Nutritional and Aroboflavinosis-curing Effects of Riboflavin-5'-monobutyrate and Monopalmitate

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Riboflavin-5'-monobutyrate has the same vitamin B₂ activity (nutritional and ariboflavinosis-curing effects) in rats as riboflavin, whereas the vitamin B₂ 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 B₂ 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,² reported in 1963 that riboflavin tetrabutyrate (R-TB) had the same vitamin B₂ activity (nutritional and ariboflavinosis-curing effects) in young rats as riboflavin, but riboflavin tetrapalmitate (R-TP) did not have vitamin B₂ activity, and rats administered R-TP showed clearly ariboflavinosis.

It was expected that the newly synthesized R-MB would have the same vitamin B₂ activity as riboflavin and R-TB, but it was not clear whether R-MP would have vitamin B₂ 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.³

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:² 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, MgSO₄·7H₂O 180, Na₂HPO₄·12H₂O 235, K₂HPO₄ 644, Ca₃(PO₄)₂ 365, Ca lactate 879, and Fe citrate·5H₂O 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, L-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.).

²) Location: Tempaku-ku, Tempaku-ku, Nagoya, 468, Japan.
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 Arboflavinosis 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 arboflavinosis. 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 arboflavinosis-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, 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 arboflavinosis i.e., nose bleeding, inflammation of the eyelids, loss of appetite and moistened hairs5) as shown in Fig. 3.

![Fig. 1. Changes in Body Weight of Group A Rats](image1)

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](image2)

The animals were fed on the riboflavin-deficient diet.

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

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**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

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**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-deficient 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.

![Graph showing changes in body weight of Group G Rats](image1)

**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 (28th day).

![Graph showing changes in body weight of Group H Rats](image2)

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

The animals were administered 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 (28th 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.

![Graph showing changes in body weight of Group I Rats](image3)

**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 (28th day).

![Graph showing changes in body weight of Group J Rats](image4)

**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

<table>
<thead>
<tr>
<th>Group</th>
<th>Liver (g)</th>
<th>Kidneys (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (10 μg of riboflavin)</td>
<td>5.2</td>
<td>1.0</td>
</tr>
<tr>
<td>B (riboflavin-deficient)</td>
<td>3.0*</td>
<td>0.8*</td>
</tr>
<tr>
<td>C (riboflavin-deficient plus 10 μg of R-MP)</td>
<td>5.1</td>
<td>1.0</td>
</tr>
<tr>
<td>D (riboflavin-deficient plus 10 μg of R-MP)</td>
<td>4.3</td>
<td>1.0</td>
</tr>
<tr>
<td>E (riboflavin-deficient plus 50 μg of R-MP)</td>
<td>5.0</td>
<td>1.1</td>
</tr>
</tbody>
</table>

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

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

† 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 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 aribo-
flavinosis. On the other hand, Masushige\textsuperscript{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\textsuperscript{8}) 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 $\mu$g of R–MB) were essentially the same as those of group A, and those of group D (10
$\mu$g of R–MP) were intermediate between those of group A (10 $\mu$g 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.\textsuperscript{19)}

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