MINIREVIEW

Cellular Assimilation of Water-Soluble Vitamins in the Mammal: Riboflavin, B_6, Biotin, and C (43534B)

DONALD B. MCCORMICK[1] AND ZUOMIN ZHANG
Department of Biochemistry, School of Medicine, Emory University, Atlanta, Georgia 30322

The discovery of vitamins resulted from the searches for the cures of diseases caused by diets deficient in such micronutrients. The further investigation of specific roles has clarified most of the metabolic alterations that are necessary to convert vitamins to functional forms, which include conversions of water-soluble B complex vitamins to coenzymes. More recent work is providing information on how these metabolic conversions are regulated. Importantly different cells have somewhat different means for facilitating uptake, often with relative specificity for any one of the different structural groups of vitamins. Closure of the knowledge gap as to how vitamins are transported into the cell and how processing of vitamins within the cell are connectedly related is the thrust of ongoing studies. Our own work in this area will be the primary base for this Minireview.

Since such vitamins as riboflavin, B_6, biotin, and C have been the focus of considerable investigation in our laboratory, they will serve as primary examples to make both detailed statements as well as comparisons that often apply broadly. In particular, we have studied the importing of these vitamins into the parenchymal liver cells and proximal tubular kidney cells from the rat as a convenient animal model, because less was known about vitamin uptake in these tissues than in the small intestine. One interesting possibility for future application of some of the knowledge gained may be to piggyback a bioactive compound, e.g., drug or signal transducer, onto a transported solute, e.g., vitamin or other nutrient, that gains facilitated entry into a cell and is, thereafter, metabolized to release the active compound.

[1] To whom requests for reprints should be addressed at Department of Biochemistry, 4001 Rollins Research Center, Emory University, Atlanta, GA 30322.

P.S.E.B.M. 1993, 202: 265–270

0037-9727/93/2023-0265$3.00/0

Cellular Uptake

In general, the subject of transport through plasma membranes of cells has progressed from a classic physiologic description to the identification of mechanisms and components of specific transporters at the biophysical and biochemical level (1). It seems appropriate to be reminded of those mechanisms that apply to vitamins as well as most nutrients and metabolites before distinguishing particular examples.

With many transportable biomolecules, one process that becomes evident at higher concentrations is nonmediated transport or simple diffusion. This process, often of more pharmacologic than physiologic meaning, is a linear function of the difference in concentration of a solute on the two sides of the membrane. This is governed by the Nernst-Planck equation relating flux to mobility and electrochemical potential gradient. For uncharged molecules, the relationship simplifies to Fick's first law of diffusion, which states that a substance diffuses in the direction that eliminates its concentration gradient at a rate proportional to the magnitude of this gradient.

Of major importance within the range of physiologic concentrations of transported molecules, including the vitamins, is the process of mediated transport, which occurs through the action of specific "carrier" proteins, e.g., transporters. One type frequently encountered is passive-mediated or facilitated diffusion. In this type of transport, a solute again flows from higher to

lower concentration as from one side of the membrane to the other. The second type of mediated process to be encountered is ATP-driven active transport, in which specific molecules are transported against a gradient from low to high concentration. Among characteristics that distinguish both types of mediated transport processes, and differentiate them from nonmediated diffusion, are the greater speed, relative specificity, and saturation kinetics. The permeability coefficient of a molecule that gains facilitated entry to a cell is expectedly much greater than that of the same molecule with a flux rate dependent only on diffusion. The initial rate of uptake in a mediated process is greater than one of passive diffusion. The aspect of specificity is of importance in that a significant number of different transport proteins decrease the chance for unwanted competition between entering solutes that are essential for the cell. Yet one reflection of the relative specificity is the susceptibility to competitive inhibition by similar structures. The common finding of saturation kinetics for mediated processes is evidence for a finite number of sites (transporters) involved.

Regarding water-soluble vitamins, an examination of suitable ranges of concentration in comparison with initial uptake rate usually reveals both the mediated, saturable transport, reflected by nonlinear behavior predominant at the lower physiologic level, and the nonmediated transport due to simple diffusion, which is evidenced by a linear continuation at higher pharmacologic vitamin level. Examples of this can be seen by the uptake of riboflavin (Fig. 1) and L-ascorbate (Fig. 2) by epithelial cells isolated from the proximal tubules of rat kidneys. The capacity of the ascorbate transporter to facilitate entry of up to ~150 μmol/liter of this very water-soluble vitamin, which occurs at relatively high

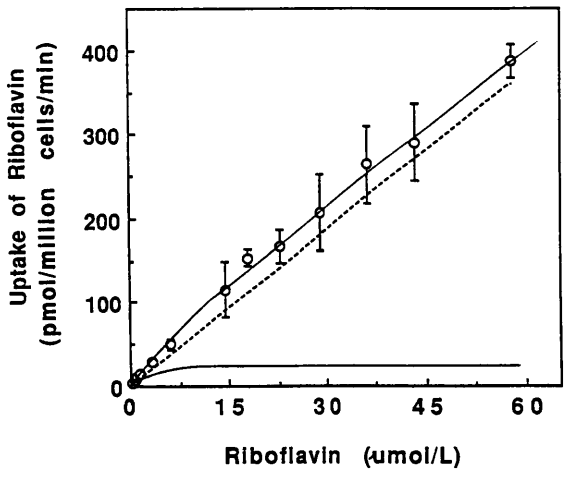

Figure 1. The effect of riboflavin concentration on its initial uptake by proximal tubular cells isolated from rat kidney (2). Results from a membrane-filtration method are the mean ± SE of six separate cell preparations. Subtracting the linear component (dashed line) reflecting diffusion from the overall curve (O) gives the saturable component (solid line) reflecting the mediated, facilitated transport.

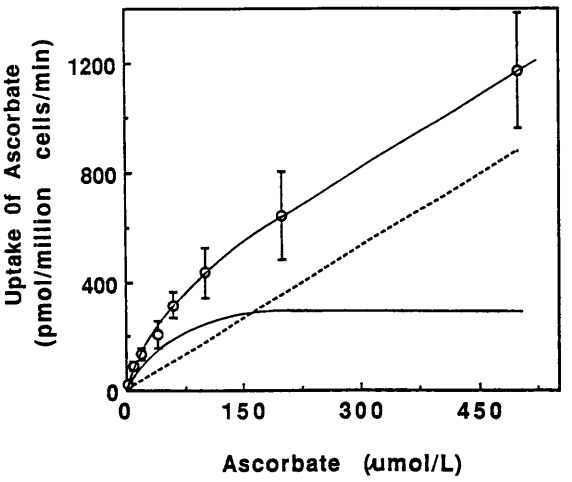

Figure 2. The effect of ascorbate concentration on its initial uptake by proximal tubular cells isolated from rat kidney (3). Results from a Percoll-centrifugation method are the mean ± SE of four separate cell preparations. Subtracting the linear component (dashed line) reflecting diffusion from the overall curve (O) gives the saturable component (solid line) reflecting the mediated, facilitated transport.

physiologic concentration (~100 μmol/liter) in rat plasma (4), considerably exceeds the more limited ability of the riboflavin transporter to facilitate cellular entry of up to ~15 μmol/liter of the less water-soluble flavin, which occurs at a lower plasma concentration (<1 μmol/liter). This correspondence of capacity, reflected by the concentration required to saturate, to the physiologic range of a given vitamin also extends to other cases. For example, hepatic (5) or renal (6) cell uptake of pyridoxine as a common and commercial form of vitamin B_6 exhibits saturation reasonably above the μmol/liter range, which is approximately the level of total B_6 vitamers found in the plasma of mammalian species (7). With biotin, which occurs in trace levels of 2–20 nmol/liter in plasma (7, 8), mediated uptake in rat hepatocytes may saturate with a few μmol/liter (9).

A summary of the kinetically related constants measured for uptake of selected vitamins in cells from the liver and kidney of rats is given in Table I. With hepatocytes from riboflavin-deficient rats, a higher V_{max} (119 pmol/10^6 cells·min) but similar K_t compared with controls suggests a greater capacity for the deficient cells to accumulate the vitamin (10). At near equilibrium conditions, the riboflavin concentration was 2.5-fold (control cells) and 5.2-fold (deficient cells) greater than the external concentration. With hepatocytes from vitamin B_6-deficient rats, however, both V_{max} (125 pmol/10^6 cells·min) and K_t compared closely with controls where vitamin B_6 concentration was twice that of external medium (5). This lack of adaptive capacity in hepatocytes in regard to B_6 is also seen in biotin deficiency (9); therefore, some, but not all, cases may have compensatory adjustments at the level of liver cell uptake during a given deficiency.

Table I. Kinetic Constants Relating to Uptake of Vitamins into Cells from the Livers and Kidneys of Normal Male Rats

Vitamin	Liver			Kidney		
	v_o[a]	V_{max}[a]	K_t[b]	v_o[a]	V_{max}[a]	K_t[b]
Riboflavin	13 (10)	82 (10)	12 (10)	9 (2)	42 (2)	8 (2)
Pyridoxine	2 (5)	106 (5)	28 (5)	6 (6)	14 (6)	1.3 (6)
Biotin	0.02 (9)	0.1 (9)				
L-Ascorbate				17 (3)	310 (3)	36 (3)

[a] Initial (v_o) and maximal (V_{max}) velocities are in pmol/10^6 cells·min; concentrations of vitamins used in incubations to obtain v_o are given in terms of molarity: riboflavin, 3 μM; pyridoxine, 0.5 μM; biotin, 20 nM; L-ascorbate, 50 μM. References to literature are in parentheses.
[b] The transport constant (K_t) is equivalent to K_m and is in μmol/liter. References to the literature are in parentheses.

Clearly there are rate-related differences in uptake for different vitamins for a given cell as well as for the same vitamin with different cells. The mediated types of mechanisms that are operable may be passive or active. For example, the proximal tubular renal cell is epithelial in nature and, based on ouabain sensitivity and ion replacement, a secondary active transport system coupled to Na^+ cotransport seems involved in the uptake of L-ascorbate (3). With uptake of pyridoxine, this renal cell (6) and vesicles prepared from the brush border membranes (11) similarly exhibit dependency on an Na^+,K^+-ATPase; however, inhibition by amiloride, a competitive inhibitor of Na^+,H^+ exchange, was also found (6). With riboflavin importing by renal cells, there is Na^+ dependency, but insensitivity to ouabain suggests that facilitated entry of this vitamin involves Na^+ cotransport by a means not dependent upon the Na^+,K^+-ATPase (2). For the bipolar hepatocyte, importing of riboflavin (10) or pyridoxine (5) seems not to be driven by Na^+,K^+-ATPase and does not exhibit Na^+ dependency; however, biotin uptake into hepatocytes is similar to other organic acid anion transporters (classic ligandins or glutathione S-transferase B) in that an Na^+ and ATP-dependent process is involved (9).

Some aspects of specificity, an earmark of mediated transport, are summarized in Table II. In all cases, the uptake of radiolabeled vitamin is expectedly decreased competitively by unlabeled vitamin. Alterations in the 10-(1′-D-ribityl) side chain of riboflavin, which in some cases is the result of metabolic action on the vitamin, e.g., the 2′-hydroxyethylflavin, lead to modest inhibitors that compete with entry. Changes in the nature of substituents within the benzenoid or pyrimidinoid portions of the ring system also result in compounds varyingly able to compete with riboflavin for cellular entry. Although pyridoxamine, as one vitaminic form of B_6, competes fairly effectively against pyridoxine for uptake, pyridoxal does not. The 4′-aldehyde form of B_6 reacts both with amines and other functional groups on proteins and with itself to form the cyclic hemiacetal with the 5′-hydroxymethyl substituent. Synthetic analogs with methyl groups replacing the hydroxymethyls at position 4′ and 5′ in pyridoxine are fairly effective competitive inhibitors against B_6 uptake. With analogs of biotin, alteration of the carboxylate terminus less severely affects competitive entry behavior than does extension of side chain length, as in homobiotin or biocytin especially. Analogs that lack either the thiolane ring sulfur, i.e., dethiobiotin, or the ureido carbonyl function, i.e., the diaminocarboxylate, still compete modestly against biotin uptake. Isoascorbic acid, a synthetic stereoisomer of vitamin C, and dehydroascorbic acid, a natural interconvertible form of C, are both able to compete with the uptake of L-ascorbate.

In connection with specificity, it should be noted that this property has already allowed the affinity purification of two B_6-binding proteins from the brush border plasma membranes of renal proximal tubular cells (12). Extension of such techniques should allow isolation of similar protein transporters, such as the riboflavin-binding protein in the plasma membrane of hepatocytes (13).

From all of the preceding, it is clear that water-soluble vitamins enter cells under physiologic conditions by mediated, saturable, and relatively specific means that for some cases, additionally reflect dependency on Na^+. Some broader examples of such comparisons are to be found in fairly general reviews (14, 15).

Cellular Disposition

Upon entry into the cell, most of the water-soluble vitamins are metabolically altered in such a manner as to trap them within the cell. Typically this "metabolic trapping" occurs as a consequence of conversion to coenzymes that are more charged, often anionic, and thereby less able to escape through the relatively hydrophobic plasma membrane. Additionally, the coenzymes combine with appropriate apoenzymic proteins to constitute catalytically active holoenzymes that remain *in situ*. Upon turnover and catabolic action on coenzyme or excess free vitamin, the latter or metabolites can then exit cells.

The above generalized processes are well reflected by considering how vitamin B_6 and riboflavin are metabolically managed upon carrier-mediated entry into a representative mammalian cell, as illustrated in Figure

Table II. Relative Specificities of Cellular Transporters for Some Water-Soluble Vitamins

Vitamin + analog	As percentage of control rate	
	Liver	Kidney
Riboflavin (3 μM)	100 (10)	100 (2)
+ Lumiflavin (23[a] or 15 [b] μM)	66 (10)	86 (2)
+ 2′-Hydroxyethylflavin (3[a] or 15[b] μM)	64 (10)	100 (2)
+ 7,8-Dichlororiboflavin (3[a] or 15[b] μM)	62 (10)	70 (2)
+ N^3-Methylriboflavin (15 μM)		75 (2)
Pyridoxine (0.5[a] or 0.3[b] μM)	100 (5)	100 (6)
+ Pyridoxamine (5 μM)		64 (6)
+ Pyridoxal (5 μM)		102 (6)
+ 5′-Deoxypyridoxal (5 μM)		57 (6)
+ 5′-Deoxypyridoxine (0.5[a] or 5[b] μM)	85 (5)	76 (6)
+ 4′-Deoxypyridoxine (0.5[a] or 5[b] μM)	65 (5)	60 (6)
Biotin (20 nM)	100 (9)	
+ Biotinol (0.2 μM)	51 (9)	
+ Homobiotin (0.2 μM)	75 (9)	
+ Biotin methyl ester (0.2 μM)	49 (9)	
+ Biocytin (0.2 μM)	86 (9)	
+ Dethiobiotin (0.2 μM)	62 (9)	
+ Diaminocarboxylate (0.2 μM)	68 (9)	
L-Ascorbate		
+ Isoascorbate	K_i = 34[c] (3)	
+ Dehydroascorbate	K_i = 13[c] (3)	

[a] Concentration with liver cells.
[b] Concentration with kidney cells.
[c] Competitive inhibitory constant in mmol/liter.
Note. References to the literature are in parentheses.

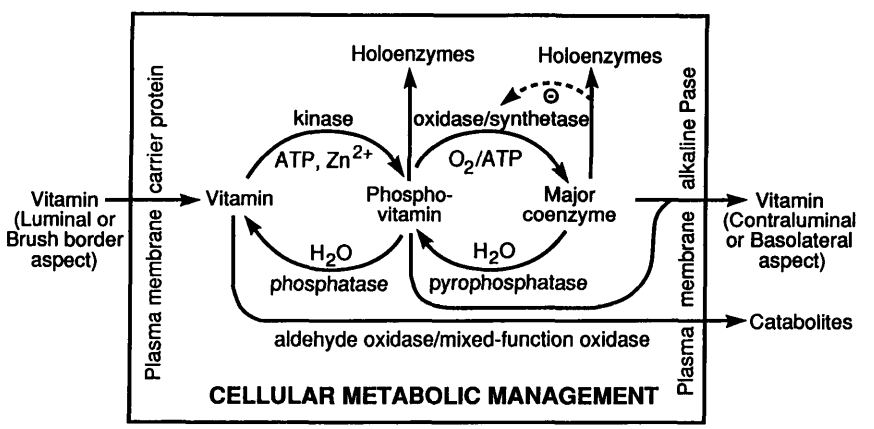

Figure 3. Principal processes in common to disposition of vitamin B_6 and riboflavin by mammalian cells.

3. After transit of these vitamins through the plasma membrane at the brush border side of an epithelial cell, they are metabolically trapped by kinase-catalyzed conversions to the 5′-phosphate esters within the cytoplasm. Both pyridoxal kinase (16, 17) and flavokinase (18, 19) preferentially utilize Zn^{2+} as a 1:1 chelate with ATP in different stereospecific presentations (20, 21) during phosphorylation of the vitamins in mammalian systems. In the case of the vitamin B_6 group, the 5′-phospho forms of pyridoxine and pyridoxamine are oxidized by molecular oxygen to form the coenzyme pyridoxal 5′-phosphate and hydrogen peroxide. The flavin mononucleotide-dependent oxidase responsible for this is not only relatively specific for the 5′-phosphate ester of 4′-substituted B_6 compounds (22–26), but obviously sensitive to riboflavin status (27, 28) and O_2 supply (23, 29). The intrinsic activity of this cytosolic oxidase is affected by supply of substrates but is critically regulated by its product, pyridoxal 5′-phosphate (30, 31). With riboflavin 5′-phosphate, which serves as coenzyme flavin mononucleotide for a modest number of enzymes, most is further converted to flavin adenine dinucleotide (FAD) by a second ATP-requiring step catalyzed by FAD synthetase. This Mg^{2+}-preferring en-

zyme is really not a phosphorylase, since it strongly favors catalysis only in the direction of FAD (32, 33). This product, the predominant flavocoenzyme of most tissues, is also inhibitory to the synthetase and thereby confers regulation (34). Hence, unlike classic biosynthetic pathways where the first committed step is usually the one regulated by feedback inhibition from the endproduct of the pathway, the two-step pathways for formation of pyridoxal 5′-phosphate or FAD are negatively controlled by endproducts at the last (second) step.

The catabolic routing of such coenzymes typically involves hydrolytic release of the vitamin and subsequent oxidative alterations of the fraction of the latter that is not recaptured by the coenzyme biosynthetic machinery. As expected, enzymes involved in catabolic events are localized in membranes and organelles as distinct from the biosynthetic enzymes of the cytoplasmic locus. Again with vitamin B_6 and riboflavin as examples (Fig. 3), nonspecific phosphatases can release pyridoxal (35) and riboflavin (36, 37) from their 5′-phosphocoenzymes. Pyridoxal is ultimately oxidized to 4-pyridoxic acid by aldehyde oxidase (38) and dehydrogenase (39) systems, and a fraction of riboflavin is oxidized in the 7- and 8-methyl functions by the action of mixed function oxidase (40). These and other interconnected relationships of B_6 and riboflavin have been reviewed elsewhere (41).

Similar metabolic management occurs with biotin in that it is trapped by conversion to the 5′-adenylate, which is both utilized to link the coenzymic biotinyl moiety to the ϵ-amino groups within a ocarboxylases and, with excess, converted to the CoA thioester that is catabolically degraded by successive β-oxidative cleavage of the valeryl side chain (42). The events with thiamin, which is pyrophosphorylated to form coenzyme, or folate, which is reduced and polyglutamated, are further examples of the ways in which metabolic trapping interconnects uptake, function, and ultimate catabolism or transfer of vitamins among mammalian cells.

Basic Questions and Application Potential

Now that a larger picture of what occurs with water-soluble vitamins during transport, metabolism, and function has emerged, it will be instructive and potentially useful to examine some of the details to see where further clarity is needed. Too little has been done to elucidate mechanisms involved with exporting vitamins from cells. Less work has been done with the basolateral and plasma membranes essential to this process than with the brush border or luminal-side membrane involved with import. At the molecular level, too little is known about the specific nature and action of membrane proteins participating in transporter function for most cases.

Since there are some qualitative as well as quantitative differences in the way in which different cells assimilate vitamins, the possibility exists for utilizing this knowledge especially as entails specificity. We have already shown that one can piggyback a bioactive compound onto a vitamin as a means for facilitating entry of the compound (43, 44). One has first to determine what substituent function of the given vitamin or other transported nutrient can be chemically altered without too markedly impairing facilitated entry of the vitamin (nutrient). This, of course, is simply a matter of circumscribing the structural specificity tolerated by the transport system. Next, one has to determine what linkage should be used to couple the bioactive compound to be delivered into a given cell with the vitamin (nutrient) that will enhance its entry. The use of a covalent link that can be metabolically unlinked inside the cell is an obvious choice.

The illustration of the above principles with vitamin B_6 was done with cells from kidney (43) and liver (44) utilizing synthetic *N*-(4′-pyridoxyl)-amines. Here it was known that the B_6 transporter would tolerate alterations at the 4′-position of the vitamin, as would both the kinase and oxidase required for internal release of pyridoxal 5′-phosphate and the originally attached amine. An example of a biologically encountered derivative of B_6 that competes with its transporter and is subsequently hydrolyzed within the cell to release free vitamin is the pyridoxine-5′-β-D-glucoside that commonly occurs in plants (45). Other cases for transporter-enhanced delivery and metabolic release of bioactive compounds include the 4-formyl function of pyridoxal with such effectors as neuroactive or signal transducing amines; the 5′-hydroxymethyl group on riboflavin and carboxyl functions of biotin and niacin offers other possible linkage sites for esterification of bioactive compounds. These and other possibilities are being considered.

The synthesis and testing of transporter-enhanced complexes (transplexes) designed to selectively deliver drugs and metabolic modulators to specific types of cells is a concept whose time has come.

1. Voet D, Voet JG. Biochemistry. New York: John Wiley & Sons, pp484–505, 1990.
2. Bowers-Komro DM, McCormick DB. Riboflavin uptake by isolated rat kidney cells. In: Edmondson DE, McCormick DB, Eds. Flavins and Flavoproteins. New York: Walter de Gruyter & Co., pp449–453, 1987.
3. Bowers-Komro DM, McCormick DB. Characterization of ascorbic acid uptake by isolated rat kidney cells. J Nutr **121**:57–64, 1991.
4. Moser U, Bendich A. Vitamin C. In: Machlin LJ, Ed. Handbook of Vitamins, 2nd ed. New York: Marcel Dekker, pp195–232, 1991.
5. Kozik A, McCormick DB. Mechanism of pyridoxine uptake by isolated rat liver cells. Arch Biochem Biophys **229**:187–193, 1984.

6. Bowman BB, McCormick DB. Pyridoxine uptake by rat renal proximal tubular cells. J Nutr **119**:745–749, 1989.
7. Combs GF Jr. The Vitamins. Fundamental Aspects in Nutrition and Health. San Diego: Academic Press, 1992.
8. Ikeda M, Hosotani T, Veda T, Kotake Y, Sakakibara B. Effect of vitamin B_6-deficiency on the levels of several water-soluble vitamins in tissues of germ-free and conventional rats. J Nutr Sci Vitaminol **25**:141–149, 1979.
9. Bowers-Komro DM, McCormick DB. Biotin uptake by isolated rat liver hepatocytes. In: Dakshinamurti K, Bhagavan HN, Eds. Biotin. New York: Ann NY Acad Sci, Vol **447**: pp350–358, 1985.
10. Aw TY, Jones DP, McCormick DB. Uptake of riboflavin by isolated rat liver cells. J Nutr **113**:1249–1254, 1983.
11. Bowman BB, McCormick DB, Smith EE. Vitamin B_6 uptake by rat kidney cells and brush-border membrane vesicles. In: Dakshinamurti K, Ed. Vitamin B_6. New York: Ann NY Acad Sci, Vol **585**: pp106–109, 1990.
12. McCormick DB, Bowers-Komro DM, Bonkovsky JL, Larsen C, Zhang Z. Characteristics of a transporter for uptake of vitamin B_6 into mammalian cells: Isolation of B_6-binding proteins from brush-border membranes of rat renal tubular epithelial cells. In: Fukui T, Kagamiyama H, Soda K, Wada H, Eds. Enzymes Dependent on Pyridoxal Phosphate and Other Carbonyl Compounds as Cofactors. New York: Pergamon Press, pp609–611, 1991.
13. Nokubo M, Ohta M, Kitani K, Zs-Nagy I. Identification of protein-bound riboflavin in rat hepatocyte plasma membrane as a source of autofluorescence. Biochim Biophys Acta **981**:303–308, 1989.
14. Rose RC. Transport of ascorbic acid and other water-soluble vitamins. Biochim Biophys Acta **947**:336–366, 1988.
15. Bowman BB, McCormick DB, Rosenberg IH. Epithelial transport of water-soluble vitamins. Annu Rev Nutr **9**:187–199, 1989.
16. McCormick DB, Snell EE. Pyridoxal kinase of human brain and its inhibition by hydrazine derivatives. Proc Natl Acad Sci USA **45**:1371–1379, 1959.
17. McCormick DB, Gregory ME, Snell EE. Pyridoxal phosphokinases. I. Assay, distribution, purification, and properties. J Biol Chem **236**:2076–2084, 1961.
18. McCormick DB. The intracellular localization, partial purification, and properties of flavokinase from rat liver. J Biol Chem **237**:959–962, 1962.
19. Merrill AH Jr, McCormick DB. Affinity chromatographic purification and properties of flavokinase (ATP:riboflavin 5′-phosphotransferase) from rat liver. J Biol Chem **255**:1335–1338, 1980.
20. Churchich JE, Wu C. Nucleoside phosphorothioates as probes of the nucleotide binding site of brain pyridoxal kinase. J Biol Chem **257**:12136–12140, 1982.
21. Nakano H, McCormick DB. Stereospecificity of the metal ATP complex in flavokinase from rat small intestine. J Biol Chem **266**:22125–22128, 1991.
22. Kazarinoff MN, McCormick DB. N-(5′-Phospho-4′-pyridoxyl)Amines as substrates for pyridoxine (pyridoxamine) 5′-phosphate oxidase. Biochem Biophys Res Commun **52**:440–446, 1973.
23. Kazarinoff MN, McCormick DB. Rabbit liver pyridoxamine (pyridoxine) 5′-phosphate oxidase: Purification and properties. J Biol Chem **250**:3436–3442, 1975.
24. DePecol ME, McCormick DB. Syntheses, properties, and use of fluorescent N-(5′-phospho-4′-pyridoxyl)amines in assay of pyridoxamine (pyridoxine) 5′-phosphate oxidase. Anal Biochem **101**:435–441, 1980.
25. Merrill AH Jr, Korytnyk W, Horiike K, McCormick DB. Spectroscopic studies of complexes between pyridoxamine (pyridoxine) 5′-phosphate oxidase and pyridoxyl 5′-phosphate compounds differing at position 4′. Biochim Biophys Acta **626**:57–63, 1980.
26. Bowers-Komro DM, McCormick DB. Single- and double-headed analogs of pyridoxamine 5′-phosphate as probes for pyridoxamine 5′-phosphate utilizing enzymes. Bioorg Chem **15**:224–236, 1987.
27. Rasmussen KM, Barsa PM, McCormick DB. Pyridoxamine (pyridoxine) 5′-phosphate oxidase activity in rat tissues during development of riboflavin or pyridoxine deficiency. Proc Soc Exp Biol Med **161**:527–530, 1979.
28. Rasmussen KM, Barsa PM, McCormick DB, Roe DA. Effect of strain, sex, and dietary riboflavin on pyridoxamine (pyridoxine) 5′-phosphate oxidase activity in rat tissues. J Nutr **110**:1940–1946, 1980.
29. Choi J-D, Bowers-Komro DM, Davis MD, Edmondson DE, McCormick DB. Kinetic properties of pyridoxine (pyridoxamine) 5′-phosphate oxidase from rabbit liver. J Biol Chem **258**:840–845, 1983.
30. Merrill AH Jr, Horiike K, McCormick DB. Evidence for the regulation of pyridoxal 5′-phosphate formation in liver by pyridoxamine (pyridoxine) 5′-phosphate oxidase. Biochem Biophys Res Commun **83**:984–990, 1978.
31. Horiike K, Merrill AH Jr, McCormick DB. Activation and inactivation of rabbit liver pyridoxamine (pyridoxine) 5′-phosphate oxidase activity by urea and other solutes. Arch Biochem Biophys **195**:325–335, 1979.
32. Oka M, McCormick DB. Complete purification and general characterization of FAD synthetase from rat liver. J Biol Chem **262**:7418–7422, 1987.
33. Bowers-Komro DM, Yamada Y, McCormick DB. Substrate specificity and variables affecting efficiency of mammalian flavin adenine dinucleotide synthetase. Biochemistry **28**:8439–8446, 1989.
34. Yamada Y, Merrill AH Jr, McCormick DB. Probable reaction mechanisms of flavokinase and FAD synthetase from rat liver. Arch Biochem Biophys **278**:125–130, 1990.
35. Lumeng L, Li T-K. Characterization of the pyridoxal 5′-phosphate and pyridoxamine 5′-phosphate hydrolase activity in rat liver. J Biol Chem **250**:8126–8131, 1975.
36. McCormick DB, Russell M. Hydrolysis of flavin mononucleotide by acid phosphatases from animal tissues. Comp Biochem Physiol **5**:113–121, 1962.
37. Daniel H, Wille U, Rehner G. Hydrolysis of FMN and FAD by alkaline phosphatase of the intestinal brush-border membrane. Int J Vitam Nutr Res **53**:109–114, 1983.
38. Schwartz R, Kjeldgaard NO. The enzymatic oxidation of pyridoxal by liver aldehyde oxidase. Biochem J **48**:333–337, 1951.
39. Stanulovic M, Jeremic V, Leskovas V, Chaykin S. New pathway of conversion of pyridoxal to 4-pyridoxic acid. Enzyme **21**:357–369, 1976.
40. Ohkawa H, Ohishi N, Yagi K. Hydroxylation of the 7- and 8-methyl groups of riboflavin by the microsomal electron transfer system of rat liver. J Biol Chem **258**:5629–5633, 1983.
41. McCormick DB. Two interconnected B vitamins: Riboflavin and pyridoxine. Physiol Rev **69**:1170–1198, 1989.
42. McCormick DB, Wright LD. The metabolism of biotin and its analogues. In: Florkin M, Stotz EH, Eds. Comprehensive Biochemistry. Amsterdam: Elsevier, pp81–110, 1970.
43. Zhang Z, McCormick DB. Uptake of N-(4′-pyridoxyl)amines and release of amines by renal cells: A model for transporter-enhanced delivery of bioactive compounds. Proc Natl Acad Sci USA **88**:10407–10410, 1991.
44. Zhang Z, McCormick DB. Uptake and metabolism of N-(4′-pyridoxyl)amines by isolated rat liver cells. Arch Biochem Biophys **294**:394–397, 1991.
45. Zhang Z, Gregory JT III, McCormick DB. Pyridoxine-5′-β-D-glucoside competitively inhibits uptake of vitamin B_6 into isolated rat liver cells. J Nutr **123**:85–89, 1993.