9. F.Z. Meerson and M.G. Pshennikova, Adaptive Protection of the Body: Major Mechanisms and Use for Prophylaxis and Therapy [in Russian], Moscow (1992).
10. F.Z. Meerson, S.A. Radzievskii, L.M. Giber, et al., Dokl. Akad. Nauk SSSR, **237**, No. 4, 977-980 (1977).
11. F.Z. Meerson, V.P. Tverdokhlib, and A.A. Nikonorov, Vopr. Med. Khim., No. 6, 104-109 (1988).
12. F.Z. Meerson, A.A. Ugolev, and L.Yu. Golubeva, Kardiologiya, No. 11, 91-95 (1990).
13. K. Miyoshi, S. Saito, H. Kawai, et al., J. Labor. Clin.Med., **92**, 341-352 (1978).
14. J. Skinner, in: Stress and Heart Disease, ed. by R.E. Beamish et al., Boston (1985), pp. 44-59.

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Influence of Polyhemoglobin Composition on Erythrocyte Aggregation and Circulation Time

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Investigations in the area of oxygen-carrying blood substitutes have resulted in the development of a variety of preparations meeting the principal requirements of a blood substitute, namely a sufficient intravascular persistence [1, 7] and effective oxygen transport [3, 5].

Works dealing with the circulation of such substances in an intravascular system faced major problems of toxicity [4], clearance mechanism [1, 6] as well as changes in the fractional composition of poly-Hb [2]. In addition, the concern about the effect of poly-Hb on the state of the formed elements of the blood is of great value in evaluating the optimum composition of poly-Hb solutions.

On this account the purpose of this study was to evaluate changes in the erythrocyte aggregation *in vivo* and in vitro in a model of 50% blood loss using chemically modified hemoglobin solutions with a varying content of the polymerized form.

TABLE 1. Main Characteristics of Chemically Modified Hemoglobins

Sample	Poly-Hb content,%	Oxygen affinity, mm Hg	Mn, thousand	Mv, thousand
PG-PF-1	2-3	28-30	-	-
PG-PF-2	25-30	26-31	100	90
PG-PF-3	40-45	27-29	230	200
PG-PF-4	70-75	25-28	600	650
Blood		26-29	-	-

MATERIAL AND METHODS

Hemoglobin modifications were prepared as previously described by the reaction with glutaraldehyde and pyridoxal-5-phosphate [1]. The hemoglobin concentration in the solutions and in the plasma samples as well as the methemoglobin concentration were measured on an IL 282 Co-Oximeter (Instrumentation Laboratory, USA). Compositions of samples of modified Hb were determined by high-performance liquid chromatography on a TSK-G3000 SW column (7.5 x 300 mm) (LKB, Sweden) in a 0.01 M phosphate buffer at pH 6.5; flow rate was 0.5 ml/min. Absorbance was measured at 280 nm. The percentage of poly-Hb was calculated using a 2220 Recording Integrator (Hewlett-Packard, USA). (M_n) and (M_w) were determined by sedimentation analysis using an analytical ultracentrifuge (Beckman Instruments, USA).

For a study of erythrocyte aggregation, samples of modified Hb were mixed 1:1 with samples of canine blood, and cells were counted in a Goryaev chamber: free, incorporated in aggregates, and total amount. Erythrocyte sedimentation rate was measured in capillaries by the standart technique: poly-Hb solution was added in a 1:1 volume ratio to fresh donor blood to simulate 50% blood loss compensation.

The experiments on dogs were carried out in a model of acute lethal (50 mg/kg body weight) blood

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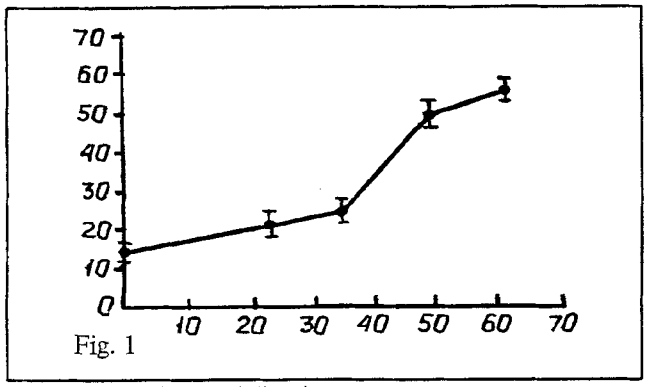

Fig. 1

Fig. 1. Effect of degree of Hb polymerization on suspension properties of blood.
Abscissa: poly-Hb content (in %). Ordinate: erythrocyte sedimentation rate (mm/h).

loss compensation using solutions of modified hemoglobin at a dose of 4 g/kg body weight. Samples were taken 10 min, 1, 2, and 4 hours after infusion. The erythrocyte aggregation in the microcirculatory system of the bulbar conjunctiva was studied with a Misura system (LOMO).

RESULTS

Samples of modified hemoglobin with different contents of poly-Hb were investigated. The main characteristics are summarized in Table 1.

As shown in Table 1, the preparations studied possess similar oxygen-affinity values; the differences observed in the content of the polymerized form are reflected in the molecular weight values. These data document that the substances developed meet the principal requirement of an artificial oxygen carrier, *i.e.*, they have oxygen release capacity similar to that of whole blood.

It was interesting to evaluate the effect of the poly-Hb content of the samples on the suspension properties of the blood. For this purpose the erythrocyte sedimentation rate was measured in capillaries using the standard technique in the model of 50% blood loss. The results obtained are presented in Fig. 1. As seen in Fig. 1, an increase in poly-Hb content from 0 to 25% resulted in a negligible and gradual increase in ESR to 25 mm/h; a further increase in poly-Hb content caused a sharp uneven ESR increase to 55-60 mm/h. A greater increase in the polymerized form content resulted in the formation of a suspension of large aggregates during the first 20 min. The differences in the two parts of the curve can be characterized by values of the angle of slope tangent" 0,28±0.08 for the gently sloping part and -21.66±0.12 for the steep part.

The results obtained on the influence of the poly-Hb content of the samples on erythrocyte aggregation are presented in Table 2.

Screening of the data obtained indicated that the formation of large aggregates depends on the poly-Hb content of chemically modified hemoglobin samples amounting to 100% in the case of the PG-PF-4 sample with 75% poly-Hb content. For this reason, only samples with a poly-Hb content up to 45%, namely PG-PF-1,2,3, were used in further study in the model of acute blood loss in dogs. As shown by testing of the microcirculatory system of the bulbar conjunctiva as well as the mesentery of dogs in the model of acute blood loss compensation using solutions if chemically modified hemoglobin under a visual control, the infusion of the PG-PF-3 sample with a 40-45% content of poly-Hb caused intensive erythrocyte aggregation. No discernible influence was found on erythrocyte aggregation compared to the pre-infusion level caused by the infusion of PG-PF-2 solution with 25-30% poly-Hb.

The study of the clearance dynamics from the intravascular system in dogs showed that, in spite of a relatively low poly-Hb content of samples, from 2-5 to 40-45%, their concentrations in the intravascular system remained on a relatively high level during the experiment, amounting to 67-73% of the quantity infused. The data obtained are presented in Table 3.

It can be concluded from the negligible decrease of the concentration of Hb-derivatives in the blood plasma, as well as the preservation of their functional full-value during the circulation time, as described previously [2, 3], that the preparations developed possess a sufficient oxygen-transport effectiveness.

The results shown in Table 3 also indicated that no significant influence was found on the intensity of

TABLE 2. Erythrocyte Aggregation as Function of the Poly-Hb Content of Samples

Sample	Number of cells, %		
	not incorporated in aggregates	incorporated in small aggregates (15-25m)	incorporated in large aggregates (50-100m)
PG-PF-1	78	22	0
PG-PF-2	53	47	0
PG-PF-3	63	18	19
PG-PF-4	0	0	100

TABLE 3. PG-PF-Content of Samples of Blood Plasma During Circulation in the Canine Intravascular System

Sample	PG-PF Concentration after infusion			
	after 2 hours		after 4 hours	
	g/dl	% of initial value	g/dl	% of initial value
PG-PF-1	3.9±0.24	80.0±7.00	3.8±0.12	68.7±1.85
PG-PF-2	4.5±0.12	86.3±5.65	4.3±0.14	62.8±4.64
PG-PF-3	4.0±0.40	88.7±8.43	4.7±0.11	72.7±4.48

sample clearance from the intravascular system due to differences in poly-Hb content.

Consequently, in this experimental model the effect on the erythrocyte aggregation is the principal criterion of the optimum fractional composition of chemically modified Hb. PG-PF-2 with 25-30% poly-Hb content is, therefore, the most promising candidate for the development of an artificial oxygen carrier, which does not cause the formation of large aggregates when injected into the intravascular system.

It can thus be concluded from the results of our investigation of a modified Hb-based blood substitute that the influence on the aggregation of the blood elements, as well as their functional full-value coupled with a sufficient intravascular persistence are the principal criteria of the optimum fractional composition of Hb-derivatives and, therefore, their compatibility.

LITERATURE CITED

1. M. A. Azhigirova, M. G. Vashkevich, N. N. Kontuganov, *et al., Byull. Eksp. Biol. Med.*, No. 4, 262-264 (1990).
2. E. P. Vyazova, M. A. Azhigirova, L. V. Fetisova, *et al., Ibid.*, No. 4, 374-376 (1991).
3. I. R. Kolonina, Yu. A. Litvinenko, and A. V. Sokolov, *Ibid.*, No. 5, 503-505 (1991).
4. E. A. Selivanov, V. N. Snabolin, I. M. Bystrova, *et al., Ibid.*, No. 4, 488-490 (1989).
5. K. L. Alsop and R. N. Condie, *Labor. Medicine*, **18**, 444-448 (1987).
6. W. K. Bleeker, P. J. Boer, J. Agterberg, *et al., Biomat. Artif. Cells Immob. Biotech.*, **19**, No. 2, 358 (1991).
7. C. J. C. Hsia, *Prog. Clin. Biol. Res.*, **319**, 339-349 (1989).

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Mechanisms of Mast Cell Modulating Effect on the Leukocyte Reaction in Inflammation

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We have earlier reported a modulating effect of mast cells (MC) on the leukocyte reaction in inflammation [1, 2]. The effect is evidently associated with many mechanisms involving the effects of a great number of transmitters released by MC.

To elucidate some of these mechanisms we studied the blockade of major mast cell products, such as histamine, serotonin, and heparin, and its effect on the functional state of neutrophils and monocytes from exudate and peripheral blood.

MATERIAL AND METHODS

Experiments were performed on 60 male Wistar rats of body weight 180-200 g. Peritonitis was induced by intraperitoneal administration of 2 billion (1/2 LD_{50}) microbial bodies of a 24-hour *E.coli* culture in 1 ml NaCl isotonic solution, which were obtained from a patient with peritonitis. Dimedrol (H_1-antagonist of histamine), cimetidine (H_2-antihistamine), cyproheptadine (anteserotonin preparation), and protamine sulfate (heparin-neutralizing agent) were used in the study. These agents were administered locally in 0.1 ml NaCl isotonic solution in the following doses: dimedrol 1 mg; cimetidine 120 μg; cyproheptadine 80 μg [4, 7, 8]. The preparations were given as a single injection 20 min to 1 hour before inflammation was induced to study the granulocyte reaction (3 h after phlogogen produced its effect) [4, 7, 8] and twice a day to study the monocyte reaction (after 3 days). In the first case protamine sulfate was administered in a dose of 0.6 mg simultaneously with the inflammatory agent and then every 30 min [5]; in the second case it was administered twice a day besides the scheme indicated. Total leukocyte number in the abdominal cavity and blood, the cellular composition of the exudate, and the leukocyte formula were calculated using standard methods. To obtain exudate the abdominal cavity was rinsed with 0.5 ml of 0.14 M NaCl containing 5 IU/ml heparin. Myeloperoxidase served as a marker of neutrophil functional activity, α-naphthylacetate esterase for that of monocyte-macrophage activity, and acid phosphatase was a marker of phagocyte lysosomes of both

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