# SOME GENERAL PRINCIPLES GOVERNING INTERACTION BETWEEN ERYTHROCYTES AND PLASMA OF HEALTHY PERSONS DURING BLOOD CLOTTING IN VITRO

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It has now been proved that destroyed erythrocytes have various influences on the process of blood clotting and possess thromboplastic activity [1-3, 5-7, 13]. In the opinion of several investigators the thromboplastic factor of the erythrocytes, unlike that of the platelets, exerts its activity in the absence of plasma contact factor [2, 13], and it is therefore considered by them to be the initiator of the clotting process.

Although investigations directed towards studying the role of hemolyzate in the process of blood clotting are necessary, it must be recognized that the study of the influence of hemolyzate does not reveal the role of the erythrocytes in this particular process, whether in physiological or in the overwhelming majority of pathological conditions. It is well known that, except in a few diseases (Marchiafava-Micheli disease), destruction of the erythrocytes takes place intracellularly, and this prevents the entry of the products of destruction into the blood stream. In addition, emphasis must be laid on the nonspecific character of the thromboplastic activity of the hemolyzate: any destroyed cell (including tumor and sex cells) possesses the same effect [10]. The role of intact erythrocytes in the process of blood clotting (thromboplastin formation) has not been adequately studied, and is the subject of lively debate [2, 5, 10].

The author was interested above all in the study of the general principles governing the interaction between intact erythrocytes and plasma in the process of clotting. Accordingly an attempt was made to solve the following problems: can intact erythrocytes exhibit thromboplastic activity, are there individual variations in the thromboplastic activity of the erythrocytes, and to what degree does the manifestation of their activity depend on the properties of the plasma, and what is the relationship between the thromboplastic factor of the erythrocytes and plasma contact factor.

## EXPERIMENTAL METHOD

The investigations were carried out on 42 healthy persons of both sexes aged 23-47 years, with a normal morphological composition of their peripheral blood. The thromboplastic activity of the intact erythrocytes was judged from the degree of acceleration of plasma containing very few platelets, after addition of an erythrocyte suspension, and also by means of the method of thrombelastography. The recalcification time was determined by Howell's method, in the modification adopted in B. A. Kudryashov's laboratory [4], but with the use of a more highly concentrated solution of $CaCl_2$ (0.05 M), prepared in isotonic NaCl solution.

The thrombelastographic investigations were carried out on a Soviet apparatus at an amplitude of 4, with the following proportions of the ingredients: 0.2 $mm^3$ oxalated plasma, 0.2 $mm^3$ physiological saline, and 0.2 $mm^3$ of 0.15 M $CaCl_2$ solution. During the addition of the erythrocyte suspension, 0.1 $mm^3$ of physiological saline was mixed with the same volume of suspension.

From the oxalated blood, after washing five times (at 1000 rpm for 10 min), a suspension of erythrocytes of constant concentration (4,000,000/$mm^3$) was prepared. This preparation was conventionally called zero (0) dilution, and dilutions of 1 : 10, 1 : 100, and 1 : 1000 were subsequently obtained from it. It was found that the use of an erythrocyte suspension of different dilutions was more successful in detecting individual differences in the thromboplastic activity of the erythrocytes. The mean number of platelets in the initial dilution of the erythrocyte suspension was 7000/$mm^3$ and the mean number of leukocytes was 200/$mm^3$.

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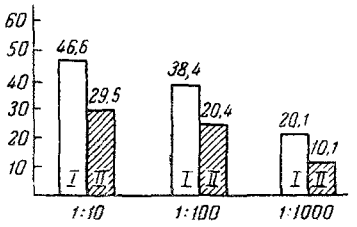


Fig. 1. Comparative acceleration of the recalcification time of plasma poor in platelets on addition of erythrocytes and hemolyzate. I) Hemolyzate; II) erythrocytes. Along the axis of abscissas – dilutions of suspension of erythrocytes; along the axis of ordinates – acceleration (in %).

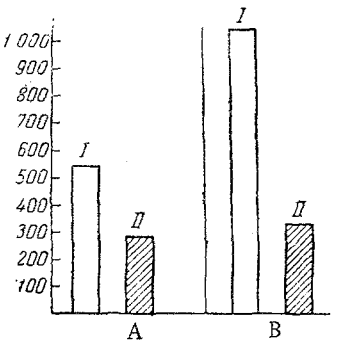


Fig. 3. Effect of erythrocytes on the recalcification time of plasma rich (A) and poor (B) in platelets in a silicone-treated tube. I) Control; II) addition of erythrocytes. On the axis of ordinates – recalcification time (in sec).

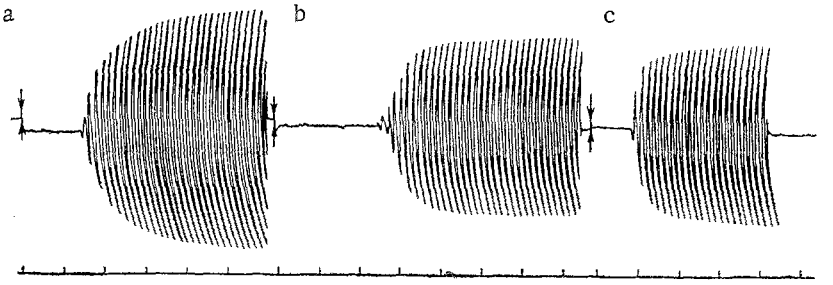


Fig. 2. Effect of a suspension of erythrocytes on results of thrombelastography of plasma poor in platelets (donor D.). a) Plasma rich in platelets (183,000/mm$^3$): $\alpha = 42°$, r = 243 sec, $k_1 = 81$ sec, $r + k_1 = 324$ sec, MA = 59 mm; b) plasma poor in platelets (8500/mm$^3$): $\alpha = 27°$; r = 378 sec, $k_1 = 90$ sec, $r + k_1 = 468$ sec, MA = 46 mm; c) plasma poor in platelets, after addition of 0.1 mm$^3$ of a suspension of erythrocytes of zero dilution: $\alpha = 48°$, r = 189 sec, $k_1 = 36$ sec, $r + k_1 = 225$ sec, MA = 44 mm.

The hemolyzate (or "osmolyzate") was prepared on the basis that each of its dilutions corresponded accurately in its erythrocyte content, before they were destroyed, to the concentration of the suspension in dilutions of 1 : 10, 1 : 100, and 1 : 1000. The osmolyzate was kept at 4° for 5 h and then diluted until its salt concentration was isotonic with physiological saline.

Plasma with very few platelets (10,000/mm$^3$) was obtained by centrifugation of plasma rich in platelets at 3500 rpm for 30 min. Plasma relatively rich in platelets (I) (65,000/mm$^3$) was obtained by centrifuging oxalated blood at 1000 rpm. Plasma rich in platelets (II) was obtained by centrifuging oxalated blood for 7 min at 700 rpm. The number of platelets in the plasma rich in platelets (II) was much greater (130,000-280,000/mm$^3$) than in the plasma relatively rich in platelets (I), but at the same time it was less stable and depended on the platelet count in the donor's blood.

The accelerating influence of the erythrocytes on the clotting time of the plasma was determined in relation to the clotting time of that same plasma in the control series (i.e., to the clotting time of the plasma after the addition of physiological saline in a volume corresponding to the hematocrit index of the initial dilution of the erythrocyte suspension).

Blood was taken by a two-syringe method. All the preparatory manipulations, and, in appropriate cases, the determination of the clotting time were carried out in silicone-treated tubes. Dimethyldichlorosilane was used for the silicone treatment. The results were analyzed by statistical methods.

## EXPERIMENTAL RESULTS

The results of the investigations of the thromboplastic activity of the erythrocytes of 20 persons, in which the erythrocytes were added to the same subject's plasma, are given below. A suspension of erythrocytes in dilutions of 0 and 1 : 10 caused acceleration of recalcification of plasma poor in platelets in all cases tested, in a dilution of 1 : 100 – in 12 cases, and in a dilution of 1 : 1000 – in only 7 cases. The erythrocyte suspension of zero dilution accelerated the recalcification time on the average by 48.0 ± 3.2% (M ± m), in a dilution of 1 : 10 by 29.54 ± 4.4%. and in the last two dilutions by 20.4 ± 4.8 and 10.1 ± 5.5%.

The effect of the hemolyzate was usually identical in character with the effect of the intact erythrocytes, but it was more marked in degree (Fig. 1). If the thromboplastic activity of the hemolyzate in a dilution of 1 : 10 is conventionally regarded as 100, the activity of the erythrocyte suspension in the same dilution was 63.3% relative to the hemolyzate. Hence, if the view of those investigators [5, 11] who consider that the thromboplastic activity of washed erythrocytes is due to partial destruction of the erythrocytes is accepted, it must be considered that in the erythrocyte suspension used more than half the total number of erythrocytes was destroyed. However, this was contrary to the results of microscopic examination of the suspension.

The results of thrombelastography (Fig. 2) showed that the thromboplastic activity of the intact erythrocytes was manifested by a decrease in the values of r and $K_1$. In these circumstances the elasticity of the clot remained unchanged, which is in agreement with the data in the literature concerning the absence from the erythrocytes of the factor capable of replacing the retractozyme of the platelets [2, 3].

It may be concluded from the foregoing account that intact erythrocytes possess thromboplastic activity, associated with the cell membrane. The destroyed erythrocyte possesses greater thromboplastic activity because substances with thromboplastic activity connected with the stroma of the cell are implicated in the process of clotting.

The accelerating effect of the erythrocytes was characterized by very considerable individual variations. For example, for an erythrocyte suspension of zero dilution $M \pm \sigma$ was $48.9 \pm 14.3\%$, i.e., the ratio between $\sigma$ and M was 29.21%. Individual variations, concerning both the character of the influence of the erythrocyte and the magnitude of the accelerating effect, could be dependent not only on the erythrocytes, but also on the individual properties of the plasma, which in turn depended on the relative proportions of coagulants, procoagulants, and physiological inhibitors. The individual properties of the plasma poor in platelets were shown, in particular, after additional dilution of the plasma with physiological saline in the control series: in 6 cases this led to acceleration of clotting, and in 14 to its retardation.

Consequently, to answer the question whether the erythrocytes of healthy persons vary in their thromboplastic activity, it was necessary to stabilize the properties of the plasma. For this purpose the experiments were conducted in such a way that the thromboplastic activity of the erythrocytes of different persons was compared with the same plasma. In each experiment erythrocytes from four persons were used, but with the plasma of only one of them (taking group compatibility into account). Three experiments were performed. In these circumstances, as in the experiments using erythrocytes and plasma from the same person, erythrocytes in dilutions of 0 and 1 : 10 in all cases caused acceleration, in a dilution of 1 : 100 they did so in 9 cases, and in a dilution of 1 : 1000 in 6 cases. The accelerating effect also exhibited individual variations, but these were only one-third as large as those in the first series of experiments.

Hence the erythrocytes of healthy persons are evidently characterized by individual variations in their thromboplastic activity.

On the other hand, the significance of the individual properties of the plasma became apparent in the third variant of the experiments, in which erythrocytes from one subject were added to plasma, poor in platelets, from 4 persons. Consequently, during the interaction between erythrocytes and plasma in the course of the clotting process the final result was determined by the properties both of the erythrocytes and of the plasma.

In order to study the relationships between the thromboplastic activity of the erythrocytes and the plasma contact factor, in 16 persons the recalcification time was determined in silicone-treated tubes (Fig. 3). These tests showed that the addition of a suspension of erythrocytes of zero dilution was consistently accompanied by acceleration of clotting of plasma, whether poor or rich in platelets (I and II), by approximately twice.

At the same time, when the silicone treatment was "ideal" in its effect, the recalcified plasma did not clot for 1 h or more, and neither erythrocytes nor hemolyzate could accelerate clotting.

It may be concluded from the results of the investigations that the thromboplastic activity of both erythrocytes and platelets can be exhibited only with the participation of the contact factor. On the other hand, the thromboplastic factor of the erythrocytes is less sensitive than that of the platelets to a deficiency of contact factor, and this accounts for the effect of the erythrocytes in the silicone-treated tube with the plasma rich in platelets, described above. It may be expected that in certain physiological and pathological conditions the thromboplastic factor of the erythrocytes plays an extremely important role in the mechanism of the intensification of subthreshold coagulatory effects, due primarily to the action of other agents.

## SUMMARY

Intact human erythrocytes are capable of considerable thromboplastic activity, which is manifested by acceleration of recalcification of plasma poor in platelets and by a decrease in the values of r and $K_1$ of the thrombelastogram. The thromboplastic activity of intact erythrocytes is characterized by individual variations. The thromboplastin factor of the erythrocytes, just as the trombocytic factor, displays its activity only in the presence of the factor of plasma contact, but at the same time the thromboplastin factor of the erythrocytes as compared to the thrombocytic factor, is less sensitive to the deficit of the factor of plasma contact.

It is possible that under certain physiological and pathological conditions the thromboplastin factor of the erythrocytes plays an important role in the mechanism of intensification of the coagulative effects of subthreshold intensity, which are initially produced by the action of other agents.

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**All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of the first issue of this year.**

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