# COAGULATORY AND FIBRINOLYTIC PROPERTIES OF ERYTHROCYTES IN RABBITS WITH EXPERIMENTAL ATHEROSCLEROSIS

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In rabbits with hypercholesteremia the thromboplastic activity of the erythrocytes was lowered and their total antifibrinolytic activity increased. Changes in the latter were due mainly to an increase in the content or activity of fibrin-stabilizing factor.

Human and animal erythrocytes are known to contain substances with a definite effect on the coagulation of blood and fibrinolysis [5, 9, 12, 22]. The suggestion has been made that these substances may be liberated not only by disintegrating, but also by intact erythrocytes [3, 13]. Erythrocytes must be considered to play a much more important part in thrombosis than has hitherto been accepted.

In atherosclerosis there are usually considerable changes in the activity of the plasma factors of the blood-clotting system. Definite abnormalities are found also in the functional state of the platelets [8, 17]. There is little information on the erythrocytes in this connection in the literature. In patients with atherosclerosis some increase in the procoagulatory activity of the erythrocytes has been found [3,4]. A similar phenomenon has also been observed in hypertensive patients [11].

The object of the present investigation was to study the coagulatory and fibrinolytic properties of the erythrocytes in rabbits with experimental atherosclerosis.

## EXPERIMENTAL METHOD

Experiments were carried out on 128 chinchilla rabbits, of which 58 formed the control and 70 the experimental series. The experimental rabbits received cholesterol with their food in a dose of 0.4 g/kg body weight daily for 4-12 months. Unwashed erythrocytes, erythrocytes washed free from plasma, hemolyzates, and their stromas were investigated. The erythrocytes were washed 5-6 times with 0.1 M sodium oxalate solution and twice with physiological saline. The number of leukocytes present was 120-130/mm$^3$. Hemolyzates were obtained by repeated freezing and thawing. The stromas of the erythrocytes were separated by centrifugation at 7,000 rpm and washed 7 or 8 times with distilled water until the benzidine test with the washings was negative or very slightly positive. The washed stroma was resuspended in physiological saline in a volume equal to the volume of the erythrocyte suspension used to prepare the hemolyzate.

The coagulatory (thromboplastic) activity of the erythrocyte samples was determined from the shortening of the recalcification time of fresh platelet-free plasma of healthy rabbits after addition of the test material. The result was expressed as a percentage of the control – the clotting time of the plasma without samples of erythrocytes. Thrombin (second platelet factor) and antithrombin accelerators were determined from the change in thrombin time of platelet-free bovine plasma substrate on addition of the erythrocyte material to it. Thrombin was used for this purpose in a strictly constant concentration (1 unit/ml).

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TABLE 1. Coagulatory and Fibrinolytic Factors of Erythrocytes of Healthy Rabbits and Rabbits Receiving Cholesterol

| Index | Group of rabbits | Unwashed erythrocytes | | | Washed erythrocytes | | | Hemolyzate | | | Stroma | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *n* | *M*±*m* | *P* | *n* | *M*±*m* | *P* | *n* | *M*±*m* | *P* | *n* | *M*±*m* | *P* |
| Thromboplastic activity in %[1] | Control | 28 | 53,19±1,81 | <0,01 | 29 | 49,36±2,08 | <0,001 | 41 | 52,38±2,31 | <0,001 | 37 | 55,50±1,99 | <0,01 |
| | Experimental | 27 | 43,63±2,44 | | 29 | 35,32±2,72 | | 51 | 36,19±2,27 | | 49 | 47,56±1,87 | |
| Second platelet factor (thrombin accelerator, in sec) | Control | 9 | 3,44±0,30 | — | 9 | 4,66±0,23 | >0,05 | 19 | 11,00±0,65 | >0,5 | 24 | 4,46±0,55 | >0,2 |
| | Experimental | 9 | 3,44±0,57 | | 9 | 3,77±0,40 | | 19 | 10,53±0,59 | | | 3,50±0,51 | |
| Antithrombins | Control | 9 | 0 | | 9 | 0 | | 19 | 0 | | 24 | 0 | |
| | Experimental | 9 | 0 | | 9 | 0 | | 19 | 0 | | 30 | 0 | |
| Antiheparin factor (in %) | Control | 27 | 43,85±4,21 | <0,01 | 26 | 55,81±3,73 | >0,1 | 25 | 44,11±2,51* | >0,1 | 34 | 62,12±2,47 | >0,1 |
| | Experimental | 16 | 60,09±3,09 | | 16 | 63,43±2,99 | | 25 | 49,54±2,22* | | 28 | 67,71±2,91 | |
| Plasmin | Control | 27 | 0 | | 26 | 0 | | 30 | 0 | | 32 | 0 | |
| | Experimental | 28 | 0 | | 16 | 0 | | 31 | 0 | | 34 | 0 | |
| Plasminogen activator | Control | 27 | 0 | | 24 | 0 | | 30 | 0 | | 32 | 0 | |
| | Experimental | 17 | 0 | | 16 | 0 | | 31 | 0 | | 34 | 0 | |
| Plasminogen pro-activator | Control | 12 | 0 | | 12 | 0 | | 14 | 0 | | 12 | 0 | |
| | Experimental | 12 | 0 | | 12 | 0 | | 14 | 0 | | 12 | 0 | |
| Antifibrinolytic activity (index) | Control | 34 | 2,61±0,17* | <0,05 | 33 | 2,08±0,11* | <0,01 | 56 | 2,35±0,11* | <0,001 | 47 | 1,41±0,06 | <0,01 |
| | Experimental | 37 | 3,24±0,27* | | 35 | 2,62±0,15* | | 72 | 2,97±0,13* | | 54 | 1,70±0,08 | |
| Antiplasmins (in %) | Control | 21 | 43,79±10,85 | >0,5 | 18 | 0 | | 33 | 0 | | 28 | 0 | |
| | Experimental | 16 | 40,62±9,37 | | 15 | 0 | | 24 | 0 | | 27 | 0 | |
| Antiactivators (in %) | Control | 11 | 87,64±8,06 | >0,5 | 11 | 76,27±9,19 | >0,5 | 18 | 80,61±5,23 | >0,2 | 18 | 27,06±3,97 | >0,5 |
| | Experimental | 13 | 84,38±7,54 | | 12 | 77,00±10,10 | | 21 | 88,24±6,92 | | 20 | 24,90±4,00 | |
| Fibrinase (index) | Control | 33 | 1,46±0,05 | <0,01 | 38 | 1,52±0,05 | <0,01 | 56 | 1,80±0,06 | <0,001 | 48 | 1,03±0,02 | >0,5 |
| | Experimental | 45 | 1,73±0,07 | | 46 | 1,86±0,10 | | 69 | 2,13±0,06 | | 69 | 1,07±0,02 | |

* Additional dilution of sample 1 : 50.

Determinations were also made of plasmin, plasminogen activator and proactivator [19], total antifibrinolytic activity [16], antiheparin activity [20], the concentration of antiplasmins and antiactivators [16], and fibrinase [6].

## EXPERIMENTAL RESULTS

A sharp increase in the concentrations of cholesterol (654.73±36.00 mg%), phospholipids (275.07±14.73 mg%), and β-lipoproteins (1360.68±110.87 mg%) was found in the plasma of the rabbits receiving cholesterol by comparison with the control group. The corresponding figures for the control animals were: 51.62±2.58($P < 0.001$), 105.94±4.74 ($P < 0.001$), and 112.39±4.77 mg% ($P < 0.001$)

The total coagulatory activity of the erythrocyte samples, which is determined mainly by the content of thromboplastic factor in the erythrocytes, was lowered in the experimental rabbits (Table 1). No antithrombins were detected in the erythrocytes. The content of the second platelet factor (thrombin accelerators) in the erythrocytes of rabbits receiving the atherogenic diet was approximately the same as in the erythrocytes of the control rabbits. No statistically significant deviations likewise were found in the indices of antiheparin activity.

The rabbit's erythrocytes contained no plasmin or plasminogen activator and proactivator. Antifibrinolytic substances inhibiting the phase of plasminogen activation (antiactivators) and fibrino-stabilizing factor were found in them. The erythrocytes of rabbits receiving cholesterol had a stronger antifibrinolytic action than the erythrocytes of the control animals. This was observed when erythrocytes washed to remove plasma, hemolyzates, and stroma were tested. The increased antifibrinolytic activity of the erythrocyte substrate of the experimental animals was due to its more marked fibrin-stabilizing property. No significant deviations in the content of antiactivators were found. No antiplasmins were found in the erythrocyte fractions, except from unwashed erythrocytes. The antiplasmin activity of the latter was due to the plasma present as an impurity, for plasma has a high content of antiplasmins [2, 15].

A decrease in thromboplastic activity and an increase in fibrin-stabilizing activity of the erythrocytes were thus found in rabbits fed for long periods with cholesterol.

It is interesting to note that frequently analogous changes are found in the blood plasma of rabbits with cholesterol atherosclerosis: a decrease in the coagulatory and fibrinolytic activity and activation of fibrinase [7, 15]. Similar changes have also been observed in the platelets [18] and in the tissues of blood vessel walls [1, 14]. In experimental atherosclerosis similar changes in the biochemical substrate evidently take place in the blood plasma and cells and in the arterial wall, and they give rise to similar changes in the coagulatory and fibrinolytic potential. The biochemical nature of these changes has not yet been discovered. Accumulation of β-lipoproteins and a change in the composition of the phospholipids are possible causes of this phenomenon. There are reports in the literature that β-lipoproteins have a marked antifibrinolytic action [10, 23]. Usually considerable changes take place in the phospholipids in atherosclerosis and, in particular, there is an increase in the content of sphingomyelin, which is known to possess antithromboplastic properties [21, 24]. The possibility cannot be ruled out that the changes observed in the coagulatory and fibrinolytic activity of the erythrocytes in experimental atherosclerosis may also be due to other causes, which require clarification.

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