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Degradation of Vitamin B_{12} in Dietary Supplements

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Abstract: Beverages and solid dietary supplements rich in various added vitamins and minerals have recently become available. It seems reasonable to consider that the intake of these foods is convenient for easy ingestion of nutrients, but problems caused by blending different nutrients in high concentrations have arisen. We focused on vitamin B_{12} (B_{12}) among vitamins and determined the B_{12} contents of beverages and solid dietary supplements purchased from a retail shop. The B_{12} contents of three of five beverages were less than stated on the labels. On the other hand, certain beverages unexpectedly contained much more B_{12} than stated on the labels. In these beverages the amount of B_{12} decreased rapidly with time, whereas B_{12} content was lower than stated on the label in only one of four solid dietary supplements. The content of B_{12} was affected by storage time, light exposure, temperature, and vitamin C. From experimental analysis with a competitive binding assay method employing a ACS Chemiluminescent B_{12} kit, examining differential binding by intrinsic factors and spectral analysis of B_{12}, it was determined that some of the B_{12} might have been converted into B_{12} analogues or small degradation products by multinutrient interaction during storage.

Key words: Content of vitamin B_{12}, dietary supplement, beverages, degradation of vitamin B_{12}, vitamin B_{12} analogue

Introduction

Vitamin B_{12} (B_{12}) is essential for hematopoiesis and normal neurological functions. It is known that deficiency of B_{12} causes megaloblastic anemia accompanied by neuropathy, including cognitive impairment [1]. B_{12} is synthesized exclusively by bacteria and is present in the normal liver but not in plants, except for some types of seaweed. Therefore B_{12} is called an animal protein factor. The B_{12} status is compromised in long-term adherents of a strict, uncooked vegan diet [2], young dieters, and old persons whose intake of animal food is decreased. In fact, B_{12} deficiency occurs in senile dementia of the Alzheimer type (SDAT) and familial Alzheimer's disease [3–6]. Sanada *et al* reported that administration of egg phosphatidylcholine combined with B_{12} to patients with dementia of the Alzheimer type caused improvement in 65% of patients [7].

Japan is now a country where people enjoy longevity and desire to maintain their health while living a creative and happy life. However, there is a trend in society towards poorer health, so there is a need to promote self-aware-

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ness of "health throughout life" to counter this trend. To this end, the Ministry of Health, Labour and Welfare established "Foods for Special Health Use", a regulatory system for health claims about food, in 1991 and "Nutrient Contents Labeling" in 1994. Since then, beverages and solid dietary supplements rich in various added vitamins, including vitamin B_{12}, and minerals have become available in ordinary retail shops. Only one of 51 liquid supplements listed contained B_{12} according to a 1993 guidebook, whereas 15 of 108 liquid supplements listed contained B_{12} according to a 2006 guidebook [8]. The proportion of supplementary diets that contain B_{12} to the total number of supplementary diets has increased remarkably. Taking these dietary supplements seems to be convenient for easy ingestion of nutrients. However, problems due to blending different nutrients in high concentrations have arisen. We must remember the classical studies by Blitz *et al* reporting that B_{12} in commercially sold vitamin B-complex injectable products was largely decomposed by thiamine and niacinamide [9]. Moreover, Kondo *et al* reported that B_{12} in a multivitamin-mineral pill could be converted to potentially harmful B_{12} analogues and that B_{12} analogues were formed by the concerted actions of vitamin C, thiamine, and copper on B_{12} [10]. Notwithstanding these findings, manufacturers of supplementary diets mix B_{12} with many nutrients in high concentrations. However, as far as we know, little attention has been paid to this problem.

In this study, we focused on B_{12} and determined the B_{12} contents of both liquid and solid dietary supplements and compared them to the contents stated on the labels. The B_{12} contents of some liquid dietary supplements were found to be less than stated on the labels and decreased in a time-dependent manner. Therefore, we examined the effects of storage conditions such as the time, temperature, light irradiation, and the effects of various nutrients on the content of B_{12}.

Materials and Methods

Materials

Liquid and solid dietary supplements were purchased from retail shops during 1996–98. Cyanocobalamin, pig intrinsic factor, and pig haptocorrin obtained from Sigma, USA, and other reagents obtained from Wako Pure Chemical Industries, Japan, were used.

Methods

Extraction of B_{12}

B_{12} of samples was extracted by heating at 100°C for 20 minutes in an acetate-buffered solution (pH 5) with an excess amount (100–1000 times as much as the expected amount of B_{12} [w/w]) of potassium cyanide added [11].

Analysis of B_{12}

B_{12} was assayed microbiologically using *Escherichia coli* 215 [11] for the experiments in Figures 1 through 4. Determination of B_{12} contents is dependent on the measurement method. A limitation of the bioassay method using *E. coli* 215 is that cobamide, cobinamide, and methionine are included in measurements. We also used a competitive binding assay method employing an ACS Chemiluminescent B_{12} assay kit (Corning) for samples [12] in a model experiment (Figure 5) to determine only genuine B_{12} content.

Investigation on storage conditions reducing the B_{12} content

The effects of storage conditions (time, temperature, and light) on the B_{12} content were investigated using samples C and E. We chose these samples because these were representative of two types of supplementary diets, one in which the actual B_{12} content decreased quite slowly with time, and the other in which it decreased rapidly with time. Samples C and E belonged to the former and the latter groups, respectively (Figures 1 and 2).

Samples bearing the same date of manufacture and the same batch number were stored for one month at –18°C in the dark, 4°C in the dark, or 4°C in the light (29W fluorescent lamp with irradiation from 30 cm). The "4°C in the light" simulated the storage conditions in a showcase in a supermarket, and the "20°C in the dark" a cool, dark place in the home.

Investigation on various nutrients reducing the B_{12} content

To investigate the degradation of B_{12} by various nutrients, we did model experiments. We mimicked the components of samples G and H in which the actual values of B_{12} content were lower than stated on the labels and sample C in which the actual value was similar to that stated on the label. Sample C was used as a control as shown in Table I. Table II shows the components of model beverages. The sources of protein, lipids, and sugars were not stated on the labels but, as the degradation of B_{12} by proteins and lipids was unlikely to occur, we did not include these constituents in the model beverages. As sugars, glucose and fructose were added to sample C at the rate of 1:1. Samples G and H were used for studies to determine the effect

Table I: Stated composition of the samples by label and container and pH of the contents (for 100 ml or 100 g)

Sample	B	C	E	G	H	BP	CM	BU	PM
Item Container	Alum. can	Steel can	Amber bottle	Trans. bottle	Amber bottle				
pH	3.46	6.14	3.03	3.6	2.52				
Carbohydrate (g)	*	*	*		*	95	53	47	49
Protein (g)		5	*	*		1	10	6.7	7.6
Lipid (g)		2.2				1.8	28	27	21
Ash (g)		0.6							0.2
Calcium (mg)	10	39					304	536	929
Sodium (mg)	28					12.5	390	430	170
Potassium (mg)	22								
Iron (mg)		0.65					4.6	32	11
Vitamin A (IU)		450			95	45 000	1140	2250	1050
β-Carotene (mg)			*					3.2	
Vitamin B_1 (mg)		0.2	0.67		0.71	20	0.5	4	0.6
Vitamin B_2 (mg)		0.3	0.76	5	1.07	27.5	0.8		0.8
Vitamin B_6 (mg)	0.22	0.5	1.05	5	1.43	50	1.3	5.4	1.1
Vitamin B_{12} (μg)	**0.75**	**1.5**	**1.43**	**5**	**4.29**	**50**	**1.9**	**5.4**	**1.1**
Vitamin C (mg)	15	12.5	95.2	300	214.3	1250	32	110	26
Vitamin D (IU)		37.5			7.86	3750	95	536	155
Vitamin E (mg)		10			0.36	200	25	27	17
Nicotinic Acid (mg)	1.3	3.25	8.57	20	8.57	350	8.2	51	9
Pantothenic acid (mg)	0.7	2.5	2.9	10	4.29	150	6.3	19	
Folic acid (μg)		150			8	5000	250	540	110
L-Asp-K (mg)				500					
L-Asp-Mg (mg)				500					
Alcohol (ml)				≤ 1					
Energy (kcal)	23	100	47.6		37.1	400	506	482	418

*Amount not stated

Table II: The composition of iron, sugars, and vitamins in model beverages.
We mimicked according to Table I, the composition of sample G and H in which the actual B_{12} contents were lower than stated on the labels, and sample C in which the actual B_{12} content was similar to that stated on the label as a control. We changed the concentration of vitamin C in sample G from 0 to 300 mg/100 mL and in sample H from 0 to 214 mg/100 mL. Effect of iron was investigated in sample C with and without sugars. (for 100 ml)

Nutritients	Imitation of Sample C	Imitation of Sample G	Imitation of Sample H
Vitamin B_{12} (μg)	1.5	5	4.29
Vitamin B_1 (mg)	0.2	0	0.71
Vitamin B_2 (mg)	0.3	5	1.07
Vitamin B_6 (mg)	0.5	5	1.43
Vitamin C (mg)	12.5	$0 \sim 300$	$0 \sim 214$
Nicotinic acid (mg)	3.25	20	8.57
Pantothenic acid (mg)	2.5	10	4.29
Folic acid (mg)	150	0	8
Iron (mg)	$0 \sim 0.65$	0	0
Sugars (g)	$0 \sim 14.4$	0	0

of vitamin C (0–300 mg/100 mL) on the B_{12} content. A mimic of sample C was utilized for studies on sugars and iron. Effects of pantothenic acid (10 mg/100mL) and nicotinic acid (20 mg/100 mL) on B_{12} were determined using pure cobalamin (B_{12}) and analysis of B_{12} was performed by a competitive binding assay method employing an ACS Chemiluminescent B_{12} assay kit. To confirm the effects of vitamin C on B_{12} degradation, a solution including 5 μg of B_{12} and 300 mg of vitamin C in 100 mL of water was used. Analysis of B_{12} was performed using a competitive binding assay method after 4-day incubation at the indicated temperature.

Fractional analysis of B_{12} using intrinsic factor

B_{12} analogues in samples were separated by incubation of B_{12} with an intrinsic factor that could specifically bind genuine B_{12}. The B_{12} analogues were removed by dialysis. The efficacy of the dialysis was checked by using haptocorrin, which can bind both genuine B_{12} and B_{12} analogues [13].

Spectral analysis of vitamin B_{12}

The spectra were recorded at 20°C with 0.5-nm steps on a spectrophotometer. Before determination of the spectra, vitamin C was removed from the experimental mixture by using a Sep-Pak Plus C18 cartridge (Waters).

Definition

In this paper, genuine B_{12} refers to corrinoid compounds that are active as B_{12} for mammalians. B_{12} analogue means the B_{12}-related compounds that are active for some bacteria such as *E. coli* 215 but are inactive for mammalians. This includes, for example, cobinamide and cobamide in addition to cobalamins.

Results

Table I shows the compositions of the samples stated on the labels or containers and pH. B_{12} concentrations in liquid dietary supplements were 1.43–5 μg/100 mL, while those in solid dietary supplements were 1.1–50 μg/100 g.

B_{12} contents of some beverages and dietary supplements and time course changes of B_{12} contents of beverages

We focused on B_{12} and determined the B_{12} contents of beverages (Figure 1A) and solid dietary supplements (Figure 1B). The B_{12} contents of some beverages (samples G and H) were less than stated on the labels. On the other hand, sample E contained about 2.5 times as much B_{12} as stated on the label shortly after being produced. On the other hand, only one of the four solid dietary supplements had lower B_{12} content than stated on the label. As decomposition of B_{12} was suggested by the fact that the B_{12} content of sample E after the use-by date was markedly decreased and was similar to that stated on the label, time course changes of B_{12} contents of beverages were determined. As shown in Figure 2, the B_{12} contents of samples B and C were not largely changed by 150 days after manufacture, while that of sample E decreased in a time-dependent manner. These results suggested that the content of B_{12} decreased during storage or transport.

Decrease of B_{12} during storage in various conditions

While beverages were usually kept at 4°C in vending machines, the temperature during their storage or transportation varied. Cans and bottles bearing the same dates of production and the same batch numbers were collected for samples C and E. In sample C, B_{12} did not decrease

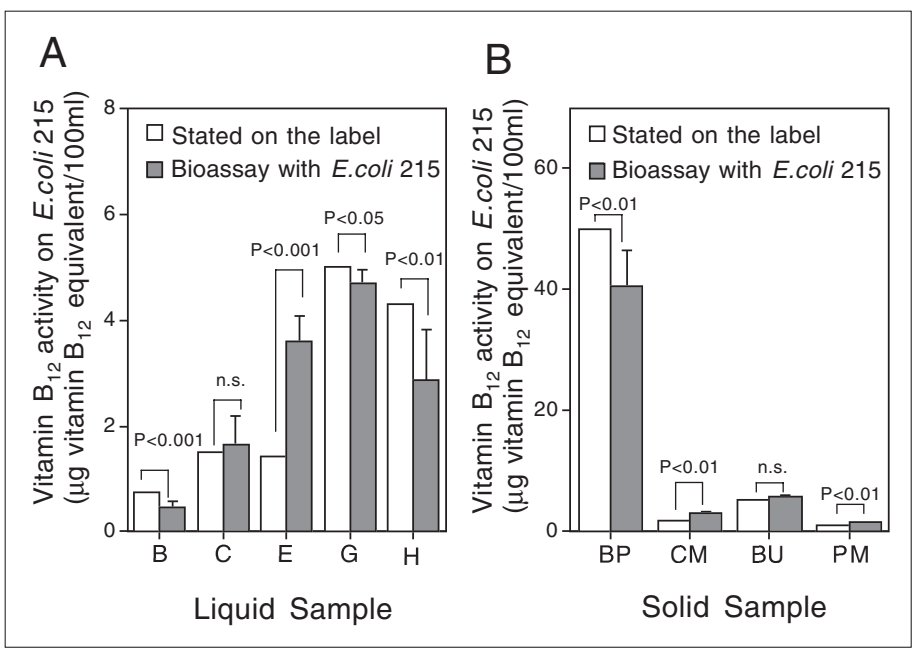

Figure 1: The contents of vitamin B_{12} of some beverages and dietary supplements. Beverages (A) and solid dietary supplements (B) were purchased from retail shops. B_{12} was extracted by heating in an acetate-buffered solution (pH 5) with a large amount of potassium cyanide added. Vitamin B_{12} was assayed microbiologically using *E. coli* 215 and the *t*-test between B_{12} content of stated on the label and that determined by bioassay with *E. coli* 215. n.s.; not significant.

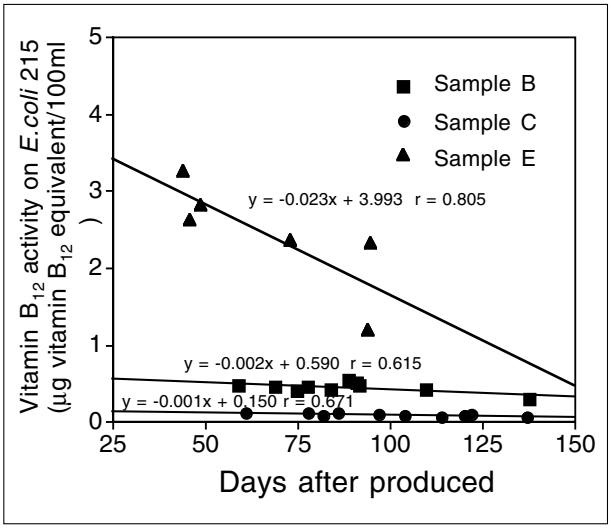

Figure 2: Time course changes of vitamin B_{12} contents of beverages.
We obtained beverages with different dates of manufacture. B_{12} was extracted by heating in an acetate-buffered solution (pH 5) with a large amount of potassium cyanide added. B_{12} was assayed microbiologically using *E. coli* 215.

markedly with time, and in sample E, B_{12} decreased quickly with time in the previous experiments. They were stored for one month under various conditions (temperature and light) and then assayed for B_{12} content. Sample C was a canned beverage, so it was not exposed to light. Figure 3 shows that the B_{12} content of sample C decreased in a temperature-dependent manner in the dark and that the B_{12} content of sample E did not decrease after storage for one month at –18°C or 4°C in the dark (Figure 3A). On the other hand, storage at 4°C in the light markedly reduced the B_{12} content despite the amber color of the beverage container (Figure 3B).

Determination of genuine B_{12} and B_{12} analogue contents in beverages after storage under various conditions

The B_{12} content obtained by the microbiological assay method using *E. coli* 215 includes both B_{12} that is active in mammals and B_{12} analogues that are inactive as B_{12} in mammals, but are B_{12}-active in microorganisms; therefore the changes of B_{12} content shown in Figure 3 are the sum of genuine B_{12} and B_{12} analogues. To determine the changes of B_{12} analogue contents under various conditions, total B_{12} and genuine B_{12} were determined separately. The difference between the former, which is bound by haptocorrin, and the latter, which is bound by intrinsic factors, was taken as the amount of B_{12} analogues. As shown in Figure 4, after samples C and E were stored at 20°C in the dark or at 4°C in the light for one month, the amount of B_{12} was less than half the amount of total B_{12}, *i.e.* B_{12} plus analogues, which was estimated by the bioassay method using *E. coli* 215. In sample E, the total amount of B_{12} after storage at 4°C in the light was less than that after storage at 20°C in the dark. The amount of genuine B_{12} bound by intrinsic factor in sample E stored at 4°C in the light also was less than that after storage at 20°C in the dark. The decrease of B_{12} binding to haptocorrin *i.e.* B_{12} plus analogues, suggested that under these conditions B_{12} in samples was degraded into products that were inactive even for *E. coli* 215.

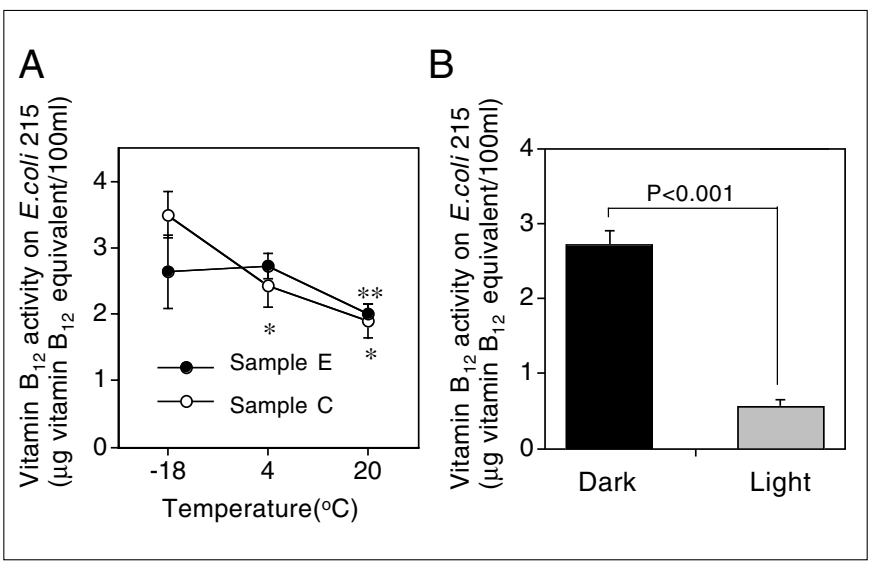

Figure 3: Decrease of vitamin B_{12} in storages in various conditions.
B_{12} contents of sample C and sample E bearing the same lot number respectively were assayed for B_{12} after 1-month storage in various conditions, temperature (A) and light exposure (B). B_{12} was extracted by heating in an acetate-buffered solution (pH 5) with a large amount of potassium cyanide added. B_{12} was assayed microbiologically using *E. coli* 215. Light exposure was not performed for sample C because the container was a steel can. A: * ; $p < 0.001$, **; $p < 0.005$, *t*-test comparing storage at –18°C storage and various temperatures. B: Light exposure was performed using a 29W fluorescent lamp with irradiation from 30 cm.

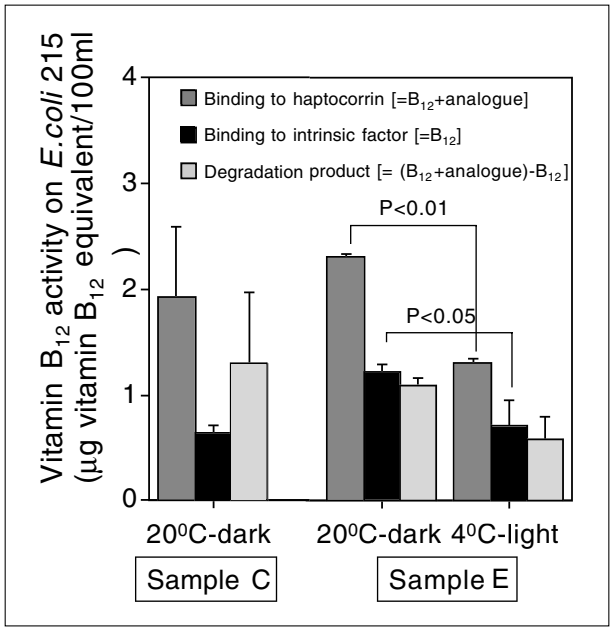

Figure 4: Separate determination of genuine B_{12} and B_{12} analogue contents after various storage conditions.
Genuine B_{12} contents of sample C and sample E after various storage conditions were determined by fractional analysis of B_{12} as described in "Material and Methods". Both genuine and B_{12} analogues can bind haptocorrin. Intrinsic factors can specifically bind genuine B_{12}.

Effects of various nutrients on the B_{12} contents of beverages and temperature- and time-dependent degradation of B_{12}

Next, we examined the effects of various added nutrients such as vitamins and minerals on B_{12} contents in model experiments. The effects of nutrients (sugar-glucose + fructose, 14.4 g/100 mL; iron, 0.65 mg/100 mL; vitamin C, 0–300 mg/100 mL; nicotinic acid, 20 mg/100 mL; pantothenic acid, 10 mg/100 mL) were investigated in a model experiment as described in Materials and Methods. Sugars, iron, and vitamin C reduced the B_{12} contents of beverages (Figures 5A and B). As the degradation of B_{12} by vitamin C occurred in a concentration-dependent manner and was larger than that by any other nutrient, further experiments were performed on this vitamin. A solution including 5 μg of B_{12} and 300 mg of vitamin C in 100 mL of water was used. As shown in Figure 5C, the B_{12} content did not decrease at –18°C and 4°C after storage for one month but the extent of vitamin B_{12} degradation by vitamin C increased in a temperature-dependent manner above room temperature. Vitamin C degraded the vitamin B_{12} in a time-dependent manner at 30°C (Figure 5D). We kinetically analyzed the results in Figure 5D (inset in Figure 5D). Degradation of vitamin B_{12} followed first-order kinetics based on the result of the linear relationship between time (day) and the vitamin C concentration (ln).

Changes in the absorbance spectrum of B_{12} (cyanocobalamin) after incubation with vitamin C

To determine the changes in the absorbance spectrum of vitamin B_{12} (cyanocobalamin) caused by vitamin C, B_{12} (5 μg/100 mL) was incubated with or without vitamin C (300 mg/100 mL) at 4°C or 30°C for 8–15 days. The spectrum of B_{12} (cyanocobalamin) did not change after the incubation at 4°C for 8 days with vitamin C. On the other hand, the incubation at 30°C with vitamin C induced a change in the peak of B_{12} in a time-dependent manner. After 15 days of incubation with vitamin C, the absorbance peak of B_{12} (cyanocobalamin) at 551 nm [14] disappeared.

Discussion

In this report, we investigated the B_{12} contents of both liquid and solid dietary supplements, compared them with the information on the labels and also investigated the effects of storage conditions and various nutrients on the B_{12} contents. The B_{12} contents of some liquid dietary supplements were less than described on the labels and decreased in a time-dependent manner. As no one takes liquid or solid dietary supplements as a staple food, thinking strictly of the real contents of nutrients in various dietary supplements may not be meaningful. However, a situation may occur in which people who are absolutely deficient in a certain nutrient take such dietary supplements to obtain the nutrient. For example, some strict vegetarians who do not eat even seaweed may ingest B_{12} from dietary supplements because of the complete lack of B_{12} in their diet. In addition, elderly persons whose intake of animal food is decreased may utilize liquid dietary supplements for the supply of B_{12}. In such cases, it is very important that the dietary supplement contain exactly the same amount as on the label for deciding how much of it should be taken to obtain the required amount of B_{12}. As dietary supplements are very convenient for easy supply of nutrients, this is highly desirable. From this point of view, it is undesirable that the contents of B_{12} of four of nine samples were less than stated on the labels. On the other hand, sample E contained about 2.5 times as much B_{12} as stated on the label shortly after being produced and the B_{12} content decreased very quickly in a time-dependent manner. We inspected the compositions of supplementary diets provided on the labels, and found that the concentration of vitamin C in

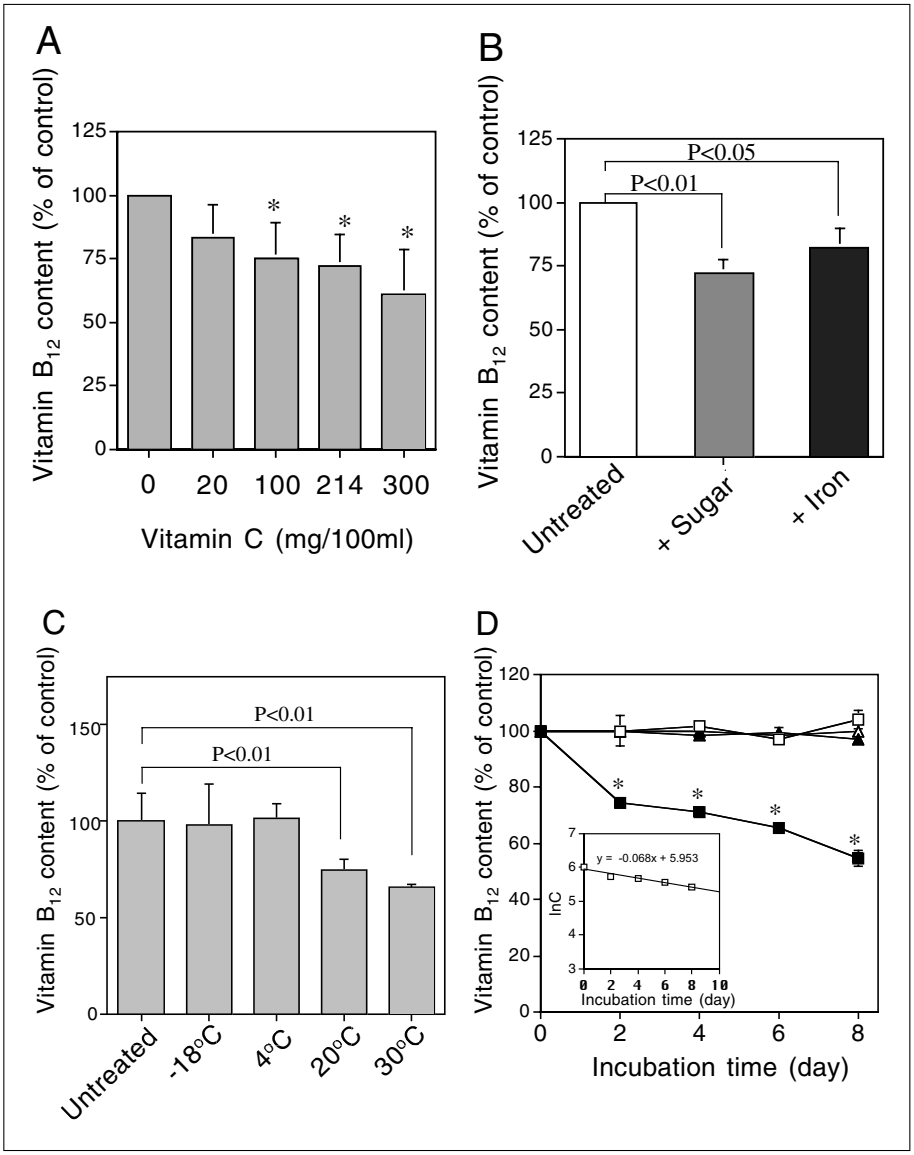

Figure 5: Effects of various conditions on the B_{12} contents of beverages.
A: Effects of vitamin C. A solution including 5 µg of B_{12} and 0~300 mg of vitamin C in 100 mL of water was used. B_{12} content without vitamin C was taken as the control. B: Effect of sugar and iron. A solution including 5 µg of B_{12} with or without sugar (glucose + fructose; 14.4 g/100 mL) and iron (0.65 mg/100 mL). B_{12} content without sugar and iron was taken as the control. C: Effect of temperature on B_{12} degradation. A solution including 5 µg of B_{12} and 0~300 mg of vitamin C in 100 mL of water was used. D: Time-dependent degradation of B_{12} by vitamin C. ■; vitamin C (+) at 30°C, □; vitamin C (+) at 4°C, ▲; vitamin C (–) at 30°C, △; vitamin C (–) at 4°C. Inset in D: Kinetic analysis of the degradation of B_{12} by vitamin C. B_{12} content was assayed using a competitive binding assay method employing an ACS Chemiluminescent B_{12} assay kit.

sample E was much higher than in samples B and C, in which B_{12} contents were not changed by 150 days after manufacture. As shown in Figure 1, the amount of B_{12} in sample H, which also contained a high concentration of vitamin C (214 mg/100 mL), was much less than stated on the label. Sample G contained a high concentration of vitamin C (300 mg/100 mL), but B_{12} content was similar to that stated on the label. A possible explanation might be a lack of carbohydrate or slightly higher pH in sample G than in samples E and H.

In spite of the rapid decrease of B_{12} in sample E, its B_{12} concentration remained above that on the label. Thus, it seems that we could obtain more than the desired amount of B_{12} safely from sample E until the expiration date. However, as shown in our previous report, in which we investigated the bioavailability of raw and dried asakusanori *(Porphyra tenera)* as sources of B_{12} and the effects of eating them on B_{12} nutriture, B_{12} in raw asakusanori could be changed into harmful B_{12} analogues by the drying process and the analogues cause a worsening of B_{12} nutriture [15]. No deleterious effects have been reported for ingesting mega-doses of B_{12}. Thus, it does not matter even if some of beverages contain as much as twice the daily allowance of B_{12}. However, it was found that a part of the B_{12} in the beverages was degraded during storage under various conditions. Some of the degradation products generated by the action of vitamin C and copper in vitamin-mineral pills have been known to inhibit the activity of B_{12}-dependent enzymes *in vitro*. Similar deleterious effects may occur for beverages rich in various added vitamins and minerals.

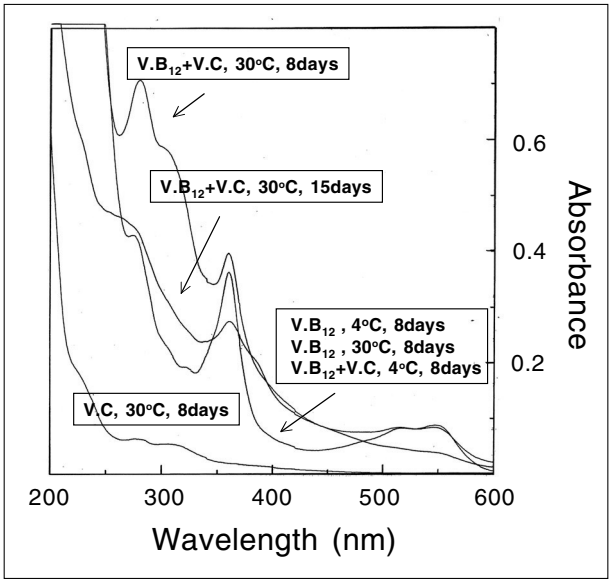

Figure 6: Changes in the absorbance spectrum of B_{12} after incubation with vitamin C.
B_{12} (5 µg/100 mL) was incubated with or without vitamin C (300 mg/100 mL) at 4°C or 30°C for 8–15 days. The spectra were recorded at 20°C with 0.5-nm steps. A Sep-Pak Plus C18 cartridge was used for the separation of B_{12} from vitamin C and the concentration of B_{12} for spectral analysis.

In our experimental model mimicking beverages, vitamin C decreased the content of B_{12} in a concentration-dependent manner. These results suggested that vitamin C might be one of the reasons for the decrease of B_{12} in samples E and H. The stability of B_{12} in the presence of vitamin C has been reported [16–19]. Hines determined the B_{12} concentration in serum of 90 subjects who took more than 500 mg of vitamin C daily. They found that possibly 2% to 3% of the subjects on the megadose regimen of vitamin C might well be at a risk for ultimate development of B_{12} deficiency [20], and the serum B_{12} level increased in these subjects within three months after cessation of the megadose regimen of vitamin C. However, low serum B_{12} levels in hospitalized patients who had been receiving 2 g of oral vitamin C daily did not necessarily reflect tissue deficiency of B_{12}, because B_{12} was extracted from serum samples without addition of KCN [21] in the study. Thus it is possible that extraction of B_{12} from sera was incomplete and the estimated B_{12} concentrations were accidentally low. Degradation of B_{12} by thiamine, nicotinic acid, or vitamin C in injectable commercial vitamin B-complex products [9] or multivitamin-mineral pills [10] has been reported.

In this study, we showed that the B_{12} contents of some beverages were affected by storage time, light, temperature, sugars, iron, and vitamin C, and that B_{12} might be converted to B_{12} analogues by multinutrient interactions during storage. Kondo *et al* also reported that, as shown in Figure 4, the content of intact B_{12} plus B_{12} analogues in sample E was decreased during storage in light even at 4ºC. The result suggested that B_{12} might be converted not only to B_{12} analogues but also to small degradation products that could not bind to haptocorrin. We also found the changes in the absorption spectrum of B_{12} after incubation with vitamin C. Intact cyanocobalamin has three absorption bands (278 nm, 361 nm, and 551 nm) attributed to π–π transition of the corrin ring [22]. The disappearance of the band of 551 nm might involve a destruction of a corrin ring. At present, the significance of the change of the absorbance spectrum is not fully clear and further study will be needed.

In this study, we did not examine the effects of all nutrients in beverages, but the coexistence of vitamin C, iron, and sugar decreased the B_{12} contents. Compared with the beverages, the changes of B_{12} contents in solid dietary supplements were small. The most remarkable difference in composition between beverage and solid dietary supplements is water content. The B_{12} in beverages, which contain more water, is easily influenced by other coexisting nutrients. Kondo *et al* reported that B_{12} in multivitamin-mineral pills could be converted to potentially harmful B_{12} analogues. They incubated pills at 37ºC for 2 hours in water and isolated B_{12}. In this context, Kondo's sample and our liquid sample are similar, and in both cases the decrease of B_{12} was marked.

Taken together, the results of this study suggest that incautious blending of various nutrients in high concentrations, especially vitamin C, is dangerous for B_{12} status. This precaution is more important for liquid dietary supplements than for natural foods owing to the following reason. When animal liver containing a high concentration of B_{12} and lemon juice containing vitamin C are ingested simultaneously, the liver is digested in the stomach and then B_{12} is released. However, B_{12} is not degraded by vitamin C because it is bound by haptocorrin, a kind of B_{12}-binding protein that is secreted by the salivary gland and travels together with the ingested liver through the esophagus [23]. We have found, in preliminary experimental results, that haptocorrin can protect B_{12} from degradation by vitamin C [24]. In liquid dietary supplements, B_{12} is in a free form, and is easily exposed to and degraded by vitamin C. In conclusion, this study confirms previous work done on the effect of ascorbic acid on B_{12} stability and is a reminder of the need for skepticism in reading ingredient labels on dietary supplements.

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