In Vitro Interaction of 1-^{14}C-Ascorbic Acid and 2-^{14}C-Thiamin with Dietary Fiber[1]

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ABSTRACT

Hard red spring (HRS), soft white winter (SWW) wheat brans, citrus pectin (CP), and alphacel cellulose (AC) were studied for their ability to influence the availability (not trapped by, bound to, or destroyed by the fiber matrix) of 1-^{14}C-ascorbic acid or 2-^{14}C-thiamin in vitro. HRS and SWW wheat brans seemed to have a stabilizing influence on preventing the degradation of ascorbic acid that diminished as the level of the fiber increased in the incubation mixture. The availability of ascorbic acid decreased with increasing amounts of HRS, SWW, or CP incubated with saline and with HRS, SWW, or CP incubated with sodium taurocholate and saline ($P < 0.005$). Availability of ascorbic acid was not related to increasing levels of AC, incubated with or without sodium taurocholate. The availability of thiamin decreased significantly with increasing HRS or SWW incubated with saline and with HRS, SWW, or CP incubated with sodium taurocholate and saline ($P < 0.05$). Free thiamin in mixtures of AC incubated with or without sodium taurocholate was not significantly different from that of mixtures containing no AC. In general, the availability of ascorbic acid decreased at pH 5.5 and above in the presence of all fiber sources. The relationship between pH and thiamin availability was more pronounced than the relationship between pH and ascorbic acid in the presence of fiber sources. The interaction between ascorbic acid and selected sources of dietary fiber may be the result of ascorbic acid being trapped by water held in the fiber matrix or adsorption (binding) to the fiber matrix and some loss due to degradation. However, the loss of availability of thiamin is more consistent with binding or being trapped by water held in the fiber matrix.

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Increased inclusion of fiber in the diets of the Western World has resulted in renewed interest in fiber by nutritionists and various health-allied professionals. Recently, the United States Department of Agriculture and the Food and Nutrition Board of the National Research Council proposed a policy recommending diets that include dietary fiber (U.S. departments of Agriculture and Health and Human Services 1980). Divergent conclusions have been reached by different groups of investigators regarding the benefits and adverse effects of high fiber consumption (Kelsay 1978, Losowsky 1977, Scala 1974). Because long-term dietary fiber treatment is often recommended, researchers wish to ascertain whether the intake of large amounts of dietary fiber may cause undesired side-effects such as lower bioavailability of certain essential metals.

One area that has received relatively little formal research is the effect of typical dietary fiber constituents on the bioavailability of vitamins. Utilization or absorption of vitamins B_{12} and B_6 have been reported to be affected adversely by diets high in fiber (Cullen and Oace 1977, Leklem et al 1980). Dietary fiber influences urinary excretion of ascorbic acid (Keltz et al 1978) with increased intake of hemicellulose, resulting in increased ascorbic acid excretion.

The objective of this in vitro study was to determine what effect, if any, typical dietary fiber sources have on selected water-soluble vitamins that are or might be consumed with cereals, cereal grain products, or other foods.

MATERIALS AND METHODS

Sources of Chemicals

L-Ascorbic acid, thiamin, methylbenzethonium hydroxide (hyamine hydroxide), and sodium taurocholate were obtained from the Sigma Chemical Co., St. Louis, MO. Citrus pectin (CP) and alphacel cellulose (AC) were obtained from ICN Nutritional Biochemicals, Cleveland, OH. Hard red spring (HRS) and soft white winter (SWW) certified wheat brans were obtained from the American Association of Cereal Chemists. L-[1-^{14}C]-ascorbic acid (9.0 mCi/mmol) was obtained from the New England Nuclear Corp., Boston, MA. Aqueous Counting Scintillant (ACS) and [thiazole-2-^{14}C] thiamin hydrocholoride (5.0 mCi/mmol) were obtained from Amersham/Searle, Arlington Heights, IL. All other chemicals were of the highest purity obtainable from commercial sources. Homogeneities of [1-^{14}C]-ascorbic acid and [2-^{14}C]-thiamin were confirmed by thin-layer chromatography to be 95 and 99%, respectively (Klain et al 1981).

[1] Reference to a company or product does not imply approval or recommendation of the product by the USDA to the exclusion of others that may also be suitable.

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Vitamin-Binding Assay

Binding of ascorbic acid and thiamin was measured by a method modified from Kritchevsky and Story (1974) that was used to measure the binding of bile salts. Substrate solutions of ascorbic acid or thiamin were made by dissolving the respective vitamin (1 mM) in 0.15M NaCl. Tracer quantities (0.1 μCi/incubation) of L-[1-^{14}C]-ascorbic acid or [thiazole-2-^{14}C] thiamin hydrochloride were included in the solution. All incubations contained 5 ml of the substrate solution with a known amount of the fiber sources being tested. Each incubation was performed in a stoppered test tube that was shaken at 37° C for 1 hr. After incubation, an aliquot of the mixture was centrifuged at 9,000 × g for 30 min (Kritchevsky and Story 1974).

A 0.4-ml aliquot of the supernatant was digested with 0.4 ml of hyamine hydroxide at 50–55° C for 60 min. A 0.2-ml aliquot was analyzed for radioactivity in ACS scintillation fluid by liquid scintillation spectrometry. The amount of vitamin bound or not available was calculated as the difference between the amount of vitamin added and the amount recovered in the supernatant. Available vitamins are those vitamins that are not trapped by, bound to, or destroyed by the fiber matrix.

Experiments were performed to investigate the binding of ascorbic acid or thiamin to natural and semipurified fiber sources, with or without the presence of bile salt, sodium taurocholate. Bran concentrations were 40, 100, and 200 mg per incubation with pectin and cellulose concentrations of 20, 60, and 120 mg per incubation (Kritchevsky and Story 1974). The pH of incubation mixtures containing sodium taurocholate was 5.1–5.2. To test the influence of pH on the interactions between dietary fiber and ascorbic acid or thiamin, each respective fiber was suspended in 5 ml of 0.05M phosphate buffer at the appropriate pH values of 1.5–8.0.

Degradation of ^{14}C-Ascorbic Acid

The determination of the amount of oxidation of ^{14}C-ascorbic acid was similar to a procedure published elsewhere (Klain et al 1981). Five-milliliter aliquots of saline solution were added to 25-ml Erlenmeyer flasks containing 0.2 μCi of 1-^{14}C-ascorbic acid and 100 mg of HRS or 60 mg of CP or AC. The flasks were stoppered with a rubber stopper from which a plastic well was suspended and were incubated with shaking at 37° C. After 1 hr of incubation, 0.2 ml of hyamine hydroxide was injected into the well and 2 ml of 2N H_2SO_4 into the flask solution. The flasks were shaken for an additional hour to trap $^{14}CO_2$. We also examined the effect of not adding 2N H_2SO_4 into the flask solution. Afterward, the wells were removed and placed into scintillation vials, and radioactivity was determined.

Statistics

Data were analyzed statistically by Student's t-test or by analysis of variance. Values are expressed as percent of initial added radioactivity.

RESULTS

Relationship Between the Concentration of Dietary Fiber Sources and ^{14}C-Vitamins

The results of the analysis for 1-^{14}C-ascorbic acid remaining after 1 hr of incubation at 37° C with specific dietary fibers are tabulated in Table I. Analysis of variance comparing the fiber substance with no fiber illustrated a significant decrease in radioactivity with increasing fiber concentration: HRS (in saline), $P < 0.001$; SWW (in saline), $P < 0.001$; pectin (in saline), $P < 0.005$; HRS (in sodium taurocholate), $P < 0.001$; SWW (in sodium taurocholate), $P < 0.005$; and pectin (in sodium taurocholate), $P < 0.005$. Analysis of variance showed no significant effect of cellulose. Both HRS and SWW wheat brans seemed to have a stabilizing influence, preventing the degradation of ascorbic acid that diminished as the concentration of the fiber increased in the incubation mixture. AC had less of this influence, and the concentration of CP was directly related to the loss of 1-^{14}C-ascorbic acid. Loss of ascorbic acid was statistically related to the concentration of all the fiber sources except AC. In general, the addition of bile as sodium taurocholate had no significant influence. The addition of bile to the 100-mg SWW wheat bran sample did result in 4.4% more loss of 1-^{14}C-ascorbic acid. The addition of 1-^{14}C-ascorbic acid to incubation solutions without fiber and to solutions without fiber but including bile resulted in 38.8 and 35.5% loss of 1-^{14}C-ascorbic acid, respectively.

The results of dietary fiber on ^{14}C-thiamin are shown in Table II. Analysis of variance comparing the fiber substance illustrated a significant decrease in radioactivity with increasing fiber concentration: HRS (in saline), $P < 0.05$; SWW (in saline), $P < 0.05$; HRS (in sodium taurocholate), $P < 0.005$; SWW (in sodium taurocholate), $P < 0.001$; pectin (in sodium taurocholate) and pectin (in saline), $P < 0.05$. Analyses of variance showed no significant effect of cellulose. In this experiment the loss in ^{14}C-thiamin was more clearly representative of binding to the fiber. Little loss of ^{14}C-thiamin occurred in incubation solutions with or without bile and containing no dietary fiber, suggesting no significant degradation of that particular vitamin. Analysis of variance comparing the fiber substances illustrated a significant decrease in radioactivity with increasing fiber concentration, with the exception of AC with or without bile. At 40 mg of bran, both HRS and SWW wheat brans showed increased loss of ^{14}C-thiamin when bile as sodium taurocholate was added. In general, the loss of ^{14}C-thiamin in the presence of fiber sources was less than that found for ^{14}C-ascorbic acid. However, CP resulted in 29.9–42.9% loss of ^{14}C-thiamin at 120 mg incubated with saline and saline plus bile, respectively, compared to 47.1–42.3% loss of ^{14}C-ascorbic acid at the same level of CP incubated with saline and saline plus bile, respectively.

Degradation of 1-^{14}C-Ascorbic Acid in Vitro

Alphacel cellulose, CP, and HRS wheat bran, representing one of the two wheat samples, were investigated at 60 mg, 60 mg, and 100 mg per incubation mixture, respectively. Table III shows that the total percentage of ^{14}C evolution as CO_2 was less than 1% of the initial radioactivity of ^{14}C-ascorbic acid added. Since the amount of $^{14}CO_2$ produced is small in relation to the amount of ^{14}C-ascorbic acid lost in the above experiment (Table I), we performed an additional experiment without the final addition of $2N$ H_2SO_4. The results shown in the third column of Table III illustrate that the amount of $^{14}CO_2$ produced is similar to the volumes produced when $2N$ H_2SO_4 was added.

Effect of pH

Incubation of ascorbic acid with CP or HRS resulted in a pH optimum of 5.5 for loss in ascorbic acid, and AC resulted in increased loss at two different pH values, 3.5 and 6.0 (Fig. 1).

The relationship between the vitamin, thiamin, and pH in the presence of HRS wheat bran, CP, and AC is shown in Fig. 2. Increases in the loss of thiamin in bran mixture became pronounced at higher pH values. CP caused some loss of thiamin at all pH values, with greater losses noted at pH 3.0 and 6.0. Alphacel cellulose was ineffective in producing a loss in thiamin.

DISCUSSION

Dietary fiber exerts several ill-defined influences on gastrointestinal functions and, subsequently, human health. Regardless of the benefits of dietary fiber, impaired absorption of fat (Beyer and Flynn 1978, Kay and Truswell 1977, Southgate et al 1976, Tarpila et al 1978, Walker 1975), protein (Beyer and Flynn 1978, Kies and Fox 1977, Southgate et al 1976), minerals, and trace elements (Bjorn-Rasmussen 1974, Dobbs and McLean Baird 1977,

TABLE I
Ascorbic Acid Remaining in Supernatant of Centrifuged Fiber Samples[a]

Fiber Substance (mg)	Ascorbic Acid Content (%)	
	Saline	Sodium Taurocholate
Hard red spring wheat bran (40)	80.9 ± 2.01	76.9 ± 0.76
Hard red spring wheat bran (100)	77.1 ± 1.43	77.5 ± 1.00
Hard red spring wheat bran (200)	69.7 ± 2.46	68.1 ± 1.70
Soft white winter wheat bran (40)	76.9 ± 1.44	75.1 ± 1.60
Soft white winter wheat bran (100)	70.3 ± 1.35	65.9 ± 0.61[b]
Soft white winter wheat bran (200)	66.4 ± 0.05	67.9 ± 1.59
Citrus pectin (20)	65.3 ± 0.03	68.3 ± 1.86
Citrus pectin (60)	62.0 ± 1.45	58.5 ± 1.14
Citrus pectin (120)	52.9 ± 2.95	57.7 ± 3.94
Alphacel cellulose (20)	72.4 ± 0.27	65.8 ± 2.55
Alphacel cellulose (60)	71.5 ± 3.53	65.6 ± 0.75
Alphacel cellulose (120)	65.4 ± 4.09	65.2 + 0.97
No fiber	61.2 ± 0.61	64.5 ± 1.25

[a]Each observation is the mean ± S.E.M. of three incubations. Each substance was incubated with 5 ml 0.15 M NaCl or 0.15 M NaCl containing 100, 240, or 480 μmol of sodium taurocholate for the three respective fiber concentrations. Values for pH of sodium taurocholate containing solutions were between 5.1 and 5.2.
[b]Significantly different ($P < 0.05$) compared to fiber substance in saline incubation.

TABLE II
Thiamin Remaining in Supernatant of Centrifuged Fiber Samples[a]

Fiber Substance (mg)	Thiamin Content (%)	
	Saline	Sodium Taurocholate
Hard red spring wheat bran (40)	94.6 ± 1.18	92.3 ± 1.50
Hard red spring wheat bran (100)	86.1 ± 3.83	82.7 ± 1.92
Hard red spring wheat bran (200)	79.1 ± 1.40	80.6 ± 2.30
Soft white winter wheat bran (40)	93.1 ± 2.01	87.6 ± 1.80[b]
Soft white winter wheat bran (100)	81.6 ± 1.43	88.1 ± 1.65
Soft white winter wheat bran (200)	82.7 ± 2.75	86.6 ± 2.43
Citrus pectin (20)	93.8 ± 2.11	90.3 ± 6.34
Citrus pectin (60)	84.6 ± 3.07	86.7 ± 3.40
Citrus pectin (120)	70.1 ± 2.83	57.1 ± 10.72
Alphacel cellulose (20)	100.7 ± 3.25	102.7 ± 3.99
Alphacel cellulose (60)	98.1 ± 3.83	100.0 ± 2.31
Alphacel cellulose (120)	95.9 ± 4.81	97.2 ± 1.61
No fiber	100.0 ± 1.72	100.0 ± 1.48

[a]Each observation is the mean ± S.E.M. of three incubations. Each substance was incubated with 5 ml 0.15 M NaCl or 0.15 M NaCl containing 100, 240, or 480 μmol of sodium taurocholate for the three respective fiber concentrations. Values for pH of sodium taurocholate containing solutions were between 5.1 and 5.2.
[b]Significantly different ($P < 0.05$) compared to fiber substance in saline incubation.

TABLE III
In Vitro Oxidation of 1-^{14}C-Ascorbic Acid[a]

Fiber Substance	Sodium Taurocholate	Saline	Saline[b]
Hard red spring wheat bran	0.50 ± 0.046	0.67 ± 0.072	0.56 ± 0.079
Citrus pectin	0.34 ± 0.011	0.17 ± 0.012	0.22 ± 0.016
Alphacel cellulose	0.41 ± 0.009	0.35 ± 0.052	0.22 ± 0.015
No fiber	0.04 ± 0.001	0.79 ± 0.039	0.46 ± 0.012

[a]The values are percentages of ascorbic acid oxidized. Each observation is the mean ± S.E.M. of three incubations.
[b]Content of reaction flask was not acidified with $2N$ H_2SO_4.

Heaton and Pomare 1974, Jenkins et al 1975) have been reported. Also, the utilization of vitamins B_{12} and B_6 has been reported to be affected adversely by diets high in fiber (Cullen and Oace 1977, Leklem et al 1980).

The observed changes in the availability of ascorbic acid or thiamin after 1 hr of incubation with HRS, SWW, and CP suggest that physicochemical interactions occur. Wheat brans may have stabilizing action, preventing ascorbic acid oxidation, perhaps through reducing compounds or through the presence of endogenous antioxidants (Kay and Strasberg 1978). At least some of the loss in availability of ascorbic acid is related to binding by fiber of wheat bran or trapping water held in the fiber matrix of wheat bran. The observation that some ^{14}C-ascorbic acid is not always recovered after incubation, even at low pH values where the degradation of ascorbic acid is minimal, supports the hypothesis that some of the loss in ascorbic acid is the result of binding or trapping by the fiber. A clearer perspective of the degree of ascorbic acid degradation to CO_2 was not possible, as the final step in the measurement of CO_2 production required acidifying of the reaction mixture, thereby releasing CO_2 for absorption by hyamine hydroxide. This final step would also favor reconversion of dehydroascorbic acid to reduced ascorbic acid (Omaye et al 1982). As shown in Fig. 3, further oxidation of dehydroascorbic acid to CO_2 would not occur. This interpretation is further supported by our observation that the amount of CO_2 generated without adding $2N$ H_2SO_4 was approximately the same as the amount of CO_2 generated with the addition of $2N$ H_2SO_4 (Table III). Ascorbic acid may be degraded to dehydroascorbic acid during incubation at 37° C with subsequent further degradation to CO_2 while samples are being centrifuged and prepared for liquid scintillation spectrometry. Ascorbic acid solutions have been known to be unstable, particularly at pHs above 7 (Omaye et al 1982).

Citrus pectin or AC (Table I) did not appreciably stabilize or prevent ascorbic acid oxidation. Addition of higher concentration of CP to the reaction mixture (Table I) resulted in more loss of availability of ascorbic acid. Interactions between ascorbic acid and the three fiber sources was, for the most part, not pH-dependent. However, more loss of availability of ascorbic acid

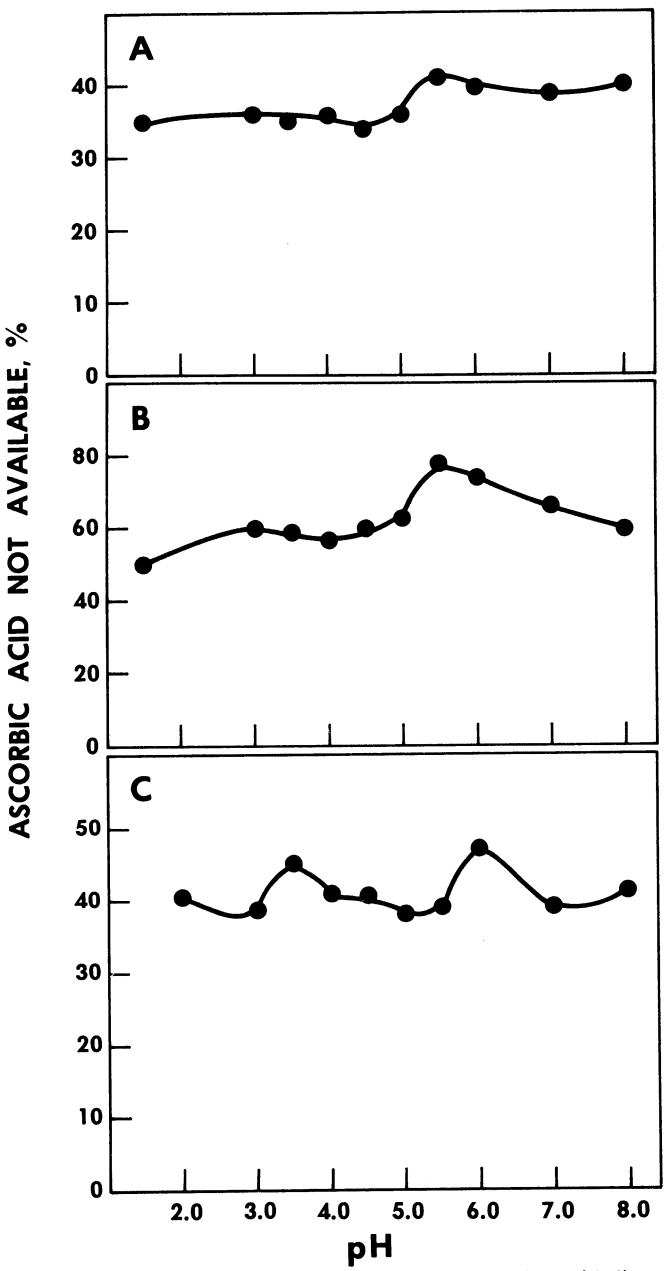

Fig. 1. Relationship between pH and ascorbic acid interactions with dietary fiber. Each point represents the mean of duplicate samples. **A,** hard red spring (HRS) wheat bran (100 mg); **B,** citrus pectin (60 mg); **C,** alphacel cellulose (60 mg).

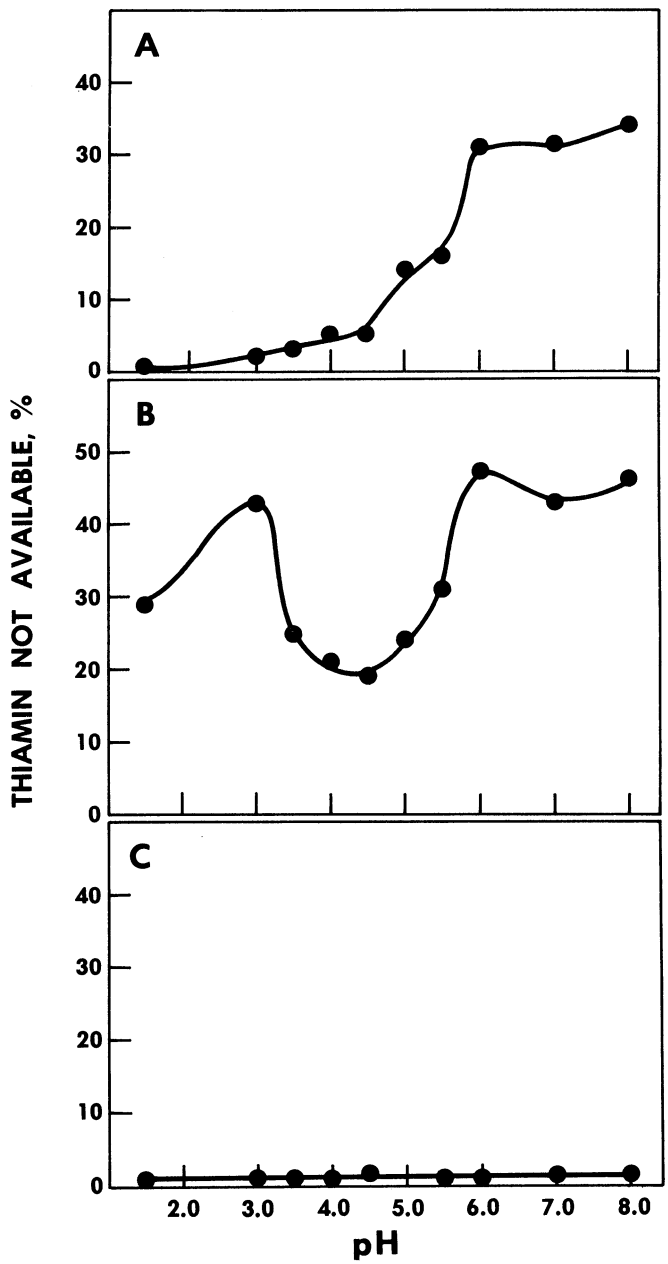

Fig. 2. Relationship between pH and thiamin interactions with dietary fiber. Each point represents the mean of duplicate samples. **A,** hard red spring (HRS) wheat bran (100 mg); **B,** citrus pectin (60 mg); **C,** alphacel cellulose (60 mg).

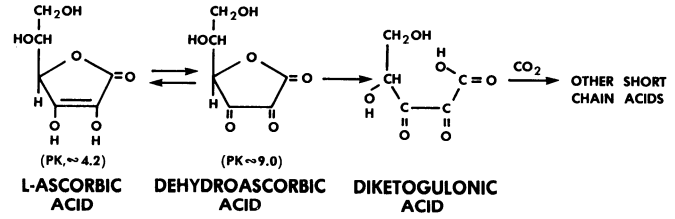

Fig. 3. Degradation of ascorbic acid (Omaye et al 1982).

occurred at pH 5.5–6.0.

Our observation of the interaction between dietary fibers and the vitamin thiamin suggests a mechanism of binding or trapping. Thiamin incubated alone with fiber was recoverable and, with the exception of CP, was recoverable at low pH values. In general, a concentration-related response existed between the amount of available thiamin and the concentration of CP added to the incubation mixture, ie, the amount of available thiamin was related indirectly to the amount of bran or pectin present. AC had little effect on the availability of thiamin at various levels or in the presence of pH changes (Table II, Fig. 2).

Because endogenous bile and the absorption of many nutrients are closely associated, we examined whether bile as sodium taurocholate would influence ascorbic acid or thiamin availability in the presence of dietary fiber. Tables I and II show that little or no influence was exerted beyond the effects observed when the vitamins were incubated with saline and the respective dietary fiber.

Several investigators have noted that bile (Eastwood and Hamilton 1968, Kritchevsky and Story 1974, Normand et al 1981) and lipids (Stasse-Wolthuis et al 1980, Baig and Cerda 1981) can be affected adversely by several sources of dietary fiber. Fat-soluble vitamins have also been implicated to be adversely affected (deLumen et al 1982), but results are conflicting (Kasper et al 1979, Phillips and Brien 1970). Information on the bioavailability of vitamins is scant. To the best of knowledge, this is the first report to consider the adverse affects that certain dietary fibers might exert on the availability of thiamin and the second report to consider the availability of ascorbic acid (Keltz et al 1978). Ellingson and Massengale (1952) found that the ingestion of methylcellulose had little statistical influence on the effectiveness of thiamin in the depleted or normal rat. Description of the mechanisms involved with these interactions can only be speculative. Considering the hydrophobic areas of ascorbic acid or thiamin molecules, interactions with dietary fibers could be similar to those suggested for lipid-dietary fiber interactions. Baig and Cerda (1981) suggested that the interaction between the pectin and lipoprotein is electrostatic, which might be influenced by the presence of divalent cations in the reaction mixture. Fiber consists of a matrix of polysaccharides; in wheat bran, it also consists of protein. Water is distributed at the surface, in the interstitial space, and as free water (Eastwoods et al 1976). Subsequently, nutrients may be adsorbed into the fiber or dissolved in the interstitial water held by the hydrated fiber. In addition, the vitamins may be influenced by cation exchange properties of the respective fiber.

Although the physicochemical system differs from the in vivo situation, the results from this study suggest that certain interactions between ascorbic acid and thiamin occur with selected dietary fibers. The implication is that such interactions may influence vitamin bioavailability, gastrointestinal absorption, or both. The seriousness of such an implication may not be consequential during short-term feeding but may have adverse effects in long-term (chronic) dietary programs.

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