INFLUENCE OF THE INTACT ERYTHROCYTES ON THE THROMBOPLASTIC ACTIVITY OF PLASMA

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After incubation of oxalated blood at 37° for 1-2 h the erythrocytes increase the thromboplastic activity of plasma containing few platelets: the "release effect."

This is observed in healthy persons and in certain pathological states, the release effect being observed somewhat more frequently and to a more marked degree quantitatively in patients with atherosclerosis than in healthy subjects. Preliminary dilution of the blood with isotonic NaCl solution in most experiments increased the release effect.

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An increase in the prothrombin consumption in plasma containing few platelets and obtained from whole blood after incubation at 37° was first found by McKellar and Dacie [12] and later by Bradlow [6], who attributed it to the release of a phospholipid by the erythrocytes into the plasma which was capable of compensating to some extent for the deficiency in platelet thromboplastic factor.

The concept introduced by these workers is in agreement with modern data indicating the considerable lability of phospholipids of the erythrocyte membrane, which can diffuse into the surrounding medium [8-10], and it suggests that the ability of intact erythrocytes to influence thromboplastic formation may not only be manifested directly in the course of blood coagulation as a result of contact with a foreign surface [1, 4, 13], but in addition, it may be a reflection of exchange of phospholipids existing under physiological conditions between erythrocytes and plasma.

The objects of these investigations were as follows: to make a comparative study of the ability of erythrocytes of incubated blood to increase the thromboplastic activity of plasma deficient in platelets in healthy persons and in certain pathological states; to analyze the relationship between this phenomenon and the relative volumes of erythrocytes and plasma (the hematocrit index), the morphological and functional properties of the erythrocytes (diameter, volume, number of reticulocytes, resistance to acid hemolysis), the biochemical composition of the plasma (total protein, ratio between the globulin fractions, fibrinogen, cholesterol), and also one of the indices of physicochemical relationships between erythrocytes and plasma, namely the erythrocyte sedimentation rate (ESR); and to study the possibility of activating this property of the erythrocytes to release into the plasma a factor with thromboplastic activity.

EXPERIMENTAL METHOD

Oxalated blood was incubated on a water bath at 37° in silicone-treated test tubes for 1-2 h. Plasma containing few platelets was then obtained from it; its thromboplastic activity was compared with that of plasma containing the same number of platelets but prepared from nonincubated blood. Determination of the thromboplastic activity of plasma deficient in platelets and obtained from incubated plasma rich in platelets was used as the control.

Thromboplastic activity was assessed by the consumption method [3]. To rule out the possibility of any effect of partial destruction of the erythrocytes on plasma thromboplastic activity, the hemoglobin concentration in the plasma was determined [5] before and after incubation of the blood. The method of obtaining blood and plasma containing different numbers of platelets was described previously [1].

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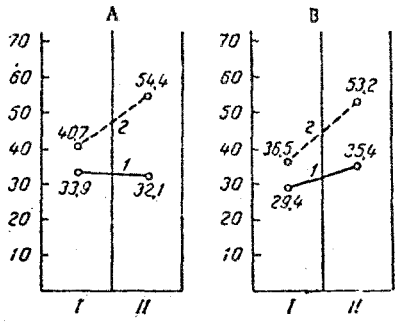

Fig. 1. Activation of release effect under the influence of preliminary dilution of the incubated blood (plasma) with isotonic saline. A) Appearance of release effect; B) intensification of existing release effect. Ordinate: prothrombin consumption time (in sec); abscissa: control (I), experiment (II). 1) Before dilution; 2) after dilution.

The acid resistance of the erythrocytes was determined by the method of Gitel'son and Terskov [2]. The indices of the normal erythrogram were obtained from the results of tests carried out on 24 healthy persons of both sexes.

The investigations were carried out on ten healthy persons (control group) and 42 patients, of whom 22 had atherosclerosis, 13 had diffuse chronic diseases of the kidney (ten had chronic diffuse glomerulonephritis) and 7 patients with various diseases constituted a mixed subgroup. It seemed logical to carry out such investigations on patients with atherosclerosis, because in this disease lipid metabolism is disturbed and there is an increased tendency toward thrombosis. In patients with kidney diseases the plasma indices which were studied showed considerable abnormalities, and their effect could therefore be studied. The results were analyzed by statistical methods.

EXPERIMENTAL RESULTS

We conventionally described the ability of erythrocytes to increase the plasma thromboplastic activity as the "release effect."

In the control group this effect was observed in three persons, and was shown by an increase in the prothrombin consumption time on the average by 16.7%. Of the 42 patients, a release effect was found in 18: in 10 patients with atherosclerosis, in 5 with diseases of the kidney, in 2 with posthemorrhagic anemia, and in one with chronic pneumonia. The release effect was observed rather more frequently in patients with atherosclerosis than in healthy subjects, and quantitatively it was more marked, with a mean value of 26%.

When the relationship between the release effect and the hematologic indices was analyzed (irrespective of the character of the disease), the following results were obtained. Whereas with changes in the hematocrit index no effect was found on the release effect, an increase in the content of young erythrocytes (reticulocytes) in the blood produced this effect. For instance, of eight patients with a high reticulocyte count in the blood (including five with a normal erythrocyte count), a release effect was observed in six patients, in agreement with Bradlow's observations [6].

The study of the distribution of erythrocytes by diameter (Price–Jones curve) revealed an increase in the content of macrocytes in patients with a release effect, and also an increase in their mean erythrocyte volume compared with that in the subjects of the other subgroup. The differences, however, were not statistically significant.

Among the biochemical indices, a significant decrease in the plasma fibrinogen level (0.25 g%) was found in the patients with a release effect compared with those in whom this effect was absent (0.32 g%). These differences occurred in patients with a normal blood erythrocyte count, including patients with atherosclerosis.

No relationship was found between the release effect, the total protein concentration, the relative proportions of the globulin fractions, and the ESR.

The acid erythrogram showed a decrease in the proportion of highly resistant erythrocytes (14.9%) in patients with a release effect compared with healthy subjects (22.48%), evidently in association with a change in the lipid content in the erythrocyte membrane.

Since the rate and direction of movement of individual phospholipids (from erythrocyte to plasma or vice versa) in the process of dynamic exchange of lipids (phospholipids) between erythrocyte and plasma are dependent, among other factors, on the quantitative ratio between phospholipids bound to the cell membrane and free in the plasma, we put forward the working hypothesis that a decrease in the concentration of phospholipids in the plasma must lead to an increase in the release effect. For this reason, in the second part of the investigation the experimental method was modified so that in each case oxalated blood was incubated parallel with blood preliminarily diluted with physiological saline (on the basis of the hematocrit index, the plasma was diluted twice).

Altogether 26 experiments were carried out (on 10 healthy subjects and 16 patients), in most of which activation of the release effect was observed, as shown by the appearance of a release effect in response to dilution in 9 persons and to a quantitative increase in this effect in 10 cases (Fig. 1).

Dilution of the blood (plasma) with no additional treatment was accompanied by an increase in thromboplastic activity of the plasma irrespective of the number of platelets it contained, a result considered to be due to lowering of the activity of heparin, the physiological anticoagulant, which is highly sensitive to dilution [11]. We observed that this occurred in 23 of 26 experiments with dilution; it was therefore necessary to discover whether the increase in the release effect after dilution is due primarily to a decrease in anticoagulant (heparin) activity.

Evidence against this hypothesis was given, however, by the fact that a release effect could be obtained in the three experiments described above in which there was no decrease in anticoagulant activity in response to dilution, the absence of a release effect in five persons whose plasma reacted to dilution by a decrease in anticoagulant activity, and finally, absence of correlation between the degree of the release effect and the degree of lowering of the anticoagulant activity of the plasma on dilution, indirectly reflecting the initial anticoagulant level.

From analysis of our own findings and those reported in the literature it can be postulated that erythrocytes play an important role in the formation, and possibly in the regulation, of the plasma thromboplastic activity.

The activating effect of dilution of the plasma on the property of erythrocytes to release a factor (phospholipid) with thromboplastic activity into the plasma, mentioned above, does not rule out the possibility of a similar situation taking place in vivo, for example, after massive blood loss as a result of the entry of a large volume of tissue fluid into the blood stream.

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