5. Yu. I. Lisunkin, *Farmakol. Toksikol.*, № 2, 175-180 (1961).
6. N. Akaike, K. Hattori, N. Inomata, and Y. Oomura, *J. Physiol. (London)*, **360**, 367-386 (1985).
7. Kam Pui Fung, *Comput. Biol. Med.*, **19**, 131-135 (1989).
8. W. Loscher, *Antiepileptic Drugs* (H.-H. Frey and D. Janz, Eds), Berlin (1985), pp. 507-536.
9. R. L. MacDonald, M. G. Weddle, and R. A. Gross, *Transmission and Anxiety* (G. Biggio and E. Costa, Eds), New York (1986), pp. 67-78.
10. R.W. Olsen, *Ann. Rev. Pharmacol. Toxicol.*, **22**, 245-277 (1982).
11. R.W. Olsen and A. Snowman, *J. Neurosci.*, **2**, 1812-1823 (1982).
12. R.E. Study and J.L. Barker, *Proc. Nat. Acad. Sci. USA*, **78**, 7180-7184 (1981).
13. R.E. Study and J.L. Barker, *J.A.M.A.*, **247**, 2147-2151 (1982).

BIOCHEMISTRY AND BYOPHYSICS

Effect of Proteoglycans on Erythrocytes in the Circulating Blood

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Among the various biological functions of proteoglycans (PG) in the animal organism, the capacity of these biopolymers to ensure spatial exclusion and cell concentration in a definite volume, preventing their dispersion, is of great importance for the organism [3,6,10,12]. The best studied in this respect are hyaluronic acid (HA), protein-chondroitin-keratansulfate (PCKS), and PG aggregates represented by natural complexes of HA, PCKS, and binding protein [4-6]. These are the very PG used in the modern treatment of various diseases [6,7].

In this connection it is important to consider the correlation between the therapeutic effect of the above mentioned biopolymers and their activity as factors of spatial exclusion. One of the approaches to this problem could be a study of the effect of HA and PCKS introduced into the blood flow on the erythrocyte (Er) spatial exclusion, i.e., aggregation. This report presents the results of such a study. The need for such studies is further dictated by the fact that they make it possible to compare the results of model experiments on the mechanism of PG-mediated cell spatial exclusion [4,7] with the data obtained at the level of the organism.

MATERIALS AND METHODS

Sodium salts of HA and PCKS were used in this study. Highly purified HA preparations of high polymerization were derived from human umbilical cord [2]. PCKS of similar parameters was obtained from bovine tracheal cartilage [1]. The data of analyses of

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TABLE 1. Results of Analysis of HA and PCKS Preparations (%) ($M \pm m$, $n = 6$).

Substance	Nitrogen	N – acetylglucosamine	N – acetylgalactosamine	Glucuronic acid	Sulfated sulfur	Protein calculated
HA	2,9±0,1	42,0±0,5	0,0	42,0±0,2	0,0	0,8
PCKS	4,1±0,1	3,7±0,6	22,0±0,5	24,0±0,3	4,3±0,4	15,7

these preparations are listed in Table 1. Outbred rabbits weighing 1.8-2.0 kg received intravenously HA (125 mg/kg body weight) or PCKS (250 mg/kg body weight) in 8 ml buffered saline (pH 7.4). Each experimental group contained 10 animals. Quantitative evaluation of the Er aggregation in the citrate blood was performed as described earlier [4]. Blood samples were taken during 24 hours after PG administration, first after 15 min and then at various intervals. In the control experiments the hematocrit (ratio of plasma to corpuscular volume) was evaluated following PG-free saline injection.

In similar experiments, outbred mice weighing 18-20 g received via the caudal vein 0.1 mg/g HA or 0.2 mg/g PCKS in 0.1 ml saline. Each group consisted of 25-30 animals. At certain intervals, within 15 min to 24 hours after injection, blood was taken from the abdominal artery of 5-7 mice under chloroform anesthesia and tested as described. Control measurements were performed using an equal number of uninjected and saline-injected mice.

RESULTS

In the control experiments on rabbits and mice, the administration of saline was followed by a transient (60-100 min) 10-15% reduction in the blood corpuscular volume, without any sign of Er aggregation. It is worth emphasizing here that the change in the ratio of liquid to corpuscular phase volumes in the absence of aggregation-promoting factors does not per se induce cell aggregation. Intravenous injection of HA (0.1 mg/g) into the rabbits induces a rapid fall in the kinetic stability of circulating Er, leading to the formation of aggregates. As a result, instead of an even Er distribution in the plasma, the blood undergoes separation into two phases, one consisting of plasma and the other of Er aggregates. Thirty minutes after HA administration, the phases reach equal volume. Aggregation slowly decreases 8 hours later, and total recovery of the dispersed (free) state of Er takes place 24 hours following HA injection (Fig. 1).

Administration of PCKS (0.25 mg/g) also induces Er aggregation in the rabbits, but to much lesser extent than in the case of HA. The relative volume of cell-free plasma reaches 25-30% of the total blood volume 15 min after injection and remains on that level for 4 hours, after which time a slow recovery sets in. The final dispersal of Er aggregates can be observed 24 hours following the PCKS challenge (Fig. 1).

The experiments with HA and PCKS carried out on mice yielded analogous results.

The long-term stability of Er aggregates formed in the circulating blood following injection of HA or PCKS shows that these PG are able to fully realize their effect as agents of spatial exclusion of the cells

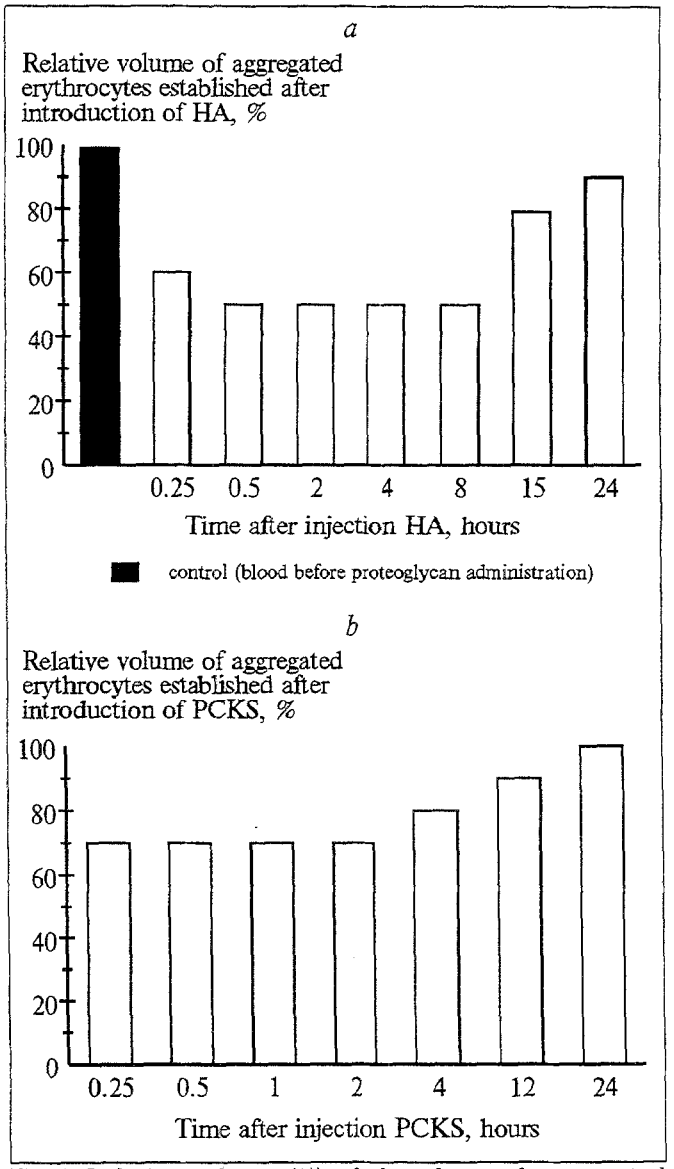

Fig. 1. Relative volume (%) of the phase of aggregated erythrocytes to the total blood volume in rabbits following intravenous injection of HA (*a*) and PCKS (*b*).

in the given environment. As a result, Er aggregated in the circulating blood partially lose their individuality, a factor which may affect their functional capacities.

One should keep in mind that there are mechanisms promoting or reducing the spatial exclusion-mediating action of HA and PCKS. Depending on the ratio of the concentrations of different plasma proteins, the action of HA and PCKS can be enhanced or inhibited by heparin fractions [5] containing three and four H_2SO_4 residues per disaccharide unit of macromolecule, respectively. Heparin fractions do not produce spatial exclusion by themselves, but neutralize the action of HA. Taken separately, each fraction given in low doses activates PCKS-produced Er aggregation and in increasing doses inhibits Er aggregation. However, the combination of fractions in the mentioned ratio has no effect on PCKS activity [4]. As the aggregated state of Er in the circulating blood remains on the same level for a long time after HA and/or PCKS administration, and then decreases slowly and smoothly, it follows that the mentioned mechanisms influencing Er aggregation are not recruited under the given experimental conditions.

The dissolution of Er aggregates in the circulating blood evidently occurs perhaps as the result of HA and PCKS clearance from the plasma, which proceeds slowly [7-9]. After injection into the blood flow of rabbits and mice, HA and PCKS are gradually cleared from the plasma and accumulate in the liver, spleen, bone marrow, and lymph nodes, and to a much lesser extent in the skeletal and heart muscles. The decomposition of HA and PCKS takes place mostly in the liver and spleen [7-9,11,13]. Total clearance of HA and PCKS from the plasma of rabbits and mice is completed 24 hours following their entry into the blood, this coinciding with the longevity of PG-produced Er aggregates in the circulation. This shows that HA and PCKS retain their spatial exclusion activity during the whole time of their persistence in the plasma. The fact that PCKS-produced Er aggregates dissociate slightly earlier than the HA-produced aggregates may be explained by the presence in the PCKS molecule of sulfate groups and of an increased quantity of covalently bound protein component, as compared to HA. This promotes the formation of electrovalent complexes with various tissue components and, correspondingly, the absorption of PCKS from the plasma and tissue fluids.

The coincidence in the times of entire Er dissociation and total PG clearance from the plasma provides an opportunity to determine the duration of circulation of these biopolymers using the first parameter (Er aggregate dissociation) without the direct measurement of PG concentration. Such a relationship provides additional evidence that the PG-mediated spatial exclusion of the cells and their concentration in a limited space is a physical phenomenon.

The levels of HA and, especially, PCKS in the normal plasma are constantly low [9], which is of profound biological significance. In various pathological conditions and during surgical procedures and the use of PG as drugs, these biopolymers would enter the bloodstream and first of all aggregate Er, which could lead to unpredictable (due to the shortage of information) consequences for the organism. This problem deserves further investigaton.

REFERENCES

1. S. M. Bychkov and V. N. Kharlamova, *Biokhimiya*, **33**, 840-846 (1968).
2. S. M. Bychkov and M. F. Kolesnikova, *Ibid.*, **34**, 204-208 (1969).
3. S. M. Bychkov and S. A. Kuz'mina, *Vopr. Med. Khimii*, № 1, 19-32 (1986).
4. S. M. Bychkov and S. A. Kuz'mina, *Byull. Eksp. Biol.*, **102**, № 12, 692-695 (1986).
5. S. M. Bychkov and S. A. Kuz'mina, *Ibid.*, **111**, № 6, 605-606 (1991).
6. S. M. Bychkov and S. A. Kuz'mina, *Usp. Sovr. Biol.*, **112**, 273-280 (1992).
7. A. Engstrom-Laurent, *Circulating Sodium Hyaluronate*, Uppsala (1985).
8. J. K. E. Fraser, T. C. Laurent, H. Pertoft, *et al.*, Biochem. J., 200, 415-424 (1981).
9. J. K. E. Fraser, L. E. Appelgren, and T. C. Laurent, *Cell Tiss. Res.*, **233**, 285-293 (1983).
10. K. M. Jon and S. Chis, *Microvasc. Res.*, **11**, 121-130 (1976).
11. D. Kaplon and K. Meyer, *J. Clin. Invest.*, **41**, 243-249 (1962).
12. J. E. Morris, *Exp. Cell Res.*, **120**, 141-153 (1979).
13. K. U. Wood, F. S.Wusteman, and C. G. Curtis, *Biochem. J.*, **134**, 1009-1013 (1973).