THE SYNTHESIS OF VITAMINS BY INTESTINAL BACTERIA IN MAN AND THE EFFECT OF CELLULOSE

I. SYNTHESIS OF RIBOFLAVIN

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Yasuda (1) has previously observed that riboflavin synthesis is increased in the mouse by administration of vegetable food while it is decreased by animal food and Iinuma (2) has reported that in human subject, riboflavin in the stool increases markedly with vegetable food while with animal food, the vitamin in the stool does not show much increase and the quantity of the synthesized vitamin decreases. Yasuda (3) has observed furthermore, in studies in the mouse, that when animal food is supplemented with cellulose, the synthesis of riboflavin by intestinal bacteria is greatly intensified and the excretion of the vitamin in the urine and stool is markedly increased. He ascribed therefore the intensification of synthesis of riboflavin by the vegetable diet to cellulose. The present study was undertaken in order to examine the finding in man.

EXPERIMENTAL

Diets

The experimental diets were similar to those of Iinuma (2) and consisted of the ordinary, vegetable and meat diets. Tables I-III give the composition, ingredients and calories of each diet. Only riboflavin contents were measured, and the others were calculated according to "the Standard Tables of Food Composition in Japan" (4). The losses in cooking were not taken into consideration. As seen from the Tables, the ordinary diet furnished 2200 Calories and 590 γ riboflavin, the vegetable diet 2200 Calories and 700 γ and the meat diet 2230 Calories and 660 γ riboflavin.

Methods

The subject used in the study was a normal, healthy male, 26 years of age. The schedule was one week of ordinary diet, one week of meat diet, one week of meat diet supplemented with filter paper and finally one week of meat diet alone. The filter paper was ground in a mortar until it became mushy and was added to white rice in concentration of 2 per cent of the total diet, and cooked. A similar schedule was followed with the vegetable diet.

The 24 hour urine (8-8 a. m.) was collected each day, stored in the dark

TABLE I
Ordinary Diet.

B, breakfast ; L, lunch ; D, dinner ; PR, proteins ; CH, carbohydrates ;
A, vitamin A ; B₁, thiamine ; B₂, riboflavin ; C, ascorbic acid.

	B	L	D	Total	Cal.	PR	Fat	CH	Vitamins			
									A	B ₁	B ₂	C
	<i>g</i>	<i>g</i>	<i>g</i>	<i>g</i>		<i>g</i>	<i>g</i>	<i>g</i>	I.U.	γ	γ	<i>mg</i>
Bread	200			200	494	14	3	103.2		300	60	
Jam	30			30	72	0.1	0.1	17.7				
Butter	10			10	73	0.06	8.1	0.02	240	1	2	
Milk	200			200	116	6	6.2	9	160	60	104.4	2
White rice		250	300	550	957	15.8	2.1	212.5		484	110	
Egg		40		40	61	5.1	4.5		320	40	116	
Salted salmon		30		30	45	7.55	1.5			30	35.4	
Pork			100	100	145	21.4	6.5	0.2		1000	100	
Cabbage			50	50	9	0.75	0.15	1.2	25	40	40	40
Soy bean oil				20	180		20					
Soy bean sauce				20	7	1.1	0.1	0.4		4	14.4	
Sauce				10	2			1.25			8	
White sugar				10	40			9.9				
Total					2201	73	53	355	745	1569	589	42

and measured by the lumiflavin method of Fujita (5). In the ordinary and meat diet periods, 8 ml was taken and in the meat plus filter paper period, 4 ml was taken, and made up to 10 ml with water, then 10 ml of 1 N NaOH was added. The mixture was centrifuged and 5 ml of the supernatant was used for photolysis to determine riboflavin. Permanganate was used to re-

TABLE II
Vegetable Diet.

	B	L	D	Total	Cal.	PR	Fat	CH	Vitamins			
									A	B ₁	B ₂	C
	<i>g</i>	<i>g</i>	<i>g</i>	<i>g</i>		<i>g</i>	<i>g</i>	<i>g</i>	I.U.	γ	γ	<i>mg</i>
Polished rice	150	200	200	550	957	15.8	2.1	212.5		484	110	
Sweet potato	100	100	100	300	354	3.9	0.6	83.1	300	450	115.5	90
Carrot	100	100	100	300	123	5.7	0.6	23.4	40500	300	120	30
Soy bean	50	30	50	130	517	44.6	22.1	41.2	13	650	260	
Cabbage		50	50	100	18	1.5	0.3	2.4	50	80	80	80
Soy bean oil				20	180		20					
Soy bean sauce				20	7	1.1	0.1	0.4		4	14.4	
White sugar				10	40			9.9				
Total					2196	72.5	45.8	372.9	40853	1968	700	200

move interfering fluorescent substances. Recovery was 98–99 per cent in the majority of cases.

TABLE III
Meat Diet.
 Supplemented with 40 mg ascorbic acid daily.

	B	L	D	Total	Cal.	PR	Fat	CH	Vitamins			
	<i>g</i>	<i>g</i>	<i>g</i>	<i>g</i>		<i>g</i>	<i>g</i>	<i>g</i>	A I.U.	B ₁ γ	B ₂ γ	C <i>mg</i>
Polished rice	150	200	200	550	957	15.8	2.1	212.5		484	110	
Egg	40	40	40	120	182	15.3	13.5		960	120	348	
Beef (fatty)	110	110	110	330	855	63.4	66.7	0.7	165	198	170	3
Soy bean oil				20	180		20					
Soy bean sauce				20	7	1.1	0.1	0.4		4	14.4	
White sugar				10	40			9.9				
Sauce				20	4			2.5			16	
Total				1070	2225	95.6	102.4	226	1125	796	658	3

The stool samples were treated in the following manner. The 24-hour stool was mixed well, 2 g were placed in water which had preliminarily been heated to 90°, warmed for 15 minutes in a 80° water bath, then ground, an adequate amount of water was added, the pH was adjusted to 4.5 with 1 *N* HCl, and the total volume was made up to 40.0 ml with water, heated for 5 minutes at 80°, cooled and centrifuged. Four ml of the supernatant was taken, and 6 ml of water and then 10 ml of 1 *N* NaOH were added and mixed well. Five ml samples were photolyzed and the riboflavin was determined. Permanganate was used to remove interfering substances. Recovery was 98–99 per cent in most of the cases. The amount of riboflavin contained in both the 24-hour urine and stool was expressed as the total excretion and the difference of it from that in the diet was expressed as the quantity synthesized.

Results

As shown in Fig. 1 and 2, the daily excretion of riboflavin in the urine after ingesting the ordinary diet was about 42 γ and that excreted in the stool was 578 γ . There was little fluctuation, the values remaining fairly constant. With the meat diet, there was little difference in the quantity of urine and stool, but compared with the ordinary diet period, there was an increase of about 40 per cent in the riboflavin excreted in the urine and a decrease of about 20 per cent in the vitamin excreted in the stool. The synthesized vitamin decreased therefore markedly to –140 γ . When the meat diet was supplemented with filter paper, the quantity of the vitamin in the urine increased to four times and that of the stool increased to about two times more than that observed with the ordinary diet. As seen in Fig. 1, the riboflavin content of the stool increased markedly day by day and a parallel rise in urinary vitamin as well as synthesized vitamin was also observed. On march 30, there was fever and diarrhea and the experiment was therefore temporarily stopped. On April 4, the meat plus filter paper diet was rein-

FIG. 1 *Metabolism with Meat Diet.*

stituted and when the vitamin content of the stool reached values roughly equal to those prior to the diarrhea, the diet was switched to the meat one. With cessation of supplementing filter paper, the vitamin content of the stool promptly dropped with a corresponding decrease to the urinary vitamin content and a marked reduction in the synthesized vitamin. The diet was returned to the ordinary one and after the urinary and fecal vitamin content had become fairly constant, the vegetable diet was instituted. There was a gradual rise in the vitamin content of the stool but little change was observed in the urine. The average riboflavin content of the stool with the vegetable diet was approximately 2 times more than that with the ordinary diet but the increase in the urinary vitamin was about 20 per cent. With addition of filter paper to the vegetable diet, there was a further increase in the fecal vitamin and the excretion

FIG. 2 *Metabolism with Vegetable Diet.*

was 3 times greater than that in the case of the ordinary diet but there was only an increase of about 30 per cent in the urine as can be clearly seen in Fig. 2.

DISCUSSION

From the above results it can be seen that cellulose markedly increases the riboflavin content of the stool and has an accerelating action on the synthesis of the vitamin by the intestinal bacteria in man. It was also found that the addition of filter paper to a vegetable diet resulted in a further increase in riboflavin excretion in the stool and an increase in the synthesis of the vitamin by intestinal bacteria. In other words, the marked increase in synthesis of the vitamin with the vegetable diet (as compared with the meat diet) is due to cellulose and it is believed that the nutritional significance of cellulose in riboaflavin metabolism has been clarified by this experiment.

SUMMARY

In experiments in human subject, it was found that there was a decrease in synthesis of riboflavin by intestinal bacteria when the diet was changed from an ordinary to a meat diet but with addition of cellulose, there was an increase in the synthesis of the vitamin.

When cellulose was added to a vegetable diet, the effect became even more marked. For increase in riboflavin content of the urine, the addition of meat besides cellulose had a greater effct.

REFERENCES

1. Yasuda, T., *Vitamins* 4, 440 (1951).
2. Iinuma, S., *ibid.* 5, 96 (1952).
3. Yasuda, T., *ibid.* 6, 7 (1953).
4. Committee on Food Composition, Resources Council, Prime Minister's Office. Standard Tables of Food Composition in Japan. Shigen-Kyokai, Tokyo (1954).
5. Fujita, A., Vitamin Assay Methods (Japanese). Nankodo, Tokyo (1955).