RELATIONSHIP BETWEEN THROMBOPLASTIN FACTOR OF ERYTHROCYTES AND THE SURFACE OF THE CELL MEMBRANE

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The results of a comparative investigation of the activity of thromboplastin factor or normal and trypsinized human erythrocytes suggest that the incorporation of erythrocyte thromboplastin factor in the process of internal thromboplastin formation is coupled with reorganization of the internal structure of the cell membrane, initiated by interaction between the membrane sialoprotein (clotting receptor?) and activated plasma clotting factors.

In the process of internal thromboplastin formation whole erythrocytes can compensate to some degree for a deficiency of platelet factor 3 by virtue of the presence of a lipoprotein with procoagulant activity in the erythrocyte membrane [12].

With the inclusion of erythrocyte thromboplastin factor (ETF) in the process the catalytic reaction of blood clotting is transformed into the second, heterogeneous phase, resulting in interaction between factors Y and X.

The mechanism of activation of ETF is not clear. Meanwhile, the increase in ETF activity with a change in the transport function of the membrane [6] suggests that ETF mobilization is dependent on the functional state of the cell membrane.

In continuation of the investigation of erythrocyte thromboplastin activity [1-5], the mechanism of incorporation of ETF into the process of internal thromboplastin formation was studied.

EXPERIMENTAL METHOD

The thromboplastin activity of erythrocytes (hemolyzate) was estimated from their ability to increase thromboplastin formation in autologous plasma deprived of platelets. The tests used: thrombin generation time (TGT) [8] with some modification (see below), recalcification time, and thromboelastogram.

The TGT was determined in samples of equal volumes (0.25 ml) of the ingredients of a clotting mixture (plasma, isotonic NaCl solution, erythrocytes or hemolyzate, 0.05 M calcium chloride solution). The thromboelastogram was recorded on the Tromb-2 apparatus with specified proportions of the ingredients [5]. A suspension of erythrocytes (hemolyzate) was prepared from a residue of washed normal and trypsinized erythrocytes (8-9 million/mm^3) in dilutions of 1:2, 1:10, and 1:100. Isotonic NaCl solution was added to the control in accordance with the hematocrit index [1]. The samples were tested from 2 min after recalcification at intervals of 1 min for 7 min. The total TGT also was studied over the period from the second to the fourth minute, the fourth to the seventh minute, and for the whole period (from the second to the seventh minute). Erythrocytes were trypsinized with a buffered 0.1% or 0.4% solution of the crystalline enzyme (pH 7.2) at 37°C for 45 min.

For a comparative analysis of the results, besides absolute values, the following relative indices also were calculated: 1) the index of comparative erythrocyte activity – the ratio between the absolute value of

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TABLE 1. Comparative Effect of Normal and Trypsinized Erythrocytes on Indices of Thromboplastin Formation (M ± m)

Series of expts.	Index studied	Recalcification time (sec)	Thrombin generation time		
			in 2-4 min	in 5-7 min	in 2-7 min
I n = 20	ICEA ICHA	1,13±0,015 0,96±0,015	1,17±0,04 0,90±0,013	1,04±0,02 1,05±0,01	1,12±0,028 0,97±0,04
II n = 15	ICEA ICHA	1,14±0,04 0,97±0,01	1,49±0,09 0,96±0,013	1,24±0,05 0,98±0,015	1,41±0,06 0,97±0,01

TABLE 1. (Continued)

Maximal thrombin activity (sec)	Thromboelastogram		
	α (deg)	r (sec)	K (sec)
1,05±0,2 0,97±0,02	0,96±0,04 —	1,07±0,13 —	1,25±0,15 —
1,24±0,04 0,97±0,015	0,88±0,08 1,13±0,095	1,32±0,098 0,83±0,07	1,40±0,21 0,94±0,095

Legend: ICEA) Index of comparative erythrocyte activity; ICHA) index of comparative hemolyzate activity.

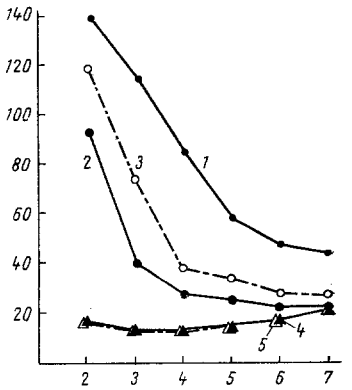

Fig. 1. Comparative effect of normal and trypsinized erythrocytes and of their hemolyzate on thrombin generation in plasma deprived of platelets: 1) thrombin generation in plasma before treatment; 2, 3, 4, 5) thrombin generation in plasma after addition of normal and trypsinized erythrocytes and of hemolyzate of normal and trypsinized erythrocytes, respectively. Dilution of erythrocyte suspension (hemolyzate) 1:10, trypsin in 0.4% solution. Abscissa, time of incubation of clotting mixture (in min); ordinate, thrombin activity (in sec).

of the index (in sec) in experiments with plasma containing trypsinized erythrocytes and the absolute value (in sec) of this index in experiments with plasma with normal erythrocytes; 2) the index of comparative activity of the hemolyzate was calculated similarly.

Blood from 35 healthy persons of both sexes aged from 20 to 44 years was tested.

EXPERIMENTAL RESULTS AND DISCUSSION

In the original variant of the experiments using a relatively concentrated cell suspension (4-4.5 million/mm^3), treatment of the erythrocyte with trypsin (0.1% solution) led to some decrease in thromboplastin activity [lengthening of TGT and the time of maximal activity of the thrombin, the recalcification time, and the r and k indices of the thromboelastogram (Table 1)]. The decrease in ETF activity was more marked

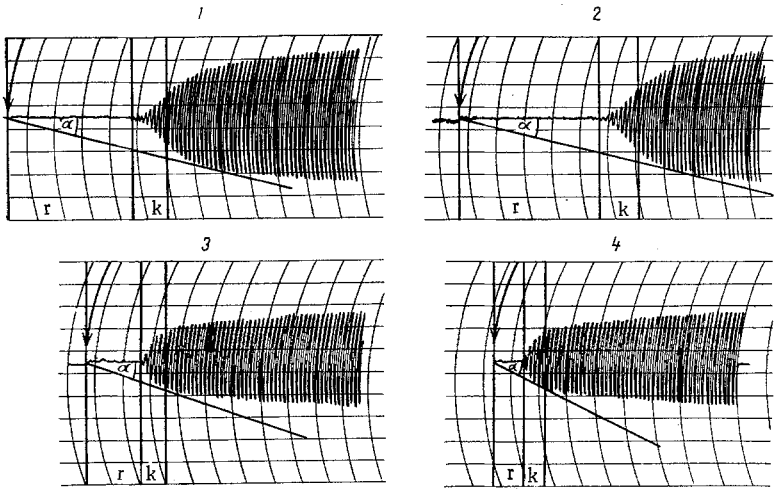

Fig. 2. Comparative effect of normal and trypsinized erythrocytes (hemolyzate) on thromboelastogram indices. Dilution of erythrocytes (hemolyzate) 1:10. Donor Dzh. Trypsin in 0.4% solution. Plasma deprived of platelets was treated with: 1) normal erythrocytes: $\alpha = 15°$, r = 258 sec, k = 78 sec; 2) erythrocytes trypsinized· $\alpha = 13°$, r = 300 sec, k = 84 sec; 3) hemolyzate of normal erythrocytes: $\alpha = 19°$, r = 120 sec, k = 54 sec; 4) hemolyzate of trypsinized erythrocytes; $\alpha = 26°$, r = 66 sec, k = 42 sec.

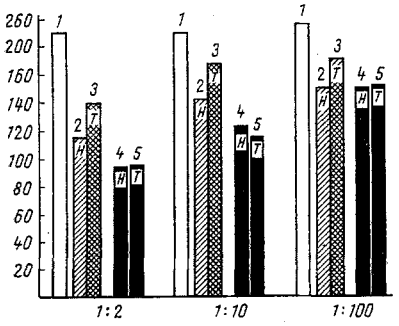

Fig. 3. Comparative effect of suspension of normal and trypsinized erythrocytes (hemolyzate) of different concentrations on recalcification time of plasma deprived of platelets (from results of 10 experiments): 1) recalcification time of untreated plasma; 2, 3, 4, 5) recalcification time of plasma after addition of normal and trypsinized erythrocytes and hemolyzate of normal and trypsinized erythrocytes, respectively. Trypsin in 0.1% solution. Abscissa, dilutions of erythrocyte suspension (hemolyzate); ordinate, recalcification time (in sec).

when a less concentrated suspension of erythrocytes (800,000-900,000/mm^3) treated with a more concentrated solution (0.4%) of trypsin was used (Table 1).

It must be emphasized that none of these differences were found when activity of the hemolyzates obtained from normal and trypsinized erythrocytes was compared (Table 1; Figs. 1 and 2).

The decrease in ETF activity after trypsinization was due to partial removal of the effect of ETF but was not connected with the acquisition of inhibitory properties with respect to actual thromboplastin formation by any particular fraction of the erythrocytes. This conclusion is supported by the absence of a negative influence of the trypsinized erythrocytes on the process of thromboplastin formation in platelet-enriched plasma.

The decrease in ETF activity under these conditions was not due to direct damage to the thromboplastin factor by the proteolytic enzyme. If the trypsin had acted directly on the protein part of the lipoprotein with procoagulant activity [9] the decrease in thromboplastin activity of the cell would have remained after its destruction [7]. It can be postulated that the disappearance of the differences discussed after destruction of the cell was due to the fact that the excess content of ETF in the hemolyzate masked the partial disappearance of ETF activity in the whole cell. Against this suggestion, however, there is the fact that when hemolyzate with lower activity from normal and trypsinized erythrocytes was used no decrease likewise was found in its activity (Fig. 3).

If trypsin does not directly damage the lipoprotein with procoagulant activity in the whole cell we are forced to consider that the ETF is located in the deeper layers of the cytoplasmic membrane and not on the surface. In that case it would be expected that removal of the sialoprotein complex under the influence of trypsin would facilitate interaction between the plasma clotting factors and the erythrocytic clotting factors as is known, in particular, with respect to the T-phenomenon [10, 14] and some other immunological reactions [11]. However, the opposite effect is observed.

ETF, if removed from the surface of the cell membrane, thus requires the sialoprotein of the outer layer of the cell membrane, which plays the role of a special type of mediator, before it can manifest its activity.

The following hypothesis can be put forward on the basis of the facts described above. The closer approximation of ETF to the surface of the membrane must correspond to optimal contact between ETF and the plasma factors. This closer contact is evidently secured by reorganization of the internal structure of the cell membrane as a result of interaction between the sialoprotein (clotting receptor?) and activated plasma clotting factors.

LITERATURE CITED

1. I. Ya. Ashkinazi, Byull. Éksperim. Biol. i Med., No. 7, 45 (1966).
2. I. Ya. Ashkinazi, Byull. Éksperim. Biol. i Med., No. 5, 27 (1968).
3. I. Ya. Ashkinazi, Byull. Éksperim. Biol. i Med., No. 1, 3 (1969).
4. I. Ya. Ashkinazi, Byull. Éksperim. Biol. i Med., No. 3, 46 (1970).
5. I. Ya. Ashkinazi, Byull. Éksperim. Biol. i Med., No. 7, 28 (1972).
6. P. Cohen, Brit. J. Haemat., 14, 141 (1968).
7. J. C. Georgatsos, C. V. Hussey, et al., Am. J. Physiol., 181, 1, 30 (1955).
8. N. D. Hicks and W. P. Pitney, Brit. J. Haemat., 3, 227 (1957).
9. S. Margolis, in: E. Tria and A. M. Scany (editors), Structural and Functional Aspects of Lipoproteins in Living Systems, London (1969), p. 370.
10. J. A. Morton and M. M. Pickles, Nature, 159, 779 (1947).
11. G. Pardoe, Nouv. Rev. Franc., 11, 863 (1971).
12. A. Quick, in: Proceedings of the Fourth International Congress on Biochemistry, Vol. 10, Vienna (1958), p. 123.
13. E. Tria and O. Barnabei, in: E. Tria and A. M. Scany (editors), Structural and Functional Aspects of Lipoproteins in Living Systems, London (1969), p. 144.
14. W. E. Wheeler, A. L. Lunby, and L. L. Schooll, J. Immunol., 65, 39 (1950).