Xanthine Oxidase in Pair-Fed Rats on Diets Marginally Deficient in Lysine. (22923)

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A number of studies relating xanthine oxidase activity in the liver of the rat to quality of dietary protein has been carried out(1,2, 3). It is well established that in inanition and during periods of restricted protein intake, animals suffer loss of liver protein as well as other tissue protein. This decrease in liver protein, however, does not represent an equivalent decrease in activity of all liver enzymes. Thus a decrease of xanthine oxidase activity(4) and an increase in succinoxidase activity has been reported in rats on reduced food intake for 3 weeks(5).

Procedure. We attempted to resolve factors associated with inanition by using paired feeding technic with weanling rats. All experimental groups were pair-fed to the group receiving the lowest amount of amino acid (0.25% lysine). The caloric intake was identical in all groups. Weanling rats were used to give a better insight on the influence of lysine on xanthine oxidase synthesis during feeding of an isocaloric diet. It was hoped that the initially low content of xanthine oxidase in weanling rats(6) would demonstrate larger differences in enzyme content with growth, rather than enzyme depletion demonstrated in adult rats(7). Composition of basal diet is given in Table I. The experimental groups were arranged so that rats in group I had 0.25%; group II, 0.5% and group III, 1.00% L-lysine supplementation. The diets were made isonitrogeneous by addition of glycine. Each group contained 9 male weanling rats of Sprague-Dawley strain from the colony of the National Institutes of Health. Our previous experiments had established the adequacy of growth on both 1.0% and 0.5% L-lysine, but the animals on the 0.25% L-lysine barely maintained an increase of 6 g per week when fed *ad libitum*. The immediate and prolonged growth response obtained on these rations when adequately supplemented with L-lysine indicates that the deficiency is of an uncomplicated nature. Animals were placed in metal cages with raised screen bottoms and had unlimited access to distilled water. After 42 days they were lightly anesthetized, decapitated and the livers quickly removed and placed on cracked ice for several minutes. The livers were blotted free of moisture and a weighed portion of each was homogenized in 2 volumes of ice-cold M sodium potassium phosphate buffer (pH 7.3). Liver xanthine oxidase was determined according to the method of Axelrod and Elvehjem(8). Each pair of Warburg flasks contained 2 ml of a liver homogenate, total volume of 3 ml. The endogenous respiration was partially exhausted by a preliminary 10 minute incubation before 0.15 ml of 0.05 M xanthine substrate was tipped into the flask. The protein content of liver homogenate was determined by the method of Lowry *et al.*(9).

TABLE I. Composition of Basal Diet.

	g
Zein	24
Salt H.M.W.	4
Cerelose	51.8
Choline chloride	.2
Vitamin mix 80*	1
" " 90†	5
" " 60‡	8
Amino acid supplement§	6

* Ascorbic acid 10 g, sucrose 490 g.

† Thiamin HCl, 20 mg; riboflavin, 20 mg; pyridoxine, 20 mg; calcium pantothenate, 50 mg; niacin, 50 mg; biotin, 2 mg; folic acid, 5 mg; inositol, 400 mg; p-aminobenzoic acid, 200 mg; vit. B_{12}, .15 mg; menadione, 5 mg; liver powder, 2.0 g; to 50 g with dextrin.

‡ Vit. A, 5,500 i.u.; vit. D, 1,100 i.u.; alpha tocopherol, 200 mg; cottonseed oil to 80.00 cc.

§ DL-tryptophan, .3 g; DL-methionine, .4 g; DL-phenylalanine, .5 g; L-histidine HCl, .4 g; DL-threonine, .6 g; DL-valine, 1.6 g; DL-isoleucine, 1.0 g; L-arginine, .2 g.

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TABLE II. Relationship of Liver Xanthine Oxidase Activity to Lysine Deficiency in the Rat.

Group	L-lysine supplementation, %	Activity/g Liver protein	Activity/g Wet liver	% protein of total wet liver sample
		—μl O_2/hr—		
I	.25	87	11	12.7
II	.5	994	170	17.1
III	1.0	689	170	24.6

Results are summarized in Table II. The findings indicate less xanthine oxidase per unit weight of liver in group I as compared to groups II and III. When expressed as activity per unit of liver protein, there is an apparent increase in activity of group II over group III. However, the activity, as per unit of protein, of group I still demonstrates a most marked decrease compared to activity of the other 2 groups. Reduction of observed activity at the 0.25% L-lysine level, even when expressed in terms of unit protein, would indicate that a preferential synthesis of at least some protein components occurred at the expense of xanthine oxidase, unlike those animals on a 0.5% L-lysine diet where reduced liver protein synthesis did not include this enzyme. In these experiments reduced xanthine oxidase appears to be directly related to amount of lysine supplementation and was uncomplicated by factors of inanition.

In general, the results correlate well with previous studies concerning effects of amino acid deficiencies on liver enzyme activities (10,11,12). The increased xanthine oxidase activity per unit protein at the 0.50% L-lysine level, however, does not indicate the same degree of lability of this enzyme as during the "sub-acute food restriction" observed by Wainio *et al.*(13).

Summary. Weanling rats maintained on diets marginally deficient in lysine appear to exhibit a defect in the ability to synthesize xanthine oxidase. These findings give further support to the importance of dietary lysine for xanthine oxidase activity.

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