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The Effect of Various Levels of Pectin on the Absorption of Vitamin B_{12} in Rats

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ABSTRACT

The effect of short term feeding of various levels of pectin on vitamin B_{12} absorption was studied. Rats fed fiber-free(FF) diet were divided into FF, 2% pectin, 5% pectin or 10% pectin diet group prior to the vitamin B_{12} absorption test. On the day of the absorption test, absorption of a single oral dose of 57-Co-vitamin B_{12} was measured while rats were consuming their assigned diet. 5 and 10% pectin diet significantly inhibited vitamin B_{12} absorption when compared with FF diet. Pectin intake was inversely correlated with the absorption of vitamin B_{12}.

KEY WORDS : vitamin B_{12} · pectin · absorption.

INTRODUCTION

Pectin, a fermentable fiber, is a polymer composed mainly of (1-4)–α-D-galacturonic acid units[1-3]. Pectin has received much attention due to its potential beneficial effects on health. Pectin was reported to decrease blood cholesterol level and flatten the post-prandial response of serum glucose and insulin[4-11]. Based on these observations, it has been suggested that pectin could be used to prevent or treat coronary heart disease and diabetes. Therefore, it is important to examine possible detrimental effects of pectin as well.

Cullen and Oace[12] reported that long term pectin feeding deteriorated vitamin B_{12} status in rats. 5 to 15% pectin diet elevated urinary methylmalonic acid(MMA) excretion in rats. Moore[13] demonstrated that the negative effect of pectin on vitamin B_{12} status could be partially reversed by administration of low levels of vitamin B_{12}. 5 and 10% pectin diet decreased liver vitamin B_{12} level in rats, but 2% pectin did not[14]. One possible mechanism of deteriorated vitamin B_{12} status of rats fed pectin for a long term could be impairment of vitamin B_{12} absorption by pectin. Therefore, the present experiment was designed to study the effect of various levels of pectin in diet on vitamin B_{12} absorption. The effect of pectin feeding on the absorption of a single oral dose of radiolabelled vitamin B_{12} was studied.

MATERIALS AND METHODS

Animals and Diets

7-week-old male Fischer strain rats(CDF(R)(F-344)/CrIBR : VAF/Plus™) were purchased from Charles River Breeding Laboratories(Wilmington, MA, USA). Rats were housed individually in stainless steel wire-bottomed cages and located in a room where temperatures(20~24°C) and lighting cycle(0700~1900 hr light and 1900~0700 hr

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dark) were controlled. Cardboard sheets that lined pans which collected urine and feces were changed twice a week. Deionized water was allowed *ad libitum*. Fresh diet and water were provided twice a week. Body weight and food intake and spillage were recorded at least twice a week. A semipurified vitamin B_{12} deficient basal diet served as the fiber-free(FF) control diet. The composition of the basal diet is shown in Table 1. Methionine was added to the diet since methionine is the limiting amino acid of soy protein. Pectin containing diet was made by adding pectin(Sigma Chemical Co., St. Louis, MO, USA) to the basal diet at 2, 5, or 10 percent of the total diet. Approximate polygalacturonic acid concentration and methoxy content of pectin were 89% and 9.9%. In vitamin B_{12} adequate FF diet, vitamin B_{12} as 0.1% trituration with mannitol was added at the level of 5 μg vitamin B_{12}/100g diet.

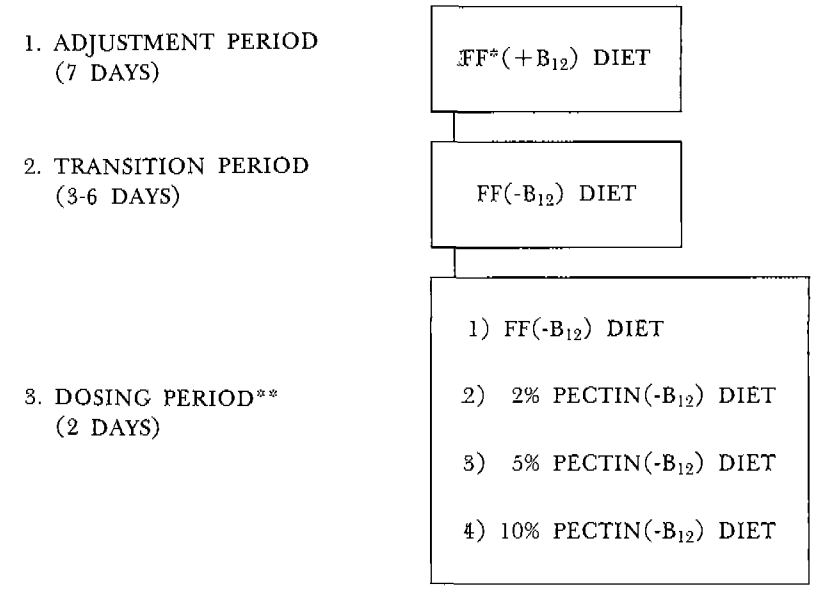
Table 1. Composition of the basal fiber-free diet

Ingredient	Amount(g/100 g diet)
Soy protein[1]	20.0
Wheat starch[2]	70.0
Corn oil[3]	5.0
Salt mix[4]	3.5
Vitamin mix[5]	1.0
D,L-methionine[6]	0.5

[1] Soya assay protein ; Teklad Test Diets, Madison, WI, USA
[2] ICN Pharmaceuticals, Cleveland, OH, USA
[3] Mazola ; Best Foods, CPC International, Englewood Cliffs, NJ, USA
[4] Rat mineral mix UCB-1Rb (Williams-Briggs, Univ. of Calif.), provided : (in mg/100g of diet) $CaCo_3$, 725 ; $CaHPO_4$, 1130 ; Na_2HPO_4, 651 ; KCl, 730 ; $MgSO_4$, 230 ; $MnSO_4 \cdot H_2O$, 15.4 ; $CuSO_4$, 1.3 ; ferric citrate(16.7% H_2O), 15.1 ; $ZnCO_3$, 2.1 ; KIO_3, 0.1.
[5] Vitamin B-12-free mix prepared in glucose, provided : (in mg/100g diet) D-biotin, 0.20 ; choline bitartrate, 100 ; folic acid, 1.00 ; nicotinic acid, 5.00 ; D-calcium pantothenate, 5.00 ; pyridoxine. HCl, 1.50 ; (in IU/100 g of diet) retinyl acetate, 1,000 ; ergocalciferol, 125 ; D,L-α-tocopherol acetate, 5.00. In vitamin B-12 adequate diet, vitamin B-12 as 0.1% trituration with mannitol was added at the level of 5 μg vitamin B-12/100 g diet.
[6] Nutritional Biochemicals Corp., Cleveland, OH, USA

Experimental Design and Vitamin B_{12} Absorption Study

Animals(mean body weight of 106 g) received vitamin B_{12} adequate FF diet *ad libitum* for one week(during adjustment period) and the vitamin B_{12} deficient FF diet for 3~6 days(during transition period)(Fig. 1). During transition period, train-feeding was developed. Rats had their food cup removed overnight and were offered 0.5ml of 10% sucrose solution with return of food cup in the morning. During transition period, any trace of dietary vitamin B_{12} was removed from the gastrointestinal(GI) tract of the rat. Therefore, absorption of radiolabelled vitamin B_{12} dose(not the mixture of the vitamin B_{12} dose and dietary vitamin B_{12}) could be measured during dosing period. On the day before a vitamin B_{12} absorption test, rats were randomly divided into FF, 2% pectin(2P), 5% pectin(5P) and 10% pectin diet(10P) group. On the following day, rats(mean body weight of 130g) received their assigned diet(2 g) after an overnight fast and then 0.5 ml of the sucrose solution containing 0.2 μCi of 100 ng 57-Co-vitamin B_{12} dose(Amersham Corporation, Arlington Heights, IL, USA). Additional food(3~4 g) was allowed after dosing. Since the assigned diet was given to rats before and after 57-Co-vitamin B_{12} dose was offered, the vitamin B_{12} dose could be well mixed with the diet in the GI tract. Rats were sacrificed at 6 hours after dosing by open-heart puncture while anaesthetized with methoxyflurane(Pittman-Moore, Inc., Washington Cross, NJ, USA). Urine and feces were collected separately with funnels attached to metabolism cages. The collection started right after dosing and continued until rats were anaesthetized. GI segme-



* Fiber-free diet

** On the second day of the dosing period, 57-Co-vitamin B_{12} dose was offered to the rat with the assigned diet.

Fig. 1. Experimental design.

nts(stomach, small intestine, cecum, and colon) and the remaining carcass were collected. The carcass was ground by a meat grinder. The collected samples(GI segments, urine, feces and the carcass) were counted by gamma counter(1197 Automatic gamma counting system ; Searle Analytic Inc., Chicago, IL, USA) to determine recovery of 57-Co. Counts recovered in various tissues were expressed as percent of total consumed dose(the sum of 57-Co recovered in all tissues collected). Vitamin B_{12} absorption was expressed as "relative absorption". 57-Co measured in the carcass was defined as "absorbed dose". 57-Co recovered in the cecum, colon, and feces(distal GI tract) was defined as "excreted dose". "Available dose" was defined as absorbed dose plus excreted dose which represented the vitamin B_{12} dose which had a chance to contact absorption sites in small intestine. "Available dose" also equals to total consumed dose minus 57-Co recovered in stomach and small intestine. "Relative absorption" was defined as the absorbed dose as a percentage of the available dose.

Statistical Analysis

Data were compiled, transformed and analyzed by the Statistical Package for the Social Sciences (SPSSX) computer program[15]. Arc sine transformation of the data was performed if it was necessary to satisfy assumptions about the variances and distribution of the observations[16)17)]. One way analysis of variance was performed on data. Tukey's multiple range test, with a procedure-wise error rate of 0.05 was used as a follow-up procedure. Linear regression was performed, with pectin intake as independent variable and "relative absorption" as dependent variable. All statistical testing was conducted at $\alpha=0.05$.

Table 2. Absorbed and available 57-Co-vitamin B-12 dose and relative absorption of the dose[1,2]

Group	(n)[3]	Absorbed	Available	Relative absorption
		% of dose		%
FF	7	31.1± 3.7^{b}	77.7± 6.5^{a}	40.7± 8.2^{c}
2P	7	28.5± 4.6^{b}	88.1± 4.5^{b}	32.6± 6.7^{bc}
5P	6	21.2± 3.7^{a}	92.8± 1.8^{b}	23.0± 4.5^{ab}
10P	6	19.3± 2.6^{a}	92.2± 1.8^{b}	21.0± 3.1^{a}

[1] Absorbed equals the percent of total recovered 57-Co that was recovered in carcass. Available equals absorbed plus distal GI recovery. Relative absorption was defined as absorbed as percent of available dose.

[2] Values are means± SD. Variables were transformed using arc sine for the analysis and then analyzed by one-way ANOVA. Tukey's multiple range test was used as a follow-up test. Means differ significantly ($P<0.05$) if they do not share a common superscript.

[3] Number of rats per group.

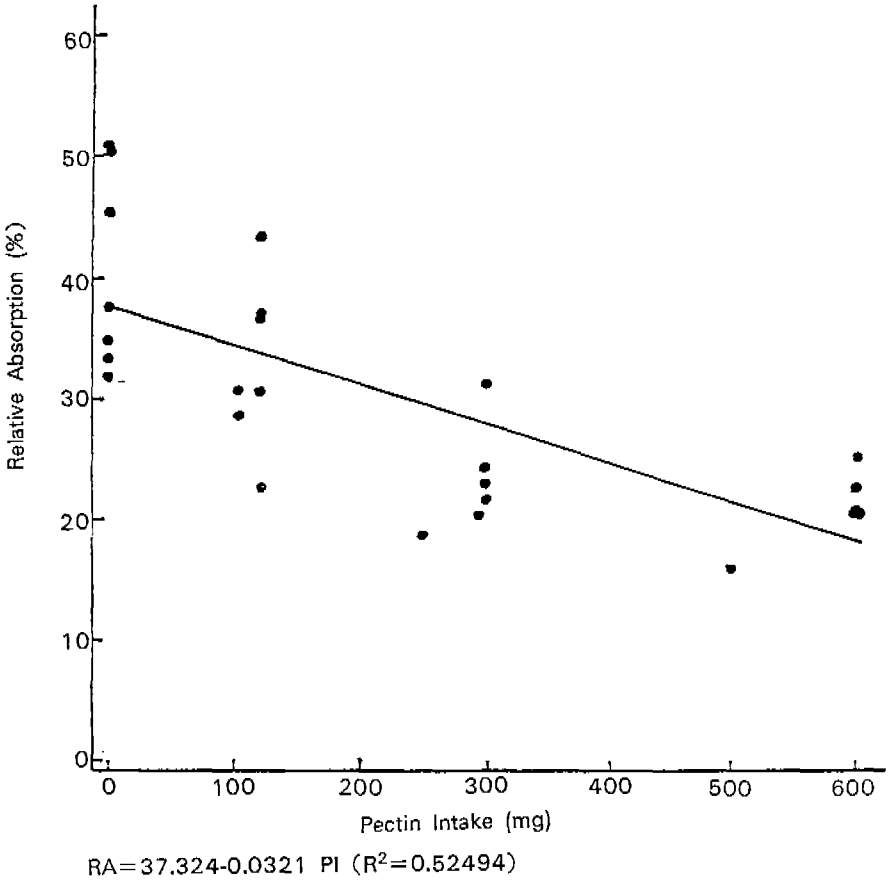


Fig. 2. Linear relationship between pectin intake and relative absorption.

RESULTS AND DISCUSSION

Vitamin B_{12} can be produced or taken up by the intestinal microflora. Therefore, a simple balance test can not measure vitamin B_{12} absorption. Cobalt labelled vitamin B_{12} was used to measure vitamin B_{12} absorption in the current study. 100ng of 57-Co-vitamin B_{12} dose was given to rats, since that amount can be considered as physiological dose of vitamin B_{12}.

Since vitamin B_{12} is exclusively absorbed in small intestine[18)19)], total consumed dose minus 57-Co-vitamin B_{12} remaining in stomach and small intestine was considered as "available dose". Available dose at 6 hours after dosing is shown in Table 2. 78% of the total consumed dose was available for the absorption in FF rats. Available doses of 2P, 5P and 10P rats were 88, 93 and 92%, respectively. Increase in available dose in pectin-fed rats suggested that more dose was available for the absorption in pectin-fed group probably due to increased upper GI transit. Absorbed doses of FF and 2P rats were 31 and 29%, respectively. Absorbed dose was lower in 5P(21%) and 10P(19%) rats than in FF and 2P rats. Relative absorption (absorbed dose/available dose) in FF rats(41%) was not significantly different from 2P rats(33%). Relative absorption of 5P(23%) and 10P rats(21%) was significantly lower than that of FF rats. In long term studies, diets containing more than 5% pectin deteriorated vitamin B_{12} status of rats[12-14)20)21)]. Cullen and Oace[12)] reported that pectin feeding at the level of 5 to 15% of a vitamin B_{12} deficient semipurified diet for 10 weeks elevated urinary MMA excretion. Wong[14)] reported 5 and 10% pectin decreased liver vitamin B_{12} level in rats fed vitamin B_{12} adequate diet, but 2% pectin did not. In the present study, pectin was fed for a short term(two days), and the effect of pectin feeding on vitamin B_{12} absorption was studied. 5 and 10% pectin diet inhibited the absorption of 100 ng vitamin B_{12} dose. 2% pectin diet tended to decrease relative absorption, but difference between 2P and FF rats was not significant. Since actual pectin intake is decided by both pectin concentration of diet and food intake, pectin intake was calculated. The regression equation between pectin intake(PI) and relative absorption(RA) was as follows ;

$RA = 37.324 - 0.0321\ PI\ (R^2 = 0.52494)$ (Fig. 2).

The coefficient of PI was significant at the 5 percent level. These data supported the conclusion that at least 5% pectin diet could inhibit vitamin B_{12} absorption. They also suggest that vitamin B_{12} absorption is negatively correlated with pectin intake. The inhibitory effect of acute pectin feeding on the absorption of vitamin B_{12} can explain the previously reported deteriorating effect of chronic pectin feeding on vitamin B_{12} status.

The effect of pectin on vitamin B_{12} absorption should be evaluated in humans. Pectin is present in some fruits and vegetables and is also consumed as a supplement and food additives. Pectin could be used therapeutically to improve coronary heart disease and diabetes. Therefore, people should consider both beneficial and possible detrimental effects of pectin.

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흰 쥐에 있어서 식이내 상이한 수준의 펙틴이 비타민 B_{12}의 흡수에 미치는 영향

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국문초록

식이내 상이한 수준의 펙틴이 비타민 B_{12}의 흡수에 미치는 영향을 조사하였다. 무섬유 식이를 섭취해온 흰쥐를 비타민 B_{12} 흡수 정도를 측정하려는 전날, 무섬유, 2% 펙틴, 5% 펙틴, 그리고 10% 펙틴 식이군으로 나누었다. 다음날, 동물이 각기 배정된 식이를 섭취하는 동안, 57-Co-비타민 B_{12}를 구강으로 섭취시켜 그 흡수 정도를 측정하였다. 무섬유 식이와 비교하여 볼 때, 5%와 10% 펙틴 식이는 비타민 B_{12}의 흡수를 유의적으로 감소시켰다. 펙틴의 섭취량은 비타민 B_{12}의 흡수와 역상관 관계를 나타내었다.