Vitamin B-6 Metabolism and Interactions with TNAP

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This research is a product of the Department of Chemistry faculty at Indiana University-Purdue University Fort Wayne.
Chapter 11
Vitamin B-6 Metabolism and Interactions with TNAP

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Abstract Two observations stimulated the interest in vitamin B-6 and alkaline phosphatase in brain: the marked increase in plasma pyridoxal phosphate and the occurrence of pyridoxine responsive seizures in hypophosphatasia. The increase in plasma pyridoxal phosphate indicates the importance of tissue non-specific alkaline phosphatase (TNAP) in transferring vitamin B-6 into the tissues. Vitamin B-6 is involved in the biosynthesis of most of the neurotransmitters. Decreased gamma-aminobutyrate (GABA) appears to be most directly related to the development of seizures in vitamin B-6 deficiency. Cytosolic pyridoxal phosphatase/chronophin may interact with vitamin B-6 metabolism and neuronal development and function. Ethanolaminephosphate phospholyase interacts with phosphoethanolamine metabolism. Extracellular pyridoxal phosphate may interact with purinoceptors and calcium channels. In conclusion, TNAP clearly influences extracellular and intracellular metabolism of vitamin B-6 in brain, particularly during developmental stages. While effects on GABA metabolism appear to be the major contributor to seizures, multiple other intra- and extra-cellular metabolic systems may be affected directly and/or indirectly by altered vitamin B-6 hydrolysis and uptake resulting from variations in alkaline phosphatase activity.

Keywords Vitamin B-6 · Seizures · Hypophosphatasia · Gamma-aminobutyrate · Phosphoethanolamine
11.1 Introduction to Vitamin B-6 Compounds and Metabolism

Studies of the interaction between vitamin B-6 and alkaline phosphatase have been reported for many years. One of the reasons for exploring vitamin B-6 metabolism in the current volume was the occurrence of pyridoxine responsive seizures in hypophosphatasia, a rare genetic disorder which inactivates tissue non-specific alkaline phosphatase (TNAP) (See Chaps. 1, 2, 3 and 14). Several reports suggest that Bethenod et al. (1967) reported pyridoxine responsive seizures in hypophosphatasia in 1967. This appears to be an error. Bethenod et al. did administer pyridoxine but it was not effective in relieving the seizures. Since then there have been multiple reports in which vitamin B-6 supplementation was beneficial (Sia et al. 1975; Litmanovitz et al. 2002; Nunes et al. 2002; Yamamoto et al. 2004; Arun et al. 2005; Baumgartner-Sigl et al. 2007; Balasubramaniam et al. 2010; Demirbilek et al. 2012; Belachew et al. 2013). Vitamin B-6 supplementation also corrected seizures in mice lacking alkaline phosphatase (Waymire et al. 1995; Narisawa et al. 2001). Finally, pyridoxal phosphate concentrations in plasma are markedly elevated in hypophosphatasia (Whyte et al. 1985) demonstrating an important physiological role for alkaline phosphatase in vitamin B-6 metabolism.

Vitamin B-6 is the name for a group of compounds with similar biological activity. The major forms in humans are pyridoxine, pyridoxal, pyridoxamine, their corresponding 5′-phosphate derivatives and the metabolic end product, 4-pyridoxic acid (Table 11.1; Fig. 11.1). Additional metabolites have been detected in humans receiving megadoses of vitamin B-6 (Mahuren et al. 1991) as well as in cats (Coburn and Mahuren 1987) and dogs (Ericson et al. 2008). The interconversion of the various forms combined with the wide variety of reactions in which vitamin B-6 is utilized makes detailed study challenging. Pyridoxal phosphate is a cofactor for over 150 enzymes representing every major class of enzymes except ligases (http://enzyme.expasy.org). This accounts for about 4% of known enzyme activity (Percudani and Peracchi 2003). These enzymes catalyze reactions including transamination, decarboxylation, racemization, aldol cleavage, and beta and gamma eliminations and replacement reactions leading Jansonius (1998) to conclude that pyridoxal phosphate is the most versatile organic catalyst known. It has been suggested that the most ancient reaction of aerobic metabolism was the synthesis of pyridoxal phosphate or pyridoxal (Kim et al. 2012). Much information about the structure, sequence and function of vitamin B-6 enzymes is contained in the vitamin B-6 database (http://bioinformatics.unipr.it/cgi-bin/bioinformatics/B6db/home.pl) (Percudani and Peracchi 2009). Although reactions involving amino acid metabolism seem to attract the most attention, 70–80% of the vitamin B-6 in mammals is located in skeletal muscle (Coburn et al. 1988a) associated with glycogen phosphorylase where the phosphate group rather than the amine or aldehyde group is the catalytic center (Palm et al. 1990). Also, while many studies of vitamin B-6 focus on pyridoxal phosphate, in most tissues other than muscle the amount of pyridoxal phosphate is equaled or exceeded by the amount of pyridoxamine phosphate.
(Coburn et al. 1988b). There is little information on the rate of interchange of these forms. Unless enzymatic activity is inhibited during homogenization, pyridoxamine phosphate in liver is quickly converted to pyridoxal phosphate (Coburn et al. 1988b). That suggests that the two compounds may be sequestered separately. It also raises questions about the accuracy of observed vitamer distribution in some studies. The commonly accepted pathway of vitamin B-6 metabolism in humans (Fig. 11.1) involves phosphorylation of the 5'-position by pyridoxal kinase (EC 3.7.1.35). The kinase can phosphorylate all three vitamers. Pyridoxine phosphate and pyridoxamine phosphate may be oxidized to pyridoxal phosphate. The oxidizing enzyme is usually named pyridoxine (pyridoxamine) phosphate oxidase. However, the name recommended by the Enzyme Commission is pyridoxal 5'-phosphate synthase (EC 1.4.3.5). The synthase can oxidize both pyridoxine phosphate and pyridoxamine phosphate. It is inhibited by its substrate, pyridoxine phosphate (Ki = 60 μM) (Kwon et al. 1991) and product, pyridoxal phosphate (Ki = 23 μM) (Kwok and Churchich 1980). Pyridoxal 5'-phosphate synthase activity varies considerably between tissues and between species (Fonda 1988). Mouse and human erythrocytes have appreciably higher activity than rat, hamster and rabbit erythrocytes. This means that the role of erythrocytes in vitamin B-6 metabolism will vary significantly between species. Pyridoxamine phosphate and pyridoxal phosphate can also be interconverted by aminotransferases. The phosphate group can be

**Table 11.1** Vitamin B-6 compounds and major enzymes associated with the interconversion of B-6 vitamers in animals

<table>
<thead>
<tr>
<th>Vitamin B-6 metabolites</th>
<th>Abbreviation</th>
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<tbody>
<tr>
<td>Pyridoxine</td>
<td>PN</td>
<td>65-23-6</td>
</tr>
<tr>
<td>Pyridoxal</td>
<td>PL</td>
<td>66-72-8</td>
</tr>
<tr>
<td>Pyridoxamine</td>
<td>PM</td>
<td>85-87-0</td>
</tr>
<tr>
<td>Pyridoxine 5'-phosphate</td>
<td>PNP</td>
<td>447-05-2</td>
</tr>
<tr>
<td>Pyridoxal 5'-phosphate</td>
<td>PLP</td>
<td>54-47-7</td>
</tr>
<tr>
<td>Pyridoxamine 5'-phosphate</td>
<td>PMP</td>
<td>529-96-4</td>
</tr>
<tr>
<td>4-pyridoxic acid</td>
<td>PA</td>
<td>82-82-6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Enzyme commision No.</th>
<th>CAS registry No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyridoxal kinase (1)(^a)</td>
<td>2.7.1.35</td>
<td>9025-42-0</td>
</tr>
<tr>
<td>Pyridoxal phosphatase (2)</td>
<td>3.1.3.74</td>
<td>9076-92-0</td>
</tr>
<tr>
<td>Alkaline phosphatase (2)</td>
<td>3.1.3.1</td>
<td>9001-78-9</td>
</tr>
<tr>
<td>Pyridoxal 5'-phosphate synthase (3)</td>
<td>1.4.3.5</td>
<td>9055-72-5</td>
</tr>
<tr>
<td>Multiple aminotransferases (4)</td>
<td>2.6.1</td>
<td>–</td>
</tr>
<tr>
<td>Aldehyde oxidase (5)</td>
<td>1.2.3.1</td>
<td>9029-07-6</td>
</tr>
<tr>
<td>Aldehyde dehydrogenase (NAD(^+)) (6)</td>
<td>1.2.1.3</td>
<td>9028-86-8</td>
</tr>
<tr>
<td>Aldehyde dehydrogenase (NADP(^+)) (6)</td>
<td>1.2.1.4</td>
<td>9028-87-9</td>
</tr>
<tr>
<td>Aldehyde dehydrogenase (NAD(P)(^+)) (6)</td>
<td>1.2.1.5</td>
<td>9028-88-0</td>
</tr>
</tbody>
</table>

\(^a\)Number in parentheses indicates the associated reaction in Fig. 11.1. In some cases the enzyme involved may vary depending on the species, tissue and cellular locations. Enzymes which have been identified only in microorganisms are not listed.
removed by non-specific phosphatases or by pyridoxal phosphatase (EC 3.1.3.74). Pyridoxal can be oxidized to 4-pyridoxic acid by aldehyde oxidase (EC 1.2.3.1) which has been detected in a variety of tissues (Moriwaki et al. 2001). Stanulovic et al. (1976) found that rats lacking aldehyde oxidase were able to produce pyridoxic acid using a NAD dependent aldehyde dehydrogenase. The identity of this dehydrogenase remains unclear. Although Stanulovic et al. (1976) suggested that it is probably the nonspecific aldehyde dehydrogenase EC 1.2.1.3, the Enzyme Nomenclature database (http://www.chem.qmul.ac.uk/iubmb/enzyme/index.html) lists three nonspecific aldehyde dehydrogenases (EC 1.2.1.3 using NAD, EC 1.2.1.4 using NADP and EC 1.2.1.5 using both) plus pyridoxal dehydrogenase (EC 1.1.1.107) described in bacteria (Burg and Snell 1969). Although Stanulovic et al. (1976) used NAD, Bode et al. (1991) detected pyridoxal dehydrogenase activity in rats using NADP which they assigned to EC 1.2.1.4. Chemical Abstracts assigned the Stanulovic rat enzyme the same CAS number as the bacterial enzyme. However, there is no obvious justification for that. Pyridoxal dehydrogenase activity was not detected in human liver using NAD or NADP (Merrill et al. 1984). Therefore, it seems unlikely to occur in human brain.
Absorption and Transport of Vitamin B-6

Intestinal absorption of unphosphorylated vitamin B-6 appears to involve diffusion and/or facilitated diffusion (Mooney et al. 2009). While it is frequently assumed that the ionic nature of pyridoxal phosphate prevents it from crossing cell membranes without prior hydrolysis, perfusion studies in rats demonstrated that pyridoxal phosphate can be transferred intact from the lumen of the intestine into the circulation (Mehansho et al. 1979). Although liver is a major site of vitamin B-6 metabolism, small doses of pyridoxine and pyridoxamine can be converted to pyridoxal in the intestine prior to release into the portal circulation in the mouse (Sakurai et al. 1987, 1988). Similar results were reported with cultured Caco-2 cells (Albersen et al. 2013). The main transport forms of vitamin B-6 in blood are pyridoxal and pyridoxal phosphate, which is released from the liver associated primarily with albumin (Lumeng et al. 1974). Hydrolysis by TNAP is important in removal of pyridoxal phosphate from plasma as indicated by a marked increase in plasma concentrations of pyridoxal phosphate when TNAP activity is limited in hypophosphatasia (Whyte et al. 1985). Significant amounts of pyridoxine and pyridoxamine do not occur in peripheral plasma with normal vitamin B-6 intake (Albersen et al. 2014). Therefore, intravenous administration of pyridoxine produces atypical metabolism. For example, skeletal muscle in mice and rats contains pyridoxal kinase but little, if any, pyridoxal 5′-phosphate synthase (Fonda 1988). Therefore, pyridoxine can be taken up by muscle and phosphorylated but cannot be converted to pyridoxal phosphate. It must be hydrolyzed and released back into the circulation. This is not necessarily harmful but it does not reflect the normal kinetic processes followed by pyridoxal and pyridoxal phosphate.

Early attempts to isolate phosphatases produced variable results. Saraswathi and Bachhawat (1963) isolated from human brain an alkaline phosphatase for which pyridoxal phosphate was the best substrate with a Km of 0.430 mM at pH 9. Magnesium was required for optimal activity. Inorganic phosphate was a competitive inhibitor with Ki of 1.65 mM. Brunel et al. (1969) and Zisapel and Levi (1980) independently isolated from bovine brain alkaline phosphatases which had little activity toward pyridoxal phosphate. Brun-Heath et al. (2011) recently demonstrated that the alkaline phosphatase expressed in human neuronal and endothelial cells corresponds to the ALPL gene driven by the bone promoter. The interactions between alkaline phosphatase, pyridoxal phosphate, pH and inorganic phosphate are complex. Examination of the membrane bound alkaline phosphatase of rat liver demonstrated that at pH 7.4 the Km for pyridoxal phosphate was about 2 µM, which is approximately the concentration of free pyridoxal phosphate in liver (Lumeng and Li 1975). The enzyme was very sensitive to substrate inhibition by pyridoxal phosphate but not by p-nitrophenyl phosphate. Inorganic phosphate is a well-recognized inhibitor of alkaline phosphatase. Under physiological conditions alkaline phosphatase activity toward pyridoxine phosphate was reduced about 50% by normal plasma phosphate concentrations and will increase or decrease significantly in response to changes in phosphate concentrations within the ranges
observed clinically (Coburn et al. 1998). The measurements utilized pyridoxine phosphate rather than pyridoxal phosphate in order to minimize substrate inhibition and non-specific protein binding. The article includes a nomogram for predicting pyridoxine phosphate phosphatase activity in undiluted serum from routine clinical measurements of inorganic phosphate and alkaline phosphatase. Seargeant and Stinson (1979) estimated that inorganic phosphate concentrations in liver would be high enough to inhibit human liver alkaline phosphatase if the phosphate was accessible to the enzyme.

Spector and Johanson (2007) noted that there are five general processes that must be understood to explain mechanistically vitamin homeostasis in brain:

(i) Transport from plasma in and out of the extracellular space of brain and cerebrospinal fluid;
(ii) Uptake and release of transported vitamers by brain cells;
(iii) Vitamer activation by kinases or other enzymes, i.e. incorporation into cofactors and transfer into appropriate compartments in the brain cells;
(iv) When appropriate, reformation of the transported vitamer for release from the brain cell and then the central nervous system; and
(v) Finally, mechanisms in kidney and gut including saturable uptake, excretion, and secretion (kidney) mechanisms that tend to keep plasma vitamin levels constant, thus contributing to brain homeostasis.

Uptake by the choroid plexus primarily involves diffusion or facilitated diffusion of non-phosphorylated vitamers followed by trapping by pyridoxal kinase (Spector and Greenwald 1978). Dual label experiments with \[^{3}H, {32}P\]pyridoxine phosphate indicated that only 10–15 % of the absorbed vitamer was absorbed without hydrolysis. Since TNAP is expressed at high levels in the choroid plexus (Nishihara et al. 1994), reduced activity in hypophosphatasia could significantly impair vitamin B-6 uptake. During a 30 min period 56 % of accumulated \[^{3}H\] was released from isolated choroid plexus and 46 % from brain slices (Spector 1978b). However, 76 % of the label released by choroid plexus was phosphorylated compared to 1 % from brain slices. Therefore, choroid plexus accounts for essentially all of the phosphorylated B-6 vitamers in the cerebrospinal fluid (CSF). The rabbit choroid plexus can also absorb unphosphorylated vitamers from the CSF. Since the non-phosphorylated vitamers are removed from the CSF faster than pyridoxal phosphate, Spector and Johanson (2007) suggested that the phosphorylated form serves as a reservoir. \[^{3}H\]pyridoxic acid or \[^{3}H\]pyridoxic acid lactone accounted for less than 10 % of the label in plasma or CSF from in vivo experiments (Spector 1978a) and less than 5 % of the label released from brain and choroid plexus slices from in vitro experiments (Spector 1978b). Spector and Johanson (2007) gave the following data from rabbits: total vitamin B-6 in plasma is 0.30 µmol/L 55 % non-phosphorylated; 0.39 µmol/L in cisternal CSF 26 % nonphosphorylated; 8.9 µmol/kg in brain 23 % nonphosphorylated; brain turnover 17 %/d; CSF turnover 24 %/3 h; permeability-surface area product <10 compared to 0.2 for mannitol (absorbed by simple diffusion) and 158 for leucine (absorbed by facilitated diffusion); Kt ~ 1–2 µmol/L (half saturation concentration for transport through the
blood brain barrier). Spector and Johanson (2007) concluded that since the Kt is similar to plasma concentration the carrier mediated process plays a crucial transport and regulatory role because simple diffusion is relatively unimportant.

In human cerebrospinal fluid pyridoxal is the dominant B-6 vitamer (Albersen et al. 2012; van der Ham et al. 2012; Albersen et al. 2014). Median values for pyridoxal and pyridoxal phosphate in the cerebrospinal fluid of infants declined over 50 % between a postmenstrual age of 30–37 and ≥42 weeks (Albersen et al. 2012). Two studies which measured only pyridoxal phosphate found that the decline continued for at least 12 months after birth (Ormazabal et al. 2008; Footitt et al. 2011). In adult subjects the median values for pyridoxal and pyridoxal phosphate were 10.5 and 55.9 nmol/L in plasma and 30.0 and 16.1 nmol/L in cerebrospinal fluid, respectively (Albersen et al. 2014). They reported a correlation of 0.629 between pyridoxal phosphate in cerebrospinal fluid and plasma. This might be interpreted to indicate direct transport of pyridoxal phosphate from plasma to cerebrospinal fluid. However, an alternative explanation could be that hydrolysis of pyridoxal phosphate in plasma is the primary source of the pyridoxal used to synthesize the pyridoxal phosphate released into the cerebrospinal fluid by the choroid plexus.

11.3 Vitamin B-6 Transporters

Evidence that vitamin B-6 uptake may involve facilitated diffusion implies the existence of transporters. A vitamin B-6 transporter has been identified in S. cerevisiae (Stolz and Vielreicher 2003). The Tpn1p protein is a member of the purine-cytosine permease family within the major facilitator superfamily and functions as a proton symporter in the plasma membrane. In S. pombe the pyridoxine transporter was a different proton symporter encoded by the car1+ (changed amiloride resistance) gene which Stolz et al. (2005) proposed renaming bsu1+ for vitamin B-6 uptake. Carrier mediated pyridoxine transport sensitive to amiloride has been reported in Caco-2 cells (Said et al. 2003; Stolz et al. 2005). However, Stolz et al. (2005) stated that no mammalian homologue of Bsu1p has been identified in database searches and Said (2011) acknowledges that nothing is known about the molecular structure of the intestinal pyridoxine transporter of any mammalian species. It has recently been proposed that the transporter could be used to enhance transfection of cancer cells by attaching a ligand to pyridoxal phosphate (Pandey et al. 2013). The hypothesis is that the pyridoxal phosphate will bind to the transporter but the large size will prevent transport resulting in endocytosis of the entire complex. This protocol did express luciferase in mouse brain suggesting that a vitamin B-6 transporter does act in brain.
11.4 Intracellular Pyridoxal Phosphatase (EC 3.1.3.74)

TNAP is a membrane bound ectoenzyme which has a major role in controlling extracellular pyridoxal phosphate concentrations. In rat neurons pyridoxal phosphatase activity at pH 5.0 was cytochemically located in a specialized region of smooth endoplasmic reticulum that is located at the trans face of the stack of Golgi elements (GERL) (Spater et al. 1978). These locations differed from the usual sites of both acid and alkaline phosphatase activity. In 1992 Fonda (1992) observed that human erythrocytes contained alkaline phosphatase activity associated with the stroma and a soluble acid phosphatase which hydrolyzed vitamin B-6 phosphates. The optimal pH was 6.5. At pH 7.4 it hydrolyzed vitamin B-6 phosphates but not nucleotide phosphates, phosphoamino acids or other phosphorylated compounds. At pH 7.4 the Km was 1.5 µM. Activity was inhibited by inorganic phosphate (Ki = 0.8 mM), fluoride and weakly by pyridoxal. It was not inhibited by levamisole, L-phenylalanine or L-tartrate. Based on tryptic fragments obtained by Gao and Fonda (1994), Jang et al. (2003) were able to identify the pyridoxal phosphatase in a cDNA library from human brain and express it in *E. coli*. The mouse enzyme was also cloned. BLAST searches identified similar proteins in organisms ranging from bacteria to mammals. The enzyme is a dimer requiring magnesium for optimal activity. The Km values for the human enzyme were 2.5, 43.4 and 80.6 for pyridoxal phosphate, pyridoxine phosphate and pyridoxamine phosphate, respectively. There was little activity with p-nitrophenylphosphate. Phosphatase mRNA was detected in essentially all tissues tested but was highest in adult and fetal brain. Liver and testis were higher than tissues other than brain. Expression in spinal cord was much lower than in brain. The structure shows no evidence of integral membrane domains which is consistent with the erythrocyte enzyme being found in the soluble fraction. Based on these data in 2004 the Enzyme Commission created EC 3.1.3.74, pyridoxal phosphatase, which has the systematic name, pyridoxal 5′-phosphate phosphohydrolase.

Kang et al. (2004) examined the relative mRNA expression of pyridoxal 5′-phosphate synthase (PNPO), pyridoxal kinase (PDXK) and pyridoxal phosphatase (PDXP) in a wide variety of human tissues which are partially listed in Table 11.2. Maximal expression of the synthase, kinase and phosphatase was in the liver, testis and cerebral cortex, respectively. Expression of pyridoxal phosphatase mRNA in most brain regions was greater than 80 % of the maximum while expression of oxidase and kinase mRNA was below 35 % of the maximum. Boe et al. (2004) identified autoantigens to pyridoxal phosphatase in patients with paraneoplastic neurologic disorders. They confirmed that the pyridoxal phosphatase gene, PDXP, was most strongly expressed in various regions of the brain. Expression was also significant in liver, kidney and testis.

Gohla et al. (2005) found that pyridoxal phosphatase also activates coflin, a key regulator of cytoskeletal dynamics. They named the pyridoxal phosphatase protein, chronophin. Lack of this enzyme causes major cell division defects. Northern blot analysis revealed high expression in brain, heart, skeletal muscle, liver and kidney.
Kim et al. (2008) found that pyridoxal phosphatase/chronophin was localized primarily in postsynaptic compartments and not in presynaptic compartments in rat hippocampus. They concluded that the interaction of pyridoxal phosphatase/chronophin with actin may have an important role in seizure activity. Hwang et al. (2007) examined the effect of ischemia produced by 5 min occlusion of the carotid arteries on vitamin B-6 metabolizing enzymes in the gerbil hippocampus. Ischemia had no significant effect on the amount of pyridoxal 5′-phosphate synthase in the CA2/3 regions. In the CA1 region relative optical density of the pyridoxal 5′-phosphate synthase spot vs sham was highest 12 h after ischemia, declined slightly until 2 days and returned to baseline by 4 days. Relative optical density of the pyridoxal phosphatase spot declined about 50 % at 3 h and peaked at about twice the sham spot after 2 days. Administration of pyridoxal phosphate 30 min prior to the ischemic event minimized the loss of pyramidal cells 4 days later. Hwang et al. suggested that this protective effect may have resulted from increased GABA. Koh (2010) examined the response of rats to middle cerebral

<table>
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<th>Tissue</th>
<th>PNPO</th>
<th>PDXK</th>
<th>PDXP</th>
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<tr>
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<td>74.2</td>
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</table>

Highest expressing tissue for each enzyme was set to 100
artery occlusion for 24 h. The pyridoxal phosphatase spot from the cerebral cortex was significantly reduced at 18% of the sham group. Koh did not cite the earlier results from Hwang et al. The difference in the response of pyridoxal phosphatase could reflect the difference in duration of ischemia and/or the portion of the brain examined. Furukawa et al. (2010) found that pyridoxal phosphatase activity was increased in the limbic forebrain of 3 month old senescence-accelerated mouse prone 10 (SAMP10) and suggested that morphological impairments in cytoplasmic processes of microglia may be related to the altered expression of pyridoxal phosphatase since this enzyme is known to be involved in brain cytoskeleton formation and associated with acute and chronic neurodegenerative conditions.

Pyridoxal phosphatase/chronophin dephosphorylates pyridoxal phosphate and actin-depolymerizing factor giving it a role in regulating both vitamin B-6 and F-actin (Kim et al. 2009). Kim et al. (2009) concluded that pyridoxal phosphatase/chronophin mediated actin dynamics may play an important role in the changes of morphological properties (dendritic spine reorganization) of the hippocampus in long-term potentiation. (Kim et al. 2008; Kwak et al. 2009).

11.5 Regulation of Vitamin B-6 Metabolism

Determining how vitamin B-6 metabolism is regulated is challenging. While it is frequently assumed that water soluble vitamins are readily lost from the body, vitamin B-6 seems to be efficiently conserved by most tissues. Pyridoxal kinase complexes with aspartate aminotransferase resulting in channeling newly formed pyridoxal phosphate to the aminotransferase (Kim et al. 1988). During vitamin B-6 deficiency in rats the large pool of pyridoxal phosphate sequestered in muscle glycogen phosphorylase was not released until anorexia and weight loss occurred (Black et al. 1978). When young adult men were placed on a controlled diet providing about 0.5 mg vitamin B-6/d, within 2 weeks their urinary excretion had dropped to approximate their intake with a loss of only 4% of their body pool (Coburn et al. 1991). While plasma content dropped markedly, muscle content showed little change after 6 weeks. If the body conserves vitamin B-6, we hypothesized that the vitamin B-6 requirement for growth could be determined by multiplying the daily gain by the average body content of vitamin B-6 (15 nmol/g) (Coburn 1994). This approach provided reasonable agreement with experimentally determined requirements for species ranging from mammals to birds and fish. The total body pool in humans is about 1,000 µmol (Coburn et al. 1988a). We proposed that the turnover is determined primarily by vitamin B-6 intake. As noted below at very low intakes brain is adversely affected. If existing tissue efficiently conserves vitamin B-6, the fact that most studies of vitamin B-6 metabolism involve growing rats raises the possibility that the observed reductions in tissue vitamers are magnified by the addition of new tissue and do not necessarily represent changes that would occur in an animal maintaining a constant weight.
Rats receiving vitamin B-6 intakes ranging from 2 to 200 times the daily requirement for 20 days showed no significant difference in the pyridoxal phosphate content of liver or brain indicating that maximal pyridoxal phosphate content is well regulated (Li et al. 1974). Since pyridoxal 5′-phosphate synthase from liver (Wada and Snell 1961; Merrill et al. 1984) or brain (Kwok and Churchich 1980) is very sensitive to product inhibition by pyridoxal phosphate, the synthase was proposed as a regulatory factor (Snell and Haskell 1971; Merrill et al. 1978). Therefore, Li et al. (1974) tested the effect of pyridoxal phosphate on pyridoxal phosphate production by rat liver cytosol in vitro. They concluded there was no effect because the total produced was similar with and without added pyridoxal phosphate. However, Merrill et al. (1978) noted that the data of Li et al. did show a slightly decreased rate of production with added pyridoxal phosphate indicating that there was inhibition. Li et al. observed that adding 80 mM sodium phosphate to inhibit phosphatase activity resulted in a marked increase in net pyridoxal phosphate production in hepatocytes or plasma membranes but not cytosol. Therefore, Li et al. concluded that under normal circumstances pyridoxal phosphate which is not protected by protein binding is rapidly hydrolyzed thus maintaining a steady state. Since the phosphatase activity was in the membrane fraction but not the cytosol, it presumably represented alkaline phosphatase rather than pyridoxal phosphatase. We now know that alkaline phosphatase is an ectoenzyme. One explanation of the results of Li et al. might be that the pyridoxal phosphate produced by the hepatocytes was released into the medium before being hydrolyzed. The description of the sample processing does not indicate that the hepatocytes were separated from the medium before pyridoxal phosphate analysis. Liver routinely releases pyridoxal phosphate into the plasma (Lumeng et al. 1974) but brain may not (Spector 1978b). Perhaps in brain pyridoxal phosphatase controls the intracellular pyridoxal phosphate concentration.

Ohkawa et al. (1994) used immunochemistry to examine the distribution of pyridoxal kinase in rabbit brain with the following results: “The cytoplasm of neuronal cells and neuroglial cells in the cerebral cortex, hippocampal region, brain nuclei and cerebellar cortex showed positive staining with various degrees of intensity. The neuronal cells and surrounding fibers in some brain nuclei, such as the area tegmentalis ventralis or the substantia nigra, showed intense staining. The neuronal cells of the hippocampal region showed somewhat weak reactivity, but some with intense reactivity were found sparsely distributed and positive staining fiber networks of a very low density were also observed.” Pyridoxal kinase activity in brain changes with dietary intake. Spector and Shikuma (1978) suggested that a report of a negative correlation between pyridoxal phosphate and pyridoxal kinase in rabbit brain (Ebadi et al. 1970) was due to errors caused by using pyridoxal rather than pyridoxine as the substrate for analyzing kinase activity. When various areas of normal rat brain were compared, there was a positive correlation between pyridoxal kinase activity and pyridoxal phosphate concentration (Ebadi and Bifano 1978). An early report suggested that dietary deficiency of vitamin B-6 in rats tended to increase kinase activity in brain slightly (McCormick et al. 1961). Later work by Meisler and Thanassi (1980) indicated that vitamin B-6 deficiency reduced both
pyridoxal phosphate and pyridoxal kinase activity in rat brain. Pyridoxal 5′-phosphate synthase and phosphatase activities did not show any consistent changes. The animals weighed about 100 g at the start of the experiment. By the 4th week brain kinase activity in both the control and deficient groups increased and the group differences were no longer statistically significant. Restoring normal vitamin B-6 intake quickly restored kinase activity to control values in the liver. As noted above kinase activity in brain had already increased and supplementation had little additional effect. Rasmussen et al. (1979) also found that pyridoxal synthase activity was not affected by vitamin B-6 deficiency. Without citing any specific references Meisler and Thanassi (1980) state that studies reported by others have shown that pyridoxal 5′-phosphate synthase is involved in regulating pyridoxal phosphate in B-6 sufficient animals and that their current results indicated pyridoxal kinase is a regulatory factor in vitamin B-6 deficiency. Those suggestions have recently been cited by Kwak et al. (2009). It is important to note that product inhibition of pyridoxal 5′-phosphate synthase will occur only when the substrate is pyridoxine phosphate or pyridoxamine phosphate. Pyridoxal 5′-phosphate synthase is not required for the production of pyridoxal phosphate from pyridoxal. In unsupplemented humans there is little or no pyridoxine or pyridoxamine in the peripheral plasma or cerebrospinal fluid (Albersen et al. 2014). Therefore, most of the pyridoxal phosphate in brain arises from pyridoxal which would limit the role of pyridoxal 5′-phosphate synthase as a regulator. In addition we would suggest that since pyridoxal kinase activity responds rapidly to changes in vitamin B-6 intake, vitamin B-6 availability rather than pyridoxal kinase is the regulator.

The vitamin B-6 analog, 4′-deoxypyridoxine, can act as either a competitive inhibitor or a competing substrate depending on the reaction in question. Unfortunately, the distinction is frequently ignored in the literature. Ebadi et al. (1970) noted that pyridoxal kinase activity was increased in the presence of deoxypyridoxine and that the increase was prevented by actinomycin D or puromycin. While Ebadi et al. (1970) interpreted the results as an inverse relationship between pyridoxal phosphate and kinase activity, we suspect that since deoxypyridoxine is a substrate for the kinase, the presence of deoxypyridoxine may have stimulated the increased synthesis of pyridoxal kinase. Pyridoxal kinase from rat brain is also inhibited by dopa, dopamine, norepinephrine, tyramine, serotonin, histamine, and GABA (Ebadi and Govitrapong 1979). Kerry et al. (1986) attributed an increase in kinase activity during isolation from sheep brain to removal of interfering substances such as amines. Based on the above discussion we suggest that vitamin B-6 intake, protein binding, phosphatase activity and biogenic amines may be the prime regulators of intracellular pyridoxal phosphate concentration in brain. Post mortem examination of over 100 human brains detected a 1000-fold range of pyridoxal kinase activity (Snell 1964). Snell noted that pyridoxal kinase is quite stable in frozen samples but does not provide any documentation that all of the brains were subject to the same post mortem protocol prior to freezing the samples.
11.6 Vitamin B-6 and Seizures

The earliest symptoms of vitamin B-6 deficiency are skin disorders, seizures and anemia. Brain does not conserve vitamin B-6 as well as muscle during severe dietary vitamin B-6 deficiency. Early studies of vitamin B-6 deficiency reported seizures in dogs (Fouts et al. 1938), pigs (Chick et al. 1938) and rats (Chick et al. 1940). In humans seizures have been observed as a result of dietary vitamin B-6 deficiency (Coursin 1955) and genetic disorders of vitamin B-6 metabolism (Footitt et al. 2013; Dakshinamurti and Dakshinamurti 2014). Electroencephalographic changes were noted within 2 weeks in women fed a diet containing essentially no vitamin B-6 (Kretsch et al. 1991). The changes were corrected with an intake of 0.5 mg vitamin B-6/d. Gregory et al. (2013) recently fed human subjects 0.37 mg vitamin B-6/d for 28 days. with no reported clinical effects. They did not report electroencephalographic data. In a milder restriction in which rats were fed 60% as much as controls for up to 40 weeks there were no significant differences in the pyridoxal phosphate or pyridoxamine phosphate content of brain (Wei 1999).

Sakurai et al. (1980b) examined the interactions between vitamin B-6 and penicillamine, thiosemicarbazide and semicarbazide in mouse brain. Doses of 2000 µmol penicillamine/kg, 165 µmol thiosemicarbazide or 1790 µmol semicarbazide/kg were lethal. Simultaneous subcutaneous administration of 250 µmol pyridoxine, pyridoxal or pyridoxamine/kg prevented both seizures and death from penicillamine or thiosemicarbazide but did not protect against semicarbazide. All three drugs reduced brain pyridoxal phosphate 20–50% from the control value of about 10 nmol/g. This decrease following penicillamine or thiosemicarbazide was prevented by administration of 250 µmol pyridoxine, pyridoxal or pyridoxamine/kg. The authors do not speculate about why the B-6 vitamers did not prevent the effects of semicarbazide. Intramuscular administration of pyridoxine or pyridoxamine alone did not increase pyridoxal phosphate above the baseline value of about 10 nmol/g. Administration of pyridoxal caused a slight decrease in pyridoxal phosphate during the peak pyridoxal concentration, possibly indicating substrate inhibition. Pyridoxamine phosphate remained about 14 nmol/g in all treatments suggesting that pyridoxamine phosphate is not in rapid equilibrium with pyridoxal phosphate and cannot compensate for a rapid decrease in pyridoxal phosphate. Peak brain content of pyridoxal was about 27, 8 and 2 nmol/g following intramuscular administration of 250 µmol/kg of pyridoxal, pyridoxine and pyridoxamine, respectively. Peak content of pyridoxamine was less than 2 nmol/g with each vitamer. Pyridoxamine was the only vitamer which increased pyridoxamine phosphate. Even though pyridoxamine alone produced very little change from the baseline vitamin B-6 content of the brain, it was just as effective as pyridoxine and pyridoxal in preventing the decrease in pyridoxal phosphate produced by penicillamine and thiosemicarbazide. It seems likely although there was not a large increase in pyridoxal, there was enough to correct the deficit produced by the drugs. Mizuno et al. (1980) found that the vitamin B-6 analogs which were the most effective competitive substrates for pyridoxal kinase also were most effective in
producing seizures in mice thus demonstrating the importance of pyridoxal phosphate in preventing seizures.

As noted earlier a role for TNAP was suggested by the occurrence of seizures in neonatal hypophosphatasia. This was confirmed in knockout mice lacking TNAP which died from seizures (Waymire et al. 1995). Pyridoxal phosphate in cerebrospinal fluid was above the normal range in three infants with hypophosphatasia (Baumgartner-Sigl et al. 2007; Hofmann et al. 2013; Belachew et al. 2013) supporting the hypothesis that low alkaline phosphatase activity restricted uptake of vitamin B-6 by the brain. Waymire et al. (1995) hypothesized that the seizures resulted from decreased concentration of the inhibitory neurotransmitter, gamma-aminobutyrate (GABA). GABA is produced from glutamic acid by glutamate decarboxylase (EC 4.1.1.15), which requires pyridoxal phosphate. After being released into the synaptic cleft GABA is taken up by adjoining astrocytes where it is converted to succinate semialdehyde and glutamate by 4-aminobutyrate-2-oxoglutarate transaminase (EC 2.6.1.19) (Baxter 2003), which also requires pyridoxal phosphate. The glutamate is then converted to glutamine by glutamate-ammonia ligase (EC 6.3.1.2) which is located primarily in the astrocytes (Suarez et al. 2002). The glutamine may return to the neurons for reuse.

Two forms of glutamic acid decarboxylase (EC 4.1.1.15) with complex regulation occur in GABA-ergic neurons (Martin and Rimvall 1993). GAD67 is cytosolic and distributed throughout the cell body. GAD65 is concentrated in the nerve terminal and is reversibly anchored to the membrane of synaptic vesicles. GAD65 in brain accounts for the large fraction of the enzyme which occurs in brain as the apoenzyme thus serving as a reserve supply. GAD67 is more highly saturated with cofactor. ATP stimulates the formation of apoenzyme while inorganic phosphate facilitates formation of the holoenzyme. Elimination of GAD67 results in lethal developmental defects including cleft palate and respiratory failure (Kash et al. 1997). Mice lacking GAD65 appear normal at birth with normal GABA and holo-GAD activity. However, the increase in activity associated with added pyridoxal phosphate is reduced indicating a decrease in total enzyme protein. The mice have increased susceptibility to seizures. Genetic studies in mice led Kash et al. (1997) to conclude that the relative contribution of GABA synthesized by GAD65 is genetically determined and that the resulting seizure activity involves the limbic region. GABA may also have a role in the synaptic integration of newly generated neurons in adult brain (Ge et al. 2006). Sharma and Dakshinamurti (1992) presented a thorough discussion of the electroencephalographic changes associated with vitamin B-6 deficiency. Seizures induced by picrotoxin, pentylenetetrazol or domoic acid in control or vitamin B-6 deficient rats could be prevented by local administration of pyridoxine or GABA in the thalamic VPL region or the hippocampal CA3 region indicating that GABA deficiency was a significant factor in seizure development.

At 10–14 days postnatal brain GABA concentrations were reduced 50 % in TNAP knockout mice compared to normal controls (Waymire et al. 1995). Similarly, in an infant with hypophosphatasia and West syndrome GABA was less than 0.005 nmol/mL in the cerebrospinal fluid (Yamamoto et al. 2004). GABA
concentrations were also low in the cerebrospinal fluid of infants with pyridoxine dependent seizures (Kurlemann et al. 1992) and West syndrome (Kurlemann et al. 1997) prior to treatment with pyridoxine. Initial attempts to rescue the mice with pyridoxine had limited success presumably because the primary metabolic pathway involved phosphorylation followed by oxidation to pyridoxal phosphate. With oral administration this occurs primarily in the liver which releases pyridoxal phosphate into the plasma. Hydrolysis of the resulting plasma pyridoxal phosphate would be limited by the lack of alkaline phosphatase thus reducing the availability of pyridoxal for transfer into the brain. Plasma pyridoxal was reduced 10-fold in the knockout mice. Use of pyridoxal in place of pyridoxine was much more effective since it could be transported directly into the brain. In liver, kidney, heart and muscle of the knockout mice pyridoxal phosphate was 50–60 % of control values. In brain it was only 35 % of control indicating that brain was affected more than other tissues. Pyridoxal phosphate concentrations in the brains of two infants with hypophosphatasia were slightly lower than in a single normal infant (Whyte et al. 1988). Litmanovitz et al. (2002) suggested that the normal concentrations in the infants might reflect higher residual alkaline phosphatase activity. The ratio of pyridoxal phosphate in plasma/cerebrospinal fluid was about 2.5 in normal subjects ranging in age from 0 to 68 years (Shin et al. 1984). Patients with vitamin B-6 related seizures tended to have lower cerebrospinal fluid values and higher plasma values yielding a plasma/cerebrospinal fluid ratio of 21. It is interesting to note that an infant with hypophosphatasia and pyridoxine responsive seizures had a ratio of 36 (Baumgartner-Sigl et al. 2007) even though the concentration of pyridoxal phosphate in the spinal fluid was 57 nmol/L compared with their cited normal range of 7–39 nmol/L (Shin et al. 1984). This suggests that the ratio may be a more reliable marker of potential seizures than the cerebrospinal fluid value alone. Baumgartner-Sigl et al. (2007) noted that all reported cases of hypophosphatasia with neonatal seizures died by 18 months of age. Di Mauro et al. (2002) found that various TNAP mutations differed in their ability to hydrolyze pyridoxal phosphate. This could help to explain the variable occurrence of seizures in hypophosphatasia.

While vitamin B-6 deficiency can cause seizures, excesses are also neurotoxic. Pyridoxal phosphate causes seizures in immature mice by blocking GABA receptors (Ishioka et al. 1995). The authors suggested the immature blood brain barrier allowed passage of pyridoxal phosphate. Such leakage does not appear to occur in hypophosphatasia. High doses of pyridoxine cause neuropathy in rats (Callizot et al. 2001), dogs (Chung et al. 2008) and humans (Gdynia et al. 2008). There is one case report of pyridoxine responsive seizures in an infant whose mother consumed 80 mg pyridoxine/d during her pregnancy (South 1999b). However, the causal relationship was questioned (Baxter and Aicardi 1999; South 1999a). It is interesting that while pyridoxamine has been proposed as a treatment for the side effects of diabetes produced by advanced glycation end products (Chen and Francis 2012), pyridoxine intoxication has been recommended as a model for diabetic sensory neuropathy (Callizot et al. 2001).
11.7 Other Aspects of Vitamin B-6 Metabolism in Brain

While reduced synthesis of GABA resulting from decreased intracellular availability of pyridoxal phosphate appears to be a major factor in the seizures observed in neonatal hypophosphatasia, it is appropriate to be aware of several additional aspects of vitamin B-6 metabolism in brain. Two good summaries of earlier work are available (Ebadi and Costa 1972; Ebadi 1981). Ebadi (1981) noted that vitamin B-6 is involved in the synthesis or metabolism of multiple neurotransmitters in addition to GABA including dopamine, norepinephrine, serotonin, tyramine, tryptamine, taurine, histamine and even acetyl choline indirectly. He also noted that vitamin B-6 is involved in the synthesis of growth hormone, follicle-stimulating hormone, luteinizing hormone in the brain as well as aldosterone, glucagon, cortisol, estradiol, testosterone and epinephrine in other tissues. The following discussion will focus on information that is more recent or particularly relevant to the focus of this book.

11.7.1 Additional Regulatory Mechanisms

In the commentary following the 1981 review (Ebadi 1981) Metzler (1981) discusses the complexities of vitamin B-6 chemistry and potential interactions. Pyridoxamine phosphate is usually bound to enzymes less tightly than pyridoxal phosphate. Glutamate decarboxylase in brain is rapidly deactivated in the presence of glutamate (Miller et al. 1978; Seligmann et al. 1978). Metzler suggests that this could be the result of a side reaction involving decarboxylative transamination which would convert pyridoxal phosphate to pyridoxamine phosphate followed by dissociation of the cofactor from the enzyme. Reactivation of the enzyme would depend on the availability of pyridoxal phosphate which in turn would depend at least in part on oxidation of pyridoxamine phosphate back to pyridoxal phosphate. Metzler also notes that the apoenzymes are often degraded more rapidly than the holoenzyme. Therefore, the interactions between pyridoxal phosphate and pyridoxamine phosphate could have some complex regulatory functions. ATP and ADP at concentrations comparable to intracellular values inhibit the association of pyridoxal phosphate with glutamate dehydrogenase raising the possibility that decarboxylase activity might be influenced by energy metabolism (Seligmann et al. 1978). Glucose deprivation of cultured hippocampal cells causes reduced ATP, increased extracellular glutamate with NMDA receptor activation and release of lactic dehydrogenase indicating cell death (Geng et al. 1997). Pyridoxal phosphate prevented these changes by increasing pyruvate presumably through increased alanine aminotransferase activity. Pyridoxal phosphate also inhibits the citrate transporter thus keeping citrate in the mitochondria where it can be used to generate ATP. Maintaining adequate ATP prevented the other consequences of glucose deprivation. Based on these observations Geng et al. (1997) suggested that pyridoxal phosphate treatment might help to minimize neuronal death in some metabolic disorders.
11.7.2 Developmental Effects

There is a variety of evidence suggesting that limiting the vitamin B-6 supply in the brain can have effects in addition to reduced production of GABA. In studies in knockout mice lacking alkaline phosphatase Narisawa et al. (2001) found that pyridoxal treatment improved the appearance of lumbar nerve roots. Creation of a dietary vitamin B-6 deficiency in normal mice also caused seizures and thinning of nerve roots. Vitamin B-6 deficiency reduces synthesis of sphingolipids and interferes with formation of myelin (Stephens and Dakshinamurti 1975) raising the possibility that limited vitamin B-6 in neonatal hypophosphatasia could have developmental effects in addition to causing seizures. Narisawa et al. (2001) cited unpublished results showing that TNAP is produced in the neonatal mouse intestine until it is replaced by the intestinal isoenzyme around post-natal day 14–15 suggesting that TNAP knockout mice might have reduced absorption of pyridoxal phosphate from milk. We could not locate any data on the pyridoxal phosphate content of mouse milk. There is an inverse relationship between alkaline phosphatase activity and the pyridoxal phosphate content of milk in rats, goats, pigs, and cows (Coburn et al. 1992) indicating that these species release significant amounts of pyridoxal phosphate into the milk. However, human milk was low in both alkaline phosphatase activity and pyridoxal phosphate suggesting that very little pyridoxal phosphate is released into the milk.

Analysis of cerebrospinal fluid in infants with hypophosphatasia detected reduced 5-hydroxyindoleacetic acid and homovanillic acid in two out of four cases (Balasubramaniam et al. 2010; Hofmann et al. 2013; Belachew et al. 2013). 3-O-methyldopa and 5-hydroxytryptophan were elevated in the only cases where they were measured (Baumgartner-Sigl et al. 2007; Balasubramaniam et al. 2010). These data suggest that multiple vitamin B-6 pathways in the brain are probably altered in hypophosphatasia.

There has been some interesting work on the role of vitamin B-6 during development in rats. When female rats were fed a diet containing 0.7 mg pyridoxine hydrochloride/kg during gestation and lactation, the pups exhibited seizures from day 12 to day 18 after which the seizures stop (Guilarte 1989). The same diet failed to produce seizures in adult rats. At day 14 the total vitamin B-6 content of most brain areas in low intake animals was about 40% of controls. However, by day 56 most areas had increased to 60% of controls with substantia nigra and cerebellum exceeding 80% of control values even though the rats remained on the low intake diet. GABA was significantly below control values in all brain regions at both 14 and 28 days even though there were no seizures at 28 days. By 56 days only the pons/medulla region was significantly below control GABA levels. These data suggest that decreased GABA concentrations may be a necessary but not sufficient factor in causing seizures. Glutamate was significantly reduced in the cortex, hippocampus, substantia nigra and pons/medulla at 14 days but not at 28 days. Glycine was significantly elevated in the hippocampus and caudate/putamen areas at 14 days but not at 28 days. Taurine was significantly reduced in the cortex,
caudate/putamen, and substantia nigra at 14 days but not at 28 days. Interactions between these and other metabolites may interact with GABA to generate seizures.

When dams and pups were fed a diet containing 0.6 mg pyridoxine hydrochloride/kg, pyridoxal phosphate content of various brain regions at 7 days postnatal was 66–90% of control values (Groziak et al. 1984). By day 15 the values declined to 15–33% of controls after which they gradually increased. In control animals pyridoxal phosphate concentration approximately doubled during day 7–15. Brain weight increased by about 80% in both groups. Therefore, total brain pyridoxal phosphate in the controls increased almost 4 times from day 7 to day 15. However, total pyridoxal phosphate in the 0.6 group remained essentially constant. This suggests that inability to supply adequate vitamin B-6 to new tissue resulted in a decline in vitamin B-6 content which may have contributed to the seizures Guilarte (1989) observed from 12 to 18 days. Pyridoxal kinase activity in rat brain increases about three times from birth to day 15 (Ebadi 1972). This increase may have allowed the tissues to accumulate sufficient vitamin B-6 to eliminate the seizures after 18 days even with limited vitamin B-6 intake. Four additional reports (Loo 1972; Bayoumi and Smith 1976; Bhagavan et al. 1977; Guilarte 1989) also found increasing pyridoxal phosphate in postnatal rat brain making it difficult to reconcile the data from Ebadi (1972) showing a marked decrease over the same period.

Pyridoxal kinase activity in human brain increases about 20-fold from 6 to 11 weeks of gestation (Bukin and Ivanova 1976). Activity in the medulla oblongata was about twice whole brain activity by 8 weeks and that difference was maintained into the second trimester. Activity in the medulla changed little from the second to third trimester while other brain areas increased to match or surpass it. By the second trimester activity in the hippocampus was also significantly higher than in the frontal lobe. By the third trimester activity in most brain areas had increased to eliminate these differences. However, activity in the cerebellar cortex was significantly below the frontal lobe. In bovine brain the cerebellar medulla rather than the cortex had low kinase activity (Table 11.3).

Although some reports (Bayoumi et al. 1972; Ruth and Morgan 1986) did not observe seizures in vitamin B-6 deficient adult rats, Sharma and Dakshinamurti

| Table 11.3 Pyridoxal kinase content of bovine brain (McCormick et al. 1961) |
|----------------|------------------|
| Tissue        | Activity (nmol/h·g) |
| Cerebrum      |                   |
| Hemisphere    | 1620              |
| Diencephalon  | 1184              |
| Mesencephalon | 1008              |
| Cerebellum    |                   |
| Cortex        | 1368              |
| Medulla       | 235               |
| Brain stem    |                   |
| Pons          | 436               |
| Medulla oblongata | 364     |
(1992) detected electroencephalographic changes indicating that seizures would likely have developed if the deficiencies were extended. Based on these observations the current author suggests that the decreased susceptibility to seizures in adult rats is influenced by the slower rate of growth and the severity of the deficiency.

11.7.3 Pyridoxamine Phosphate

As noted earlier pyridoxamine phosphate is a significant component of most tissues other than muscle. Perhaps the most extreme example is in the neocortex of 30 days old rat pups in which pyridoxamine phosphate was 4–5 times higher than pyridoxal phosphate (Groziak and Kirksey 1987). In mouse brain most of the pyridoxamine phosphate was in the mitochondria while pyridoxal phosphate was found primarily in the cytosol as was most of the pyridoxal kinase activity (Rott et al. 1960). Pyridoxal phosphatase activity was mainly in the mitochondria. Lyon et al. (1962) found that 75% of the pyridoxamine phosphate in mouse brain was associated with the mitochondria. When I-strain mice, which are susceptible to audiogenic seizures, received a vitamin B-6 deficient diet, pyridoxal phosphate in the brain declined but pyridoxamine phosphate remained constant. Lyon et al. concluded that pyridoxamine phosphate was not serving as a storage form since it was not released during vitamin B-6 deficiency. Similarly, after growing, pair-fed rats received a diet completely lacking vitamin B-6 for 2 weeks total brain B-6 declined about 26% (Sampson and O’Connor 1989). However, pyridoxal phosphate declined about 46% while pyridoxamine phosphate declined only 13%. In the brains of TNAP deficient knockout mice pyridoxal phosphate was decreased to 35% of control values while pyridoxamine phosphate was 65% of controls (Waymire et al. 1995). While pyridoxamine phosphate may be more resistant to depletion, when rats were exposed to a low vitamin B-6 diet throughout gestation and lactation, all B-6 vitamers were significantly reduced in the neocortex at 30 days postnatal (Groziak and Kirksey 1987). Since tracer studies in mice indicated very limited production of pyridoxamine, Colombini and McCoy (1970) concluded that pyridoxamine phosphate was produced primarily from pyridoxal phosphate.

In Guinea pig cortex the major B-6 vitamers were pyridoxamine phosphate (7.5 nmol/g), pyridoxal phosphate (4.75 nmol/g) and pyridoxal (1.4 nmol/g) (Loo and Mack 1971). The subcellular distribution for pyridoxamine phosphate was nucleus (18.1%), crude mitochondria (63.4%) and supernatant (18.5%) while pyridoxal phosphate was nucleus (12.4%), mitochondria 39.1% and supernatant 48.5%. Fractionation of the crude mitochondria indicated that both pyridoxamine phosphate and pyridoxal phosphate were divided about equally between the synaptosomes and the extra terminal mitochondria. In the synaptosomal organelles pyridoxamine phosphate (2.83 nmol/g) was detected primarily in the cytoplasm (19.6%), presynaptic membranes (29.3%) and the mitochondria (47.3%) while pyridoxal phosphate (2.40 nmol/g) was distributed in the cytoplasm (41.6%), presynaptic membranes (12.0%) and mitochondria (41.6%). Rat tissue gave
similar results. Loo et al. (1971) suggested that the occurrence of pyridoxamine phosphate and pyridoxal phosphate in the membranes might indicate a carrier function for amino and keto acids. Sakurai et al. (1980a) provided a detailed examination of the subcellular distribution of vitamin B-6 in mouse brain (Table 11.4). Using microbiological analysis pyridoxal phosphate was the dominant vitamer in whole brain. In the crude mitochondrial fraction pyridoxamine phosphate was highest in the extraterminal mitochondria while in the synaptosomal fraction it was higher in the synaptoplasma than in the intraterminal mitochondria. Although pyridoxine phosphate concentrations were no more than 5% of pyridoxal phosphate or pyridoxamine phosphate, the fact that pyridoxine phosphate concentrations were highest in the myelin and synaptic vesicle fractions led Sakurai et al. to suggest that pyridoxine phosphate may have a role in nervous system conduction.

### Table 11.4  Subcellular distribution of vitamin B-6 in mouse brain (Sakurai et al. 1980a)

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Protein (mg/g brain)</th>
<th>PL (pmol/mg protein)</th>
<th>PLP (pmol/mg protein)</th>
<th>PM (pmol/mg protein)</th>
<th>PMP (pmol/mg protein)</th>
<th>PN (pmol/mg protein)</th>
<th>PNP (pmol/mg protein)</th>
</tr>
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<tbody>
<tr>
<td>Whole brain</td>
<td>122</td>
<td>5.26</td>
<td>80.7</td>
<td>0.638</td>
<td>60.6</td>
<td>0.091</td>
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<tr>
<td>Nucleus</td>
<td>43.2</td>
<td>3.87</td>
<td>60.9</td>
<td>0.484</td>
<td>41.2</td>
<td>0.079</td>
<td>0.371</td>
</tr>
<tr>
<td>Crude mitochondria</td>
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<td>5.67</td>
<td>104.7</td>
<td>0.619</td>
<td>62.9</td>
<td>0.096</td>
<td>0.413</td>
</tr>
<tr>
<td>Microsomes</td>
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<td>6.73</td>
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<td>27.0</td>
<td>0.194</td>
<td>0.634</td>
</tr>
<tr>
<td>Cytosol</td>
<td>22.6</td>
<td>23.68</td>
<td>128.3</td>
<td>3.17</td>
<td>42.2</td>
<td>0.251</td>
<td>0.866</td>
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**Fractionation of crude mitochondria**

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<tr>
<th></th>
<th>PL (pmol/mg protein)</th>
<th>PLP (pmol/mg protein)</th>
<th>PM (pmol/mg protein)</th>
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<td>Myelin</td>
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<td>1.53</td>
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<td>Extraterminal</td>
<td>11.36</td>
<td>6.06</td>
<td>129.6</td>
<td>3.82</td>
<td>129.8</td>
<td>0.173</td>
</tr>
</tbody>
</table>

**Fractionation of synaptosomes**

<table>
<thead>
<tr>
<th></th>
<th>PL (pmol/mg protein)</th>
<th>PLP (pmol/mg protein)</th>
<th>PM (pmol/mg protein)</th>
<th>PMP (pmol/mg protein)</th>
<th>PN (pmol/mg protein)</th>
<th>PNP (pmol/mg protein)</th>
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</thead>
<tbody>
<tr>
<td>Synaptoplasma</td>
<td>2.89</td>
<td>48.2</td>
<td>172</td>
<td>4.61</td>
<td>147</td>
<td>0.966</td>
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<tr>
<td>Synaptic vesicles</td>
<td>1.21</td>
<td>9.77</td>
<td>53</td>
<td>14.7</td>
<td>67.7</td>
<td>1.43</td>
</tr>
<tr>
<td>Intraterminal</td>
<td>5.35</td>
<td>3.65</td>
<td>105</td>
<td>2.72</td>
<td>75.6</td>
<td>0.241</td>
</tr>
</tbody>
</table>

Abbreviations for vitamers are listed in Table 11.1

### 11.7.4 Phosphoethanolamine Metabolism

One of the consequences of hypophosphatasia is increased phosphoethanolamine in serum and/or urine. Phosphoethanolamine excretion can be influenced by vitamin B-6 as well as alkaline phosphatase. Ethanolamine-phosphate phospho-lyase (EC 4.2.3.2) utilizes pyridoxal phosphate to convert phosphoethanolamine to acetaldehyde, ammonia and inorganic phosphate (Fleshood and Pitot 1970). Enzyme isolated from rabbit liver had an optimal pH of 7.8, Km of 0.61 mM for
phosphoethanolamine, $K_m$ of 0.27 μM for pyridoxal phosphate and $K_i$ of 1.3 mM for inorganic phosphate. Rats fed a high protein, vitamin B-6 deficient diet developed osteoporotic changes and markedly increased excretion of phosphoethanolamine with no changes in serum alkaline phosphatase activity (Benke et al. 1972). Recently, AGXT2L1, a pyridoxal phosphate brain protein related to alanine glyoxylate aminotransferase, has been shown to be an ethanolamine-phosphate phospho-lyase (Veiga-da-Cunha et al. 2012; Schiroli et al. 2013). AGXT2L1 was the most consistently upregulated gene in the brains of deceased patients with schizophrenia or bipolar disorder (Shao and Vawter 2008) and was the most upregulated gene in mouse brain after treatment with lithium (McQuillin et al. 2007). This is an example of an enzyme from a generalist family [class II aminotransferases (Mehta and Christen 2000)] which has become highly specific. Nam et al. (2012) noted that specialist enzymes are frequently essential, maintain higher metabolic flux, and require more regulation to control metabolic flux in dynamic environments. Schiroli et al. (2013) suggested that the need for strict regulation in the case of AGXT2L1 might result from the fact that the reaction is irreversible and generates potentially toxic products. They noted that phosphoethanolamine is a product of sphingolipid degradation and a substrate for glycerophospholipid biosynthesis. Ethanolamine and phosphoethanolamine can interfere with mitochondrial function (Modica-Napolitano and Renshaw 2004). The fact that AGXT2L1 requires pyridoxal phosphate raises the possibility that the increased phosphoethanolamine observed in hypophosphatasia could be exacerbated by low vitamin B-6 in addition to the lack of alkaline phosphatase particularly in infants where seizures suggest the existence of vitamin B-6 deficiency. We are unaware of any data on AGXT2L1 activity in hypophosphatasia. Although alterations in phosphoethanolamine metabolism have been associated with neuropsychiatric disorders (Miller et al. 2012) and most of the previous references to AGXT2L1 deal with such disorders, there is little evidence that neuropsychiatric problems are increased in hypophosphatasia. Since signs of vitamin B-6 deficiency in hypophosphatasia seem to be limited to infants, the duration of any reductions in AGXT2L1 might be too short to cause neuropsychiatric problems in surviving infants. The Uniprot data base (http://www.uniprot.org/uniprot/?query=ethanolamine-phosphate+phospho-lyase&sort=score) contains 5 reviewed entries for AGXT2L1 from human, mouse, bovine, zebrafish and African clawed frog all indicating the subcellular location as mitochondrion. Sia et al. (1975) reported that pyridoxine supplementation reduced phosphoethanolamine excretion in the parents of an infant with neonatal hypophosphatasia by 25–69 %. Administration of pyridoxine to the infant reduced the seizures but did not reduce phosphoethanolamine excretion. Prior to pyridoxine therapy neither the infant nor the parents had detectable degradation of phosphoethanolamine in serum. In vitro degradation of phosphoethanolamine was maximally stimulated by addition of $10^{-5}$ M pyridoxal phosphate. Control serum required $10^{-3}$ M to obtain a comparable effect. Addition of pyridoxal phosphate had no effect on the degradation of p-nitrophenol-phosphate. Based on these results Sia et al. (1975) recommended further study of the benefits of pyridoxine supplementation in hypophosphatasia.
11.7.5 Inflammation

It has been suggested that inflammation increases the demand for vitamin B-6 from plasma for increased metabolism of tryptophan to anthranilic acid and 3-hydroxyanthranilic acid (Paul et al. 2013; Reginaldo et al. 2013). A study of inflammation in hypophosphatasia showed that calcium pyrophosphate but not pyridoxal phosphate increased prostaglandin E2 synthesis in cultured skin and dura mater cells (Girschick et al. 2006). The authors concluded that high pyridoxal phosphate in hypophosphatasia did not cause inflammation. However, their data show that prostaglandin production by hypophosphatasia tissues in the presence of calcium pyrophosphate tended to be slightly lower when pyridoxal phosphate was administered with calcium pyrophosphate. This raises the possibility that the pyridoxal phosphate may have reduced rather than enhanced the response. Since the hypophosphatasia tissues presumably have limited ability to hydrolyze pyridoxal phosphate, it would be interesting to repeat the studies using pyridoxal in place of pyridoxal phosphate to determine whether increased availability of pyridoxal would reduce the inflammatory response.

11.7.6 Long Term Potentiation

Vitamin B-6 appears to be associated with long-term potentiation which in turn is associated with learning and memory. Immunoreactivity of pyridoxal 5′-phosphate synthase but not pyridoxal kinase was increased in the dentate gyrus of rats after induction of long-term potentiation (Kwak et al. 2009). Injection of pyridoxine, pyridoxal or pyridoxamine had no effect on development of long term potentiation in rat brain. Injecting pyridoxal phosphate produced multiple population spikes which were attributed to disruption of GABA_A receptor mediated inhibition. Transduction of Tat-pyridoxal kinase did not affect induction of long-term potentiation. However, transduction of Tat-pyridoxal 5′-phosphate synthase increased the efficiency of long-term potentiation induction. The authors concluded that because Tat-pyridoxal 5′-phosphate synthase injection did not affect fast or late paired-pulse inhibitions, the upregulation of pyridoxal 5′-phosphate synthase may not be related to GABAergic inhibition. They did not report any measurements of pyridoxal phosphate concentrations. Serine racemase (EC 5.1.1.18) provides D-serine for the N-methyl-D-aspartate receptor which mediates neurotransmission, synaptic plasticity, cell migration and long term potentiation. Altered D-serine concentrations are associated with neuropathologies, aging deficits, and disorders including schizophrenia, amyotrophic lateral sclerosis, Alzheimer’s disease and stroke (Campanini et al. 2013). The involvement of pyridoxal phosphatase/chronophin was mentioned earlier. It is interesting that alkaline phosphatase activity has also been associated with activity dependent functions in the cortex (Fonta et al. 2004).
11.7.7 Homocysteine

Vitamin B-6 is also related to homocysteine metabolism. A genome-wide assessment of the relationship between vitamin B-6, folate, vitamin B-12 and homocysteine detected a significant relationship between the C allele of the ALPL gene and reduced vitamin B-6 (Tanaka et al. 2009). Recent results showed that supplementation with folate, vitamin B-6 and vitamin B-12 reduced cerebral atrophy in gray matter in elderly subjects with increased dementia risk and elevated homocysteine (Douaud et al. 2013).

11.7.8 Lysosomal Enzymes

Vitamin B-6 deficiency increased lysosomal enzymes in rat brain (DiPaolo et al. 1975). Faber et al. (1991) suggested that vitamin B-6 might be involved in the synthesis of mannose phosphate marker or receptor proteins on lysosomes which dictate the disposition of lysosomal enzymes.

11.8 Pyridoxal Phosphate and Purinoceptors

The interaction between alkaline phosphatase and purinoceptors is discussed elsewhere in this book (See Chaps. 4 and 13). Pyridoxalphosphate-6-azophenyl-2′,4′-disulphonic acid is widely used as a purinoceptor antagonist raising the possibility that there might be some physiological interactions between purinoceptors and pyridoxal phosphate. In discussing metabolic errors in vitamin B-6 metabolism Surtees (2006) asked whether pyridoxal phosphate might have a noncofactor role, possibly interacting with ion channels or pumps. Trezise et al. (1994) concluded that pyridoxal phosphate acts as a specific inhibitor of P2 purinoceptors in the vagus nerve and vas deferens of rats. Pyridoxal phosphate had an effect at concentrations equal to those of α, β-methylATP. Wang et al. (1999) found that pyridoxal phosphate was an ATP receptor antagonist in rat cardiomyocytes. Binding of 10 μM ATP-S to low affinity binding sites was significantly reduced by 1 μM pyridoxal phosphate. Wang et al. (1999) concluded that since normal plasma concentrations of pyridoxal phosphate range from 24 to 81 nM, pyridoxal phosphate probably would not function as an inhibitor under physiological conditions. However, they also noted that the higher ATP concentrations that were needed in the intact heart and cardiomyocytes than in the sarcolemmal membranes might result from ATP hydrolysis thus suggesting that the effective concentration of ATP might be lower than the administered amount. P2X2 and P2X2/3 receptors expressed in HEK293 cells were inhibited by pyridoxal phosphate with an IC50 of 7 and 13 μM, respectively (Theriault et al. 2014). In a phase 2 clinical trial pyridoxal phosphate (250 mg
or 750 mg/d) was administered orally beginning 3 to 10 h prior to coronary bypass
graft surgery and continuing for 30 days (Tardif et al. 2007). The 250 mg treatment
did not significantly affect the prespecified primary end point but was associated
with a significant reduction in perioperative myocardial infarction with creatine
kinase-myocardial band ≥100 ng/mL. The 250 mg dose was more effective than
750 mg. An editorial discussing those results suggested that improved efficacy may
require targeting multiple receptors or perhaps a central control point in the relevant
metabolic pathway (Verrier 2007). Pyridoxal phosphate may also interact with
calcium channels (Dakshinamurti et al. 1998). Microinjection of pyridoxal phos-
phate causes seizures which were attributed to extracellular pyridoxal phosphate
interacting with GABA<sub>A</sub> receptor (Salazar and Tapia 2001).

11.9 Hyperphosphatasia

Considering the marked elevation of plasma pyridoxal phosphate in hypophos-
phatasia, one might expect markedly reduced concentrations in hyperphosphatasia
resulting from a defect in the biosynthesis of the phosphatidylinositol glycan anchor
which attaches the enzyme to the membrane (Thompson et al. 2012) (see also
Chap. 16). However, that does not appear to be the case. Thompson et al. (2010)
reported only one out five patients with low pyridoxal phosphate and pyridoxine
responsive seizures. That patient had the lowest relative phosphatase activity of any
of the patients. Hydrolysis of pyridoxal phosphate was increased in plasma of
subjects with increased alkaline phosphatase activity due to liver or bone disorders
(Anderson et al. 1980). There was a negative correlation between endogenous
plasma pyridoxal phosphate and alkaline phosphatase activity (r = −0.893).
Increased hydrolysis of plasma pyridoxal phosphate is not necessarily a problem
unless it increases the net loss of vitamin B-6 from the body. Additional data will be
needed to clarify vitamin B-6 metabolism in hyperphosphatasia.

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