Chapter 6
Disturbances in the Control of Blood System During Posthypoxic Period

One of the pivotal problems in experimental and clinical medicine is adaptation to hypoxia. Almost any pathologic process is more or less accompanied with the development of a particular type of hypoxia. The hypoxic stimulation exerts a powerful effect on the transport of blood gases resulting in the functional and then the structural rearrangements in the mechanisms supplying oxygen for an organism. On the whole, these changes sustain the energy metabolism [1, 10, 14, 124, 126, 147, 159, 235].

The changes evolving in the peripheral blood and in the bone marrow during hypoxic stimulation are described in detail [14, 108, 121, 133, 196, 198, 268]. Generally, the blood system reacts to hypoxia with an increase of oxygen capacity by increasing the number of erythrocytes and elevating the hemoglobin content. These adaptive changes results from the reflex release of the mature cells from depot as well as from activation and increase in the count of erythroid lineage in the hemopoietic organs [14, 109, 142, 198]. In its turn, activation of erythropoiesis results from elevation of the count of HSC in the bone marrow and peripheral blood [178, 201, 214, 222, 275, 321, 360], as well as from up-regulation of erythropoietin (erythrogenin) production by the renal juxtamerular apparatus [142, 175, 198, 236]. As a rule, these processes are accompanied with the development of leukocytosis in the peripheral blood (mostly of lymphocytic type) which is explained by migration of T-lymphocytes known as the regulators of hemopoiesis into hematopoietic tissue [53, 198]. In the early terms after exposure to hypoxia, neutrophilosis is less often observed, and it is viewed as a manifestation of the general adaptation syndrome [53, 154–156, 175, 196].

Irrespective to its cause, hypoxia is always accompanied by the development of unidirectional reactions in the hematopoietic tissue. However, the mode and length of hypoxic stimulation can determine many features of the specific changes formed at various organization strata of the blood system. We carried out a comprehensive study of the role of individual elements in HIM and that of the distant neurohumoral hemopoietic control mechanisms during hypoxia of various genesis and severity [43, 101–105].

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The experiments were conducted on CBA/CaLac mice. The experimental model were based on hypoxic-hypercapnic normobaric hypoxia (hypoxic hypoxia) and two variants of hemic hypoxia, which developed under (1) hemolytic or (2) post-hemorrhagic anemia. The hypoxic hypoxia was simulated with single or double (10 min prior to the second exposure) placing the mice in a 500-ml hermetic chamber. The hemic hypoxia was modeled either with intraperitoneal injection of phenylhydrazine hydrochloride (30 or 150 mg/kg) or puncture of retro-orbital sinus for blood withdrawal (30 % circulation blood volume in a single procedure or 70 % with triple bleedings performed during 2–3 h). The intact mice were used to obtain the control indices.

A single exposure to hypoxia in the hermetic chamber, injection of the hemolytic toxin (30 mg/kg), or withdrawal 30 % circulating blood (volume percentage) produced no significant changes in the psychoneurological status. Severe oxygen deficiency (double hypoxia in the hermetic chamber, injection of 150 mg/kg hemolytic toxin, or withdrawal 70 % circulating blood) provoked the encephalopathia documented according to the development of amnesia assessed according to the disturbances in the conditioned passive avoidance test and in orienting-exploratory behavior tested in the 'open field' environment [13, 369].

The experiments showed that hypoxia of various geneses that provoked no 'overt' disorders in the psychoneurological status induced a pronounced hyperplasia of the erythroid hemopoietic lineage. During the entire period of such experiments, there was a pronounced elevation of the erythrocaryocyte count in hematopoietic tissue, which was especially great during hypoxic hypoxia (up to 438.8 % baseline on experiment day 5). Activation of the medullar erythropoiesis was echoed in the peripheral blood by elevation in the reticulocyte count observed on days 1–3, 5, 8 and 9 after hypoxic hypoxia and during the entire observation period under any type of hemic hypoxia attaining the maximum level on day 10 after injection of the hemolytic toxin (671.5 % baseline value). The changes in erythrocyte content were mostly determined by specificity of hypoxic stimulation; they were characterized by the drop in erythrocyte count coupled with decreased hematocrit after withdrawal 30 % circulation blood (days 1–5) or injection of phenylhydrazine (days 1–10). In contrast, hypoxic hypoxia induced erythrocytosis developed on experiment days 1–6. The qualitative analysis of the blood formed elements revealed a slight increase in the volume of the mature erythrocytes resulting probably from release of a great number of young erythrocytes into the circulating blood [26, 98]. However, this index decreased in the early terms of hemolytic anemia (days 1–3). Evidently, this phenomenon resulted from destruction of the largest cells by the toxin during their transport in the microcirculatory bed. In addition to the changes in the size of erythrocytes observed on days 1 and 3 after hemoexfusion, there was elevation of the mean corpuscle content of hemoglobin probably related to the fact that a pronounced share of hematocrit was comprised by the erythrocytes released from the depot. Formation of these erythrocytes was going on under the conditions of balanced hemopoiesis characterized by a rather high activity of the hemoglobin-producing processes and a rather low maturation rate of erythroid precursors. Moreover, all

three groups displayed a decreased osmotic resistance of erythrocytes, which attained the minimum level on day 1 after injection of the hemolytic toxin.

As for granulocytopoiesis, any model of hypoxia was characterized with different increase in the count of immature and mature neutrophilic granulocytes in hematopoietic tissue, which attained the maximum values in the blood loss hypoxia model. This phenomenon was accompanied with increase in the count of the rod neutrophils in peripheral blood.

The described changes in hematopoiesis were preceded by up-regulation of the colony-forming potency of the bone marrow. Irrespective of its mode, hypoxia was accompanied with increase in formation of CFU-E and CFU-GM in the methyl cellulose medium. In all cases, the proliferative activity of the committed precursors of both types increased, and maturation of erythroid precursors accelerated virtually during entire period of examination. In addition, differentiation of the precursor cells of granulomonocytopoiesis was up-regulated after hemic hypoxia (hemolytic anemia), while it was down-regulated after hypoxic hypoxia.

The state of the pool of clonogenic cells is known to be mainly determined by the level of production of a wide spectrum of the humoral hemopoietic regulators (first of all, the hemopoietic growth factors) by the cellular components in HIM [37, 255]. Among the hormone-like agents, these growth factors are the most powerful hemopoietic regulators [57, 134, 299], and their combined effect can be assessed as EPA and CSA [56].

The study of secretion activity of some bone marrow fractions under various types of hypoxia showed an increase of EPA in the conditioned media harvested from the adherent and non-adherent nucleated cells in all groups and virtually at any term of examination. Elevation of CSA in the supernatants harvested from the adherent elements was observed during various terms of experiments under hypoxic hypoxia, hemolytic anemia, and after blood loss. It was also observed in the conditioned media of the non-adherent myelocaryocytes after injection of the hemolytic toxin. In contrast, the hypoxic hypoxia and blood loss down-regulated CSA production by the non-adherent fraction of the bone marrow.

The key role in the control of hemopoiesis (especially under the stressful conditions) is played by the serum humoral factors – specifically, the hormones of adrenal cortex and medulla, opioid peptides, eicosanoids, and other endogenous biologically active substances [53, 90, 299]. The experiments showed that irrespective of its type, hypoxia increased serum CSA, which attests to activation of the stress-mediating systems during hypoxia.

However, the development of adaptive reactions in the hematopoietic tissue during oxidative failure of various geneses is mainly determined by enhancement of functional activity of the erythropoietin-producing renal apparatus [26, 349]. It is an established fact that the products of erythrocyte degradation stimulate erythropoiesis [37, 136, 180]. It is also known that hypoxic stimulation activates the processes of erythrodiaeresis.

When studying the role of humoral factors in producing the hypoxia-induced hematological alterations, all the employed models revealed elevation in the contents of erythropoietically active substances in the blood serum. However, while

EPA dynamics was similar in all hypoxia models, the content of erythropoietin significantly differed in these models both in the periods and in the degree of EPA elevation. It is noteworthy that no model displayed a close conformity between EPA dynamics and the erythropoietin content, which shows that erythropoietin does not play any pronounced role in determining the level of total serum EPA.

Really, the up-regulation of colony formation in the test system induced by the serum derived under hypoxic hypoxia was observed on experiment days 1–5, 9, and 10 (attaining maximum 299.7 % baseline value on day 3), while increase in erythropoietin content was recorded in 12 h after hypoxia and on experiment days 1, 2, and 6–9 with the largest value of 363.3 % attained in 12 h after stimulation. In contrast, hemolytic anemia increased EPA in the blood serum, while the blood content of erythropoietin was significantly elevated only on experiment day 7. Hemoexfusion of 30 % circulating blood volume increased serum EPA and the content of serum erythropoietin virtually at the same time: on experiment days 1–3, 7, and 9 (EPA), and in 12 h and on experiment days 1–3 and 9 (erythropoietin). However, these indices significantly differed by the degree of elevation.

In all hypoxia models, the degree of hemolysis greatly increased in the periods when there was no correlation between EPA and the serum level of erythropoietin (on experiment days 3, 5; 1–10; and days 4, 6 after hypoxic hypoxia, hemolytic anemia, and blood loss, correspondingly). This fact indicates a pronounced contribution of the products of erythrocyte degradation into formation of serum EPA.

Investigation of cooperation between various HIM elements and the hemopoietic cells under diverse hypoxia models revealed enhancement of the potency of the supplementary elements in the bone marrow to form the cell associations. Specifically, hypoxic hypoxia increased the count of macrophage-positive and macrophage-negative associations. The qualitative analysis showed elevation in the count of erythroid HI in the hematopoietic tissue on experiment das 2, 3, 5, 6, and 8–10, although the significant elevations in the counts of their mixed and granulocytic types were observed only on days 2 and 3.

Similar alterations in the structure-functional organization of the bone marrow were also observed after hemic hypoxia. Both variants of hemic hypoxia enhanced the adhesive potency of macrophagal elements and the fibroblasts towards the hemopoietic precursors. These changes in activity of the adherent myelocaryocytes were regularly accompanied by a pronounced elevation of the content of erythroid HI in the bone marrow during virtually entire observation period attaining maximum of 386.2 % baseline value on day 7 after injection of hemolytic toxin or 359.0 % on day 5 after hemoexfusion. Elevations in the score of granulocytic and mixed cell associations were far less pronounced.

Taking into consideration the prominent role of lymphoid elements (specifically, Thy-1,2^+-cells) in the development of adaptive reactions in the blood system provoked by stressful stimulation [44, 90], we examined activity of these cells in the control of hemopoiesis during hypoxia. The experiments showed that various by their nature stimuli triggered the development of unidirectional changes in the count of Thy-1,2^+-cells in the bone marrow. In all cases their number in the hematopoietic tissue significantly increased on experiment days 2–4 after hypoxic hypoxia and on

days 1–4 after hemolytic anemia. All hypoxia models revealed a pronounced stimulation of functional activity of hemopoietic precursors under the effect of the above regulator elements accompanied by elevation in the count of medullar T-cells. Especially pronounced were the feeder activity of Thy-1,2^{+}-cells towards the hemopoietic precursor cells during their interaction with the elements of HIM adherent fraction. It is worthy to note that after hypoxic hypoxia or blood loss, this activating effect was more pronounced in respect to proliferation and differentiation of the erythroid precursors than to those of CFU-GM.

The data obtained proved the important role of T-cells with Thy-1,2^{+} phenotype in the development of hyperplasia of the hematopoietic tissue during hypoxia of diverse geneses. The research showed that Thy-1,2^{+}-cells exerted the stimulatory effect on the committed erythropoietic precursors both directly and indirectly via interaction with the adherent elements of the bone marrow, while in respect to the granulocyte-macrophage precursors, they displayed the feeder activity only indirectly via cooperation with the stromal elements.

An extra mathematical processing of the data with analysis of significant rank correlation coefficients (r) under diverse hypoxic stimulation revealed a marked increase in the number of signal correlations between the individual compartments of erythron system in all employed hypoxia models reflecting a high degree of coupling in the erythropoiesis-stimulating performance of various regulator systems such as HIM or erythropoietin system. In this orchestrated activity, the coordinating role in shaping the response of the hematopoietic tissue is evidently given to the central (neuroendocrine) regulatory subdivision. It is noteworthy that the hemolytic anemia was characterized with a positive correlation between serum EPA and hemolysis. At the same time, the data reduction performed with the factor analysis for any type of hypoxia, revealed the predominant dependence of blood profile on the formation of the extra structure-functional units in the bone marrow, *i.e.* on erythroid HI. However, no hypoxia model displayed any significant changes in the correlation matrix of the data characterizing the processes of granulomonocytopoiesis. The load factor analysis revealed a significant enhancement of serum CSA role in the response of granulomonocytic hemopoietic lineage indicating the growing importance of the long-range humoral mechanisms in the control of granulomonocytopoiesis during various types of hypoxia and under sustaining the initially low coordination level in the performance of individual HIM elements.

Thus, hypoxia of various geneses, which produces no damage to CNS, triggers the development of clearly manifested compensation-adaptation reactions in the blood system such as pronounced hyperplasia of erythroid hemopoietic lineage responsible for the oxygen supply to the tissues and stimulation of granulocytopoiesis reflecting activation of the stress-mediating systems [40]. These alterations are determined by migration of hemopoietic regulator T-cells into the bone marrow, their cooperation with the stromal elements in hematopoietic tissue, enhancement of feeder activity of the cellular components in HID, and elevation of the content of hematopoietically active serum substances. The data obtained attest to profound role of not only erythropoietin, but also other substances in forming serum EPA and consequently, in shaping the response of erythron system to hypoxia (Fig. 6.1).

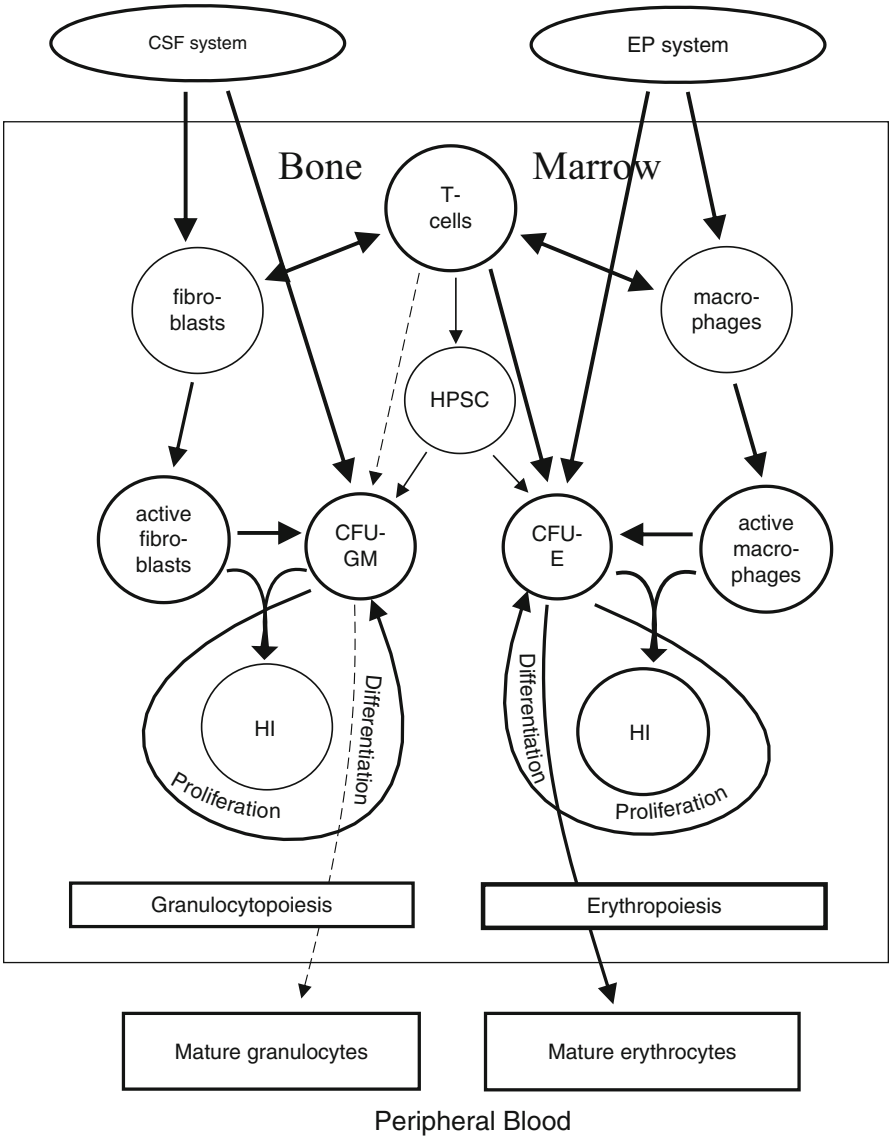

Fig. 6.1 Control of hematopoiesis during mild hypoxia provoking no damage to CNS. Absence of any significant changes is marked with fine continuous lines, while the *dash* and *thick solid lines* indicate inhibition and activation, correspondingly

The comparative analysis of hematological alterations in the employed hypoxia models showed that the most efficient reaction of erythropoiesis was observed under hemic hypoxia. It is explained by a high rate of maturation of the erythroid precursors and rapid release of erythrocytes from the hemopoietic organs. Certain differences

were also observed in the reactions of granulomonocytic lineage. In all hypoxia models, intensity of proliferation of hemopoietic precursors increased, which however was not accompanied (or little accompanied) by activation of their differentiation after hypoxic hypoxia and blood loss. Hemolytic anemia did not uncouple these processes, which probably resulted from a high oxygen deficiency during this type of hypoxia and production of a large amount of phlogogenic substances in hypoxia-damaged tissues [96, 303].

However, literature reports that pronounced inhibition of aerobic oxidation in cerebral tissues significantly reorganizes CNS, modifies the integrative-triggering activity of the neurons resulting in a qualitatively novel pattern of interaction of individual cerebral subdivisions. In case of decompensation of the adaptive mechanisms, a chain of pathologic process is triggered leading to progressive neurological disorders and abnormalities in the work of numerous visceral organs and systems [5, 119, 132, 138, 139].

Further stage of our studies was examination of possible influence of encephalopathia as a hematopoiesis-dysregulating process on the formation of hematological alterations during severe hypoxia.

Investigation of the reactions of hematopoietic tissue in animals with hypoxia-induced cerebral pathology revealed a number of interesting features. Irrespective to the triggering cause, encephalopathy was accompanied with a delayed decrease in the level of hyperplasia of hemopoietic lineage resulting from a decrease in the count of hemopoietic precursors and moderation of their proliferative activity. In particular, the hypoxic hypoxia diminished CFU-E content on experiment days 4, 5, 8, and 9, while the count of mitotically active precursors decreased as early as day 1 of experiment. The development of cerebral pathology provoked by hemolytic agent or 70 % blood volume loss was accompanied with a decrease in the colony-forming potency of the bone marrow on, respectively, experiment days 4, 6, 7, 9 or 4, 9 as well as with down-regulation of DNA synthesis in CFU-E observed on days 5–7 (hemolytic anemia) or on days 3, 8, 9 (blood loss) in comparison to the control values measured in the animals without encephalopathy.

In all hypoxia models and during entire observation period, there was a compensatory activation of the processes of CFU-E differentiation related to up-regulation of the secreting function of the adherent myelocaryocytes, elevated serum EPA, accompanied by up-regulation of the formation of the erythroid cell associations after injection of 150 mg/kg phenylhydrazine or extensive blood loss.

Severe hypoxia produced quite equivocal effect on the humoral mechanisms of erythropoiesis control. While after hypoxic hypoxia or blood loss the increase in EPA was greater than elevation of erythropoietin content, the severe hemolysis pronouncedly elevated erythropoietin content almost during entire observation period, but decreased serum EPA at the late terms of experiment. In addition, irrespective to the exciting cause of encephalopathy, all animals with this pathology demonstrated dramatic elevation in the degree of hemolysis.

The study of T-lymphocytic mechanisms of hematopoietic control under severe oxygen deficiency of diverse genesis showed that the development of CNS pathology in all cases led to pronounced and long-term decrease in the count of Thy-1,2^{+}-cells

in the bone marrow practically to the level characteristic of the intact animals. At the same time, even a small amount of these cells efficiently stimulated CFU-E in the culture, but this effect was observed only when they interacted with the elements of adherent fraction of myelocaryocytes. These data conclude that preservation of activity of one of the trigger elements of erythropoietic stimulation (T-cells of hematopoietic tissue) during severe hypoxia of various geneses is related not to the changes in the count of medullar population of Thy-1,2$^+$-cells, but to their functional state. However, virtually in all cases with severe encephalopathia, Thy-1,2$^+$-lymphocytes lost the ability to individually affect the proliferation-differentiation status of the erythroid precursors without cooperation with the resident HIM cells.

The changes in examined parameters of granulomonocytic hemopoietic lineage were in many respects similar to the dynamics of erythropoietic indices. Specifically, the medullar count of granulomonocytic progenitors decreased after hypoxic hypoxia (on day 4), hemolytic anemia (on days 2, 4, 5, 7 and 9), and blood loss (days 9–10). In all cases, these changes were preceded by (1) a decrease in CFU-GM division rate, which was most pronounced after injection of 150 mg/kg phenylhydrazine (down to 54.4 % control value on postinjection day 7) and (2) an increase in maturation rate of granulomonocytic precursors. The latter phenomenon resulted from an increased level of CSA in the tested biological fluids. Severe hypoxic stimulation (hypoxic hypoxia) and a high volume of blood loss up-regulated formation of CSA by the adherent and non-adherent nucleated cells of the bone marrow; in addition, it elevated the content of serum hemopoietins after hypoxic hypoxia and posthemorrhagic encephalopathia. In contrast, the development of cerebral pathology provoked by the hemolytic toxin significantly enhanced CSA in the conditioned media of the bone marrow cells without any discernible effect on serum CSA.

However, an increase of hypoxia severity in all cases was accompanied with a marked enhancement of ability of Thy-1,2$^+$-cells to stimulate the growth of granulomonocytic precursors either in the pool of medullar cells cultured on adhesive cell sublayer or (in contrast to hypoxia not accompanied with encephalopathia) in individually cultured non-adherent elements. Efficiency of the influence of these cells on colony formation mediated via their interaction with the adherent myelocaryocytes was pronouncedly greater than that observed during naturally similar hypoxic states that did not disturb the psychoneurological status (especially in the hemolytic variant of hypoxia). Taking into consideration the results of the study, one can conclude that a severe degree of oxygen deficiency determined the following change in the vector of the direct feeder influence of Thy-1,2$^+$-cells from predominant action on erythroid precursors after compensated hypoxia to stimulation of granulomonocytopoiesis after severe hypoxia aggravated with developing encephalopathia.

Despite the same kind of alterations in the state of granulocyte-macrophage precursors and spectacular similarity in the changes of regulatory mechanisms in all hypoxia models, there were significant differences between the experimental groups in the content of morphologically identifiable cells of granulocytic moiety. For example, while massive hemolysis and blood loss decreased the count of immature and mature granulocytes in the bone marrow relative to the baseline values, the hypoxic hypoxia elevated the counts of mature granulocytes on experiment days

3, 7, 8 and 10. In all hypoxia models, we observed the development of neutrophilic leukocytosis in the peripheral blood, which attained the maximum level (up to 437.4 % baseline value on experiment day 1) in the cases with encephalopathia caused by the hemolytic toxin. Probably, this phenomenon resulted not so much from accelerated differentiation of the granulomonocytic precursors, but rather from the disturbances in the efflux of the toxically damaged leucocytes from the tissues [26].

Irrespective to its cause, the damage to CNS was accompanied with the changes in the parameters of peripheral part of the erythron system. The hypoxic animals demonstrated either the development (hypoxic hypoxia) or aggravation of anemia. At the high doses of phenylhydrazine or after a massive blood loss, this anemia was related to specificity of stimulation characterized with the maximum drop in the count of erythrocytes to 35.1 % (day 6) or 54.4 % (day 3) baseline value, correspondingly. In addition, despite the clearly displayed reticulocytic reaction, one of the reasons of anemia was abnormality of the recovery dynamics of erythrocyte content due to the development of macrocytosis and degradation in osmotic resistance of the cells virtually during entire observation period resulting in rapid dieresis of the newly formed large erythrocytes. In the cases with encephalopathia provoked by hypoxic hypoxia, anemia was delayed and hypochromic, which can be probably explained by extreme enlargement of the mature erythrocytes and by a decrease in their hemoglobinization during the overstressed erythropoiesis.

In all encephalopathia models, the correlation and factor analyses revealed a pronounced decrease in the number of causal relationships between the numerical parameters of erythron and the functional activity of the erythropoietic control systems during the changes in the factor loads of the correlation matrix. This fact reflects a diminished role of cell-cell cooperation in mediating the response of the erythron system in contrast to that of serum EPA, which probably attests to dysregulation of hematopoiesis during hypoxia inducing the pathologic changes in CNS. In addition, all cases of severe hypoxia were characterized with a positive correlation between increase of serum EPA and intensity of hemolysis.

However, in the model of phenylhydrazine-induced encephalopathia the mathematical analysis of granulomonocytopoiesis revealed an increase in the number of signal relationships between the individual compartments of granulomonocytic lineage, which attested to enhancement of system stress. In all cases, reduction of the correlation bonds performed with the factor analysis revealed an increasing role of the humoral regulators produced by the stromal HIM components, which in the cases of hypoxic hypoxia and blood loss was also accompanied with decreasing role of serum hemopoietins in shaping the reactions of leucocytes to hypoxia. These facts indicate the changes in the character of granulocytopoiesis control during severe hypoxic stimulation.

Overall, the data obtained make it possible to describe the state of the blood system resulting from failure of the compensation-adaptation hemopoietic mechanisms during hypoxia as a kind of ‘erythropoietic distress’ manifested by disadaptation of the hematopoietic tissue and production of the pathological forms of erythrocytes.

At this state, the decrease in the count of hematopoietic precursors accompanied with enhancement of functional activity of the relatively resistant stromal components in HIM can be related to their damage due to extreme activation of the sympathoadrenal and pituitary-adrenal systems. As we mentioned in the above, such inverse (negative) effect of surplus of the catecholamines on the hemopoietic precursor cells was observed in the model of cytostatic myelosuppression provoked by antimetabolite injection [53]. As for the pathologic hyperactivity of sympathoadrenal system during hypoxia, it can result from dysfunction of inhibitory mediator systems known for their high sensitivity to oxygen deficiency [5, 132].

To test the hypothesis about the central genesis of the revealed hematologic phenomena in severe hypoxia, the exposed mice were treated with a single intraperitoneal injection of sodium oxybutirate (500 mg/kg), which in all cases eliminated the psychoneurological signs of encephalopathia and significantly corrected the manifestations of disadaptation in the blood system.

The pharmacological protection of the brain elevated the count of progenitor cells in the bone marrow tissue observed in the cases of severe hypoxic hypoxia (experiment days 3–5, 8), toxin-induced hemolysis (day 6), and massive blood loss (day 3). In all cases, these changes were accompanied with increasing proliferative activity of the precursor cells up the levels characteristic of the mice subjected to the milder variants of the corresponding hypoxic stimulation producing no dramatic disturbances in CNS. Logically, the compensation for the disturbances in the committed progenitor cells resulted in hyperplasia of erythroid hemopoietic lineage accompanied by arresting the development of anemia resulted from hypoxic hypoxia. Injection of sodium oxybutirate during severe hemolytic anemia and 70 % blood volume loss was accompanied by an increase in the count of erythrocytes in the peripheral blood. In this case, the size of mature erythrocytes was significantly decreased, which was not accompanied by any significant changes in the release of reticulocytes into the blood.

Examination of secretory function of individual HIM components after neuroprotective treatment and exposure of the mice to severe hypoxia revealed down-regulation of EPA production by the adherent myelocaryocytes on experiment day 4 (hypoxic hypoxia), on days 1, 7, 10 (hemolytic anemia), and on day 7 (massive blood loss). However, injection of sodium oxybutirate produced virtually no effect on (1) feeder activity of Thy-1,2^{+}-cells for CFU-E, (2) production of EPA by non-adherent cells in the bone marrow, and (3) serum EPA. These facts show that during total oxygen deficiency, hemopoiesis is mostly affected by the direct effects of hypoxia on HIM mobile elements (T-cells included) and on the distant humoral mechanisms of hematopoietic control in contrast to indirect effects of hypoxia on hematopoiesis mediated via CNS.

Injection of sodium oxybutirate also elevated the count of granulomonocytopoiesis precursors in the hematopoietic tissue in mice subjected to hypoxic hypoxia, hemolytic anemia, and blood loss, which was accompanied by an increase in their division rate. However, CFU-GM maturation index decreased in all cases. In all groups, the above alterations resulted from down-regulation of CSA production by the adherent medullar nucleated cells, and after hypoxic hypoxia or blood loss they were

accompanied by a decrease in the serum content of granulomonocytopoiesis inducers. Nevertheless, intensity of differentiation of the granulomonocytic precursors under the antihypoxant action of sodium oxybutirate significantly surpassed this parameter in animals subjected to the corresponding types of hypoxia that did not provoke lesion to CNS. In all cases, a comparatively high maturation rate of the progenitor cells of granulomonocytopoiesis was related to retention of feeder activity of Thy-1,2^{+}-cells for CFU-GM, up-regulation of CSA production by the non-adherent myelocaryocytes during the early posthypoxic period, and an enhanced level of serum hemopoietins during the late posthypoxic period. Finally, the changes in proliferation-differentiation status of CFU-GM resulted in elevation in the count of mature neutrophilic granulocytes in the bone marrow on experiment days 5, 7 (hemolytic anemia), and on days 3, 4, 6 (blood loss), but they produced no significant effect on the score of morphologically identifiable cells of granulocytopoiesis in the case of hypoxic hypoxia in comparison with the animals not treated with the antihypoxant agent.

In all hypoxia models, the described ambiguous alterations in the medullar granulocytopoiesis in mice treated with sodium oxybutirate were reflected in the peripheral blood by a decrease in the count of segmented neutrophils relatively to the control values as assessed on experiment days (3–7), (1, 2, 6, 7), and (7–9) after hypoxic hypoxia, hemolytic anemia, and blood loss, correspondingly. However, this index remained enhanced in comparison with the corresponding values in the animals exposed to the same types of hypoxia, which did not provoke encephalopathia. In our opinion, maintenance of a high level of neutrophil production is underlain by physiologically reasonable necessity to 'clear' the tissues from detritus [78, 303], whose production is pronouncedly increased with aggravation of hypoxia.

The experimental data unequivocally attest to interrelation between cerebral pathology caused by 'global' hypoxia of diverse geneses with decrease in the number of hemopoietic precursors in the hematopoietic tissue, enhancement of the feeder activity of the stromal components in HIM, and up-regulation of production of the pathological forms of erythrocytes (Fig. 6.2).

Under severe hypoxia, the most probable reason of the alterations in the blood system especially manifested by the disturbances in hematopoiesis is the damage to the hematopoietic cells by the adrenergic overstimulation. In such cases, the effects of catecholamines are predominantly mediated via β-adrenergic receptors [47, 53].

Examination of the role of the adrenergic hemopoietic control mechanisms in shaping the hematological alterations during hypoxia aggravated by encephalopathia showed that blockade of β-adrenergic receptors with a single subcutaneous injection of propranolol (5 mg/kg) made after double hypoxic hypoxia elevated the count of erythrocytes in the peripheral blood (experiment days 4, 7, 9), increased the hemoglobin content (day 5), and abrogated the development of hypochromic anemia in posthypoxic period. Moreover, propranolol markedly improved the recovery dynamics of erythrocyte indices in the cases of severe hemolytic and hemorrhagic anemia. In the cases of hemic hypoxia caused by phenylhydrazine (150 mg/kg) and hemorrhagic anemia provoked by massive blood loss, the count of erythrocytes was elevated correspondingly on experiment days 6–9 and 5, 6, 9, while hematocrit increased on days 6–8 and 4, respectively. In these experiments, the qualitative analysis

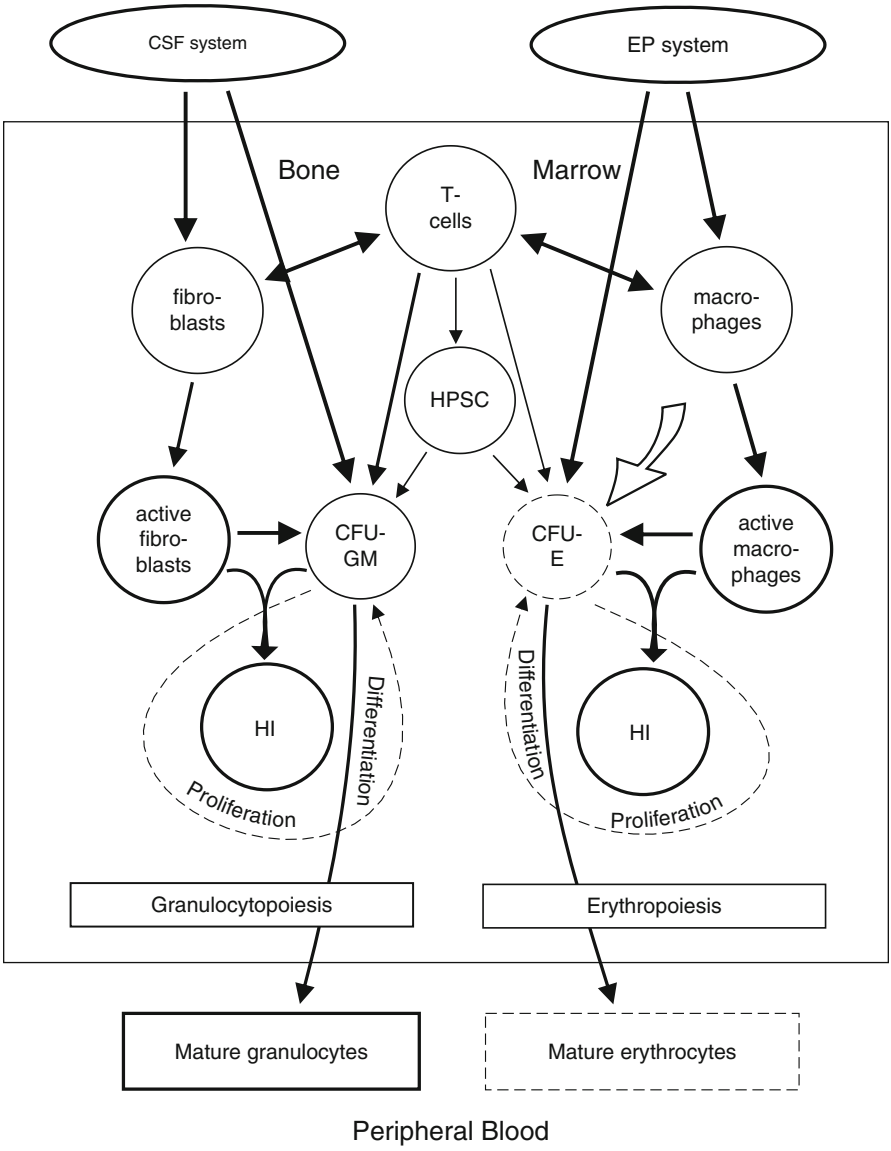

Fig. 6.2 Control of hematopoiesis during severe hypoxia provoking encephalopathia. Absence of any significant changes is marked with fine continuous lines, while the *dash* and *thick solid lines* indicate inhibition and activation, correspondingly. The *open arrow* indicates inhibitory effect by the adrenergic systems

of the blood formed elements revealed a marked decrease in the size of erythrocytes in mice treated with propranolol in comparison with similar value in mice subjected to various types of hypoxia but not treated with this adrenergic blocker.

The revealed alterations in the peripheral blood logically reflected the dynamics of medullar erythropoiesis. For example, treatment of hypoxic mice with propranolol increased the count of erythrocaryocytes in the bone marrow after double hypoxic hypoxia (observed on experiment days 5, 6, 9), hemic hypoxia caused by 150 mg/kg phenylhydrazine (days 6, 7), and hemoexfusion of 70 % circulation blood volume (days 6, 7) in comparison with the animals whose adrenergic mechanisms had not been corrected.

The cell culture studies of the effects of the adrenergic stimuli on erythropoiesis revealed dependence of the above reactions in the blood systems on the state of the progenitor cells in hematopoietic tissue. For instance, propranolol significantly elevated the count of erythroid precursors in the bone marrow on experiment day 4 after hypoxic hypoxia and on days 3–4 after the blood loss. The study of proliferative activity of the hemopoietic progenitor cells revealed an increase of their division rate in all hypoxia models, although it was significant only in the model of hypoxic hypoxia on experiment day 3. At the same time, no group of mice demonstrated significant changes in the rate of CFU-E differentiation despite down-regulation of production of the erythropoietically active substances by the adherent fraction of the bone marrow observed on experiment day 4 after hypoxic hypoxia or blood loss. In these cases, injection of an antagonist of β-adrenergic receptors produced no effect on the secretory function of HIM non-adherent elements and on serum EPA.

However, the alterations in granulomonocytic hemopoietic lineage induced by β-adrenergic antagonist were mostly redistributive in character: they were manifested by the disturbances in the release of immature neutrophils in the blood, which agree with the data obtained in other pathology model [53]. The limited effects of propranolol were manifested only by insignificant accumulation of the mature neutrophilic granulocytes in the bone marrow in the models of hemolytic and severe hemorrhagic anemia, and by a decrease in the count of the rod neutrophils in the peripheral blood observed in the models of hypoxic hypoxia and hemorrhagic anemia. No hypoxia models with the signs of encephalopathia displayed any marked differences in the counts of other morphologically differentiated granulomonocytic cell elements in the bone marrow and peripheral blood or significant diversities in the count and state of CFU-GM pool. Propranolol did not change CSA levels in the conditioned media of the non-adherent myelocaryocytes and in the blood serum, although it down-regulated CSA production by HIM adherent cells on experiment day 5 in the models of hemic hypoxia caused by 150 mg/kg phenylhydrazine and hemorrhagic anemia induced by hemoexfusion of 70 % circulation blood volume.

On the whole, our experiments showed that hyperactivation of the adrenergic systems in an organism subjected to severe hypoxia exerted a negative influence on erythropoiesis. This ‘inverse’ effect of a surplus of the catecholamines results from the damage to erythroid precursors mediated via β-adrenergic receptors located on their membranes [47, 53].

Thus, the damage to cerebral structures caused by hypoxia and the related alterations in activity of the adrenergic mechanisms of hemopoietic control pronouncedly disturb the development of adequate adaptive reactions in the blood system, which in its turn aggravates the oxygen supply to the tissues in an organism during hypoxia.