AGGREGATION OF ERYTHROCYTES AND THROMBOPLASTIN FORMATION

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The increase in aggregation of the erythrocytes from healthy and sick persons in vitro under the influence of dextran was accompanied by changes in the procoagulant activity of the cells of the healthy persons, while in patients with no clinical manifestations of atherosclerosis an increase in the procoagulant activity of the erythrocytes was observed more frequently than a decrease; in patients with atherosclerosis no such differences were observed. The possibility of an increase in the activity of the thromboplastin factor of the erythrocytes at a time of increased aggregation of the cells suggests a phylogenetic connection between the oldest mechanism of hemostasis (aggregation of the erythrocytes) and the process of true coagulation.

Increased aggregation of erythrocytes is ascribed an important role both in hemostasis [8] and in the pathogenesis of thrombosis in connection with the secondary disturbance of the hemodynamics in the microcirculation with the development of local stasis and hypoxia, contributing to hypercoagulation [3, 5, 6].

Increased aggregation of erythrocytes reflects physicochemical changes in their membrane, which must influence its properties. The question arises as to what extent increased aggregation of the erythrocytes can influence the activity of their thromboplastin factor, which is considered to be connected with the cell membrane [1, 2] and which possesses definite functional lability.

The object of the investigation described below was to study relations between the increased aggregation of the erythrocytes and their thromboplastin activity.

EXPERIMENTAL METHOD

Increased aggregation of erythrocytes suspended in autologous plasma containing few platelets was produced by preliminary addition of dextran (mean molecular weight about 80,000) to the plasma up to a final concentration of 500, 1000, or 1500 mg%.

The degree of aggregation was assessed by an original method. The residue of erythrocytes, washed three times with isotonic NaCl solution (hematocrit 74) was added to plasma (with or without dextran) in the ratio of 1:1; the number of erythrocytes in the plasma suspension was 4.4± 0.11 million/mm^3. After uniform stirring the suspension of erythrocytes was poured into a mixer, diluted with the same plasma, agitated gently for 3 min, and a sample was then introduced into a Goryaev chamber. The number of nonaggregated erythrocytes per mm^3 was counted and the size and shape of the aggregates determined at the same time. To count the total number of erythrocytes per mm^3 of suspension, the latter was diluted with isotonic NaCl solution to reverse aggregation of the cells. Knowing the total number of erythrocytes per mm^3 of suspension and the number of cells which had not aggregated, the number of aggregated erythrocytes could be obtained.

Thromboplastin activity was determined by the prothrombin consumption method [11] in a glass tube with additional activation of the internal clotting system by Diatomite. The thromboplastin activity was de-

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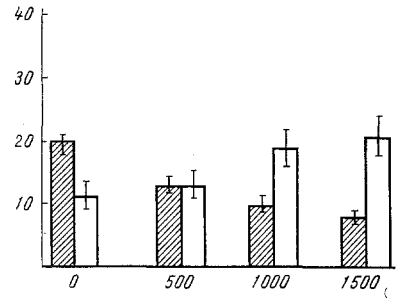

Fig. 1. Effect of dextran on aggregation of erythrocytes and on ESR. Ordinate, number of unaggregated erythrocytes (in %) and ESR (mm in 1 h); abscissa, dextran concentration in plasma (in mg%). Shaded columns represent unaggregated erythrocytes (M±m); unshaded columns represent ESR (M±m).

TABLE 1. Changes in IEA under the Influence of Dextran

Group of subjects	Overall frequency of changes in IEA (in %) for all concentrations of dextran	Character of changes in IEA for all concentrations of dextran (N %)		
		increase	decrease	no change
1	54,0	21 (33,0)	13 (21,0)	29 (46,0)
2	71,6	29 (48,3)	14 (23,3)	17 (28,3)
3	70,7	27 (36,0)	26 (34,7)	22 (29,3)

termined in plasma with a low platelet count and also after addition of erythrocytes to it. Similar investigations were carried out in the presence of dextran in the above concentration. The prothrombin consumption was measured in this way in eight samples, each of which was tested three times. The relative proportions of the ingredients of the clotting mixture were: plasma deficient in plates 0.3 ml, 1% suspension of diatomite 0.06 ml, residue of washed erythrocytes 0.3 ml, 0.05 M calcium chloride (in isotonic NaCl solution) 0.3 ml. Incubation on a water bath continued for 40 min.

After determination of the prothrombin consumption time in absolute figures the relative index, conventionally termed the index of erythrocytic activity (IEA) was calculated as the ratio

$$\frac{\text{prothrombin consumption time in plasma (with or without dextran) with erythrocytes (in sec)}}{\text{prothrombin consumption time in plasma (with or without dextran), (in sec)}}$$

Determination of IEA, reflecting changes in prothrombin consumption in plateletdeficient plasma in the presence of erythrocytes, enables the thromboplastin activity of erythrocytes in the different samples to be compared.

Tests were carried out on 66 persons of both sexes who were distributed into three groups: healthy subjects (21) with a mean age of 30.3± 1.68 years (group 1); patients with no clinical manifestations of atherosclerosis (mainly with peptic ulcer) (20), with a mean age of 30.3±1.64 years (group 2), and patients with clinical manifestations of atherosclerosis (25), mean age 60.1±1.56 years (group 3). Because of the tendency toward hypercoagulation and thrombosis in atherosclerosis, comparative investigations were deemed necessary.

EXPERIMENTAL RESULTS

Under the influence of dextran the number of aggregated cells in the suspension of erythrocytes was increased, and this was accompanied by an increase in size of the aggregates and elevation of the ESR (Fig. 1). With all concentrations of dextran the overall frequency of changes in IEA was least in the control group (Table 1). The IEA was increased more often than decreased in groups 1 and 2, as could be seen with dextran in a concentration of 1000 and 1500 mg%, while in group 3 the frequency of both tendencies was about equal.

No significant differences were found between the mean values of the increase in IEA with the different dextran concentrations in the various groups or between the overall values for each group: they all averaged 23.3%. Significant differences between the mean overall increases (26.3±3.37; $P = 0.001$) and decreases (10.2±0.9; $P = 0.001$) in IEA were observed only in group 2.

Analysis of the changes in IEA gave the following picture. The increase in IEA in all subgroups (77 observations) corresponded in most cases (62) also to an absolute increase in the prothrombin consumption time in the plasma suspension of erythrocytes containing dextran (on the average by 26%), whereas the prothrombin consumption in plasma alone after the addition of dextran remained unchanged in 46 cases. The decrease in IEA (53 observations) was connected in 20 cases with an absolute decrease in the prothrombin

consumption time in the plasma with erythrocytes in the presence of dextra, whereas the prothrombin consumption time in plasma in most of these cases (16) was unchanged or slightly increased. In the other cases (33) the reason for the decrease in IEA was the predominantly increased prothrombin consumption time in the plasma coupled with the constant prothrombin consumption time in plasma with erythrocytes, or its relatively smaller increase compared with that in plasma alone. The change in prothrombin consumption observed in the presence of dextran in plasma with erythrocytes, independent of the character of prothrombin consumption in plasma alone, is evidence in support of a change in the thromboplastin activity of the erythrocytes under these circumstances. These group differences evidently reflect characteristics of both erythrocytes and plasma.

Turning to data in the literature on the effect of dextran on procoagulant activity of the blood cells, it must be pointed out that this has been studied only with respect to platelets. The decrease in activity of factor III observed under these circumstances did, however, depend on the duration of incubation of the platelets with dextran, on its mean molecular weight, and on the method used to investigate the thromboplastin activity [7, 10]. Results indicating an increase in the thromboplastin activity of whole blood under the influence of dextran, whereas no such effect was exhibited by blood plasma [9], are noteworthy. Although the author cited does not stress this fact, in the present writer's view it indicates a role of the blood cells in the mechanism of the stimulation of thromboplastin formation by dextran.

The results of the present investigation support such a possibility.

The increase in the intesnsity of aggregation of the erythrocytes under the influence of dextran reproduces to some extent the phenomenon also observed in vivo with an increase in the blood levels of globulins and fibrinogen [4]. The possibility therefore cannot be ruled out that the observed changes in thromboplastin activity of the erythrocytes at the time of their increased aggregation may also take place in the microcirculation in vivo.

The possibility of an increase in the activity of the thromboplastin factor of the ryethrocytes during increased aggregation of the cells suggests that aggregation of erythrocytes, the oldest mechanism of hemostasis, is linked phylogenetically with the process of true coagulation.

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