IMPORTANCE OF INTACT ERYTHROCYTES FOR THE ADHESIVENESS OF THE PLATELETS AND FOR FIBRINOLYSIS

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An important role in the process of hemostasis is played by the adhesive function of the platelets. By their adhesion after trauma to form conglomerates, the platelets rapidly seal the wound surface and create favorable conditions for thrombus formation. With an increased tendency of the blood to form thrombi, the adhesiveness of the platelets is also increased [2-5].

Various authors [4,5,8] consider that when the number of adhesive platelets is increased, the formation of a blood clot is accelerated.

Meanwhile, several investigators [6,11,12] have shown that the adhesiveness of the platelets is dependent on the erythrocyte count; the higher the number of red cells in the blood, the more marked the ability of the platelets to stick to the foreign surface. This is associated with the presence of the "adhesiveness factor" in the erythrocytes.

The presence of intact erythrocytes is known to increase the time taken by a blood clot to dissolve [6,9]. The reason for this, however, is not clear.

The object of this investigation was to determine whether intact blood cells have the property of secreting an adhesive factor directly into the plasma, and whether the action of the erythrocytes on fibrinolysis is not associated with the liberation of antifibrinolysin into the plasma.

EXPERIMENTAL METHOD

Blood was poured in a volume of 4.5 ml into each of two silicone-coated tubes and mixed at once with glucose-citrate mixture (0.5 ml). One of the tubes was placed for 1 or 2 h in a water bath (37°) or in a refrigerator (4°). The other tube of blood was centrifuged for 10 min at 1500 r.p.m., after which the plasma was aspirated from it and placed in identical conditions. After the given length of time had elapsed, the blood was centrifuged and the plasma drawn off. Hence, the plasma in both tubes contained approximately the same number of platelets.

Next the plasma (but not the blood, as in the classical method of Moolten and co-workers [13]) was filtered through glass wool and the number of adhesive platelets and the percentage of adhesiveness were determined. For this purpose, the number of platelets was counted before and after filtration.

The fibrinolytic activity of the plasma was determined by the authors' modification of the method of Kowarzyk and Buluk [10]. To discover the role of the erythrocytes in the process of fibrinolysis, the dissolved residue of the euglobulin fraction of the plasma was treated, in the experimental series with 0.5 ml of a suspension of washed erythrocytes, and in the control series with the same volume of physiological saline or of hemolyzate. The rate of solution of the clot was determined in the usual way.

TABLE 1. Effect of Intact Erythrocytes on Adhesiveness of Platelets

Number of observations	Incubation period (in h)	Substrate tested	Statistical index	Platelets		% adhesiveness
				total	adhesive	
10	1	Plasma incubated without erythrocytes (37°)	M	497,990	109,060	21.9
		Plasma incubated with erythrocytes (37°)	M	497,420	206,480	41.7
			m±	11,878	11,427	1.8
			P	0.5	< 0.001	<0.001
10	2	Plasma incubated without erythrocytes (37°)	M	514,920	106,590	20.7
		Plasma incubated with erythrocytes (37°)	M	480,390	221,000	46.0
			m±	16,674	14,772	2.3
			P	< 0.05	< 0.001	<0.001
10	1	Plasma incubated without erythrocytes (4°)	M	512,190	120,160	23.4
		Plasma incubated with erythrocytes (4°)	M	500,960	198,980	39.7
			m±	7,317	8,930	1.8
			P	0.5	< 0.001	<0.001

Note: Statistical analysis was carried out for a small number of related observations.

TABLE 2. Effect of Intact Erythrocytes on Rate of Solution of Blood Clot (in min)

Number of observations	Statistical index	Control 1 (physiological saline added)	Control 2 (hemolyzate added)	Experiment (intact erythrocytes added)
10	M	205	165	762
	m		12	50
	P		< 0.05	< 0.001

TABLE 3. Rate of Solution of a Clot Formed from Plasma Preliminarily Incubated with Erythrocytes (in min)

Number of observations	Statistical index	Duration of incubation (in min)					
		30 Control	(37°) Experiment	60 Control	(37°) Experiment	60 Control	(4°) Experiment
15	M	228	195	241	213	245	236
	m ±		2.7		2.8		5.8
	P		< 0.05		< 0.05		> 0.05

To ascertain whether intact erythrocytes can secrete an antifibrinolysin into the plasma, the rate of solution of the euglobulin fraction was determined after preliminary incubation of the erythrocytes with plasma (4 and 37°) for 30-60 min.

EXPERIMENTAL RESULTS

Preliminary incubation of plasma with erythrocytes led to a considerable increase in the number of adhesive platelets and in the percentage of adhesiveness (Table 1).

The results of the observations were approximately the same when incubation was carried out at 37° and at 4°. When the erythrocyte count was normal, the adhesive platelets were fairly numerous. It seems that the erythrocytes secrete the adhesiveness factor into the plasma while circulating.

The presence of intact erythrocytes considerably increased the time required for solution of a blood clot (Table 2). In some experiments, hemolysis was ill defined, yet as the results in Table 2 show, the hemolyzate not only did not retard, but slightly accelerated solution of the clot.

To examine the problem of the mechanism of the antifibrinolytic activity of intact erythrocytes, observations were made in which the effect of incubation of erythrocytes with plasma (at 37° and 4°) on the rate of solution of the blood clot was studied (Table 3).

Rather unexpected results were obtained. If the plasma was first incubated with erythrocytes, the time for solution of a formed clot was shortened slightly. It may be supposed that during incubation the erythrocytes adsorbed on to their surface an antifibrinolysin, so that as a result the fibrinolysin exhibited a stronger action. Adsorption took place more intensively at 37°, leading to a more rapid solution of the clot in these experiments.

The data described confirm the important role of intact erythrocytes in the process of hemostasis. Erythrocytes contain an adhesiveness factor, causing the platelets to adhere more intensively to the injured area of the blood vessels [11]. Adenosinediphosphate is a compound known to act in this manner [12]. The present experiments indicate that adhesiveness factor may enter the plasma directly from the erythrocytes. This factor is of great importance for the arrest of bleeding, and it must be taken into account in clinical practice during blood transfusion.

Erythrocytes greatly prolong the time required for solution of a formed clot. Bayerle and Kammenhuber [9] were the first to show that erythrocytes contain an antifibrinolytic factor. Their findings were subsequently confirmed by B. I. Kuznik [7] and by V. P. Baluda and I. B. Tsynkalovskii [1]. The antifibrinolysin is evidently located directly on the surface of the erythrocytes, which explains their action on the rate of solution of the blood clot. It follows from the results of the present experiments that antifibrinolysin is adsorbed by the erythrocytes from the plasma.

The erythrocytes also contain a fibrinolytic factor. For instance, if hemolyzate is added, the clot liquefaction time is shortened. It has been suggested that this last factor is a plasminogen activator [14].

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