STUDIES ON FATTY ACID ESTERS OF FLAVINS

III. NUTRITIONAL AND ARIBOFLAVINOSIS-CURING EFFECTS

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Recently, the authors synthesized fatty acid esters of riboflavin, fat-soluble riboflavin derivatives (1, 2). They are riboflavin-2',3',4',5'-tetrapalmitate (R-PAL), -tetracaprate (R-CAP), -tetrabutryrate (R-BUT), and -tetrapropionate (R-PROP)². Hydrolysis of these esters by pancreatic lipase and digestive juice showed that, among these esters, R-BUT was most easily hydrolysed to riboflavin, whereas R-PAL was hardly hydrolysed (3, 4).

These findings suggested that these esters could be hydrolysed to riboflavin and fatty acids in higher animals, thus serving as the vitamin source. To ascertain this assumption, the nutritional effects of R-BUT and R-PAL on healthy rats were studied. Then, the curing effects of these esters were examined using riboflavin-deficient rats. This paper deals with the results of the experiments in detail, some parts of which have been preliminarily reported (5, 6).

EXPERIMENTAL

Materials

1. Riboflavin

Recrystallized from the commercial sample.

2. Fatty Acid Esters of Riboflavin

Both R-BUT (mp, 145—147°; m.w., 656.7) and R-PAL (mp, 78.5°; m.w., 1329.8) were synthesized by our method described in the previous papers (1, 2).

3. Animals

White Wistar male rats (average weight, 60 g) which had received a standard diet for a week were used.

4. Standard Diet

The diet had the following composition (7), in per cent: riboflavin-free casein 22.0, sucrose (powdered, containing 3 per cent corn-starch) 66.5, butter 9.0, and the following salt mixture 2.5. The salt mixture was composed of, in mg, NaCl 117,

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² Abbreviations: R-PAL, riboflavin-2',3',4',5'-tetrapalmitate; R-CAP, riboflavin-2',3',4',5'-tetracaprate; R-BUT, riboflavin-2',3',4',5'-tetrabutryrate; R-PROP, riboflavin-2',3',4',5'-tetrapropionate.

MgSO₄·7H₂O 180, Na₂HPO₄·12H₂O 235, K₂HPO₄ 644, Ca₃(PO₄)₂ 365, Ca-lactate 879, and Fe-citrate·5H₂O 80.

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

Crystalline vitamin B components were dissolved in distilled water (0.05 ml) so as to provide each rat daily with the following, in mg: thiamine hydrochloride, pyridoxine hydrochloride, folic acid, each 0.005, riboflavin 0.01, calcium pantothenate, *p*-aminobenzoic acid, each 0.025, inositol 0.625, choline chloride 1.25.

Fat-soluble vitamins (vitamin A and D) were provided in the form of cod liver oil (5 mg), to which 0.1 mg tocopherol was added per rat daily.

To administer R-BUT or R-PAL, the ester was dissolved in ether, and the solution was added to the cod liver oil, followed by evaporation of ether at low temperature *in vacuo*. The resultant ester suspension in cod liver oil was used.

5. Riboflavin-Deficient Diet

It was the same as the standard diet except for the omission of riboflavin.

Methods

1. Nutritional Effects of Fatty Acid Esters of Riboflavin on Normal Rats

A colony of 30 Wistar male rats fed on the standard diet for a week was used. The animals were kept individually in a separate cage in a temperature-controlled

FIG. 1 Change in Body Weight of Group A
The animals were fed on the standard diet.

FIG. 2 Change in Body Weight of Group B
The animals were fed on the riboflavin-deficient diet.

room. All the animals were then divided into 6 groups of 5 rats: *Group A* was continued to place on the standard diet, *Group B* was fed on the riboflavin-deficient

FIG. 3 Rat Fed on the Riboflavin-Deficient Diet for 20 Days

FIG. 4 Change in Body Weight of Group C The animals were fed on the riboflavin-deficient diet plus 10 μ g (as riboflavin) of R-BUT suspended in cod liver oil.

FIG. 5 Change in Body Weight of Group D The animals were fed on the riboflavin-deficient diet plus 50 μ g (as riboflavin) of R-BUT suspended in cod liver oil.

diet, *Group C* on the riboflavin-deficient diet plus 10 μg (expressed as riboflavin, the same is applicable to all the following descriptions) of R-BUT dissolved in the cod liver oil, *Group D* on the riboflavin-deficient diet plus 50 μg of R-BUT suspended in the cod liver oil, *Group E* on the riboflavin-deficient diet plus 50 μg of R-BUT suspended in water, *Group F* on the riboflavin-deficient diet plus 50 μg of R-PAL dissolved in the cod liver oil.

To compare the nutritional effect of R-BUT or R-PAL with that of riboflavin, the changes in the weights of the animals were observed for 30 days. The diet and water were given freely, and vitamins were compulsorily given with a pipette.

2. Cure of Ariboflavinosis of Rats by Fatty Acid Esters of Riboflavin

A colony of 20 Wistar male rats fed on the standard diet for a week was used. They were also kept in separate cages in a temperature-controlled room. All the animals were then placed on a riboflavin-deficient diet until they almost ceased to gain weight. It took about 25 days.

They were then divided into 4 groups of 5 each: *Group G* was continued to place on the riboflavin-deficient diet, *Group H* was fed on the riboflavin-deficient diet plus 50 μg of riboflavin suspended in the vitamin B solution, *Group I* on the riboflavin-deficient diet plus 50 μg of R-BUT suspended in the cod liver oil and *Group J* on the riboflavin-deficient diet plus 50 μg of R-PAL dissolved in the cod liver oil.

FIG. 6 Change in Body Weight of Group E. The animals were fed on the riboflavin-deficient diet plus 50 μg (as riboflavin) of R-BUT suspended in water.

FIG. 7 Change in Body Weight of Group F. The animals were fed on the riboflavin-deficient diet plus 50 μg (as riboflavin) of R-PAL dissolved in cod liver oil.

To compare the ariboflavinosis-curing effect of R-BUT or R-PAL with that of riboflavin, the changes in the weights of the animals were observed for 60 days.

RESULTS

1. Nutritional Effect of Fatty Acid Esters of Riboflavin on Normal Rats

Group A (riboflavin group)—From the beginning of the test, the group was fed on the standard diet to which 10 μ g of riboflavin had been added in the form of suspension in the vitamin B solution. Normal weight gain, about 1.7 g per day, was observed and it continued until the end of the test as shown in Fig. 1.

Group B (riboflavin-deficient)—The test was started by changing the standard diet for the riboflavin-deficient one. Just a few days after the beginning of the test, the weight gain remained unchanged. However, the rate was decreased day by day, reaching a plateau within about two weeks after the beginning of the test. The condition was continued until the 20th day as shown in Fig. 2.

At this period, the animals showed the characteristic symptoms of ariboflavinosis, *i.e.*, nose bleed, inflammation of eyelids, loss of appetite, and moistened hairs as shown in Fig. 3. Then, the weight of the survived animals slowly decreased and three of the five animals died.

FIG. 8 Change in Body Weight of Group G
The animals were fed on the riboflavin-deficient diet.

Group C (receiving 10 μ g R-BUT dissolved in oil)—10 μ g of R-BUT and the riboflavin-deficient diet were given to each rat. The weight gain was the same as that of *Group A*, as shown in Fig. 4. The average weight reached to 107 g at the end of the test.

Group D (receiving 50 μ g R-BUT suspended in oil)—The weight of the group was increased more rapidly than that of *Group A* or *C* as shown in Fig. 5, and the average weight reached to 124 g at the end of the test.

Group E (receiving 50 μ g R-BUT suspended in water)—The weight gain was nearly the same as that of *Group D* as shown in Fig. 6.

Group F (receiving 50 μ g R-PAL dissolved in oil)—The weight of the group was scarcely increased as shown in Fig. 7. The symptoms of ariboflavinosis, as described above, were observed at the end of the test.

2. Cure of Ariboflavinosis of the Rats by Fatty Acid Esters of Riboflavin

Group G (riboflavin-deficient)—The weights of the rats increased normally during the first week when they were fed on the standard diet. The test was begun by changing the standard diet for the riboflavin-deficient one. Just a few days after the beginning of the test, the weight gain remained unchanged. It was decreased, however, day by day and reached a plateau to the extent that the animal

FIG. 9 Change in Body Weight of Group H

50 μ g riboflavin was administered daily after feeding on the riboflavin-deficient diet. The arrow shows the start of the administration.

failed to gain more than 6 g in a period of 14 days. At the end of this period (25th day from the beginning), the rats were supposed to be in riboflavin deficiency, as Lambooy *et al.* (8) described. After 30 days from the beginning, the average weight of the rats began to decrease gradually as shown in Fig. 8 and the animals showed, as in the case of *Group B*, the characteristic symptoms of ariboflavinosis. Some of them died after 40 days from the beginning, and two of the five survived until the end of the test (60th day).

Group H (receiving 50 μ g riboflavin)—The riboflavin-deficient diet was given to the rats for 25 days as in the case of *Group G*, then 50 μ g of riboflavin was given daily to each rat in the form of suspension in the aqueous vitamin B solution. A rapid increase of weight was observed, and it continued until the end of the test, as shown in Fig. 9.

All the animals gained the average weight of about 170 g in this case, no death occurring during the test, and seemed to remain in healthy condition.

Group I (receiving 50 μ g R-BUT)—As in the case of *Group H*, 50 μ g of R-BUT was given to each of the riboflavin-deficient rats which had been fed on the deficient diet for 25 days. A rapid increase of the weight was observed as in the case of *Group H* (see Fig. 10). The growth curves of *Group I* are nearly the same

FIG. 10 Change in Body Weight of Group I

50 μ g R-BUT (suspended in cod liver oil) was administered daily after feeding on the riboflavin-deficient diet. The arrow shows the start of the administration.

as those of *Group H*.

Group J (receiving 50 μ g R-PAL) — 50 μ g of R-PAL was given to each of the deficient rats as in the case of *Group H*. As shown in Fig. 11, a small increase of the weight was observed in a few days after beginning the administration of R-PAL and then the weight was not increased until the end of the test. One in the five animals died at the 54th day of the test, and the others did not survive long. The animals seemed to be in riboflavin deficiency.

DISCUSSION

The nutritional effect of R-BUT or R-PAL on normal rats was compared firstly with that of riboflavin. In these experiments, R-BUT had the same nutritional value as riboflavin, but it was not the case with R-PAL, showing that R-BUT, but not R-PAL, was utilized as riboflavin by the animals. The difference in the nutritional effect between the two esters could be attributed to the difference in the acyl group attached to riboflavin.

As described previously (3, 4), R-BUT is rather easily hydrolysed to riboflavin, but R-PAL is hardly hydrolysed. This difference in the rate of hydrolysis between

FIG. 11 Change in Body Weight of *Group J*
50 μ g R-PAL (dissolved in cod liver oil) was administered daily after feeding on the riboflavin-deficient diet. The arrow shows the start of the administration.

the two esters by pancreatic lipase and by duodenal juice may cause the difference in nutritional effects between the two esters.

Although R-BUT was proved to possess vitamin action and preventive effects on ariboflavinosis, it could not be decided whether these esters can cure ariboflavinosis or not. Thus, the curing-effects of R-BUT and R-PAL on ariboflavinosis were studied, and it became clear that ariboflavinosis-curing effect was found in R-BUT, but not in R-PAL.

These results indicate that R-BUT is active as the vitamin in both normal and riboflavin-deficient rats. It should be noted that R-BUT, having a prolonged action in the animal body, has also ariboflavinosis-curing effect.

SUMMARY

It was proved that riboflavin-tetrabutyrates has the same vitamin action as riboflavin, but the action of riboflavin-tetrapalmitate was not observed clearly.

The ariboflavinosis-curing effect of riboflavin-tetrabutyrates was also demonstrated, but riboflavin-tetrapalmitate did not cure the ariboflavinosis.

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