AN EFFECT OF DIETARY SELENIUM ON HEMOLYSIS AND LIPID AUTOXIDATION OF ERYTHROCYTES FROM VITAMIN E DEFICIENT RATS

S. Krishnamurthy and J. G. Bieri

Laboratory of Nutrition and Endocrinology
National Institute of Arthritis and Metabolic Diseases
National Institutes of Health, Bethesda, Maryland

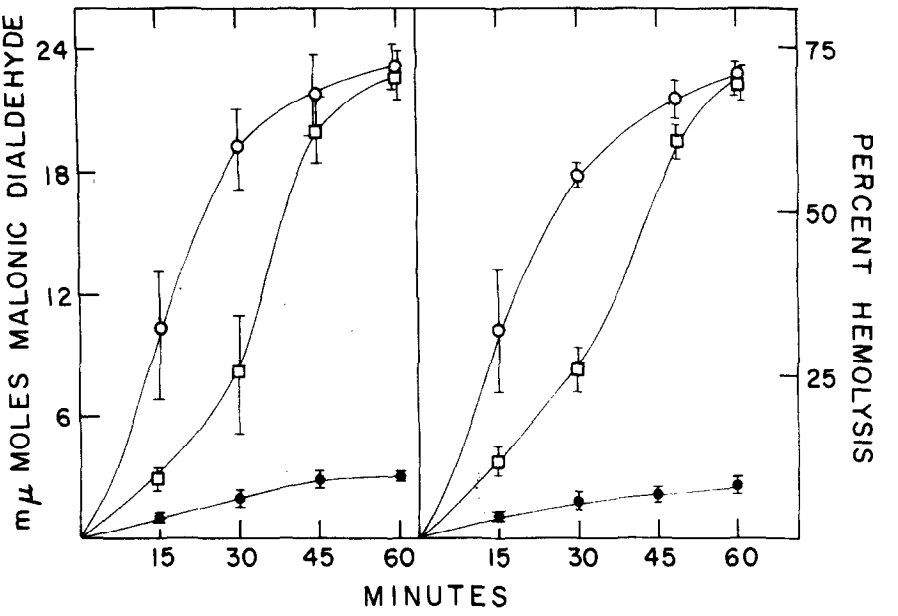
Received February 23, 1961

The susceptibility of erythrocytes to dialuric acid-induced hemolysis (Rose and Gyorgy, 1952) is a recognized characteristic of vitamin E deficiency in rats. The extent of hemolysis by dialuric acid has been shown to be paralleled by the formation of thiobarbituric acid-reacting products of lipid autoxidation (Tsen and Collier, 1960; Bunyan, Green, Edwin and Diplock, 1960). Dietary antioxidants (Moore and Sharman, 1959) as well as tocopherol prevent the hemolysis. Inasmuch as dietary selenium significantly reduces malonic dialdehyde formation in incubated tissue homogenates (Bieri, 1959; Zalkin, Tappel and Jordan, 1960) it seemed appropriate to determine if a similar effect of selenium could be shown in erythrocytes. These cells rapidly fix selenium after its administration (McConnell and Cooper, 1950).

Three groups of male weanling albino rats were fed the vitamin E-deficient Torula yeast diet of Schwarz (1951). The supplements for the respective groups were (1) none, (2) 0.5 ppm selenium as sodium selenite and (3) 200 mg. dl, α-tocopheryl acetate per kg. diet. After six weeks they were bled freely from the tail into a spot-plate containing dried heparin. One ml. of blood was centrifuged and the erythrocytes washed twice with one ml. portions of cold 0.9 per cent saline and resuspended in 1.5 ml. of saline-phosphate buffer, (pH 7.4, 0.1 M). Hemolysis was determined essentially according to Friedman, Weiss, Wherry and Kline (1958). Replicate flasks containing 0.5 ml. of the erythrocyte suspension and 1.0 ml. of freshly prepared dialuric acid solution (0.2 per cent in saline buffer) were

incubated in a metabolic shaker at 37°. At intervals one ml. of the reaction mixture was pipetted into 1.5 ml. of 10 percent trichloracetic acid, and the amount of malonic dialdehyde (as an estimate of lipid autoxidation) determined in the deproteinized extract by means of thiobarbituric acid (Bieri and Anderson, 1960). The remainder of the cells in the incubation flask were rinsed out with 1 ml. saline, centrifuged and the per cent hemolysis determined colorimetrically at 415 mμ in an aliquot of the supernatant, after suitable dilutions with distilled water.

The mean values obtained from 6 animals from each group are presented in Fig. 1.



Per cent hemolysis and malonic dialdehyde production in erythrocytes incubated with dialuric acid. Dietary supplements: ○ , none; □ , 0.5 ppm selenium; ● , 200 mg. α-tocopheryl acetate per kg. diet. Vertical lines show standard error of means.

It can be seen that the extent of hemolysis closely follows the production of malonic dialdehyde at all time intervals in confirmation of the report of Tsen and Collier (1960). There was a marked reduction in the degree of both hemolysis and malonic dialdehyde formation in the selenium-fed animals as compared to the unsupplemented group, especially at the 15 and 30 min.

incubation periods. After one hr. of incubation, however, the values for the two groups were similar. It is apparent that dietary selenium affords a definite degree of protection although less than that from dietary tocopherol.

Table I shows the extent of lipid autoxidation (malonic dialdehyde) which occurred on incubation without dialuric acid of water-lysed erythrocytes, or of the stroma and cell contents from the blood of the three groups. It can be seen that the production of malonic dialdehyde is predominantly a function of the stroma.

Table I

Lipid autoxidation in water-lysed erythrocytes and in the erythrocyte fractions

Incubation Sample	Additions to Diet	mμ moles malonic dialdehyde		
		15 min.	30 min.	60 min.
Whole Hemolysate	None	3.0	9.2	11.5
	Se	1.5	4.5	10.0
	Tocopherol	Nil	1.3	3.0
Stroma	None	4.0	12.1	14.7
	Se	2.5	6.3	10.0
	Tocopherol	Nil	1.1	2.5
Cell Contents	None	0.05	2.5	4.5
	Se	Nil	1.2	2.5
	Tocopherol	Nil	Nil	Nil

Concentration of cells and other conditions same as given in text. Hemolysis effected with distilled water and incubations done at 37° in a metabolic shaker. Stroma separated by centrifugation at 2,000 x g for 10 min. Values represent mean of 3 animals in each group.

The inhibitory effect of dietary selenium is apparent just as with the intact cells in the presence of dialuric acid.

The failure of previous workers (Gitler, Sunde and Baumann, 1958; and Friedman et al., 1958) to obtain an influence of selenium on hemolysis probably was due to their incubating for periods over one hour, by which time the antioxidant-like effect disappeared.

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