BLOOD CLOTTING IN VITAMIN C DEFICIENCY II.

PROTHROMBIN AND THROMBOTROPIN CONCENTRATION CHANGES AND THE REASONS FOR THE REDUCED BLOOD CLOTTING IN VITAMIN C DEFICIENCY IN GUINEA PIGS

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At present, nothing is known of the cause of the bleeding and petechial hemorrhages occurring in scurvy. The general opinion [5, 8, 12] is that the hemorrhage is due to an increased permeability of the capillary walls. Others [14, 15] consider that lack of vitamin C causes a disturbance of the blood clotting mechanism and that there is a fall in the amount of prothrombin which slows the clotting process. However, this has frequently been denied [16].

In previous experiments [1] we have shown that when guinea pigs are fed on a vitamin C deficient diet, besides the vitamin C deficiency, there are also blood changes in which the blood clotting rate is 30–40% below normal. It may be restored by daily injections of 2.5 mg of ascorbic acid. It has been shown that the effect on blood clotting is specific.

In the present work, a study has been made of the reduced clotting power of the blood in vitamin C deficiency, and prothrombin and thrombotropin have also been investigated.

METHOD

The experiments were carried out on guinea pigs weighing 300–370 g maintained on a vitamin C deficient diet consisting of rye straw, oats, oatmeal and water. After 12–15 days there was a fall in weight, and the animals died after 18–22 days. Post mortem examination revealed hemorrhages in the knee joints and ribs and the teeth were easily removed. Control animals on the same diet receiving daily injections of vitamin C gained weight and developed no symptoms. Samples of venous blood were collected from the jugular vein before, and at set times after being put on a vitamin C deficient diet, and changes in the concentration of the thrombogenic components, and in clotting times were determined.

The blood-clotting power was measured by the method of B. A. Kudryashov and P. D. Ulitina [7] as modified by us for guinea pigs [1]. Prothrombin concentration was measured by the method of B. A. Kudryashov, P. D. Ulitina and A. A. Pugacheva [6], and thrombotropin by B. A. Kudryashov's method [5].

Because the above methods were worked out on blood and thrombokinase taken from white rats, the clotting time of 80 normal guinea pigs was determined for different prothrombin and thrombotropin concentrations. The latter were varied by diluting the plasma with physiological saline. The thrombokinase was prepared from guinea pig brain tissue by a method described previously [6]. The results are shown in Fig. 1,a,b. The activity of the prothrombokinase of the blood platelets was determined by a method described by T.M. Kalishevskaya in which the platelets are separated by fractional centrifugation, and the prothrombokinase extracted with cold physiological saline. When activated by plasma thrombotropin active thrombokinase is formed which in the presence of $CaCl_2$ causes the plasma of normal animals to clot in 12–13 sec. The blood-platelet count was made by Fonio's method.

RESULTS

The results in Table 1 show that 13–15 days after the start of the experiment besides a fall in the clotting rate there is also a reduction in prothrombin and thrombotropin concentrations. There is less change in these concentrations than in the clotting rate, and the thrombotropin level falls by 38–35%. This result could hardly account for the fall in blood clotting rate of 62–61% (experiments 14 and 22).

Knowing that the blood clotting rate depends on the concentration both of thrombotropin and of prothrombokinase or platelet factor III [7], we have compared the levels of prothrombokinase in the blood platelets of experimental and control animals.

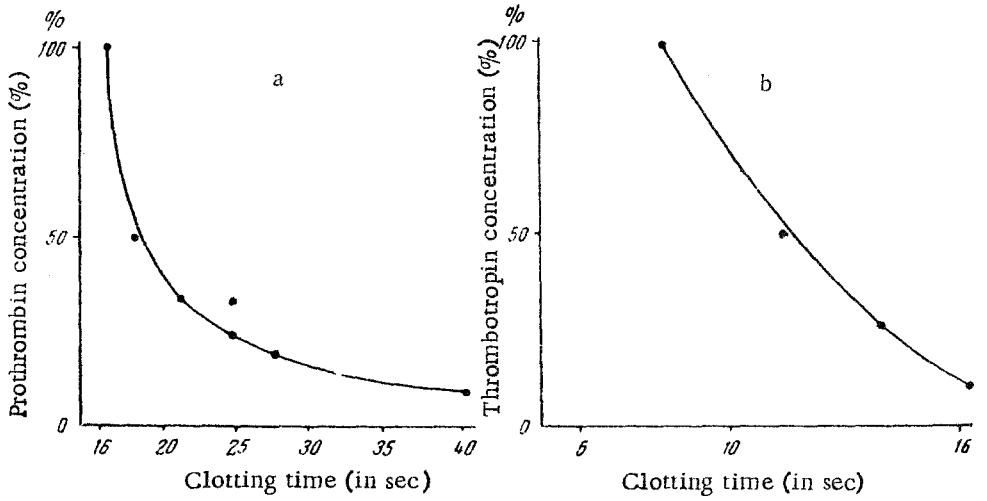

Fig. 1 Clotting time of normal guinea pig blood plasma. As related to (a) prothrombin concentration, and (b) thrombotropin concentration.

TABLE 1. Change in the Concentration of Prothrombin, Thrombotropin, and Clotting Rate in Vitamin C Deficient Guinea Pigs

No. of Experiment	Group of animals	No. of animals	Mean concentration in %: before experiment			after 13 -- 15 days			after 20 -- 21 days		
			prothrombin	thrombotropin	blood clotting rate	prothrombin	thrombotropin	blood clotting rate	prothrombin	thrombotropin	blood clotting rate
10	Experimental	5	91	92	100	61	65	58	67	75	21
	Control	3	90	90	100	84	96	90	100	100	98
14	Experimental	3	92	100	100	59	62	39	76[1]		44[1]
	Control	2	88	100	100	75	80	86	100		93
22	Experimental	5	88		93	69		38	58		38
	Control	5	90		95	87		100	100		100
Mean	Experimental	13	89	95	97	62	64	50	68	75	32
	Control	10	84	94	97	84	86	95	100	100	97

[1] Results from the one surviving animal.

The results of these experiments, given in Table 2 and Fig. 2, show that prothrombokinase activity is greatly reduced in the experimental group. After activating the platelet extract with thrombotropin from the blood of normal animals, in the presence of calcium chloride the active thrombokinase formed clots of normal plasma in 24 – 25 sec, i.e. it takes 10 – 11 sec longer than does thrombokinase obtained from normal platelets. To determine the difference in the activity (in sec), an activated platelet extract from normal animals was diluted in physiological saline until the activity was the same as that of the control group. It was found that a three- to fourfold dilution was required.

Figure 2 shows clearly the relationship between blood-clotting rate and the activity of the thrombokinase from the platelet extract. For 19 days, while the daily injections of ascorbic acid were given, both quantities remained at the normal level, but when the injection ceased there was at first a drop in blood clotting rate, and then a reduction in the activity of the thrombokinase obtained after activation of the platelet extracts of prothrombokinase by thrombotropin.

TABLE 2. Changes in Blood Clotting Rate and Thrombokinase Activity of the Blood Platelets in Vitamin C Deficient Guinea Pigs

No. of Experiment	Group of animals	No. of animals in group	Before the experiment				After 15 days			
			blood clotting rate (%)	clotting time of control plasma with calcium added			blood clotting rate (%)	clotting time of control plasma with added calcium		
					+ platelet extract	+ activated extract			+ extract of platelet	+ activated extract
21	Experimental. . .	6	98	59	43	14	53	55	39	25
	Control . .	6	98	58	45	14	92	55	38	14
22	Experimental. . .	5	93	55	39	14	38	52	33	24,5
	Control . . .	5	95	54	36	13	100	53	36	12

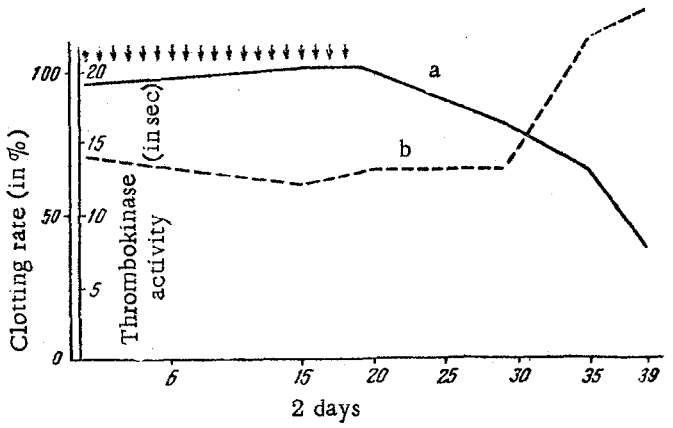

Fig. 2. Changes in blood clotting rate and prothrombokinase activity of the platelets in guinea pigs as affected by vitamin C. a – Clotting rate (in %); b – activity of thrombokinase obtained from platelets. ( ) shows moment of parenteral injection of 2.5 mg of ascorbic acid.1) Clotting rate (in %); 2) 2 days; 3) thrombokinase activity (in sec).

It remains to be found whether the reason for the reduction in the prothrombokinase of the vitamin C deficient animals was due to a fall in the number of thrombocytes, or whether there was a qualitative change with a fall in their prothrombokinase content.

It can be seen from Table 3 that the number of platelets is reduced in vitamin C deficiency, and that this fall results in a reduced amount of prothrombokinase in the blood stream.

The results of these experiments show that one of the principal reasons for hemorrhages in vitamin C deficiency is lack of thrombokinase, which reduces the clotting power of the blood. Thrombokinase activity may be reduced on account of a disturbance in the synthesis of thrombotropin, or by lack of prothrombokinase in the platelets. Because the reduction in the concentration of thrombotropin is comparatively small, the fall in the thrombokinase activity is probably mainly due to a reduction in the amount of prothrombin, which in turn is partly caused by the considerable reduction in the number of platelets. Our results have been confirmed by others [3, 10, 14, 15]. There is also evidence [2] that ascorbic acid injections increase the number of platelets. However, the reduction in the activity in the thrombokinase obtained from the platelets of the experimental animals cannot be ascribed to the reduction in the number of the platelets themselves. The thrombokinase activity is reduced by a factor of at least three to four, while the number of the platelets is reduced by only a factor of two. It is probable that besides reducing platelet numbers, vitamin C deficiency also causes qualitative changes, and a reduction in the amount of thrombokinase per platelet.

The reduction in the prothrombin and thrombotropin levels is evidently the effect of vitamin C deficiency interfering with the synthesis of the substances. Both proteins are synthesized by liver cells. Ascorbic acid exerts a marked influence on the power of the liver to form protein [10]. In vitamin C deficient animals there is a marked fall in the protein concentration of the plasma, as well as a change in the relative amounts of proteins [14]. McCraw [14] found a reduction of approximately 50% in prothrombin concentration and attributed it to secondary vitamin K deficiency following lack of vitamin C. This explanation is well founded, because in the absence of ascorbic acid there is a complete absence of certain bacteria from the gut, including certain strains of Bacillus coli and B. paratyphus which are probably concerned in the synthesis of vitamin K. Probably the reduction in prothrombin and thrombotropin is not only caused by interference with their synthesis in the liver, but is also a secondary effect due to vitamin K deficiency.

TABLE 3. Change in the Blood Clotting Rate, Thrombokinase Activity, and No. of Blood Platelets in Vitamin C Deficient Guinea Pigs

No. of experiment	No. of animals	Before experiment			After seventeen days		
		blood clotting rate (in %)	mean thrombokinase activity (in sec)	no. of platelets per ml of blood	blood clotting rate (in %)	mean thrombokinase activity (in sec)	no. of platelets per ml of blood
22	4	100	13	487 931	61	23	235 640

SUMMARY

Vitamin C deficiency in guinea pigs causes a disturbance of the blood-clotting mechanism due to reduction of prothrombin, thrombin, and thrombokinase concentrations. The decreased amount of thrombotropin and the reduced thrombokinase content of the platelets reduce the amount of thrombokinase with the result that the clotting time is increased. The reduction in the amount of prothrombokinase is due to both a thrombocytopenia and changes in the platelets.

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