EFFECT OF A WATER-SOLUBLE ANALOGUE OF VITAMIN K (VICASOL) ON CHANGES IN THE SARCOPLASMIC PROTEINS OF MUSCLE DEVELOPING IN VITAMIN E DEFICIENCY

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In recent years new concepts have arisen concerning the participation of the substituted p-quinones: vitamin E (α-tocopherol) and its oxidation product, vitamin K (phylloquinone and its products) and ubiquinone [2, 6, 7] in the processes of tissue respiration and oxidative phosphorylation. The close relation in chemical nature of the two groups of substituted para-quinones (phylloquinone and tocopherol) also conforms to data on the possible relation to the participation of para-benzoquinones and 1,4-naphthoquinones in tissue respiration and oxidative phosphorylation. There is a certain similarity in their effect on chemical and physiological processes in muscle tissue. It is known that for vitamin E deficiency there are characteristic developments of chemical, morphologic and functional changes in the striated and cardiac musculature. According to data from our laboratory, in vitamin K deficiency changes occur in the contractile and ATP-ase properties of skeletal muscle myosin [3, 4], and a decrease in the tonic tension of smooth muscle [1]. Thus, certain lines of similarity in the action of this group of substituted p-quinones on muscle tissue stand out clearly. Studies we have previously carried out have shown that vitamin E deficiency leads to changes in the fractional composition of the muscle sarcoplasmic proteins [5].

The aim of the present study was more accurate definition of data we had obtained earlier and elucidation of the problem: does the suggested synergism obtain between the actions of vitamins E and K on water-soluble proteins of striated muscle?

METHODS AND RESULTS

The experiments were performed on 27 guinea pigs of weight 250-300 g, divided into three groups. All animals were maintained on a vitamin E-deficient diet of the following composition (in %): Casein 18, cornstarch 51, sucrose 5, cellulose 10, lard 3, dry yeast 7, fish oil 2, salt mixture * (recommended by the Nutrition Institute of the USSR Academy of Medical Sciences) 4; a second group of animals received also 1.5 mg vicasol and the third group—two mg of tocopherol per day. In addition, animals in all three groups were given 20 mg of ascorbic acid daily.

In animals from groups 1 and 2, vitamin deficiency developed in the middle of the third month of maintenance on this diet. They became sluggish, poorly reactive; lay on their backs and turned over with difficulty; some were not in a state to take their usual position. Control guinea pigs remained active and reactive.

The increase in the creatine coefficient in the urine (ratio of the sum of the creatine and creatinine to creatinine) was used as the objective index for development of vitamin E deficiency. The creatine and creatinine were measured according to Bonsnes and Toski. In animals maintained on the laboratory ration before transfer to the diet, and in the animals which were maintained on the diet with the addition of vitamin E (group 3—the controls), this coefficient varied within the limits of 1-2. In the group of guinea pigs receiving the diet alone (group 1) and in animals which in addition to the diet received vicasol (group 2) the creatine coefficient rose to 3.5-4.2).

*Composition of the mixture (in g): NaCl—234; KH_2PO_4—653.2; $MgSO_4$—96.4; $CaCO_3$—640.8; $FeSO_4 \cdot 7H_2O$—44.4; KI—1.288; $MnSO_4$—7.48; $ZnSO_4 \cdot 7H_2O$—0.92; $CuSO_4 \cdot 5H_2O$—0.8; $CoCl_2$—0.04; NaF—0.84; $K_2SO_4Al_2(SO_4)_3 \cdot 2H_2O$—0.188.

Fractional Composition of Sarcoplasmic Proteins of Striated Muscle in Three Groups of Guinea Pigs in % (M ± m)

Animal group	Myoalbumin (h)	Protein with phosphorylase activity		Protein with aldolase and other vitamin activity (m, n, l)
		(α)	(β)	
1st (diet)	17.4 ± 1.5	19.0 ± 1.2	12.9 ± 1.0	47.9 ± 2.4
2nd (diet + vicasol)	18.0 ± 1.2	19.0 ± 0.8	14.0 ± 0.8	45.4 ± 2.0
3rd (diet + vitamin E)	5.9 ± 0.4	10.3 ± 1.5	9.3 ± 0.04	75.7 ± 1.3

The sarcoplasmic proteins of striated muscle were studied in animals from all three groups by the method of paper electrophoresis. Guinea pigs were killed by decapitation. To obtain the muscle extracts the soleus muscles were taken and freed on ice from the fatty tissue, fascia and tendons. Then the muscle was extracted with physiologic saline(1 : 2), centrifuged and 0.04 ml of the extract placed on the paper. Electrophoresis was carried out for 18 h in boracic borate buffer at pH 8.6, ionic strength 0.14, potential 6.5 V/cm; current strength 0.4 milliamp/cm. The electrophoregrams were read on a photoelectrocolorimeter. On the paper the proteins fell into three main groups (two of them in turn were subdivided into two fractions each): 1) rapid-moving—myoalbumin (h); 2) moderate mobility proteins, to which is related the phosphorylizing activity; this group was represented by two peaks which we have designated α and β; 3) a group of proteins with extremely slow electrophoretic mobility, containing the aldolase and triosephosphate dehydrogenase activity (m, n, l); this group in some of our experiments was divided into two fractions and in others appeared as one peak. Comparison of the electrophoregrams of the three groups of animals showed that the sarcoplasmic proteins of muscle in vitamin E-deficient guinea pigs (group 1) and in guinea pigs which had received vicasol (group 2) underwent identical changes in comparison with the control (see table).

The results of the experiments are presented in the table. It is seen that in animals from groups 1 and 2 there is a significant increase in the rapidly moving fraction—myoalbumin. In animals from these groups the amount of proteins has increased above the moderately mobile fraction, being in greater degree at the expense of the most mobile component, α; the protein content with aldolase and triose phosphate dehydrogenase activity in vitamin E deficient guinea pigs and in animals receiving vicasol was lower than the level in the control animals. These differences were statistically valid. The differences between the first and second groups were negligible.

Thus, these investigations have shown that vitamin E deficiency evokes the following changes in the ratios of protein fractions in striated muscle: an increase in the fast-moving peak—myoalbumin; an increase in the fraction with phosphorylase activity; a decrease in the slow moving fraction. Vicasol in the indicated dose does not appear to have a modifying effect on the changes in muscle or on the course of the vitamin E deficiency.

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