EFFECT OF HYPERCOAGULATION ON LIFE SPAN OF Cr^{51}-LABELED ERYTHROCYTES

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Besides the activating effect of erythrocytes on thromboplastin formation, the reverse effect is also observed: repeated activation of the internal blood clotting system in rabbits shortens the life span of the erythrocytes.

With the discovery in experiments in vitro of a definite effect of intact erythrocytes on thromboplastin formation [1-3], the possibility of an opposite process must be considered: does activation (mobilization) of the thromboplastin factor of the erythrocytes (a lipoprotein connected with the cell membrane) lead to secondary changes in the cell.

The object of the present investigation was to study in vivo the effect of hypercoagulation produced by activation of the intrinsic coagulatory system on the life span of Cr^{51}-labeled erythrocytes.

EXPERIMENTAL METHOD

Male chinchilla rabbits weighing 2-3 kg were used in the investigation.

The intrinsic coagulatory system was activated by intravenous injection of a suspension of diatomite or activated plasma [12, 13]. Changes in the blood clotting system developing as a result of this procedure are described elsewhere. Three injections, each of 2 ml/kg of a diatomite suspension, were given at intervals of 1 day. Activated plasma was first injected in a dose of 2 ml/kg 4 times on alternate days. Later, the dose of plasma injected on each occasion was increased to 9 ml/kg (each dose was given in three portions at intervals of 1 min). A triple injection of this type was given three times on alternate days. In control experiments isotonic NaCl solution or intact plasma was injected [12].

Autologous erythrocytes were labeled in vitro. Blood was obtained by cardiac puncture (3 ml/kg) and stabilized with 5% acid sodium citrate solution (9:1). After centrifugation of the blood (1000 rpm for 15 min) the plasma was removed, and a sterile solution of sodium chromate ($Na_2Cr^{51}O_4$) was added to the residue of erythrocytes so the activity introduced with the labeled cells did not exceed 50 μCi/kg [4]. After exposure to the isotope for 1 h at room temperature, the erythrocytes were washed three times with isotonic NaCl solution, after which they were injected as a suspension into the marginal vein of the ear. The mean level of incorporation of Cr^{51} into the erythrocytes was 85%. The first blood sample was obtained after 72 h and its activity was taken as 100% [11], and subsequent determinations were made at intervals of 2-3 days for 27-33 days. Constancy of the conditions of measurement of activity was ensured by addition of distilled water to the blood samples; volume of hemolysate 1 ml.

Activity of Cr^{51} γ rays was recorded by a scintillation counter, consisting of a standard USD-1 detector having a crystal with a well. The radioactivity of the samples was measured as a rule on the day after they were obtained. A correction for decay and possible fluctuations in the efficiency of recording was introduced by measuring the radioactivity of a control Cr^{51} source. The experimental background

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TABLE 1. Life Span of Erythrocytes and Daily Loss of Cr^{51} in Intact Rabbits after Injection of Activated Plasma (2 ml/kg), Intact Plasma (9 ml/kg), and Isotonic NaCl Solution (M±m)

Conditions	Whole blood		1 ml packed erythrocytes	
	$T_{1/2}$	n/day %	$T_{1/2}$	n/day %
Intact rabbits (n = 10).	12.5±0.69	5.65±0.31	13.37±0.68	5.35±0.31
Injection of 2 ml/kg activated plasma (n = 6).	12.1±0.66	5.80±0.35	12.4 ±0.63	5.70±0.31
Injection of 9 ml/kg intact plasma (n = 5).	12.0±0.80	5.86±0.38	11.5±0.56	6.68±0.42
Injection of isotonic NaCl solution (n = 3)	11.0±0.65	6.36±0.57	11.7±1.04	6.03±0.56

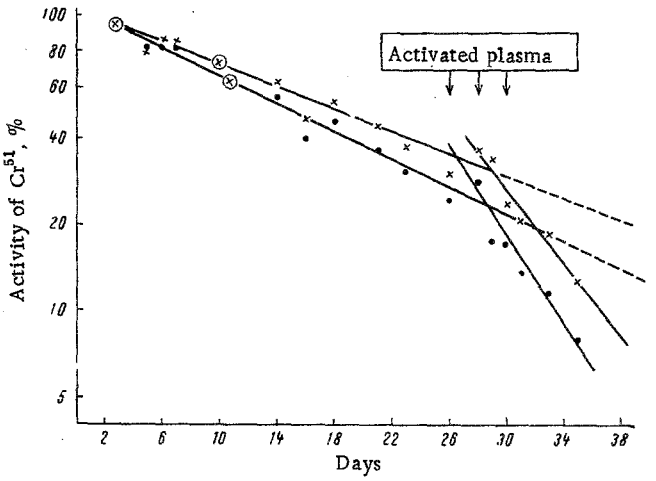

Fig. 1. Shortening of life span of labeled erythrocytes after injection of activated plasma: 9 ml/kg (experiment No. 9, April 22 – May 29, 1967). •–• activity for whole blood; x–x activity per ml packed erythrocytes; ⊕ activity for whole blood and per ml packed erythrocytes coincides.

averaged 250 pulses/min. The statistical error of determination did not exceed 5%, and was usually 1-2%. The criteria of life span of the erythrocytes were the biological half-elimination time ($T_{1/2}$) and the daily loss of Cr^{51} in percent (n/day %):

$$n/\text{day } \% = \left(1 - e^{\frac{-0.693}{T_{1/2}}}\right) \times 100 \quad (5).$$

The indices were expressed separately for 1 ml whole blood and 1 ml packed erythrocytes.

Hypercoagulation was produced after determination of $T_{1/2}$ or in the period from the 3rd to the 7th day after labeling the erythrocytes. In the latter case, the results were compared with the life span of erythrocytes of intact rabbits.

The number of erythrocytes per mm^3 blood, the hemoglobin concentration, hematocrit index, and reticulocyte count were also determined.

EXPERIMENTAL RESULTS

The life span of labeled erythrocytes ($T_{1/2}$) in 10 intact rabbits was 12.5 days when determined for whole blood and 13.4 days for 1 ml packed erythrocytes (mean 13 days), in agreement with data in the literature [6, 11]. Four injections of activated plasma (2 ml/kg) in 6 experiments had no effect on the life span of the erythrocytes (Table 1).

TABLE 2. Shortening of Life Span of Erythrocytes after Injection of Diatomite and Activated Plasma

Expt.	Intact rabbits (n = 10)	Whole blood				After injection			
		whole blood		1 ml packed erythrocytes		whole blood		1 ml packed erythrocytes	
		$T_{1/2}$	n/day %	$T_{1/2}$	n/day %	$T_{1/2}$	n/day %	$T_{1/2}$	n/day %
8	Activated plasma	9,8	7,1	11,9	5,8	5,7	12,1	6,5	10,7
9	Activated plasma	12,3	5,6	15,6	4,4	3,8	16,7	4,8	14,4
11*	Diatomite	11,8	5,9	11,8	5,9	3,8	16,7	4,2	16,5
12*	"	12,7	5,5	12,0	5,8	4,9	14,1	4,9	14,1
13*	"	13,2	5,3	13,2	5,3	2,3	25,4	2,3	25,4
22†	"					9,0	7,7	8,2	8,5

*Diatomite injected from 27th day after labeling erythrocytes; before this, in period from 3rd to 10th day after labeling, activated plasma injected (2 ml/kg) without shortening $T_{1/2}$.
†Diatomite injected from 5th day after labeling.

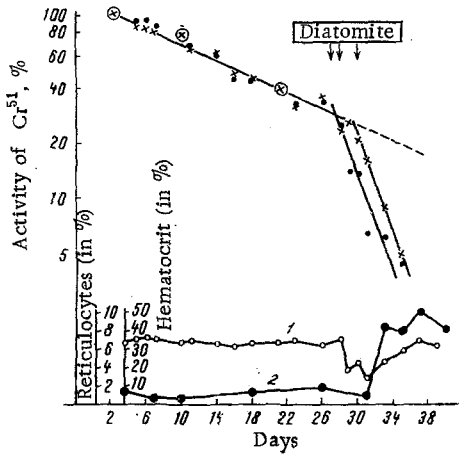

Fig. 2. Shortening of life span of Cr^{51}-labeled erythrocytes and changes in peripheral blood indices after injection of 2 ml/kg of diatomite suspension (experiment No. 13, April 22 – May 29, 1967). Legend as in Fig. 1. 1) Hematocrit index; 2) reticulocytes.

An increase in the quantity of activated plasma to 9 ml/kg led in 2 of the 6 experiments to a definite shortening of the life span of the erythrocytes (Fig. 1) when determined both for whole blood and for 1 ml packed erythrocytes (Table 2).

Injection of intact plasma in the same volume into 5 rabbits had no effect on the life span of the labeled cells (Table 1). Injection of diatomite (Table 2) had a marked effect on the life span of the erythrocytes. In 4 of the 6 experiments, considerable shortening of the life span was observed (Fig. 2). Injection of isotonic NaCl solution into 3 animals was not accompanied by this effect (Table 1). Of 6 animals with increased erythrocyte destruction, 3 developed normochromic anemia, accompanied in two cases by reticulocytosis (4-10%).

Repeated hypercoagulation due to activation of the intrinsic blood coagulation system thus led in some experiments to marked stimulation of hemolysis.

While this experiment was in progress, a group of British investigators [7-10] published their work showing that during hypercoagulation produced by intravenous injection of thrombin and snake venom increased destruction of erythrocytes also was observed. They attribute this to mechanical injury to the cells in the microcirculation through the deposition of fibrin.

The possibility that vascular thrombi may appear during hypercoagulation cannot rule out the possible role of a mechanical factor in the pathogenesis of hemolysis. At the same time, mechanical injury cannot be regarded as the sole cause of increased cell destruction, and the writers postulate the role of changes in the membrane of the circulating erythrocytes due to participation of thromboplastin factor in the process of blood clotting.

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