PRESENCE OF COMPONENTS OF THROMBOPLASTIN IN ERYTHROCYTES

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Blood coagulation is a complex biological process, requiring the participation of a large number of factors present in the plasma, the blood platelets, and the tissues. A number of workers [1 – 3, 4, 5, 11, 12] have shown that a number of factors take part in the process, other than fibrinogen (Factor 1), prothrombin (Factor 2), thromboplastin (Factor 3), and calcium salts (Factor 4). It is known, moreover, that thromboplastin is present in an inactive form in the blood platelets, leucocytes, and body tissues [1, 8]. Blood thromboplastin originates from a number of factors present in the blood platelets and the plasma. Special importance in the formation of thromboplastin has been ascribed to antihemophilic globulin (Factor 8), to the plasma component of thromboplastin (Factor 9), and to Factor 10. Ac-globulin (Factor 5), pro convertin (Factor 7), and calcium chloride (Factor 4) appear also to take part in the formation of thromboplastin, since none of these factors has any effect on the activity of formed thromboplastin [9]. Blood thromboplastin originates from the interaction of platelet and plasma factors. The existence of a relatively large number of factors which are active in the first phase is supported by the fact that deffeiency of each individual factor, except Factor 4, results in a specific form of hemorrhagic diathesis.

Evidence has been reported in the literature that the thromboplastin activity of blood depends on the presence of fully active blood prothrombokinase (blood platelets), and on the concentration of plasma thromboplastin factors [1, 11]. The more thromboplastin is formed in the first phase of clotting, the more prothrombin is converted into thrombin in the second phase, for conversion of fibrinogen into fibrin in the third phase.

It follows from the above that the most complex phase of blood clotting is that of thromboplastin formation. Very little study has been devoted to the relations of erythrocytes to this process.

A number of authors have established the presence in human erythrocytes of factors participating in the formation of thromboplastin [10, 13].

The present paper presents evidence of the presence of thromboplastin factors in dog erythrocytes.

EXPERIMENTAL

The experiments were performed on male dogs. Four ml of blood were taken from a superficial vein of a forelimb of healthy dogs, without anesthesia; 2 ml were immediately placed in a centrifuge tube containing 0.5 ml of 1.34% sodium oxalate solution, and the remaining 2 ml were placed into another centrifuge tube. Two series of experiments were performed.

In the first series evidence of the presence of thromboplastin factors in erythrocytes was based on measurements of recalcification times of oxalated dog plasma in the presence of aqueous hemolyzates of washed erythrocytes. As controls we measured recalcification times in systems containing physiological saline.

Plasma recalcification time was determined in the following way: 0.2 ml of 0.277% $CaCl_2$ solution and 0.1 ml of hemolyzate were added to a 8 × 100 mm test tube, followed quickly by 0.1 ml of plasma, a stopwatch being started at the same time. The test tube was shaken, and the time of appearance of fibrin floccules

was noted. The measurements were made in a water bath at 38°. Recalcification time was taken to be the interval between introduction of plasma and appearance of fibrin floccules [3].

In the second series of experiments we examined the effect of hemolyzate on prothrombin requirement. The prothrombin requirement for clotting was determined by the method of Quick [14]. The prothrombin requirement was calculated from the difference between its content in the plasma and serum when blood was allowed to clot in the normal way, after addition of hemolyzate or of the same volume of physiological saline (control). The data so obtained served for the estimation of total thromboplastin activity [6].

EXPERIMENTAL RESULTS

Our control experiments showed that the recalcification time of oxalated dog plasma varied within the range 80-142 seconds. This time was shortened when a diluted hemolysate of dog erythrocytes was added. A statistically treated presentation of the results is given in Table 1.

TABLE 1

Effect of Hemolyzate on Recalcification Time of Oxalated Dog Plasma

Statistical coefficients	Plasma recalcification time (seconds)	
	plasma and physiological saline	plasma and red cell hemolyzate
M	100	37
σ±	21	4
m±	5	1
t		12.6
P		< 0.001

Note: M is the mean arithmetic value, σ is the standard deviation, m the mean error, t is the index of significance of deviations, and P is the probability of difference, calculated in relation to the recalcification time found in the presence of physiological saline.

It appears from the data of Table 1 that when physiological saline is added to the plasma the recalcification time, as calculated from the arithmetic means, is equal to 100 seconds (σ = ± 21 seconds); with red cell hemolyzate this time is decreased to 37 seconds (σ = ± 4 seconds.) Hence the coagulability of oxalated plasma in the presence of hemolyzate and with subsequent recalcification is increased, with reference to the control (P < 0.001).

The shortening of recalcification time of plasma in the presence of hemolyzate is related to raised thromboplastin activity of the plasma. This may have been due to introduction of blood platelets together with the hemolyzates, or to the presence of thromboplastin components within the red cells. Special examinations showed that platelets were absent from erythrocyte hemolyzates. It may hence be assumed that the increase in thromboplastin activity of plasma observed after addition of hemolyzate is due to the presence of thromboplastin factors in erythrocytes (Table 2).

The data of Table 2 show that addition of hemolyzate raises the prothrombin requirement, as compared with the control and with normal values (P < 0.001), and this points to a raised thromboplastin activity of plasma mixed with hemolyzate. It may hence be concluded that factors possessing thromboplastin activity are present in erythrocytes.

Recalcification time in the presence of hemolyzate was accordingly shortened, and prothrombin requirement raised. It has been shown by a number of workers [1, 12] that the thromboplastin activity of blood is determined by the amount of platelet and plasma factors present, contributing to the formation of thromboplastin. The higher the content of such factors, the higher will be the thromboplastin activity of the blood, and this, in turn, determines the prothrombin requirement for the blood coagulation process. The raised thromboplastin activity and the shortened recalcification time of plasma containing hemolyzate may thus be ascribed to the presence within erythrocytes of thromboplastinogenic components participating in the formation of blood thromboplastin.

TABLE 2

Effect of Hemolyzate on Prothrombin Requirement

Statistical coefficients	Prothrombin requirement (%)		
	under ordinary conditions	in presence of hemolyzate	in presence of physiological saline
M	68	84	65
σ±	2	4	3
m±	0.5	1	1
t		14.4	0.4
P		<0.001	>0.05

Our finding of thromboplastin factors within dog erythrocytes is in agreement with those of Quick et al. and of Ottoviani and Dettori for human erythrocytes. The presence of thromboplastin factors in human and dog erythrocytes supports the view that this may be a general biological phenomenon.

SUMMARY

Data concerning the presence of thromboplastin factors in dog erythrocytes are presented. The presence of these factors was judged by the influence of water hemolyzate of washed erythrocytes on the time of recalcification of the oxalated plasma and on utilization of prothrombin. The average time of recalcification of oxalated plasma equalled 100 seconds ($\sigma = \pm 21$), while utilization of prothrombin was 68% ($\sigma = \pm 2$). Addition of hemolyzate of erythrocytes to the plasma was associated with reduced duration of recalcification ($P < 0.001$). Utilization of prothrombin was increased in presence of hymolyzate ($P < 0.001$).

Reduced time of recalcification and increased utilization of prothrombin are connected with the presence of thromboplastin factors in erythrocytes.

The presence of thromboplastin factors in erythrocytes of human beings and dogs supports the view that this phenomenon has a general biological character.

LITERATURE CITED

[1] G. V. Andreenko and B. A. Kurdryashov, Doklady Akad. Nauk SSSR, 102, 787-790, 1955.

[2] B. A. Kudryashov, Doklady Akad. Nauk SSSR, 60, No. 8, 1469-1472.

[3] B. A. Kudryashov and P. D. Ulitina, Doklady Akad. Nauk SSSR, 98, No. 5, 815-818, 1954.

[4] B. Alexander, A. de Vries, Blood, 1949, N. 4, p. 747-751.

[5] B. Alexander, A.de Vries, and R. Goldstein, Blood, 1949, N. 4, p. 739-746.

[6] H. Bergerhof and L. Z. Roka, Vitamin-Hormon u. Fermentforsch., 1954, 6, N. 1, S. 25-39.

[7] H. Brodthagen, Scand. J. Clin. a. Lab. Invest., 1953, N. 4, p. 376-377.

[8] R. Biggs and A. Douglas, J. Clin. Path., 1953, N. 6, p. 23.

[9] B. Duckert, P. Flückiger, H. Isenschmid, M. Matter, J. Vogel-Meng and F. Koller, Acta Haematol., 1954, v. 12, N. 3, 197-202.

[10] P. Ottaviani, A. Dettori and G. Manai

[11] P. Owren, Scand. J. Clin. Labor. Invest., 1949, 1, p. 81-83.

[12] A. Quick, Am. J. Physiol., 1943, v. 140, p. 212-220.

[13] A. Quick, J. Georgatsos and C. Hussej

[14] A. Quick and C. Hussej, Amer. J. Dis. Children, 1953, v. 85, N. 6, p. 698-705.