EFFECT OF ADMINISTRATION OF α-TOCOPHEROL TO ALBINO RATS ON CHANGES IN CONTENT OF ATP, ADP, AND INORGANIC PHOSPHORUS IN THE SKIN AND SKELETAL MUSCLES DUE TO AVITAMINOSIS K

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The content of ATP+ADP in the skin and skeletal muscles was reduced by one-third in rats with primary and secondary vitamin K deficiency, while the level of inorganic phosphorus was raised. Administration of the synthetic vitamin K substitute vikasol prevented these changes and the hypoprothrombinemia. Intramuscular injection of α-tocopherol did not prevent hypoprothrombinemia in the animals with avitaminosis K but it completely prevented the decrease in content of ATP+ADP and the increase in inorganic phosphorus in their skin and muscles.

Experiments showing that in vitamin K deficiency the creatine kinase activity of the skeletal muscles and blood is substantially reduced were described in a previous paper [10]. This decrease in the muscles is completely prevented by administration of vikasol or, equally of α-tocopherol to the animals.

With these results in mind, and also considering that with increased doses of α-tocopherol the decrease in activity of myosin ATPase and of the alkaline phosphatase of skeletal muscles and skin, characteristic of vitamin K deficiency, can be prevented [9], the present investigation was carried out in order to study changes in the content of these high-energy nucleotides in these tissues in the presence of vitamin K deficiency and the effect of α-tocopherol on these changes.

EXPERIMENTAL METHOD

Experiments were carried out on 158 male albino rats weighing 140-180 g. Primary avitaminosis K was produced by keeping the animals on a modified semisynthetic diet as described previously [10], while secondary avitaminosis K was produced by ligation followed by division of the bile duct as described by Kudryashov et al. [4]. The development of avitaminosis K was judged on the basis of an increase of 3 times or more in the prothrombin time, determined by Tugolukov's method [1]. By the end of the 3rd week of the experiment, from an initial 12-15 sec the prothrombin time was lengthened to 55-120 sec. At this time the animals were killed by decapitation, and weighed samples of the tissues (skin of the abdominal wall carefully freed from hair and fatty areolar tissue, and the quadriceps femoris muscle freed from tendons and membranes) were taken without delay, placed on ice, homogenized, and extracted with 4 volumes of 5% TCA solution. ATP and ADP in the extracts were determined by Seits's method [11] and inorganic phosphorus by the method of Uzbekov and Uzbekov [12].

EXPERIMENTAL RESULTS

The results given in Table 1 show that in both types of vitamin K deficiency the content of ATP+ADP in the skin and muscles was sharply reduced ($P < 0.001$), while the content of inorganic phosphorus was increased ($P < 0.01$) by comparison with the intact animals kept on the ordinary animal house diet.

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TABLE 1. Content of ATP+ADP and of Inorganic Phosphorus (in mg% P) in Skin and Skeletal Muscles of Rats with Avitaminosis K (M±m)

Nature of experiment	Skin		Skeletal muscles	
	ATP+ADP	$P_{inorg.}$	ATP+ADP	$P_{inorg.}$
Animal house diet (25)	18.1±1.04	24.5±1.09	54.8±2.80	33.1±1.79
Ligation of bile duct (18)	12.3±0.72	33.0±2.02	39.5±2.03	41.2±2.53
Ditto, 1 mg vikasol by mouth daily (18)	17.2±1.40	23.8±1.94	52.8±2.46	30.1±1.44
Ditto, 20 mg α-tocopherol intramuscularly on alternate days (13)	21.4±1.31	19.6±1.40	58.1±2.23	24.1±0.31
Vitamin K-deficient diet (33)	12.6±0.49	29.1±0.62	32.1±1.40	40.3±1.89
Vitamin K-deficient diet, 1 mg vikasol daily by mouth (32)	18.9±0.69	25.9±0.83	52.7±2.14	33.3±1.67
Ditto, 20 mg α-tocopherol intramuscularly on alternate days (19)	24.6±1.12	18.8±1.11	60.6±1.90	26.8±1.51

Note. Number of animals in parentheses.

Administration of the water-soluble vitamin K analog (vikasol) to the animals with a ligated bile duct or animals on a vitamin K-deficient diet maintained the same level of ATP+ADP and of inorganic phosphorus as in the skin and muscles of the intact animals, and also a normal prothrombin time (12-15 sec).

Administration of α-tocopherol did not prevent hypoprothrombinemia in the animals (their prothrombin time varied between 48 and 126 sec), but it completely prevented a decrease in the ATP+ADP concentration in the tissues and an increase in the inorganic phosphorus level. Further, in animals with primary avitaminosis K and receiving α-tocopherol, the content of ATP+ADP in the skin was actually higher ($P < 0.05$) than in rats on a vitamin K-deficient diet and receiving vikasol, and in animals receiving the normal animal house diet.

A decrease in the content of ATP+ADP in animals with avitaminosis K has been described previously with respect to the skeletal and smooth muscles [14] and blood plasma [2]. The present experiments show that their content in the skin also falls sharply (by one-third) and that this is accompanied by an increase in the level of inorganic phosphorus.

It is particularly interesting that administration of α-tocopherol to the animals in a dose 13 times above the mean antisterility dose, which is 0.75 mg for rats [18], completely prevented the decrease in the ATP+ADP content and the increase in the inorganic phosphorus level in the tissues studied. This phenomenon corresponds to the fact that increased doses of α-tocopherol prevent a decrease in activity of myosin ATPase [13], creatine kinase [10], and alkaline phosphatase in the skeletal muscles and blood [9] of animals with avitaminosis K, and also prevent the decrease in activity of certain enzymes in their alimentary tract [5]. These facts are in good agreement with the view [6, 8] that vitamin K, besides its effect on the biosynthesis of procoagulants, also possesses an extracoagulatory anabolic action, dependent on its participation in energy generation in high-energy nucleotides. The rejection of Martius's hypothesis [15] of the role of vitamin K in oxidative phosphorylation by a number of workers [3, 17] must evidently be regarded as premature. The results of the present investigation are also in good agreement with the suggestion [7, 8] that it is not vitamin K itself, but its metabolite (or metabolites), which may be formed in the animal body not only from vitamins of the K group but also from related substituted p-quinones, such as the E vitamins, which plays a role in transphosphorylation and production of high-energy compounds. Indirect confirmation of this view may be given by the discovery of many forms of vitamin K, some of which are unstable [16], in the animal organism.

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