Role of Hyaluronidase in the Regulation of Hemopoiesis

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We evaluated the possibility of hemopoiesis stimulation with hyaluronidase. The response of the blood system depended on the dose of this enzyme. Functional activity of hemopoietic precursor cells increased under the influence of hyaluronidase in a dose of 20 arb. units, which was accompanied by an increase in the secretion of hemopoietins by adherent myelokaryocytes and elevation of hemopoietic activity in blood plasma. The number of erythrokaryocytes and mature neutrophilic granulocytes in the bone marrow, as well as the count of reticulocytes and neutrophils in the peripheral blood increased under these conditions. Administration of hyaluronidase in a high dose (100 arb. units) led to uncoupling of proliferation and differentiation of hemopoietic precursors and produced no considerably changes in the blood.

Key Words: *hemopoiesis; hyaluronic acid; hemopoietic precursor cells*

Hyaluronic acid (HA) is one of the major components of the intercellular matrix. Published data show that HA molecules with different length of the polysaccharide chain produce various effects on biological processes and functional activity of cells [6,8,9,11]. Low- and medium-molecular-weight forms of HA stimulate angiogenesis and proliferation, differentiation, and migration of cells. High-molecular-weight molecules of HA suppress angiogenesis, inhibit cell proliferation, and decrease migration ability of cells [7,8,11,12]. Hyaluronidase plays an important role *in situ* in the metabolism and maintenance of balance between various forms of HA. This enzyme catalyzes hydrolysis of polymers [10,11]. Previous studies showed that changes in the intercellular matrix of the hemopoietic tissue, which mainly consists of HA [6], produce a strong effect on hemopoiesis [2]. However, the role of hyaluronidase in regulation of hemopoiesis remains unknown.

Here we studied the effect and mechanism for action of hyaluronidase in various doses on the blood system.

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MATERIALS AND METHODS

Experiments were performed on 164 male and female CBA/CaLac mice aging 2 months and weighing 18-20 g. The animals (class I conventional strain) were obtained from the nursery of the Institute of Pharmacology (Tomsk Research Center). Hyaluronidase (Lidaza, NPO Mikrogen, Russian Ministry of Health) was dissolved in 0.5 ml physiological saline. Intact animals received intraperitoneal injections of hyaluronidase in a single dose of 20 or 100 arb. units for 2 days. Control animals received an equivalent volume of physiological saline. The number of reticulocytes, count of various forms of leukocytes in the peripheral blood, and parameters of bone marrow hemopoiesis were estimated by routine blood tests on days 3, 5, and 8 after the start of treatment [5]. We estimated the number of bone marrow erythroid precursors (CFU-E) and granulomonocytic precursors (CFU-GM), proliferative and differentiation activity of cells, and erythropoietic (EPA) and colony-stimulating (CSA) activity of conditioned media from adherent and non-adherent cells of the hemopoiesis-inducing microenvironment and blood plasma [3]. The results were analyzed using Student's t test and nonparametric Mann—Whitney U test.

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RESULTS

The number of peripheral blood reticulocytes significantly increased at various stages after treatment with hyaluronidase in a dose of 20 arb. units. These changes were most pronounced on day 5 after hyaluronidase injection (203.7% of the control). We revealed a significant increase in the number of band (day 8) and segmented neutrophils (days 3 and 5). The number of band neutrophils in the peripheral blood did not increase during the initial period of study, which was probably associated with higher rate of cell efflux from the vascular bed due to the increased permeability of tissue barriers after treatment with hyaluronidase [11]. This mechanism did not play an important role in regulating the number of segmented neutrophils. The number of these cells in the blood is much higher and, therefore, has a lower reactivity. Moreover, the number of circulating lymphocytes significantly increased on days 5 and 8 (Fig. 1).

These changes reflected the dynamics of bone marrow hemopoiesis. Administration of hyaluronidase in a dose of 20 arb. units was followed by severe hyperplasia of the erythroid hemopoietic stem. The number of erythrokaryocytes in hemopoietic tissue increased on days 5 and 8 after treatment (190.8 and 140.6% of the basal level), respectively). Various changes were found in granulomonocytopoiesis. The number of immature neutrophilic granulocytes in the bone marrow did not differ from the basal level. The number of mature cells in all periods of study exceeded the basal level, but was maximum on day 5 (164.7% of the control). Administration of the enzyme was also accompanied by an increase in the number of lymphoid cells in the hemopoietic tissue (days 5 and 8, Fig. 1). However, the number of other morphologically distinct forms of myelokaryocytes did not differ from the basal level.

Cell culture study showed that the response of the blood system depends on activity of committed precursor cells. Degradation of HA was followed by an increase in the number of CFU-E and CFU-GM in the bone marrow (days 3, 5, and 8), rise in proliferative activity (CFU-E, day 3; CFU-GM, days 3 and 5), and accelerated maturation of erythroid (days 5 and 8) and granulomonocytic hemopoietic precursors (day 8, Fig. 2). These changes resulted from the increase in the secretion of EPA by adherent myelokaryocytes (days 3 and 5), production of CSA by nonadherent nucleated cells of the hemopoiesis-inducing microenvironment (days 5 and 8), and hemostimulating activity of blood plasma (Fig. 3).

Hyaluronidase-induced change in the intercellular matrix were followed by severe hyperplasia of hemopoietic tissue. It was determined not only by the formation of medium- and low-molecular-weight forms of HA *in situ* capable of stimulating the proliferation and differentiation of cells [7,9, 11], but also by stimulation of cells in the hemopoiesis-inducing microenvironment and influence of distant (humoral) regulatory mechanisms.

In the next series, we studied the effect of hyaluronidase in high dose (100 arb. units) on hemopoiesis. Hyaluronidase in this dose cleaves hyaluronic acid and chondroitin sulfate [11], which serves as another major component of glycosaminoglycans [2,4]. The increase in the disintegrating effect on the intercellular matrix was accompanied by significant changes in the blood. We revealed a sharp decrease in the number of peripheral blood reticulocytes (54.8 and 53.8% of the level observed on days 3 and 5 after administration of hyaluronidase in a dose of 20 arb. units, respectively). However, the number of neutrophils did not increase under these conditions (as differentiated from the experiment with low dose of hyaluronidase, Fig. 1).

Examination of bone marrow hemopoiesis revealed a slight increase in the number of erythrokaryocytes by the end of the study. It was probably associated with high-intensity metabolism of HA in the organism. One-third of the total volume of HA can be renewed over 24 h [11]. Hence, normalization of HA metabolism in this period was accompanied by the appearance of polymers that have a positive effect on cell proliferation and differentiation [7-9,11]. The increase in the effect was also followed by a decrease in the number of immature neutrophilic granulocytes in the bone marrow (day 8, Fig. 1). However, the number of mature cells did not differ from that in animals receiving hyaluronidase in a dose of 20 arb. units. It should be emphasized that significant increase in the effect abolishes neutrophilic leukocytosis. These data suggest that the observed changes are associated with rapid efflux of neutrophils from the blood to peripheral tissues, but not with impaired migration of granulocytic cells from the bone marrow. These specific features were probably related to the increased permeability of tissue barriers [11] 4and necessity for recovery of damaged intercellular substance in various organs. Perilous studies revealed an important role of neutrophils in the maintenance of tissue homeostasis [1,4].

The increase in the degree of hydrolytic cleavage was followed by a decrease in the number of bone marrow CFU-E (days 3 and 8) due to a significant reduction of proliferative activity (day 3).

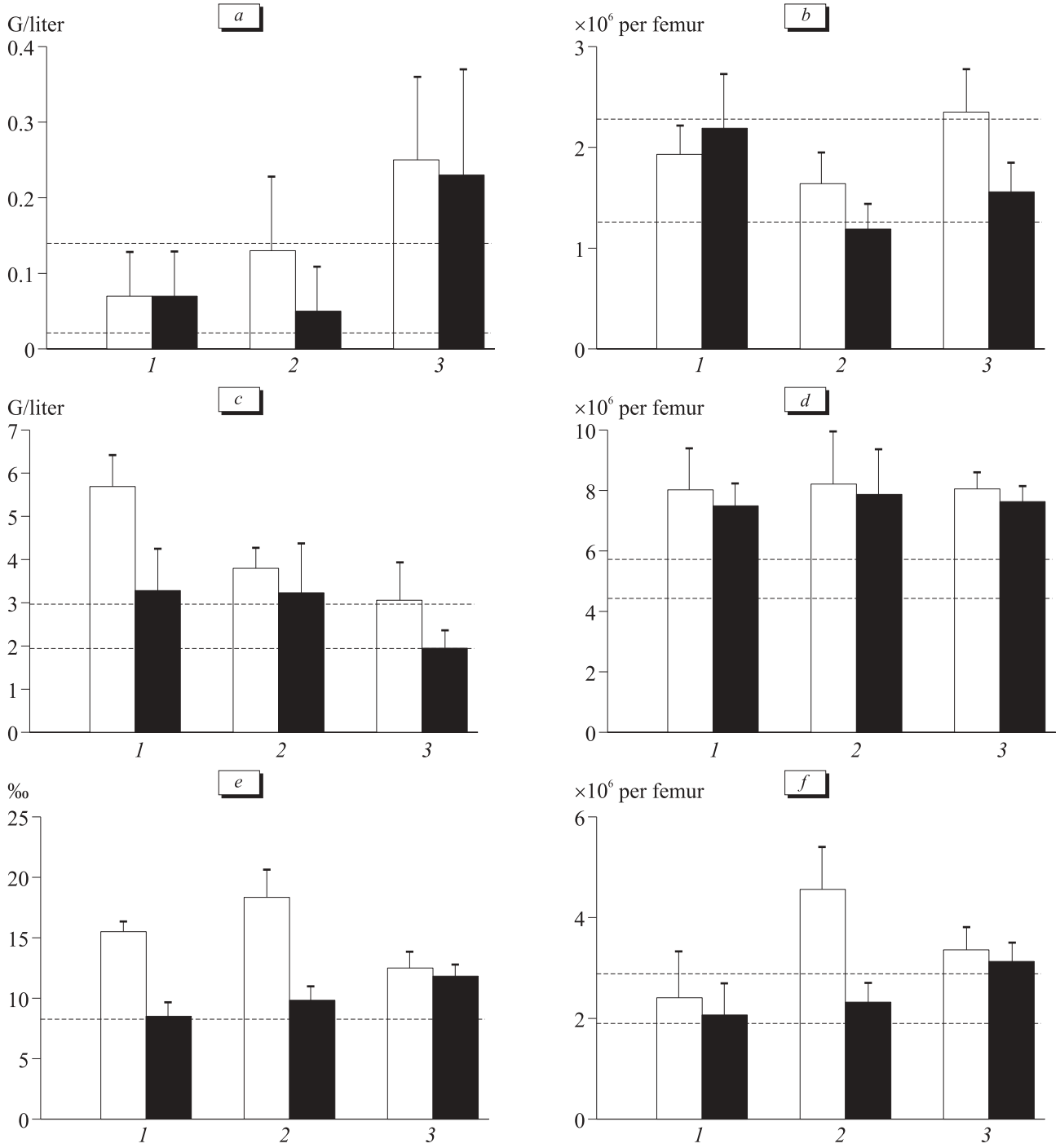

Fig. 1. Number of bands (*a*) and segmented neutrophils (*b*) and reticulocytes in the peripheral blood (*c*) and content of immature (*d*) and mature neutrophilic granulocytes (*e*) and erythrokaryocytes in the bone marrow (*f*) of CBA/CaLac mice after administration of hyaluronidase in doses of 20 (light bars) and 100 arb. units (dark bars). Here and in Figs. 2 and 3: days 3 (*1*), 5 (*2*), and 8 (*3*). Confidence intervals at $p<0.05$. Area between dotted lines: confidence interval in intact mice at $p<0.05$.

Moreover, the rate of CFU-E differentiation decreased on day 5. However, the rate of precursor cell differentiation remained unchanged in this period. The number of CFU-GM in hemopoietic tissue increased on days 3 and 5. Proliferative activity of these structures did not differ from the control (hyaluronidase in a dose of 20 arb. units). This phenomenon was probably associated with the increase in maturation of early hemopoietic precursors (multipolar cells) into CFU-GM under *in situ* conditions. At the same time, the rate of CFU-GM differentiation remained unchanged or decreased on day 8

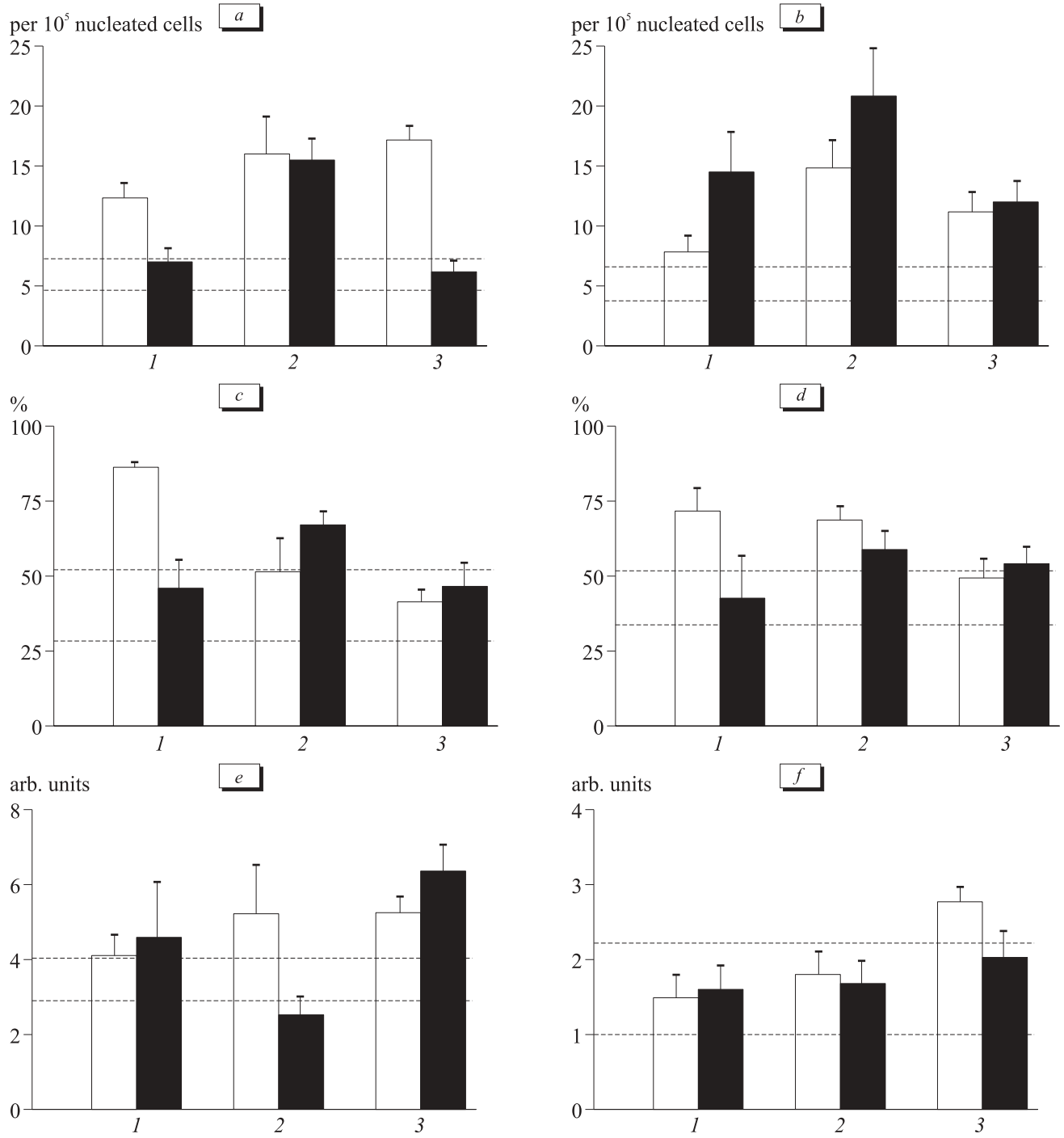

Fig. 2. Number of erythroid (*a*, *c*, *e*) and granulomonocytic (*b*, *d*, *f*) precursors cells in the bone marrow (*a*, *b*), ratio of cells in S-phase of the mitotic cycle (*c*, *d*), and maturation of cells (*e*, *f*) in CBA/CaLac mice after administration of hyaluronidase in doses of 20 (light bars) and 100 arb. units (dark bars).

(as compared to the animals receiving hyaluronidase in a dose of 20 arb. units, Fig. 2). Increasing the dose of hyaluronidase had little effect on secretory activity of cells in the hemopoiesis-inducing microenvironment and concentration of hemopoietically active substances in blood plasma. The exception was greater increase in EPA and CSA of blood plasma (Fig. 3).

Our results indicate that disintegration of components in the intercellular matrix (HA and chondroitin sulfate [7-9,11]) was accompanied by pronounced disorganization of hemopoiesis. It was manifested in uncoupling of proliferation and dif-

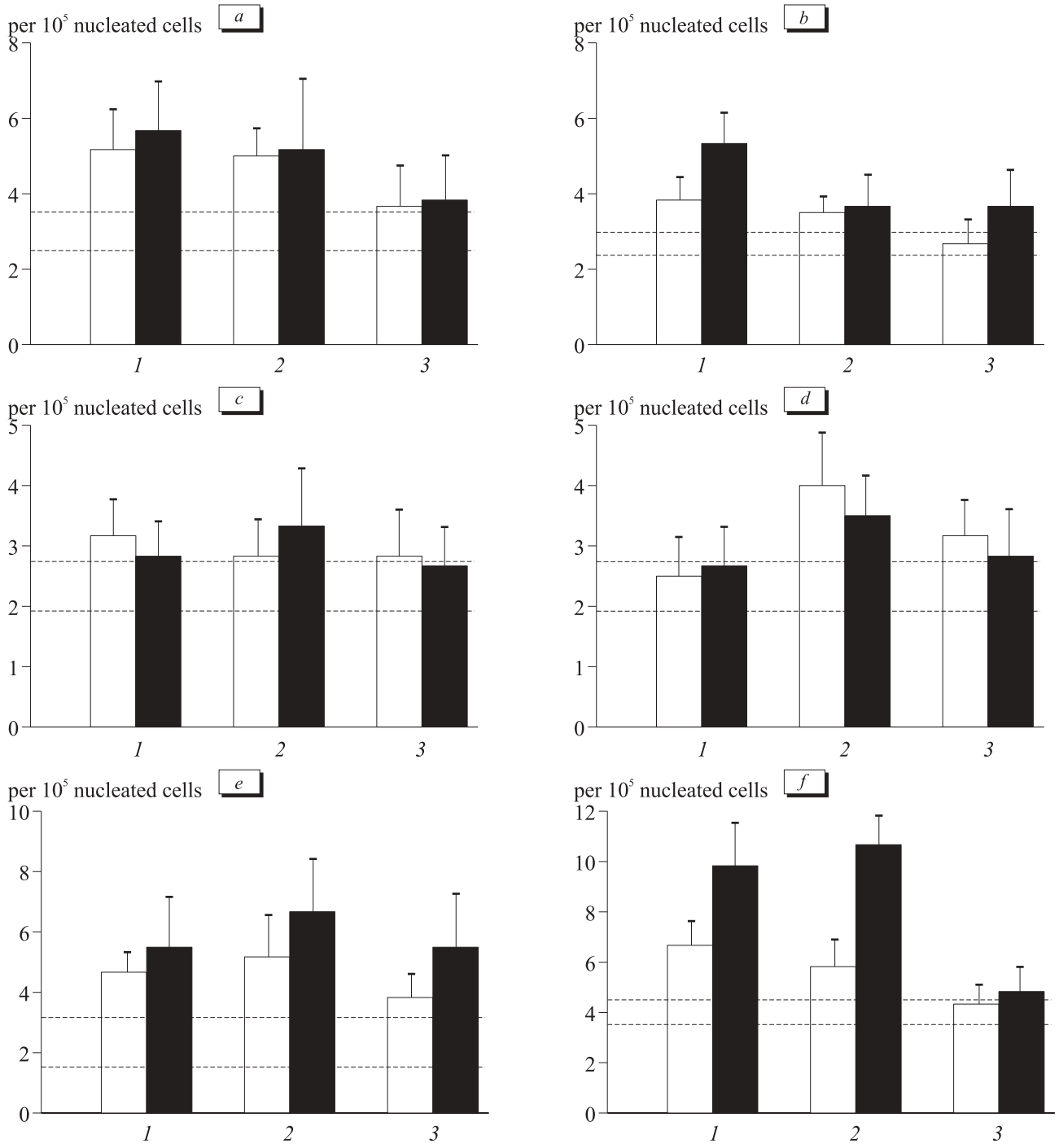

Fig. 3. Erythropoietic (*a*, *c*, *e*) and colony-stimulating activity (*b*, *d*, *f*) of conditioned media from adherent (*a*, *b*) and nonadherent myelokaryocytes (*c*, *d*) and blood plasma (*e*, *f*) of CBA/CaLac mice after administration of hyaluronidase in doses of 20 (light bars) and 100 arb. units (dark bars).

ferentiation of various types of hemopoietic precursor cells and change in the blood system. The enzyme in high dose produce a potent stimulating effect on hemopoiesis. HA and ratio between molecular forms of this compound *in situ* determine the regulation of hemopoiesis [2,7,9,11]. Pharmacological stimulation of the erythroid and granulo-monocytic hemopoietic stem can include changes in properties of hemopoietic tissue by hyaluronidase in relative low doses.

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