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Nutritional and Ariboflavinosis-curing Effects of Riboflavin-5′-monobutyrate and Monopalmitate[1)]

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Riboflavin-5′-monobutyrate has the same vitamin B_2 activity (nutritional and ariboflavinosis-curing effects) in rats as riboflavin, whereas the vitamin B_2 activity of riboflavin-5′-monopalmitate was less than that of riboflavin or riboflavin-5′-monobutyrate.

Keywords——nutritional effect of riboflavin monoester; ariboflavinosis curing by riboflavin monoester; riboflavin-5′-monobutyrate; riboflavin-5′-monopalmitate; vitamin B_2 activity; riboflavin; ariboflavinosis of rat

In the previous paper,[1)] we reported the syntheses of monoesters of riboflavin, *i.e.*, riboflavin-5′-monobutyrate (R–MB) and riboflavin-5′-monopalmitate (R–MP). Yagi and Okuda[3)] reported in 1963 that riboflavin tetrabutyrate (R–TB) had the same vitamin B_2 activity (nutritional and ariboflavinosis-curing effects) in young rats as riboflavin, but riboflavin tetrapalmitate (R–TP) did not have vitamin B_2 activity, and rats administered R–TP showed clearly ariboflavinosis.

It was expected that the newly synthesized R–MB would have the same vitamin B_2 activity as riboflavin and R–TB, but it was not clear whether R–MP would have vitamin B_2 activity or not. To investigate this, the nutritional and ariboflavinosis-curing effects of R–MB and R–MP were studied using young rats.

Experimental

Materials

Riboflavin——This was recrystallized from a commercial sample.

R-MB and R-MP——These esters were synthesized by our method, as described in the previous paper.[1)]

Animals——Wistar male rats weighing between 40 and 50 g which had received a standard diet for a week were used.

Standard Diet——The diet had the following composition:[3)] riboflavin-free casein 22.0%, sucrose (powdered, containing 3% corn-starch) 66.5%, butter 9.0%, and the following salt mixture 2.5%. The salt mixture was composed of, in mg, NaCl 117, $MgSO_4 \cdot 7H_2O$ 180, $Na_2HPO_4 \cdot 12H_2O$ 235, K_2HPO_4 644, $Ca_3(PO_4)_2$ 365, Ca lactate 879, and Fe citrate$\cdot 5H_2O$ 80.

Riboflavin-free casein was prepared as follows. Commercial milk casein (85 g) was dissolved in 600 ml of 1% NaOH, and the pH of this solution was adjusted to pH 4.6 (bromocresol green pH-test paper) with 25% acetic acid. Casein was thus precipitated, then filtered and washed with water three times to remove acetic acid, and finally washed with 50% ethanol and 100% ethanol. Riboflavin-free casein thus obtained was dried at room temperature overnight (yield 80%).

Vitamin B components and fat-soluble vitamins were separately prepared and administered as follows.

Crystalline vitamin B components were dissolved in distilled water (0.05 ml) so as to provide each rat with the following daily amounts (mg): thiamine hydrochloride, pyridoxine hydrochloride, folic acid, each 0.005, riboflavin 0.01, calcium pantothenate, *p*-aminobenzoic acid, each 0.025, DL-α-tocopherol acetate 0.09, inositol 0.625, and choline chloride 1.25. Vitamin A (10000 I.U.) and vitamin D (1000 I.U.) were dissolved in 5 g of olive oil and 5 mg (1 drop) of vitamin-olive oil was administered to each rat with a glass micropipette daily, to provide vitamin A (10 I.U.) and vitamin D (1 I.U.).

1) J. Okuda and N. Horiguchi, *Chem. Pharm. Bull.*, **28**, 181 (1980).
2) Location: *Tempaku-cho, Tempaku-ku, Nagoya, 468, Japan.*
3) K. Yagi, J. Okuda, and M. Kobayashi, *J. Vitaminol.*, **9**, 168 (1963).

Methods

1. Nutritional Effects of R-MB and R-MP——A colony of 25 Wistar male rats fed on the standard diet for a week was used. The animals were maintained in separate cages in an air-conditioned room. They were divided into 5 groups of 5 rats. Group A comprised control rats fed on the standard diet, group B was fed on the riboflavin-deficient diet, group C on the riboflavin-deficient diet plus 10 μg (expressed as riboflavin here and subsequently) of R-MB suspended in olive oil, group D on the riboflavin-deficient diet plus 10 μg of R-MP suspended in olive oil, and group E on the riboflavin-deficient diet plus 50 μg of R-MP suspended in the olive oil.

Changes in body weight of the rats were observed for 30 days to compare the nutritional effects of R-MB and R-MP with that of riboflavin. The rats were housed with free access to food and vitamins were compulsorily given with a micropipette.

2. Effects of R-MB and R-MP on Ariboflavinosis of Rats——A colony of 25 Wistar male rats was fed on the standard diet for a week before the test, then placed on a riboflavin-deficient diet for 27 days until they showed ariboflavinosis. They were then divided into 5 groups of 5 rats. Group F was fed on the standard diet, group G on the riboflavin-deficient diet, group H on the riboflavin-deficient diet plus 10 μg of R-MB suspended in olive oil, group I on the riboflavin-deficient diet plus 10 μg of R-MP suspended in olive oil, and group J on the riboflavin-deficient diet plus 50 μg of R-MP suspended in olive oil.

To compare the ariboflavinosis-curing effects of R-MB and R-MP with that of riboflavin, the change in body weight of the rats was observed for 60 days.

3. Weights and Amounts of Vitamin B_2 in the Livers and Kidneys of Rats after Administration of R-MB and R-MP——After the experiment on the nutritional effects of R-MB and R-MP, rats of groups A, B, C, D, and E were killed by decapitation and the livers and kidneys were removed and weighed. The total flavin contents and three types of flavins (FAD, FMN, and riboflavin) were determined by the methods of Yagi *et al.*[4]

Results

1. Nutritional Effects of R-MB and R-MP

Group A (10 μg of riboflavin)——The initial mean body weight of this group, 42.8 g, increased to 101.4 g after 30 days. The mean weight gain per day was 1.9 g. The results are shown Fig. 1.

Group B (riboflavin-deficient)——The initial mean body weight of this group was 43.4 g. Two rats of this group died, one on the 24th and one on the 28th day. After 30 days, the mean body weight of the remaining three rats was 55.3 g. The results are shown in Fig. 2. During this period, the rats showed the characteristic symptoms of ariboflavinosis *i.e.*, nose bleeding, inflamation of the eyelids, loss of appetite and moistened hairs,[3,5] as shown in Fig. 3.

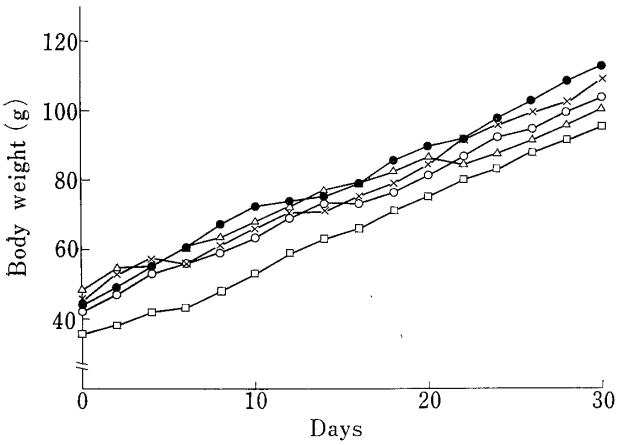

Fig. 1. Changes in Body Weight of Group A Rats

The animals were fed on the riboflavin-deficient diet plus riboflavin (10 μg per rat per day).

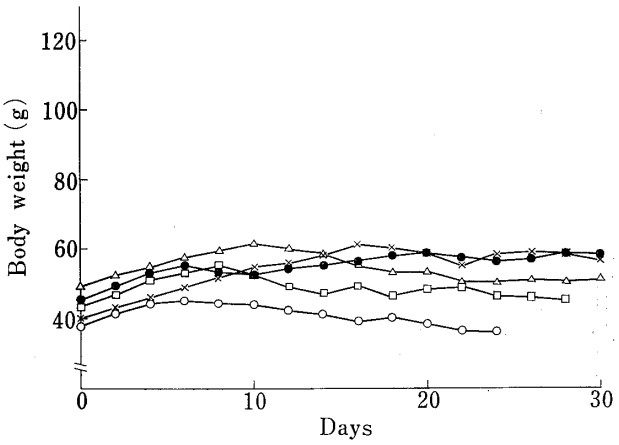

Fig. 2. Changes in Body Weight of Group B Rats

The animals were fed on the riboflavin-deficient diet.

4) K. Yagi, M. Yamada, and J. Okuda, *J. Vitaminol.*, **15**, 155 (1969).
5) J.P. Lambooy and H.V. Aposhian, *J. Nutrition*, **47**, 539 (1952).

Fig. 3. A Rat with Ariboflavinosis

it had reached 105.6 g, as shown in Fig. 6. same as that of group A.

Group C (10 μg of R-MB)——The initial mean body weight was 43.2 g. After 30 days, it had increased to 101.8 g. The daily gain was 1.9 g, the same as that of group A. The results are shown in Fig. 4.

Group D (10 μg of R-MP)——The initial mean body weight of the rats was 45.6 g. After 30 days, it had reached 79.4 g. The weight gain per day was 1.1 g. The results are shown in Fig. 5.

Group E (50 μg of R-MP)——The initial mean body weight was 44.6 g. After 30 days, The daily weight gain of this group was the

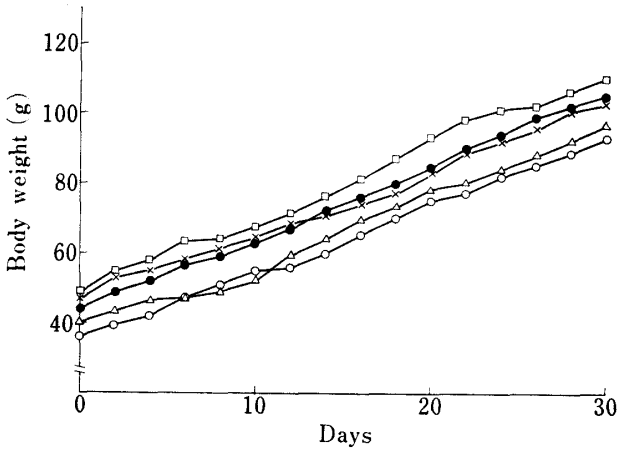

Fig. 4. Changes in Body Weight of Group C Rats

The animals were fed on the riboflavin-deficient diet plus R-MB (10 μg as riboflavin per rat per day).

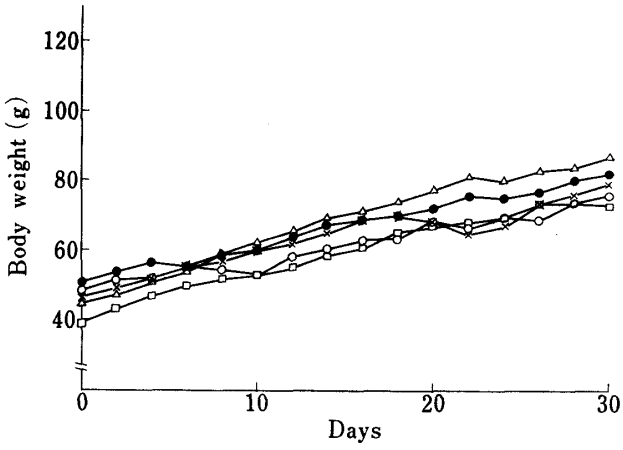

Fig. 5. Changes in Body Weight of Group D Rats

The animals were fed on the riboflavin-deficient diet plus R-MP (10 μg as riboflavin per rat per day).

2. Effects of Riboflavin Monoesters on Ariboflavinosis of Rats

Group F (Riboflavin-deficient)——The riboflavin-deficient diet was given to the rats for an initial period of 27 days, as in the case of group B, and then they were fed on the same

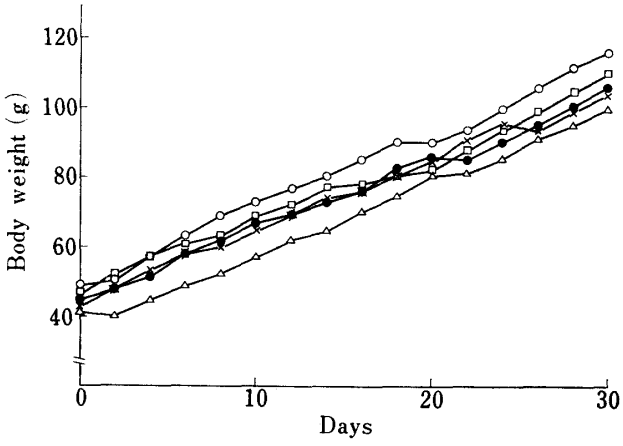

Fig. 6. Changes in Body Weight of Group E Rats

The animals were fed on the riboflavin-deficient diet plus R-MP (50 μg as riboflavin per rat per day).

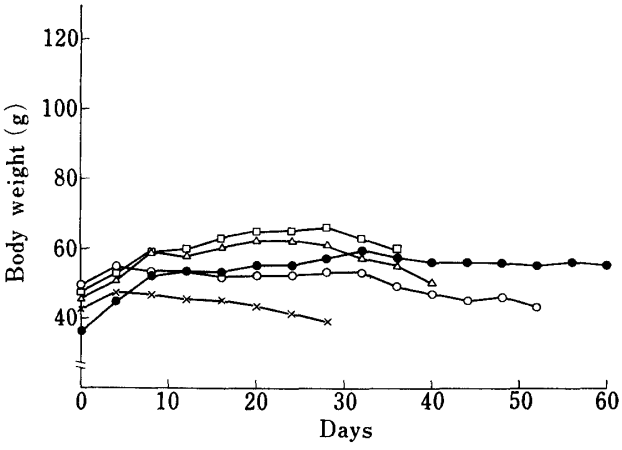

Fig. 7. Changes in Body Weight of Group F Rats

The animals were fed on the riboflavin-deficent diet.

riboflavin-deficient diet. During the test, rats died on the 28th, 36th, 40th, 52nd, and 60th days, as shown in Fig. 7.

Group G (10 μg of Riboflavin)——After feeding the riboflavin-deficient diet for 27 days, 10 μg of riboflavin was given to each rat. Rapid increases of their body weights were observed, as shown in Fig. 8, and typical symptoms of riboflavin deficiency completely disappeared.

Group H (10 μg of R-MB)——After feeding the riboflavin-deficient diet for 27 days, 10 μg of R–MB was given to each rat of this group daily. Body weight changes of this group were the same as those of group G, as shown in Fig. 9, and the characteristic signs of riboflavin deficiency completely disappeared after the administration of R–MB.

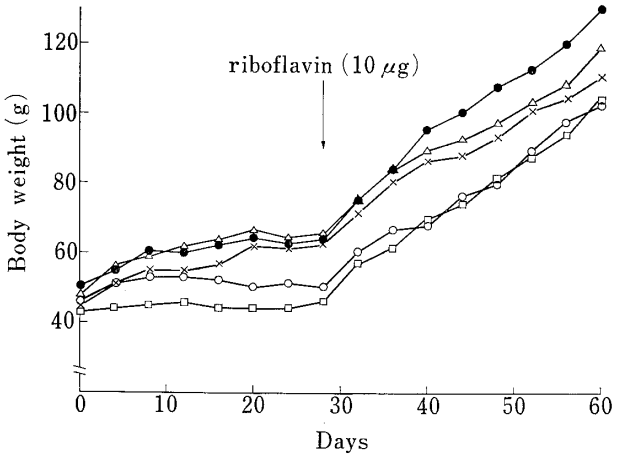

Fig. 8. Changes in Body Weight of Group G Rats

The animals were administered riboflavin (10 μg per rat per day) after they had received the riboflavin-deficient diet for 27 days. The arrow shows the start of the administration of riboflavin (28 th day).

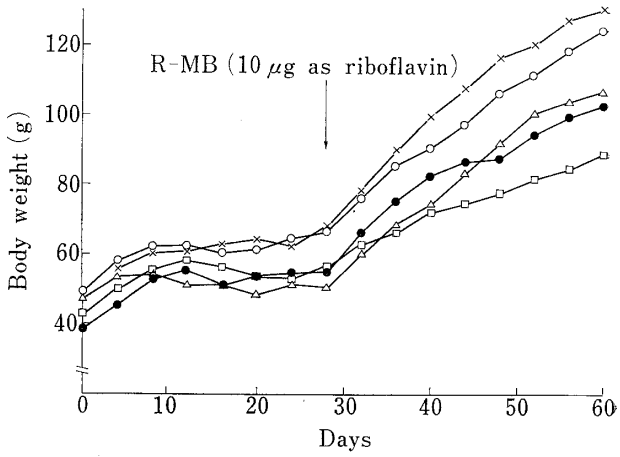

Fig. 9. Changes in Body Weight of Group H Rats

The animals were administred R–MB (10 μg as riboflavin per rat per day) after they had received the riboflavin-deficient diet for 27 days. The arrow shows the start of the administration of R–MB (28 th day).

Group I (10 μg of R-MP)——After feeding the riboflavin-deficient diet for 27 days, 10 μg of R–MP was given to each rat of this group daily. Body weight changes of this group were slightly improved, as shown in Fig. 10. The characteristic signs of riboflavin deficiency did not completely disappear.

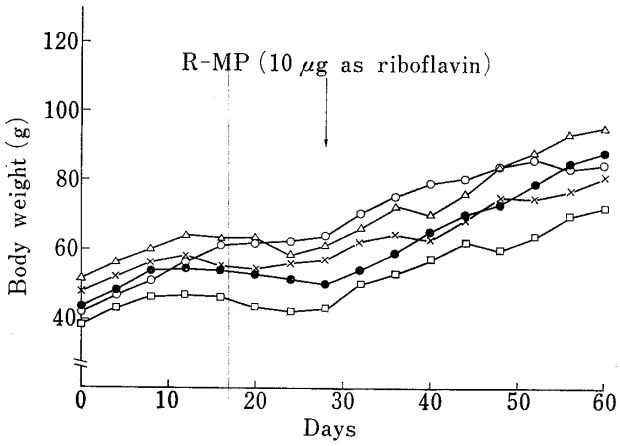

Fig. 10. Changes in Body Weight of Group I Rats

The animals were administered R–MP (10 μg as riboflavin per rat per day) after they had received the riboflavin-deficient diet for 27 days. The arrow shows the start of the administration of R–MP (28 th day).

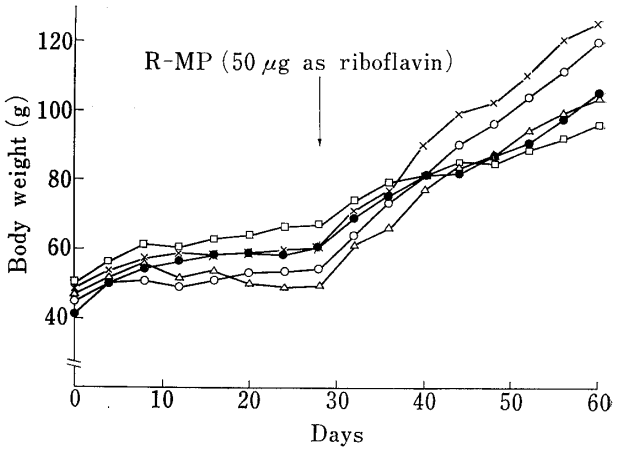

Fig. 11. Changes in Body Weight of Group J Rats

The animals were administered R–MP (50 μg as riboflavin per rat per day) after they had received the riboflavin-deficient diet for 27 days. The arrow Shows the start of the administration of R–MP (28th day).

Table I. Mean Weights of the Livers and Kidneys in Each Experimental Group

Group	Liver (g)	Kidneys (g)
A (10 μg of riboflavin)	5.2	1.0
B (riboflavin-deficient)	3.0*	0.8*
C (riboflavin-deficient plus 10 μg of R–MB)	5.1	1.0
D (riboflavin-deficient plus 10 μg of R–MP)	4.3	1.0
E (riboflavin-deficient plus 50 μg of R–MP)	5.0	1.1

$n=5$, *$n=3$.

Table II. Mean Contents of Total Flavins and Percentages of FAD, FMN, and Riboflavin in the Livers and Kidneys in Each Experimental Group

Group	Tissue	Total flavin (μg/g wet weight)	FAD %	FMN %	R† %
A (10 μg of riboflavin)	Liver	28.9	74.4	23.7	1.9
	Kidney	30.8	68.3	29.4	2.3
B (riboflavin-deficient)	Liver	21.8*	59.3*	36.4*	4.3*
	Kidney	21.1*	59.0*	37.0*	4.0*
C (riboflavin-deficient plus 10 μg of R–MB)	Liver	29.8	70.4	28.5	1.1
	Kidney	29.4	66.0	31.1	2.9
D (riboflavin-deficient plus 10 μg of R–MP)	Liver	27.2	64.5	30.0	5.5
	Kidney	28.9	60.6	36.7	2.7
E (riboflavin-deficient plus 50 μg of R–MP)	Liver	33.4	76.0	22.7	2.3
	Kidney	33.5	68.7	30.0	1.3

† riboflavin. $n=5$, *$n=3$.

Group J (50 μg of R-MP)——After feeding the riboflavin-deficient diet for 27 days, 50 μg of R–MP was given to each rat of this group daily. Body weight changes of this group were similar to those of group G, as shown in Fig. 11, and the characteristic signs of riboflavin deficiency completely disappeared.

3. Weights of, and Vitamin Contents in the Livers and Kidneys of Groups A, B, C, D, and E

The weights of the livers and kidneys of each group are listed in Table I. The weights of the livers in groups A, C, and E were 5.2, 5.1, and 5.0 g, respectively. The weights of the kidneys of groups A, C, D, and E were *ca.* 1.0 g, while those of the livers and kidneys of group B (riboflavin-deficient group) had decreased to 3.0 and 0.8 g, respectively. Total riboflavin contents in the livers and kidneys, and the percentages of the three types of flavins in these tissues are shown in Table II. The amounts of total flavins in the livers and kidneys of groups A and C were almost the same, but those of group D were rather lower than those of group A, while those of group B had decreased to about 75% of those of group A. On the other hand, the values of total flavins in the livers and kidneys of group E were about 15% and 8% higher than those of group A, respectively.

The percentages of the three types of flavins in each organ of groups A, C, and E were almost the same; the proportion of FAD was over 70%. Group B, which had received a riboflavin-deficient diet showed a low percentage of FAD and a high percentage of FMN. The percentages of FAD in group D were decreased compared to those of group E and were rather similar to those of group B.

Discussion

Yagi and Okuda reported[3] in 1963 that R–TB was able to replace riboflavin in the metabolism of the rat with respect to growth, survival, and optimal physical appearance,

but the rats that received R–TP instead of riboflavin clearly showed the symptoms of ariboflavinosis. On the other hand, Masushige[6] reported in 1969 that riboflavin dibutyrates (2′,3′-dibutyrate and 4′,5′-dibutyrate) showed the same nutritional effect as riboflavin.

In this paper, we have described the nutritional and ariboflavinosis-curing effects of R–MB and R–MP. As it had been shown in the previous paper[3] that R–TB has the same nutritional effect and ariboflavinosis-curing effect as riboflavin, and that R–TP has no vitamin B_2 activity, it was expected that R–MB would have the same effect as riboflavin, and that R–MP might have low vitamin B_2 activity.

The vitamin B_2 activity of R–MB in rats was the same as that of riboflavin, but the vitamin B_2 activity of R–MP was clearly lower than that of riboflavin or R–MB. The weights, total amounts of flavins and percentages of the three flavins in the livers and kidneys of group C (10 μg of R–MB) were essentially the same as those of group A, and those of group D (10 μg of R–MP) were intermediate between those of group A (10 μg of riboflavin) and those of group B (riboflavin-deficient).

These results suggest that R–MB was easily hydrolyzed to riboflavin, while R–MP was slowly hydrolyzed to riboflavin, possibly by an esterase (carboxylic ester hydrolyase EC 3.1.1.1), in the animal body, as reported in the previous paper.[1]

6) S. Masushige, T. Suzuki, and Y. Sahashi, *J. Vitaminol.*, **15**, 266 (1969).