# CONTENT OF ATP, ADP, AND INORGANIC PHOSPHORUS IN BLOOD PLASMA OF RATS WITH PRIMARY AND SECONDARY VITAMIN K DEFICIENCY

L. M. Bronshtein, T. S. Barkagan, and I. I. Matusis

UDC 616.391.04:577.161.5-092.9-07:616.153.963.32+616.152.18-074

In rats with primary and secondary vitamin K deficiency the prothrombin time was prolonged 7-8 times, the blood level of ATP + ADP was lowered by more than half, and the concentration of inorganic phosphorus was increased. Administration of synthetic vitamin K substitute vikasol to these animals completely prevented all the observed changes.

* * *

Previous investigations showed that vitamin K participates in blood coagulation not only by stimulating biosynthesis of some procoagulants, but also by acting on the final phase of the process of blood clotting: in animals with avitaminosis K the elastic properties of fibrin plasma clots are considerably reduced, and administration of the synthetic vitamin K substitute vikasol sharply increases their elasticity [5]. More recently, results have been obtained confirming that the process of fibrin formation is accompanied by the splitting of organic phosphorus compounds [10] and by the breakdown of ATP [12].

In face of these findings and of our earlier demonstration of the importance of vitamin K in metabolism of high-energy phosphorus compounds in muscle [2-4, 8, 9], the present investigation was carried out with the aim of determining whether vitamin K deficiency is accompanied by changes in the concentrations of these compounds in the blood plasma.

## EXPERIMENTAL METHOD

Experiments were carried out on 80 adult male albino rats. Secondary avitaminosis K was produced by ligation and excision of the bile duct [1]. To produce primary avitaminosis K the rats were kept on a semisynthetic diet [11], slightly modified by ourselves: instead of diets of soy protein recommended by other authors, the same quantity of casein was used. This was treated for 3 h with boiling ethanol, allowed to stand overnight in the same solvent, and then dried at room temperature. Coprophagy was prevented by keeping the animals in cages with grated floors, through which the excreta could fall. By the end of the 3rd week vitamin K deficiency had developed in the animals receiving this diet and also in the animals with a ligated bile duct, as shown by a marked increase in their prothrombin time. At this period the animals were sacrificed by decapitation and blood quickly taken for investigation. ATP and ADP in the plasma were determined by Seits's method [6], and inorganic phosphorus by the method of Uzbekov and Uzbekov [7].

## EXPERIMENTAL RESULTS

The experimental results are given in Table 1.

Ligation of the bile duct led to the development of vitamin K deficiency in the rats, as shown by the prolongation of their prothrombin time to 8 times that of the intact animals. The content of ATP +ADP in these animals was reduced by more than half, while at the same time the inorganic phosphorus concentration was increased. In the group of rats with ligated bile duct and receiving the water-soluble analog of vitamin K (vikasol) by subcutaneous injection in a dose of 1 mg daily for 3 weeks, the prothrombin time and inorganic phosphorus concentration were the same as in the intact animals, while the ATP + ADP concentration were the same as in the intact animals, while the ATP + ADP concentration was slightly higher.

Department of Physiology and Biochemistry of Man and Animals and Biological Research Division, Institute of Chemistry, Gor'kii University. (Presented by Academician of the Academy of Medical Sciences of the USSR S. E. Severin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 67, No. 3, pp. 48-50, March, 1969. Original article submitted February 26, 1968.

TABLE 1. Prothrombin Time, Total ATP + ADP, and Inorganic Phosphorus in Blood Plasma of Rats (M±m)

| Group and number of animals | Prothrombin time (in sec) | ATP + ADP (in μg/ml) | $P_{inorg}$ (in μg/ml) |
|---|---|---|---|
| Intact rats on normal vivarium diet (23) | 14±0.3 | 18.0±1.02 | 18.4±0.57 |
| Rats with secondary avitaminosis K produced by ligation of bile duct (20) | 113±9.6 | 8.7±0.54 | 26.5±0.75 |
| Rats with ligated bile duct receiving vikasol (15) | 14±0.5 | 22.9±0.97 | 18.8±0.70 |
| Rats with primary avitaminosis K (10) | 101±8.9 | 6.7±0.86 | 26.3±1.61 |
| Rats receiving vitamin K-deficient diet + vikasol (12) | 14±0.6 | 17.2±0.84 | 18.3±0.55 |

Note. Differences between mean data obtained for experimental (avitaminosis) and corresponding control rats (intact animals, rats receiving vikasol) are statistically significant: $P < 0.001$.

In the animals receiving a vitamin K-deficient diet, avitaminosis K also developed: the prothrombin time was 7 times longer than in the intact rats. The ATP + ADP concentration in the blood plasma fell by 60% compared with the intact animals and the inorganic phosphorus concentration was increased. In the animals receiving a vitamin K-deficient diet and vikasol in a daily dose of 1 mg throughout the experiment, all the indices studied remained at the same level as in intact animals on a normal vivarium diet.

It thus follows that primary alimentary vitamin K deficiency and secondary avitaminosis K produced by ligation and excision of the bile duct led to a sharp decrease (by more than 50%) in the plasma ATP + ADP concentration with a simultaneous increase in that of inorganic phosphorus. Administration of vikasol, the water-soluble analog of vitamin K, completely prevented these changes.

## LITERATURE CITED


1. B. A. Kudryashov, P. D. Ulitina, and A. A. Pugacheva, Byull. Éksperim. Biol. i Med., 11, No. 2, 99 (1941).
2. P. V. Lidina, Biokhimiya, No. 3, 209 (1951).
3. I. I. Matusis, in: Proceedings of the 16th Scientific Session of the Institute of Nutrition, AMN SSSR [in Russian], No. 1, Moscow (1966), p. 75.
4. I. I. Matusis, N. G. Bogdanov, M. I. Pechenina, et al., Vopr. Med. Khimii, No. 6, 613 (1966).
5. I. I. Matusis and L. M. Bronshtein, Byull. Éksperim. Biol. i Med., No. 2, 57 (1967).
6. I. F. Seits, Byull. Éksperim. Biol. i Med., No. 2, 119 (1957).
7. G. A. Uzbekov and M. G. Uzbekov, Lab. Delo, No. 6, 349 (1964).
8. I. I. Ulasevich, in: Proceedings of the First Scientific Biochemical Conference to Celebrate the 50th Anniversary of the Great October Socialist Revolution [in Russian], Omsk (1967), p. 149.
9. N. I. Yalovaya and N. G. Bogdanov, Abstracts of Proceedings of an All-Union Conference on Musclw Biochemistry [in Russian], Moscow-Leningrad (1966), p. 157.
10. C. V. Born, Biochem. J., 68, 659 (1958).
11. M. S. Mameesh and B. C. Johnson, Proc. Soc. Exp. Biol. (New York), 101, 467 (1959).
12. J. Marr, J. J. Barboriak, and S. A. Johnson, Nature, 205, 259 (1965).