EFFECT OF 8-AMINO-8-DEMETHYL-D-ARABOFLAVIN, 
8-AMINO-8-DEMETHYL-D-RIBOFLAVIN AND 
8-HYDROXY-8-DEMETHYL-D-RIBOFLAVIN 
ON MICE¹

Kunio Matsui and Sabu Kasai²

Division of Biology, Research Institute for Atomic Energy, Osaka 
City University, Sumiyoshi-ku, Osaka 558, Japan
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Diets containing AAF or ARF instead of RF were administered to 
RF-deficient mice and growth was followed, and after 9 days liver and 
kidneys were removed and weighed. No significant difference in 
growth was found between experimental and control (RF-deficient) 
groups. AAF significantly increased the weight percentage of liver to 
body weight, and the liver of some mice administered AAF or ARF had 
the appearance of fatty liver. In kidneys no difference in weight per-
centage and appearance was found between experimental and control 
group. The hair of mice administered ARF was nearly as clean as that of 
mice administered RF, although that of mice deficient in RF or ad-
ministered AAF was dirty and had the appearance of being drenched. 
Mice administered HF per os or by subcutaneous injection were not 
distinguished from RF-deficient mice in growth, weight of liver and 
kidneys, and in appearance of hair.

Nishida (1) reported that 8-amino-8-demethyl-D- and L-araboflavin showed 
a growth-promoting effect on RF-deficient rats 5 days after the beginning of oral 
administration and that the activity of D-compound was about a half of RF and 
that of L-compound was less than that of the D-compound. Natural isallopoxazine 
compounds have a D-ribityl side chain at the 10-position with the exception of 
L-lyxoflavin, the occurrence of which is questioned by some workers (2). Moreover, 
the D-ribityl compound has higher biological activity than its diastereoisomers (3). It would, therefore, be reasonable to expect higher biological activity 
in ARF than in AAF. Recently Ghisla and Mayhew (4) reported the occurrence 
of an 8-hydroxy-8-demethylflavin in a bacterial coenzyme. Though the exact

¹ Abbreviations used: AAF, 8-amino-8-demethyl-D-araboflavin; ARF, 8-amino-8-de-
methy1-D-riboflavin; RF, D-riboflavin; HF, 8-hydroxy-8-demethyl-D-riboflavin.
² 松井邦夫，笠井佐夫
structure of the 10-side chain of the new natural flavin has not yet been determined, it would be reasonable to assume that it is the D-ribityl chain, and expect that HF may have any physiological effect on animals. We have synthesized ARF, AAF and HF as related compounds of roseoflavin which is 8-dimethylamino-8-demethyl-D-riboflavin (5) and tested the physiological effect on mice. Although rats are usually used in experiments on RF deficiency, we used mice (6), because mice show a decrease in body weight, one of the signs of RF deficiency, earlier than rats, about 5 days after the beginning of feeding RF-free diet in contrast with about 3–4 weeks for rats. However, we could not find the growth-promoting effect of AAF and ARF on mice in spite of Nishida’s report, but found a toxic effect on liver. Curiously enough, ARF repaired the hair of RF-deficient mice and kept them rather clean, though similar phenomenon was reported by Nishida with AAF (1). HF did not show any effect apparently on growth, liver, kidneys and hair.

**EXPERIMENTAL**

*Flavins.* AAF was prepared by the method of NISHIDA (1). It was purified by acetylatyng the crude preparation with acetic anhydride and pyridine to tetraacete, recrystallizing it from ethanol, hydrolyzing the acetate with 0.1 m NaOH, neutralizing it with acetic acid, and washing with water and ethanol. Mp, 293–295°C (containing 1.5 H2O). Mp of tetraacetate, 288–289°C.

ARF was prepared in a similar way, that is, by condensing 2-nitro-4-amino-toluene with D-ribose in the presence of NH4Cl, reducing the riboside with H2 in the presence of Raney nickel, and then condensing the ribitylamine with violuric acid. It was purified in the same way as AAF. The details of synthesis will be published elsewhere in near future. Mp, >300°C. Mp of tetraacetate, 248–252°C.

HF also was prepared in a similar way to roseoflavin (5), from 2-hydroxy-4-aminotoluene, D-ribose, and violuric acid, and purified with tetraacetate. Mp, >300°C (containing 1.5 H2O). The details of synthesis also will be published in near future.

RF was obtained from National Institute of Hygienic Sciences ("Riboflavin Standard").

*Animals.* DD and DM male mice were obtained from Nihondobutsu, Inc. They were kept in wire cages to prevent coprophagy and in a dark room under a sodium lamp to retard the photolysis of flavins. Room temperature was maintained at 26–28°C. Before experiments the mice were fed a CE-2 diet for several days obtained from Clea Japan, Inc.

*Diets.* The composition of basal diet (RF-free) was as follows (7): sucrose 67.6 g, casein (vitamin-free) 18.0 g, cotton seed oil 8.0 g, DL-methionine 0.3 g, choline·HCl 0.1 g, salt mixture 4.0 g, powdered cellulose 1.5 g, vitamin A acetate 2,000 IU, vitamin D3 200 IU, α-DL-tocopherol acetate 10 mg, vitamin K3 0.5 mg,
Effect of 8-amino and 8-hydroxyflavin on mice

Experiment 1. DD Mice maintained on CE-2 for 6 days were fed a basal diet, and changes of their body weight were followed (Fig. 1). After 9 days they were divided into six groups, and fed for 9 days with test diets. Figure 1 shows that

Effect of AAF and ARF on the growth of mice

Fig. 1. Effect of AAF and ARF on the growth of mice (experiment 1). DD Mice (male, 83 animals), fed on CE-2 for 6 days (x-x), were fed with basal diet (RF-free) (o-o). After 9 days they were divided into 6 groups and fed with test diets for 9 days. o-o, Control group (basal diet, 14 animals); o-o, RF group (RF was added to basal diet, 8 μg/g of diet, 13 animals); □-□, AA8 group (AAF, 8μg/g, 14 animals); △-△, AR1 group (ARF, 1μg/g, 14 animals). Mean body weights are plotted.

Fig. 2. Effect of HF on the growth of mice (experiment 2, oral administration). DD Mice (male; 51 animals), fed on CE-2 for 6 days (x-x), were fed with basal diet (o-o). After 10 days they were divided to 3 groups and fed with test diets for 9 days. o-o, Control group (basal diet; 17 animals); o-o, RF group (RF was added to basal diet, 8 μg/g of diet; 17 animals); △-△, HF group (HF, 8 μg/g; 17 animals). Mean body weights are plotted.
mice began to lose body weight 5 days after the beginning of experiment because of RF deficiency and that RF group gained weight rapidly, but the other 5 groups (control, AA8, AA1, AR8, AR1 groups) lost weight, and an insignificant difference in time course of weight loss was found among the latter 5 groups. That is, contrary to NISHIDA's report (1) which was concerned with rats, we could not demonstrate growth-promoting effect of AAF and ARF on mice.

**Effect of HF on the growth of mice**

**Experiment 2.** DD Mice were pretreated similarly as in experiment 1. After being fed a basal diet for 10 days, they were divided into three groups, and fed test diets. The growth curves are shown in Fig. 2, which indicates that the RF group shows normal growth and that there is no significant difference between growth curves of the control and HF group. Similar results were obtained in the next experiment.

![Graph](image)

**Fig. 3.** Effect of HF on the growth of mice (experiment 3, administration by injection). DM Mice (male, 34 animals) were fed with basal diet (○). After 8 days they were divided to 3 groups and injected with test solutions once everyday. Control group (injected with 0.1 ml of saline; 12 animals); ○, RF group (injected with 30 µg of RF in 0.1 ml saline; 11 animals); Δ, HF group (injected with 30 µg of HF in 0.1 ml saline; 11 animals). Mean body weights are plotted.

**Experiment 3.** DM Mice fed a basal diet for 8 days were divided to 3 groups and were injected with test solutions (saline, RF and HF in saline) everyday. Figure 3 demonstrates that mice began to lose body weight 5 days after the beginning of administration of the basal diet and that the RF group showed normal growth curve, but there is no significant difference between the growth curves of the control and HF group.

These 2 experiments demonstrate that HF has no growth-promoting effect on mice.
Effect of AAF and ARF on the liver of mice

At the end of experiment 1, mice were killed by cervical dislocation and their liver weighed. Figure 4 shows that the liver of RF group mice is apparently heavier than that of control group, but the differences in liver weight between the control group and the other four groups (AA8, AA1, AR8, and AR1) are stochastically insignificant. However, if the percentages of liver weight to body weight are compared, the difference in weight percentage between the control and RF group is insignificant, but the difference between the control and AA8 group is significant (Snedecor’s F-test, \( F = 2.405 < F_{1213}(0.05) = 2.60 \); Student’s t-test, \( t = 2.455 > t_{12}(0.05) = 2.060 \)). The difference in weight between the control and AR8 group is insignificant (F-test, \( F = 2.88 > F_{1213}(0.05) = 2.58 \); t-test, \( t = 1.864 > t_{12}(0.1) = 1.706 \)). These results mean that the difference in liver weight between the control and RF group is parallel to the difference in body weight, and that between the control and AA8 group is not parallel to the difference of body weight. That is, AAF has some effect to increase the relative weight of liver to body weight. Three of 13 livers of the AA8 group and 2 of 13 of the AA1 group had the appearance of fatty liver, and none of control and RF group did. It is probable that AAF is toxic to liver and increases fat content of liver. And in actuality, one animal in both the AA8 and AA1 groups died during feeding on AAF diets.

Three of 14 livers of the AR8 group and one of 14 of AR1 group had the appearance of fatty liver. Therefore, ARF also would be toxic to liver, though the
difference in weight percentage of liver between the control and AR8 group is stochastically insignificant. However, the toxicity of ARF may be lower than that of AAF, because none of the mice, administered ARF, died during the experiment, though 2 of the mice administered with AAF died. The lower toxicity of ARF is understandable because ARF differs from RF only in one group, 8-amino, in contrast with AAF which differs in 2 groups, 8-amino and 10-arabityl.

**Effect of AAF and ARF on the kidneys of mice**

The weight and weight percentage of the kidneys of mice at the end of experiment 1 are shown in Fig. 5. The difference in weight of kidneys between the control and RF group is significant (F-test, $F=1.551<F_{13,12}(0.05)=2.67$; t-test, $t=4.487>t_{35}(0.001)=3.726$), but the difference in weight percentage of kidneys between two groups is insignificant similarly to liver. The differences in weight and weight percentage between control and other four groups (AA8, AA1, AR8, AR1) are insignificant. This suggests that AAF and ARF have no apparent effect on the kidneys of mice.

**Effect of HF on the liver of mice**

The weights and weight percentages of the liver of mice at the end of experiment 2 and 3 are shown in Fig. 6. The effect of RF is similar to that in experiment 1. The differences in weight and weight percentage of the liver between the
Effect of 8-amino and 8-hydroxyflavin on mice

Fig. 6. Effect of HF on the liver of mice. At the end of experiment 2 and 3 (Figs. 2 and 3), the mice were killed and the livers were weighed. a, experiment 2; b, experiment 3. Mean and standard deviation are shown.

Fig. 7. Effect of HF on the kidneys of mice. At the end of experiment 2 and 3 (Figs. 2 and 3), the mice were killed and kidneys were weighed. a, experiment 2; b, experiment 3. Mean and standard deviation are shown.

control and HF group are insignificant. This demonstrates that HF has no apparent effect on liver of mice independently of the way of administration.

Effect of HF on the kidneys of mice

The weight and weight percentage of kidneys of mice at the end of experiment 2 and 3 are shown in Fig. 7. The effect of RF is similar to that in experiment 1. The differences in weight and weight percentages of kidneys between the control and HF group are insignificant. Therefore, it is concluded that HF has no apparent effect on the kidneys of mice.

Effect of AAF, ARF and HF on the hair of mice

Figure 8 shows the hair of the mice at the end of experiment 1. The hair of mice of control group is unclean and look as if it is drenched, though that of RF group is clean. The hair of AA groups, especially of AA8 group, also is unclean; that of the AA8 group is less clean than that of the control group. The hair of the AA1 group is not so unclean as that of AA8 group, but some of the hairs in the head-neck region have fallen out. The hair of the ARF groups is clean, especially that of the AR1 group is nearly as clean as that of the RF group.
Fig. 8. Effect of AAF and ARF on the hair of mice. Def. means the control group, and RF, A8, A1, R8, R1 mean RF, AA8, AA1, AR8, AR1 groups, respectively. Typical mice of groups are shown.

Though the hair of the mice at the end of experiments 2 and 3 is not shown in the photograph, the hair of the HF groups was as unclean as that of the control group.

As AAF is toxic to liver, it is reasonable to assume that AAF is bad for hair. HF has apparently no effect on either liver, kidneys or hair. It is nevertheless rather curious that ARF, toxic to liver, is apparently good for hair. However, the hair of the AR8 group is more unclean than that of the AR1 group which is nearly as clean as that of the RF group. This phenomenon means that a small amount of ARF is good for hair, but a large amount is not good. The unfavorable effect of large amounts of ARF on hair may be secondary to the damage to liver.

DISCUSSION

There are many investigations on the biological activity of various isoalloxazine compounds, but few on 8-amino- and 8-hydroxy-isoalloxazines. Only NISHIDA (I) reported the growth-promoting activity of AAF on rats, while Upjohn Co.
reported an antivitamin activity of several 8-aminoisoalloxazines (8). However, as described above, we could not find the growth-promoting activity to mice of AAF as well as ARF which more closely resembles RF, but we did find the toxicity of both compounds to the animals. The 8-aminoisoalloxazines are more reddish than RF, and fairly different in chemical properties. The Upjohn Co. (8) reported an antivitamin activity of 8-amino compounds e.g. 7-methyl-8-amino-10-hydroxyethylisoalloxazine. Otani et al. (9) also reported an antivitamin activity to Lactobacillus casei of roseoflavin. From these facts, it would be difficult to expect vitamin activity in AAF and ARF. The curious result in Nishida’s experiment is that the vitamin activity of AAF was manifested 5 days after the beginning of administration. This may suggest that the activity of AAF to rats is secondary. A possible explanation of the phenomenon is that AAF might have promoted the production of RF by intestinal bacteria and a lot of RF might be absorbed or feces containing a lot of RF might be ingested by rats. It is regrettable that Nishida didn’t describe his way of keeping rats.

Our results (experiment 1) suggest that fat might be accumulated in the liver of AAF and ARF groups. There are many reports that lipid was accumulated in the liver of RF-deficient animals fed with fat-rich diets, and that RF prevented the accumulation of lipid and was useful for treatment of fatty liver (10, 11, 12). These phenomena are understandable enzymologically, that is, fatty acid metabolizing enzymes require FAD (13, 14). From these facts and our results it would be deduced that AAF and ARF have toxicity and inhibit metabolism of fat. Whether the aminoisoalloxazines inhibit the metabolism by competing with RF or by any other mechanism should be decided by further experiments in future.

One of the physiological activities of RF is to prevent dermatitis and the loss of hair (15). Nishida reported that AAF also made the hair of RF-deficient rats clean in a few days after the beginning of administration. But in our experiments AAF didn’t make the hair of RF-deficient mice clean, and this phenomenon was consistent with the toxicity of AAF to liver; but ARF made the hair clean. However, it is difficult to explain by present knowledge how ARF clean the hair of RF-deficient mice because ARF also is toxic to liver. Does ARF promote the production of RF by intestinal bacteria? Or is ARF converted to RF or any similar compound, or vice versa, which make the hair clean? Our results have brought out a new interesting subject of investigation.

HF is supposed to be a constituent of a new bacterial coenzyme (4). It would be not surprising even though HF has physiological activity in animals, because many coenzymes and vitamins are common in bacteria and animals. In our experiments it was apparently inert to mice, and neither favorable nor toxic effect was found. But it is not reasonable to conclude that HF has no influence on mice. It might have other physiological activities which could not be detected by our experiment, or an enough amount of HF might be stored in mice and symptom(s) of deficiency might not be manifested during the period of our experiment,
or administered HF might be ineffective and HF only, synthesized where it is utilized, might be useful. The occurrence of HF in animal tissues should be established at first.

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