PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 151, 770–774 (1976)

Vitamin E Supplementation and Glutathione Peroxidase Activity[1] (39304)

N. Y. JACK YANG, IAN B. MACDONALD AND INDRAJIT D. DESAI
(Introduced by Melvin Lee)

Division of Human Nutrition, University of British Columbia, Vancouver, B.C., Canada V6T 1W5

Vitamin E and selenium have been recognized as physiological antioxidants in the prevention of oxidative damage to tissues. Rotruck *et al.* (1) found that selenium was able to help prevent oxidative damage to rat erythrocytes incubated with glucose, as evidenced by decreased hemolysis and decreased hemoglobin oxidation. Vitamin E, however, protected only against hemolysis, whether with or without glucose, but did not prevent hemoglobin oxidation. These results indicated that the effect of selenium was specific and distinct from that of vitamin E. Later, it was demonstrated by Rotruck *et al.* (2) that selenium was an integral and essential part of glutathione (GSH) peroxidase. Glutathione peroxidase was reported to be responsible for the destruction of lipid peroxides in cells (3, 4). Reduced GSH is a hydrogen donor for the GSH peroxidase reaction and is itself regenerated through the reaction of GSH reductase, using NADPH from the oxidation of glucose in the phosphogluconate pathway (2, 3, 5). Noguchi *et al.* (6) showed that GSH peroxidase was present mainly in cytosol and the plasma while the action of vitamin E was thought to be primarily within the membrane. A new hypothesis (2, 5, 6) is now being presented that vitamin E functions by neutralizing the free radicals at the site of their formation whereas GSH peroxide appears to be involved in the intracellular decomposition of lipid hydroperoxides formed. However, the relationship between dietary vitamin E supplementation and activity of GSH peroxidase in tissues has not been thoroughly investigated. The present study was undertaken to investigate the effects of a wide range of dietary vitamin E supplementation (from 0 to 25,000 IU vitamin E/kg diet) on the activity of GSH peroxidase in some tissues, such as the liver, plasma, and uterus of female rats.

Materials and methods. Female weanling rats of Wistar strain weighing approximately 50 g were randomly divided into six groups with four rats in each group. Food and water were supplied *ad libitum*. The basal tocopherol-deficient diet of Draper *et al.* (7), with supplements of 0, 25, 250, 2500, 10,000, and 25,000 IU vitamin E (*dl*-α-tocopheryl acetate)/kg diet was fed to six groups of rats for 8 months. Blood samples were taken from the inferior vena cava after anesthetizing the animals with ether, and the liver and uterus were removed. The plasma, liver, and uterus were frozen and stored until further analysis. The liver and uterus were minced and then homogenized in 5 vol of KCl (0.154 *M*) first with a Sorvall microhomogenizer attached to an omnimixer and then with a motor-driven Potter–Elvehjem homogenizer. The homogenate was subjected with osmotic shock to facilitate release of GSH peroxidase from mitochondria. The activity of GSH peroxidase in diluted homogenate of liver, uterus, and diluted plasma was determined by Mills' procedure 2 (8) with the modification of Hafeman *et al.* (9). The protein was measured by the method of Miller (10). A unit of GSH peroxidase activity was expressed as a decrease in log[GSH] of 0.001/min after deducting the decrease in log[GSH]/min of the nonenzymatic reaction (8, 9). The data were analyzed by Duncan's new multiple range test (11).

Results. The results presented in Figs. 1–3 indicate that the tissue activity of GSH peroxidase changes in response to the level of vitamin E supplementation in the diet of rats. The GSH peroxidase activity in liver was significantly (Fig. 1) decreased ($P < 0.05$) either by excess supplementation or by depletion of dietary vitamin E compared to rats on the 25 or 250 IU vitamin E/kg

[1] Supported by the National Research Council of Canada, Grant No. A-4686 and University of British Columbia Research Grant.

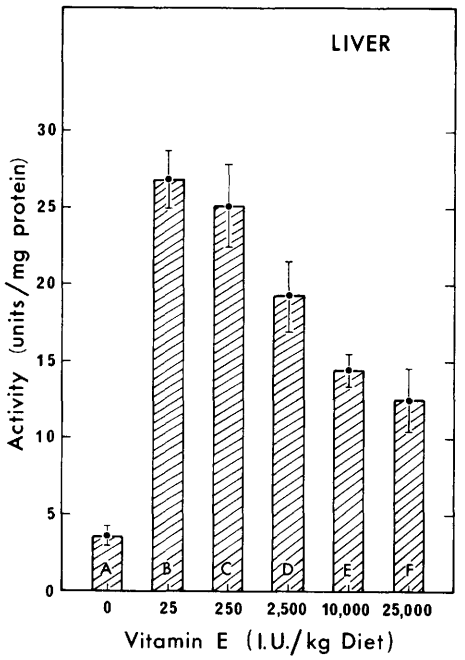

FIG. 1. Glutathione peroxidase activity in liver of rats on different dietary levels of vitamin E for 8 months. Each point represents mean ± SEM of four rats. Groups B and C are significantly higher than other groups ($P < 0.05$); group A is significantly lower than all other groups ($P < 0.01$); group F is significantly lower than groups C, B, and D ($P < 0.05$); group E is significantly lower than groups C and B ($P < 0.01$).

diet. The liver GSH peroxidase activities of animals treated with 0 or 25,000 IU vitamin E/kg diet were only 14 and 46%, respectively, of the liver GSH peroxidase activity of animals receiving 25 IU vitamin E/kg diet. The activity of GSH peroxidase in liver of vitamin E-depleted rats was significantly lower ($P < 0.01$) than that of all vitamin E supplemented groups. Similar effects of dietary vitamin E supplementation of GSH peroxidase activity were also observed in plasma (Fig. 2). Because the plasma protein level of vitamin E-deficient rats was significantly lower than of rats on vitamin E supplements, the GSH peroxidase activity was expressed per milliliter of plasma. Again the rats on 25 or 250 IU vitamin E/kg diet had higher GSH peroxidase activity in plasma than rats of other groups. The GSH peroxidase activity in the uterus of rats treated with no vitamin E or excess levels of vitamin E showed lower GSH peroxidase activity than rats treated with 25 IU vitamin E/kg diet (Fig. 3). The decrease in GSH peroxidase activity of the uterus of deficient and high vitamin E supplemented groups is not as marked as that of liver and plasma. When the enzyme activity in the uterus was calculated per gram of uteral tissue, there was no significant difference among these groups of rats. The data above indicate that the dietary level of vitamin E required to maintain the maximum GSH peroxidase activity in tissues of female rats is around 25 to 250 IU vitamin E/kg diet. During long-range treatment, either excess dietary vitamin E or vitamin E deficiency would cause a significant decrease in GSH peroxidase activity of the tissues. The response to vitamin E treatment can be quite variable depending on the tissue being examined. The results were not any different when the activity of GSH peroxidase was expressed per gram of liver or per 100 mg of protein in plasma.

Body and tissue weight and concentration of soluble proteins in tissue homogenates and in plasma of rats on different levels of dietary vitamin E for 8 months are presented in Table I. The weights of the body

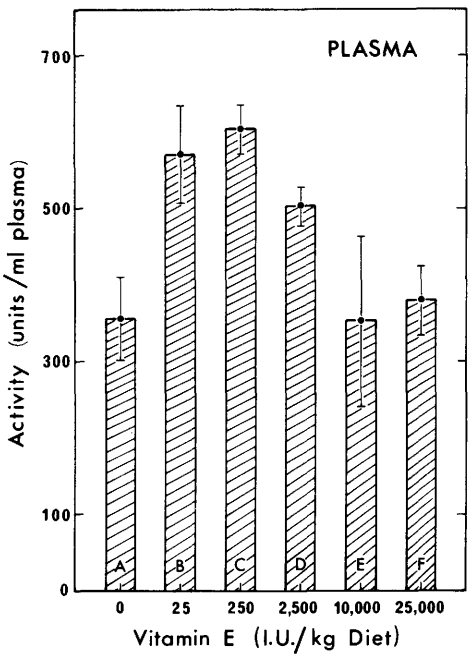

FIG. 2. Glutathione peroxidase activity in plasma of rats on different dietary levels of vitamin E for 8 months. Each point represents mean ± SEM of four rats. Groups B and C are significantly higher ($P < 0.05$) than groups A, E, and F.

and uterus of vitamin E deficient rats were approximately half that of vitamin E-supplemented groups. The weights of the body, liver, and uterus decreased in rats given 10,000 and 25,000 IU vitamin E/kg diet. The concentrations of soluble protein in homogenate of liver and uterus and in plasma were not significantly different among the vitamin E-supplemented groups. However, lower plasma protein and higher soluble protein concentrations in homogenate of liver and uterus of vitamin E-deficient rats were observed.

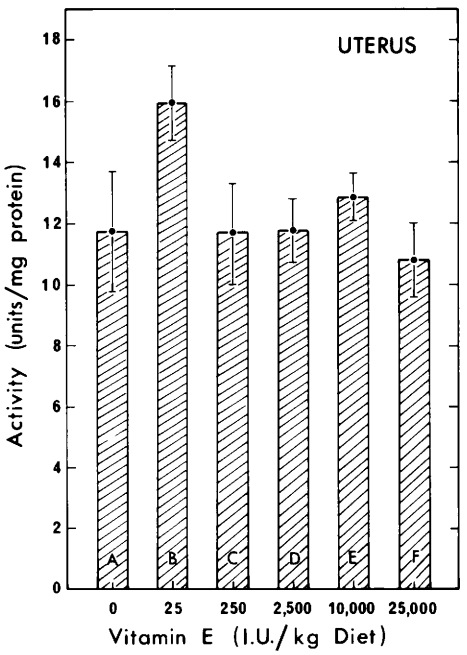

FIG. 3. Glutathione peroxidase activity in uterus of rats on different dietary levels of vitamin E for 8 months. Each point represents mean ± SEM of four rats. Group B is significantly higher ($P < 0.05$) than groups A, C, D, and F.

Discussion. Many factors, such as dietary levels of selenium (5, 6) vitamin A, and ethoxyquin (12), cholesterol (13), trio-*o*-cresyl phosphate (14), tissue lipid peroxides (15), and a number of nucleotides (16), have been found to affect the activity of GSH peroxidase. Pinto *et al.* (17) also reported that age affects the liver GSH peroxidase activity in rat and that the enzyme activity is about 80% higher in female rats than in males.

Little *et al.* (16) recently suggested that GSH peroxidase is an allosteric enzyme. Our results indicate that the activity of GSH peroxidase in liver, plasma, and uterus is readily modified by dietary vitamin E. The mechanism of the repressive effect of excess dietary vitamin E on GSH peroxidase activity is not clear yet, but based on the known biological function of vitamin E and GSH peroxidase some possible mechanisms can be proposed. As mentioned before, both vitamin E and GSH peroxidase are able to prevent the oxidative damage to tissues. Vitamin E is believed to prevent the formation of lipid peroxides, whereas GSH peroxidase decomposes the lipid peroxides formed in the tissues. A high level of dietary vitamin E might result in lowering the concentration of lipid peroxides in the tissues, thereby sparing the need for GSH peroxidase, the decomposer of lipid peroxides. Substrate induction is known to be an important factor for the synthesis of certain enzymes (18); for example, the activity of tryptophan pyrrolase in liver has been shown to increase in rats in response to the administration of its substrate, tryptophan (19, 20). It has been shown (15, 21, 22) that the accumulation of fluoresent products (believed to be products of lipid peroxidation) in tissues is related to

TABLE I. BODY AND TISSUE WEIGHT AND CONCENTRATION OF SOLUBLE PROTEINS IN TISSUE HOMOGENATES AND IN PLASMA OF RATS ON DIFFERENT LEVELS OF DIETARY VITAMIN E FOR EIGHT MONTHS[a]

Groups	Dietary vitamin E (IU/kg diet)	Body weight (g)	Tissue weight		Soluble protein in homogenate (mg/g)		Protein in plasma (mg/ml)
			Liver (g)	Uterus (mg)	Liver	Uterus	
A	0	^{a}165.9 ± 12.5	^{fg}8.8 ± 1.1	^{h}308.6 ± 59.9	^{k}125.0 ± 4.8	^{m}42.3 ± 3.3	^{o}5.5 ± 0.29
B	25	^{bd}366.1 ± 31.4	^{g}10.2 ± 0.8	^{i}677.6 ± 41.8	^{l}105.9 ± 5.0	^{n}32.3 ± 2.5	^{p}6.6 ± 0.19
C	250	^{cde}332.2 ± 11.9	^{fg}8.2 ± 0.5	^{i}679.5 ± 34.2	^{kl}115.7 ± 2.7	^{n}33.3 ± 2.4	^{p}6.6 ± 0.18
D	2,500	^{bde}356.3 ± 16.4	^{fg}8.8 ± 0.7	^{i}700.4 ± 21.2	^{l}108.1 ± 5.3	^{n}35.3 ± 0.4	^{p}7.1 ± 0.32
E	10,000	^{ce}312.5 ± 8.0	^{f}8.0 ± 0.4	^{j}551.4 ± 64.4	^{kl}112.4 ± 6.1	^{n}30.4 ± 1.5	^{p}7.0 ± 0.35
F	25,000	^{c}301.0 ± 15.5	^{f}7.4 ± 0.6	^{j}586.4 ± 21.6	^{kl}117.3 ± 3.1	^{n}33.7 ± 2.3	^{p}7.2 ± 0.20

[a] Each value is the mean ± SEM for four rats. Values within each column which are not identified by the same preceding superscript letter are significantly different ($P < 0.05$).

the level of dietary vitamin E. The reduced oxidative stress might be part of the reason for lower activity of GSH peroxidase in tissues of rats given high levels of vitamin E.

March *et al.* (23) have reported that excess dietary vitamin E depressed the activity of thyroid gland in the chick. Regulation of protein synthesis is one of the major effects of thyroid hormone (24). It has been shown that a normal amount of thyroxine is essential for protein synthesis in liver, muscle, kidney, and reticulocytes. For example, physiological levels of thyroxine are required for increasing the activity of certain enzymes such as α-glycerophosphate dehydrogenase in liver. But the role of thyroxine in the biosynthesis of GSH peroxidase has not yet been studied. Whether the repressive effect of excess dietary vitamin E on GSH peroxidase activity is due to decreased *de novo* synthesis or increased degradation or due to some other inhibitory effect on this enzyme remains to be investigated. Although the mechanism of the repressive effect of excess dietary vitamin E on GSH peroxidase activity is still not known, the data presented in Table I show that change in enzyme activity is not due to the alterations in cell fragility. If the cell fragility was being affected, the concentrations of soluble protein in the tissue homogenates also would be different. But, there is no significant difference in protein concentrations of tissue homogenates of various vitamin E-supplemented groups.

Our results also showed that activities of GSH peroxidase are significantly lower in the liver, uterus, and plasma of vitamin E-depleted rats than those receiving 25 IU vitamin E/kg diet, considered to be the adequate dietary level of vitamin E. It has to be pointed out that all four vitamin E-depleted rats showed severe signs of vitamin E deficiency, such as paralysis of legs, ceroid pigment formation in uterus, deformed skeleton, rough greasy coat, and bleeding nose. When liver weight was expressed as a percentage of body weight, the liver weight of vitamin E-depleted rats was two times higher than that of rats in other groups. Liver necrosis has been recognized as a typical symptom of vitamin E deficiency in rats. Liver necrosis may contribute to the lowering of GSH peroxidase activity in liver of vitamin E-depleted rats. Murty *et al.* (25) found that the activities of some hepatic heme-containing enzymes such as catalase, tryptophan pyrrolase, and microsomal cytochromes b_5 and P-450 were significantly reduced in rats receiving vitamin E-deficient diets for 120 days, but no differences were observed in the activities of some nonheme protein enzymes. Not only was the GSH peroxidase activity significantly lower in plasma of vitamin E-depleted rats than in those given 25 IU vitamin E/kg diet, but also a significantly lower total plasma protein was found. Chio and Tappel (26) showed that lipid peroxides are able to inactivate enzymes and that sulfhydryl enzymes, such as GSH peroxidase, are most susceptible to inactivation. The real mechanism of lower GSH peroxidase in rats of long-term vitamin E deficiency is still unknown. However, Chow *et al.* (15) found that the activities of GSH peroxidase were significantly increased in perirenal adipose tissue, paraepididymal adipose tissue, and musle, but not in liver, lung, and kidney after being given a 15.7% tocopherol-stripped corn oil diet for 2 months compared to rats on 45 IU vitamin E/kg diet.

Summary. Both excess dietary vitamin E and vitamin E deficiency in rats can significantly depress the activity of GSH peroxidase in liver and plasma of rats. Of all the six levels of vitamin E tested in this study, the dietary level of vitamin E found to maintain the maximum activity of GSH peroxidase in tissues of rats was somewhere between 25 and 250 IU/kg diet. This study conclusively indicates that the excess dietary vitamin E represses GSH peroxidase activity.

1. Rotruck, J. T., Pope, A. L., Ganther, H. E., and Hoekstra, W. G., J. Nutr. **102,** 689 (1972).
2. Rotruck, J. T., Pope, A. L., Ganther, H. E., Swanson, A. B., Hafeman, D. G., and Hoekstra, W. G., Science **179,** 588 (1973).
3. Little, C., and O'Brien, P. J., Biochem. Biophys. Res. Commun. **31,** 145 (1968).
4. Christopherson, B. O., Biochim. Biophys. Acta **164,** 35 (1968).
5. Chow, C. K., and Tappel, A. L., J. Nutr. **104,** 444 (1974).
6. Noguchi, T., Cantor, A. H., and Scott, M. L., J.

Nutr. **103,** 1502 (1973).
7. Draper, H. H., Bergan, J. G., Chiu, M., and Csallany, A. S., J. Nutr. **84,** 395 (1964).
8. Mills, G. C., J. Biol. Chem. **234,** 502 (1959).
9. Hafeman, G. D., Sunde, R. A., and Hoekstra, W. G., J. Nutr. **104,** 580 (1974).
10. Miller, G. L., Anal. Chem. **31,** 964 (1959).
11. Steel, R. G. D., and Torrie, J. H., *in* "Principles and Procedures of Statistics," p. 107, McGraw-Hill, New York (1960).
12. Combs Jr., G. F., and Scott, M. L., J. Nutr. **104,** 1297 (1974).
13. Tsai, A. C., J. Nutr. **105,** 946 (1975).
14. Shull, L. R., and Cheeke, P. R., Nutr. Rep. Int. **11,** 39 (1975).
15. Chow, C. K., Reddy, K., and Tappel, A. L., J. Nutr. **103,** 618 (1973).
16. Little, C., Olinescu, R., Reid, K. G., and O'Brien, P. J., J. Biol. Chem. **245,** 3632 (1970).
17. Pinto, R. E., and Bartley, W., Biochem. J. **112,** 109 (1969).
18. White, A., Handler, P., and Smith, E. L. *in* "Principles of Biochemistry," 5th ed., p. 775, McGraw-Hill, New York (1973).
19. Civen, M., and Knox, W. E., J. Biol. Chem. **234,** 1787 (1959).
20. Greengard, O., and Feigelson, P., J. Biol. Chem. **236,** 158 (1961).
21. Desai, I. D., Fletcher, B. L., and Tappel, A. L., Lipids **10,** 307 (1975).
22. Raychaudhuri, C., and Desai, I. D., Science **173,** 1028 (1971).
23. March, B. E., Wong, E., Sim, S. J., and Biely, J., J. Nutr. **103,** 371 (1973).
24. Sawin, C. T. *in* "The Hormones," p. 104, Little, Brown and Co., Boston (1969).
25. Murty, H. S., Caasi, P. I., Brooks, S. K., and Nair, P. P., J. Biol. Chem. **245,** 5498 (1970).
26. Chio, K. S., and Tappel, A. L., Biochemistry **8,** 2827 (1969).

Received October 17, 1975, P.S.E.B.M., 1976; Vol. 151.