THE PRESENCE OF A HEPARIN INHIBITOR IN THE RED BLOOD CELLS

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(Received July 25, 1958. Presented by Active Member AMN SSSR V. N. Chernigovskii)

The coagulation of the blood is a complex biological process requiring the participation of a large number of factors of the plasma, the blood cells and the tissues. The interaction of all these factors leads to the formation of a fibrin clot. There are, in addition, several inhibitors which prevent this process (natural anticoagulants).

A number of workers have demonstrated the presence of thromboplastin components in the red blood cells of man and animals [1, 4, 6].

In the present communication we describe findings showing the presence of a heparin inhibitor in the red blood cells of man and animals.

EXPERIMENTAL METHOD

Blood from human subjects, rabbits and dogs was investigated. In order to prevent the blood from clotting, a 1.34% solution of sodium oxalate was added in a volume of 0.5 ml to each 2 ml of blood. The plasma was separated by centrifugation of the blood for 5 minutes at 1000 rpm.

To obtain a hemolyzate of red cells, the blood was washed five times with physiological saline during centrifugation; 0.5 ml of the washed red cells was hemolyzed in 3 ml of distilled water.

The hemolyzate was centrifuged for 10 minutes at 1500 rpm. The top layers of hemolyzate, free from stroma of the red cells, were used in the experiment.

Two series of experiments were carried out. In the first series of experiments the presence of a heparin inhibitor was judged by the effect of the hemolyzate on the plasma heparin tolerance, and in the second by the effect of the hemolyzate on the coagulation of heparinized, deprothrombinized plasma by means of thrombin.

The plasma heparin tolerance was determined by a modification of Poller's method [8]. To 0.5 ml of plasma was added one unit of heparin, 0.1 ml of hemolyzate and 1 ml of 0.277% $CaCl_2$ solution. The time of delaying of clotting of the recalcified plasma by heparin was estimated.

In control experiments the hemolyzate was replaced by the same volume of physiological saline.

To determine the clotting time of the deprothrombinized plasma, 0.1 ml of hemolyzate, 0.1 unit of heparin, 0.1 ml of thrombin solution and 0.3 ml of deprothrombinized plasma were taken. The time of appearance of the fibrin clot was observed.

In the first group of control experiments of this series, hemolyzate was replaced by an equal volume of physiological saline, and in the second, by an equal volume of toluidin blue, which has the property of inactivating heparin.

A further series of experiments was carried out to study the effect of the hemolyzate on the tolerance of the plasma to different concentrations of heparin (0.5, 1.0, 1.5 and 2.0 units).

TABLE 1

The Effect of a Hemolyzate of Red Cells on the Tolerance of Rabbits' and Dogs' Plasma to Heparin*

Species of animal	Statistical indices	Tolerance to heparin (time in sec)	
		hemolyzate	physiological saline (control)
Rabbit	M	122	192
	σ±	39	38
	m±	15	14
	P		<0.01
Dog	M	135	179
	σ±	21	22
	m±	10	11
	P		<0.05

* M – arithmetic mean; σ -square deviation; m – mean error; P – significance of difference.

EXPERIMENTAL RESULTS

The control investigations in the first group of experiments showed that one unit of heparin delayed the clotting of recalcified rabbits' plasma for 192 seconds (σ ± 38, m 14 seconds) and dogs' plasma ± for 179 seconds (σ ±22, m±11 seconds).

Addition of the hemolyzate to recalcified plasma increased its tolerance to heparin (Table 1).

From the figures given in this table it can be seen that addition of the hemolyzate shortened the clotting time of the rabbits' plasma by 36% (P< 0.01), and of dogs' plasma by 25% (P< 0.05).

Results showing the influence of the hemolyzate on the tolerance of the plasma to different concentrations of heparin are given in the figure. As may be seen from the figure, the addition of hemolyzate to plasma containing different concentrations of heparin produced a more rapid clotting of the plasma by comparison with control experiments.

The difference in the clotting time of the plasma was most clearly expressed when two units of heparin were present in the plasma.

The results obtained in the first series of experiments suggested that the shortening of the clotting time of the plasma in the presence of hemolyzate was due to a heparin inhibitor contained in the red cells and possessing the property of combining with the added heparin.

In order to confirm this suggestion a second series of experiments was performed to determine the clotting time of deprothrombinized plasma in the presence of hemolyzate (Table 2).

It can be seen from the results in Table 2 that the addition of an equal volume of hemolyzate in place of physiological saline accelerated the clotting of the heparinized, deprothrombinized plasma.

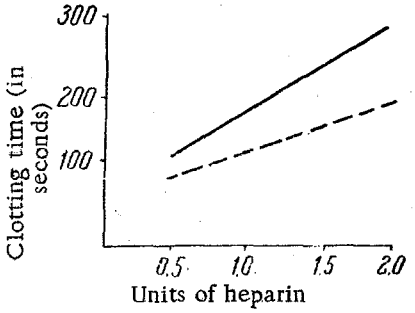
Experiments with toluidin blue showed that the clotting time of heparinized, deprothrombinized plasma was also shortened by thrombin in the presence of toluidin blue.

Addition of the red cell hemolyzate to plasma was accompanied by an increase in its tolerance to heparin

TABLE 2

The Effect of the Hemolyzate on the Clotting Time of Deprothrombinized Plasma

Species of blood	Statistical indices	Clotting time (in seconds)		
		hemolyzate	control	
			physiological saline	toluidine blue
Rabbit	M	17	26	17
	σ±	0.6	1.5	0.5
	m±	0.3	0.7	0.3
	P		<0.001	<0.001
Dog	M	76	152	63
	σ±	7	8	3
	m±	3.5	3.0	1.5
	P		<0.001	<0.001
Man	M	37	46	—
	σ±	3.5	1.5	—
	m±	1.8	0.8	—
	P		<0.01	



The effect of the hemolyzate on the tolerance of the plasma to different concentrations of heparin. —Tolerance to different concentrations of heparin in the presence of physiological saline. - - - Tolerance to different concentrations of heparin in the presence of hemolyzate.

and by acceleration of the clotting of heparinized, deprothrombinized plasma under the influence of thrombin.

On what are these changes based? We know that the heparin tolerance test of the plasma is a measure of the general coagulability of the blood, and depends on the concentration of the factors which take part in the clotting of the blood [7]. The heparin tolerance test is very sensitive to a change in the platelet count [5]. An increase in the platelet count is accompanied by an increase in the heparin tolerance of the plasma, and a fall in the platelet count by a decrease.

The change in the heparin tolerance of the plasma under the influence of variations in the platelet count is mainly dependent on platelet factor III, which is the prothrombokinase of the blood, and platelet factor IV, which is antiheparin, and inhibits the antithromboplastin and antithrombin action of heparin. The red cells of man and animals contain a thromboplastin component, similar to factor III of the blood platelets [4, 9]. This explains the fact that the addition of red cell hemolyzate to plasma considerably shortens the recalification time of the plasma and increases the prothrombin utilization in the process of clotting of the blood. In our experiments, however, the addition of hemolyzate to heparinized plasma considerably raised its tolerance to heparin.

On the basis of the data in the literature and of our previous work [1] it may be assumed that the increase in the heparin tolerance of the plasma after the addition of hemolyzate depends either on the presence of a

thromboplastic component in the red cells, or on the presence of a new factor – antiheparin – similar to the antiheparin factor of the blood platelets.

If the increase in the heparin tolerance of the plasma is associated with a thromboplastic component of the red cells, in these conditions the tolerance is therefore determined by the amount of blood thromboplastin formed, since heparin possesses the property of destroying thromboplastin. In the presence of a large amount of thromboplastin, part of it may remain intact, and this may be accompanied by an increase in the heparin tolerance of the plasma.

The heparin tolerance of the plasma may also depend, however, on the presence in the red cells of a heparin inhibitor – antiheparin. This hypothesis was confirmed by the second series of experiments, in which the action of the thromboplastic component of the red cells was excluded. In this series we studied the effect of the hemolyzate on the clotting time of heparinized, deprothrombinized plasma under the influence of addition of excess thrombin. The absence of prothrombin from the plasma excluded the effect of the thromboplastic component on the clotting time of the plasma, which depended purely on the activity of the added thrombin and the amount of heparin.

As may be seen from the data in Table 2, the addition of red cell hemolyzate to heparinized plasma shortened its clotting time. The increased coagulability of the plasma in these conditions may be explained by a decrease in the antithrombin activity of the heparin, which is evidently associated with the presence of heparin inhibitor in the red cells.

The control experiments with toluidin blue, which possesses the property of inactivating heparin, confirmed this hypothesis. In the presence of toluidin blue the clotting time of heparinized plasma under the influence of thrombin was shortened by the same degree as after the addition of the red cell hemolyzate.

From the results obtained, it may thus be concluded that in the red blood cells of man, rabbits and dogs a heparin inhibitor is present. A similar hypothesis has also been put forward by Cappeletti [2].

It has recently been shown that phospholipids, isolated from the soya bean, possess an antiheparin action [3]. It is possible that the heparin inhibitor is the phospholipids of the red cells.

SUMMARY

The paper contains data on the presence of heparin inhibitor in erythrocytes of man, rabbits and dogs. The presence of heparin inhibitor in the erythrocytes was judged by the effect of the water erythrocytic hemolyzate on the tolerance of the plasma to heparin and coagulation time of the heparinized deprothrombinized plasma, with the thrombin in excess. It was established that the plasma tolerance to heparin rises when erythrocyte hemolyzate is added to the former. The coagulation ability of heparinized deprothrombinized plasma (caused by the thrombin action) rises in the presence of erythrocytic hemolyzate. The above phenomena are explained by the presence of heparin inhibitor in erythrocytes.

LITERATURE CITED

[1] V. P. Baluda, Byull. Ekspti. Biol. i Med., 44, No. 9, 7-9 (1957).*

[2] G. Cappeletti, Arch. Med. interna, 1957, v. 9, p. 35-57.

[3] W. Connor, Carter J., Proc. Soc. Exper. Biol. a. Med., 1958, v. 97, p. 38-43.

[4] J. Georgatsos, C. Hussey, A. Quick, Am. J. Physiol., 1955, v. 181, p. 30-34.

[5] P. Meneghini, Minerva med., 1956, v. 1, p. 2013-2023.

[6] L. Pocora, M. Fusco, Riforma med., 1953, N. 3, p. 69-72.

[7] Poller, Angiology, 1954, v. 5, S. 21-26.

[8] A. Quick, C. Hussey, J. Lab. a. Clin. Med., 1955, v. 46, p. 940.

*Original Russian pagination. See C. B. Translation.