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The Effect of Diet on Riboflavin and Xanthine Oxidase in Rat Liver and Intestine.* (19570)

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The effect of low protein and low riboflavin diets on liver riboflavin has been reported previously(1,2). The present study shows a proportional reduction in the riboflavin content of the sedimentable particles and the supernatant fractions of a liver homogenate in both of these types of dietary restrictions. The corresponding changes in intestinal riboflavin, and the independent fluctuation of total riboflavin vs. the flavin enzyme, xanthine oxidase, in both liver and intestine have also been determined.

Methods. Male weanling rats were placed on 4 diets that varied as follows:

Diet	A	B	C	D
GBI Casein (vit. test)	31%	8%	31%	28.5%
Riboflavin (mg/100 g)	.5	.5	0	.5
Soy flour (50% protein)†	0	0	0	5

† A. E. Staley Mfg. Co., Decatur, Ill. Extracted soya flour.

The remainder of each diet consisted (in g/100 g) of: Crisco 4, Wesson oil 2, cod liver oil 1, Phillips and Hart salt mixture 4, and glucose (89—casein and soy flour). The vitamin supplement added (mg/100 g); nicotinic acid 2.5, calcium pantothenate 1.0, thiamine 0.4, pyridoxine 0.4, biotin 0.05, inositol 50.0, p-aminobenzoic acid 10.0, vit. B_{12} 0.05, mixed tocopherols 50.0, ascorbic acid 25.0, 2-methyl-1,4-naphthoquinone 0.25, choline chloride 100.0. In comparison with the control diet A, diet B was low in protein, C was low in riboflavin, and D contained soy flour as a source of the unidentified xanthine oxidase (X.O.) factor(3). After 2 or 4 weeks of *ad libitum* feeding, groups of rats from each diet were sacrificed by decapitation for analysis of the livers and small intestines. Riboflavin was determined microbiologically, using *L. casei* as the test organism and a Difco synthetic medium. The tissues were homogenized with cold 0.04 M phosphate buffer, pH 7.4, and aliquots were prepared and assayed by the procedure of Strong(4). The liver homogenates obtained at 2 weeks were also separated into a combined particulate (nuclei plus mitochondria plus microsomes) and supernatant fractions by centrifuging at 0-5° and approximately 30,000 x g for 90 minutes(5). The precipitate was washed by resuspending it in buffer and centrifuging again for 90 minutes; the wash was added to the super-

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TABLE I. Liver and Intestinal Riboflavin and Xanthine Oxidase Values Obtained When Weanling Rats Were Fed the Indicated Diets for 2 to 4 Weeks.

	Tissue analyzed	Diet A Body weights (avg at 28 days) 176	Diet B 88	Diet C 91	Diet D 174
Riboflavin (μg/g wet wt)	Liver: Whole homogenate	29.8 ±2.6	17.3 ±1.1	12.4 ± .8	26.9 ±1
	Precipitate	16.2 ± .7	10.7 ± .8	8.3 ± .7	16.1 ±1
	Supernatant	9.6 ±1.5 (3)	5.9 ± .5 (6)	3.6 ± .3 (5)	8.6 ± .9 (4)
	Intestine	5.15± .21 (5)	4.30± .24 (8)	2.35± .12 (7)	4.93± .27 (9)
Xanthine oxidase* (mm^3 O_2/283 mg/ 20 min)	Liver	25 ±3 (6)	.6 ± .6 (8)	19 ±2.4 (9)	32 ±2.4 (7)
	Intestine	10 ±1.5 (4)	1.5 ± .8 (8)	6.4 ±1.3 (9)	27 ±2.8 (6)

Values given are mean ± stand. error for the number of rats indicated in parentheses. Values for 8 weanling rats at start of experiment were: Body wt 59 g, liver riboflavin 24.2, intestinal riboflavin 4.65, liver xanthine oxidase 15.

* Values for xanthine oxidase are given as net oxygen consumption with hypoxanthine substrate per 20 min per Warburg flask containing 283 mg fresh tissue.

natant. Both the supernatant and precipitate fractions were assayed for riboflavin. An attempt was made to fractionate the livers obtained at 4 weeks in a sucrose medium(2). Insofar as could be determined the results were comparable with those obtained in phosphate buffer, but the large amount of sucrose created difficulties in some of the riboflavin assays. The liver and intestinal homogenates obtained at 4 weeks were also analyzed for xanthine oxidase(6).

Results. The results are recorded in Table I. The decreased values for liver riboflavin on a low protein or low riboflavin diet agreed with and confirmed previous reports(1,2). Intestinal riboflavin showed a similar response to these dietary deficiencies, but a low protein diet had less effect on intestinal than on liver riboflavin. Addition of soy flour as a source of the X.O. factor had no measurable effect on liver or intestinal riboflavin, though it did affect the xanthine oxidase content of these tissues. Riboflavin was lost proportionately from both the particulate and supernatant fractions as a result of feeding either a low protein or low riboflavin diet. This is in agreement with previous studies(1,2). Riboflavin was not lost selectively from the supernatant fraction, as occurred with the flavin enzyme, xanthine oxidase(5).

Liver and intestinal xanthine oxidase levels again(6) showed: 1) a marked loss of this enzyme from both the liver and intestine on a purified low protein diet, 2) a marked loss of this enzyme from the intestine when the diet contained adequate protein and riboflavin but inadequate X.O. factor, and 3) a marked influence of the X.O. factor on the intestinal xanthine oxidase and a much smaller effect on the liver enzyme. Liver xanthine oxidase was not depleted extensively by a riboflavin deficient diet (diet C vs. A). Previous studies indicating a more marked effect of riboflavin deficiency(7) were carried out under more prolonged dietary restrictions, and the results could have been influenced by other factors, such as inanition(6) and a prolonged deficiency of the X.O. factor. In the present studies, where an acute riboflavin deficiency was evidenced by growth failure and low liver riboflavin values, the liver xanthine oxidase was decreased, but the effect was not marked. The effect of diet C in depleting intestinal xanthine oxidase could be attributed to the absence of the X.O. factor, and the effect of a riboflavin deficiency *per se* could not be determined.

There was no correlation between the effect of diet on liver or intestinal total riboflavin and the xanthine oxidase activity of the tissue. This was especially clear in a comparison of intestinal values on diets A and D; total

riboflavin was essentially the same, while xanthine oxidase on diet D was almost 3 times as high as on diet A. Similarly a comparison of the liver supernatant fractions, which contained all the xanthine oxidase(5), showed approximately a 3-fold difference between diets C and A in riboflavin content but only a 30% difference in xanthine oxidase activity. Hence, xanthine oxidase did not constitute a major fraction of the total riboflavin present in either liver or intestine, nor was it affected by diet in a manner characteristic of other flavin constituents.

Summary. 1. Weanling rats fed diets deficient in protein or riboflavin for 2 to 4 weeks had liver riboflavin levels that were reduced approximately 40% and 55% respectively; the corresponding reduction in intestinal riboflavin was approximately 15% and 55%. About 60% of the riboflavin in normal liver was in the sedimentable particles (nuclei plus mitochondria plus microsomes), and the above loss of riboflavin was proportionately the same from both the particulate and supernatant fractions. 2. The incorporation of soy flour in the diet as a source of the unidentified xanthine oxidase factor had no effect on the total riboflavin content of the liver or intestine, but did increase the liver and intestinal xanthine oxidases 30% and 170% respectively. The amount of riboflavin bound in xanthine oxidase represents only a very small fraction of the total riboflavin present. Liver xanthine oxidase was decreased only 25% by a riboflavin deficient diet.

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Growth of Newcastle Disease Virus in the Embryonated Egg. (19571)

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There have been many approaches to the study of the factors involved in the multiplication of viruses. The early investigators were obliged to study virus propagation in terms of the reaction that followed the introduction of the agent into animals, isolated organs, or tissues. The growing interest in the phenomenon of virus propagation has given rise to many new avenues of approach and numerous ingenious technics. Although a large body of data have been collected, it appears that much basic information regarding the activity of the virus particle is still lacking. Especially is this true in regard to the mechanism of the development of the infectious and the hemagglutinating properties of some viruses. While considerable data regarding the influenza virus have been accumulated(1-5) the literature revealed but limited information on the growth and behavior of the Newcastle disease virus. However, some of its physical, immunological, and infectious properties have been described (6-10).

Since Burnet has reported a similarity between the viruses of Newcastle disease and influenza(11) this study was undertaken to apply to the Newcastle disease virus some concepts of host-virus interplay that had been described for influenza. This was ac-

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