# ANALYSIS OF THE INHIBITORY EFFECT OF WHOLE HUMAN RED BLOOD CELLS ON PLASMA HEPARIN ACTIVITY

I. Ya. Ashkinazi

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The accelerating effect of whole human red blood cells (RBC) on the thrombin time of plasma (deprothrombinized, platelet-deprived) is manifested regardless of the presence or absence of calcium ions or of preincubation of the RBC with plasma and it is not accompanied by a fall of the free heparin level in the plasma. Acceleration of the transformation of the plasma fibrinogen into fibrin under the influence of whole RBC is unconnected with inhibition of endogenous heparin but reflects the fibrinoplastic effect of the cell. The experimental results do not support the view expressed in the literature that whole erythrocytes have a regulating effect on the blood level of endogenous heparin.

KEY WORDS: whole red blood cells; endogenous heparin.

Whereas the inhibitory effect of whole red blood cells (RBC) on exogenous heparin under certain experimental conditions has been proved [14, 15], the question of the relations between RBC and endogenous heparin remains a matter for discussion. In confirmation of the inhibitory effect of whole RBC on the endogenous anticoagulant the observed shortening of the thrombin time of plasma (not only heparinized, but also ordinary [7, 9]) has been cited, although the effect has not been observed by other workers [4, 5] and, besides, this could reflect a different effect on the RBC. Incidentally, only some of these workers cited added calcium chloride together with thrombin to the plasma [5], and considering that the inhibition of exogenous heparin by whole RBC depends on the presence of calcium ions in the medium (see below), this is a significant factor. However, it must be remembered that the plasma clotting time after addition of thrombin was measured in seconds and, consequently, the duration of contact of the RBC with the endogenous anticoagulant in the presence of calcium ions was restricted to an equally short interval. The question arises whether the absence of an antiheparin effect of the RBC is connected with too short a period of contact of the cells with the endogenous anticoagulant in recalcified plasma.

The object of the present investigation was to clarify whether whole human RBC can exhibit inhibitory properties toward endogenous heparin (and heparinoids with anticoagulants activity). Three main series of experiments were carried out: 1) to study the effect of RBC on the thrombin time of ordinary and recalcified plasma – with incubation of the plasma with and without RBC; 2) determination of the free heparin level in ordinary and recalcified plasma – with and without preincubation of the plasma with RBC; 3) to compare the accelerating effect of RBC on the thrombin time of plasma and of a solution of fibrinogen.

## EXPERIMENTAL METHOD

Experiments were carried out with oxalated unheparinized plasma. Nevertheless, the established rules for interaction between whole RBC and exogenous heparin [14, 15] were taken into account, for these rules could also extend to interaction between RBC and the endogenous anticoagulant. This rule applies to the dependence of manifestation of the antiheparin activity of RBC on the optimal temperature (37°C), the presence of calcium ions in the medium, and a sufficiently long incubation of the RBC with exogenous anticoagulant [14, 15]. Satisfaction of the two last conditions during determination of the thrombin time and the free heparin of ordinary plasma is impossible, for when calcium chloride is added to plasma, the process of internal thromboplastin and thrombin formation is activated. To avoid this, the plasma was first freed from prothrombin and factor IX (adsorption on barium sulfate at 4°C for 30 min). The same method was used previously when studying the inhibitory properties of a hemolysate against exogenous heparin [3]. The use of platelet-free plasma prevented

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TABLE 1. Changes in Thrombin Time of Deprothrombinized, Platelet-Deprived Plasma under the Influence of RBC (M ± m, n = 26)

| Series of experiments | Without preincubation | | | After incubation with RBC | | |
|---|---|---|---|---|---|---|
| | control | experiment | IEA | control | experiment | IEA |
| Without addition of calcium chloride | 32,7±0,98 $P<0,001$ | 26,0±0,81 $P<0,001$ | 1,26±0,02 $P<0,001$ | 36,7±1,22 $P<0,001$ | 28,3±0,99 $P<0,001$ | 1,30±0,02 $P<0,001$ $P_1>0,05$ |
| With addition of calcium chloride | 30,9±0,92 $P<0,01$ | 26,5±0,91 $P<0,001$ | 1,17±0,02 $P<0,001$ | 33,2±1,14 $P<0,001$ | 28,0±1,03 $P<0,001$ | 1,19±0,02 $P<0,001$ $P_1>0,05$ |

Legend. $P_1$) significance of differences between mean values of IEA in experiments with and without incubation.

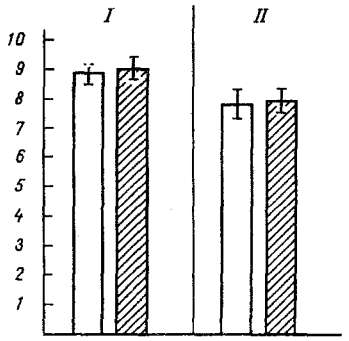


Fig. 1. Changes in free heparin level in deprothrombinized, platelet-deprived plasma after incubation with RBC (M ± m, n = 26). I) Without addition of calcium chloride; II) with addition of calcium chloride. Unshaded column – control; shaded column – experiment. Ordinate, free heparin in plasma (in sec).

any possible effect of platelet factor 4.

The thrombin time and free heparin were determined by the known method [10]. The effect of RBC on the plasma (fibrinogen) thrombin time was estimated on an equal volume of ingredients of the clotting mixture (0.1 ml). The ability of the RBC to adsorb endogenous heparin from plasma was judged from the change in the concentration of free heparin (and of heparinoids with anticoagulant activity) in the plasma after incubation with RBC. The ingredients of the incubation mixture (plasma, 0.05 M calcium chloride in isotonic NaCl solution, a suspension of RBC in a constant concentration of 4 million cells/$mm^3$, hematocrit index 37) were taken in equal volume (1 ml). The incubation mixture was kept on a water bath at 37°C for 10 min and then freed from RBC by centrifugation. In the control, isotonic NaCl solution was added to the plasma in a volume equal to its concentration in the volume of RBC suspension taken.

As in previous investigations [1, 2], to facilitate the comparative analysis of the data, besides absolute values a relative index – the index of erythrocytic activity (IEA) – was calculated as the ratio between the indices (thrombin time of plasma and fibrinogen, free heparin time in the plasma) in absolute values and the values of the corresponding index after addition of RBC to the plasma (fibrinogen).

The 26 subjects studied included ten healthy people aged 20-46 years (mean 30.0 ± 2.86 years) and 16 patients with various diseases of the internal organs, aged 21-67 years (mean 48.7 ± 3.52 years). By carrying out the investigation on a group of mixed composition, the aim was to clarify the patterns observed in healthy subjects.

## EXPERIMENTAL RESULTS

The results of the two subgroups of experiments were identical, so that they could be analyzed together.

In the presence of RBC the transformation of the plasma fibrinogen into fibrin under the influence of thrombin took place faster (Table 1), in agreement with observations by other workers [6-9] but not in confirmation of conclusions drawn by some workers to the effect that RBC do not accelerate this process [4, 5]. Neither the addition of calcium chloride to the plasma nor an increase in the duration of contact of the plasma with RBC had any visible effect on the intensity of the accelerating action of the RBC. This follows from a comparison

of IEA in the different variants of the experiment (Table 1). This must be stressed, for in experiments with exogenous anticoagulant, not only was the presence of calcium ions necessary for manifestation of the antiheparin effect of the RBC, but the RBC had to be incubated for a certain critical time with the heparinized medium. Incubation for 10 min at 37°C (as was the case in the present experiment) was sufficient for the RBC to exhibit their antiheparin effect [14, 15].

Let us assume that there are differences in the conditions under which RBC can exhibit their antiheparin activity against exogenous and endogenous heparin, as a result of which the inhibitory effect of RBC against the endogenous anticoagulant is exhibited independently of the factors mentioned above. However, in that case also, if the shortening of the plasma thrombin time under the influence of RBC is considered in fact to reflect the antiheparin effect, a fall in the plasma level of endogenous heparin (and heparinoids) would be expected as a result of their binding with RBC. A second series of experiments was carried out to study this problem, but in none of the variants of the experiment was the endogenous anticoagulant level lowered: The free heparin time of the plasma was unchanged after its incubation with RBC (Fig. 1).

It can be concluded from the facts described above that shortening of the plasma thrombin time under the influence of RBC was unconnected with the antiheparin effect, but reflected some other effect of the RBC. In the writer's view, in this case what was found was a "fibrinoplastic" effect. This is confirmed, in particular, by the equal degree of shortening of the thrombin time of plasma (IEA = 1.26 ± 0.02; $P < 0.01$) and of fibrinogen (IEA = 1.27 ± 0.03; $P < 0.001$) under the influence of the RBC suspension. The fibrinoplastic effect of whole RBC is nonspecific and can be induced by various colloids [13]. The effect of the RBC examined above is connected in all probability with an external glycoprotein complex of the cell membrane or with plasma components adsorbed on its surface.

It can be concluded from the results of these experiments that whole human RBC do not exhibit inhibitory properties toward endogenous anticoagulant. The stimulant effect of whole RBC on thromboplastin formation discovered previously in platelet-free plasma (unheparinized!) thus fully reflects the effect of the thromboplastin factor of the cell. The data given above and an analysis of the literature provide no grounds for accepting the view that circulating RBC have a regulatory effect on the endogenous heparin level in the plasma under physiological [9, 11] and pathological [12] conditions.

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