

Effects of combination of functional food materials on body weight, body fat percentage, serum triglyceride and blood glucose

Verification of opuntia ficus-indica + mushroom chitosan containing foods and white kidney bean + coleus forskohlii + mushroom chitosan containing foods

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Introduction

Following the changes in the eating habits of the Japanese people in recent years, the intake of fats and easy to digest sugars has increased leading to an excessive intake of calories (energy). In addition people with obesity and obesity accompanied life-style related diseases such as diabetes and hyperlipedemia, etc. are also increasing due to various factors such as lack of exercise and mental stresses, etc. This has become a national problem¹⁾ and concrete action plan “Health Japan 21” has been designed that first and foremost aims at inculcating a habit of exercising, limiting the intake of calories by improving the eating habits and improving the nutritional balance. However, in today’s busy life, there are many people who very often have irregular mealtimes or irregular meal contents or have many opportunities of eating out and as such find it difficult to improve their dietary habits. The nutritional food supplements are expected to undertake the role of helping such people in improving their dietary habits. These nutritional food supplements are useful in improving the dietary habit as they replenish the insufficient nutrients or contain non-nutrient functional components, and there is a demand that their effectiveness and safety be scientifically proven. However, only *in vitro* examinations or *in vivo* examinations using animals are not sufficient and effectiveness and safety test involving humans are essential.

Barrious Laboratories has developed “SUPER BOWS DIET TYPE A” and “SUPER BOWS DIET TYPE B” as health supplements that are useful in improving the dietary habits to avoid risks such as obesity and diabetes, hyperlipedemia, etc. and has conducted tests on humans to evaluate the characteristics and examine the results on humans and to verify the safety. An outline of the test results obtained up to now has been introduced in this report.

1. “SUPER BOWS DIET” Series products on market and products under test

“SUPER BOWS DIET TYPE A” is a granular food and its main ingredients include the opuntia ficus-indica powder “Neopuntia (R)” which is obtained by drying young leaves of the opuntia ficus-indica cactus and then crushing them to make a powder and mushroom derived complex polysaccharide mushroom chitosan “Plus fort Barrious ®”. The insoluble dietary fiber “Neofiber TM” contained in opuntia ficus-indica powder has a lipase inhibition action and it is expected to suppress the decomposition/absorption of fats²⁾. The mushroom chitosan has a combined action of emulsifying and enveloping the fats and demonstrates an effect of suppressing the absorption of fats from the meals and it has been verified that it results in reduction of weight and body fat percentage on long-term intake³⁻⁵⁾. Therefore, since it contains opuntia ficus-indica powder and mushroom chitosan that have the above characteristics as the main components, continuous intake of “SUPER BOWS DIET TYPE A” is expected to prevent major health problems such as metabolic syndromes like obesity, hyperlipedemia, high blood pressure and diabetes, etc.

On the other hand “SUPER BOWS DIET TYPE B” is a granular food and its main ingredients include white kidney bean extract powder “Phase 2”, coleus forskohlii extract and mushroom chitosan “Plus fort Barrious ®”. The white kidney bean extract powder has the a-amylase inhibition action and it has been verified that it suppresses the decomposition and absorption of fats⁶⁾. Moreover, the coleus forskohlii extract has a-glucosidase and lipase inhibition action in addition to a-amylase inhibition and it has been confirmed that it suppresses

the absorption of sugar and fats from the meals^{7,8)}. Thus, since it contains white kidney bean extract powder, coleus forskohlii extract and mushroom chitosan as the main components, continuous intake of “SUPER BOWS DIET TYPE B” is expected to have curative properties against obesity, hyperlipidemia, high blood pressure and diabetes, etc.

In the exploratory studies of nutrient functions described below, “SUPER BOWS DIET TYPE A” and “SUPER BOWS DIET TYPE B” were used as experimental foods A and B respectively. Granular food products with dextrin, colorants and flavoring agents were used as placebo. The tests were commissioned to TTC as the third party organization and (1) Weight/body fat tests and (2) Lipid absorption test were conducted in Shinjuku Oiwake Clinic (Doctor in-charge: Hiroshi Ito) and (3) Glucose tolerance tests were conducted in Mareesia Garden Clinic (Doctor in-charge: Watanabe Miwako). All the tests followed the spirit of the Helsinki Declaration and review/approval was obtained in advance from the Ethics Committee set up in the medical institutions. All the test subjects were explained the objectives of the test, description, safety and personal information protection matters in advance by the Doctors in-charge and their written consent was obtained.

“Plus fort Barrious ®” is the registered trademark of Barrious Laboratories Ltd.
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2. Nutrient Functions Exploratory Study

Barrious Laboratories had planned the tests given in Table 1 at the time of development of “SUPER BOWS DIET TYPE A” and “SUPER BOWS DIET TYPE B”. First, the effect of 8 weeks of continuous intake of both experimental foods A and B on the body weight and body fat percentage was examined. In addition, taking into consideration the characteristics of the raw materials of each food stuff, (2) study of the triglyceride level increase inhibitory action after fat loading was carried out for “experimental food A” and (3) study of the blood glucose level increase inhibitory action after glucose loading was carried out for “experimental food B” to examine the mechanism of the weight/body fat percentage reduction effect obtained in this test (Table 1).

(1) Study of Body weight/body fat percentage reducing effect

25~60 years old 47 adult men and women were randomly allocated into 2 groups, i.e. experimental food A intake group (average age 48.7 years \pm 9.9 years, 7 men, 17 women) and experimental food B intake group (average age 46.4 years \pm 9.2 years, 6 men, 17 women). The test subjects of the respective groups were prescribed the experimental food twice a day for a continuous period of 8 weeks, wherein, experimental food A group were asked to take 1 packet with 1 glass of water within 30 minutes after lunch and dinner and the experimental food B group were asked to take 1 packet with 1 glass of water within 20 minutes before lunch and dinner.

The test subjects were asked to come to the hospital before start of intake, after 4 weeks of intake and after 8 weeks of intake and their body weight, body fat percentage (impedance method), blood glucose levels, triglyceride levels, total cholesterol and other clinical microscopy items were checked. The test subjects were asked to maintain a diary of their daily food intake during the examination period and a national registered dietician calculated the amount of intake of calories from the contents of these diaries.

Body weight and body fat percentage were the major evaluation items and the changes in the values after 8 weeks as compared to the values before start of the tests were evaluated by one-sample t-test. The values after 4 weeks were evaluated the same. Calorie intake, fasting blood glucose, triglyceride levels and total cholesterol were the minor evaluation items and the changes in all the values after 8 weeks as compared to the values before the start of the tests were evaluated by one-sample t-test. Moreover, in safety evaluation, the principal investigator made the judgments regarding the

relationship between the experimental foods and the adverse events that were generated during the examination period. Also, the changes in the values of clinical microscopy items after 8 weeks as compared to the values before the start of the examination were also evaluated by one-sample t-test. All the statistical analysis tests were conducted for both sides and the level of significance was set at 5% and the numerical values were indicated in average value \pm standard deviation.

From the results of the tests, it was observed that the body fat percentage decreased significantly in the experimental food A group (BMI₂₂, n = 24) after 4 weeks ($-1.33 \pm 0.31\%$, $p < 0.01$) and after 8 weeks ($-1.75 \pm 0.35\%$, $p < 0.01$) of intake. There was a significant reduction in weight after 4 weeks of intake ($0.39 \pm 0.16\text{kg}$, $p < 0.05$) (Figure 1). The calorie intake during the examination period calculated from the diet check was almost constant, i.e. there was no significant change. A significant drop in triglyceride levels was seen in the blood tests after 4 weeks of intake ($21.7 \pm 10.2\text{mg/dl}$, $p < 0.05$) and the drop trend continued in the blood tests after 8 weeks ($-21.8 \pm 11.2\text{mg/dl}$, $p = 0.064$). There was a significant drop in the total cholesterol values after 8 weeks of intake ($-10.0 \pm 4.4\text{ mg/dl}$, $p < 0.05$). Moreover, there was a significant drop in the fasting blood glucose after 4 weeks of intake ($-5.8 \pm 2.1\text{mg/dl}$, $p < 0.05$) and after 8 weeks of intake ($-5.01 \pm 1.6\text{mg/dl}$, $p < 0.05$) even in test subjects who had a BMI of over 24 as well as high fasting blood glucose (over 95 mg/dl) at 0 weeks (n=8). Similarly, in test subjects who had a BMI of over 24 as well as high triglyceride levels (over 150 mg/dl) at 0 weeks (n=8), the triglyceride levels dropped significantly after 4 weeks ($-59.4 \pm 16.0\text{mg/dl}$, $p < 0.01$) and after 8 weeks ($-63.9 \pm 23.2\text{mg/dl}$, $p < 0.05$).

In case of experimental food B group, there was a significant drop in the body weight and body fat percentage of the test subjects (BMI₂₂, n=23) after 4 weeks of intake (body weight: after 4 weeks $-0.63 \pm 0.18\text{ kg}$, $p < 0.01$; body fat percentage: $-0.91 \pm 0.35\%$, $p < 0.05$) and after 8 weeks (body weight: $-0.78 \pm 0.20\text{ kg}$, $p < 0.01$; body fat percentage: $-1.19 \pm 0.37\%$, $p < 0.01$) (Figure 2). Furthermore, the calorie intake during the examination period calculated from the diet check was almost constant, i.e. there was no significant change. There was a significant drop in the total cholesterol values after 4 weeks of intake ($-10.2 \pm 4.5\text{ mg/dl}$, $p < 0.05$). Moreover, in the test subjects who had a BMI of over 24 as well as high total cholesterol values (over 220 mg/dl) before the start of the tests (n=10), there was a significant drop in the total cholesterol after 4 weeks ($-25.3 \pm 7.1\text{ mg/dl}$, $p < 0.01$) and after 8 weeks ($-11.3 \pm 4.0\text{ mg/dl}$, $p < 0.05$).

At the same time, there were no changes in any of the test values of safety evaluation items that could be clinically problematic. Though temporary gastrointestinal symptoms such as bloating sensation (abdomen enlarged feeling), and constipation, etc. were observed in both the experimental food A and B intake groups, all the symptoms were of a low grade and disappeared within a short period during the continuous intake of the experimental foods, and were judged by the principal investigator to have no cause-and-effect relation with the experimental foods and as such there were no safety issues.

Table 1 Experimental foods used in the 3 human tests conducted up to now

Test	Target experimental food	Number of test subjects	Intake period	Test design
(1) Body weight / body fat percentage test	A*, B**	47	8 weeks	Open test
(2) Lipid absorption test	A*	12	Single intake	Placebo comparison Double blind cross over test
(3) Glucose tolerance tests	B**	13	Single intake	Placebo comparison Double blind cross over test

*: "SUPER BOWS DIET TYPE A"

** : "SUPER BOWS DIET TYPE B"

Figure 1 Changes in the body weight and body fat percentage in the test subjects of the experimental food A intake group (n = 24)

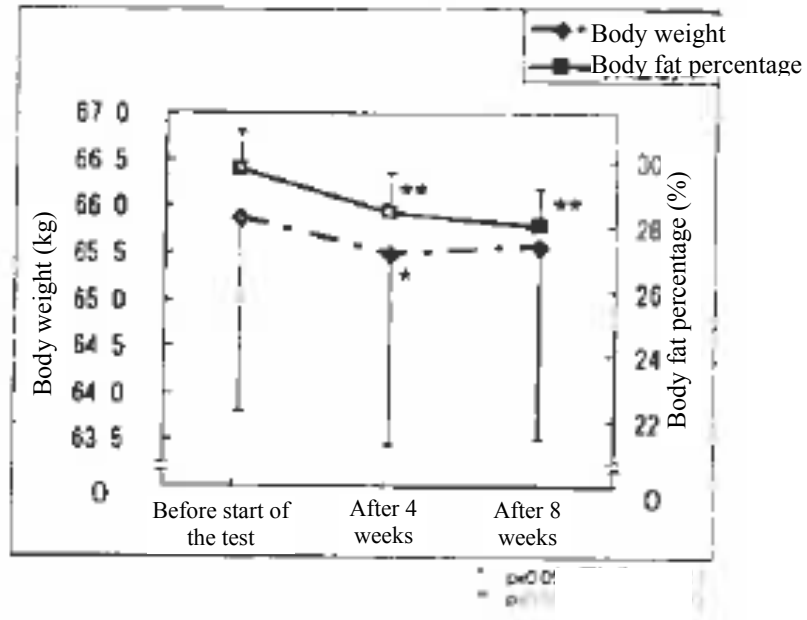


Figure 2 Changes in the body weight and body fat percentage in the test subjects of the experimental food B intake group (n = 23)

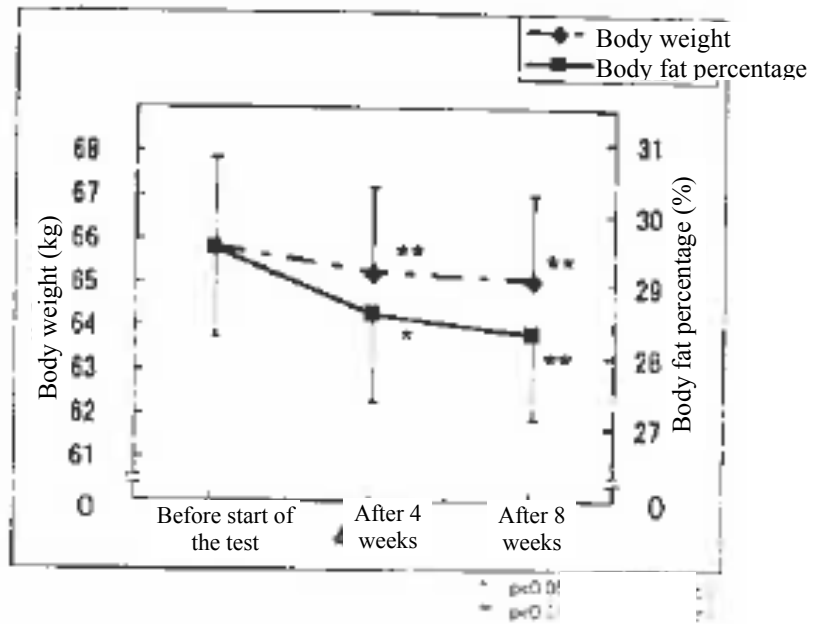
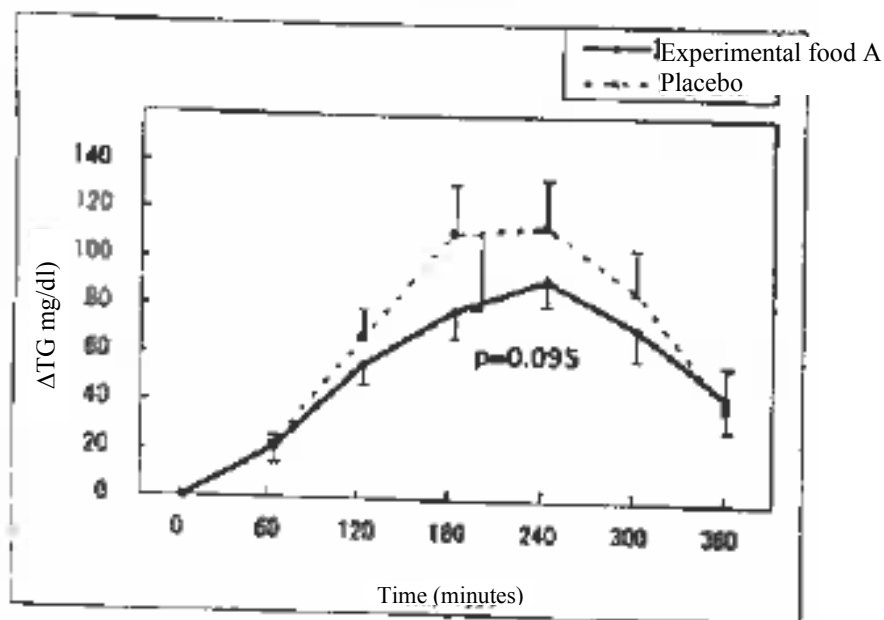


Figure 3 Changes over time in the post-prandial triglyceride levels (TG) in lipid absorption test subjects on single intake of experimental food A and placebo



The above test results demonstrate that experimental food A (“SUPER BOWS DIET TYPE A”) works to reduce the body weight and body fat percentage in persons having a BMI of 22 or more and are predisposed to obesity and also works to reduce the fasting blood glucose levels in persons with a BMI of 24 or more and having high fasting blood glucose (more than 95 mg/dl). Correspondingly, the experimental food B (“SUPER BOWS DIET TYPE B”) works to reduce the body weight and body fat percentage in persons having a BMI of 22 or more and also works strongly to reduce the total cholesterol levels in person with a BMI of 24 or more and having high total cholesterol levels (more than 220 mg/dl).

The following crossover tests were conducted in comparison to placebos to examine the mechanism of body weight and body fat percentage reduction effect of long-term intake of “SUPER BOWS DIET TYPE A” and “SUPER BOWS DIET TYPE B”.

(2) Study of Lipid absorption inhibitory action

As mentioned above, the “SUPER BOWS DIET TYPE A” has a prospective inhibitory action against the absorption of lipids when taken with meals. Consequently, a double blind crossover test in comparison to placebo was conducted targeting test subjects who had absorbed lipids to examine whether single intake of “SUPER BOWS DIET TYPE A” (experimental food A) demonstrates an inhibitory action against the rise of triglyceride levels after meals.

12 adult men and women in the age bracket of 23-57 having triglyceride levels below 250mg/dl were asked to take foodstuffs loaded with fats (butter and lard added corn soup: fats 40g) and then 5 minutes later take experimental food A or placebo with 1 glass of water. The experimental food A and placebo were crossed over alternatively with a respite of 1 week in between. Blood samples were collected just before the intake of the fat loaded foodstuff (0 minutes) and after 60, 120, 180, 240, 300 and 360 minutes and the triglyceride levels were measured.

Out of the 12 test subjects, the efficacy was analyzed for 10 subjects who had usual meals on the day before the tests and 2 subjects who had totally different meals were excluded. The order effect and time effect were not considered to be significant and the cross over method was judged to be appropriate. It was seen in the test results, that the area under the concentration curve of the triglyceride levels in blood 0 - 360 minutes after intake (AUC₀₋₃₆₀) and the amount of rise in the triglyceride levels at 120-300 minutes after intake were lower in case of intake of experimental food A, as compared to the intake of placebo and a rise inhibiting trend was seen after 180 minutes after intake ($p = 0.095$) (Figure 3).

This result suggests the possibility that "SUPER BOWS DIET TYPE A" has an action of easing off the absorption of fats contained in the meals.

(3) Study of Glucose absorption inhibitory action

"SUPER BOWS DIET TYPE B" has a prospective inhibitory action against the absorption of glucides when taken with meals. Consequently, a double blind crossover test in comparison to placebo was conducted targeting test subjects who had absorbed excessive sugar to examine whether single intake of "SUPER BOWS DIET TYPE B" (experimental food B) demonstrates an inhibitory action against the rise of blood glucose levels and insulin values after meals.

13 adult men and women in the age bracket of 29-57 having fasting blood glucose levels above 126mg/dl were asked to take experimental food B and placebo with 1 glass of water and then 5 minutes later take foodstuff loaded with sugar (polished rice 300g). The experimental food A and placebo were crossed over alternatively with a respite of 1 week in between. Blood samples were collected just before the intake of the sugar loaded foodstuff (0 minutes) and after 30, 60, 90 and 120 minutes and the blood glucose levels and insulin levels were measured.

Out of the 13 test subjects, the efficacy was analyzed for 12 subjects and 1 subject who was observed to have abnormal glucose tolerance was excluded. The order effect and time effect were not considered to be significant and the cross over method was judged to be appropriate. It was seen in the test results, that the blood glucose levels and insulin levels 30 minutes after intake were significantly lower in case of intake of experimental food B as compared to the intake of placebo ($p < 0.01$) (Figure 4 and 5). Moreover, it was also seen that the insulin value AUC₀₋₁₂₀ was significantly lower in case of intake of experimental food than in case of placebo ($P < 0.01$) (Figure 5).

This result indicates that "SUPER BOWS DIET TYPE B" has an action of easing off the absorption of glucose after meals as well as inhibitory action against rapid secretion of insulin.

Figure 4 Changes over time in the post-prandial blood glucose levels (Glc) in lipid absorption test subjects on single intake of experimental food B and placebo (n = 12)

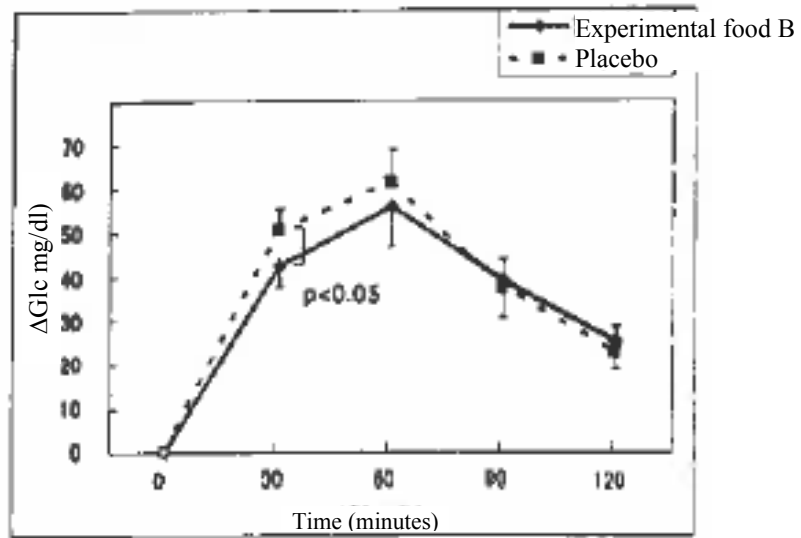
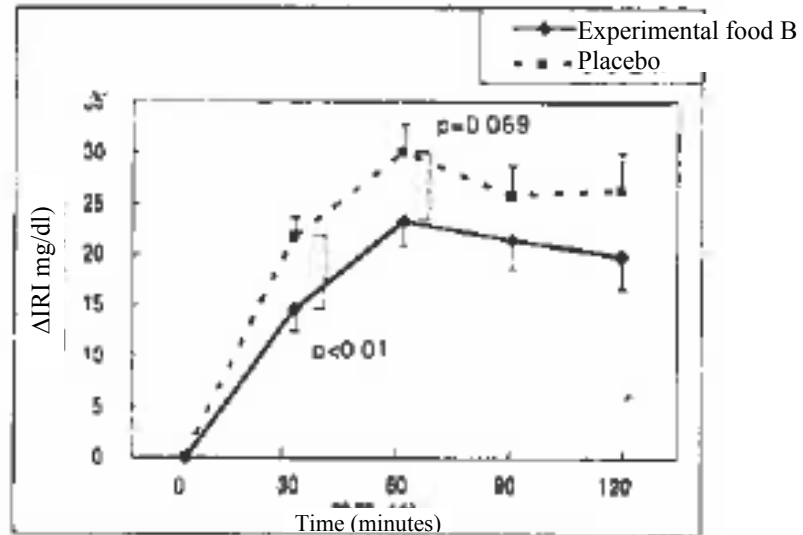


Figure 5 Changes over time in the post-prandial insulin levels (IRI) in lipid absorption test subjects on single intake of experimental food B and placebo (n = 12)



3. Examination of Safety

No adverse events caused by either of the experimental foods and no anomalous changes in laboratory data that could cause clinical problems were observed in the above 8 weeks intake test and the 2 single intake crossover tests. From these results, it is believed that “SUPER BOWS DIET TYPE A” and “SUPER BOWS DIET TYPE B” have no safety related problems as long as they are taken correctly. However, since there were cases of temporary gastrointestinal symptoms such as bloating sensation, etc. in some of the test subjects, care is required against excessive intake.

Summary

The results obtained from the 3 test conducted on humans, supported that “SUPER BOWS DIET TYPE A” has a body weight/body fat percentage reducing action and an inhibitory action against the rise of triglyceride levels after meals and “SUPER BOWS DIET TYPE B” has body weight/body fat percentage reducing action and an inhibitory action against the rise of blood glucose levels after meals. Their safety was also simultaneously confirmed. Therefore, it can be scientifically said that “SUPER BOWS DIET TYPE A” is a foodstuff that is useful for improving the dietary habits of people who are striving to cure/prevent obesity caused by high intake of fats and that “SUPER BOWS DIET TYPE B” is useful for improving the dietary habits of people who are striving to cure/prevent obesity caused by high intake of sugar. Even more wide-scale and detailed search and investigation in the functionality of “SUPER BOWS DIET TYPE A” and “SUPER BOWS DIET TYPE B” is expected in the future.

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