of pyridoxal and pyridoxal phosphate were measured in 3 patients and were low in all cases.

The *PNPO* gene is situated on chromosome 17q21.2. The newborns with epileptic encephalopathy and the biochemical changes reported above were shown to be homozygous for missense, splice site and stop codon mutations Expression studies showed that the splice site (IVS3−1g>a) and stop codon (X262Q) mutations were null activity mutations and that the missense mutation (R229W) markedly reduced pyridox(am)ine-phosphate oxidase activity.

Diagnostic tests

The least-invasive test for PNPO deficiency is measurement of urinary vanillyllactate, but the sensitivity and specificity of this determination in neonates with seizures has yet to be determined. High CSF (and plasma) threonine, and low CSF HVA and 5HIAA, with high CSF 3-methoxytyrosine are important indicators of possible PNPO deficiency. A tentative diagnosis of PNPO deficiency might be made if a neonate has seizures that respond dramatically to PLP having failed

to respond to pyridoxine (but therapeutic trials must be undertaken with great care—see below).

Pyridoxal phosphate for intravenous use is not readily available. Fortunately, PLP is very effective when given via a nasogastric tube (in a sick neonate) or orally following recovery from the seizures. A trial of treatment with PLP should only be undertaken in a setting where full resuscitation and intensive care facilities are available. In a PNPO-deficient patient, nasogastric administration of 50 mg of PLP led to cessation of seizures within an hour, but this was associated with profound hypotonia and unresponsiveness and also some hypotension. His neurology only normalized after 4 days of treatment. Continuing control of seizures was achieved with 10 mg/kg of PLP 6-hourly. Without PLP treatment, PNPO deficiency was rapidly fatal in all affected infants. The one case who was treated with PLP after suffering from severe seizures for 2 weeks is alive at 2 years 8 months but has severe dystonia, acquired microcephaly and moderate to severe developmental delay. Treatment with L-dopa and carbidopa was helpful in controlling his dystonic spasms. The effect of earlier treatment remains to be established.

Hypophosphatasia

Hypophosphatasia was described by Rathbun in 1948. He reported paradoxically low levels of alkaline phosphatase (ALP) activity in blood and in several tissues of an infant who died with 'rickets' and epilepsy. Hypophosphatasia is now known to be an inborn error of metabolism–deficient activity of the tissue nonspecific isoenzyme of ALP (TNSALP) caused by deactivating mutations in the *ALPL* gene (McKusick 171760). Waymire and colleagues in 1995 showed that the *ALPL* knockout mouse developed fatal seizures shortly after birth and that these were associated with elevated serum PLP levels and reduced levels of GABA in the brain. Seizures

Fig. 3 Mechanism of pyridoxal 5′-phosphate deficiency in hyperprolinaemia type II
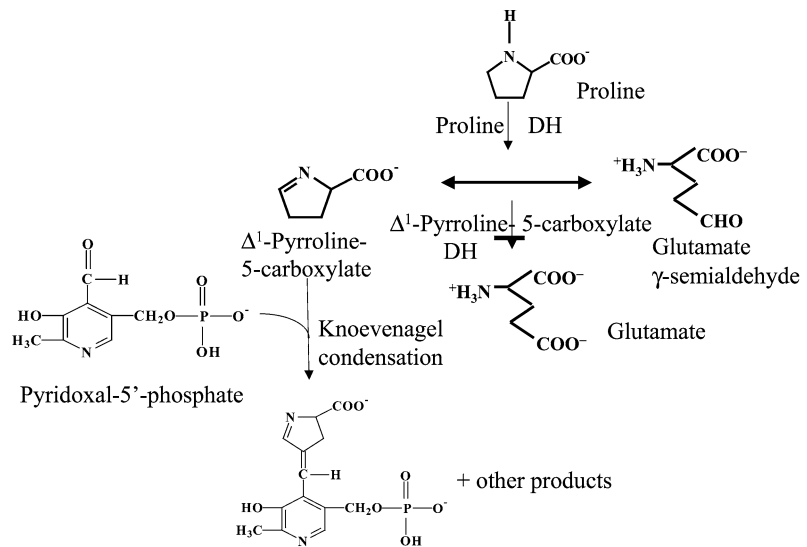
could be prevented by the administration of pyridoxal. Several case reports have indicated that some human infants with hypophosphatasia develop neonatal convulsions with burst suppression on EEG and/or infantile spasms with hypsarrhythmia on EEG (Litmanovitz et al 2002; Whyte et al 1988). Good responses to treatment have been described with pyridoxine 100 mg intravenously or oral pyridoxal phosphate (30 mg/kg per day). Affected individuals have high plasma concentrations of PLP (Iqbal et al 1998) but low plasma concentrations of pyridoxal (Whyte et al 1988). These observations are consistent with the scheme proposed in Fig. 1 which indicates that TNSALP is an ecto-enzyme required for the dephosphorylation of circulating PLP to form pyridoxal, which can then enter the brain (and other tissues).

Drugs that affect enzymes involved in B$_6$ vitamer metabolism

In the brain of the seizure-prone gerbil, vigabatrin has been shown to inhibit pyridox(am)ine oxidase (An et al 2004). This effect is separate from the known inhibition of GABA transaminase. Enzyme-inducing anticonvulsants probably increase the rate of catabolism of B$_6$ vitamers. Adults on anti-epileptic medication have hyperhomocysteinaemia and subnormal plasma concentrations of PLP; these can be corrected with a supplement of folic acid, pyridoxine and riboflavin (Apeland et al 2002). Apeland and colleagues used a dose of pyridoxine of 2 mg/kg per day but stressed that little is known about the relative effectiveness of high versus low doses of pyridoxine hydrochloride in patients on anticonvulsants.

Pyridoxal kinase is inhibited by methylxanthines (Ubbink et al 1990a); consequently it can be shown that pyridoxal phosphate levels fall when a patient is given a theophylline infusion for treatment of asthma (Ubbink et al 1990b). With prolonged treatment with xanthines there is a compensatory increase in pyridoxal kinase activity. Seizures are a serious complication of theophylline therapy. In rabbits, theophylline-induced EEG changes can be reversed with pyridoxine (Glenn et al 1995).

Inborn errors causing accumulation of metabolites that inactivate PLP

Hyperprolinaemia type II (Δ^1-pyrroline-5-carboxylate dehydrogenase deficiency)

In this disorder, mutations in the gene encoding L-Δ^1-pyrroline-5-carboxylic acid (P5C) dehydrogenase lead to accumulation of P5C. Walker et al (2000) observed xanthurenic acid in the urine of a child with hyperprolinaemia type II who had severe seizures complicating pneumonia. They deduced that the seizures and increased excretion of xanthurenic acid might be due to PLP deficiency and found that, indeed, the plasma PLP concentration was low. Farrant and colleagues (2001) proposed that PLP had been inactivated by reacting with P5C (Fig. 3). They showed that P5C could indeed react with PLP by a novel condensation reaction. This involved a Claisen condensation (or Knoevenagel type of reaction) between the activated C-4 carbon of the pyrroline ring of P5C and the aldehyde carbon of PLP.

Pyridoxine-responsive epilepsy

Pyridoxine-responsive epilepsy (PDE) was first described in 1954 (Hunt et al 1954). The diagnosis is based on the clinical observation of cessation of seizures after the administration of 50–100 mg (rarely 500 mg) of pyridoxine, continuing

Fig. 4 Proposed mechanism of pyridoxal 5′-phosphate deficiency in pyridoxine-dependent epilepsy
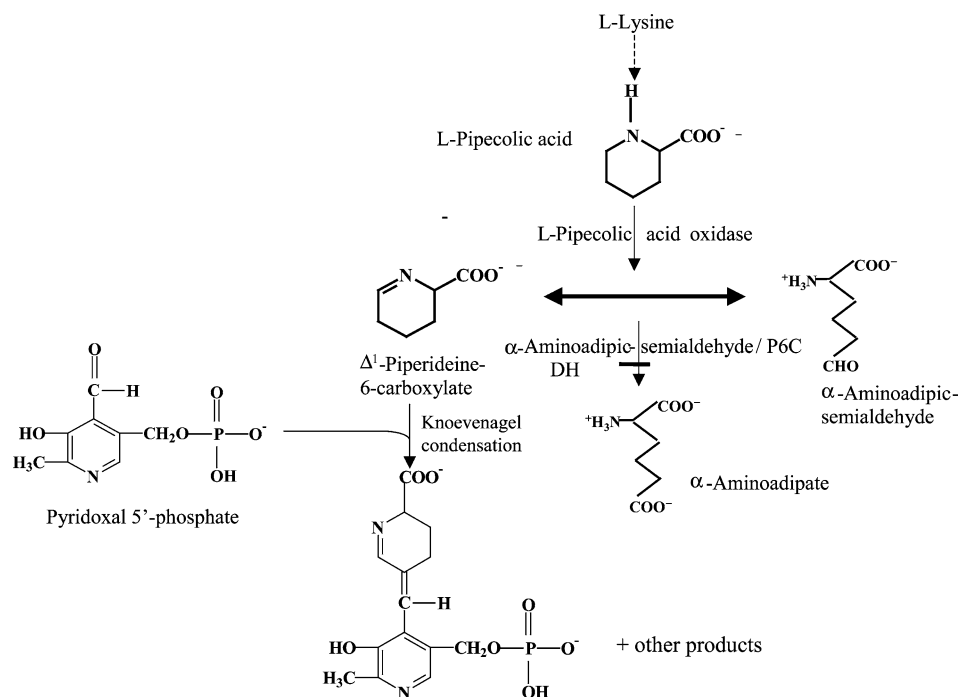
seizure control on pyridoxine 10 mg/kg per day and recurrence of fits when the pyridoxine is stopped. The response to pyridoxine may be dramatic, not only in terms of the seizure control but also in terms of additional effects that can include severe unresponsiveness, hypothermia, hypotension and respiratory arrest. These latter effects are more likely if the infant has been treated with anticonvulsants. The EEG prior to administration of pyridoxine frequently shows burst suppression; 20 min after administration, pyridoxine the EEG may be flat; after 24 h normal activity can be seen.

Pyridoxine dependency is often classified as 'typical' or 'atypical' (Baxter 2001). Typical seizures are of perinatal onset, are refractory to anticonvulsants, respond rapidly to pyridoxine and recur soon after the pyridoxine is stopped. Atypical pyridoxine dependency is of later onset (up to 2 years); the patient may only respond after repeated administration of pyridoxine and patients may be seizure-free for up to 5 months when treatment is stopped. Additional features that may be seen in infants with pyridoxine dependency include jitteriness, hypothermia, neonatal dystonia, and a prodrome of restlessness, irritability and emesis preceding the seizures. Imaging may show cerebellar dysplasia, hemispheric hypoplasia or atrophy, neuronal dysplasia, periventricular hyperintensity, and intracerebral haemorrhage. Patients occasionally develop hydrocephalus on follow-up. In 2000, Cormier-Daire and colleagues mapped the gene for PDE to 5q31.

Estimates of the incidence of PDE vary considerably. In one centre in Germany where a trial of pyridoxine is a formal part of the protocol for neonatal seizures, an incidence of 1 in 20 000 has been described (Ebinger et al 1999). In the UK

and Ireland (where there is considerable variation in the use of a trial of pyridoxine for neonates and infants with seizures) a poll of paediatricians suggested an incidence of 1 in 700 000 for definite and probable cases of PDE (Baxter 2001). However, there was marked geographical variation, suggesting the possibility of under-recognition and under-reporting. A similar nationwide survey in The Netherlands suggested an incidence of 1 in 396 000 for definite and probable cases and 1 in 252 000 when possible cases were included (Been et al 2005).

In 2004, I developed a hypothesis for the pathogenesis of PDE, based on two main observations: (1) the known ability of PLP to react with small molecules (in particular the observations of Walker et al (2000) and Farrant et al (2001) on the reaction between PLP and P5C that occurs in hyperprolinaemia type II (see above)); and (2) the observation of increased pipecolic acid concentrations in CSF, plasma and urine of patients with PDE (Plecko et al 2000). The hypothesis was that PDE may be caused by a defect in the catabolism of pipecolic acid. Metabolism of pipecolic acid occurs via pipecolic acid oxidase, which generates Δ^1-piperideine 6-carboxylate (P6C). P6C is structurally similar to P5C and might be expected to undergo a Knoevenagel condensation with PLP, with the aldehyde group of the PLP condensing with the activated C5 methylene of the piperideine ring of P6C. Thus, PDE might be caused by a defect in the metabolism of P6C (which is in equilibrium in solution with α-aminoadipic semialdehyde (α-AASA); Fig. 4). The piperideine-6-carboxylate dehydrogenase from *Streptomyces clavuligerus* had been cloned (Perez-Llarena et al 1998). A BLAST search of the human genome was

undertaken with an amino acid sequence that is highly conserved between the P6C dehydrogenase of *S. clavuligerus* and aldehyde dehydrogenases from *Caenorhabditis elegans* and *Pisum sativum* (and conserved to a lesser degree in the pyrroline-5-carboxylate dehydrogenase from *Bacillus subtilis*) (Mills et al 2006). This search led to a single human gene encoding a protein called antiquitin (because the homology between the protein from humans and that from the pea suggested it arose early in evolution) (Lee et al 1994). The gene was known as *ALDH7A1* because of its homology with other aldehyde dehydrogenases. *ALDH7A1* mapped to 5q31 (Skvorak et al 1997) and this was the known locus for PDE (Cormier-Daire et al 2000). Thus it seemed a good candidate gene. We were able to show that antiquitin is indeed an α-aminoadipic semialdehyde/P6C dehydrogenase and that children with PDE have *ALDH7A1* mutations that abolish this enzyme activity. We were further able to show that α-AASA is present in increased concentration in CSF, plasma and urine of children with PDE and that P6C can undergo a Knoevenagel condensation with PLP at physiological temperature and pH (Mills et al 2006).

Drugs that react with PLP and inactivate it

Hydrazines

The pyridoxal phosphate hydrazide complex cannot act as a cofactor (Biehl and Vilter 1954) and may also inhibit pyridoxal kinase (McCormick and Snell 1961).

During isoniazid treatment of tuberculosis, symptomatic pyridoxine deficiency tends to occur only in patients who are slow isoniazid inactivators or who have renal insufficiency. The commonest effect is peripheral neuropathy; this and other side-effects can be prevented by giving pyridoxine in a dose 50–100 mg of pyridoxine daily (for an adult).

Penicillamine

Symptomatic pyridoxine deficiency became rare when treatment with D, L-penicillamine was replaced by treatment with D-penicillamine. Nevertheless, most people treating Wilson disease with penicillamine give pyridoxine at a dose of 25 mg daily (for an adult).

Inborn errors affecting PLP-dependent enzymes

Some patients with inborn errors affecting enzymes that use PLP as cofactor are pyridoxine responsive. One obvious explanation could be that these patients have mutations that impair the binding of PLP to the enzyme. However, the nature of the mutations in some pyridoxine-responsive patients ren-

ders this explanation unlikely. Thus it is important to consider other possible mechanisms. In particular we need to think about a possible role of PLP in assisting protein folding and/or reducing the likelihood of degradation of misfolded proteins. For any particular mutation, more than one mechanism may contribute to cofactor responsiveness as shown by the detailed studies of the molecular basis of tetrahydrobiopterin responsiveness in phenylketonuria (Erlandsen et al 2004).

Pyridoxal phosphate as chaperone

As early as 1963, Greengard and Gordon provided evidence indicating that the amount of tyrosine aminotransferase (TAT) protein in the liver could be increased by treatment with pyridoxine. They measured the liver TAT activity (using a saturating concentration of PLP) before and 4 h after pyridoxine administration. There was a 3-fold rise in the enzyme activity following B_6 administration, and this increase was blocked by puromycin and was therefore dependent on protein synthesis. More recently, Gross-Mesilaty and colleagues (1997) showed that the increased overall synthesis of TAT in the presence of PLP could be explained by reduced catabolism of nascent protein by the ubiquitin-proteasome pathway. In their model system, TAT conjugation and degradation required ubiquitin, ATP and the 26S proteasome and could be inhibited by association of TAT with PLP.

Among the inborn errors affecting enzymes that have PLP as cofactor, there is considerable variation in the proportion of affected patients who are pyridoxine-responsive. Thus, estimates of pyridone responsiveness range from 90% of patients with deficiency of erythroid δ-aminolevulinate synthase (pyridoxine-responsive anaemia), through 50% of patients with cystathionine β-synthase deficiency (classical homocystinuria), to 5% of patients with ornithine δ-aminotransferase deficiency (ornithinaemia with gyrate atrophy) and 0% of patients with TAT deficiency (despite the chaperone effect demonstrated in the rat).

Pyridoxine-responsive anaemia

Pyridoxine-responsive anaemia (or X-linked sideroblastic anaemia) is caused by a defect in the erythroid-specific form of δ-aminolevulinate δ-ALA synthase, a PLP-dependent enzyme that catalyses the reaction between succinyl-CoA and glycine to produce δ-aminolevulinic acid, coenzyme A and CO_2. It usually presents in the second decade of life with a microcytic, hypochromic anaemia with a sideroblastic marrow and other problems caused by iron overload. Improvement with pyridoxine is usual but may not be seen in patients with a severe degree of iron overload.

Most of the (more than 24) published mutations in the gene are missense and are in the catalytic domain as opposed to

the mitochondrial-targeting or regulatory domains. When the mutations are expressed in *E. coli*, they have reduced enzyme activity that can be increased in the presence of pyridoxine.

In 1979, Aoki and colleagues showed that newly synthesized δ-ALA-synthase from patients with pyridoxine-responsive anaemia was more rapidly broken down by erythroid protease than wild-type enzyme. In 1995, Cotter and colleagues showed that two pyridoxine-responsive patients had reduced apo-enzyme stability.

Furuyama and colleagues (1997) studied a patient with δ-ALA synthase deficiency who was pyridoxine-resistant. Expression studies suggested that the B_6 resistance might be due to failure of mitochondrial import of the mutant enzyme, but iron overload may also have contributed to the pyridoxine resistance of this patient.

Homocystinuria (cystathionine β-synthase deficiency)

Cystathionine β-synthase (CBS) catalyses the PLP-dependent condensation of serine with homocysteine. A defect in this reaction causes classical homocystinuria. In 1967, Barber and Spaeth reported 3 patients with CBS deficiency who responded to very high doses of pyridoxine (250–500 mg daily) with decreases of plasma methionine levels to normal and virtual elimination of homocystine from plasma and urine. Subsequent reports suggested that approximately 50% of CBS-deficient patients showed a marked or a partial response to pyridoxine *in vivo* (Mudd et al 2001). This effect may not be detectable if the patient is folate-depleted. The response tends to be consistent between siblings. In general there is a correlation between residual hepatic enzyme activity and pyridoxine responsiveness, but there are some exceptions–individuals with high residual activity and no response to pyridoxine. An interesting observation that suggests the possibility of a chaperone action of PLP came from measurement of CBS activity in liver biopsies from patients taken before and after *in vivo* pyridoxine supplementation. The B_6 administration led to 1.3- to 4.5-fold increase in the hepatic CBS activity (Mudd et al 2001).

Ornithine δ-aminotransferase deficiency

PLP is the cofactor for ornithine δ-aminotransferase (OAT). Between 1978 and 1981 six patients from 5 sibships were described who showed significant reduction in plasma ornithine in response to high doses of pyridoxine (500–100 mg/day) (Berson et al 1978, 1981; Weleber et al 1978, 1981). Weleber and colleagues found that 15–20 mg/day was as effective as the higher doses in some of their patients. Fibroblast OAT activity in these patients increased when high concentrations of PLP were added to the assay medium (Kennaway et al 1980). These experiments suggested that the K_m for PLP in patients with pyridoxine-responsive OAT deficiency might be in the order of 80–310 μmol/L in contrast to a figure of 20 μmol/L in controls. However, the experiments were done in an intact cell system and it is possible that chaperoning by PLP led to reduced degradation of newly synthesized enzyme and that this contributed to the increased enzyme activity. Molecular modelling has been used to try to explain pyridoxine responsiveness in OAT deficiency. PLP is covalently bound to the lysine-292 residue of the enzyme, but other residues are also important in noncovalent interactions between the enzyme and its cofactor. Patients with pyridoxine-responsive OAT deficiency due to the A226V mutation could have an impaired interaction between the E230 residue and the 3′-hydroxyl group of PLP. Molecular modelling does not provide an explanation for the pyridoxine responsiveness of the V332M or E318K missense mutations causing B_6-responsive OAT deficiency.

Dangers of an excessive intake of vitamin B_6

There may be significant differences between pyridoxine and PLP in terms of adverse effects of high doses. Supplementation of pyridoxine intake to 1–10 mg/kg per day (for treatment of premenstrual syndrome) caused subtle neurological symptoms in 60% of the women taking the supplement (Dalton and Dalton 1987). Objective evidence of dorsal root ganglionopathy has been documented in individuals taking a similar dose (Baxter 2001; Bender 1999). In young rats, doses of pyridoxine of 100–400 mg/kg per day given intraperitoneally caused EEG changes and sometimes frank seizures (Veresova et al 1998). Six hundred mg/kg twice daily for 3–4 days consistently produced neurotoxicity in rats (Levine and Saltzman 2004). This effect is exacerbated when the rats are given a low-protein diet and is not seen with B_6 vitamers other than pyridoxine.

One neonate treated with PLP showed an unexpected increase in seizure frequency (Hammen et al 1998) and PLP injected into the cerebral ventricles of rats can induce seizures (Salazar and Tapia 2001). One infant with homocystinuria treated with pyridoxal phosphate at a dose of 300 mg/kg per day showed evidence of hepatotoxicity (Yoshida et al 1985).

Against these observations it is important to note that both pyridoxine and PLP have been used quite extensively for treatment of children with idiopathic epilepsy without obvious side-effects (Wang et al 2005).

References

An SJ, Park SK, Hwang IK, et al (2004) Vigabatrin inhibits pyridoxine-5′-phosphate oxidase, not pyridoxal kinase in the hippocampus of seizure prone gerbils. *Neurochem Int* **44**: 133–137.

Aoki Y, Muranaka S, Nakabayashi K, Ueda Y (1979) delta-Aminolevulinic acid synthetase in erythroblasts of patients with

pyridoxine-responsive anemia. Hypercatabolism caused by the increased susceptibility to the controlling protease. *J Clin Invest* **64**: 1196–1203.

Apeland T, Mansoor MA, Pentieva K, McNulty H, Seljeflot I, Strandjord RE (2002) The effect of B-vitamins on hyperhomocysteinemia in patients on antiepileptic drugs. *Epilepsy Res* **51**: 237–247.

Barber GW, Spaeth GL (1969) The successful treatment of homocystinuria with pyridoxine. *J Pediatr* **75**: 463–478.

Baxter P (2001) Pyridoxine dependent/responsive seizures. In: Baxter P, ed. *Vitamin Responsive Conditions in Paediatric Neurology*. London: MacKeith Press, 109–165.

Been JV, Bok LA, Andriessen P, Renier WO (2005) Epidemiology of pyridoxine-dependent seizures in The Netherlands. *Arch Dis Child* **90**: 1293–1296.

Bender DA (1999) Non-nutritional uses of vitamin B$_6$. *Br J Nutr* **81**: 7–20.

Berson EL, Schmidt SY, Shih VE (1978) Ocular and biochemical abnormalities in gyrate atrophy of the choroid and retina. *Ophthalmology* **85**: 1018–1027.

Berson EL, Shih VE, Sullivan PL (1981) Ocular findings in patients with gyrate atrophy on pyridoxine and low-protein, low-arginine diets. *Ophthalmology* **88**: 311–315.

Biehl JP, Vilter RW (1954) Effects of isoniazid on pyridoxine metabolism. *J Am Med Assoc* **156**: 1549–1552.

Bilski P, Li MY, Ehrenshaft M, Daub ME, Chignell CF (2000) Vitamin B$_6$ (pyridoxine) and its derivatives are efficient singlet oxygen quenchers and potential fungal antioxidants. *Photochem Photobiol* **71**: 129–134.

Cormier-Daire V, Dagoneau N, Nabbout R, et al (2000) A gene for pyridoxine-dependent epilepsy maps to chromosome 5q31. *Am J Hum Genet* **67**: 991–993.

Cotter PD, May A, Fitzsimons EJ, et al (1995) Late-onset X-linked sideroblastic anemia. Missense mutations in the erythroid delta-aminolevulinate synthase (ALAS2) gene in two pyridoxine-responsive patients initially diagnosed with acquired refractory anemia and ringed sideroblasts. *J Clin Invest* **96**: 2090–2096.

Coursin DB (1954) Convulsive seizures in infants with pyridoxine-deficient diet. *J Am Med Assoc* **154**: 406–408.

Dalton K, Dalton MJ (1987) Characteristics of pyridoxine overdose neuropathy syndrome. *Acta Neurol Scand* **76**: 8–11.

Ebinger M, Schultze C, Konig S (1999) Demographics and diagnosis of pyridoxine-dependent seizures. *J Pediatr* **134**: 795–796.

Ehrenshaft M, Bilski P, Li MY, Chignell CF, Daub ME (1999) A highly conserved sequence is a novel gene involved in de novo vitamin B$_6$ synthesis. *Proc Natl Acad Sci USA* **96**: 9374–9378.

Eliot AC, Kirsch JF (2004) Pyridoxal phosphate enzymes: mechanistic, structural and evolutionary considerations. *Annu Rev Biochem* **73**: 383–415.

Erlandsen H, Pey AP, Gámez A, et al (2004) Correction of kinetic and stability defects by terahydrobiopterin in phenylketonuria with certain phenylalanine hydroxylase mutations. *Proc Natl Acad Sci USA* **101**: 16903–16908.

Farrant RD, Walker V, Mills GA, Mellor JM, Langley GJ (2001) Pyridoxal phosphate de-activation by pyrroline-5-carboxylic acid. Increased risk of vitamin B$_6$ deficiency and seizures in hyperprolinemia type II. *J Biol Chem* **276**: 15107–15116.

Fouts PJ, Lepkovsky S (1942) A green pigment-producing compound in urine of pyridoxine-deficient dogs. *Proc Soc Exp Biol Med* **50**: 221–222.

Furuyama K, Fujita H, Nagai T, et al (1997) Pyridoxine refractory X-linked sideroblastic anemia caused by a point mutation in the erythroid 5-aminolevulinate synthase gene. *Blood* **90**: 822–830.

Glenn GM, Krober MS, Kelly P, McCarty J, Weir M (1995) Pyridoxine as therapy in theophylline-induced seizures. *Vet Hum Toxicol* **37**: 342–345.

Greengard O, Gordon M (1963) The cofactor-mediated regulation of apoenzyme levels in animal tissues. I. The pyridoxine-induced rise of rat liver tyrosine transaminase level *in vivo*. *J Biol Chem* **238**: 3708–3710.

Gross-Mesilaty S, Hargrove JL, Ciechanover A (1997) Degradation of tyrosine aminotransferase (TAT) via the ubiquitin-proteasome pathway. *FEBS Lett* **405**: 175–180.

Hallert C, Grant C, Grehn S, et al (2002) Evidence of poor vitamin status in coeliac patients on a gluten-free diet for 10 years. *Aliment Pharmacol Ther* **16**: 1333–1339.

Hammen A, Wagner B, Berkhoff M, Donati F (1998) A paradoxical rise of neonatal seizures after treatment with vitamin B$_6$. *Eur J Paediatr Neurol* **2**: 319–322.

Hunt AD Jr, Stokes J Jr, McCrory WW, Stroud HH (1954) Pyridoxine dependency: report of a case of intractable convulsions in an infant controlled by pyridoxine. *Pediatrics* **13**: 140–145.

Iqbal SJ, Brain A, Reynolds TM, Penny M, Holland S (1998) Relationship between serum alkaline phosphatase and pyridoxal-5′-phosphate levels in hypophosphatasia. *Clin Sci (Lond)* **94**: 203–206.

Kennaway NG, Weleber RG, Buist NRM (1980) Gyrate atrophy of the choroid and retina with hyperornithinaemia: biochemical and histological studies and response to vitamin B$_6$. *Am J Hum Genet* **32**: 529–541.

Kowlessar OD, Haefner LJ, Benson GD (1964) Abnormal tryptophan metabolism in patients with adult celiac disease, with evidence for deficiency of vitamin B$_6$. *J Clin Invest* **43**: 894–903.

Kretsch MJ, Sauberlich HE, Newbrun E (1991) Electroencephalographic changes and periodontal status during short-term vitamin B-6 depletion of young, nonpregnant women. *Am J Clin Nutr* **53**: 1266–1274.

Lee P, Kuhl W, Gelbart T, et al (1994) Homology between a human protein and a protein of the green garden pea. *Genomics* **21**: 371–378.

Levine S, Saltzman A (2004) Pyridoxine (vitamin B$_6$) neurotoxicity: enhancement by protein-deficient diet. *J Appl Toxicol* **24**: 497–500.

Litmanovitz I, Reish O, Dolfin T, et al (2002) Glu274Lys/Gly309Arg mutation of the tissue-nonspecific alkaline phosphatase gene in neonatal hypophosphatasia associated with convulsions. *J Inherit Metab Dis* **25**: 35–40.

Mackey AD, Lieu SO, Carman C, Gregory JF (2003) Hydrolytic activity towards pyridoxine-5-β-D-glucoside in rat intestinal mucosa is not increased by vitamin B-6 deficiency: effect of basal diet composition and pyridoxine intake. *J Nutr* **133**: 1362–1367.

McCormick DB, Snell EE (1961) Pyridoxal phosphokinases. II. Effects of inhibitors. *J Biol Chem* **236**: 2085–2088.

McKinley MC, McNulty H, McPartlin J, et al (2001) Low-dose vitamin B-6 effectively lowers fasting plasma homocysteine in healthy elderly persons who are folate and riboflavin replete. *Am J Clin Nutr* **73**: 759–764.

Mills PB, Surtees RAH, Champion MP, et al (2005) Neonatal epileptic encephalopathy caused by mutations in the *PNPO* gene encoding pyridox(am)ine 5′-phosphate oxidase. *Hum Mol Genet* **14**: 1077–1086.

Mills PB, Struys E, Jakobs C, et al (2006) Mutations in antiquitin in individuals with pyridoxine-dependent seizures. *Nature Medicine* **12**: 307–309. Epub 2006 Feb 19.

Morrison LA, Driskell JA (1985) Quantities of B$_6$ vitamers in human milk by high-performance liquid chromatography. Influence of maternal vitamin B$_6$ status. *J Chromatogr* **337**: 249–258.

Mudd SH, Levy HL, Kraus JP (2001) Disorders of transsulfuration. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. Childs B, Kinzler KW, Vogelstein B, assoc, eds. *The Metabolic and Molecular Bases of Inherited Disease*, 8th edn. New York: McGraw-Hill, 2007–2056.

Namazi MR (2003) Pyridoxal 5′-phosphate as a novel weapon against autoimmunity and transplant rejection. *FASEB J* **17**: 2184–2186.

ParkYK, Linkswiler H (1971) Effect of vitamin B$_6$ depletion in man on the plasma concentration and the urinary excretion of free amino acids. *J Nutr* **101**: 185–191.

Perez-Llarena, FJ, Rodriguez-Garcia A, Enguita FJ, et al (1998) The pcd gene encoding piperideine 6-carboxylate dehydrogenase involved in biosynthesis of alpha-aminoadipic acid is located in the cephamycin cluster of *Streptomyces clavuligerus. J Bacteriol* **180**: 4753–4756.

Plecko B, Stockler-Ipsiroglu S, Paschke E, Erwa W, Struys EA, Jakobs C (2000) Pipecolic acid elevation in plasma and cerebrospinal fluid of two patients with pyridoxine-dependent epilepsy. *Ann Neurol* **48**: 121–125.

Rathbun JC (1948) Hypophosphatasia: a new developmental anomaly. *Am J Dis Child* **75**: 822–831.

Salazar P, Tapia R (2001) Seizures induced by intracerebral injection of pyridoxal-5′-phosphate: effect of GABAergic drugs and glutamate receptor antagonists. Neuropharmacology **41**: 546–553.

Salhany JM, Schopfer LM (1993) Pyridoxal 5′-phosphate binds specifically to soluble CD4 protein, the HIV-1 receptor. *J Biol Chem* **268**: 7643–7645.

Sauberlich HE (1999) Vitamin B-6 (pyridoxine). In: Sauberlich HE, ed. *Laboratory Tests for the Assessment of Nutritional Status*, 2nd ed. Boca Raton: CRC Press, 71–102.

Skvorak AB, Robertson NG, Yin Y, et al (1997) An ancient conserved gene expressed in the human inner ear: identification, expression analysis, and chromosomal mapping of human and mouse antiquitin (ATQ1). *Genomics* **46**: 191–199.

Takagi M, Fukui T, Shimomura S (1982) Catalytic mechanism of glycogen phosphorylase: pyridoxal(5′)diphospho(1)-α-D-glucose as a transition-state analogue. *Proc Natl Acad Sci USA* **79**: 3716–3719.

Tully DB, Allgood VE, Cidlowski JA (1994) Modulation of steroid receptor-mediated gene expression by vitamin B$_6$. *FASEB J* **8**: 343–349.

Ubbink JB, Bissbort S, Vermaak WJ, Delport R (1990a) Inhibition of pyridoxal kinase by methylxanthines. *Enzyme* **43**: 72–79.

Ubbink JB, Delport R, Bissbort S, Vermaak WJ, Becker PJ (1990b) Relationship between vitamin B-6 status and elevated pyridoxal kinase levels induced by theophylline therapy in humans. *J Nutr* **120**: 1352–1359.

Veresova S, Kabova R, Velisek L (1998) Proconvulsant effects induced by pyridoxine in young rats. *Epilepsy Res* **29**: 259–264.

Walker V, Mills GA, Peters SA, Merton WL (2000) Fits, pyridoxine, and hyperprolinaemia type II. *Arch Dis Child* **82**: 236–237.

Wang HS, Kuo MF, Chou ML, et al (2005) Pyridoxal phosphate is better than pyridoxine for controlling idiopathic intractable epilepsy. *Arch Dis Child* **90**(5): 512–515.

Waymire KG, Mahuren JD, Jaje JM, Guilarte TR, Coburn SP, MacGregor GR (1995) Mice lacking tissue non-specific alkaline phosphatase die from seizures due to defective metabolism of vitamin B-6. *Nature Genetics* **11**: 45–51.

Weleber RG, Kennaway NG, Buist NR (1978) Vitamin B$_6$ in management of gyrate atrophy of the choroids and retina. *Lancet* **2**: 1213.

Weleber RG, Kennaway NG (1981) Clinical trial of vitamin B$_6$ for gyrate atrophy of the choroid and retina. *Ophthalmology* **88**: 316–324.

Whyte MP, Mahuren JD, Fedde KN, Cole FS, McCabe ER, Coburn SP (1988) Perinatal hypophosphatasia: tissue levels of vitamin B$_6$ are unremarkable despite markedly increased circulating concentrations of pyridoxal-5′-phosphate. Evidence for an ectoenzyme role for tissue-nonspecific alkaline phosphatase. *J Clin Invest* **81**: 1234–1239.

Yoshida I, Sakaguchi Y, Nakano M, Yamashita F, Hitoshi T (1985) Pyridoxal phosphate-induced liver injury in a patient with homocystinuria. *J Inherit Metab Dis* **8**: 91.