Time Course of Erythrocyte Aggregation in Various States of the Animal Organism

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The ability of proteoglycans to act as factors of steric exclusion of cells from the space filled with these biopolymers in tissues and solutions, to concentrate them in a certain volume, and to prevent dispersion of the formations emerging during this process is among the most distinguishing of their biological functions [3,4,7,12,13]. Studies on steric exclusion mediated by these biopolymers with the use of an erythrocyte suspension as a model of isolated cells have revealed that proteoglycans realize nonspecific aggregation of these formed elements both in saline and in their natural medium, blood plasma, with some differences determined by the composition of these liquid phases [6,7].

We thought it interesting to investigate in this connection the possibility of proteoglycan participation in erythrocyte aggregation in circulating blood, because in health the proteoglycan plasma concentration is extremely low, whereas during erythrocyte aggregation their levels increase for one reason or another. For this reason we examined the ratio between the plasma concentration of hexosamine-containing biopolymers and erythrocyte aggregation in rabbits during pregnancy, malignant growth, and experimental sepsis.

MATERIALS AND METHODS

Outbred rabbits weighing 2.0 to 2.5 kg were used in the experiments, eight to ten animals in each series. Blood from females fertilized in the vivarium was collected every 3-4 days over the entire course of pregnancy. In experiments with malignant tumor growth only males were used; a suspension of preminced Brown-Pierce carcinoma cells in 0.15 M NaCl solution was injected into the testicles. Blood for analysis was collected 2-3 days before the animals' death. Sepsis was induced by subcutaneous injection of 1 ml of *Str. haemolyticus* (Group A, type 27, strain 7) suspension (1×10^3 cells) in 0.15 M NaCl solution without hyaluronidase. After the animals' death at the end of the experiment bacteriological analysis of organs and tissues was carried out which detected *Str. haemolyticus* in the blood, liver, spleen, and other organs. Blood sampling was performed every 24 h. The plasma hexosamine concentration and erythrocyte aggregation in the blood were measured before the experiments in all the animals. Animals with an already increased erythrocyte aggregation occurred among those selected for the experiments; they were left as controls, and similar blood analyses were carried out in them.

The plasma hexosamine concentration was determined after hydrolysis in 2.5 N HCl [1]. Quantitative assessment of erythrocyte aggregation was carried out in fresh citrate blood [2]. In some experiments the total nitrogen content was measured by Kjeldahl's micromethod in the serum precipitate forming in the presence of trichloroacetic acid and including all serum protein components and proteoglycan complexes forming with them under such conditions [4].

RESULTS

The experiments with pregnant rabbits showed the plasma hexosamine concentration to be within the

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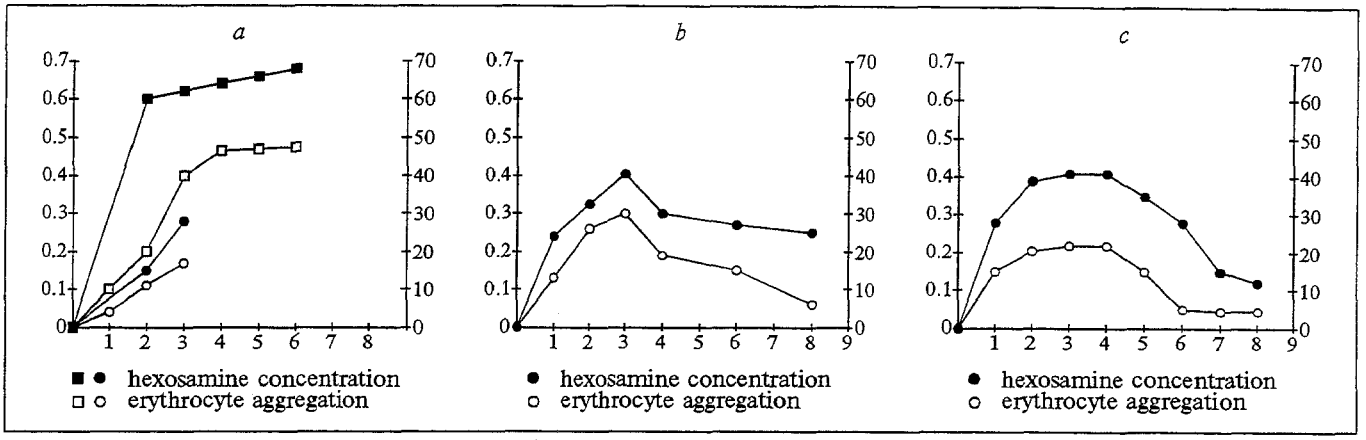

Fig. 1. Changes of hexosamine concentration and erythrocyte aggregation in circulating blood plasma of rabbits infected with *Str. haemolyticus*. Abscissa: time elapsing after infection, days; ordinate (left): changes in plasma hexosamine concentrations (mg×ml^{-1}); (right): increase of plasma volume above aggregated erythrocytes, % of total volume of blood collected. *a*: These rabbits died 3 and 6 days after infection. *b* and *c*: These rabbits did not die after infection.

normal range over the entire pregnancy term and no erythrocyte aggregation in the blood. The same was seen in rabbits with carcinomas starting from the onset of malignant growth until death of the animal.

A marked rise of the plasma hexosamine concentration and a simultaneous onset of erythrocyte aggregation in the blood were observed as soon as 24 h after rabbit infection with *Str. haemolyticus*. Typical curves reflecting the time course of plasma hexosamine concentrations and intensity of erythrocyte aggregation in the blood of some rabbits infected with *Str. haemolyticus* are presented in Fig. 1. A comparison of changes in the above values (Fig. 1) with the relationship between the erythrocyte aggregation and hexosamine concentration in the same coordinate system for all experimental rabbits (Fig. 2) revealed a general quantitative relationship between hexosamine concentration and erythrocyte aggregation despite some variations in these values in some animals. During the increase of the initially low blood hexosamine concentration, the rate of erythrocyte aggregation linearly depends on these concentrations in all the rabbits, whatever the outcome of the infection. Later these parameters increase and remain high for some time. Then, before death the hexosamine concentration may sharply rise, whereas the erythrocyte aggregation level is unchanged. In rabbits not dying of the infection the hexosamine concentrations and erythrocyte aggregation levels drop after they reach their peak, the erythrocyte aggregation level falling more rapidly than the hexosamine concentration. One more difference between rabbits dying from the infection and survivors is that the total nitrogen content in the serum protein sediment is significantly increased as compared to the normal value in the former and is within the normal range in the latter (Table 1).

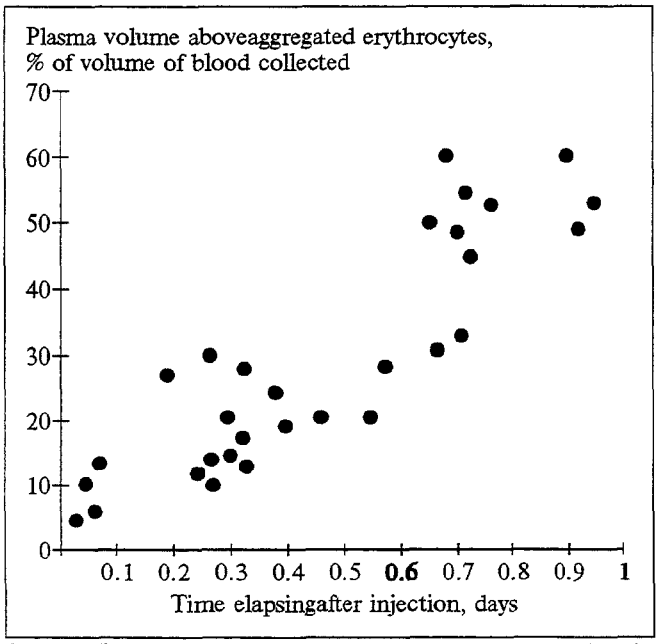

Fig. 2. Relationship between erythrocyte aggregation in circulating blood and plasma hexosamine concentration in 10 rabbits infected with *Str. haemolyticus*. The data refer only to the period when these parameters were increased (see text).

In rabbits without apparent disease but with an elevated erythrocyte aggregation level in the blood the changes in plasma hexosamine concentrations and erythrocyte aggregation had a parallel course. Two such rabbits died without their hexosamine concentrations or erythrocyte aggregation level dropping and the rest survived with these parameters reduced to normal values.

It is evident that the development of a number of pathological processes of an infectious origin is paralleled by synchronous changes in plasma hexosamine concentration and erythrocyte aggregation intensity in the blood. If the hexosamine concentrations are unchanged, aggregation is not observed.

TABLE 1. Total Nitrogen (mg×ml^{-1}) Content in Blood Serum of Rbbits before and after Experimental *Str. haemolyticus* Infection

Rabbit №	Duration of experiment, days	Nitrogen content		Changes, % (reduction)
		before infection	at end of experiment	
1	2	10.0	6.6	34.0
2	2	8.3	6.3	24.0
3	3	10.1	4.4	56.0
4	6	9.7	5.0	48.0
5	7	11.4	10.0	12.0
6	8	11.0	10.7	–
7	8	9.3	10.0	–
8	7	11.8	11.0	–

Note. Rabbits Nos. 6, 7, and 8 did not die after *Str. haemolyticus* infection. The rest died as shown in the table.

Hence, we may conclude that hexosamine-containing biopolymers are the principal factor determining erythrocyte aggregation in the blood.

Such biopolymers are mainly represented in the blood plasma of rabbits and other animals by glycoproteins that are unable to mediate steric exclusion. That is why an increase of the plasma concentrations of hexosamines in some cases may not be the result of increased glycoprotein concentrations. In health blood plasma contains very low concentrations of such proteoglycans as hyaluronic acid and protein-chondroitin-keratin-sulfate (PCKS), characterized by a marked capacity for steric exclusion [8,11]. Our findings permit us to assume that the increase of hexosamine concentration in the plasma of rabbits infected with *Str. haemolyticus* and in some other cases is due to the appearance of hyaluronic acid or PCKS or both into the bloodstream. In such a case the reduced nitrogen content in the serum protein precipitate and high serum hexosamine concentrations in rabbits dying of sepsis cannot be explained solely by a possible hypoproteinemia. If the serum contains hyaluronic acid of PCKS, then, upon entering the precipitates, they reduce the nitrogen content in them, for these proteoglycans contain much lower levels of this element than protein substances. In serum precipitates of surviving rabbits whose blood plasma hexosamine concentrations are close to the normal ones the nitrogen content is the same as before infection, this being in line with our hypothesis.

Comparison of relationship between erythrocyte aggregation in the blood of infected rabbits and concentrations of hyaluronic acid and PCKS at the peak of aggregation and the similar relationship during erythrocyte aggregation in saline has shown this relationship to be linear in both cases. Model experiments with erythrocyte aggregation have demonstrated that besides hyaluronic acid, PCKS, and other proteoglycans performing their major function, many other factors and substances contribute to this process. The combined effect of hyaluronic acid and PCKS on erythrocyte aggregation is greater than their individual effects in the same concentrations [7]. Blood plasma albumins and (α+β)- and γ-globulin fractions reduce the effect of hyaluronic acid and enhance that of PCKS as steric exclusion factors [6]. Heparin fractions, one of which contains three (HP-3) and the other four (HP-4) sulfuric acid residues per replicative disaccharide structural macromolecule unit, in some concentration suppress hyaluronic acid aggregating effect. A similar PCKS effect is enhanced by HP-3 and HP-4 in relatively low PCKS concentrations, whereas in higher concentrations the effect of HP-4 is suppressive. When HP-3 and HP-4 are present together, they do not influence PCKS action as an erythrocyte aggregation factor [5]. And, one more point, the erythrocyte aggregation rate increases as the cell concentration in the liquid phase decreases.

The contribution of these mechanisms to the erythrocyte aggregation process realized by hyaluronic acid and PCKS evidently makes the relationship between the aggregation rate and the concentration of these proteoglycans more intricate than it is without these mechanisms. We have already mentioned that in rabbits surviving a streptococcal infection the erythrocyte aggregation rate was at first linearly related to the hexosamine concentration and after aggregate dispersion this concentration was still rather high for some time, this undoubtedly being due to mechanisms suppressing the effects of hexosamine-containing biopolymers as steric exclusion factors. This explanation is in line with the fact that, once in the circulating blood, hyaluronic acid and PCKS are absorbed from it extremely slowly [9-11].

Thus, proteoglycans appear in the blood of animals in a number of diseases; circulating in the blood, these proteoglycans show their characteristic steric exclusion property manifested by erythrocyte aggregation. It is possible that low plasma concentrations of hyaluronic acid and PCKS represent a certain constant of the body's internal medium, because even a slight increase of the plasma concentrations of these proteoglycans may interfere with the physiologically vital dispersion of erythrocytes in the blood.

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Comparison of the Antioxidant Activity of Carnosine in Different Chemical and Biological Models

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The antioxidant activity of carnosine has been demonstrated in several laboratories by different methods [2,3,9-11]. However various authors have obtained diverse values of carnosine efficiency as an antioxidant [4,8,10,11], which could be due to various sources of carnosine preparations differing in purity, as well as to differences in methodology. For this reason we compared the efficacy of carnosine manufactured by Sigma and Serva with that obtained by column chromatography from meat extract at a Russian drug factory. The comparison was performed with the use of different chemical and biological models.

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MATERIALS AND METHODS

Different methods of inducing free-radical reactions were used to compare the interaction of carnosine with different active forms of oxygen: chemical reactions (interactions with H_2O_2, ClO^-, and OH formed in the Fenton reaction); model processes in which superoxide anion formation in the biological structures was induced by iron ions; and free-radical generation in leukocyte suspension provoked by $BaSO_4$ (mainly O^-_2 and ClO^- and, to a lesser extent, $OH^\bullet$).

The products of carnosine interaction with active forms of oxygen in chemical reactions were measured spectrophotometrically (Hitachi-557); in model processes malonic dialdehyde was measured as the major thiobarbituric acid reaction product whose formation was induced by the addition of Fe^{2+} ions to rat

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