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.

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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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types. Myeloperoxidase and α-naphthylacetate esterase in leukocytes of exudate and blood was determined cytochemically [3]; acid phosphatase in the supernatants of the peritoneal smear and blood serum was determined after Bessey-Lowry-Broke [3] on an FP-901 biochemical analyzer (Labsystem, Finland) using Lachema reagent kits (Czechoslovakia). Functional activity of granulocytes was assessed by the HCT-test [6].

RESULTS

The study of the leukocyte number in the abdominal cavity 3 h after the phlogogen effect demonstrated that dimedrol, cimetidine, and cyproheptadine produced a marked influence on neutrophil accumulation: it increased 1.7-fold with cimetidine, and 2.4- and 2.1-fold with dimedrol and cyproheptadine, respectively (Fig. 1, a). Protamine sulfate failed to exert any appreciable effect. Myeloperoxidase activity in exudate leukocytes decreased 1.6-fold with protamine sulfate and was found to be unchanged with cimetidine and cyproheptadine (Fig. 1, b). Cimetidine caused a 2.4-fold rise in the activity of acid phosphatase in the supernatants of the peritoneal smear, whereas the other preparations had no effect on it (Fig. 1, d). An increase in the indexes of the HCT-test was observed after cimetidine administration and a decrease after dimedrol (Fig. 1, e).

The assay of the leukocyte number in the exudate 3 h after inflammation onset revealed that with dimedrol, cyproheptadine, and protamine sulfate monocyte accumulation was considerably decreased by a factor of 1.9, 1.8, and 1.9, respectively, while a slight 1.3-fold increase was noted with cimetidine application (Fig. 1, a). Alfa-naphthylacetate esterase activity in the leukocytes diminished due to dimedrol and cyproheptadine and rose when cimetidine was applied (Fig. 1, c). Protamine sulfate failed to produce any notable effect. As for the activity of acid phosphatase in the peritoneal smear supernatants, it was reliably increased (2.15-fold) only with dimedrol (Fig. 1, d).

Three hours after the action of the inflammatory agent, dimedrol and cyproheptadine brought about a 2.2- and 4-fold decrease in leukocyte number, whereas no considerable changes were noted upon cimetidine and protamine sulfate administration as compared with the natural conditions of inflammation (Fig. 2, a). Cimetidine and protamine sulfate were found to significantly decrease (1.5-fold) myeloperoxidase activity in neutrophils (fig. 2. b). Acid phosphatase activity in the blood serum increased when either of the antagonists was used (Fig. 2, d). There was an increase in the HCT-test indexes with cimetidine and a decrease with dimedrol. Cyproheptadine and protamine sulfate had no reliable effect (Fig. 2, e).

Cimetidine, cyproheptadine, and protamine sulfate caused a 1.6-, 1.9-, and 2.4-fold drop in leukocyte number 3 days after the onset of inflammation versus the natural conditions of inflammation, whereas dimedrol had no perceptible effect (Fig. 2, a). Both dimedrol and cyproheptadine decreased activity of alfa-naphthylacetate esterase in the blood monocytes by a factor of 3.1 and 2, 6, respectively (Fig. 2, c). The activity of acid phosphatase in the serum was lowered only by cimetidine (Fig. 2, d).

The results of the study indicate that histamine, acting via H_2-receptors, inhibits the functional activity of neutrophils and their influx into the focus. Histamine and serotonin were found to stimulate neutrophil accumulation via H_1-receptors, and serotonin appeared to increase monocyte accumulation in the focus and the

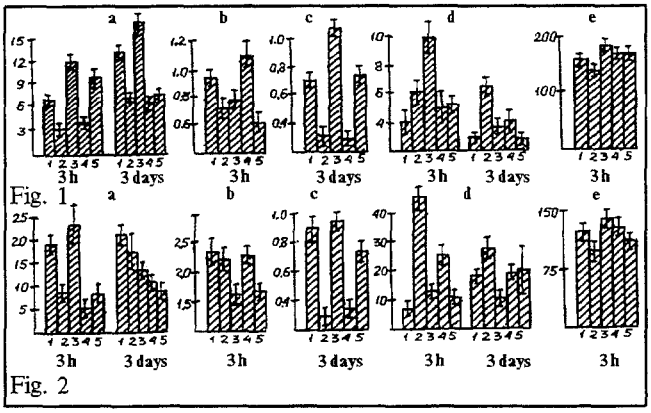

Fig. 1. Total number of leukocytes (a) in the abdominal cavity ($x10^7$); activity of myeloperoxidase (b) and alfa-naphthylacetate esterase (c) in leucocytes; acid phosohatase activitty (d) in peritoneal swear (μcat/liter); index of HCT-test (e) of rat exudate leukocytes (in conventional units) under natural conditions of inflammation (1) and due to dimedrol (2), cimetidine (3), cyproheptadine (4), and protamine sulfate (5).

Fig. 2. Total number of leukocytes (a) in blood(10^9/liter); activity of myeloperoxidase (b) and alfa-naphthylacetate esterase (c) in leukocytes (mean cytochemical coefficient) and acid phosphatase activity (d) in blood serum (μcat/liter); indexes of HCT-test (e) in rat blood leukocytes (in conventional units) under natural conditions of inflammation (1) and due to dimedrol (2), cimetidine (3), cyproheptadine (4), and protamine sulfate (5).

activity of alfa-naphthylacetate esterase in monocytes. Histamine produced the opposite effect acting via H_2-receptors. Heparin also promoted monocyte accumulation in the focus. Histamine (via H_2 receptors) and heparin reduced functional activity of blood neutrophils, producing an effect on the H_1-receptors; histamine, as well as serotonin, stimulated blood monocytes.

The results of the study also demonstrated that in the main cimetidine and protamine sulfate produced effects similar to those aimed at elimination of MC [1, 2], such as enhancement of the neutrophil influx into the focus, neutrophilia, and an increase in granulocyte functional activity in the focus and blood, whereas dimedrol and cyproheptadine decreased monocyte influx into the focus and diminished alfa-naphthylacetate esterase activity in the monocytes of the focus and blood. This indicates that the modulating effect of MC on the neutrophil reaction in inflammation is mainly due to the action of histamine and heparin, the histamine effect being exerted via H_2-receptors. As

for the monocyte reaction, the MC effect is due primarily to histamine, acting via H_1-receptors, as well as to serotonin.

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Biophysics and Biochemistry

Erythropoetin Activity of Plasma in Healthy Children and Children with Iron-Deficiency Anemia

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Erythropoetin (EP) - a glycoprotein hormone - is the main factor of erythropoesis regulation. EP controls proliferation and differentiation of the erythroid precursors; data are available on its stimulating influence on the proliferation of erythroblasts, hemoglobin synthesis, and the release of reticulocytes from the bone marrow into the blood [2,4,8]. In the mid-1980's recombinant erythropoetin was obtained [4], the use of which immediately yeilded promising results in iron-deficiency anemia therapy [5].

Erythropoetin therapy proved to be especially successful in the case of anemia accompanying chronic kidney failure [6] and early anemia in premature infants [7]. Recently a proposal was made by V. I. Gudim et al. [2] to use recombinant erythropoetin in iron-deficiency anemia (IDA) therapy in adults, in connection with the discovery that EP biosynthesis is reduced in moderately severe and severe IDA [2].

In the present work the activity of EP in the plasma of healthy children and children with IDA was studied.

MATERIAL AND METHODS

The method of biological testing of erythropoetin activity in vitro [9, 10] was used in modification [3] with some changes. Nucleus-containing spleen cells from anemic BALB/c mice weighing 20-30 g were used as the target cells for EP activity determination. Anemia was caused by the administration of phenylhydrazine hydrochloride at a rate of 60 mg/kg. Three days after the second administration of phenylhydrazine, a suspension of cells was prepared in the following manner: 3 ml of alfa-MEM medium

TABLE 1. Erythropoetin Activity in Plasma of Healthy Children (M±m)

Indicator	Age, month								
	2	3	6	9	12	18	24	30	36
Number of children examined	7	6	7	6	9	6	8	8	9
EP activity, mU/ml	6.3±0.7	6.9±1.4	12.5±1.7	18.3±2.5	16.9±2.8	14.8±1.9	13.9±2.6	11.8±3.7	12.5±2.6
	(5.0-10.2)	(5.5-11.6)	(6.9-19.1)	(10.2-22.	(8.8-24.8)	(8.0-22.6)	(6.2-20.8)	(5.0-22.5)	(6.4-21.5)

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