PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 173, 568–573 (1983)

Effect of Dietary Fiber on Absorption of B-6 Vitamers in a Rat Jejunal Perfusion Study[1] (41688)

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Abstract. Previous research has indicated that dietary fiber may affect the absorption and utilization of certain nutrients. To determine the effect of certain fiber materials on the absorption of B-6 vitamers, jejunal segments from young male adult rats were perfused *in situ* with a control solution containing 0.02 m*M* pyridoxine (PN), 0.02 m*M* pyridoxal (PL), and 0.02 m*M* pyridoxamine (PM), followed by a test solution containing the same vitamin B-6 mixture and one of five fiber-rich test materials (cellulose, pectin, lignin, homogenized fresh carrot, or carrot homogenized after 10 min boiling) added at a concentration of 1–3%. The mean absorption rates of PL, PN, and PM from the control solution were, respectively, 3.66 ± 0.23, 2.06 ± 0.23, and 1.74 ± 0.37 nmole/min/20 cm jejunal segment. There were no significant differences between the absorption rates of B-6 vitamers from control and test solutions containing cellulose, pectin, and lignin. The absorption rates of PM and PL were significantly depressed ($P < 0.05$ and $P < 0.01$, respectively) by the presence of fresh or cooked carrot. The absorption rate of PN in presence of cooked carrot was also decreased relative to the control value but the difference was only marginally significant ($P < 0.10$). When the concentration of fresh carrot in the test solution was increased to 10% by weight and the perfusion rate was decreased from 1.91 to 0.49 ml/min in a second perfusion experiment, there was a significant increase in variability and the differences between absorption rates of the B-6 vitamers in control and test solutions were not statistically significant. The limited evidence of adverse effect of carrot on absorption of vitamin B-6 suggested the need for further clarification of the influence of dietary fiber in an unrefined state on the bioavailability of vitamin B-6.

An assessment of the adequacy of a dietary intake of vitamin B-6 requires not only knowledge of the vitamin B-6 content of the foods ingested but also the extent to which the vitamin present in the diet is available for absorption and utilization. Because several studies have indicated a marginal vitamin B-6 status in certain groups of the American population (1–3), it is important to investigate factors which may affect the bioavailability of dietary vitamin B-6. Research has shown that dietary fiber may retard the absorption of certain cationic materials (4, 5). Luther *et al.* (6) reported *in vitro* interactions of folacin with various polysaccharides. Since it has been suggested that dietary fiber consumption be increased (7, 8), it is important that its effects on the absorption and utilization of other nutrients be fully examined. Previous research has suggested that the bioavailability of vitamin B-6 may be reduced by nondigestible polysaccharides in foods (9–12) although, in these studies, the relative effects of dietary fiber materials and other food components could not be determined.

In this study, the relative absorption rates of B-6 vitamers in absence and presence of dietary fiber materials were determined using a rat jejunal perfusion procedure. The test materials were selected to include dietary fibers which differ in chemical and physical properties and which are naturally occurring or added food or feed components. Cellulose is used as a fiber source in many semipurified diets for scientific investigation and as an ingredient of certain foods; pectin is often used as a food additive for textural or other physical properties; lignin is part of plant cell walls and one of the main components of "dietary fiber." Carrot was included as a source of fiber which is high in hemicellulose in its unrefined state.

[1] Florida Agricultural Experiment Stations Journal Series No. 4420.

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0037-9727/83 $1.50

Materials and Methods. *Test materials.* Test materials, representing dietary fiber as semipurified and natural food forms were: cellulose (Aircel, Hercules, Inc.), lignin (ATR, Indulin, Westvaco), citrus pectin (rapid set, No. 3342, Sunkist Growers, Inc.), and carrots (purchased at a local supermarket). The carrots were peeled, diced, and homogenized with a blender for 2 min either immediately or after boiling for 10 min. Pyridoxine hydrochloride (PN), pyridoxamine dihydrochloride (PM), and pyridoxal hydrochloride (PL) were purchased from ICN Pharmaceuticals, Inc.

Control perfusion solutions contained a mixture of the three free-base forms of vitamin B-6, each at a concentration of 0.02 m*M*. The vitamin B-6 level used reflects the vitamin B-6 content of many common foods (13). Test perfusion solutions contained the same concentrations of vitamin B-6 plus 1% pectin, cellulose, or lignin; or 3% of either fresh or cooked and homogenized carrot. The estimated concentration of vitamin B-6 provided by the carrot (ca. 0.9 μM) was negligible relative to the concentration of the added vitamers. All solutions were adjusted to pH 6.5 with NaOH and 300 mOsm with NaCl, as measured by freezing point depression. In a second experiment, 10% fresh carrot solution was perfused in presence of vitamin B-6 at a slower rate than that used in the first experiment. Difficulty in perfusing more concentrated carrot homogenates precluded a comparison with the semipurified fiber materials on an equal solids basis.

Perfusion procedure. Male Sprague–Dawley rats [strain Crl:CD(SD)BR, Charles River Breeding Laboratories], weighing 300–500 g and maintained in accredited animal care facilities on a lab chow diet, were fasted but allowed water *ad libitum* 12–16 hr before the perfusion operation. Each rat was anesthetized with ketamine hydrochloride intramuscularly (Kataset, Bristol Laboratories), 87 mg/kg. The abdomen was shaved and cleansed with povidone iodine. A midventral abdominal incision extending 5–6 cm anteriorly to the pectoris minor was made, and the small intestine exposed. The ligament of Trietz was located and marginal vessels were doubly ligated before a small incision was made 4–6 cm below the ligament of Trietz. A distance of 20 cm was measured along the antimesenteric border of the bowel and a second small incision similar to the first was made. Polyvinyl catheters were inserted into the proximal and distal ends of the isolated jejunal segment and fixed with double ligatures of 4-O Ethicon suture. The jejunal segment was gently flushed with 15–20 ml saline solution until the effluent was clear and free of particulate matter. The small intestine was returned to the peritoneal cavity and the incision covered with saline-moistened gauge sponges warmed with a lamp. The procedure was carefully monitored to avoid obstruction and distention of the perfused segment.

The tubing of the proximal end was attached to an infusion pump (Model 940, Harvard Apparatus Co.) and the perfusate was collected at the distal end by gravity. All solutions were warmed to 37°C before perfusion, and protected from light with aluminum foil. Groups of four to eight rats were used for each test material and each rat was perfused with the control solution prior to perfusing with a test solution. Perfusion rates of 1.91 and 0.49 ml/min were used in the first and second experiments, respectively. A 20- to 30-min equilibration period preceded collection of duplicate 10-ml aliquots of the perfusate. No significant differences in flow rates were detected between the duplicate fractions collected. Collected samples were kept frozen at −20°C until analyzed for vitamin B-6 by high-performance liquid chromatography (HPLC).

Water absorption was monitored by recording collected volumes at timed intervals and comparing the output flow rate with the pumping rate. Middleton (14) reported no differences in results of water absorption calculated by this method or determined by the use of ^{14}C-dextran, a nonabsorbable marker. The absorption rates of the B-6 vitamers in presence or absence of test material were determined according to the formula

$$Cl_A = C_{in} \times F_1 - C_{out} \times F_2$$

where Cl_A = absorption clearance rate, nanomoles per minute; C_{in} and C_{out} = concentrations of B-6 vitamers, nanomoles per milliliter, of perfusion solutions before and after perfusion, respectively; F_1 = flow rate set by the infusion pump, milliliters per minute; and F_2 = measured output flow rate, milliliters per minute.

Vitamin B-6 analysis by HPLC. The reversed phase HPLC method developed by Gregory and Kirk (15) was used in the first experiment to determine the B-6 vitamer concentrations of control and test solutions before and after the perfusion operation. The HPLC apparatus included an Altex Model 312 chromatograph, an octadecylsilica column (Partisil 10 ODS-3, 25 cm × 4.6 mm i.d.), Whatman, In.), and an Aminco Fluoro-Monitor detector. The mobile phase, 0.033 *M* potassium phosphate, pH 2.2, was pumped at a flow rate of 1.5 ml/min and a 30-min equilibration period preceded the injection of 100-μl samples. Fluorometric detection of the B-6 vitamers was performed using a 70-μl flow cell, a Germicidal lamp (General Electric Model C4T41), a 295-nm Baird Atomic excitation filter (American Instrument Co.), and a 405-nm narrow pass emission filter (Turner Associates) in the Fluoro-Monitor. An internal standard, 4-deoxypyridoxine (4DPN), was added to standard solutions and samples at a concentration of 0.12 m*M*. Results were recorded and computed by an electronic integrator (Hewlett-Packard Model 3388A). Figure 1 shows a typical chromatogram of a calibration mixture of B-6 vitamers obtained under the described operating conditions.

In the second experiment, the B-6 vitamers were analyzed by the same HPLC apparatus but using the ion-pair chromatography method developed by Tryfiates and Sattsangi (16), with minor modifications. The calibration mixture contained PL, PN, and PM (each at a concentration of 0.59 nmole/ml) and 4DPN (0.8 μg/ml). Control and test perfusates were diluted 1:20 with solvent A (10% 2-propanol and 0.09% glacial acetic acid) after addition of 4DPN as internal standard (4.22 nmole/ml). The column (Partisil 10 ODS-3, Whatman, Inc.) was equilibrated with solvent B (solvent A with addition of sodium salts of 1-octane sulfonic acid and 1-heptane sulfonic acid, each at 0.004 *M*) for 15 min prior to injection and elution of samples with solvent A at a flow rate of 0.8 ml/min. Detection of B-6 vitamers was performed fluorometrically (Perkin Elmer, Model LS-5) at 291 nm excitation and 412 nm emission wavelengths. The ion pair HPLC method provide an alternate mechanism of separation which yielded greater retention of PM and greater resolution of all sample components. A direct comparison of the conventional reverse-phase (15) and the ion-pair methods (16) on selected perfusates from the first experiment yielded comparable results.

Statistical analysis. A paired *t* test was used to compare vitamin B-6 absorption rates in the presence and absence of fiber materials. Differences in absorption rates between PN, PL, and PM in control solutions were evaluated by one-way analysis of variance followed by the Tukey procedure for multiple comparisons (17). All data are expressed as mean and standard error.

Results. The monitoring of water absorption/secretion during the perfusion operation showed that most animals were either in equilibrium or in a slightly absorptive state. The mean rate of water absorption was 0.067 ± 0.045 ml/min/20 cm jejunal segment in the first experiment and 0.001 ± 0.003 ml/min/20 cm segment in the second experiment. Twenty-six rats from four test groups (cellu-

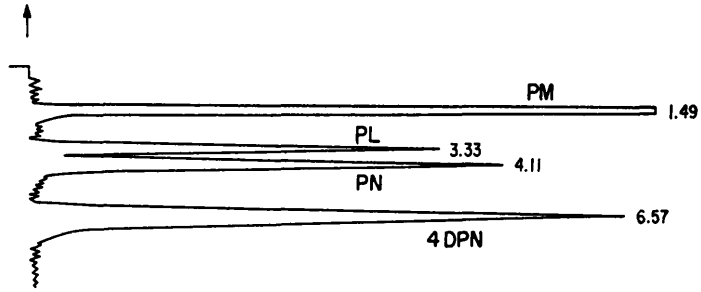

FIG. 1. Chromatogram of a calibration mixture of B-6 vitamers analyzed by reverse-phase HPLC. Numbers indicate the retention time in minutes. The sample mixture (100 μl) was composed of 20 μ*M* pyridoxamine (PM), pyridoxal (PL), and pyridoxine (PN), and 120 μ*M* 4-deoxypyridoxine (4DPN), the internal standard.

lose, lignin, pectin, and cooked carrot) were pooled for the determination of differences in rat absorption of PN, PL, and PM from the control solution. The mean absorption clearance rates of PL, PN, and PM were, respectively, 3.66 ± 0.23, 2.06 ± 0.23, and 1.74 ± 0.37 nmole/min/20 cm jejunal segment. The differences between absorption rates of the three B-6 vitamers from the control solution were statistically significant ($P < 0.005$).

Table I presents absorption clearance rates of PM, PL, or PN in test solutions containing cellulose lignin, pectin, cooked carrot, or raw carrot as compared to clearance rates of the B-6 vitamers in blank control solutions in the first perfusion experiment. Although there was no significant difference ($P > 0.05$) in absorption clearance rates of blank control and test solutions containing either cellulose, lignin, or pectin, the absorption rates of PM, and PL were significantly depressed ($P < 0.05$ and $P < 0.01$, respectively) by the presence of either raw or cooked carrot. The absorption rate of PN in the presence of cooked carrot was also decreased relative to the control value but the difference was only marginally significant ($P < 0.10$). Except with the cooked carrot group which showed a higher absorption rate of PM relative to PN, the order of absorption rates of the three vitamers in presence of test dietary fiber was the same as with the control solution, e.g., PL > PN > PM.

When the concentration of raw carrot in the test solution was increased in 10% by weight and the perfusion rate was decreased to 0.49 ml/min in the second experiment, there was much variability among the absorption rates, and the differences between absorption rates of the B-6 vitamers in control and test solutions were not significant (Table II).

Discussion. In this rat jejunal perfusion study, each rat served as its own control. The time required to perfuse both control and test solutions did not exceed the 3 hr that Savina *et al.* (18) demonstrated to be the time limit after which a physiological deterioration of the animal under prolonged anesthesia was observed. The absorption rates observed (Table I) were higher than the rate of *in vitro* uptake of PN by rat jejunal everted sacs in the study by Middleton (19), who observed a ^{3}H-PN uptake rate of 0.0255 nmole/4 min/cm when the concentration of ^{3}H-PN in the incubation medium was only 2 μM. Data from the *in vivo* study of PN absorption in the rat by the same author (20) indicated an *in vivo* PN absorption rate equivalent to about 0.006 nmole ^{3}H-PN/min/20-cm jejunal loop when the solution injected into the jejunal loop was as low as 0.2 μM. The uptake of PN hydrochloride has been demonstrated to be linear over a range of 0.2 to 75 μM due to absorption by passive diffusion (20), and the higher ab-

TABLE I. ABSORPTION RATES OF B-6 VITAMERS IN PRESENCE AND ABSENCE OF VARIOUS TYPES OF DIETARY FIBER IN THE FIRST RAT JEJUNAL PERFUSION EXPERIMENT

Dietary fiber tested[a]	N	Vitamin B-6 absorption rates,[b] nmole/min/20 cm jejunal loop					
		PM		PL		PN	
		Control	Test	Control	Test	Control	Test
Cellulose	(8)	0.98 ± 0.77	0.48 ± 0.99	3.88 ± 0.53	4.04 ± 1.70	2.40 ± 0.46	2.41 ± 1.32
Lignin	(6)	1.08 ± 0.19	0.98 ± 0.35	2.94 ± 0.37	3.22 ± 0.46	1.79 ± 0.40	1.41 ± 0.44
Pectin	(4)	1.29 ± 1.36	2.90 ± 1.68	3.88 ± 0.81	6.08 ± 1.62	2.06 ± 0.73	2.50 ± 0.87
Carrot, cooked	(8)	3.21 ± 0.40	1.42 ± 0.68[c]	3.87 ± 0.23	1.94 ± 0.32[d]	1.92 ± 0.40	1.29 ± 0.63
Carrot, raw	(8)	8.27 ± 1.00	4.35 ± 1.46[c]	4.63 ± 0.42	1.21 ± 0.43[d]	3.44 ± 0.88	2.03 ± 0.77

[a] Concentrations of dietary fiber in test perfusion solutions were 1% for cellulose, lignin, and pectin, and 3% for raw and cooked carrot. Numbers in parentheses indicate number (N) of animals in various groups.

[b] Mean ± SE. Concentrations of PM, PL, and PN in control and test solutions were 0.02 mM. Perfusion performed at a flow rate of 1.91 ml/min. Concentrations of B-6 vitamers before and after perfusion analyzed by reverse-phase HPLC. Mean water absorption for all groups: 0.067 ± 0.045 ml/min (differences in water absorption values for various groups not significant).

[c] Significantly different from control value ($P < 0.05$).

[d] Significantly different from control value ($P < 0.01$).

TABLE II. ABSORPTION RATES OF B-6 VITAMERS IN PRESENCE AND ABSENCE OF RAW CARROT IN SECOND PERFUSION EXPERIMENT

B-6 vitamers	N	Absorption rates,[a] nmole/min/20 cm jejunal loop	
		Control	Test
PM	6	0.64 ± 1.18	1.19 ± 0.95
PL	8	1.53 ± 1.46	3.26 ± 1.11
PN	5	1.75 ± 1.46	2.90 ± 1.19

[a] Mean ± SE. Flow rate of perfusion pump was 0.49 ml/min. Control solutions contained a mixture of PN, PM, and PL, each at a concentration of 0.02 m*M*. Test solutions contained 10% carrot in addition to the vitamin mixture. B-6 vitamers concentrations analyzed by ion-paired reverse-phase HPLC. Differences between absorption rates of B-6 vitamers from control and test solutions not significant ($P > 0.05$). Mean water absorption rate ± SE = 0.001 ± 0.003 ml/min (no significant differences in water absorption rates between control and test perfusion periods).

sorption rates observed in this study are consistent with the higher concentration of PN used in the perfusion solution (20 μM). Mehansho *et al.* (21) and Hamm *et al.* (22) suggested 0.02 μmole B-6 vitamer in a 1.0-ml adminstered volume as a physiological amount for the rat. This is equivalent to the B-6 vitamer concentration used in this study, although the perfusion rates and total volume of perfusate of this study were higher.

The rats perfused with the raw carrot solution showed an unusually high absorption rate of PM from both control and test solutions which could not be adequately explained. To eliminate the possibility of artifact in the results of this group, only the four other rat groups in the first experiment (cellulose, pectin, lignin, and raw carrot) were pooled in the comparison of absorption rates of various B-6 vitamers from control solutions. The mean absorption rates of PL were approximately double the absorption rates of PM (3.66 ± 0.23 and 1.74 ± 0.37 nmole/min/20 cm jejunal segment, respectively). These observed absorption rates of PL relative to PM are in general agreement with findings by other authors. Mehansho *et al.* (21) reported absorption of 40% of amount of PL or PLP injected in the lumen of rat small intestine after 10 min while Hamm *et al.* (22) indicated 17–20% absorption of PM or PMP in the same length of time. The order of absorption rates of the three B-6 vitamers (PL > PN > PM) in this study is also supported by reports from earlier research work (23).

A comparison of absorption rates of B-6 vitamers in blank control solutions and dietary fiber-containing test solutions showed that the differences are not statistically significant ($P > 0.05$) for cellulose, lignin, and pectin, which are dietary fiber components in purified or semipurified form. The presence of carrot in the test solution in both cooked and raw forms decreased the absorption rates of PM ($P < 0.05$) and PL ($P < 0.01$) by half.

In the second rat perfusion experiment, significant differences in absorption rates of B-6 vitamers in presence and absence of carrot were expected since the concentration of carrot, the test material that had been shown to have an inhibitory effect on vitamin B-6 absorption, was increased in the test solution, and the infusion rate was slowed to 0.49 ml/min. The differences in absorption rates between control and test solutions observed in this experiment were not statistically significant, mostly as a result of the high variability in responses within the rat groups (Table II). The infusion rate of 0.49 ml/min used in this study is close to the optimal perfusion rate (0.59 ml/min) recommended by Savina *et al.* (18) for *in situ* intestinal absorption studies in rats. Middleton (14), however, has reported high variability in rat responses with this perfusion rate and chose to use a much smaller perfusion rate in his studies. Although effort was made to avoid uneven flow of the test fluid through the intestinal segment, the viscosity of the homogenate in the second experiment caused intermittent partial obstruction, which may have contributed to the high variability in results in this study. Increased variability also was observed in controls at the low flow rate, however.

The effects of dietary fiber in unrefined form from various plant-derived foods on vitamin B-6 absorption need to be further elucidated. Previous research in our laboratory has indicated little or no adverse effect of purified or partially refined dietary fiber materials on the utilization of PN by rats and chicks (24) and no binding of PN, PM, or PL to a wide variety of dietary fiber components *in vitro* (25). The results of this perfusion study suggest

that certain naturally occurring fiber components may reduce the bioavailability of vitamin B-6. These results support those of Nelson *et al.* (12) which indicated that certain unidentified components of orange juice may retard the absorption of B-6 vitamers in the human intestine. Further study will be directed toward this phenomenon in order to more fully elucidate the factors influencing the bioavailability of this vitamin.

This research was supported by Grant 5901-0410-9-0305-0 from the Competitive Research Grants Office, USDA-SEA.

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Received December 7, 1982. P.S.E.B.M. 1983, Vol. 173.