EFFECT OF ACTIVATED HAGEMAN FACTOR ON THE BLOOD CLOTTING SYSTEM IN VIVO

I. Ya. Ashkinazi and I. K. Klemina

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Activation of Hageman factor by diatomite in rabbits not only accelerates blood (plasma) clotting in a silicone-treated tube, shortens the R and K intervals of the thromboelastogram, and increases prothrombin consumption, but it can also lead to abnormalities of prothrombin consumption. Despite marked activation of the Hageman factor, no increase in fibrinolysis was observed.

On the appearance of activated Hageman factor in the blood stream the process of blood clotting in a silicone-treated tube is accelerated [6, 7, 12, 13]. Meanwhile, the results of investigations of thromboplastin activity are highly conflicting [9, 10, 14]. Studies of fibrinolysis under these conditions have not revealed any increase in its intensity [10], and this cannot be reconciled with the view that fibrinolysis is regularly induced by activated Hageman factor [11].

The object of this investigation was to study the clotting system of the blood after activation of Hageman factor by a silica-containing substance, diatomite, in vivo and after injection of activated plasma.

EXPERIMENTAL

Male chinchilla rabbits weighing 2-3 kg were used in the investigation. A suspension of diatomite (8 mg/kg) in isotonic NaCl solution [13] was allowed to stand at room temperature for 10 min; the supernatant layer was withdrawn and injected into the marginal vein of the ear (2 ml/kg body weight on the course of 20 sec. Activated plasma was obtained from homologous platelet-free plasma after incubation with diatomite in the proportion of 2 mg/ml [13], and intact plasma by excluding all contact between blood (plasma) and the glass, using a silicone-coated vessel for this purpose. The plasma (3 ml/kg) was injected over a period of 10 sec.

The following parameters were determined to estimate the coagulating activity of the blood: 1) the rate of blood clotting by the Lee-White method; 2) the plasma recalcification time; the components of the clotting mixture (0.05 M $CaCl_2$, plasma, isotonic NaCl solution) were taken in equal volumes (0.3-0.5 ml); 3) the thromboelastogram of recalcified plasma (0.3 ml plasma, 0.2 ml 0.05 M $CaCl_2$ solution); the thromboplastic activity of the plasma from the prothrombin consumption [2]; 5) the prothrombin complex [3]; 6) factor V [8]; 7) factor VIII [3]; 8) thrombin activity; 9) free heparin [5]; [10] fibrinogen [1]; 11) platelets; 12) fibrinolytic activity of whole blood [4].

Indices 1, 2, 3, and 4 were determined in parallel tests for contact blood (plasma), conventionally designated as "glass," and blood which had not been in contact with glass – "silicone." The index of contact activation (ICA) was determined in all investigations with parallel tests using glass and silicone plasma (the ratio between corresponding indices in silicone and glass plasma).

In 5 series of experiments the group of indices (except 1, 11, 12) was studied over a period of time after injection of the following substances into rabbits: 1) a suspension of diatomite (2 ml/kg); 2) isotonic

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TABLE 1. Changes in Index of Contact Activation after Injection of Diatomite and Activated Plasma (M± m)

Character of procedure	Time of investigation of blood		Recalcification time, sec	Prothrombin consumption time (in sec)	Thromboelastogram						
					r	*k*	*t*	*S*	*T*	*ma*	*α*
Injection of diatomite	Before procedure	22	3,4±0,40*	0,74±0,03*	2,43±0,22*	1,98±0,5*	1,05±0,06	1,09±0,05	1,22±0,05	1,02±0,03	0,72±0,03*
	After "		1,67±0,17	0,95±0,04	1,16±0,07	1,04±0,12	1,18±0,12	1,14±0,11	1,13±0,10	1,08±0,03	0,99±0,03
Injection of isotonic solution (control)	Before "	10	2,86±0,26	0,67±0,03	2,0±0,13	1,43±0,11	0,93±0,07	0,94±0,07	1,05±0,07	1,03±0,05	0,81±0,04
	After "		3,12±0,20	0,68±0,02	1,71±0,16	1,32±0,20	0,84±0,07	0,85±0,07	0,94±0,06	1,0±0,02	0,87±0,05
Injection of of activated plasma	Before "	12	3,1±0,20*	0,74±0,03	2,26±0,22*	1,40±0,15	1,05±0,13	1,05±0,12	1,11±0,12	1,01±0,11	0,74±0,04*
	After "		1,9±0,07	0,89±0,07	1,32±1,14	1,04±0,14	0,87±0,06	0,79±0,07	0,90±0,06	0,97±0,02	0,94±0,04
Injection of intact plasma (control)	Before "	11	3,24±0,36	0,69±0,05	2,44±0,27	1,71±0,22	1,11±0,10	1,13±0,15	1,26±0,11	1,09±0,06	0,71±0,04
	After "		3,45±0,16	0,67±0,03	2,61±0,19	1,86±0,31	1,0±0,23	1,02±0,22	1,14±0,23	1,0±0,02	0,64±0,05
Withdrawal of blood (control)	Before "	13	3,68±0,25	0,78±0,06	2,06±0,12	1,48±0,12	1,09±0,08	1,09±0,08	1,25±0,08	0,99±0,01	0,74±0,02
	After "		3,42±0,20	0,71±0,05	2,18±0,09	1,42±0,12	0,96±0,10	0,91±0,08	1,05±0,07	1,02±0,02	0,78±0,02

*Differences between mean values of ICA before and after test procedures are statistically significant.

TABLE 2. Comparative Decrease in Activity (M± m) of Factor I, V, VIII of Antithrombin Activity (in percent of initial level) after Injection of Diatomite and Isotonic NaCl Solution

Agent injected	Factor V	Factor VIII	Factor I	Thrombin time
Diatomite	18,2±3,8	26±6,0	36,7±2,1	15,3±1,9
Isotonic saline	4,6±1,3	6,1±1,3	9,0±1,8[1]	8,6±1,2

[1] Index combined for two control series of experiments (II and V).

TABLE 3. Comparative Effect of Diatomite and Isotonic NaCl Solution on Fibrinolysis

Time of testing blood after injection of agent (in min)	Fibrinolysis					
	increased		lowered		unchanged	
	diatomite	isotonic saline	diatomite	isotonic saline	diatomite	isotonic saline
3 min	2	7	8	2	—	1
6 »	3	7	7	3	—	—
12 »	3	4	7	4	—	2
Total . . .	8	18	22	9	—	3

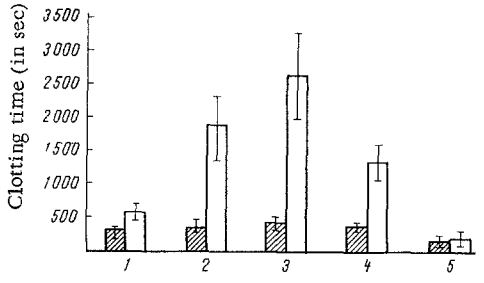

Fig. 1. Comparative rate of blood clotting in glass and silicone-treated tube after injection of diatomite and activated plasma (M±σ). 1) Activated plasma (3 mg/kg, N = 9); 2) intact plasma (3 mg/kg, N = 9); 3) intact rabbits (N = 10); 4) isotonic NaCl solution (2 mg/kg, N = 7); 5) suspension of diatomite (2 mg/kg, N = 11). Unshaded columns denote silicone-treated tube, shaded columns plain glass tube.

NaCl solution (2 ml/kg); 3) activated plasma (3 ml/kg); 4) intact plasma (3 ml/kg), and also after repeated blood sampling. These substances were injected 45-60 min after determination of the initial indices, for which 7 ml blood was required; the transient acceleration of blood clotting due to removal of the blood disappears during this period. Because of differences between the rates of appearance of hypercoagulation and its duration [13], blood was investigated on the average 51.78 sec after injection of plasma and 163 ± 10.6 sec after injection of diatomite. The blood was obtained by puncture from the heart. A 1.34% solution of sodium oxalate was used as stabilizer.

EXPERIMENTAL RESULTS

The clotting time of whole blood from intact rabbits in a silicone-treated tube was much slower than in a plain glass tube, the ICA value being 6. Injection of diatomite and of activated plasma was accompanied by marked acceleration of blood clotting in the silicone-treated tube, with a decrease of ICA to 1.8 and 1.1, respectively, indicating marked acceleration of the process of internal thromboplastin formation following the appearance of active Hageman factor in the blood stream (Fig. 1).

The results of the comparative tests described above were confirmed by a closer analysis of the indices over a period of time. Injection of diatomite was accompanied by shortening of the recalcification time, shortening of the R and K intervals of the thromboelastogram with an increase in the angle α, and an increase in the prothrombin consumption in plasma contained in the silicon-treated tube (Fig. 2). The initial prothrombin consumption time averaged 46 ± 3.51 sec in the glass tube and 31 ± 1.5 sec in the silicone-treated tube (ICA 0.74); after injection of diatomite the prothrombin consumption time in the glass tube was unchanged (43 ± 2.77 sec), and in the silicone-treated tube it was increased to 41 ± 2.72 sec (ICA 0.95). In response to injection of activated plasma (3 ml/kg), shortening of the R interval and of the recalcification time also was observed, but the other indices did not exhibit such regular changes as in the experiments with diatomite. The clot elasticity (ma), and also the values of t, S, and T were substantially unchanged in both series of experiments (Table 1).

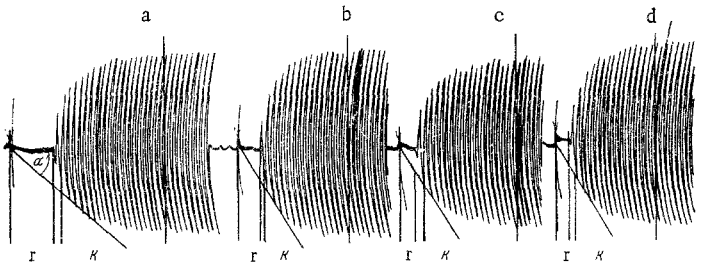

Fig. 2. Changes in thromboelastogram after injection of suspension of diatomite (experiment 17). a) Silicone plasma before injection of diatomite, r 225 sec, k 36 sec, t 522 sec, S 540 sec, T 765 sec, ma 51 mm, α 40°; b) silicone plasma after injection of diatomite, r 99 sec, k 18 sec, t 450 sec, S 468 sec, T 567 sec, ma 52 mm, α 58°; c) glass plasma before injection of diatomite, r 81 sec, k 36 sec, t 504 sec, S 540 sec, T 621 sec, ma 49 mm, α 57°; d) glass plasma after injection of diatomite, r 72 sec, k 27 sec, t 432 sec, S 459 sec, T 531 sec, ma 52 mm, α 58°.

Analysis of the frequency of deviations in the content of individual clotting factors in the blood revealed no significant differences compared with the control experiments. Meanwhile, the intensity of the decrease in content of factors I, V, and VIII of antithrombin activity in the blood was definitely greater in the experiments in which diatomite was injected (Table 2), and taking into account the absence of stimulation of fibrinolysis (see below), this is evidence of the development of mild disturbances of prothrombin consumption. The fact that under these circumstances the number of platelets in the blood did not diminish is evidently due to their liberation from the depots.

Fibrinolysis was studied in 90 experiments (45 controls) when blood was tested 3, 6, and 12 min after injection of diatomite and 2 min after injection of activated plasma; no increase in fibrinolysis was observed; 3 and 6 min after injection of diatomite, fibrinolysis was inhibited (Table 3).

In a study of histological preparations of the viscera (kidneys, lungs, liver) of 14 animals sacrificed at the height of development of hypercoagulation after injection of diatomite, and from 11 animals after injection of activated plasma (3 ml/kg, 3 injections at intervals of 1 min), no thrombus formation was observed.

The results of these investigations indicate that activation of the Hageman factor in vivo not only causes marked acceleration of blood clotting but can also lead to intravascular thromboplastin formation. The absence of increased fibrinolysis, despite the marked activation of Hageman factor, is evidence of exaggeration of the role of this factor in the mechanism of stimulation of fibrinolysis in vivo.

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