COMPONENTS OF THE PROTHROMBIN MOLECULE DURING DEVELOPMENT OF AVITAMINOSIS K

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Recent investigations have shown that vitamin K participates in reactions of post-translation formation of calcium-binding zones of protein (γ-carboxylation of glutamic acid residues) [5, 12] and carbohydrate components [7, 11] of prothrombin. The role of vitamin K in the glycosylation of prothrombin has been studied only in rats, without taking into account the dynamics of development of avitaminosis K, and the results have proved ambiguous [13].

The object of this investigation was to determine the content of protein and carbohydrate (hexosamines) components of prothrombin during the development of avitaminosis K in pigeons and rats.

EXPERIMENTAL METHOD

A state of avitaminosis K was induced in pigeons (weighing 240-300 g) and rats (weighing 150-200 g) by keeping them on a special diet [1, 10] for 20 and 30 days, respectively. Control animals received the same diet but with the addition of vikasol (containing vitamin K, in a dose of 10 mg/kg diet). The development of avitaminosis K in the experimental rats was monitored by recording the thromboplastin time (by Quick's method). The animals were killed at various times of development of avitaminosis K after brief ether anesthesia. Prothrombin was isolated from the animals' blood plasma [8, 9] and protein [9] and hexosamines [6] were determined in it.

EXPERIMENTAL RESULTS

Keeping the pigeons on a vitamin K-deficient diet for 7, 14, and 20 days led to an increase in the thromboplastin time from 28 ± 0.5 sec to 42 ± 3, 64 ± 4, and 138 ± 19 sec, respectively. In the rats similar changes were found in the thromboplastin time from a normal 26 ± 0.7 to 46 ± 4, 54 ± 3, and 95 ± 9 sec 10, 20, and 30 days, respectively, after the beginning of the experiment. These results are evidence of differences in the duration of development of alimentary avitaminosis K in pigeons and rats.

The results of investigation of prothrombin components in the control animals are given in Table 1. They are similar to the corresponding figures given in the literature [7, 11, 13].

These experiments showed that during the development of avitaminosis K in both species of animals a gradual decrease was observed in the concentration of the components of prothrombin studied, except in the case of protein, the level of which was virtually restored to normal in the rats on the 20th day of the experiment. This may be linked with the appearance in the blood of prothrombin with low activity, on account of blocking of γ-carboxylation of glutamic acid residues in the protein moiety of the prothrombin molecule [5, 12].

Changes in the prothrombin molecule were judged according to the ratio of the protein concentration to the hexosamine content. This ratio in prothrombin from intact pigeons was 26.4. In pigeons kept on a vitamin K-deficient diet it was increased by 9, 33, and 51%, respectively, on the 7th, 14th, and 20th days of the experiment. The ratio of the protein concentration to the hexosamine content changed in a similar way in the prothrombin of the experimental rats: It was increased by 4, 50, and 47%, respectively, on the 10th, 20th and

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TABLE 1. Protein and Hexosamine Content (in μg/100 ml plasma) in Prothrombin during Development of Avitaminosis K (M ± m)

Test object	Experimental conditions	Number of experiment	Protein	Hexosamines
Pigeons	Control	10	4220±144	160±6,2
	Avitaminosis K			
	7 days	12	3837±150	134±4,0*
	14 days	10	3050±120*	87±4,0*
	20 days	9	2556±109*	64±2,9*
Rats	Control	10	5100±162	184±4,4
	Avitaminosis K			
	10 days	13	4012±119*	139±5,2*
	20 days	11	4856±94	116±5,6*
	30 days	10	2170±112*	53±2,2*

*P < 0.05 compared with control.

30th days of the experiment. These calculations indicate a decrease in the number of residues of these hexosamines in prothrombin during the course of avitaminosis K.

The results are evidence of inhibition of glucosamine biosynthesis in the liver and a reduction in its content in the prothrombin of rats with vitamin K deficiency [4, 7, 11]. This disturbance of glycosylation of the prothrombin molecule may be induced by blockade of glucosamine transport to the protein moiety of prothrombin [7]. However, a more likely factor in this disturbance may be inhibition of the reaction of biosynthesis of glucosamine, for this precedes its transport. Indirect support in favor of this suggestion is the decrease in content of other hexosamine-containing biopolymers and the decrease in the hexosamine content in tissue seromucoids of rats with avitaminosis K [3, 4], synthesis of which depends on the presence of glucosamine.

According to existing views [2], a carbohydrate deficiency in the composition of glycoproteins shortens their half-life in the tissues. From this standpoint, the changes observed in the hexosamine content may lead to a decrease in the blood prothrombin concentration, such as is observed *in vivo* in vitamin K deficiency.

These experiments thus showed that during the development of avitaminosis K in pigeons and rats a decrease is observed in the hexosamine content in the blood prothrombin, possibly on account of inhibition of glucosamine biosynthesis.

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