Hydrolysis of Pyridoxine-5′-β-D-Glucoside by a Broad-Specificity β-Glucosidase from Mammalian Tissues[1] (43142)

PAULA R. TRUMBO,[2] MELANIE A. BANKS, AND JESSE F. GREGORY III
Food Science and Human Nutrition Department, University of Florida, Gainesville, Florida 32611-0163

Abstract. **Research was conducted to evaluate the ability of a broad-specificity β-glucosidase in mammalian tissues to catalyze the hydrolytic release of free pyridoxine from pyridoxine-5′-β-D-glucoside, a naturally occurring form of vitamin B6 in plant-derived foods. Activity was detected in liver and intestinal mucosa using tritiated pyridoxine glucoside as a substrate. In the rat and guinea pig, enzyme activity was greater in intestine than in liver or kidney while even greater activity was detected in human intestinal tissue. Reaction rates were, however, low in all tissues. Hydrolysis of the synthetic substrate 4-methylumbelliferyl-β-D-glucoside was also greatest in intestinal tissue. The characteristics of the enzymatic hydrolysis of pyridoxine glucoside to pyridoxine included: (i) most activity in the soluble tissue fraction, (ii) a pH optimum of approximately 6.0, and (iii) inhibition caused by the addition of sodium taurocholate. These characteristics are very similar to those of the broad-specificity β-glucosidase in mammalian tissues with respect to the hydrolysis of a variety of naturally occurring and synthetic substrates. The apparent K_m was greater than 2 m*M* for pyridoxine glucoside hydrolysis by intestinal preparations of each species, which is much greater than expected intestinal concentrations derived from dietary sources. *In vivo* studies have indicated that the intestine is involved in the metabolic utilization of dietary pyridoxine glucoside. The results observed here suggest that an alternate process, possibly involving intestinal microorganisms, may also be involved in the *in vivo* hydrolysis of pyridoxine glucoside.** [P.S.E.B.M. 1990, Vol 195]

A major form of vitamin B_6 in plant-derived foods is the glycosidic conjugate 5′-*O*-(β-glucopyranosyl) pyridoxine (PN-glucoside) (1, 2). The extent of utilization of dietary PN-glucoside in vitamin B_6 metabolism is 20–30%, relative to pyridoxine, in the rat (3–5) and approximately 60% relative to pyridoxine in the human (6). Studies employing alternate routes of administration of radioisotopically or stable isotopically labeled PN-glucoside and PN have shown that the intestine is responsible, in part, for the release of biologically active pyridoxine from PN-glucoside (5, 6). Hydrolysis of the β-glycosidic bond of PN-glucoside to release free pyridoxine is a requisite step in the utilization of PN-glucoside in vitamin B_6 metabolism. The extent of involvement of β-glucosidases of the intestinal microflora and/or the intestinal mucosa in the utilization of PN-glucoside has not been determined, although evidence of enzymatic hydrolysis of PN-glucoside by extracts of rat tissues has been reported previously (7).

Two β-glucosidases have been identified in mammalian tissues that catalyze the hydrolysis of a variety of nonphysiologic aryl-β-D-glucosides as well as several naturally occurring substrates. One of the enzymes is located in the lysosomes and is responsible for the hydrolysis of the β-glycosidic linkage of glucocerebrosides. This enzyme is associated with the lysosomal membrane and requires nonionic detergents for extraction into soluble form (8). Neutral detergents or ionic lipids, such as sodium taurocholate, are required for maximal activity. The second major β-glucosidase is a cytosolic enzyme of broad specificity (9, 10) that is inhibited by sodium taurocholate and certain other polar lipids (11). The cytosolic enzyme catalyzes the hydrolysis of several synthetic aryl-β-D-glucosides (10–

[1] Florida Agricultural Experiment Station Journal Series Number R00110.

[2] Present address: Department of Foods and Nutrition, Purdue University, West Lafayette, IN 47907.

Received July 17, 1989. [P.S.E.B.M. 1990, Vol 195]
Accepted June 21, 1990.

0037-9727/90/1952-0240$2.00/0
Copyright © 1990 by the Society for Experimental Biology and Medicine

12), although its physiologic action is unclear. The broad-specificity cytosolic β-glucosidase from guinea pig liver has been shown to catalyze the hydrolysis of L-picein, a naturally occurring aryl glycoside in certain plants (13). The cytosolic enzyme from rabbit liver, kidney, and small intestine will hydrolyze various steroid-3-β-glucosides *in vitro* (14, 15) and may act in this manner *in vivo.*

The research described here was conducted to identify and to partially characterize the β-glucosidase activity of mammalian tissues which may be relevant to the utilization of PN-glucoside in vitamin B_6 metabolism. The main objective of this research was to clarify further the factors influencing the bioavailability of dietary vitamin B_6.

Materials and Methods

Materials. [^{3}H]Pyridoxine HCl (1.4 Ci/mmol) was purchased from Amersham Corp. (Arlington Heights, IL). [^{3}H]Pyridoxine-glucoside was prepared from [^{3}H] pyridoxine by biologic synthesis with alfalfa sprouts and purified by ion-exchange and gel filtration chromatography as described previously (4). The purity of these compounds was determined by high-performance liquid chromatography (HPLC) as described below. The specific radioactivity of purified [^{3}H]PN-glucoside was 4.0 mCi/mmol, which reflects isotopic dilution with unlabeled PN-glucoside during the biologic synthesis.

Tissue samples were obtained from adult male guinea pigs (250–300 g; Harlan Sprague-Dawley, Indianapolis, IN) and Sprague-Dawley rats (150–200 g; Crl:CD(SD)BR; Charles River Breeding Laboratories, Inc., Wilmington, MA). The animals were killed by decapitation after anesthesia, and tissues were collected immediately. Liver and kidneys were packed in crushed ice, while the small intestine was flushed with cold isotonic saline before being packed in ice. These tissues were homogenized, and fractionation was begun immediately. Human intestinal mucosa was obtained from small samples of jejunal tissue obtained during elective jejunoileal bypass surgery and was provided by Dr. James Cerda, University of Florida College of Medicine. The human tissue was stored either as scraped, unfractionated mucosa in liquid nitrogen or as intact intestine at −20°C until homogenization and analysis.

Tissue Preparation and Fractionation. Tissue samples were homogenized (60 sec, setting 6; Polytron PT10/35, Brinkmann Instruments, Westbury, NY) in two to three volumes of 0.2 *M* sodium acetate buffer (pH 5.5) containing 1 m*M* phenylmethylsulfonyl fluoride and 10 m*M* 2-mercaptoethanol in the presence or absence of 0.1% (w/v) sodium taurocholate. For comparisons of the specific activity of tissues homogenates were first subjected to centrifugation at 20,000*g* for 20 min at 4°C. For determination of the subcellular distribution of activity, guinea pig small intestine was subjected to differential centrifugation. Nuclei, whole cells, and cell debris were sedimented by centrifugation of the homogenate at 1000*g* for 15 min and were discarded. The supernatant was centrifuged at 20,000*g* for 20 min to sediment the "mitochondrial fraction," and the "microsomal fraction" was then sedimented by centrifugation at 100,000*g* for 60 min (Beckman LS-50 ultracentrifuge, type Ti50 rotor; Beckman Instruments, Inc., Fullerton, CA). The sedimented fractions were resuspended and homogenized in the homogenization buffer. Protein content in all preparations was determined according to the method of Bradford (16) with bovine serum albumin as the standard.

Enzyme Assays. Standard enzyme reactions involved the incubation of [^{3}H]PN-glucoside (ca. 5 μM), and a sample of the tissue fraction (1–10 μg of protein/μl of reaction mixture) for various reaction times at 37°C. For routine assays, this low concentration of PN-glucoside was used to conserve this substrate. In later studies, variation in substrate concentration was achieved by adding a constant amount of [^{3}H]PN-glucoside and varying the amount of unlabeled PN-glucoside added. All reactions were conducted in 0.2 *M* sodium acetate buffer (pH 5.5) containing 1 m*M* phenylmethylsulfonyl fluoride and 10 m*M* 2-mercaptoethanol and were terminated by the addition of trichloroacetic acid (final concentration, 5% w/v) followed by centrifugation. The supernatant was collected for HPLC analysis. The precision of replicate assays was typically within 5%. Routine assays, including incubations and HPLC analyses, were conducted in duplicate with a concurrent blank. Under the conditions of this assay, linear rates of product formation were observed over the entire 21-hr period studied.

β-Glucosidase activity was also assayed at 37°C, with 1.75 m*M* 4-methylumbelliferyl-β-D-glucoside (MUG) (Sigma Chemical Co. St. Louis, MO) as a substrate as described by Daniels *et al.* (12). The buffer was identical to that used in assays with PN-glucoside as substrate. The concentration of the product, 4-methylumbelliferone, was measured fluorometrically according to the method of Peters *et al.* (17). Linear standard curves were observed over the range of 0–25 μM 4-methylumbelliferone.

HPLC Methods. The reverse-phase ion-pair HPLC procedure used to separate unhydrolyzed [^{3}H]PN-glucoside from [^{3}H]pyridoxine was a modification of the procedure of Gregory and Ink (1), as reported previously (18). This separation was performed using a binary gradient chromatographic system (Rainin Instruments) with an Ultrasphere IP octadecylsilyl column (4.6 × 25 cm; Beckman Instruments, Inc.), fluorometric detector (model LS-5; The Perkin-Elmer Corp., Norwalk, CT). A fraction collector (Cygnet; ISCO Corp., Lincoln, NE) was attached to the detector outlet to collect 0.75-ml fractions. The elution profile consisted

Table I. β-Glucosidase Activity in Rat and Guinea Pig Liver, Kidney, and Small Intestine and Human Small Intestinal Mucosa Determined with PN-Glucoside as Substrate[a]

Group	pmol of PN/hr/mg protein		
	Liver	Kidney	Jejunum[b]
Guinea pig ($n = 4$)	1.47 ± 0.46	ND	3.78 ± 0.62a
Rat ($n = 5$)	ND	ND	3.41 ± 0.60a
Human ($n = 3$)	NA	NA	22.0 ± 7.8b

[a] Analysis of 20,000*g* supernatants. Results are mean ± SE.
[b] Values followed by the same letter were not significantly different, $P < 0.01$.
[c] ND, not detected; NA, not analyzed.

Table II. Distribution of β-Glucosidase Activity among Guinea Pig Tissues (20,000*g* Supernatant Fraction) as Determined with MUG and PN-glucoside as Substrate[a]

Tissue	Specific Activity (pmol/hr/mg protein)	
	MUG	PN-glucoside[b]
Liver	7.37 ± 1.76	1.47 ± 0.46
Kidney	14.2 ± 4.3	Not detected
Jejunum	155 ± 86	3.78 ± 0.62

[a] Results are mean ± SE of four animals.
[b] Values in this column were significantly different from the corresponding specific activity for MUG, $P < 0.05$.

Table III. Distribution of β-Glucosidase Activity in Subcellular Fractions of Guinea Pig Jejunum Determined with PN-Glucoside as Substrate[a]

Fraction	Specific activity (pmol/hr/mg)	Total protein (mg)	Total activity (pmol/hr)
Pellet (20,000*g*)	7.0	4.4	31
Pellet (100,000*g*)	1.0	3.5	3.5
Supernatant (100,000*g*)	2.7	97.5	263

[a] Values are results of analyses of fractions from a single intestine sample.

of a nonlinear gradient from 100% buffer A (0.033 *M* H_3PO_4 [pH 2.2], 8 m*M* octane sulfonic acid) to 100% buffer B (0.033 M H_3PO_4 [pH 2.2], 17.5% v/v 2-propanol, and no octane sulfonic acid).

Measurement of Radioactivity. Radioactivity in HPLC fractions was determined by liquid scintillation counting (model LS-2800; Beckman Instruments, Inc.) with a commercial scintillation fluid (Aqualyte Plus; J. T. Baker Chemical Co., Phillipsburg, NJ). A channel ratio procedure and quench curve were employed to correct for quenching.

Specific enzyme activity was expressed as picomoles of pyridoxine liberated/hr/mg of protein on the basis of the specific radioactivity of [^{3}H]PN-glucoside in the reaction assay mixture. Because of the high specific activity of the substrate and the complete separation obtained between substrate and product, this assay provided high sensitivity and specificity. The lower limit of quantification with this assay depends on the incubation time and enzyme concentration. As an example of a low reaction rate under typical assay conditions, an observed 2% hydrolysis of [^{3}H]PN-glucoside in a 15-hr incubation with 3 mg of protein yields specific enzymatic activity of 0.18 pmol/hr/mg of protein.

Statistical Analysis. Differences between species with respect to the specific activity of intestinal β-glucosidase toward PN-glucoside were evaluated by using one-way analysis of variance and the Tukey procedure for multiple comparisons. The effectiveness of MUG and PN-glucoside as substrates for β-glucosidase of guinea pig liver and intestine was determined by one-way analysis of variance. These procedures were conducted as described by Neter and Wasserman (19).

Results and Discussion

Enzymatic Hydrolysis of Pyridoxine-5′-β-Glucoside. β-Glucosidase activity capable of hydrolyzing PN-glucoside was detected in the small intestine of rats, guinea pigs, and humans, as well as in rat liver, while little or no activity was detected in kidneys (Table I). The rates of hydrolysis of PN-glucoside were very low in all samples, although the intestine exhibited the highest specific activity of the tissues examined in the rat and guinea pig. A similar difference in apparent β-glucosidase activity between crude homogenates of rat liver and small intestine was observed by Tsuji *et al.* (7) under substantially different assay conditions. The specific activity of human intestinal samples toward PN-glucoside was significantly greater than that observed for rat and guinea pig ($P < 0.05$).

It should be noted that this study was conducted with portions of whole small intestine in the fractionation and analysis of rat and guinea pig tissues, while only human intestinal mucosa, rather than whole intestine, was available for use (because of the availability of samples). To determine whether this difference (whole intestine versus mucosa) could account for the greater specific activity observed in the human, an additional study was conducted with samples of rat intestine. Supernatant fractions (20,000*g*) were prepared from homogenates of both whole intestine and mucosa from the same tissue samples, followed by assay of β-glucosidase with PN-glucoside and MUG as substrates. The β-glucosidase activity with respect to each substrate was similar between these two types of supernatant preparations, with only a trend toward higher specific activities (20% ± 17%, mean and SD; $n = 4$) in the supernatants of mucosal samples. Thus, the

Table IV. Effect of sodium Taurocholate on the Hydrolysis of PN-Glucoside by β-Glucosidase of Guinea Pig Intestine[a]

Treatment[b]	NaTC (% w/v)	Specific activity (pmol of PN/hr/mg of protein)
NaTC added during homogenization	0.0	3.86
(20,000*g* supernatant)	0.1	1.55
NaTC added to reaction mixture	0.0	6.00
(100,000*g* supernatant)	0.1	2.80

[a] Taurocholate was added either to the buffer used in tissue homogenization or to the reaction mixture immediately before assay.
[b] Guinea pig jejunum (20,000*g* supernatant). NaTC, sodium taurocholate.

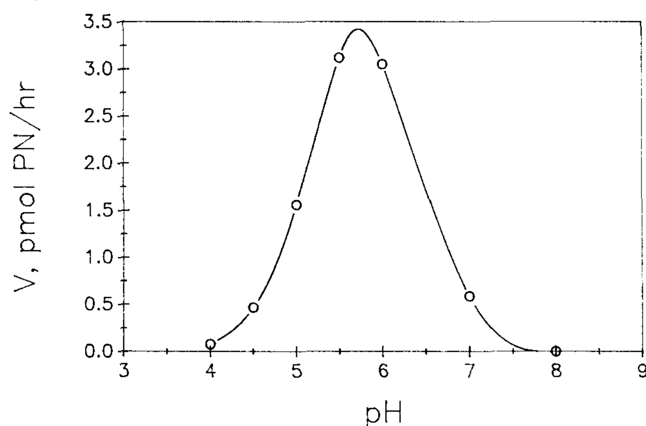

Figure 1. pH activity profile of β-glucosidase activity of 20,000*g* supernatant of guinea pig intestine with PN-glucoside as substrate. The buffer was 0.2 *M* sodium acetate—0.1 m*M* phenylmethylsulfonyl fluoride—10 m*M* 2-mercaptoethanol, with pH adjustment by addition of HCL or NaOH.

evaluation of β-glucosidase activity showing greater specific activity in the human small intestine (Table I) represents a valid comparison of these species. Although a relatively small number of samples was evaluated in the comparison of intestinal enzymatic activity ($n = 3$, 5, and 4 for humans, rats, and guinea pigs, respectively), the human intestinal tissue consistently exhibited greater activity with respect to PN-glucoside hydrolysis ($P < 0.05$).

Liver, kidney, and small intestine preparations from guinea pigs were evaluated to compare the effectiveness of PN-glucoside and MUG as substrates (Table II). MUG is frequently used as a substrate for the convenient fluorometric assessment of β-glucosidase activity. As observed with PN-glucoside, the specific activity toward MUG was highest in the small intestine preparation. The reaction rates were significantly greater for MUG than for PN-glucoside ($P < 0.05$), indicating that MUG was a preferred substrate under the conditions of this study. It should be noted that PN-glucoside was employed in the assay under non-saturating conditions in this study. Although MUG appears to be a superior substrate, further study would be required to provide a direct comparison of kinetic constants. Differences have been reported in comparisons of MUG, other aryl-β-glucosides, and steroid-β-glucosides (12).

Because of the limited supply of human intestinal tissue, guinea pig small intestine was studied in greater detail to evaluate further the properties of the intestinal enzyme activity. Guinea pig intestine was subjected to differential centrifugation to permit evaluation of the subcellular distribution of PN-glucoside hydrolytic activity (Table III). Essentially all of the activity was detected in the high-speed supernatant, which indicates the soluble nature of the enzyme. Little activity was noted in either of the particulate fractions, which would contain mixed brush border and basolateral membranes and other organelles of the intestinal epithelial cells. Similar results were reported by Mellor and Layne (15) concerning the cytosolic nature of small intestinal "steroid-β-glucosidase," in contrast to large intestine, which exhibits significant activity in the 11,000*g* and 105,000*g* particulate fractions (15).

Further information concerning the subcellular locus of PN-glucoside hydrolytic activity was obtained in studies concerning the effect of sodium taurocholate on PN-glucoside hydrolysis by guinea pig intestine preparations. These studies were based on the observations that sodium taurocholate, at millimolar concentrations, is an inhibitor of the cytosolic broad-specificity β-glucosidase, while it markedly stimulates the lysosomal glucocerebrosidase (9, 12, 20). As shown in Table IV, sodium taurocholate inhibited the hydrolysis of PN-glucoside whether it was added before tissue homogenization or before the assay of the 20,000*g* supernatant. Thus, the lysosomal glucocerebrosidase appears to have no activity toward PN-glucoside.

The pH activity profile of guinea pig small intestinal PN-glucoside hydrolytic activity exhibited a maximum at pH 5.5–6.0 (Fig. 1). A similar pH optimum has been reported for the steroid β-glucosidase and broad-specificity β-glucosidase activity of liver (8, 12).

PN-glucoside hydrolytic activity in rat, guinea pig, and human small intestinal fractions was found to be linearly related to substrate concentration over the physiologically relevant micromolar concentration range (Fig. 2). These results, i.e., approximate linearity over the range of PN-glucoside concentration exam-

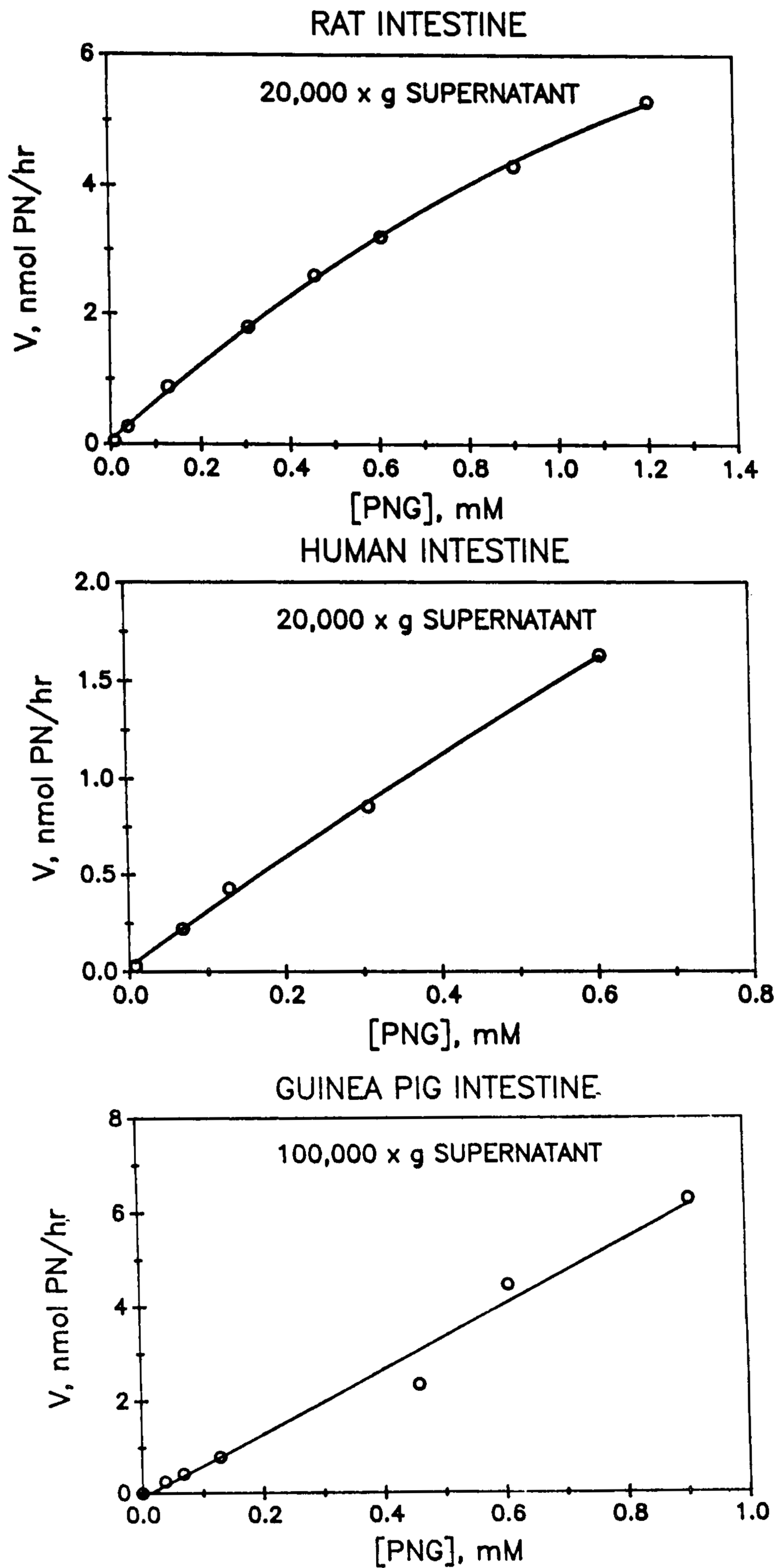

Figure 2. Rate of hydrolytic release of PN as a function of PN-glucoside concentration for rat, human, and guinea pig intestine. Incubations were performed using the buffer described for Figure 1 at pH 5.5.

ined, indicate an apparent K_m greater than 2 mM for each species. These K_m values in the millimolar range are substantially greater than those reported for various MUG (e.g., K_m for MUG, 0.06 mM) (11, 12), steroid-3-β-glucosides (e.g.,K_m, 0.03 mM for 17α-estradiol-3-glucoside) (15), and a naturally occurring aryl-glucoside L-picein (K_m, 0.63 mM) (13). The very low velocities observed for PN-glucoside in this study are similar to those reported for steroid glucosides with comparable tissue fractions (15).

Role of Cytosolic β-Glucosidase in the *In Vivo* Utilization of Dietary Pyridoxine-5′-β-Glucoside. The results of this study indicate that the cytosolic broad-specificity β-glucosidase in mammalian small intestine and liver is capable of catalyzing the hydrolysis of PN-glucoside. Several lines of evidence are pertinent to an evaluation of the *in vivo* significance of the hydrolysis of dietary PN-glucoside by this enzyme. The specific activity of intestinal β-glucosidase was greater in the human than in the rat or guinea pig. Whether this difference in activity among species is physiologically significant with respect to the utilization of dietary PN-glucoside is presently unclear, although this trend is consistent with the results of *in vivo* studies concerning the biological activity of PN-glucoside in the rat and human. We have found that dietary PN-glucoside exhibits approximately 20–30% of the vitamin B_6 potency of free pyridoxine in the rat (1, 4, 5), while recent stable-isotopic studies indicate that humans are capable of a mean of 60% utilization of dietary PN-glucoside, relative to the utilization of pyridoxine (6). In addition, studies comparing the utilization of PN-glucoside administered to rats and humans orally or by injection have shown that the intestine plays a significant role in the metabolic utilization of this compound (5, 6). Further studies concerning the relationship between intestinal β-glucosidase activity and the *in vivo* utilization of dietary PN-glucoside in various species are in progress.

In spite of the apparent relationship between intestinal activity of β-glucosidase and the previous *in vivo* results, it is unclear from the very low rates of hydrolysis observed in this study whether the intestinal broad-specificity β-glucosidase exhibits sufficient activity toward PN-glucoside to be functionally significant in the metabolic utilization of this compound. Mellor and Layne (14, 15) reported that rabbit small intestine, as well as liver and kidney, contains β-glucosidase activity with specificity toward steroid-β-glucosides. As observed in the present study, the rates of hydrolysis were sufficiently low that the *in vivo* significance of these β-glucosidase-catalyzed reactions is difficult to ascertain.

This study has shown that β-glucosidase activity of mammalian intestine and other tissues is capable of catalyzing the hydrolysis of PN-glucoside, a naturally occurring form of vitamin B_6 in human diets. In view of the low reaction rates and very high K_m observed *in vitro*, the physiologic significance of this enzyme with respect to the utilization of PN-glucoside remains unclear. β-Glucosidases have been isolated from various bacteria, and it will be of interest to determine the contribution of the intestinal microflora to the total hydrolysis of PN-glucoside. Differences observed in the bioavailability of PN-glucoside in the rat and human may be related to differences in intestinal microbial populations, in addition to the difference in intestinal cytosolic β-glucosidase activity.

This study was supported by NIH Grants DK37481 and F32-DK08179. Preliminary discussions with Dr. Robert H. Glew, University of Pittsburgh, are gratefully acknowledged.

1. Gregory JF, Ink SL. Identification and quantification of pyridoxine-β-glucoside as a major form of vitamin B6 in plant-derived foods. J Agric Food Chem **35**:76–82, 1987.
2. Kabir H, Leklem JE, Miller LT. Measurement of glycosylated vitamin B6 in foods. J Food Sci **48**:1422–1425, 1983.
3. Ink SL, Gregory JF, Sartain DB. Determination of pyridoxine β-glucoside bioavailability usine intrinsic and extrinsic labeling in the rat. J Agric Food Chem **34**:857–862, 1986.
4. Trumbo PR, Gregory JF, Sartain DB. Incomplete utilization of pyridoxine-β-glucoside as vitamin B6 in the rat. J Nutr **118**:170–175, 1988.
5. Trumbo PR, Gregory JF. Metabolic utilization of pyridoxine-β-glucoside in rats: Influence of vitamin B6 status and route of administration. J Nutr **118**:1336–1342, 1988.
6. Gregory JF, Trumbo PR, Bailey LB, Toth JP, Cerda JJ, Baumgartner TG. Bioavailability of pyridoxine-5′-β-D-glucoside determined in humans by stable-isotopic methods. J Nutr (in press).
7. Tsuji H, Okada J, Iwami K, Yasumoto K, Mitsuda H. Availability as vitamin B6 and small intestinal absorption of pyridoxine-β-D-glucoside in rats. Vitamins (Kyoto) **51**:153–159, 1977.
8. Pentchev PG, Brady RO, Hibbert SR, Gal AE, Shapiro D. Isolation and characterization of glucocerebrosidase from human placental tissue. J Biol Chem **248**:5256–5261, 1973.
9. Chester MA, Hultberg B, Ockerman PA. The common identity of five glycosidases in human liver. Biochim Biophys Acta **429**:517–526, 1973.
10. Ben-Yoseph Y, Shapira E, Elelman D, Burton BK, Nadler HL. Purification and properties of neutral β-galactosidases from human liver. Arch Biochem Biophys **184**:373–379, 1977.
11. LaMarco KL, Glew RH. Galactosylsphingosine inhibition of the broad-specificity cytosolic β-glucosidase of human liver. Arch Biochem Biophys **236**:669–676, 1985.
12. Daniels LB, Coyle PJ, Chiao Y-B, Glew RH, Labow RS. Purification and characterization of a cytosolic broad specificity β-glucosidase from human liver. J Biol Chem **256**:13004–13013, 1981.
13. LaMarco KL, Glew RH. Hydrolysis of a naturally occurring β-glucoside by a broad specificity β-glucosidase from liver. Biochem J **237**:469–476, 1986.
14. Mellor SD, Layne DS. Steroid β-D-glucosidase activity in rabbit tissues. J Biol Chem **246**:4377–4380, 1971.
15. Mellor SD, Layne DS. Steroid β-D-glucosidase: Purification of the enzyme from rabbit liver. J Biol Chem **249**:361–365, 1974.

16. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem **72**:248–254, 1976.
17. Peters SP, Lee RE, Glew RH. A microassay for Gaucher's disease. Clin Chim Acta **60**:391–396, 1975.
18. Trumbo PR, Gregory JF. The fate of dietary pyridoxine-β-glucoside in the lactating rat. J Nutr **119**:36–39, 1989.
19. Neter J, Wasserman W. Applied Linear Statistical Models. Homewood, IL: Richard D Irwin 1974.
20. Peters SP, Coyle P, Glew RH. Differentiation of β-glucocerebrosidase from β-glucosidase in human tissues using sodium taurocholate. Arch Biochem Biophys **175**:569–582, 1976.