THE ANTIHEPARIN ACTIVITY OF HEMOLYZED HUMAN RED CELLS

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Hemolyzed red cells, like platelets, have been shown to possess heparin-neutralizing properties [1, 4, 5, 6, 8, 11, 12], although the mechanism of this action has not yet been explained. Quick and Hickey [11] have pointed out that a hemolyzate can increase the prothrombin consumption only up to a limiting concentration of heparin. They concluded from their experiments that the antiheparin action of hemolyzed red cells is associated with increased thrombin formation under the influence of the thromboplastic factor of the red blood cells.

A different view is taken by V. P. Baluda and N. A. Gorbunova [1], who found that after the addition of hemolyzate the thrombin time of heparinized, deprothrombinized plasma was considerable shortened. The absence of prothrombin and calcium in these experiments made it impossible for heparin to be combined on account of thrombin formation. Although these investigations demonstrated that hemolyzed red cells contain a heparin inhibitor, the possibility remains that this compound may be the thromboplastic factor of the red blood cells.

The object of the present investigation was to examine the mechanism of the antiheparin action of hemolyzed red cells.

EXPERIMENTAL METHOD

The effect of hemolyzed human red cells on the recalcification time, the thrombin time, and the prothrombin consumption of heparinized plasma was studied.

Hemolyzates were prepared as follows. Blood taken from the cubital vein was mixed with sodium oxalate (1.34% solution) in a ratio of 4:1, and immediately centrifuged at 800-1000 rpm for 5-10 min. The plasma was drawn off and the red cells washed five times with physiological saline, and precipitated each time by centrifugation. The resulting packed red cells were frozen at −25 to −30°. After thawing, the resulting hemolyzate was used for the experiments.

The recalcification time was investigated by the method of Bergerhof and Roka [7], the heparin tolerance by Poller's method [10], and the prothrombin consumption was determined by a slight modification of the method described by M. A. Kotovshchikova and Z. D. Fedorova [3]. In the experiments hemolyzate was added to the plasma in proportions of 1:1 and 1:2, and in the control determinations an equal volume of physiological saline was used.

The experimental results were analyzed by the method of variational statistics, and in the present report we give only those results the significance of which is not in doubt.

EXPERIMENTAL RESULTS

Our own results and the experimental findings of other workers [1, 8, 12] showed that the hemolyzed red cells significantly shortened the thrombin time of ordinary and heparinized plasma. This action is only partly explained by neutralization of heparin. Whole hemolyzates shortened the thrombin time to a greater degree than toluidine blue, a specific neutralizer of heparin. For instance, toluidine blue shortened the thrombin time from 29.2 to 19.3 sec, and hemolyzate to 13.4 sec ($P < 0.001$).

It is difficult at first glance to account for these facts. Meanwhile, it is known that platelets also shorten the thrombin time to a greater degree than toluidine blue. This action is explained by the presence of a compound in the platelets which is capable of accelerating the change from fibrinogen into fibrin. This substance, which is called platelet factor 2, acts independently of the antiheparin substance, but requires the presence of thrombin.

TABLE 1. Effect of Hemolyzates on the Thrombin Time (in seconds) of Heparinized Plasma

Ingredients added to plasma	Initial value	Dose of heparin (in units/ml)						
		0,25	0,5	0,75	1,0	1,5	2,0	3,0
Physiological saline	20	46	110	360	900	900	900	900
Hemolyzate (1:1)	10	10	10	13	16	—	80	165
Hemolyzate (2:1)	—	—	—	—	—	—	11	61
Physiological saline	25	60	320	900	900	900	900	900
Hemolyzate (1:1)	10	10	10	12	15	50	180	—
Hemolyzate (2:1)	—	—	—	—	12	17	55	
Physiological saline	27	85	432	900	900	900	900	
Hemolyzate (1:1)	11	10	11	14	15	43	75	
Hemolyzate (2:1)	—	—	—	—	—	15	33	

TABLE 2. Effect of Hemolyzates on the Recalcification Time and Prothrombin Consumption of Heparinized Plasma

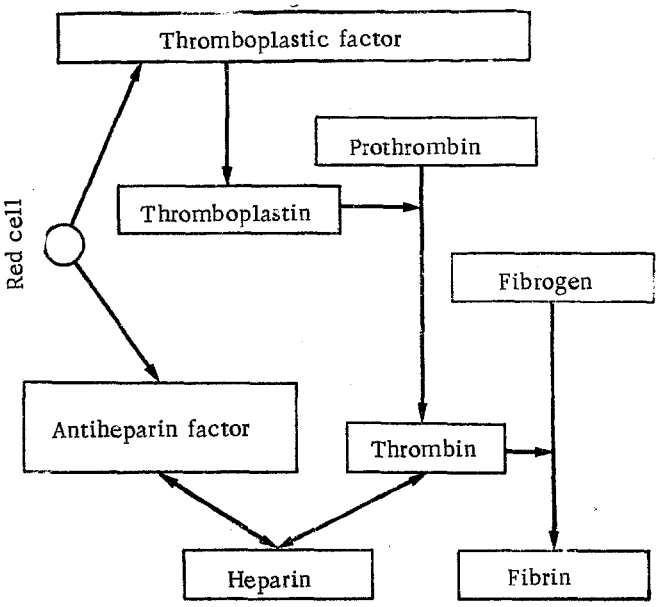
Experiment No.	Criterion studied	Ratio of hemolyzate to plasma	Initial value	Dose of heparin (in units/ml)				
				0,5	1,0	1,5	2,0	3,0
1	Recalcification time (in seconds)							
	Control		115	165	230	310	480	720
	Experiment	1:1	80	110	130	170	200	240
	Experiment	2:1	90	100	101	110	151	179
	Prothrombin consumption (in seconds)							
	Control		82	80	75	67	33	28
	Experiment	1:1	105	105	101	94	83	40
	Experiment	2:1	90	90	91	92	91	85
2	Recalcification time (in seconds)							
	Control		135	180	301	428	540	812
	Experiment	1:1	105	125	145	160	221	334
	Experiment	2:1	110	115	116	116	170	221
	Prothrombin consumption (in seconds)							
	Control		120	100	45	40	40	—
	Experiment	1:1	170	170	115	105	70	—
	Experiment	2:1	152	152	156	150	125	—

Note. In experiment No. 1 the prothrombin consumption was determined after 1 h, and in experiment No. 2, after 2 h.

Our results demonstrate that red cells contain a compound resembling platelet factor 2. Hemolyzate without thrombin does not lead to the coagulation of citrated or oxalated plasma.

The action of hemolyzate was weakened if the plasma was heparinized to a considerable degree. If, however, the dose of hemolyzate were increased without changing the amounts of plasma and heparin, the thrombin time was shortened much more. This confirmed the view that the heparin inhibitor is actually in the red cells, and that its action (at least up to a certain level of heparinization) is not connected with the formation of compounds arising in the course of blood clotting. Some typical experimental results are shown in Table 1.

These results, however, did not answer the question whether the thromboplastic and antiheparin factors are the same or different substances. To shed light on this aspect a special series of investigations was carried out, in which the effect of different concentrations of hemolyzates was studied on the recalcification time and the prothrombin consumption of heparinized plasma. Some typical results of these experiments are given in Table 2.



Scheme of the process of neutralization of heparin under the influence of hemolyzate

These results suggest that the antiheparin and thromboplastic factors are, in fact, different compounds. This is confirmed by the following findings: 1) only large doses of heparin can lower the prothrombin consumption of the plasma after addition of hemolyzate; 2) an increase in the dose of hemolyzate leads to an increase in the prothrombin consumption of heparinized plasma.

Had heparin inhibitor and thromboplastic factor been the same substance, with small doses of heparin neutralization of thromboplastic factor would have taken place, and this would have been reflected significantly in the prothrombin concentration in the test serum. If, on the other hand, heparin combined only with the thrombin formed in the process of clotting of the blood, an increase in the concentration of hemolyzate would not lead to an increase in the prothrombin consumption of the heparinized plasma. We know that the yield of thrombin is determined by the concentration of its inactive precursor – prothrombin – and is independent of the amount of added activators and, in particular, of thromboplastin [2].

Do the facts we have described mean that the thromboplastic factor of the red cells plays no part in the neutralization of heparin. We believe that this is not so. Under the influence of thromboplastin the yield of thrombin, a powerful antagonist of heparin, is accelerated. Prolongation of the recalcification time after the addition of heparin and hemolyzate in our experiments may be explained by the fact that heparin partially combined with the newly formed thrombin, preventing it from acting on fibrinogen.

By taking the above statements into account, the process of neutralization of heparin under the influence of hemolyzate may be represented by the following scheme (see figure).

In our experiments our attention was drawn to the fact that large doses of hemolyzate had a smaller effect on the recalcification time and the prothrombin consumption of ordinary plasma. Several investigations [10, 13] have shown that besides a thromboplastic factor, red cells also contain an antithromboplastic factor, capable of reducing to some extent the prothrombin consumption. The more hemolyzate was added, the more strongly was the action of the antithromboplastic factor shown, reducing the prothrombin consumption.

The difference between our experimental results and those of Quick may apparently be due to the slightly different experimental conditions. By adding a large volume of hemolyzate to heparinized rabbit's plasma, Quick found no increase in the prothrombin consumption, possibly as a result of the action of antithromboplastic factor.

SUMMARY

As established, the antiheparin and thromboplastic erythrocyte factor are different compounds. However, both substances play an important role in heparin neutralization. Besides, erythrocytes contain a compound accelerating

(together with thrombin) the change of fibrinogen into fibrin. Evidently this substance is analogous to factor 2 of the platelets. A scheme reflecting the participation of disintegrated erythrocytes in heparin neutralization is presented.

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