Vitamin E Deficiency and Xanthine Oxidase in Rabbits.* (20680)

DAN A. RICHERT AND W. W. WESTERFELD.

From the Department of Biochemistry, State University of New York, Medical College, Syracuse.

Dinning has reported(1) that vit. E-deficient rabbits have an unusually high concentration of xanthine oxidase (XO) in the liver. This has been confirmed in the present study, and it has also been found that this increase occurs only in the liver and is not accompanied by any parallel change in the molybdenum concentration. The latter point is of interest because of the recently demonstrated(2) relationship between molybdenum and xanthine oxidase.

Experimental. White male New Zealand rabbits, weighing approximately one kg, were separated into groups and fed various diets until muscular dystrophy appeared in the vit. E-deficient animals(3). The deficient and control rabbits were then sacrificed and analyzed. In the first experiment a large number of rabbit tissues were analyzed for xanthine oxidase in the presence and absence of methylene blue by the methods previously described(4). In the second experiment, only the liver was analyzed for xanthine oxidase in the presence of methylene blue, since the methylene blue procedure gave the most reliable results; in addition, a large number of tissues were dried extensively at 80°, digested with sulfuric-nitric-perchloric acids, and analyzed for molybdenum colorimetrically(5,6).

The 15% casein diet described by Young and Dinning(7), and also used by Dinning(1) in his studies on liver xanthine oxidase during vit. E-deficiency, was used. Both experiments employed this diet with and without the addition of 50 mg of mixed tocopherols per 100 g diet. In the second experiment 2 additional diets containing 2 mg Mo (as Na_2MoO_4) per kg diet were used, and a third vit. E-Mo-deficient diet, containing 24% casein and 30% sucrose, was also studied. Purina rabbit chow, containing 15% protein and 1.84 mg Mo per kg, was fed to another control group of rabbits in each experiment.

Results. In the normal chow-fed rabbits no xanthine oxidase could be detected in the presence or absence of methylene blue in heart, lung, kidney, spleen, brain or skeletal muscle. Nor could xanthine oxidase be detected in these tissues during vit. E-deficiency in those rabbits which showed a marked elevation in lixer XO. Liver and small intestine from normaly chow-fed rabbits contained small amounts of this enzyme, which could be detected in the presence of methylene blue, but not regularly in its absence.

The results obtained with liver and intestinal xanthine oxidases in the first experiment are shown in Table I. Although there was considerable variation in the magnitude of the increase in liver XO during vit. E-deficiency, there was no question of its occurrence; 4 of the 7 deficient rabbits had liver XO values (as determined in the presence of methylene blue), between 20 and 50, and these far exceeded any of the control values. The diets had little or no effect on the low intestinal xanthine oxidase levels.

The effect of vit. E-deficiency on the endogenous respiration of a rabbit liver homogenate is shown in Fig. 1. These curves are reminiscent of those previously published(8) for rat liver, and show that the endogenous oxygen uptake was low when the liver contained little XO (controls); the endogenous respiration increased with the elevated liver xanthine oxidase found in vit. E-deficiency. The effect of methylene blue on the determination of xanthine oxidase in both normal and vit. E-deficient rabbit liver was similar to its effect in a variety of normal tissues (8-12), and was apparently unrelated to any effect that methylene blue might have on vit. E deficiency.

Table II shows the results of the second experiment. Liver xanthine oxidase was again elevated on all vit. E-deficient diets; the pres-

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TABLE I. Effect of Vitamin E Deficiency on Liver and Intestinal Xanthine Oxidase Activity in the Rabbit.

	Days on diet	Body wt, g Start	Body wt, g End	No. of rabbits	Liver XO* —	Liver XO* +MB	Intestine XO* —	Intestine XO* +MB
Chow	39	1130	2045	3	2 ± 1.0	5 ± 2.5	0	4 ± 0.7
Purified diet with vit. E	32	1086	1086	5	0	7 ± 2.5	0	9 ± 3.0
Purified diet without vit. E	29	1001	851	7	12 ± 6.0	26 ± 6.9	0	7 ± 2.9

* Liver and intestine xanthine oxidase activities were determined in the presence and absence of methylene blue, and have been recorded as net mm³ O_2 per 20 min. per flask containing 283 mg fresh tissue; mean ± stand. error.

TABLE II. Effect of Dietary Vitamin E and Molybdenum on Xanthine Oxidase and Molybdenum Concentrations in Rabbit Tissues.

Diet: Vit. E	Diet: Mo	Avg days fed	Body wt, g: Start	Body wt, g: End	No. of rabbits	Liver xanthine oxidase†	Mo content—γ/g dry wt: Kidney	Liver	Intestine	Stomach	Colon	Lung	Skin	Muscle
—	—	33	1161	979	6	9 ± 1.9	1.3	.9	.6	.6	.5	.7	.2	.2
+	—	37	1099	1265	5	1 ± 0.5	1.7	.7	—	—	.5	.8	.3	.2
—	+	28	1154	1012	6	17 ± 6.0	2.6	1.4	1.5	1.2	1.1	1.4	.6	.3
+	+	43	1089	1228	6	3 ± 2.8	3.6	1.6	1.6	1.1	1.2	1.5	.6	.4
—	—*	28	1095	1051	5	23 ± 6.0	1.5	1.0	.7	.4	.6	.8	.4	.2
Chow		40	1134	2470	6	5 ± 2.5	2.0	1.5	1.1	.8	.8	.6	.3	.2

* 24% casein diet; all others contained 15% casein; chow contained 15% protein.

† Xanthine oxidase activities were determined in the presence of methylene blue, and have been recorded as net mm³ O_2 consumed per 20 min. per flask containing 283 mg fresh tissue; mean ± stand. error.

ence or absence of molydenum and the use of 24% instead of 15% casein in the diets did not prevent this increase. Nor did the addition of Mo to the diet influence the control level of liver xanthine oxidase or the appearance of the muscular dystrophy deficiency symptoms. Comparison of the magnitude of the increase obtained with different diets was not justified, because the change in liver XO has been reported(1) to occur late in the deficiency and the variability among rabbits within the deficient groups might be influenced by the severity of the deficiency at the time of sacrifice. It is possible that the purified diet contained enough molybdenum, as an impurity, to meet the requirements of the rabbit.

The molybdenum content of the rabbit tissues reflected the Mo content of the diet, and was not affected by the presence or absence of vit. E or by the difference in protein content. Vit. E deficiency did not alter the molybdenum content of the liver or the distribution of Mo in the other tissues, and there was no correlation between the xanthine oxi-

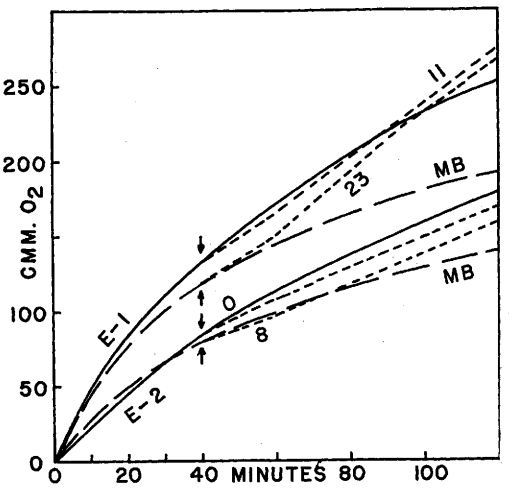

FIG. 1. The avg endogenous respiration (solid line) of liver homogenates from 6 vit. E-deficient (E-1) and 5 control (E-2) rabbits. Dash lines were obtained in presence of methylene blue (MB), dotted lines show effect of tipping in hypoxanthine substrate at the 40 min. point. Numerical values along the dotted lines are corresponding xanthine oxidase activities in net mm³ O_2 per 20 min. per flask containing 283 mg fresh tissue.

dase activity of the liver and its molybdenum concentration.

Summary. Vit. E-deficient rabbits with muscular dystrophy had elevated liver xanthine oxidase activity. This increase was observed in the presence or absence of added dietary molybdenum, and with 15 or 24% casein in the diet. Low intestinal xanthine oxidase levels were not increased during vit. E deficiency. Xanthine oxidase was not detected in rabbit heart, lung, kidney, spleen, brain, or skeletal muscle normally or during vit. E deficiency. The molybdenum content of various rabbit tissues was influenced by the Mo content of the diet, but was not altered by the presence or absence of vit. E.

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Effect of Whole Body X-Irradiation on Ascorbic Acid of Rat Tissues.* (20681)

H. L. OSTER, A. L. KRETCHMAR, AND F. H. BETHELL. (Introduced by C. C. Sturgis.)
From Atomic Energy Commission Biological Effects of Irradiation Laboratory, University of Michigan, Ann Arbor.

Interest in the fate of ascorbic acid in irradiated tissues has been stimulated by several reports. It has been shown(1) that X-irradiation of ascorbic acid in aqueous solutions results in a loss of from 1.7 to 2.4 μ moles/1000 r. This is the order of magnitude of the effect of X-irradiation on a number of organic compounds in dilute solution(2). The destruction of ascorbic acid by X-irradiation, unlike thiamine and most organic compounds, is not inhibited by the presence of albumin and, indeed, proceeds to the same extent in blood plasma as in aqueous solution. However, only slight destruction of ascorbic acid occurred in minced rat muscle exposed to 22,000 r *in vitro* (1). Favorable clinical reports on the use of ascorbic acid in the treatment of X-ray sickness led to the study of the effect of X-ray therapy on the serum ascorbic acid of patients by Kretzschmar and Ellis(3). A reduction was noted during and after treatment. These workers also reported an immediate and sustained (12-30 days) reduction in plasma ascorbic acid of rabbits which had received 1500 r on one side of the body. Moreover, they found a reduction in muscle and kidney ascorbic acid in rats 1-48 hours after irradiation of these tissues. These reports indicate the possibility that cell ascorbic acid might be destroyed by the production of oxidizing radicals in cell water during X-irradiation(4). This type of immediate *in vivo* destruction has been reported by Skoog(5) to occur in the auxin of plants receiving from 600-2100 r. The prolonged effect on plasma ascorbic acid found by Kretzschmar and Ellis suggests that besides the possible primary oxidative destruction, there are also secondary factors which influence the *in vivo* ascorbic acid levels after

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