Adrenergic Control of Production of Humoral Regulators of Hemopoiesis in Cytostatic Myelodepression

A. M. Dygai, I. A. Khlusov, S. G. Aksinenko,
B. Yu. Gumilevskii, E. D. Gol'dberg

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 119, No. 2, pp. 135-140, February, 1995
Original article submitted April 25, 1994

It is established that the sympathetic nervous system controls the production of humoral regulators of hemopoiesis by bone marrow cells forming a hemopoiesis-inducing microenvironment in mice treated with 5-fluorouracil. The suppressive effect of catecholamines on the erythropoietic and colony-stimulating activities of the adhesive, nonadhesive, as well as the whole fraction of the bone marrow is shown using catecholamines and ganglioblocker in the period of restoration of hemopoiesis after cytostatic treatment.

Key Words: *hemopoietins; hemopoiesis-inducing microenvironment; bone marrow; adrenergic antagonists; 5-fluorouracil*

As was established previously, the sympathicoadrenal structures play an ambiguous role in the response of the hemopoietic tissue to cytostatic action [10]. In the present investigation the role of the sympathetic nervous system was studied in the regulation of hemopoietin production by bone marrow cells in myelodepression caused by the administration of a massive dose of 5-fluorouracil.

MATERIALS AND METHODS

Experiments were carried out on 670 male CBA mice (Rassvet nursery, Tomsk) weighing 18-20 g. For modeling the state of myelodepression the animals were i.p. injected one time with half of the maximum permissible dose (114 mg/kg) of 5-fluorouracil. The ganglioblocker pentamine (6 mg/kg), the α-adrenoblocker dihydroergotamine (3.9 mg/kg), or the b-adrenoblocker propranolol (5 mg/kg) was injected s.c. in combination with 5-fluorouracil 3-5 min before and 5 h after the cytostatic or on the 3rd day following its injection (twice at an interval of 5 h). Control animals were injected with an equal volume of saline (0.2 ml). Mice were killed by dislocation of the neck at different times after cytostatic treatment. The spontaneous production by bone marrow cells of erythropoietic activity (EPA) and colony-stimulating activity (CSA) was tested as described previously [13] in a modification reported elsewhere [4]. For this purpose, unseparated, adhesive or nonadhesive nucleated bone marrow cells were incubated for 24h in culture medium of the following composition: 90% RPMI-1640 (Sigma); 10% FCS (Flow); 10 mM HEPES (Flow); 40 mg/liter gentamicin (Serva); 280 mg/liter L-glutamine (Sigma); 2.5×10^{-5} M 2-mercaptoethanol (Sigma). Upon completion of the incubation, supernatants were collected and stored at -20°C for no more than one month.

The data were processed statistically using the nonparametric Wilcoxon *U* and *T* tests [6]. An integral index characterizing the influence of the test preparation on the production of humoral fac-

Institute of Pharmacology, Tomsk Research Center, Siberian Division of the Russian Academy of Medical Sciences

0007-4888/95/0002-0127$12.50

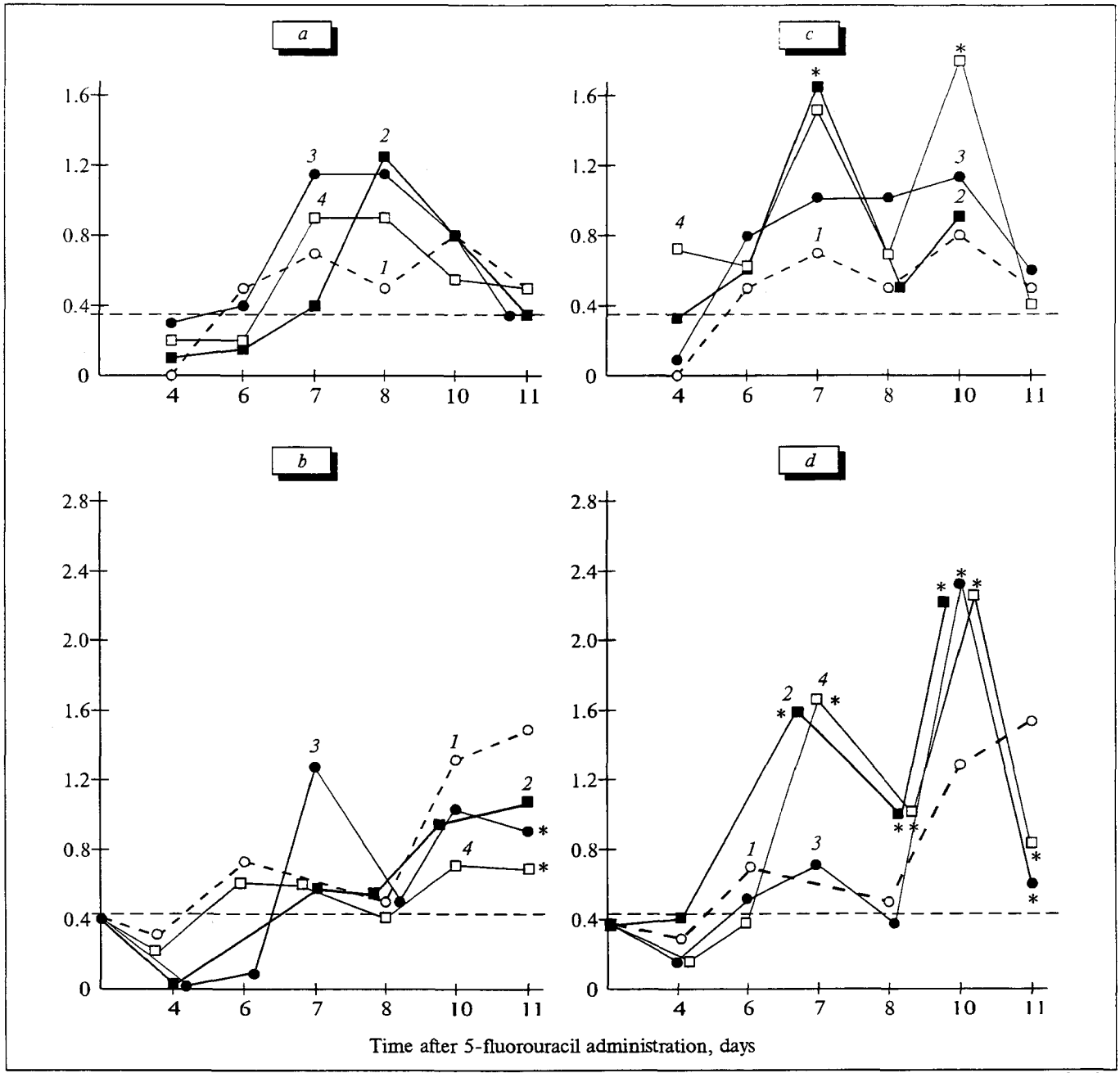

Fig. 1. Levels of CSA (*a*, *c*) and EPA (*b*, *d*) production by cells of whole bone marrow fraction of CBA mice treated with pharmacological antagonists of the sympathetic nervous system at different times after 5–fluorouracil administration. Here and in Figs. 2 and 3: *Ordinate:* Level of activity, $\times 10^5$ cells; *a*, *b*) combined administration of preparations and 5–fluorouracil; *c*, *d*) on the 3rd day after 5–fluorouracil. *1*) 5–fluorouracil; *2*) ganglioblocker; *3*) α–adrenolytic; *4*) β–adrenolytic. * denotes significance of differences compared with data of *1* according to the Wilcoxon *U* test.

tors was calculated as reported elsewhere [8] according to our modification:

$$K_j = \frac{\sum_{i=1}^{n} M_{ij}}{n \times M_j\ (0)} \times 100\%,$$

where n is the number of periods of measurement; M_{ij} is the value of the the jth index at the ith time of measurement; and M_j (0) is the initial value.

RESULTS

The present investigation established that, upon injection in mice in a high dose, 5-fluorouracil significantly boosts the secretory activity of bone marrow cells which make up the hemopoiesis-inducing microenvironment (HIM). Thus, among other processes an increase of EPA and CSA in supernatants from the whole bone marrow fraction was noted from the 6th day of the experiment

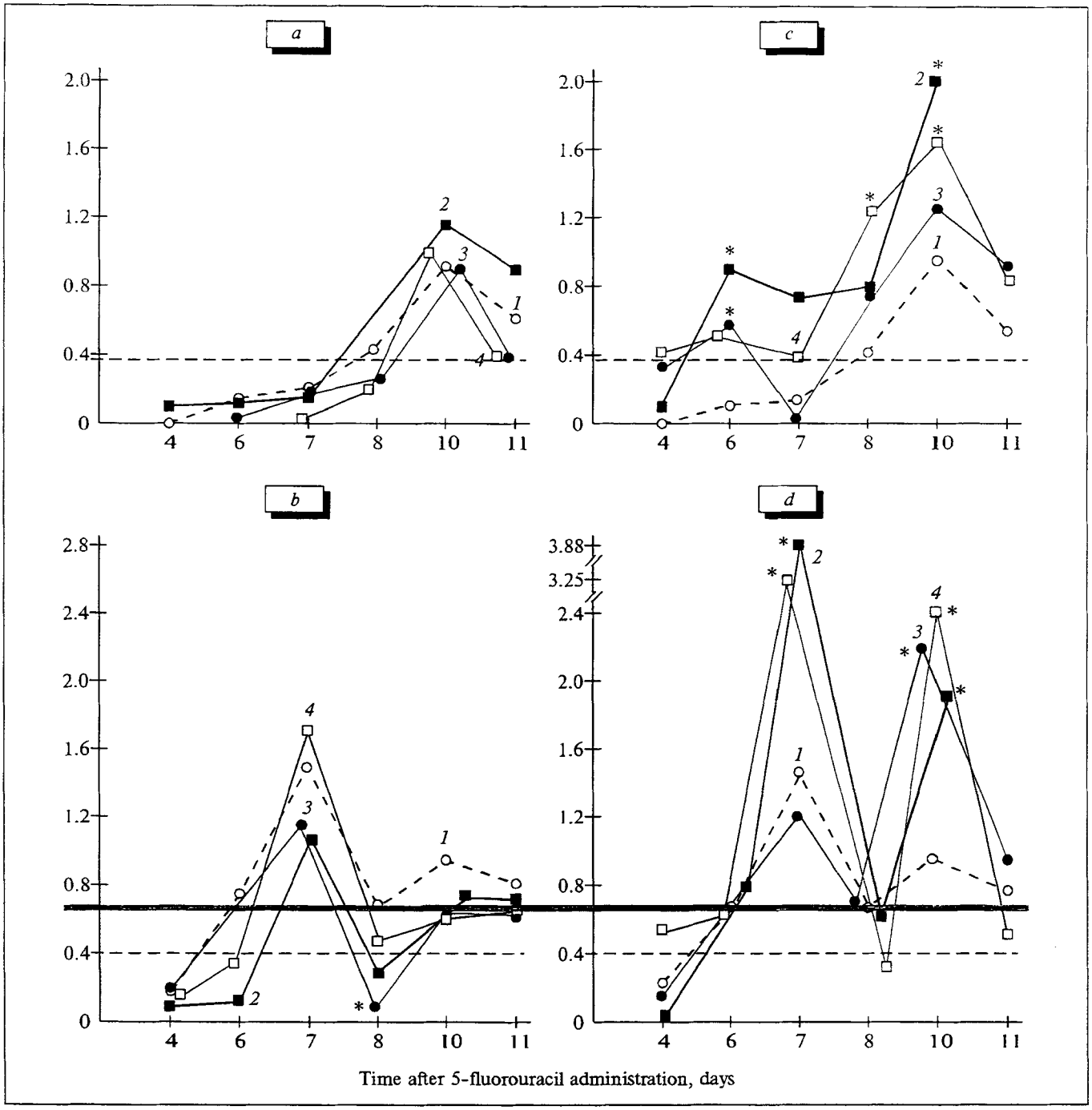

Fig. 2. Levels of EPA production by adhesive (*a*, *c*) and nonadhesive (*b*, *d*) bone marrow cells in CBA mice treated with pharmacological antagonists of the sympathetic nervous system at different times after 5–fluorouracil administration.

(Fig. 1). This is attested to by the elevation (by 88 and 30% of the baseline level, respectively) of the integral index values (Table 1). Nevertheless, the capacity of cells to produce humoral regulators of erythro- and granulocytopoiesis attained maximal values only toward the 10th-11th days of the experiment (Fig. 1). Here, only EPA production was reliably elevated (3-3.5 times). Separation of bone marrow nucleated cells into fractions showed that the secretory activity of nonadhesive cells was ahead of the others (Figs. 2, 3) and exceeded the baseline level 2-4-fold on the 7th-10th days after 5-fluorouracil administration. This may be partly due to the accretion of lymphocytes, which reportedly [7] migrate from the thymus to the bone marrow, on the 6th and 9th days. At the same time, the levels of EPA and CSA production by adhesive nucleated cells were reliably higher (2- and 5-fold, respectively) than the initial value by the 10th-11th days (Figs. 2, 3). It should be noted

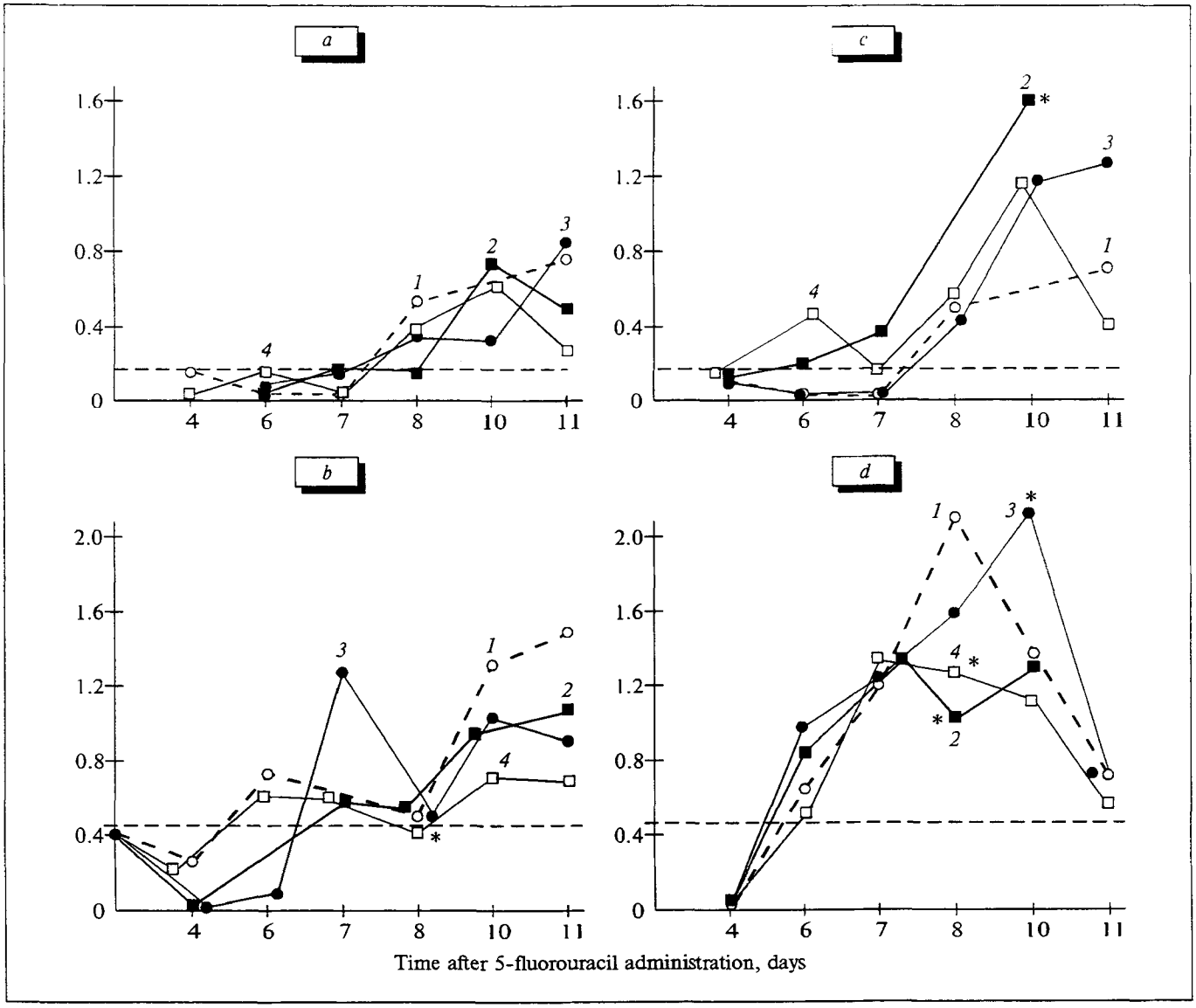

Fig. 3. Levels of CSA production by adhesive (*a*, *c*) and nonadhesive (*b*, *d*) bone marrow cells in CBA mice treated with pharmacological antagonists of the sympathetic nervous system at different times after 5–fluorouracil administration.

that the EPA content in adhesive cell-conditioned medium is rather low virtually throughout the period of observations (the value of the integral index is 91%, Table 1). There is no doubt that the enhanced hemopoietic activity of HIM cells on the 7th-11th days after 5-fluorouracil administration is largely responsible for the reported [7,10,12] dynamics of postcytostatic repair of the hemopoietic tissue in the bone marrow. The elevation of EPA and CSA production is of a nonspecific nature and is an obligatory component of the adaptive reactions of stress-realizing systems of the macroorganism subjected either to a myeloinhibiting or to a myelostimulating action [3].

The modulating effect of sympathetic transmitters on the functional activity of HIM cells for 5-fluorouracil administration is also evident from our experiments with adrenergic pharmacological antagonists. For instance, for combined administration of α- or β-adrenoblockers with 5-fluorouracil (the 1st scheme) the levels of EPA and CSA production by nucleated cells of different bone marrow fractions either did not differ or were significantly below the control values (Figs. 1, 2, 3, Table 1). Similar changes were found previously by us when adrenoblockers were administered according to an analogous scheme under conditions of immobilization stress [2,5], which are known to be characterized by enhanced sympathetic activity [9] and boosted control of HIM by the sympathetic nervous system [11].

On the other hand, treatment with adrenergic antagonists on the 3rd day after cytostatic administration (the 2nd scheme) resulted mainly in

TABLE 1. Values of the Integral Index (in % of Baseline Level) Characterizing the Effect of Preparations on EPA and CSA Production by Bone Marrow Cells of Different Fractions

Integral index	5 - Fluorouracil	5-Fluorouracil + ganglioblocker		5-Fluorouracil + α-adrenoblocker		5-Fluorouracil + β-adrenoblocker	
		Scheme I	Scheme II	Scheme I	Scheme II	Scheme I	Scheme II
EPA production, whole fraction	188	130*	296**	147	194	125*	253
CSA production, whole fraction	130	135	224**	186	213*	139	260*
EPA production, nonadhesive fraction	200	124*	363	138*	230	161	323
CSA production, nonadhesive fraction	197	137	186	118**	226	83	168
EPA production, adhesive fraction	91	117	229**	69**	157**	67	204
CSA production, adhesive fraction	223	159	428**	191	324	154	313

Note. * indicates $p<0.01$, ** indicates $p<0.05$ for the reliability of differences in comparison with the control (cytostatic alone) according to the Wilcoxon T test.

stimulation of the hemopoietic activity of cells forming the hemopoietic microenvironment throughout the observation period (Table 1). At individual times (on the 7th and 10th days) the CSA and, especially, EPA content was above (2-fold and more) the corresponding values in the control (cytostatic alone) (Figs. 1, 2). The described processes evidently underlie the markedly accelerated restoration of cell number in the erythroid and granulocytic components of myelopoiesis which we established previously using the 2nd scheme of administering adrenergic antagonists [10].

Thus, it may be concluded that for 5-fluorouracil administration the hemopoietic function of bone marrow cells forming HIM is significantly controlled by the sympathetic nervous system. This is also confirmed by the results of experiments with pentamine, which mainly blocks autonomic ganglia [1] and, like adrenoblockers, affects the activity levels in conditioned media (Figs. 1, 2, and 3, Table 1). The negative effect of catecholamines on the HIM production of humoral regulators of myelopoiesis during periods of cytostatic-induced devastation with subsequent repair of hemopoietic tissue (3rd-11th days) is beyond question. Furthermore, the initial functional integrity of the sympathetic nervous system is essential to the formation of an adequate HIM response to the administration of massive doses of 5-fluorouracil.

REFERENCES

1. S. V. Anichkov, *Neuropharmacology* [in Russian], Leningrad (1982).
2. E. D. Gol'dberg, A. M. Dygai, I. A. Khlusov, *et al., Pat. Fiziol.*, № 3, 14-17 (1991).
3. E. D. Gol'dberg, A. M. Dygai, I. A. Khlusov, *et al., Byull. Eksp. Biol. Med.*, **116**, № 9, 244-246 (1993).
4. E. D. Gol'dberg, A. M. Dygai, and V. P. Shakhov, *Methods of Tissue Culture in Hematology* [in Russian], Tomsk (1992).
5. A. M. Dygai, I. A. Khlusov, V. P. Shakhov, *et al., Pat. Fiziol.*, № 3, 17-20 (1991).
6. G. F. Lakin, *Biometry* [in Russian], Moscow (1990).
7. E. V. Melik-Gaikazyan, *Probl. Gematol.*, № 8, 48-50 (1980).
8. V. V. Novitskii, V. A. Fokin, and V. E. Gol'dberg, *A Method for Integrative Evaluation of the Toxic Effect of Antitumorigenic Preparations on Circulatory System* [in Russian], Moscow (1990).
9. E. B. Khaisman, V. A. Arefolov, L. A. Malikova, *et al., Farmakol. Toksikol.*, № 4, 18-21 (1991).
10. I. A. Khlusov, A. M. Dygai, S. G. Aksinenko, *et al., Byull. Eksp. Biol. Med.*, **115**, № 4, 372-375 (1993).
11. I. A. Khlusov, A. M. Dygai, and E. D. Gol'dberg, *Ibid.*, **116**, № 12, 570-572 (1993).
12. C. Lerner and D. Harrison, *Exp. Hematol.*, **18**, 114-118 (1990).
13. D. Metcalf, *Hemopoietic Colonies*, Berlin (1977).