THE MECHANISM OF CHANGES IN THE THROMBOPLASTIC ACTIVITY OF THE BLOOD IN WHITE RATS UNDER THE INFLUENCE OF LARGE DOSES OF VITAMIN C AND B_{12}

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In an earlier publication [5] B. A. Kudryashov and P. D. Ulitina have shown that the thromboplastic activity of the human and animal blood depends on an adequate source of prothrombokinase (3rd platelet factor) and on the concentration of thrombotropin in the plasma: this latter factor is necessary for the activation of prothrombokinase into thrombokinase. In some pathological conditions which cause a decrease in the number of platelets circulating in the blood or cause changes in the functional state of the platelets (e.g. in case of radiation sickness), as well as

TABLE 1. Changes in the Thromboplastic Activity of the Blood in White Rats After Injection of Vitamin B_{12} in Doses of 7.5 μg for 5 Days.

Group of animals	Number of animals	Before the injection of vitamin B_{12}		5 days after the injection of vitamin B_{12}		
		Thromboplastic activity (in %)	Activity of the activated platelet extract (in sec)	Thromboplastic activity (in %)	Activity of the activated platelet extract (in sec)	Activity of the activated platelet extract, diluted 4 times (in sec)
Experimental group...	26	100	12.8	150	9	14
Control group.....	24	100	12.9	98	14	–

TABLE 2. Changes in the Thromboplastic Activity, the Platelet Prothrombokinase Activity, the Red Cell and the Platelet Count After Injection of Vitamin B_{12} in Doses of 7.5μg for 11 Days.

Group of animals	Before the injection of vitamin B_{12}					After the injection of vitamin B_{12}									
						After 5 days				After 10 days		After 11 days			
	Number of animals	Thromboplastic activity (in %)	Number of red cells (in millions)	Number of platelets	Thrombokinase activity (in sec)	Thromboplastic activity (in %)	Number of red cells (in millions)	Number of platelets	Thrombokinase activity (in sec)	Thromboplastic activity (in %)	Thrombokinase activity (in sec)	Thromboplastic activity (in %)	Thrombokinase activity (in sec)	Number of red cells (in millions)	Number of platelets
Experimental group	5	100	7,943	295 718	12,8	145	9,45	485 440	8	84	16	69	16	8,63	269 463
Control group	5	100	7,818	301 397	12,9	100	9,078	252 423	14	95	13	97	14	7,806	310 823

TABLE 3. Changes in the Thromboplastic Activity of the Blood and in the Platelet Prothrombokinase Activity in Rats After the Injection of Ascorbic Acid in a Dose of 7 mg per 100 g Body Weight.

Group of animals	Number of animals	Before the injection of ascorbic acid		24 hours after the injection of ascorbic acid	
		Thromboplastic activity (in %)	Activity of the activated patelet extract (in sec)	Thromboplastic activity (in %)	Activity of the activated patelet extract (in sec)
Experimental group	28	100	12.8	51	19.7
Control group	27	100	13.1	100	13.9

TABLE 4. Changes in the Thromboplastic Activity, in the Platelet Prothrombokinase Activity, in the Red Cell and in the Platelet Count Caused by a Single Injection of Ascorbic Acid in a Dose of 7 mg per 100 g Body Weight.

No. of experiment	Group of animals	Number of animals	Before the injection of ascorbic acid				24 hours after the injection of ascorbic acid			
			Thromboplastic activity (in %)	Activity of activated platelet extract (in sec)	Number of red cells (in millions)	Number of platelets	Thromboplastic activity (in %)	Activity of activated platelet extract (in sec)	Number of red cells (in millions)	Number of platelets
38	Experimental group. . . .	6	100	12.8	8.81	330 700	51	16.5	6.71	240 264
	Control group.	6	100	13.1	8.01	312 286	96	11.	6.7	245 200
1	Experimental group. . . .	5	—	—	6.63	415 610	—	—	4.94	313 370
	Control group.	4	—	—	6.99	400 470	—	—	5.06	312 400

in those cases in which the blood thrombotropin level decreases (in liver disorders and in case of treatment with anticoagulants) the thromboplastic activity of the blood falls to a considerable degree, and this leads to disorders in the process of blood coagulation, followed by hemorrhagic symptoms [4, 6].

Changes in the thromboplastic activity of the blood may, however, occur in healthy animals under the influence of large doses of certain vitamins; e. g. it has been shown that injection of vitamin B_{12} in a dose of 7.5 μg for 5 consecutive days, leads to a marked increase in the thromboplastic activity of the blood beyond the usual physiological level [1]. A single injection of ascorbic acid in a dose of 7 mg/100 g weight caused in white rats, within one day, a 50% decrease in the thromboplastic activity [2]. The mechanism of these changes is still obscure. The assumption has been voiced, however, that vitamin B_{12} stimulates the hemopoietic function of the bone marrow and in this manner enhances the formation of prothrombokinase by the blood platelets.

In the present paper we describe the results of experiments carried out with the aim of studying the factors responsible for the increase in the thromboplastic activity, observed after injection of vitamin B_{12} and the factors responsible for the fall in the thromboplastic activity, observed after injections of ascorbic acid.

EXPERIMENTAL METHOD

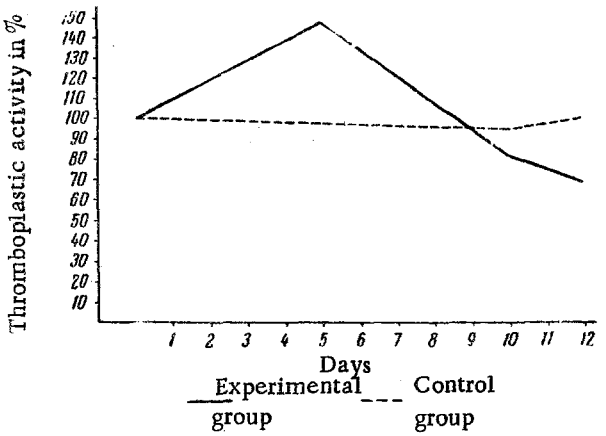
The experiments were carried out on white rats, weighing between 170 and 200 g and kept on a fully adequate normal diet. One group of experimental animals were given intramuscular injections of 7.5 μg vitamin B_{12} from the 1st to the 5th day inclusively; the second group were given the vitamin in similar doses for 11 days. The control animals in the same period received doses of 0.5 ml of physiological saline. In another series of experiments the animals were given a single intramuscular injection of 7 mg ascorbic acid per 100 g body weight.

At certain intervals blood samples were taken from the jugular vein to estimate the thromboplastic activity of the blood, the thrombotropin level, and the activity of the platelet prothrombokinase by the corresponding methods [3, 5]. The activity of the prothrombokinase was assessed on the basis of the time required for the coagulation of oxalated control plasma in the presence of $CaCl_2$ and of an extract of washed platelets, activated by the plasma

thrombotropin. This method is highly sensitive. Changes of 2-3 sec in the coagulation time indicated considerable changes in the activity of the platelet extract. Besides, we counted the number of red cells in Goryaev's chamber and the platelets by the method of Fonio.

EXPERIMENTAL RESULTS

The data set forth in Table 1 show that the increase in the thromboplastic activity of the blood, caused by the injection of large doses of vitamin B_{12}, is accompanied by a considerable increase in the activity of the platelet prothrombokinase (3rd factor). The activated extract of the platelets from the control rats causes coagulation of the control plasma within 14 sec, whereas the same activated extract of platelets from the experimental animals coagulates the control plasma within 9 sec. If one dilutes this extract 4 times with normal saline its activity becomes equal to the activity of the platelets from the control animals. This increased activity of the platelet extract may be caused by an increase in the number of platelets, circulating in the blood, and apparently also by an increase in the prothrombokinase content of the platelets themselves.



Changes in the thromboplastic activity of the blood after prolonged injection of vitamin B_{12} (by intramuscular injection in doses of 7.5 μg per day).

Platelet counts showed that after the administration of vitamin B_{12} for 5 days the number of platelets increases by 35 %. This increase is particularly marked if one compares the number of platelets per 1000 red cells in the experimental and in the control animals respectively. In the former the number of platelets increases by 17, whereas in the control animals the number of platelets decreases to a considerable degree.

The figure shows that after prolonged administration of vitamin B_{12} the stimulating effect upon the formation of prothrombokinase subsides, and the thromboplastic activity of the blood decreases: this is caused by the fall in the number of platelets and the consequent lack of platelet prothrombokinase (Table 2).

Unpublished findings, obtained in our laboratory by O. V. Kiseleva, have shown that injection of vitamin B_{12} causes no changes in the plasma thrombotropin level. Our experiments thus showed the relative dependence of the thromboplastic activity on both the number of platelets and – to an even higher degree – on the prothrombokinase content of the platelets.

Our experiments concerning the injection of vitamin C in large doses suggest that the consequent changes in the thromboplastic activity of the blood are based on a similar mechanism.

The data set forth in Table 3 show that the decrease in the thromboplastic activity of the blood by 51%, caused by a single injection of ascorbic acid is accompanied by a considerable fall in the prothrombokinase content of the platelets (3rd factor).

This decrease can, however, not be explained with a fall in the number of platelets, as a similar decrease, caused by the collection of relatively large blood samples for the analysis, can be observed in both the experimental and the control animals (Table 4).

As injections of ascorbic acid cause no changes in the plasma thrombotropin activity, the fall in the thromboplastic activity of the blood in the platelet prothrombokinase level can be explained with qualitative changes in the platelets: namely a fall in their prothrombokinase content.

SUMMARY

Experiments were staged on healthy white rats kept on a natural standard diet. As shown, a rise of the earlier recorded blood thromboplastic activity upon the administration of 7.5 microgram of vitamin B_{12} for a period of 5 days was due to a rise of blood platelet count by 35%, mainly because of a 4-fold increase of prothrombokinase therein (3rd factor). With further vitamin B_{12} administration in the same doses for a period of 11-12 days there occurs a gradual reduction of the thromboplastic activity down to 65% with a simultaneous decrease of the platelet count and a reduction of the platelet prothrombokinase activity. Diminution of 7 mg of ascorbic acid per 100 g of body weight is due to the quantitative changes in the blood platelets, namely to the reduced prothrombokinase content therein.

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