EFFECT OF AVITAMINOSIS C ON THE STATE OF THE ANTICLOTTING SYSTEM OF THE BLOOD IN GUINEA PIGS

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During the development of avitaminoses C and P in guinea pigs, a gradual and progressive depression of the anticlotting system of the blood is observed, accompanied by activation of fibrinolysis in the early stages of scurvy and by absence of fibrinolytic activity on the appearance of external manifestations of the avitaminosis. In hypovitaminosis the changes in the anticlotting system of the blood are less marked. Administration of vitamin P to animals kept on a scorbutogenic diet somewhat alters the time of development of changes in the state of the anticlotting system of the blood, but does not affect their character.

The role of vitamin C in the activity of the clotting system of the blood contains a number of contradictions, and these are responsible for inconsistency in recommendations given on the use of this vitamin [4].

Only 10-15 years ago many investigators considered that the bleeding tendency in scurvy is not connected with changes in the clotting system of the blood, but is due to increased permeability of the vessel walls [5, 9]. It has now been proved beyond doubt that in scurvy there is a disturbance of hemostasis due to a marked decrease in the thromboplastic activity of the blood, caused by a decrease in the activity of platelet factor [1, 6], a decrease in the adhesiveness of the platelets, [7], and a decrease in the activity of factor XIII [2].

The functional state of the anticlotting system in avitaminosis C has not been studied. The investigation described below was undertaken to consider this problem.

EXPERIMENTAL METHOD

Experiments were carried out on 65 male guinea pigs weighing 250-350 g, kept on Lecocq's diet in N. N. Berezovskaya's modification, free from vitamins C and P, and consisting of wheat flour, bran, brewers' yeast, sunflower oil, and common salt [3]. The animals were divided into five groups. The first four groups received Lecocq's diet with the addition of vitamins: group 2) 10 mg vitamin P, group 3) 25 mg vitamin C and 10 mg vitamin P, group 4) 25 mg vitamin C. The vitamins were given by mouth. The animals of group 5 were kept on the ordinary winter diet of the animal house, deficient in vitamins C and P.

Blood for investigation was taken from the jugular vein before the experiment and 5, 10, 15, and 25 days after it began. The blood samples were mixed with sodium citrate in the ratio of 9 : 1, centrifuged for 10 min at 1500 rpm, and kept until the time of the investigation in an ice bath. The state of the anticlotting system of the blood was characterized by determining the fibrinogen concentration (by Lazar's method), the fibrinolytic activity (by Astrup's method), the plasma heparin tolerance (by Gormsen's method), and the concentrations of heparin (by Sirmai's method), antiplasmin (by Nordoy's method in G. D. Andreenko's modification), and of ascorbic acid in the blood (by Berizovskaya's method).

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TABLE 1. State of the Anticlotting System of the Blood in Guinea Pigs Kept on Lecocq's Diet

Index studied	Statistical index	Animals kept on diet + vitamin C					Animals kept on vitamins C and P				
		before expt.	5	10	15	25	before expt.	5	10	15	25
			days of experiment					days of experiment			
Fibrinolytic activity (in mm^2)	M $\pm m$ P	178 7,1	187 11,7 >1,1	199 9,7 >0,1	185 15,6 <0,1	144 9,7 <0,02	212 10,6	198 6,1 >0,1	184 3,4 <0,02	181 4 >0,05	192 4 >0,05
Fibrinogen (in mg %)	M $\pm m$ P	416 20,6	409 22,6 <0,1	442 8,4 >0,1	472 17,7 >0,05	468 5,3 <0,05	320 16,3	390 18,9 >0,05	340 12,1 <0,1	380 18,9 <0,05	360 9,65 >0,05
Antiplasmin activity (in sec)	M $\pm m$ P	78 1,77	71 1,77 <0,01	89 2,87 <0,01	89 1,44 <0,01	71 1,42 <0,01	74 1,78	78 2,11 >0,05	88 2,4 <0,05		80 2,82 >0,05
Plasma heparin tolerance (in min)	M $\pm m$ P	3,6 0,71	8 0,71 <0,01	10 0,28 <0,01	14 0,85 <0,01	20 0,85 <0,01	4,7 0,78	9,8 0,49 <0,01	13,6 0,23 <0,01		20 0,49 <0,01

Index studied	Statistical index	Animals kept on winter diet of animal house				Animals kept on Lecocq's diet without vitamins C and P					Animals kept on diet + vitamin P						
		before expt.	5	15	25	before expt.	5	10	15	25	before expt.	5	10	15	20	30	35
			days of experiment				days of experiment					days of experiment					
Fibrinolytic activity (in mm^2)	M $\pm m$ P	68 5,54	54 1,85 <0,05	184 7,7 <0,01	154 8,74 <0,01	129 3,55	236 10 <0,01	326 5,95 <0,01	224 12,08 <0,01	49 3,55 <0,01	186 6,2	212 6,78 <0,01	191 7,12 >0,1	200 5,5 >0,1	243 4,62 <0,01	320 8,7 <0,01	158 1,78 <0,01
Fibrinogen (in mg %)	M $\pm m$ P	498 17,3	365 13,3 <0,01	361 6,1 <0,01	586 7,56 <0,01	406 10,3	415 11,4 >0,1	465 12,4 <0,01	633 16,5 <0,01	641 7,3 <0,01	353 7,9	388 5,4 <0,01	478 3,89 <0,01	615 14,7 <0,01	596 5,95 <0,01		840 9,5 <0,01
Antiplasmin activity (in sec)	M $\pm m$ P	96 1,53	64 0 >0,05	72 0,51 <0,01	74 0,51 <0,01	75 1,42	63 1,77 <0,01	114 1,77 <0,01	116 2,81 <0,01	109 2,81 <0,01	71 5,95	73 8,95 >0,05	100 0,2 <0,01	132 1,48 <0,01		147 2,31 0,01	
Plasma heparin tolerance (in min)	M $\pm m$ P	3,8 0,51	5,4 0,41 <0,01	6,5 0,103 <0,01	8,8 0,615 <0,01	9,1 0,49	9,4 0,49 >0,1	7 0,35 <0,01		7 0,28 <0,01	5,1 0,24	3,3 0,17 <0,01	5,3 0,24 0	3,3 0,149 <0,01	2,5 0 <0,01	2,0 0,059 <0,01	2,0 0,059 <0,01

EXPERIMENTAL RESULTS

After the guinea pigs had been kept in Lecocq's diet for 15-18 days, their body weight was reduced, their teeth became loose and fell out, and hemorrhages developed in the intercostal spaces and ankles.

During the first five days of absence of vitamins C and P in the animals' diet, an increase in the fibrinogen concentration, a marked increase in the fibrinolytic activity, and a decrease in the antiplasmin concentration of the blood were observed. By the 10th day, despite the absence of external signs of avitaminosis, the fibrinolytic activity was increased to more than twice its initial level (Table 1).

Meanwhile the antifibrinolytic activity of the blood was increased and its heparin concentration reduced. A disturbance of the coagulability of the blood appeared at this time. By the 15th-18th day, the fibrinolytic activity of the blood was reduced, the antifibrinolytic activity was increased, and the fibrinogen concentration was raised. The plasma heparin tolerance was also raised at this time. These changes, indicating depression of the function of the anticlotting system of the blood, subsequently increased further in severity, and before death of the animals a high fibrinogen concentration and absence of fibrinolytic activity were observed.

Changes observed in the animals of the experimental group were due to the absence of ascorbic acid in their diet. This is shown by the results obtained in the remaining four groups of animals. In the guinea pigs receiving an adequate dose of ascorbic acid in addition to their diet, just as in the animals receiving vitamins C and P, no appreciable changes in any of the indices determined were observed. In the animals receiving vitamin P only, the same changes were found as in the experimental animals, but the time when they occurred was considerably altered and they were less severe. In the guinea pigs of group 5, kept on the winter diet of the animal house, insufficient in vitamins C and P, a state of hypovitaminosis evidently developed. The dynamics of the changes in fibrinogen concentration and in fibrinolytic activity and antifibrinolysin showed that same tendency as if vitamins C and D were completely absent from the diet, but it was much less marked.

The maximal increase in fibrinolytic activity was accompanied by an increase in antifibrinolysin activity. Consequently, it could be due to presence of an activator in the blood. In fact, when fibrinolytic activity was determined in relation to fibrin disks by Astrup's method, a higher level of activity in the blood of the animals of the experimental group was observed in unheated disks because of the increased level of activator.

Absence of vitamin C from the diet of guinea pigs thus causes severe changes in the clotting system of the blood as the result of a decrease in the number of platelets [1], a decrease in the thromboplastic activity of the blood due to a decrease in the change of the platelet thrombokinase, a decrease in adhesiveness of the platelets and a decrease in the activity of factor XIII [2]. In the beginning these changes are accompanied by an increase in fibrinolysin activity.

The increase in the fibrinolytic activity of the blood was caused by the passage of activators of fibrinolysis from the tissues into the blood. On the first days this was evidently the result of a disturbance of the permeability of the blood vessels, for the addition of vitamin P alone to the diet prevented this elevation of fibrinolytic activity. However, later the protective action of vitamin P was no longer sufficient. The process of tissue changes reached a high level and led to the appearance of large quantities of tissue activator in the blood stream. As a result of changes in the state of the liver function which are observed in scurvy, the elimination of the activator was probably disturbed.

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