PRESENCE OF A FIBRIN-STABILIZING FACTOR IN ERYTHROCYTES

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Blood clotting is a complex biological process involving many factors of the plasma, the blood cells, and the tissues [1, 3-5, 8, 10].

Formation of an insoluble blood clot during coagulation of the blood takes place with the participation of the Laki-Lorand fibrin-stabilizer factor (fibrinase), present in the plasma [6, 7, 9]. It has recently been shown that fibrinase is present not only in the plasma, but also in the brain, liver, kidneys, lungs, and muscles [10].

A decrease in the fibrinase activity leads to disturbance of the process of fibrin formation.

The object of the present investigation was to study the presence of fibrinase in the erythrocytes of man and the rat.

EXPERIMENTAL METHOD

Investigations were made of human erythrocytes (15 experiments) and on erythrocytes of "August" rats (20 experiments).

To obtain a hemolyzate, blood taken from a vein was mixed with a 1.34% solution of sodium oxalate in proportions of 9:1. The blood was then centrifuged for 10 min at 2000 rpm in a type K14A refrigerator centrifuge, and the plasma was withdrawn.

The erythrocytes of the blood were washed three times with physiological saline by centrifugation; 1 ml of the washed erythrocytes was hemolyzed in 9 ml distilled water.

Fresh hemolyzate and hemolyzate heated for 5 min at 56° were used in the experiments.

The fibrinase activity was determined by the method described in the literature [2]. The principle of the determination was based on the study of the rate of solution of the fibrin clot in urea when the fibrinase was blocked by monoiodoacetate [9]. In control experiments the hemolyzate was replaced by an equal volume of physiological saline.

In another series of investigations the effect of a hemolyzate containing fibrinase was studied on the process of formation of a fibrin clot from solutions of fibrinogen, containing different concentrations of monoiodoacetate (0.4-0.05%).

The numerical results obtained were analyzed by the usual statistical methods.

EXPERIMENTAL RESULTS

The fibrin clot obtained in the control experiments from fibrinogen after the addition of thrombin and calcium chloride did not form in the presence of monoiodoacetic acid (0.4-0.1%). In solutions of fibrinogen containing monoiodoacetate in a concentration of 0.05%, loose, transparent fibrin clots appeared.

On the addition of hemolyzate of washed human and rats' erythrocytes to solutions of fibrinogen containing monoiodoacetate in concentrations of 0.4-0.5%, the fibrin clots were firm, opaque, and occupied the whole cross-section of the tube.

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Effect of Hemolyzate of Human and Rats' Erythrocytes on Lysis of a Fibrin Clot

Erythrocytes		Physiological saline (control)	Time of lysis of fibrin clot (sec)		
			physiological	hemolyzate	
				fresh	heated
Human	M	11		29	15
	P			< 0.001	< 0,001
Rats'	M	11		37	18
	P			< 0.001	< 0,001

Note. P was calculated by comparison with the results obtained with fresh hemolyzate.

As the table shows, in the control experiments the fibrin clot dissolved in the urea oxalate on the average in 11 sec. The activity of the fibrinase present in the fibrinogen solution was taken as 100.

On the addition of fresh hemolyzate of washed human erythrocytes to the fibrinogen solution, the time of lysis of the fibrin clot was increased by 2.6 times, and on the addition of hemolyzate of rats' erythrocytes the increase was by 3.3 times. The increase in the time of lysis of the fibrin clot was evidently due to the presence of fibrinase in the erythrocytes.

Heating the hemolyzate for 5 min at 56° lowered the fibrinase activity, and the fibrin clot then dissolved more quickly.

The rate of lysis of the fibrin clot when the fibrinase was blocked by monoiodoacetate bore a direct relationship to the concentration of fibrinase inhibitor [9]. The higher the fibrinase activity, the more monoiodoacetate had to be added to the test mixture to cause lysis of the fibrin [9].

Analysis of data in the literature [6, 7, 9] on the role of fibrinase in the mechanism of formation of insoluble fibrin during coagulation of the blood, and the results of the authors' own investigations suggest that the formation of firm fibrin clots in solutions containing hemolyzate is due to the presence of fibrinase in the erythrocytes. Investigations were carried out with fibrinogen solutions free from prothrombin and other procoagulants, on which the blood clotting factors contained in the erythrocyte (thromboplastin, etc.) might have some influence.

The results of experiments with heated hemolyzate showed that fibrinase contained in the erythrocyte is thermolabile and its activity is lowered at 56°. Its degree of thermolability is therefore the same as that of plasma fibrinase, evidence that they share common properties.

The results of these investigations confirm recently published information [5] regarding the presence of a fibrin-stabilizing factor (fibrinase) in the erythrocytes. It may be concluded from the discovery of fibrinase in the erythrocytes of man and animals that this phenomenon is of a general biological character.

It is possible that the serious consequences of hemolysis of the erythrocyte, especially during blood transfusion, may be connected with the liberation of several factors influencing blood clotting from the erythrocytes, including fibrinase. Under the action of active fibrinase, a fibrin clot more resistant to plasma may be formed, preventing its lysis during the simultaneous activation of the fibrinolytic system.

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