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The Antiriboflavin Effect of Isoriboflavin.*

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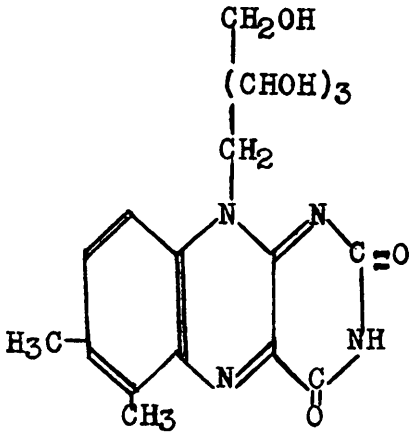
Several investigators have reported on the antagonism existing between certain B vitamins and structurally related compounds. Following the discovery of the antagonism between sulfanilamide and para-aminobenzoic acid by Woods,[1] pyrithiamine,[2] pyridine-3-sulfonic acid,[3] and pantoyltaurine[4] have been reported as inhibitors of thiamine, nicotinic acid and pantothenic acid respectively. Kuhn and co-workers[5] have recently reported that 6,7-dichlor-9-d-riboflavin inhibits the growth of riboflavin requiring microörganisms.

An isomer of riboflavin, namely, 5,6-dimethyl-9-(*d*-1′-ribityl)-isoalloxazine (termed isoriboflavin) when assayed for riboflavin activity was found to suppress growth both in riboflavin-deficient rats and in rats receiving a sub-optimal intake of the vitamin.

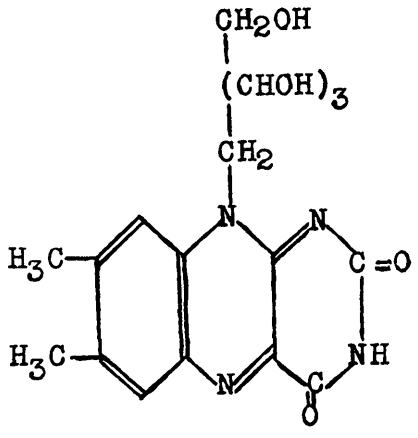
Forty-two male weanling rats were placed on the riboflavin-deficient ration and segregated into 6 like groups treated as follows:

1. No riboflavin.
2. No riboflavin and 2 mg of isoriboflavin daily.
3. Sub-optimal level of riboflavin (10 μg daily).
4. Sub-optimal level of riboflavin (10 μg daily) and 2 mg of isoriboflavin.
5. Optimal level of riboflavin (40 μg daily).
6. Optimal level of riboflavin (40 μg daily) and 2 mg of isoriboflavin.

The basal ration consisted of vitamin-free casein 18%; dextrose 68%; salt mix U.S.P. XI No. 1 4%; Crisco 8%; cod liver oil 2%; supplemented with 0.8 mg each of thiamine and pyridoxine, 5 mg of Ca pantothenate, 10



Isoriboflavin



Riboflavin

* We are indebted to Dr. Karl Pfister and Miss Eleanor Smart of Merck & Co., Inc., for the synthesis of this compound. The synthesis of this compound will be described elsewhere.

1 Woods, D. D., *Brit. J. Exp. Pathol.*, 1940, **21**, 74.

2 Woolley, D. W., and White, A. G. C., *J. Biol. Chem.*, 1943, **149**, 285.

3 McIlwain, H., *Brit. J. Exp. Pathol.*, 1940, **21**, 136.

4 Snell, E. E., *J. Biol. Chem.*, 1941, **139**, 975.

5 Kuhn, R., Weyand, F., and Möller, E. F., *Ber.*, 1943, **76**, 1044.

TABLE I.
Growth Response of Rats to Riboflavin and Iso-riboflavin (Both compounds were dosed as a suspension in 0.5 ml gum acacia).

No.	Group	Gain in wt 28 days (Avg initial wt, 46 g) Period I g	Gain in wt Succeeding 21 days Period II* g
1.	Riboflavin deficient 0.5 ml gum acacia	15	
2.	2 mg Isoriboflavin	5	
3.	10 μg riboflavin	69	94
4.	10 μg riboflavin + 2 mg isoriboflavin	29	118
5.	40 μg riboflavin	96	
6.	40 μg riboflavin + 2 mg isoriboflavin	92	

* Riboflavin increased to 80 μg daily between periods I and II.

mg of nicotinamide, and 100 mg of choline chloride per 100 g of diet.

The riboflavin and isoriboflavin were fed by stomach tube as a suspension in gum acacia.

Isoriboflavin inhibited the growth of rats in the absence of riboflavin (Group 2, Table I, Period I) although the manifestations of deficiency (greasiness of the fur and alopecia) and the survival time were not significantly different between the animals in groups 1 and 2. The analog, in the dosage employed, markedly depressed the growth of the rats receiving a sub-optimal intake of the vitamin (Groups 3 and 4). The growth-depressing effect of isoriboflavin was almost completely overcome by the feeding of 40 μg of riboflavin (Groups 5 and 6).

Thus, it would appear that the effect of isoriboflavin was that of an inhibitor of riboflavin.

The counteraction of large doses of the vitamin upon the inhibiting effect of the analog was further shown in the animals of Groups 3 and 4. After 28 days on test the level of riboflavin was increased from 10 μg to 80 μg for these groups: the feeding of isoriboflavin to the rats in group 4 was continued at the level of 2 mg. As evidenced by the growth response (Table I, Period II), as well as by the general improvement in the condition of the animals, the inhibiting effects of isoriboflavin were overcome by the curative feeding of 80 μg of riboflavin.

Summary. The feeding of isoriboflavin to rats receiving a sub-optimal intake of riboflavin inhibited the growth-promoting action of the vitamin. The antagonistic effect of the isomer was prevented or overcome by the feeding of an adequate level of riboflavin.

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Influence of Bacteriologic Peptones on Hydrogen Sulfide, Indol and Acetyl Methyl Carbinol Production by Enterobacteriaceæ.

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The production of hydrogen sulfide (H_2S) by members of the family Enterobacteriaceæ is regarded by microbiologists as an important differential diagnostic criterion. In addition, the production of indol and acetyl methyl carbinol (A.M.C.) is used as a means of separating two significant genera, namely, the Aerobacter from the Escherichia. Bacteriologic peptones serve as sources of (a) sulfur compounds for H_2S formation, (b) tryptophane for indol formation, and (c) compounds possessing the guanidine nucleus necessary for the detection of A.M.C. That peptones vary between manufacturers is almost axiomatic.