EFFECT OF α-TOCOPHEROL ON CREATINE KINASE ACTIVITY OF SKELETAL MUSCLES AND BLOOD IN PRIMARY AND SECONDARY VITAMIN K DEFICIENCY

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The creatine kinase (2.7.3.2) activity in the blood serum of rabbits with pelentan-induced avitaminosis K and in the skeletal muscles of rats with primary (alimentary) avitaminosis K is considerably reduced, while the extractability of the muscle proteins with tris-buffer is increased. This decrease in enzyme activity can be prevented in rats by administration of the synthetic vitamin K preparation vikasol and of α-tocopherol.

Vitamin K not only stimulates the synthesis of certain procoagulants in the liver, but also contributes to the transphosphorylation and synthesis of high-energy phosphorus compounds, as is particularly evident in muscle tissue [2, 3, 5, 6, 9, 10]. In rats with secondary vitamin K deficiency, produced by ligation of the bile duct, creatine kinase activity is reduced in skeletal and smooth muscles [8, 11].

The object of this investigation was to study creatine kinase activity in the muscles and blood in primary (alimentary) avitaminosis K and in secondary avitaminosis K produced by administration of pelentan, an antivitamin K, to the animals. The effect of vitamin E on muscle creatine kinase activity was also investigated in animals with primary avitaminosis K.

EXPERIMENTAL METHOD

Experiments were carried out on 100 adult male albino rats and 15 adult male rabbits. Primary avitaminosis K was induced by keeping the rats on the semisynthetic diet of Mameesh and Johnson [13], as described previously [8]. Secondary avitaminosis K was produced in rabbits by feeding the animals with pelentan, which was given for the first two days in doses of 50 mg/kg body weight, and subsequently in daily doses of 10 mg/kg. The criterion of development of avitaminosis K was a persistent lengthening of the prothrombin time to three or more times its initial value. The rats were sacrificed by decapitation, blood was collected, the quadriceps femoris muscle was quickly removed and the tissue minced without delay and extracted on ice for 20-30 min with four volumes of 0.1 M tris-buffer, pH 8.8-9.0. Creatine kinase (2.7.3.2) activity was measured for the reaction proceeding in the direction to the right as described by Maksimova [4]. Creatine phosphate (CP) was determined by Alekseeva's method [1] and phosphorus by Lowry's method [12].

EXPERIMENTAL RESULTS

Both primary avitaminosis K in rats and secondary pelentan avitaminosis K in rabbits led to a significant decrease in the creatine kinase activity of the blood serum (Table 1).

Creatine kinase activity in the skeletal muscles (Table 2) was expressed in micromoles of CP formed, and the result was calculated in two ways: per gram protein of muscle extract and per gram fresh weight of muscle tissue.

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TABLE 1. Serum Creatine Kinase Activity in Primary and Secondary (Pelentan) Avitaminosis K (M±m)

Animals	Nature of experiment	Prothrombin time (in sec)	Enzyme activity (in μmoles CP/ml serum)	P
Rats (18)	Vivarium diet	14.3±0.34	12.0±1.21	
" (13)	Vitamin K deficient diet	112.5±6.39	7.2±0.47	< 0.001
Rabbits (15)	Before administration of pelentan	23.8±0.48	15.6±1.11	
" (15)	After course of pelentan	102.2±7.06	3.5±0.76	< 0.001

Note. Here and in Tables 2 and 3, number of animals given in parentheses.

TABLE 2. Creatine Kinase Activity of Skeletal Muscles (M±m)

Nature of experiment	Prothrombin time (in sec)	Creatine kinase activity (in μmoles CP)	
		per gram protein of extract	per gram fresh weight of muscles
Vitamin K deficient diet (42) . .	14±0.2	638±39.6	7.7±0.50
Before administration of pelentan (23)	111±4.6	280±44.0	4.6±0.44
Vitamin K deficient diet + 1 mg vikasol daily by mouth (25) . . .	15±0.5	592±53.0	6.8±0.49
Vitamin K deficient diet + 20 mg α-tocopherol by mouth every other day (10)	105±6.2	613±116.6	7.3±0.83

TABLE 3. Protein Concentration in Extracts of Skeletal Muscles of Albino Rats (M±m)

Nature of experiment	Protein (in %)
Vitamin K deficient diet (42) .	1.29±0.086
Before administration of pelentan (23)	1.67±0.058
Vitamin K deficient diet + vikasol (25)	1.28±0.051
Vitamin K deficient diet + tocopherol (10)	1.30±0.97

In the animals with avitaminosis K, produced by both methods, activity of the enzyme was significantly reduced ($P < 0.001$–0.01) compared with its level in rats kept on the normal vivarium diet and in rats receiving the vitamin K deficient diet supplemented with vikasol. Although α-tocopherol did not protect the animals from hypoprothrombinemia, it completely prevented the decrease in creatine kinase activity due to avitaminosis K.

The degree of depression of enzyme activity in the animals with avitaminosis K, when calculated per weight of muscle tissue, was less than when calculated per gram protein of the muscle extract: by the first method of calculation, the activity of the enzyme in the group of rats receiving the standard diet was lower than in the other three groups by 1.48–1.67 times, and by the second method it was lower by 2.11–2.28 times. This difference can be explained by the higher rate of extraction of creatine kinase from the muscles of the animals with avitaminosis K (Table 3).

The reason for the increased extractability of muscle proteins in animals with avitaminosis K requires special investigation. It must be emphasized that, despite the observed differences, whatever method was used to express enzyme activity (relative to protein of extract and to fresh muscle), its decrease in the animals with avitaminosis K and the action of vikasol and α-tocopherol in preventing this phenomenon were both statistically significant.

The decrease in creatine kinase activity in the muscle tissue of the animals with avitaminosis K cannot be explained by discharge of the enzyme into the blood, because activity of the enzyme in blood was also reduced.

The decrease in creatine kinase activity of the skeletal muscles and blood serum discovered in both secondary and primary forms of vitamin K deficiency is in good agreement with the published data cited above indicating disturbance of other components of the transphosphorylation system and a decrease in synthesis of high-energy phosphorus compounds in this avitaminosis. The effect of α-tocopherol in preventing the decrease in creatine kinase activity in avitaminosis K is in agreement with the corresponding effect of vitamin E in preventing the decrease in muscle ATPase activity in avitaminosis K [7]. This fact is further evidence of the synergism of action of two groups of substituted p-quinones (vitamins K and E) on transphosphorylation.

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