Sparing of Riboflavin in Rats by 6,7-Dimethyl-9-(ω-Hydroxyalkyl)isoalloxazines.* (31266)

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Analogs of riboflavin in which the 1′-d-ribityl substituent in position 9 is replaced by ω-hydroxyalkyl chains of 3 through 6 carbons in length were synthesized and found inactive as substrates in the flavokinase system from rat liver(1), which catalyzes phosphorylation of riboflavin to flavin mononucleotide (FMN). Also the chemically synthesized phosphates of the ω-hydroxyalkyl analogs are not good substrates with the pyrophosphorylase from rat liver(2), which catalyzes conversion of riboflavin 5′-phosphate to flavin-adenine dinucleotide (FAD). Hence, ω-hydroxyalkylflavins do not appear to be converted to coenzymatically active forms *in vitro*.

Microbiological assays have demonstrated that ω-hydroxyalkylflavins are inactive in totally replacing riboflavin for growth of *Lactobacillus casei*, and at high concentration in the medium, they can antagonize the utilization of riboflavin *in vivo*(3).

The present study was undertaken to elucidate the behavior of 6,7-dimethyl-9-(ω-hydroxyalkyl)isoalloxazines in the rat. It is demonstrated that these riboflavin analogs do not functionally replace the natural vitamin, but appear to spare suboptimal amounts of riboflavin by being absorbed and perhaps destroyed in its place.

Materials and methods. Riboflavin was purchased from Eastman Organic Chemicals; D-riboflavin-2-C^{14} was from Nuclear-Chicago Corp. The following analogs were synthesized from appropriate ω-hydroxylamines and 1,2-dinitro-4,5-dimethylbenzene as described previously(1): 3′-hydroxypropylflavin[6,7-dimethyl-9-(3′-hydroxypropyl)isoalloxazine], 4′-hydroxybutylflavin[6,7-dimethyl-9-(4′-hydroxybutyl)isoalloxazine], 5′-hydroxypentylflavin[6,7-dimethyl-9-(5′-hydroxypentyl)isoalloxazine], and 6′-hydroxyhexylflavin[6,7-dimethyl-9-(6′-hydroxyhexyl)isoalloxazine].

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Young, 50 to 55 g, male Sprague-Dawley rats from Holtzman Co. were maintained on a riboflavin-deficient test diet† obtained from Nutritional Biochemicals Corp. Varying amounts of riboflavin or analog were admixed with the diet which was fed *ad libitum* to 5 to 8 rats in each group. The weights of animals were recorded before and during the experimental periods, and growth response is indicated by the average weight gain in grams.

Rats which were used for determination of urinary excretion of radioactive riboflavin were maintained for 2 weeks on the riboflavin-deficient diet or this diet supplemented with 20 μg of riboflavin per 15 g of diet. Both deficient and normal animals separately were divided into 3 groups for an intraperitoneal injection with 1 ml containing 10 μg (0.625 μc) of riboflavin-2-C^{14} in isotonic saline alone, in saline plus 40 μg of unlabeled riboflavin, or in saline plus 40 μg of 5′-hydroxypentylflavin. The combined urine from each of 3 rats per group was collected for 24 hours with 1 ml of acetic acid as preservative. Volumes of urine were measured and 0.1 ml aliquots pipetted into 10 ml of Bray's scintillation fluid(4). Radioactivity was determined in a Packard Liquid Scintillation Spectrometer.

Results. The rate of weight gain in rats which received varying amounts of riboflavin with and without the ω-hydroxyalkylflavins is shown by the data in Fig. 1. The very poor growth found when no riboflavin was added is partly overcome by 5 μg of riboflavin per

† The diet contains the following in %: vitamin-free casein, 18; vegetable oil, 10; U.S.P. salt mixture No. 2, 4; sucrose and vitamins, 68. Vitamins are the following per 100 lb of diet: vit. A, 900,000 units; vit. D, 100,000 units; α-tocopherol, 5 g; ascorbic acid, 45 g; inositol, 5 g; choline chloride, 75 g; menadione, 2.25 g; *p*-aminobenzoic acid, 5 g; niacin, 4.5 g; pyridoxine hydrochloride, 1 g; thiamine hydrochloride, 1 g; calcium pantothenate, 3 g; biotin, 20 mg; folic acid, 90 mg; vit. B_{12}, 1.35 mg.

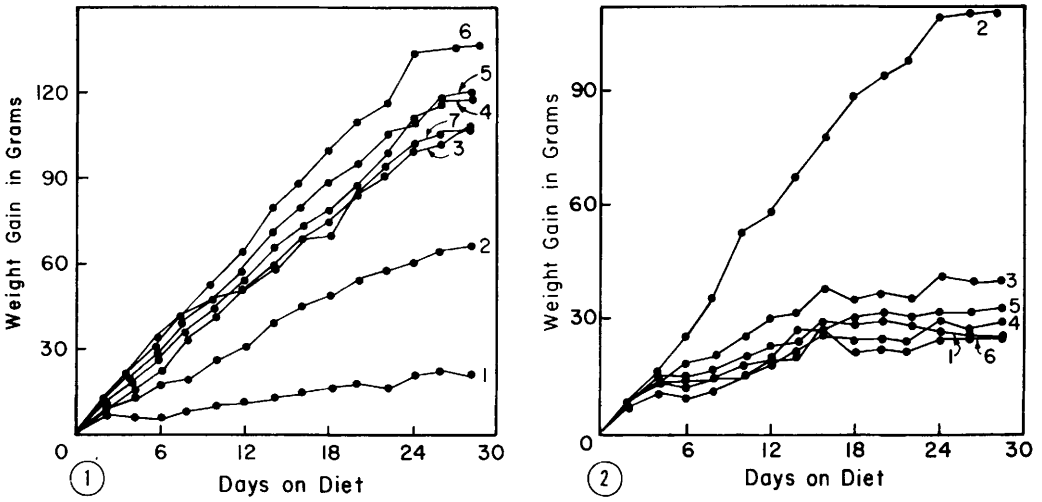

FIG. 1. Rate of weight gain in rats which received varying amounts of riboflavin with and without ω-hydroxyalkylflavins. The following were added per 15 g of diet: (1) no flavin, (2) 5 μg riboflavin, (3) 20 μg riboflavin, (4) 5 μg riboflavin plus 20 μg 4′-hydroxybutylflavin, (6) 5 μg riboflavin plus 20 μg 5′-hydroxypentylflavin, and (7) 5 μg riboflavin plus 20 μg6′-hydroxyhexylflavin.

FIG. 2. Rate of weight gain in rats which received either riboflavin or ω-hydroxyalkylflavins. The following were added per 15 g of diet: (1) no flavin, (2) 20 μg riboflavin, (3) 20 μg 3′-hydroxypropylflavin, (4) 20 μg 4′-hydroxybutylflavin, (5) 20 μg 5′-hydroxypentylflavin, and (6) 20 μg 6′-hydroxyhexylflavin.

15 g of diet and approaches normal with 20 μg. The effect of including 20 μg of any one of the ω-hydroxyalkyl analogs with 5 μg of riboflavin per 15 g of diet is to increase the rate of growth from that seen with this suboptimal level of the vitamin alone to a rate which is generally at least as satisfactory as that obtained with 20 μg of riboflavin. Thus, these analogs seem to increase very effectively the utilization of amounts of riboflavin which are below those usually required for optimal growth in the rat.

The rate of weight gain in rats which received the same amounts of either riboflavin or the ω-hydroxyalkylflavins singly is illustrated in Fig. 2. None of the analogs were effective in replacing riboflavin at the level of 20 μg per 15 g of diet. Generally growth with 20 μg of each analog was little or no better than that obtained with the riboflavin-deficient diet. Hence, these analogs cannot adequately replace the vitamin *per se*.

The excretion of C^{14}-activity into urine from normal and riboflavin-deficient rats over a 24-hour period following an intraperitoneal injection of riboflavin-2-C^{14} with and without additional riboflavin or 5′-hydroxypentylflavin is indicated by the data in Table I. Compared with rats injected with 10 μg of radioactive riboflavin alone, those animals which received an additional 40 μg of either riboflavin or 5′-hydroxypentylflavin excreted more C^{14}-activity in the urine. These effects are considerably more pronounced in rats which were made deficient in riboflavin and are somewhat greater with the vitamin than its analog. Also slightly less total C^{14}-activity appeared in the urine from each group of deficient animals when compared with a similarly injected group which had received 20 μg of riboflavin per 15 g of diet.

Discussion. Causes for the sparing of riboflavin elicited in the rat by the ω-hydroxyalkylflavins can be considered. The chief loss of riboflavin in the rat has been shown to be through destruction not specifically related to use, and loss by excretion is negligible compared to such destruction except with excessively large levels of intake of the vitamin (5). These studies were extended to the riboflavin-deficient rat where nearly 2 μg of riboflavin can be accumulated in the tissues per day; a quantity equal to the rate of fecal excretion and superimposed on a relatively con-

TABLE I. Urinary Excretion of C^{14}-Activity from Normal and Riboflavin-Deficient Rats During 24 Hours Following an Intraperitoneal Injection of D-Riboflavin-2-C^{14} With and Without Additional Riboflavin or 5'-Hydroxypentylflavin.

Riboflavin added to diet* (μg/15 g diet)	Avg wt gain during 2 wk on diet† (g)	Compound injected with 10 μg of D-riboflavin-2-C^{14}‡ (40 μg/ml/rat)	Urinary excretion of C^{14}-activity/rat (c/m-background)	Increase in urinary excretion of C^{14}-activity due to compound injected (%)
0	12.5	none	14,652	0
		riboflavin	18,972	30
		5'-hydroxypentylflavin	17,680	21
20	54.2	none	18,500	0
		riboflavin	19,861	7
		5'-hydroxypentylflavin	18,752	2

* Rats were divided into 2 groups of 9 each. † Initial average weight was 69 g. ‡ Each injection group was comprised of 3 rats.

stant rate of urinary excretion(6). The ω-hydroxyalkyl analogs may decrease destruction of riboflavin as evidenced by its increased excretion when either an analog or excess vitamin is injected, especially in a deficient animal. The inability of ω-hydroxyalkylflavins to be converted to physiologically active coenzyme forms(1,2), but marked sparing efficiency for riboflavin in the rat, is somewhat similar to the behavior of L-lyxoflavin. This latter riboflavin analog was found not to serve as a partial substitute for riboflavin in *L. casei* or chicks, but enhances the efficiency with which limited supplies of riboflavin are utilized(7). Related findings have been reported for certain ring-substituted analogs of riboflavin in microbiological and rat assays (8-10).

Summary. A series of ω-hydroxyalkylflavins with 2 to 6 carbon chain lengths are shown to markedly enhance the utilization of normally suboptimal amounts of riboflavin given to growing rats. These analogs cannot function in place of the vitamin, but may decrease its metabolic destruction.

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Effect of Cortisol on Nuclear RNA-Synthesis *in vitro*.*(31267)

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Although it is now well accepted that steroid hormones cause a stimulation of RNA synthesis followed by formation of specific proteins in target organs(1,2), the primary reaction of the steroid molecule with a speci-

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