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Original Articles

The Kinetics of Hematopoietic Stem Cells During and After Hypoxia

A Model Analysis

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Summary. A previously described mathematical model of the hematopoietic stem cell system has been extended to permit a detailed understanding of the data during and after hypoxia. The model includes stem cells, erythroid and granuloid progenitors and precursors. Concerning the intramedullary feedback mechanisms two basic assumptions are made: 1) The fraction "a" of CFU-S in active cell cycle is regulated. Reduced cell densities of CFU-S, progenitors or precursors lead to an accelerated stem cell cycling. Enlarged cell densities suppress cycling. 2) The self renewal probability "p" of CFU-S is also regulated. The normal steady state is described by $p = 0.5$, indicating that on statistical average each dividing mother stem cell is replaced by one daughter stem cell, while the second differentiates. Diminished cell densities of CFU-S or enlarged densities of progenitors and precursors induce a more intensive self renewal ($p > 0.5$), such that the stem cell number increases. The self renewal probability declines ($p < 0.5$) if too many CFU-S or too few progenitors and precursors are present. The model reproduces bone marrow data for CFU-S, BFU-E, CFU-C, CFU-E, 59 Fe-uptake and nucleated cells in hypoxia and posthypoxia. Although the ratio of differentiation into the erythroid and granuloid cell lines is kept constant in the model, a changing ratio of CFU-E and CFU-C results. The model suggests that stem cells and progenitor cells are regulated by a regulatory interference of erythropoiesis and granulopoiesis.

Key words: Hematopoiesis – Stem cells – Mathematical model – Hypoxia – Posthypoxia

The behaviour of hematopoietic stem cells and progenitor cells in murine bone marrow during and after hypoxia has been examined by various investigators. During hypoxia, Lord and Murphy (1973) [18] measured CFU-S numbers, CFU-S cycling and nucleated cells.

BFU-E and CFU-E were determined by Dunn et al. (1980) [8]. 59 Fe-uptake was measured by Turner et al. (1967) [27] and Kubanek et al. (1968) [14]. During the post-hypoxic phase Peschle et al. (1977) [23] and Wagemaker et al. (1977) [28] examined the behaviour of BFU-E, CFU-C and CFU-E. In this communication we show further posthypoxic data for CFU-S and nucleated cells which were obtained according to the method described by Lord and Murphy (1973) [18].

The characteristics of these findings are: During hypoxia the number of CFU-S remains relatively normal. Their cycling as well as the number of BFU-E and CFU-C seem to decrease while the erythroid precursors show an increase. A different behaviour is, however, observed in animals which do not respond with an appropriate erythropoietic cell production to a severe hypoxia [5, 8, 10, 14]. Therefore, in this study only data from BDF_1, $BCDA_1$, CD_1 and $C_3H \times AKR$ mice are considered, which are responsive to hypoxic stimulation.

During the posthypoxic phase, CFU-S shows a steep increase to values above 150% of normal after an initial time-lag of several days. Similarly, the BFU-E and CFU-C numbers rise from below normal to clearly above normal levels. Stem cell cycling is slightly increased as is the nucleated cell count.

There are several models of murine stem cell regulation which consider the influences of erythropoietin (Epo) [1, 2, 31], but only the qualitative model of OKunewick et al. (1969) [22] discusses hypoxia and posthypoxia. That model suggests an hypoxic decrease of pluripotent stem cells, due to enlarged differentiation, and an overshoot in post-hypoxia.

The following analysis is performed with a mathematical model of intramedullary stem cell regulation. It represents an extended version of a simpler model which did not include granulopoiesis [15]. The model distinguishes two dominant regulatory mechanisms. First, the cycling of stem cells is regulated. It is accelerated if the density of any intramedullary cell stage falls below normal while increased cell densities suppress cycling. Second, the self renewal probability of stem cells is regulated independently of the cyclic status of the cells. The normal steady state, with constant stem cell numbers, is characterized by a self renewal probability of $p = 0.5$ indicating that all dividing mother stem cells are on average replaced by 50% of their daughter cells. A larger self renewal probability ($p > 0.5$) initiates a growth of the stem cell population, a smaller "p" accompanies a stem cell reduction.

It is a basic model assumption that the cycling and the self renewal of stem cells are regulated by the cell densities of stem cells, erythroid and granuloid progenitors and precursors. In this study we investigate what kind of interaction and which type of demand regulation might be detectable in the present set of experiments. A novel hypothesis will be derived which can explain the data on hypoxia and especially post-hypoxia.

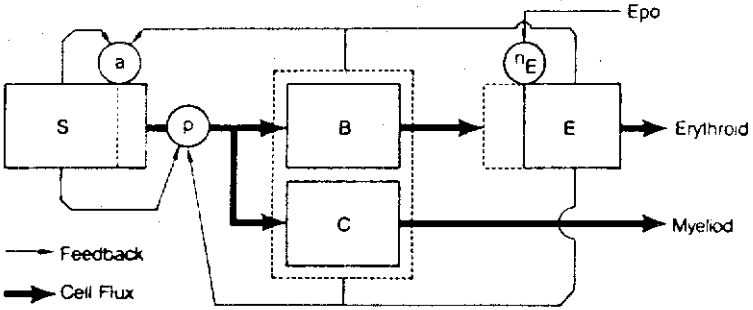

Fig. 1. Block diagram of the model. S: pluripotent stem cells; B: Epo-independent erythroid progenitors; E: Epo-dependent erythroid progenitors; C: granuloid progenitors; Epo: Erythropoietin; a: proliferative fraction of S; p: self renewal probability in S; n_E: number of divisions in E. Regulatory influences on B and C are not considered. The dashed line around B and C indicates that the relative numbers of B and/or C modulate "p" and "a". The dashed line in S indicates that "a" changes the ratio of resting to cycling S cells. The dashed lines in E illustrate how additional mitoses "n_E" may lead to an expansion of the compartment

Mathematical Methods

A schematic review of the model is given in Fig. 1. For the analysis, the following mathematical description is used for the four cell compartments S, B, C and E. (S, B, C, E stand for both the name and the size of the compartments): t is time in hours

$$\dot{S}(t) = (2\,p - 1)\,a\,S/t_S \tag{1}$$

$$\dot{B}(t) = \dot{B}^{in}(t) - \dot{B}^{out}(t) \tag{2a}$$

$$\dot{C}(t) = \dot{C}^{in}(t) - \dot{C}^{out}(t) \tag{2b}$$

$$\dot{E}(t) = \dot{E}^{in}(t) - \dot{E}^{out}(t) \tag{3}$$

The compartment inflow and outflow rates $\dot{S}^{out}$, $\dot{B}^{in}$, $\dot{B}^{out}$ etc. are described in equations (4)–(6):

$$\dot{S}^{out}(t) = 2(1 - p)\,a\,S/t_S \tag{4}$$

$$\dot{B}^{in}(t) = \alpha_E{}^* N_B{}^* \dot{S}^{out}(t);\quad \dot{B}^{out}(t) = B(t)/T_B \tag{5a}$$

$$\dot{C}^{in}(t) = \alpha_G{}^* N_C{}^* \dot{S}^{out}(t);\quad \dot{C}^{out}(t) = C(t)/T_C \tag{5b}$$

$$\dot{E}^{in}(t) = N_E{}^* \dot{B}^{out}(t);\quad \dot{E}^{out}(t) = E(t)/T_E \tag{6}$$

The meaning and value of the different parameters is given in the following:

Minimal cell cycle time in S: $t_S = 8$ h (7a)

Compartment transit time in B:
$T_B = 40$ h, in E: $T_E = 40$ h, in C: $T_C = 80$ h. (7b)

The values for t_S, T_B, T_E are identical with those in the previous model [15] and are known from experiment. T_C is chosen to equal $T_B + T_E$.

The fraction of all differentiating cells which undergo erythroid or granuloid determination are called: α_E, α_G. The amplification factors in B, C and E are called N_B, N_C, N_E. In steady state the amplification factor equals the ratio of compartment outflow to inflow rates. The amplification factor N_E in compartment E will depend on erythropoietin (Epo) (see below). The constant parameters α_E, α_G, N_B and N_C need not be specified for the model calculations since only relative compartment sizes are considered. (For practical calculations they can e. g. be given the values 0.1, 0.9, 5, 10.)

The regulatory functions are influenced by the normalized cell numbers: $S' = S/S_{normal}$, $C' = C/C_{normal}$, $B' = B/B_{normal}$, $E' = E/E_{normal}$.

The proliferative fraction "a" of S-cells is considered as a regulated quantity. It is reasonable to assume that it can reach both the maximum value and the minimum value in asymptotic ways. There exist a number of mathematical possibilities to represent such sigmoidally shaped curves. The following one is chosen for its handiness:

$$a = a(S', C', E') = \frac{a_1 \exp(X) - a_2 \exp(-X)}{\exp(X) + \exp(-X)} \tag{8a}$$

(Note: For $a_1 = a_2$ the formula simplifies to $a = a_1 * \tanh(x)$) with

$$X = X(S', C', E') = a_3 [1 + \sigma * \ln(S') + \gamma * \ln(C') + \varepsilon * \ln(E')] + a_4 . \tag{8b}$$

The values of a_1, a_2, a_3, a_4 are determined from the values

$$a_{max} = 1.0,\ a_{norm} = 0.1,\ a(0.5, 1, 1) = 0.4,\ a_{min} = 0.01 . \tag{8c}$$

σ can be set to equal 1.

By setting $C' = E' = 1$ one can show that the function a (S', 1, 1) is very similar to the proliferative function described in the previous model [15]. Thus eq. (8) represents an extension of the previous model now also including C' and E'.

The self renewal probability "p" of pluripotent stem cells also depends on S', C' and E' in a bi-asymptotic way. Again the mathematically representation is chosen for its handiness:

$$p = p(S', C', E') = \frac{p_1 \exp(Y) - p_2 \exp(-Y)}{\exp(Y) + \exp(-Y)} \tag{9a}$$

$$Y = Y(S', C', E') = p_3 * [f_S (1-S') - f_C (1-C') - f_E (1-E')] \tag{9b}$$

p_1, p_2, p_3 are determined by.

$$p_{max} = 0{,}6,\ p_{min} = 0.4 \text{ and } p(0.5, 1, 1) = 0.55 . \tag{9c}$$

The inclusion of C' in eq. (9) represents an extension of the previous model [15], f_S can arbitrarily be set to equal 1.

It is assumed that Epo influences the number, n_E, of mitoses in E. The number of mitoses determines the amplification factor N_E as follows:

$$N_E = N_E(Epo) = 2^{n_E(Epo)},\ n_E(Epo) = c_1 - c_2 * \exp(-c_3 * Epo) \tag{10a}$$

c_1, c_2, c_3 are determined by $n_E^{max} = 7$, $n_E^{norm} = 5$ and $n_E^{min} = 2$. (10b)

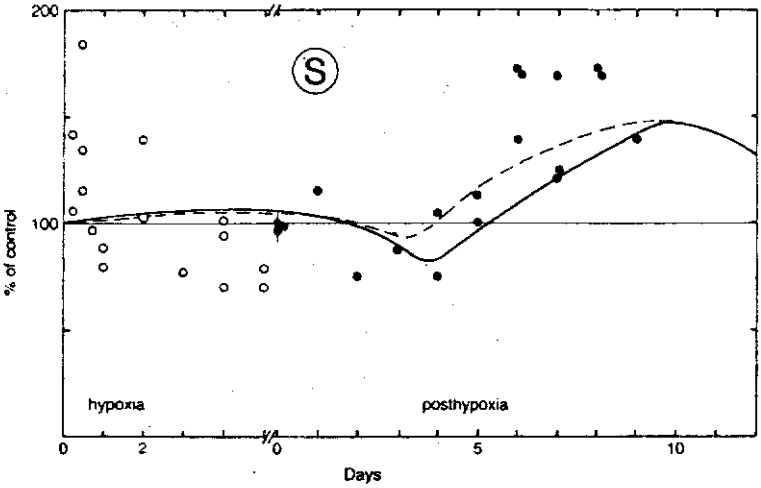

Fig. 2. Pluripotent stem cells in hypoxia and posthypoxia. Comparison of experimental data for CFU-S in the bone marrow [○: hypoxia, [18]; ●: posthypoxia, Murphy and Lord (1973 unpublished results)] and model curves for compartment S (——: feedback by S, C, E; – – –: feedback by S, B, E)

As can be seen, the number of mitoses n_E and the amplification factor N_E asymptotically reach their extreme values for high Epo-levels.

The model contains a total of 24 parameters. Five of them are irrelevant because they only affect absolute compartment contents and will not be considered (α_E, α_G, N_B, N_C, n_E^{norm}).

Eleven parameters can be estimated from independent experiments (t_S, T_B, T_E, T_C, a_{max}, a_{norm}, $a(0.5, 1, 1)$ a_{min}, P_{max}, n_E, n_E^{min}) [15, 17]. Four parameters (P_{min}, $p(0.5, 1, 1)$, f_S, σ) are chosen to reproduce the corresponding regulatory functions of the previous model. All these parameters were known and could be kept at fixed values. No distributions were taken into account. The remaining free parameters ε, γ, f_C, f_E had to be chosen. We call them 'feedback intensities' because they determine how strongly changes in the differentiated compartments C or E affect the values of the stem cell qualities "a" and "p". In all calculated model curves of Figs. 2–7 they were given the values

$$f_C = 3.0,\ f_E = 1.0,\ \varepsilon = 0.3,\ \gamma = 0.1 \tag{11}$$

Values differing from these by up to 20% would, however, still yield a similar model behaviour.

The only external input fed into the model was an Epo-time curve simulating an hypoxic stimulation equivalent to 6 km altitude for 5 days and a subsequent posthypoxic suppression of 10 days with consecutive recovery (see [30]).

In the following, comparisons are made between the model compartment S and CFU-S data, B and BFU-E, C and CFU-C, E and CFU-E (or 59Fe-uptake) in the bone marrow. Concerning CFU-S and BFU-E, the spleen's contribution to the whole

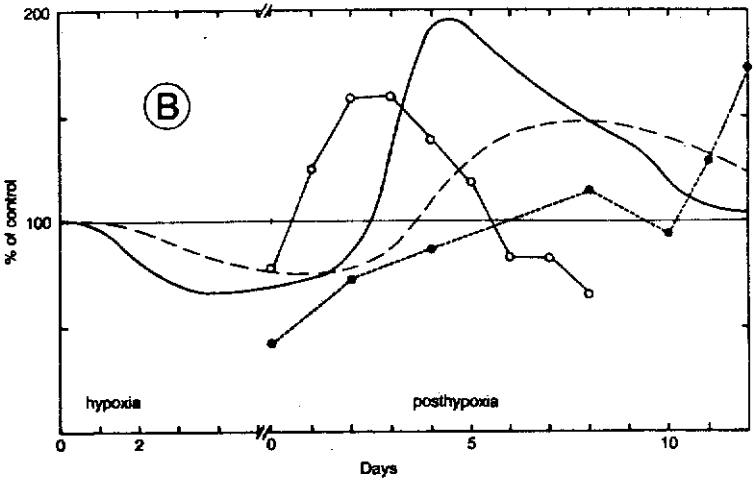

Fig. 3. Erythropoietin-independent erythroid progenitors in hypoxia and posthypoxia. Comparison of experimental data for BFU-E [○: posthypoxia; [28], after 72 h hypoxia in 9 days, BCDA-F1 mice; ●: posthypoxia; [23], after 209 h hypoxia in 11 days, CD1 mice] and model curves for compartment B (symbols as in Fig. 2)

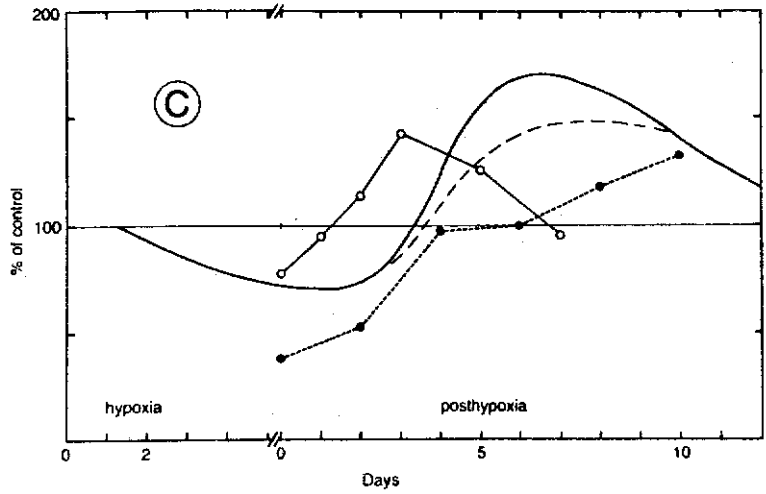

Fig. 4. Granuloid progenitors in hypoxia and posthypoxia. Comparison of experimental data for CFU-C and model curves for compartment C (symbols as in Fig. 3)

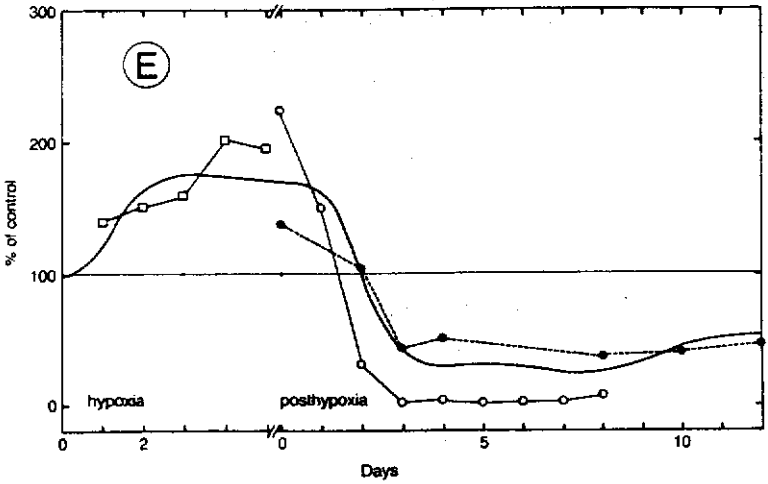

Fig. 5. Erythropoietin-dependent erythroid progenitors in hypoxia and posthypoxia. Comparison of experimental data [□: hypoxia; 59 Fe-incorporation shifted by two days, [27] C3H × AKR mice; ○, ●: posthypoxia, CFU-E, symbols as in Fig. 3] and model curves for compartment E (symbols as in Fig. 2, both curves coincide)

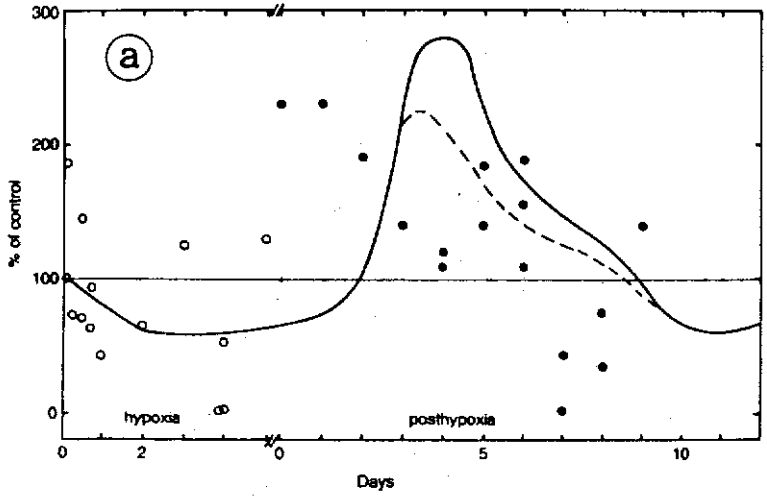

Fig. 6. Proliferative fraction of pluripotent stem cells in hypoxia and posthypoxia. Comparison of experimental data for 3 HTdR-suicide of CFU-S [○: hypoxia, [18]; ●: posthypoxia, [Murphy and Lord (1983 unpublished results)] and model curves for the parameter "a" (symbols as in Fig. 2)

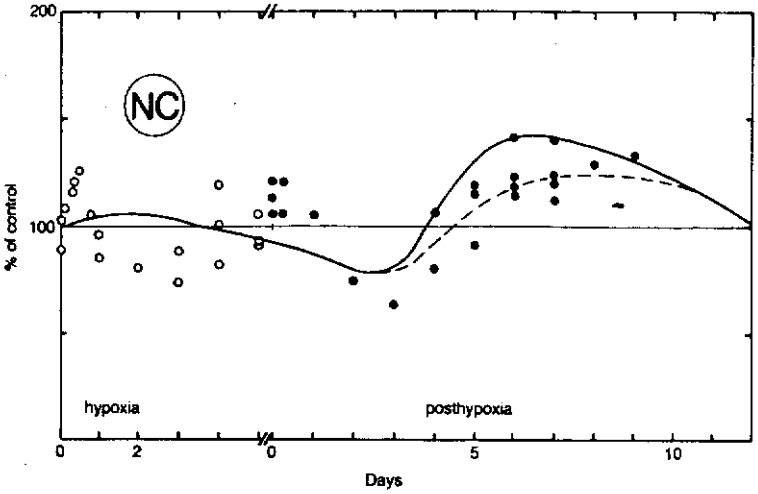

Fig. 7. Bone marrow cellularity in hypoxia and posthypoxia. Comparison of nucleated cell count [○: hypoxia, [18]; ●: posthypoxia, Murphy and Lord (1983 unpublished results)] and model curves (symbols as in Fig. 2)

animal's CFU-S and BFU-E never exceeds 20% in hypoxia or posthypoxia [5, 6, 12, 14, 18, 23, 25]. During hypoxia, splenic CFU-E account for not more than 33% of the whole animal's cells [23]. Furthermore, marrow and spleen never show antagonistic behaviour so that the cells in the bone marrow are always quantitatively dominating. It is therefore permitted to consider the bone marrow alone as representative of the whole animal's erythropoiesis.

In order to compare model calcultions with data on total nucleated cell counts (see Fig. 7) the weighted sum of E and C plus lymphoid cells has been estimated. It is assumed that the lymphoid cells behave in a manner parallel to C (according to the data from Beran & Tribukait (1971 a) [3]; Turner et al. (1967) [27] and that the ratio of erythroid versus granuloid plus lymphoid cells is 1:4.

Results

The basic regulatory functions of this model are the proliferative fraction of pluripotent stem cells "a" and their self renewal probability "p". Both depend on cell numbers in the compartments S, C (or B) and E. A reduction in any of these numbers increases the proliferative fraction "a", i. e., brings pluripotent stem cells S from the quiescent state into the active cell cycle. For the self renewal probability, the pattern is different. Here a reduction in S elevates "p", so that more cells remain in S. A reduction in C (or B) and E, however, reduces "p" so that more cells differentiate from S to C (or B) and E, satisfying their need. For the following, it is important to remember that the efflux from S to B and C is proportional to $(1 - p)$ *a*S.

Figs. 2–7 show calculations for hypoxia and posthypoxia with this generalized stem cell model. Typical qualitative and quantitative characteristics of the experimental data are reproduced by the model where the feedback depends either on S, C, E (full line) or on S, B, E (dashed line):

Hypoxia

Days 0–2. The hypoxic increase of erythropoietin (Epo) directly stimulates additional mitoses in E. The consequences of high E-values are twofold: first there is a reduction in pluripotent stem cell proliferation ("a" decreases) and there is an initial rise of stem cell numbers because the self renewal probability "p" rises above 0.5. Both effects reduce the efflux from S and lead to subnormal values in B and C.

Days 3–5. The diminished C (or B) now compensates for the influences of the still enlarged E on "p" until a new steady state is approached. This hypoxic steady state ($p = 0.5$) has elevated E, decreased B and C, reduced proliferative activity in S, and normal S values. The nucleated cell numbers remain relatively normal.

Posthypoxia

Days 0–4. Epo levels drop drastically, followed by a steep decrease in E to low values within two days. This has the following consequences. First the proliferative fraction "a" in S increases, and second, the self renewal probability "p" falls below 0.5, with a subsequent fall of the S values. Both effects increase the efflux from S so that B and C overshoot. The nucleated cell numbers drop to subnormal values due to the drastic decrease of E.

Days 5–8. As a consequence, the high C (or B) values lead to a (relative) reduction of the stem cell proliferation ("a" falls again) and second, as a very important effect, elevated C (B) values increase the self renewal probability "p" to near maximum values. Therefore, after a few days in posthypoxia, S values increase steeply, while B and C diminish again but stay supranormal. An intermediate steady-state ($p = 0.5$) is reached with reduced E, elevated B, C and S. The proliferative activity shows normal values after a temporary increase.

Days 8–12. Data from several studies [4, 7, 21, 26] indicate that the hematocrit approaches normal values by days 8–12. Therefore, Epo slowly normalizes. Accordingly, E starts to recover. This leads to a decrease in the proliferative fraction "a" and in the self renewal probability "p" and thus to decreases of S, B and C. In total, the system turns back to normal values.

In the model, changes in E following the Epo-time curve are the leading motor and driving force for the regulatory activities. The behaviour of B, C and S in posthypoxia are closely correlated. In the initial posthypoxic phase, S shows a slight temporary dip. During this phase, B and C increase. The duration of the S-dip is correlated with the time interval where B, C and E are below normal. As a result of the constant determination rates α_E and α_G, B and C behave in parallel. Nevertheless C and E behave in an antiparallel manner. Comparison of these calculations with data shows a good agreement for S, B, C and E with CFU-S, BFU-E, CFU-C and CFU-E.

should be emphasized, that the model does not provide a realistic description of the cell cycle. E. g. cell kinetic labelling experiments (PLM, LI) could not be simulated at this stage of modelling. A connection of a compartment description with a realistic description of the cell cycle in each of these compartments would be a further step of model refinement. Technically one would either have to introduce further subcompartments or to switch to a description in partial differential equations. In any case the numerical effort would increase considerably, many parameters (G_1, S, G_2-phase durations) would be unknown and finally for hypoxia and posthypoxia no comparable cell kinetic data exist. Therefore the cell kinetic simplifications adopted here seem adequate to the data situation.

A second simplification is the description of the G_0-phase in the stem cell compartment. It proved advantageous in the previous model [15]. It is nevertheless conceptually similar to other models [29], and avoids the speculative incorporation of many cell phase parameters which are all unknown. But even if they could be estimated thereby giving rise to a more realistic description of the G_0-phase the above conclusions would qualitatively not change.

Third, measurements suggesting a variable ratio of erythroid versus non-erythroid differentiation of CFU-S after Ara-C treatment [19] do not necessarily contradict the model assumptions on fixed ratios. On one hand the mathematical model analysis of cytotoxic drug effects indicates that they can considerably alter the regulation mechanisms of stem cell cycling, self renewal and differentiation (to be published). On the other hand it has been well documented that BFU-E and CFU-C show parallel development in many circumstances (hypertransfusion: [9, 13]; posthypoxia: [23, 28]; bleeding: [13, 24], after acute irradiation: [16, 24]) which, in our eyes, supports the assumption of constant differentiation rates.

Forth, evidence for variable transit times in BFU-E [11, 13] and CFU-C [20] compartments exists. Changes in transit times may affect compartment contents. But the magnitude of these effects is small compared with the effects induced in compartment B and C by the changes of the influx rates. They can be increased drastically by acceleration of the stem cell cycling. Therefore incorporation of realistic variable transit times would not significantly change the model results.

The present model has two further advantages compared with the earlier version [15]. First, it retains all positive capabilities including a good data reproduction of the system after acute and during chronic irradiation, following bleeding anemia and Epo-injection. The reproduction of hypertransfusion (especially CFU-S) data is even better than in the old model. Second, this version exhibits a deeper symmetry. Although originally designed for the erythroid lineage, the model now allows expansion to the granuloid (and other) pathways. Pursuing the parallel relation between BFU-E and CFU-C mentioned above it is easy to understand that in this generalized model, B and C can take over each others role. For the model calculations, it makes no significant difference whether B or C is used in the regulatory functions "a" and "p" [Eqs. (8) and (9)]. Furthermore, it cannot be finally decided whether E and C have to represent CFU-E and CFU-C only or whether they also may represent more mature bone marrow cells like erythroblasts and myeloblasts.

In summary, the model analysis suggests that two biologically different feedback mechanisms can explain the stem cell behaviour during and after hypoxia. One mechanism is a simultaneous (but heterogeneous) interaction of erythroid and granuloid

progenitors (CFU-E, CFU-C) or precursors (erythroblasts, myeloblasts) on CFU-S. The other possible mechanism is a simultaneous (but heterogeneous) interaction of early (BFU-E) and late (CFU-E) erythroid committed cells on CFU-S. But interestingly in both cases, the ratio of differentiation from CFU-S into the erythroid and the granuloid cell lines can be kept constant. The present results should, in our opinion, focus the search for growth and regulation factors to the question whether the proposed system of refined imbalance of erythroid versus granuloid feedback influences on stem cell cycling and self-renewal really exists.

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