Adrenergic Regulation of Erythropoiesis during Cytostatic-Induced Myelosuppressions

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The role of central adrenergic structures in the regulation of the erythroid hematopoietic stem was studied during administration of cyclophosphamide and 5-fluorouracil. The central nervous system contributed to suppression of erythropoiesis during cytostatic treatment. The suppressive effect of brain adrenergic structures on the erythron after treatment with cyclophosphamide and 5-fluorouracil was related to dysfunction of adherent cells in the hemopoiesis-inducing microenvironment (formation of hemopoietic islets and secretion of erythropoietic activity) and production of growth factors by myelokaryocytes, respectively. Brain norepinephrine had an inhibitory effect on proliferative activity and differentiation of erythroid precursors that were associated with the erythropoietin and peripheral α-adrenergic structures. However, stimulation of β-adrenergic structures was followed by an increase in the rate of erythroid cell maturation.

Key Words: *adrenergic system; precursors; hemopoiesis-inducing microenvironment; erythropoiesis; cytostatics*

Previous studies showed that central and peripheral dopaminergic and serotoninergic structures are involved in the regulation of the erythroid hematopoietic stem during myelosuppression [6,7]. However, the regulation of hematopoiesis involves not only dopamine and serotonin. After cytostatic treatment, α-adrenoceptors and β-adrenoceptors on committed hematopoietic precursors and cells of the hemopoiesis-inducing microenvironment (HIM) are involved in the regeneration of hematopoiesis [3]. It should be emphasized that activity of peripheral adrenergic mechanisms is regulated by the sympathetic nervous system. It cannot be excluded that brain adrenergic structures had a modulatory effect on the blood system during myelosuppressions. This assumption is confirmed by published data that sympatholytic reserpine suppresses the hematopoietic tissue during neuroses [2].

Here we studied the regulatory effect of central adrenergic structures on erythropoiesis during cytostatic treatment.

MATERIALS AND METHODS

Experiments were performed on 896 female CBA/CaLac mice (class I conventional mouse strain) aging 2-2.5 months and obtained from the nursery of the Institute of Pharmacology.

Erythropoiesis was inhibited by single intraperitoneal injection of an alkylating agent cyclophosphamide (CP, one-third of the maximum tolerated dose, 83 mg/kg) or fluoropyrimidine antimetabolite 5-fluorouracil (FU, 76 mg/kg). The animals of treatment groups received intraperitoneal injection

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of sympatholytic reserpine in a single dose of 2 mg/kg (Polfa) 30 min before cytostatic treatment. Control animals received an equivalent volume of physiological saline (0.2 ml) under similar conditions. The number of peripheral blood reticulocytes was measured on days 1, 2, 3, 4, 5, 6, and 7 after cytostatic treatment. The mice were euthanized by cervical dislocation under ether anesthesia. The count of bone marrow erythrokaryocytes was evaluated. The content of erythroid colony-forming (CFU-E) and cluster-forming units (ClFU-E) in the bone marrow was estimated by *in vitro* cloning of nonadherent myelokaryocytes in methylcellulose culture medium [4]. Proliferative activity of erythroid precursors was studied by the method of cell suicide using hydroxyurea. Cell differentiation was assayed by the index of maturation (cluster/colony ratio in the well) [4]. Structural and functional characteristics of the bone marrow were estimated by enzymatic isolation of hemopoietic islets and study of their quantitative and qualitative composition [8]. Erythropoietic activity of conditioned media from adherent and nonadherent cells of HIM and blood serum was tested with intact mouse myelokaryocytes [4].

The final concentration of an α-adrenergic agonist mezaton (GNTsLS, Kharkov) and β-adrenergic agonist isoproterenol (Sigma) in the bone marrow culture was 10^{-8} M. The final concentration of erythropoietin (Sigma) was 0.5 U/ml.

The results were analyzed by standard methods of variational statistics. The significance of differences was evaluated by parametric Student's *t* test and nonparametric Mann—Whitney *U* test.

RESULTS

Cytostatic treatment was followed by a significant decrease in the number of bone marrow erythrokaryocytes and peripheral blood reticulocytes (Fig. 1). Studying the local mechanisms for regulation of hematopoiesis showed that long-term suppression of the erythroid stem is associated with various factors. It was primarily related to changes in structural and functional characteristics of the bone marrow (abnormal formation of hemopoietic islets, variations in the production of erythropoietic activity by HIM cells, and decrease in serum activity) and suppression and/or inhibition of division and maturation of erythroid precursors. The inhibition of erythropoiesis in FU-treated mice was more pronounced compared to animals of the CP group. Probably, this antimetabolite had a stronger toxic effect on hematopoietic tissue that the alkylating agent.

Catecholamine depletion in the central nervous system (CNS) after injection of reserpine increased the degree of CP-induced suppression of bone marrow erythropoiesis (day 2, Fig. 1). However, the number of erythrokaryocytes in hematopoietic tissue of FU-treated animals increased on days 4 and 5 after sympatholytic treatment (*vs*. day 6 in the cytostatic control). The sympatholytic contributed to reticulocyte accumulation in the peripheral blood of mice from both groups. This effect was most pronounced in CP-treated animals.

It should be emphasized that reserpine not only decreases norepinephrine concentration in CNS, but also inactivates the dopaminergic system. However, the neuroleptic haloperidol had a less pronounced modulatory effect on the bone marrow and peripheral blood than the sympatholytic [6]. Therefore, the effects of reserpine on the erythroid hematopoietic stem are mainly realized via adrenergic structures of the brain.

Committed hematopoietic precursors determine the type of blood changes under extreme conditions (stress, cytostatic treatment, and neuroses) [2,3,5]. Functional activity of these cells is regulated by not only erythropoietin, but also peripheral α-adrenoceptors and β-adrenoceptors on hematopoietic precursors. It cannot be excluded that the modulatory effect of reserpine on cytostatic-suppressed erythropoiesis is realized via erythropoietin-sensitive and adrenergic structures.

Under conditions of CP-induced myelosuppression, the sympatholytic significantly decreased the rate of division and differentiation of erythroid precursors after addition of isoproterenol to the test system (days 1 and 7). These changes contributed to a decrease in the content of CFU-E in methylcellulose medium to $1.5\pm0.2\times10^5$ and $2.0\pm0.1\times10^5$ cells, respectively (*vs*. $3.5\pm0.5\times10^5$ and $3.8\pm0.4\times10^5$ cells in the cytostatic control, respectively; and $0.5\pm0.2\times10^5$ cells before treatment; Fig. 2). *In vitro* stimulation of erythropoietin-sensitive and α-adrenergic structures was also followed by inhibition of proliferative activity (days 7 and 5, respectively) and decrease in the index of precursor maturation (days 1, 3, and 7). However, recombinant erythropoietin and adrenoceptor agonists differed in the effect on erythroid cells. On days 1 and 3 after injection of the alkylating agent, addition of Recormon to the culture induced a significant increase in the ClFU-E/CFU-E ratio. This ratio in mice of the treatment group was 367-2680% higher than in control animals (CP, $p<0.001$). Mezaton also increased the rate of erythroid cell maturation (days 3 and 7).

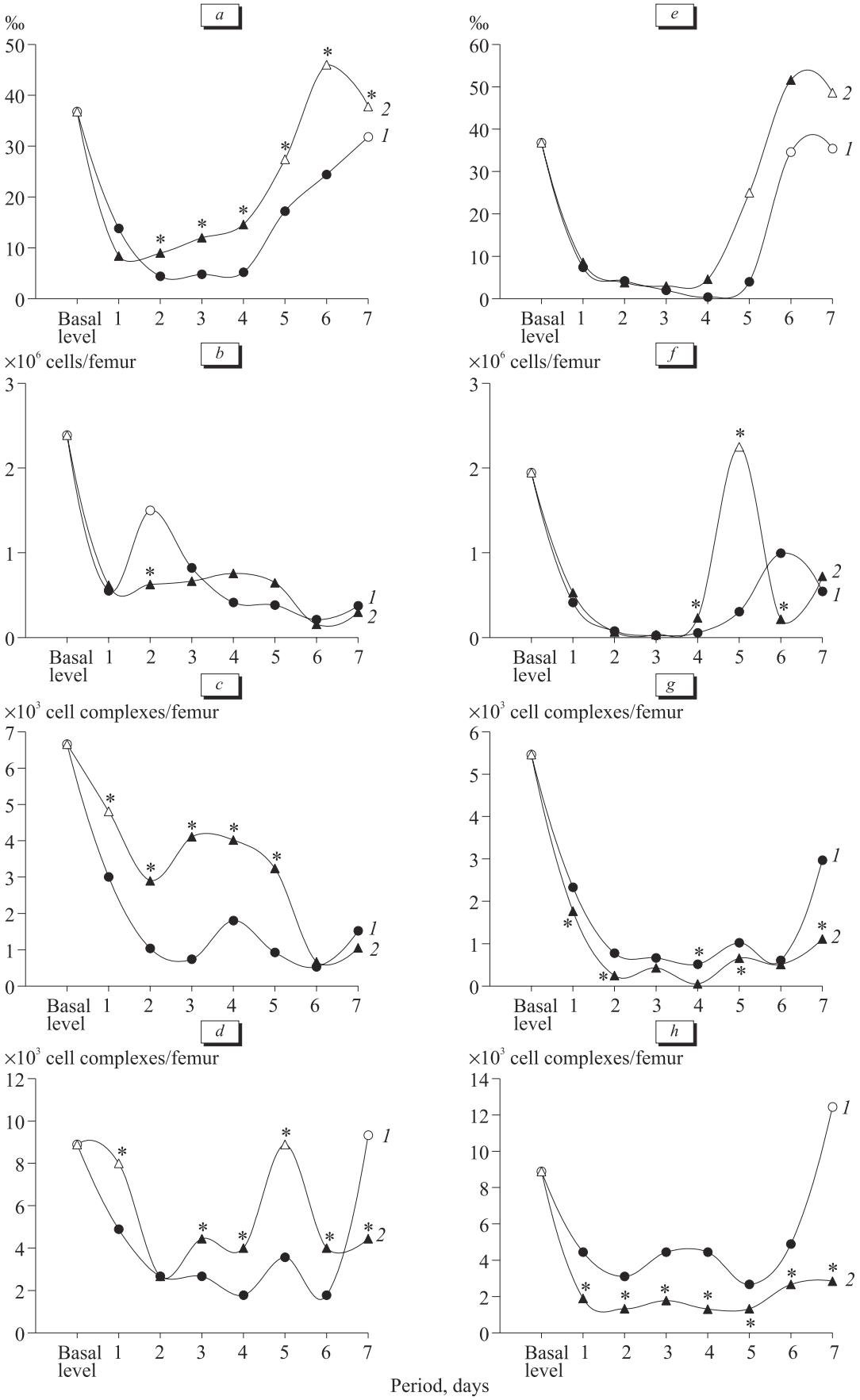
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TABLE 1. Effect of Reserpine on Erythropoietic Activity ($\times 10^5$ Cells) in Biological Fluids of CBA/CaLac Mice after Treatment with CP or 5-FU ($X \pm m$, $n=9$)

Period, days		*In vivo* preparation	Supernatants of adherent myelokaryocytes	Supernatants of nonadherent myelokaryocytes	Blood serum
Intact control		Physiological saline	0.25±0.15	0.38±0.18	0.25±0.10
CP	1	Physiological saline	0.15±0.12	0.25±0.25	0.4±0.25
		Reserpine	0.25±0.25	0.38±0.26	1.0±0.1
	3	Physiological saline	0.25±0.10	0.50±0.28	0.25±0.25
		Reserpine	0.35±0.16	0.35±0.25	0.25±0.15
	5	Physiological saline	0.13±0.10	0.25±0.25	0.25±0.25
		Reserpine	1.00±0.25	0.75±0.25	0.50±0.28
	7	Physiological saline	1.50±0.12*	1.55±0.15*	0.38±0.15
		Reserpine	2.75±0.25*+	1.0±0.1	0.25±0.14
5-FU	1	Physiological saline	1.25±0.15*	2.75±0.25*	0.25±0.15
		Reserpine	2.75±0.25*+	1.0±0.1+	1.4±0.1*+
	3	Physiological saline	1.50±0.28*	0.25±0.22	0.25±0.25
		Reserpine	1.0±0.2	0.75±0.25	0.5±0.25
	5	Physiological saline	0.25±0.25	1.0±0.2	0.50±0.28
		Reserpine	0.2±0.1	0.25±0.15	0.25±0.15
	7	Physiological saline	0.25±0.22	0.50±0.28	0.35±0.20
		Reserpine	0.38±0.23	0.5±0.3	0.38±0.22

Note. $p<0.05$: *compared to the intact control; +compared to cytostatic-treated animals with no reserpine treatment.

Under conditions of catecholamine depletion, injection of FU, and addition of erythropoietin to the test system, changes in functional activity of precursor cells did not differ from those in animals receiving the alkylating agent. We revealed a decrease in the count of clonogenic cells. Mezaton *in vitro* decreased the content of ClFU-E and CFU-E by 86 and 50%, respectively, compared to the cytostatic control (day 1). The observed changes were related to inhibition of division and maturation of precursor cells (Fig. 3). These parameters increased on days 5 and 7. Addition of isoproterenol to the bone marrow culture was followed by various changes in erythroid precursors. The β-adrenergic agonist increased proliferative activity of CFU-E, but had an inhibitory effect on ClFU-E proliferation and decreased the index of erythroid cell differentiation on days 1 and 3 after antimetabolite injection. The content of CFU-E in S phase of the mitotic cycle decreased in the follow-up period. The content of mitotically active ClFU-E increased on days 5 and 7.

After CP injection, reserpine decreased the division rate of committed erythroid precursors. The maturation index of precursor cells did not decrease, but even increased in certain periods after treatment. These changes were most pronounced after *in vitro* addition of recombinant erythropoietin. During FU-induced myelosuppression, the sympatholytic had an inhibitory effect on the erythropoietin system and modulated the adrenergic mechanisms for regulation of proliferative activity and differentiation of clonogenic erythroid cells. Brain norepinephrine probably contributes to a significant increase in proliferative activity of erythroid precursors after cytostatic treatment, which is realized via the erythropoietin system and peripheral adrenergic receptors. Stimulation of β-adrenergic structures also increases the rate of erythroid cell maturation. The interaction of CNS with erythropoietin-sensitive and α-adrenergic structures may be followed by the increase or inhibition of precursor cell differentiation, which depends on the period of study.

We evaluated the effect of reserpine on HIM after cytostatic treatment. After injection of CP, reserpine increased the content of macrophage-positive (days 1 and 3-6) and erythroid cell com-

Fig. 1. Number of peripheral blood reticulocytes (*a*, *e*), erythrokaryocytes (*b*, *f*), and macrophage-positive (*c*, *g*) and erythroid hemopoietic islets (*d*, *h*) in the bone marrow of CBA/CaLac mice receiving CP (*a-d*) or 5-FU (*e-h*). Administration of physiological saline (*1*) and reserpine (*2*). Dark symbols: significant differences compared to the pre-treatment parameter ($p<0.05$). *$p<0.05$ compared to physiological saline.

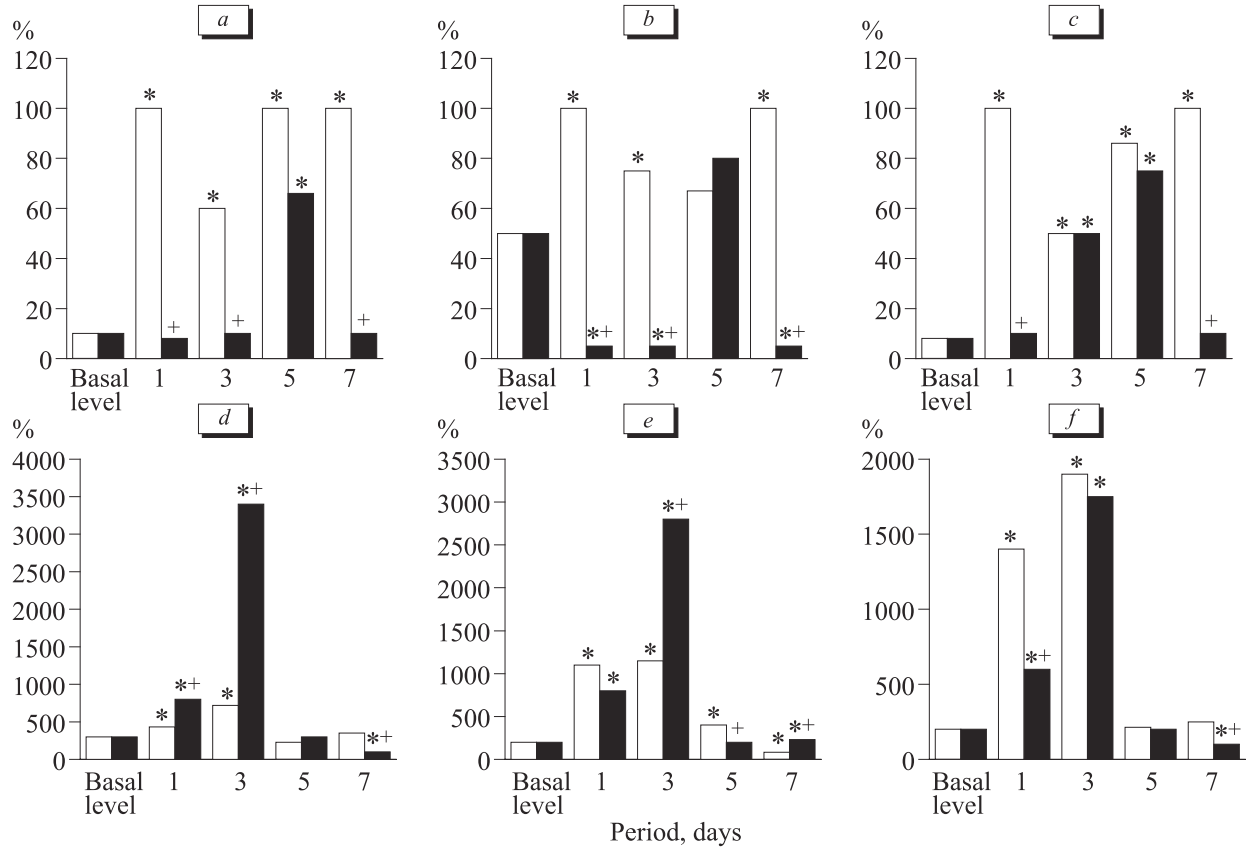

Fig. 2. Ratio of CFU-E in S phase of the mitotic cycle (*a-c*) and maturation of erythroid precursors (*d-f*) in the bone marrow of CP-treated CBA/CaLac mice. Here and in Fig. 3: *in vitro* stimulation with recombinant erythropoietin (*a*, *d*), mezaton (*b*, *e*), or isoproterenol (*c*, *f*). Light bars, physiological saline; dark bars, reserpine. $p<0.05$: *compared to the pre-treatment parameter; +compared to physiological saline.

plexes (days 1-5) in the bone marrow. Moreover, the secretion of EPA by adherent cells of HIM increased under these conditions (days 5 and 7; Fig. 1, Table 1). After injection of FU, this sympatholytic inhibited the formation of hemopoietic islets with the central macrophage (days 1-5 and 7) and erythroid cell complexes (days 1, 2, 4, 5, and 7). The observed changes were more pronounced than in the cytostatic control. Activity of conditioned media from myelokaryocytes and blood serum increased under these conditions (day 1).

After CP treatment, reserpine contributes to the restoration of structural and functional integrity of the bone marrow (*de novo* formation of hemopoietic islets and secretion of EPA by adherent myelokaryocytes). It may be suggested that central adrenergic structures decrease activity of stromal cells in HIM. After injection of FU, the sympatholytic induced a greater decrease in the number of hemopoietic islets in hematopoietic tissue and caused an increase in EPA of biological fluids. These data indicate that CNS plays a role in the formation of erythroid cell complexes and inhibition of EPA in microenvironmental cells.

We conclude that central adrenergic structures of the brain are involved in cytostatic-induced hypoplasia of bone marrow erythropoiesis. CNS regulates the erythron through the erythropoietin system and adrenergic structures on erythroid precursors. An indirect regulatory effect is realized via HIM cells. Suppression of the erythroid stem and decrease in the rate of regeneration differ in animals of the CP and FU groups. These differences are related to specific interaction between distant and local regulatory systems, which depends on the toxic effect of thecytostatic drugs on the blood system. However, cytostatic-induced hypoplasia of hematopoiesis is mediated by a variety of mechanisms. After treatment with the antimetabolite and alkylating agent, functional activity of HIM cells is regulated by dopaminergic and serotoninergic systems of the brain [6,7]. The regulatory effect of dopaminergic structures on structural and functional integrity of the brain is similar to that of norepinephrine. However, serotonin inhibits the formation of hemopoietic islets after injection of FU. Moreover, serotonin abolishes the suppressive effect of cytostatic treatment in CP-treated animals.

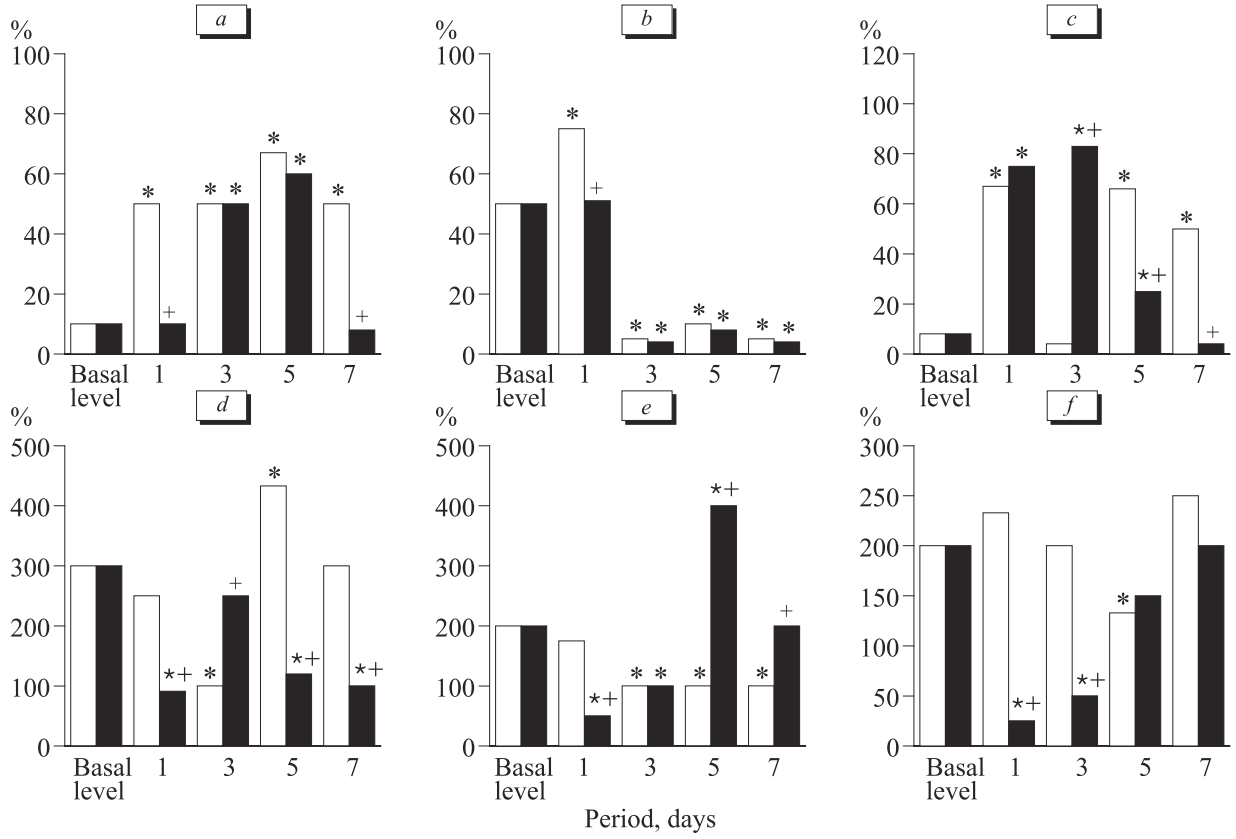

Fig. 3. Ratio of CFU-E in S phase of the mitotic cycle (*a-c*) and maturation of erythroid precursors (*d-f*) in the bone marrow of FU-treated CBA/CaLac mice.

REFERENCES

1. E. D. Gol'berg, A. M. Dygai, and V. V. Zhdanov, *Role of the Hemopoiesis-Inducing Microenvironment in the Regulation of Hematopoiesis during Cytostatic-Induced Myelosuppressions* [in Russian], Tomsk (1999).
2. E. D. Gol'dberg, A. M. Dygai, N. V. Provalova, *et al.*, *Role of the Nervous System in the Regulation of Hematopoiesis* [in Russian], Tomsk (2004).
3. E. D. Gol'dberg, A. M. Dygai, and I. A. Khlusov, *Role of the Autonomic Nervous System in the Regulation of Hematopoiesis* [in Russian], Tomsk (1997).
4. E. D. Gol'dberg, A. M. Dygai, and V. P. Shakhov, *Tissue Culture Methods in Hematology* [in Russian], Tomsk (1992).
5. Yu. M. Zakharov, *Ros. Fiziol. Zh.*, **90**, No. 8, 987-1000 (2004).
6. M. Yu. Minakova, E. G. Skurikhin, and O. V. Pershina, *Byull. Eksp. Biol. Med.*, Suppl. 1, 79-86 (2007).
7. M. Yu. Minakova, E. G. Skurikhin, O. V. Pershina, and E. V. Udut, *Ibid.*, Suppl. 1, 86-91 (2007).
8. P. R. Crocker and S. Gordon, *J. Exp. Med.*, **162**, No. 3, 993-1014 (1985).