Nutritional Quality of Detoxified Guar (*Cyamopsis tetragonoloba*) Meals

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Defatted guar meal was detoxified by extracting with 1 or 0.25 N HCl or autoclaving, and the nutritional quality of these processed meals was evaluated by various techniques. Although the in vitro digestibility of the meal proteins was improved, the amino acid composition, available lysine content, and chemical score of the meal were not affected appreciably by these treatments. The defatted guar meal did not support the growth of weanling rats, and its protein efficiency ratio (PER), net protein ratio (NPR), and net protein utilization (NPU) were all negative. On the other hand, the PER, NPR and NPU of the detoxified meals ranged from 1.23 to 1.98 (casein value 3.77), 3.38 to 3.82 (casein value 5.67), and 44 to 56 (casein value 71), respectively. Also, the PER and NPU of the acid-extracted meals were significantly higher than those of the autoclaved meal, indicating that acid treatments were superior to autoclaving in detoxifying the meal and improving its nutritive value.

INTRODUCTION

Guar meal, the byproduct of the guar gum industry, is rich in proteins (50%) and has been shown to contain certain antinutritional factors such as trypsin inhibitors (Couch et al., 1966; Hooper and Couch, 1971), hemagglutinins (D'Souza, 1972), saponins (D'Souza, 1972; Subramanian and Parpia, 1975), polyphenols (Kaushal and Bhatia, 1982), and some unpleasant flavor components. Hence, its use for food and feed purposes is limited. However, over the past several years numerous attempts have been made to detoxify the meal. Extraction of the meal with dilute acid (Kawatra et al., 1969, 1974; D'Souza, 1972; Khopkar and Rege, 1984), aqueous or acidic alcohols (D'Souza, 1972; Subramanian and Parpia, 1975; Misra et al., 1984), and autoclaving or heat treatment (Kawatra et al., 1969; D'Souza, 1972; Subramanian and Parpia, 1975) are among the treatments that have shown some promise. In this investigation, the nutritional quality of the 1 and 0.25 N HCl extracted and autoclaved guar meals has been evaluated by chemical and in vivo techniques.

MATERIALS AND METHODS

Guar seed (variety Durgapur safed) was procured from an established guar gum industry. Casein, hydrogenated fat, shark liver oil, sugar, and corn starch were purchased from the local market. Male albino rats of Wistar strain were obtained from CFTRI Animal House. The sources of the chemicals used are as follows: N^{α}-benzoyl-L-arginine-*p*-nitroanilide hydrochloride (BAPA), pepsin, and trypsin were from Sigma Chemical Co., and methyl chloroformate and 1-fluoro-2,4-dinitrobenzene (FDNB) were from E. Merck. All the other chemicals used were of analytical grade purchased from M/s BDH or Sarabhai Merck Chemicals.

Detoxification of Guar Meal. Processing of guar seeds to obtain guar meal and the preparation of 1 N HCl extracted, 0.25 N HCl extracted, and autoclaved guar meals, starting from the defatted guar meal, were done according to the methods reported earlier (Tasneem et al., 1982).

Polyphenol Estimation. Extraction of polyphenols was done by refluxing the meal with 80% ethanol, meal to solvent ratio being 1:100, for 2 h. The method of Swain and Hillis (1959) was used for the estimation of polyphenols in the extracts. A standard curve, prepared with gallic acid, was used to determine the polyphenol content of the various samples.

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Table I. Free Polyphenol Content and Trypsin Inhibitor Activity of Processed Guar Meals

sample	free polyphenol content, %	trypsin inhibit act. TUI/mg protein
defatted guar meal	0.36	7.6
1 N HCl extr guar meal	0.06	nil
0.25 N HCl extr guar meal	0.05	nil
autoclaved guar meal	0.32	2.5

Trypsin Inhibitor Activity. The trypsin inhibitor activity was determined by the method of Kakade et al. (1969) using a synthetic substrate, BAPA. The protein (N × 6.25) content of the meal extract was determined by the micro Kjeldahl method, and the trypsin inhibitor activity was expressed as number of trypsin units inhibited (TUI)/milligram of protein.

Hemagglutinating Activity. The hemagglutinating activity of the samples was tested by the serial dilution method of Liener and Hill (1953) using 0.9% saline extract of the meal and a 2% suspension of trypsinized rabbit erythrocytes (Lis and Sharon, 1972).

Amino Acid Analyses. Amino acid analyses was performed with a LKB 4150 α amino acid analyzer previously calibrated with a standard mixture of amino acids using acid hydrolysates of the meals, which were prepared according to the method of Moore and Stein (1963). A sample containing about 3 μg of nitrogen was loaded on the sulfonated polystyrene resin column by means of an autoloader, and analysis was performed. The reaction of ninhydrin with amino acids was made use of in the detection and quantitation of the latter. Nitrogen content of the hydrolysate was estimated by the micro Kjeldahl method.

Tryptophan content of the various meals was estimated by the microbiological assay (Barton-Wright, 1952) using the organism *Lactobacillus arabinosus* 17/5 and alkali hydrolysates of the samples. A standard curve was prepared against tryptophan in the range 0.2–2.0 μg.

Available Lysine Estimation. Available lysine content of the samples was estimated by the modified method of Carpenter and Ellinger (1955) using the acid hydrolysates containing DNP-lysine.

Chemical Score. The chemical score of the processed guar meal proteins was calculated against the FAO/WHO (1973) reference pattern. The content of each essential amino acid in the sample was expressed as a percentage

0021-8561/86/1434-0853$01.50/0

Table II. Amino Acid Composition, Available Lysine, Chemical Score, and Limiting Amino Acids of Processed Guar Meals[a]

amino acid	defatted guar meal	1 N HCl extr guar meal	0.25 N HCl, extr guar meal	autoclaved guar meal
aspartic acid	8.9	10.0	9.2	9.8
threonine	2.7	3.2	3.7	2.7
serine	4.4	4.5	5.0	4.6
glutamic acid	21.9	19.4	17.4	23.0
proline	3.5	3.7	4.1	3.6
glycine	4.0	4.0	4.0	4.0
alanine	3.1	3.9	4.1	3.0
cysteine	0.7	0.6	0.8	0.6
valine	3.3	4.1	4.4	3.0
methionine	0.9	0.9	1.0	1.0
isoleucine	2.7	3.0	3.6	2.7
leucine	5.4	5.9	7.1	5.4
tyrosine	3.2	3.0	3.6	2.3
phenylalanine	3.5	4.0	4.7	3.7
histidine	2.3	2.6	2.8	2.4
lysine	3.6	3.6	4.0	3.6
arginine	12.2	10.9	10.4	12.6
tryptophan	1.3	1.0	1.0	1.1
avail lysine	3.0	2.8	2.8	2.9
chem score	46	43	51	46
lim amino acids I		total sulfur amino acids		
II		lysine		

[a] Units: g/16 g of N.

of the content of the same acid in the reference protein, and the minimum was taken as the chemical score.

In Vitro Digestibility. In vitro digestibility was determined according to the method of Villegas et al. (1968) with some modifications as detailed below. After the peptic followed by pancreatic digestions, the digest was diluted with water and the undigested proteins and any protein intermediary products stable to enzyme attack were precipitated by adding an equal volume of 10% TCA solution to the diluted digest (Birk and Bondi, 1955). The dispersion was centrifuged, and nitrogen in the supernatant was estimated by the micro Kjeldahl method and corrected for blank. Casein was used as a standard. The in vitro digestibility was calculated as the percent of total nitrogen hydrolyzed by the enzymes.

All chemical determinations were carried out in duplicate, and mean values are given.

Net Protein Ratio (NPR) and Protein Efficiency Ratio (PER). The basal diet contained 10% hydrogenated fat, 10% sugar, 2% salt mixture (Hubell et al., 1937), 2% vitamin mixture (Chapman et al., 1959), and 10% protein, derived from casein or guar samples. Young weanling rats of Wistar strain, 21–23 days old, were allotted to the various groups of 10 rats each by the randomized block design. Each rat was housed in an individual cage, and the diet, as a paste with water and 2 drops of shark liver oil, and water were given ad libitum. A 7- and 10-day record of food consumption and body weights of rats was maintained. The NPR and PER values were calculated after feeding for 10 and 28 days, respectively.

Net Protein Utilization (NPU). For NPU determination, 28-day-old rats (eight per group, allotted by randomized block design), maintained on a stock diet for 1 week, were fed the test diet for 10 days and the body nitrogen, after drying the carcass thoroughly at 110 °C, was estimated by the Kjeldahl method. The NPU values were estimated from the body nitrogen and nitrogen intake of rats fed test diet and the body nitrogen of rats fed the nonprotein diet.

RESULTS AND DISCUSSION

In order to study the effects of these detoxification treatments on the antinutritional factors, polyphenol content, trypsin inhibitor activity, and hemagglutinating activity of the processed guar meals were determined. Hemagglutinating activity could not be detected in any of the samples. Nath et al. (1978) also reported that the meal extract did not show any hemagglutinating activity. Defatted guar meal contained 0.36% free polyphenols (Table I) while Kaushal and Bhatia (1982) reported that the polyphenol content of whole guar seeds ranged from 0.69 to 1.26% depending on the stage of maturity. The low value obtained in this investigation might be due to the fact that the polyphenols are mostly present in the seed coat (Singh and Jambunathan, 1981), and the meal was free from this fraction. The free polyphenol content of the autoclaved meal was comparable while those of the acid-extracted meals were significantly lower than that of the defatted meal.

Defatted guar meal showed a trypsin inhibitor activity of 7.6 TUI/mg of protein (Table I). Nath et al. (1978) in their studies using the FS 277 variety of guar seeds and casein as substrate for the assay reported a lower value of 5.5 TUI/mg of protein. Kaur and Bhatia (1984) have, however, reported a much higher value of 24.5 TUI/mg of protein in the phosphate buffer (0.1 M, pH 7.6) extract of FS 277 guar meal. Autoclaving the meal reduced the trypsin inhibitor activity by 67% due to the partial thermal inactivation of the trypsin inhibitors. Reduction in the trypsin inhibitor activity of guar meal by heat was reported by others also (Couch et al., 1966; Kaur and Bhatia, 1984). The acid-extracted samples did not show any trypsin inhibitor activity, which could be due to the inactivation and/or leaching out of the trypsin inhibitor during the acid treatments. In the case of soybean also extraction with dilute acid was reported to reduce the trypsin inhibitor activity (Birk and Gertler, 1961).

Amino Acid Composition. The contents of the various amino acids of the defatted guar meal (Table II) were comparable to the literature values (VanEtten et al., 1961). Guar meal, like many other legumes, was found to be rich in glutamic acid, aspartic acid, and arginine and deficient in sulfur-containing amino acids. The detoxification treatments did not cause a major change in the amino acid composition of the meal; however, the acid-extracted meals had higher contents of threonine, valine, leucine, and

Table III. Diet Intake and Gain in Weight of Rats and NPR and PER of Processed Guar Meals and Casein

gp	protein source	av protein intake, g		av wt gain, g		NPR[a] (mean)	PER[b,c] (mean)
		10 days	28 days	10 days	28 days		
A	defatted guar meal	1.1	6.6	−6.5	−5.5		
B	1 N HCl extr guar meal	4.1	16.7	9.4	32.7	3.82	1.98
C	0.25 N HCl extr guar meal	2.9	12.8	3.5	20.4	3.38	1.57
D	autoclaved guar meal	3.0	10.5	4.8	13.9	3.73	1.23
E	casein	5.4	21.4	24.5	80.9	5.67	3.77
F	nonprotein diet			−6.2			

[a] Standard error 0.2. [b] Standard error 0.1. [c] Note: This rat bioassay included three more groups that are not included in this paper. Results of test of significance (average NPR): *C (3.38)*; *D (3.73)*; *B (3.82)*; E (5.67). Results of test of significance (average PER): D (1.23); C (1.57); B (1.98); E (3.77). Any two means not italicized are *significantly* different.

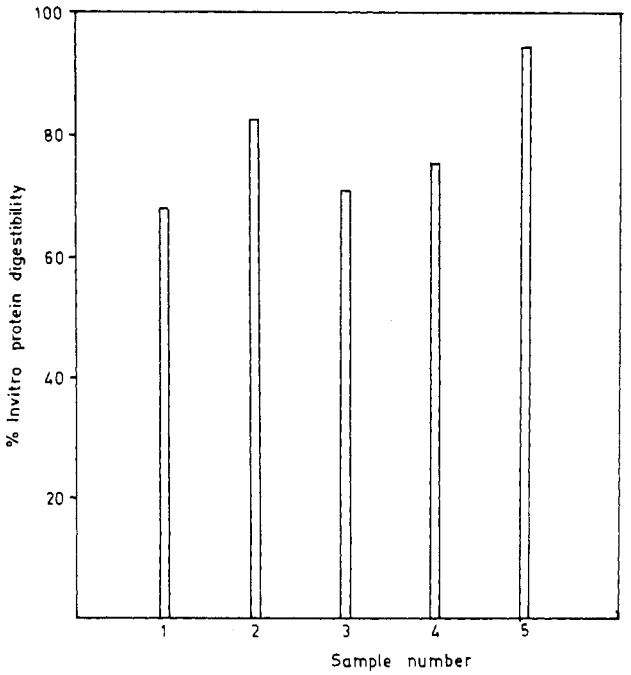

Figure 1. In vitro protein digestibility of processed guar meals and casein: (1) defatted guar meal; (2) 1 N HCl extracted guar meal; (3) 0.25 N HCl extracted guar meal; (4) autoclaved guar meal; (5) casein.

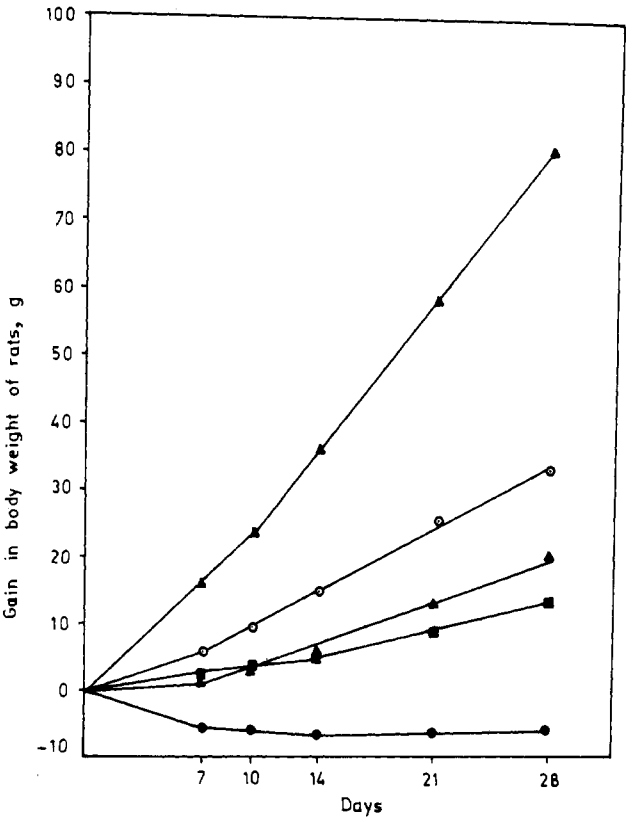

Figure 2. Growth curves of rats maintained on guar meals and casein: (●) defatted guar meal; (⊙) 1 N HCl extracted guar meal; (△) 0.25 N HCl extracted guar meal; (■) autoclaved guar meal; (▲) casein.

phenylalanine and lower contents of arginine, glutamic acid, and tryptophan, compared to the defatted meal. The most limiting amino acids in all the samples were found to be the total sulfuramino acids, with chemical scores ranging from 43 to 51, followed by lysine (Table II).

The total and the available lysine contents of autoclaved guar meal were comparable with those of the defatted meal (Table II). Nath (1980) however reported that autoclaving reduced the available lysine content of guar meal by about 10%. The 0.25 N HCl extracted meal gave a higher value for total lysine than the 1.0 N HCl extracted meal; the available lysine content of these meals was the same and much lower than the total lysine contents. Nath (1980) also reported a decrease in the available lysine content of guar meal after extraction with dilute acid.

In Vitro Digestibility. The factor that is most likely to affect the amino acid availability is the protein digestibility. The in vitro digestibility of the defatted guar meal proteins by the enzymes pepsin and pancreatin was 68% (Figure 1). Detoxification treatments increased the protein in vitro digestibility; the value for the 1 N HCl extracted meal (83%) was the highest among the various guar samples, although it was low compared to that of casein (94%). The improvement in the digestibility of legume protein, in general, seems to parallel the destruction of trypsin inhibitors and hemagglutinins (Jaffe, 1969; Liener and Kakade, 1980). However, our results on in vitro digestibility of processed guar meals did not show a linear correlation with their trypsin inhibitor activities.

Evaluation of Nutritive Value by in Vivo Methods. Figure 2 presents the growth curves of rats maintained on processed guar meals as compared to those fed casein. The defatted guar meal, as reported by others (Kawatra et al., 1969; D'Souza, 1972), did not support growth of rats; instead, a loss in their body weight was observed. The average loss in body weight of rats was 5 g in 1 week. The loss in body weight of rats was observed during the second week also, and after two weeks the change in body weight was not appreciable. In contrast to the very high mortality of rats as reported in literature (Kawatra et al., 1969; D'Souza, 1972; Misra et al., 1984), only 1 out of 10 rats maintained on defatted guar meal diet died during the fourth week of feeding.

The detoxified meals on the other hand promoted greater diet intake and growth of rats; the growth response did not however show a linear correlation with diet consumption. The average gain in body weight of rats (14–33 g in 4 weeks) fed detoxified guar meals was, however, low compared to that produced by feeding casein (81 g).

Table IV. Net Protein Utilization (NPU) of Processed Guar Meals and Casein

gp	protein source	av wt gain, g (10 days)	av N intake, g (10 days)	carcass N, g	NPU[a] (mean ± SE)
A	defatted guar meal	−6.0	0.583	1.448	
B	1 N HCl extr guar meal	14.3	1.415	2.260	53.85 ± 3.0
C	0.25 N HCl extr guar	6.4	1.248	2.204	56.43 ± 3.0
D	autoclaved guar meal	8.4	1.324	2.083	43.86 ± 3.0
E	casein	33.8	1.447	2.752	70.78 ± 3.0
F	nonprotein diet	−4.5	0.078	1.572	

[a] Note: In the NPU study, three more groups were included that are not reported here. Results of test of significance, group (average NPU): D (43.86); *B (53.85)*; *C (56.43)*; E (70.78). Any two means not italicized are *significantly* different.

Kawatara et al. (1969) also observed low growth of rats fed hot water or 1 N HCl extracted guar meal as compared to those fed casein and attributed this to the deficiency of methionine and lysine in the meal proteins. However, in the present study the 1 N HCl extracted guar meal gave significantly higher growth than the 0.25 N HCl extracted meal, though the chemical score of its protein was the lowest among the detoxified samples.

Data on the NPR and PER assay of processed guar meals as compared with those of casein aree given in Table III. Both the NPR and PER values of defatted guar meal were negative because of loss in weight of rats in this group. The presence of antinutritional factors in the defatted meal was responsible for the negative growth of rats. Detoxification treatments improved the nutritive value of the meal as indicated by the NPR and PER values of detoxified meals. The acid treatments were found to be better than autoclaving in improving the nutritive value of the meal. This could be due to the fact that autoclaving inactivates mainly the heat-labile antinutritional factors such as trypsin inhibitors while during acid treatments substances such as saponins, polyphenols, etc., which affect the nutritional value of proteins, are also leached out, in addition to acid denaturation of proteinacious antinutritional factors. Although the NPR values of the variously processed meals were comparable, their PER values showed significant difference, indicating that the variation in the growth-promoting quality of detoxified meals was more evident in the long-term (28-day) feeding trial. In this study, the PER of casein (3.77) was much higher than the normally reported value of 2.5–3.0; such higher values have been reported in literature (Sosulski and Fleming, 1977; Thompson, 1977).

The NPU data of the various samples are presented in Table IV. It was observed that the carcass nitrogen of the defatted guar meal fed rats was lower than that of nonprotein group, thereby giving negative NPU values for defatted guar meal. The NPU values of the detoxified meals also indicated that the acid treatments were better than autoclaving in improving the nutritional quality of guar meal proteins. The 1 N HCl extracted guar meal diet resulted in significant higher growth of rats (14.3 g) as compared to 0.25 N HCl extracted meal diet (6.4 g). However, their NPU values were comparable (53.9 and 56.4, respectively). This shows that though the proteins in these meals differ in their growth-promoting ability, they are utilized to the same extent by rats.

Thus, all biological parameters indicated that extraction of guar meal with 1 N HCl was more effective in improving the protein quality of guar meal. This was particularly true for the growth-promoting quality of guar proteins. This could be attributed to the very effective inactivation/removal of antinutritional factors in this method of detoxification.

ACKNOWLEDGMENT

We thank Indira A. S. Murthy for her help in the statistical analysis of the biological data and M. C. S. Sastry for carrying out amino acid analyses of the samples.

Registry No. HCl, 7647-01-0; trypsin inhibitor, 9035-81-8; L-lysine, 56-87-1.

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Received for review June 13, 1985. Accepted May 12, 1986.

Determination of Pyridoxine β-Glucoside Bioavailability Using Intrinsic and Extrinsic Labeling in the Rat

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A major form of vitamin B_6 in plant-derived foods is 5′-*O*-(β-D-glucopyranosyl)pyridoxine (PN-β-glucoside). In this study, the bioavailability of vitamin B_6 as tritiated PN-β-glucoside in purified form and in intrinsically enriched alfalfa sprouts was examined relative to ^3H- and ^{14}C-labeled pyridoxine (PN). Twenty-four hours after administration of the isotopes in a single test meal, isotopic contents of tissues and excreta and distribution of vitamin B_6 metabolites were determined. The extent of intestinal absorption of PN-β-glucoside was approximately half that of PN. PN-β-glucoside, when fed in purified form or in sprouts, was partially hydrolyzed in vivo and utilized vitamin B_6. Over 80% of urinary ^3H derived from dietary PN-β-glucoside was in the form of the intact glucoside, however. These results indicate incomplete bioavailability of vitamin B_6 as a glucose conjugate.

The vitamin B_6 content of various plant-derived foods has been determined following hydrolytic treatment to release bound forms of the vitamin. This process may not be reflective of the digestive process in the human gastrointestinal tract, and the analytical results may not represent the amount of vitamin available for metabolic utilization. A conjugated form of pyridoxine and glucose (pyridoxine β-glucoside) has been isolated from rice bran and identified as 5′-*O*-(β-D-glucopyranosyl)pyridoxine (Yasumoto et al., 1977). The amount of vitamin B_6 present as a glycosidic conjugate has been determined in several foods and was found to represent 50% or more of the total vitamin B_6 in some fruits, vegetables, and legumes (Kabir et al., 1983a). Tsuji et al. (1977) reported that chemically synthesized pyridoxine β-glucoside both is biologically available as vitamin B_6 to rats that are B_6 deficient and is permeable to everted sacs of rat small intestine. In contrast, Kabir et al. (1983b) reported an inverse relationship between the glycosylated vitamin B_6 content of a food and its vitamin B_6 bioavailability in humans as indicated by measurement of urinary 4-pyridoxic acid and plasma pyridoxal phosphate.

The purpose of the present study was to investigate the distribution of tritium in the tissues and excreta of rats fed [^3H]pyridoxine β-glucoside in a purified form or in alfalfa sprouts and to identify any [^3H]vitamin B_6 compounds resulting from metabolism of pyridoxine β-glucoside. Thus, the bioavailability of pyridoxine β-glucoside in the rat could be determined on the basis of the metabolism and retention of the compound and its presence in the excreta. A unique aspect of this study was the intrinsic enrichment of a plant-derived food, alfalfa sprouts, for the evaluation of vitamin B_6 bioavailability.

MATERIALS AND METHODS

Animals and Diets. Male Sprague–Dawley rats (approximately 200 g) (Crl:CD(SD)BR) from Charles River Breeding Laboratories, Wilmington, MA, were individually housed in stainless-steel metabolism cages with wire mesh floors and were fed a commercially pelletted diet (#5001, Ralston Purina, St. Louis, MO) ad libitum. In addition, the rats were fed daily approximately 10 g of 1% w/w calcium alginate gel and Kelco Co., San Diego, CA) that was made 17% w/w sucrose. This gel was fed to the rats between 9:00 a.m. and 11:00 a.m. each day. Rat chow pellets were removed from the cage until the rat had consumed most of the gel. After 6–7 days of conditioning the rats were fed a weighed portion of an alginate gel (8 g) containing the appropriate sources of radiolabeled vitamin B_6. Two hours after the gel had been fed, any gel remaining in the cage was removed and the pelletted chow supplied ad libitum. The rats were decapitated 24 h after the radiolabeled gel had been fed. Livers were rapidly excised and frozen along with carcass, intestinal contents, plasma, and urine for subsequent analysis.

All experimental gels were extrinsically enriched with [^{14}C]pyridoxine (0.246 μCi/g, 4.20 nmol/g). The control diet was also enriched with [^3H]pyridoxine as the source of tritiated vitamin B_6 (0.243 μCi/g, 0.170 nmol/g). Alfalfa sprouts, which had been intrinsically enriched with [^3H]-pyridoxine as described later, served as a source of [^3H]-PN-β-glucoside in crude or purified form for enrichment of gels. The gelled diets were formulated with tritium sources as follows: purified [^3H]PN-β-glucoside, 0.253 μCi/g, 4.7 nmol/g; 12.5% w/w raw intrinsically enriched alfalfa sprouts, 0.201 μCi/g, 5.0 nmol/g; 12.5% w/w intrinsically enriched alfalfa sprouts heated in an autoclave at 121 °C for 20 min, 0.240 μCi/g, 5.80 nmol/g. All of these data refer to microcuries or nanomoles per gram of hydrated alginate gel as fed to the rats.

All unlabeled forms of vitamin B_6 used in this study were obtained from Sigma Chemical Co. (St. Louis, MO). [^3H]Pyridoxine hydrochloride (1.4 Ci/mmol) was purchased from Amersham Corp. (Arlington Heights, IL). The distribution of tritium reported by the manufacturer was as follows: methyl, 61.9%; 5-methylene, 2.0%; 4-methylene, 21.9%; C-6, 11.9%. [4,5-^{14}C]Pyridoxine hy-

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0021-8561/86/1434-0857$01.50/0