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A mathematical model of erythropoiesis in mice and rats

Part 1: Structure of the model

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Abstract. A mathematical model has been developed which describes the regulation of erythropoiesis in mice and rats. The main model assumptions are: (1) Regulation is mediated by erythropoietin (EPO). (2) The production of EPO depends exponentially on the tissue oxygen pressure (e.g. in the renal production sites). (3) There are sigmoidal dose-response curves relating the EPO concentration in the plasma to the mitotic activity of CFU-E and proliferative erythropoietic precursors. For maximum stimulation two to four additional mitoses may occur, while for an absent stimulus three to five mitoses may be omitted. (4) The normal precursor transit time of three to four days may be shortened by more than 50% during maximum stimulation. (5) The erythrocytes have a normal lifespan of 42–56 days, which may be reduced to 15–20 days under erythropoietic stimulation.

Among these assumptions, the dose-response relationships between EPO and the mitotic activity of CFU-E and the proliferative erythropoietic precursors are the most important hypotheses of the model. This is the first of a series of three papers and gives a description of the mathematical formalism and the parameters used. In the subsequent papers computer simulations on erythropoietic stimulation and suppression are presented.

It is generally accepted that erythropoietin (EPO) is the essential hormone in a feedback system regulating erythropoietic cell production. Most of the models of erythropoiesis in man and in rodents make the assumptions that the production of erythropoietin depends on the demand for red blood cells (RBC) and that EPO stimulates the proliferation of erythropoietic progenitors and precursors (see review in Wichmann, 1983).

Mary *et al.* (1980) and Tarbutt & Blackett (1968) developed mathematical models for normal steady state erythropoiesis in mice and rats. Regulatory models have been described by Mylrea & Abbrecht (1971) and Leonard *et al.* (1980) for hypoxia, by Pabst *et al.* (1981, 1985) for anaemia, hypertransfusion and administration of hydroxyurea and by Aarneas (1978) for irradiation, chemotherapy and injection of erythropoietin in mice. In the following a

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mathematical model will be discussed which is able to describe the normal steady state situation and several erythropoietic manipulations. The first part will present the basic assumptions and the mathematics of the model. In the two subsequent papers (Part 2 and 3: Wichmann *et al.*, 1989; Wulff *et al.*, 1989) the validity of the model will be tested during and after several kinds of stress which stimulate or suppress erythropoiesis in mice and rats. Comparing the theoretical curves with the experimental data it will be shown which experimental findings can be reproduced by the model and which are not yet understood.

THE MODEL

The model describes the whole erythropoietic series but neglects haemopoietic stem cell regulation which has been analysed separately in detail (Wichmann & Loeffler, 1985). This is justified, since situations of stem cell manipulations such as those induced by irradiation or cytotoxic drugs will not be considered subsequently. The erythropoietic differentation lineage is described by five compartments, each representing one cell-type (CFU-E, proliferative erythroid precursors (PEP), non-proliferative erythroid precursors (NPEP), reticulocytes (RETI), erythrocytes (ERY)). These compartments are connected by cell fluxes.

The model is a demand system where changes of the oxygen-supply lead to an increased or decreased production of the erythropoietic progenitors and precursors. The regulation of these processes are mediated by the hormone erythropoietin (EPO). The block diagram and the parameters of the model are given in Fig. 1 and Table 1 respectively, the model equations are presented in the appendix.

Late erythropoietic progenitors (CFU-E)

The erythropoietic cells originate from pluripotent haemopoietic stem cells (CFU-S). In the model, CFU-S and the early erythropoietic progenitors (BFU-E) are neglected because it is known experimentally that their status will change little during the situations analysed here and in the subsequent two papers. Therefore, a constant cell differentiation rate into the first model compartment (the late progenitors (CFU-E)) is assumed.

The *in vivo* dose-response curves of the *amplification* factor f of CFU-E (corresponding to the number of mitoses m with $f = 2^m$) are assumed to depend on EPO. These curves describe the most important assumptions of the model because they determine the erythropoietic reaction on stimulating and suppressing stress. Four parameters are necessary to determine the sigmoidal dose-response curve used (see Appendix).

For mice, it is assumed that cells in the CFU-E compartment perform five divisions under normal steady state conditions. This corresponds to an amplification factor $f_{CFU\text{-}E} = 2^5 = 32$ and has been derived from experiments in which erythropoiesis was suppressed by hypertransfusion (Gregory *et al.*, 1973; Hara & Ogawa, 1977; Iscove, 1977; Monette, 1983). On the other hand, from experiments with stimulated erythropoiesis (e.g. in severe anaemia) it follows that one to two additional mitoses may occur which corresponds to an additional amplification factor of two to four (Adamson *et al.*, 1978; Iscove, 1977). In the model, a factor of 4 is assumed leading to $f_{CFU\text{-}E} = 4*32 = 128$ for maximum stimulation. On the fourth parameter describing the steepness of the dose-response curve, no direct experimental data are available.

Since no CFU-E data are available for rats, the erythropoietic amplification for these animals can only be found by using the data about morphologically recognizable precursors. Subtracting the erythropoietic proliferation due to the mitotic activity of erythroblasts from the total erythroid proliferation, we find approximately a normal amplification of $f_{CFU\text{-}E} = 2^3 = 8$

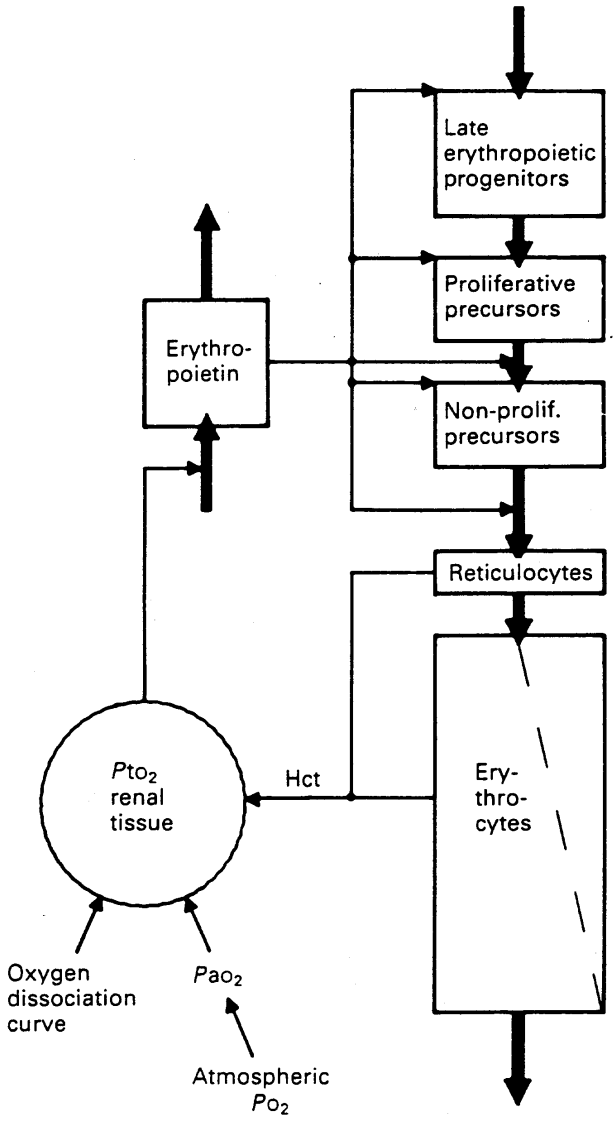

Fig. 1. Block diagram of the proposed mathematical model of erythropoiesis. ➡ = transition between the compartments; → = regulatory influences.

and a maximum amplification of five times normal, corresponding to $f_{CFU\text{-}E} = 40$ (Lamerton & Lord, 1964; Travnicek & Neuwirt, 1968).

The transit time of CFU-E is estimated to be 50 hours both for mice and rats (Udupa & Reissmann, 1979), as a constant value independent of stimulation or suppression.

Proliferative erythropoietic precursors (PEP)

This compartment represents proerythroblasts, basophilic and proliferative polychromatic erythroblasts.

For mice, the normal amplification in the PEP compartment has been chosen as 64 which corresponds to six mitoses ($f_{PEP} = 2^6 = 64$), according to measurements of Fe^{59}-uptake. For maximum stimulation, $f_{PEP} = 96$ is assumed and no mitoses for an absent stimulus (Erslev

Table 1. Model parameters during normal, minimum and maximum stimulation of erythropoiesis

Parameter	Abbreviation	Mice			Rats		
		Minimum Stimulation	Normal	Maximum	Minimum Stimulation	Normal	Maximum
Bone marrow							
Late progenitors (CFU-E)	$Y_{CFU\text{-}E}$						
transit time (h)	$\tau_{CFU\text{-}E}$	50	50	50	50	50	50
amplification factor	$f_{CFU\text{-}E}$	8(1)$^{++}$	32	128	2·5(1)$^{++}$	8	40
Proliferative precursors (PEP)	Y_{PEP}						
transit time (h)	τ_{PEP}	65	54	27	72	60	30
amplification factor	f_{PEP}	10(1)$^{++}$	64	96	40(25)$^{++}$	100	200
Non-proliferative precursors (NPEP)	∇_{NPEP}						
transit time (h)$^{+}$	τ_{NPEP}	20	18	6	33	30	10
erythroblasts	$Y_{NPEP'}$						
transit time (h)	$\tau_{NPEP'}$	6	6	6	10	10	10
BM-reticulocytes	$Y_{NPEP''}$						
transit time (h)	$\tau_{NPEP''}$	14	12	0	23	20	0
Total marrow precursor transit time (h)	$\tau_{PEP+NPEP}$	85	72	33	105	90	40
total amplification factor	$f_{CFU\text{-}E}{}^{*}f_{PEP}$	80(1)$^{++}$	2048	12288	100(25^{++})	800	8000
Blood							
Reticulocytes in blood:	Y_{RETI}						
transit time (h)	τ_{RETI}	16	18	30	17	20	40
Erythrocytes:	Y_{ERY}						
life span (d)	τ_{ERY}	45	42·2	15	60	56·2	20
age-dependent influx in ERYA (%)	Y_{ERYA}	100	90·5	0	100	90·5	0
random-loss influx in ERYR (%)	Y_{ERYR}	0	9·5	100	0	9·5	100
Erythropoietin:	Y_{EPO}						
turnover time (h)	τ_{EPO}	3	3	3	3	3	3
production rate(/h)	P_{EPO}	0·1(0)$^{++}$	1	200	0·1(0)$^{++}$	1	200
Oxygen dissociation curve:							
Hill's exponent	n		3			3·5	
*P*50 (mmHg)	*P*50		40			38	
Art. o_2 pressure (mmHg)	Pao_2		80			95	
Art. o_2 saturat. (%)	Sao_2		89			91	
o_2 desaturation (%)$^{+++}$	dSo_2		30			40	
Tissue o_2 saturat. (%)$^{+++}$	Sto_2		59			51	
Tissue-Po_2 (mmHg^{+++})	Pto_2		45			39	

$^{+}$ For the regulated amplification factors and transit times BR = 0·7 has been chosen (see also equations 7a, b).

$^{++}$ Realistic minimum for EPO = 0·1 or Hct of about 1·5* normal (hypothetical minimum for EPO = 0 in brackets).

$^{+++}$ of the whole body, but for simplicity also assumed for the sites of EPO production in the kidney.

et al., 1978; Mary *et al.*, 1980; Wagemaker *et al.*, 1977). For rats, the corresponding model values are 6·6 mitoses (or $f_{PEP} = 100$) under normal stimulation, one additional mitosis under maximum stimulation and two mitoses less under absent stimulation (Deiss *et al.*, 1966; Reissmann, 1964; Stohlmann *et al.*, 1964; Tarbutt & Blackett, 1968). The dose-response curve relating the transit time of the erythropoietic precursors to EPO is not known. Similar to the amplification factors f a sigmoidal dependence of the erythropoietic precursor transit time on EPO is assumed (see equation 7b in the Appendix). From differential cell counts in the bone marrow a normal transit time of the proliferative precursors (τ_{PEP}) of 54 h follows for mice and of 60 h for rats (Mary *et al.*, 1980; Papayannopoulou & Finch, 1975; Ganzoni *et al.*, 1969; Tarbutt & Blackett, 1968). For erythropoietic stimulation it is assumed that τ_{PEP} can be reduced to half its normal value (mice 27 h; rats 30 h). For absent stimulus, a slight prolongation by 20% is assumed (mice 65 h; rats 72 h). Both values have been adapted from corresponding measurements in man (Hillman & Finch, 1967; Erslev, 1971).

Non-proliferative erythropoietic precursor (NPEP)

This compartment contains the non-proliferative polychromatic and orthochromatic erythroblasts and the marrow reticulocytes. The amplification factor is 1 because the cells do not proliferate anymore.

The transit time τ_{NPEP} is derived from the difference between the total transit time of all erythropoietic precursors and τ_{PEP} resulting in 18 h for mice and 30 h for rats (Ganzoni & Hillman, 1969; Naga & Hara, 1968; Papayannopoulou & Finch, 1975; Tarbutt, 1969). τ_{NPEP} may be separated into the transit time of the non-proliferating blasts (mice 6 h; rats 10 h) and of the marrow reticulocytes (mice 12 h; rats 20 h) according to Mary *et al.* (1980), Tarbutt & Blackett (1968) and Mylrea & Abbrecht (1971).

Reticulocytes in the blood (RETI)

One can estimate the transit time of blood reticulocytes from the lifespan of all red cells and the proportion of reticulocytes. We assume that τ_{RETI} equals 18 h for mice and 20 h for rats (Ganzoni *et al.*, 1969; Hanna, 1967; Gurney *et al.*, 1961; Huff *et al.*, 1975) and that about 1·5%–2% of the red blood cells are reticulocytes.

During erythropoietic stimulation the marrow reticulocytes are shifted into the blood where they continue their maturation (Griffiths *et al.*, 1970). In the model, we assume that the total maturation time of the reticulocytes in the bone marrow and the blood is constant (see equations 4b and 4c in the Appendix). Thus, under erythropoietic stimulation the maturation time in the blood increases to 30 h in mice and 40 h in rats while it is slightly reduced to 16 h in mice and 17 h in rats for suppressed erythropoiesis.

Erythrocytes (ERY)

In equilibrium, the destruction of erythrocytes occurs mainly age-dependent with a mean lifespan of about 42 days in mice and 56 days in rats. This follows from labelling experiments with ^{59}Fe or other markers (Bentley, 1977; Tarbutt, 1967; Jirtle & Clifton, 1978; Landaw & Winchell, 1970). Both the type of destruction and the lifespan are changed for erythrocytes produced during severe stimulation. These so-called stress erythrocytes are mainly destroyed at random and their lifespan is reduced to less than half the normal value (Berlin & Lotz, 1951; Card *et al.*, 1969; Landaw & Winchell, 1970; Stohlman *et al.*, 1964). In contrast, during absent stimulation the lifespan of the erythrocytes is almost normal.

To incorporate this experimental knowledge into the model two parallel subcompartments are constructed. The first one called ERYA has a first-in-first-out structure with a lifespan of 45 d for mice and 60 d for rats. It represents the cells which die in an age-dependent manner after

a fixed lifespan. The second subcompartment called ERYR represents the erythrocytes formed under stress conditions and has a random structure with a mean lifespan of 15 d for mice and 20 d for rats.

In equilibrium, 90% of the newly formed erythropoietic cells enter ERYA and only 10% ERYR. During maximum stimulation all new cells may enter ERYR, but for missing stimulus all of them enter ERYA. The switch directing the flux of cells into ERYA or ERYR should be regulated by the 'stress history' of the regulatory systems. For technical reasons, this is simplified and the efflux of the compartment of reticulocytes regulates the switch parameter (see equations 5a, 5b and 5c in the Appendix).

Erythropoietin (EPO)

Erythropoietin (EPO) is activated by a renal erythropoietin inducing factor (RIF), which is produced in the kidney (Fisher *et al.*, 1975). In the model, RIF is not considered explicitly and for EPO an exponential dependence of the production rate on the tissue oxygen pressure is assumed with a maximum of 200 times normal and a minimum close to zero (see References in Part 2). The exponential form of this regulatory function has been taken from a model on erythropoiesis in man (Wichmann *et al.*, 1976). It is assumed that the EPO-production depends on the partial tissue oxygen pressure in the renal EPO production sites (Demopoulos *et al.*, 1965; Dunn *et al.*, 1976; Fisher *et al.*, 1975). Several factors influence Pto_2 as the number of red blood cells (RBC), the oxyhaemoglobin dissociation curve (ODC), the arterial oxygen pressure Pao_2 the arterio-venous difference dso_2 and the local blood flow. This relation has been discussed in detail elsewhere (Wulff, 1983; Wichmann, 1983) and is given in the Appendix.

The ODC has been measured for mice and rats. It can be described by Hill's formula with its parameters $P50$ (oxygen pressure for 50% desaturation) and n (steepness of the linear part of the curve) which depend on several factors especially the pH or the concentration of 2,3 diphosphoglycerate (Miller *et al.*, 1973). Information about changes in these factors due to an experimental treatment (e.g. hypoxia) will be taken into account (Part 2). In all other circumstances the model value for $P50$ equals 40 or 38 mmHg and n equals 3 or 3·5 for mice or rats, respectively (Table 1) (Hammond *et al.*, 1968; Leonard *et al.*, 1983). The normal oxygen desaturation (dso_2) of 30% for mice and 40% for rats has been taken for the whole body and for the kidney. These values lead to good reproduction of the experimental data during hypoxia and anaemia and fit the values of Pto_2 measured in skin pockets (see Part 2).

Erythropoietic feedback loop

How the oxygen supply influences the regulation of erythropoiesis in the model is shown in Fig. 2 for mice. Under normal conditions, the arterial O_2-pressure in mice equals 80 mmHg which corresponds to an arterial saturation of 89%. Desaturation by 30% leads to a tissue saturation of 59% and a tissue oxygen pressure Pto_2 of 45 mmHg. The Pto_2 is the regulatory parameter in the model. If Pto_2 is decreased the production rate of erythropoietin is stimulated; if it is increased, the production of erythropoietin is suppressed.

In anaemia, the capacity available to transport oxygen from the lung to the tissues is reduced and a further desaturation of haemoglobin is necessary to meet the constant oxygen demand. Thus, if the red cell mass is diminished to 50% (Fig. 2a) the desaturation has to be doubled if other compensatory mechanisms are neglected. In this situation, tissue saturation is reduced to 29% and Pto_2 to 29 mmHg.

During the initial period of hypoxia, the red cell mass is normal but the arterial oxygen pressure is lowered. For a Pao_2 of 40 mmHg which corresponds to an altitude of 5 km one finds

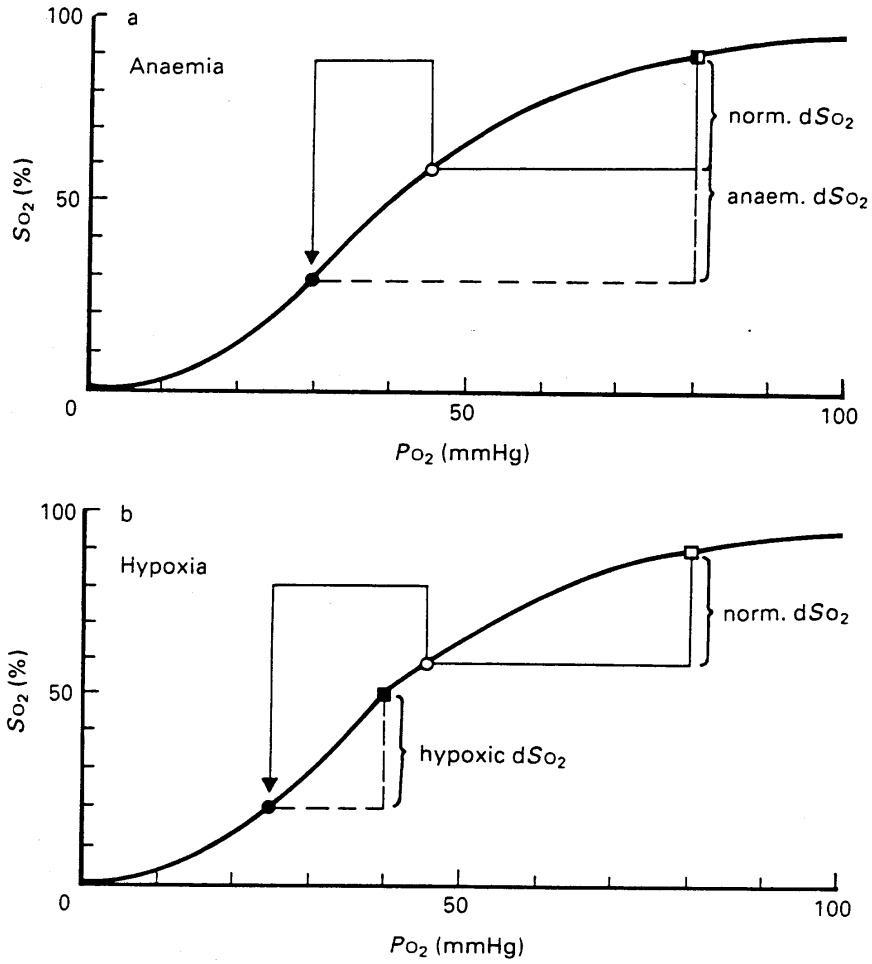

Fig. 2. Oxygen dissociation curve (ODC) for mice in the model. Symbols represent the arterial O_2 saturation (squares) and the tissue O_2 saturation (circles) under steady state conditions (open symbols) or under stimulation of erythropoiesis (closed symbols), respectively. (a) *Anaemia*: reduction of the oxygen transport capacity in anaemia leads to a higher desaturation (anaem. dSo_2) compared to steady state conditions (norm dSo_2) (dSo_2 = arterial o_2-saturation – tissue O_2 saturation). (b) *Hypoxia*: lower atmospheric pressure reduces the arterial o_2 saturation but keeps the desaturation constant (hypoxic dSo_2 = norm dSo_2). Thus, both anaemic and hypoxic results in a reduction of the tissue oxygen saturation and pressure (arrow) which stimulates EPO production (see Fig. 1). (For simplicity, plasma volume and ODC are kept constant.)

an arterial Sao_2 of 50%. A desaturation by 30% leads to a tissue saturation of 20% and a reduced Pto_2 of 25 mmHg (Fig. 2b).

The regulation of the whole erythropoietic system is summarized in Fig. 3. If the Hct is reduced, erythropoietin increases up to 200 times normal and stimulates erythropoietic amplification. Such maximum stimulation increases the number of (proliferative + non-proliferative) erythroblasts up to four times normal in mice and up to 7·3 times normal in rats. The precursor transit time is reduced from 72 h to 33 h in mice and from 90 h to 40 h in rats. Total production reaches a plateau of six times normal in mice and 10 times normal in rats.

On the other hand, an elevation of Hct above normal leads to a decrease in erythropoietic production. For an Hct of 1·5 times normal, the number of erythroblasts is reduced to 2% in mice

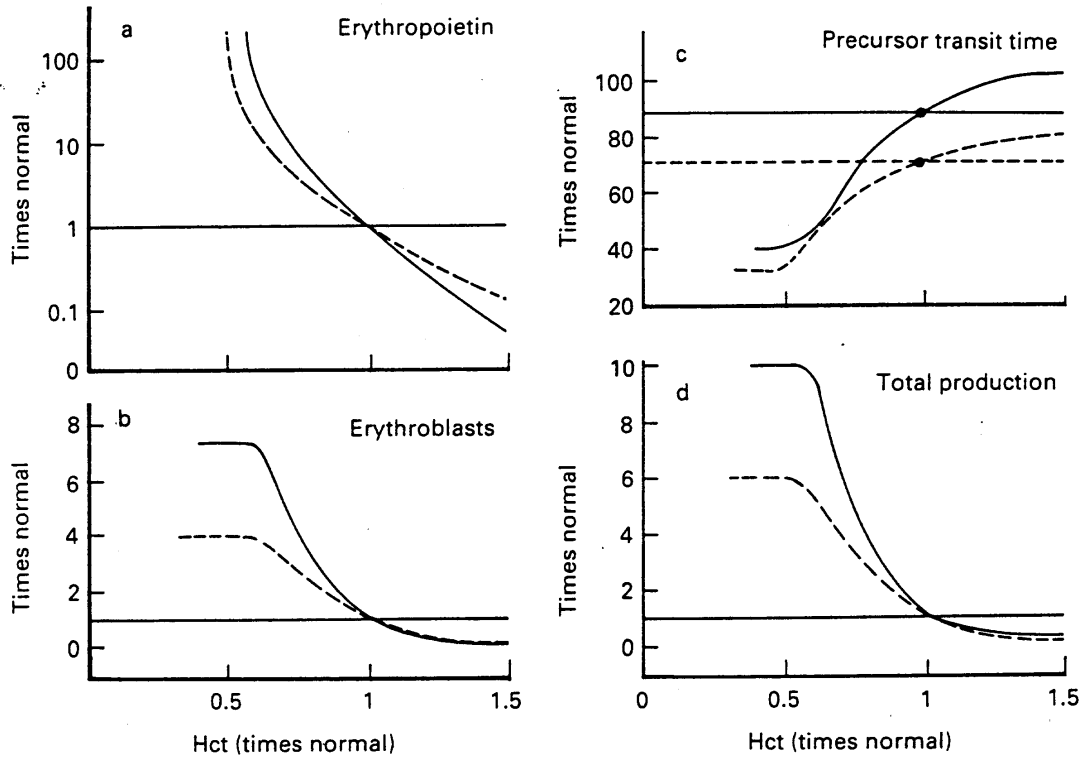

Fig. 3. Model curves of (a) erythropoietin concentration, (b) number of proliferative and non-proliferative erythroblast precursors, (c) precursor transit time and (d) total amplification factor (production) of the late erythropoietic progenitors and the precursors together depending on the haematocrit (Hct) calculated for mice (---) and rats (——).

and to 13% in rats. The precursor transit time is slightly prolonged from 72 h to 85 h in mice and from 90 h to 105 h in rats and total erythropoietic cell production may decrease significantly to 0·4% in mice and to 13% in rats.

The model curves in Fig. 3 terminate at their lower end of a Hct of 0·33 times normal in mice and 0·4 times normal in rats as under these circumstances a complete desaturation of haemoglobin is reached. The curves are not continued beyond a Hct of 1·5 times normal because such situations are not tolerated due to haemodynamic reasons.

The cell kinetic properties of the model in normal and stimulated steady state are summarized in Fig. 4 (for mice). Under normal steady state conditions, about 2000 mature erythrocytes are derived from each cell entering the CFU-E compartment (Fig. 4a). These erythropoietic cells leave the bone marrow after a precursor transit time of 72 hours and serve for another 42 days as functional cells in the peripheral blood. Under severe stimulation, about 12000 cells are derived from each cell entering the CFU-E compartment (Fig. 4b) which corresponds to 2·6 additional mitoses. In addition, the precursor transit time is reduced to 33 hours. The advantage that a large number of mature cells is available within a relatively short period has to be payed by the 'disadvantage' that these 'stress erythrocytes' have a shorter lifespan (15 days). For missing erythropoietic demand (Fig. 4c), proliferation is reduced and the amplification factor is only 80 (e.g. reached in severe hypertransfusion). The transit times and the lifespan of erythrocytes are nearly normal under these circumstances.

Table 2. Number of independent model parameters and experimental evidence for their choice

Parameters with good data base:
Transit times: τ_{PEP}(max, min,norm), τ_{NPEP}(norm), τ_{RETI}, τ_{ERYR}, τ_{ERYR}, τ_{EPO}, switch alpha (between ERYA and ERYB)
Amplification rates: $f_{CFU\text{-}E}$(max,min), f_{PEP}(max,min)
Oxygen dissociation curve: n, *P*50, Pa_{O_2}

Poorer data base but plausible choice possible:
Transit times: $\tau_{CFU\text{-}E}$, τ_{NPEP}(max,min)
Production rate: p_{EPO}(max,min)
Oxygen desaturation in the kidney: dSo_2

Speculative:
Steepness factor BR in f and τ functions

Parameter sensitive for the model behaviour:
$f_{CFU\text{-}E}$(max,min), f_{PEP}(max,min), BR, dSo_2

Number of parameters
Total: 14 (transit times) + 12 (amplification rates) + 4 (oxygen dissociation curve) = 30
Redundant: 4 (BR in f and τ)
Irrelevant after standardization to normal: 3 (normal values of $f_{CFU\text{-}E}$ f_{PEP}, p_{EPO})
Relevant parameters: 23 (known by data or estimate: 22, speculative: 1)

Parameter estimation

In total, the model requires specification of 30 parameters: 14 for transit times, 12 for amplification rates and 4 for the oxygen dissociation curve. Since the model has been constructed to simulate the reaction on perturbation relative to the steady state (and not in terms of total cell numbers) the absolute values of the normal amplification factors are irrelevant. Furthermore we decided to chose the steepness factor BR in all dose response-relationships for f and τ alike (BR = 0·7). This reduces the number of relevant parameters to 23. Of these most are supported by a good data base. For six parameters, the data base is poorer, amongst them the parameters associated with oxygen desaturation in the kidney and those related to the erythropoietin production rate. The steepness parameter BR is the only parameter where no information is available at present. It was chosen according to the fit of computer simulations to data (see Parts 2 and 3).

Of the 23 parameters involved six turn out to influence the model behaviour in particular. These sensitive parameters are the ones involved in the amplification dose response curves (minimum and maximum of $f_{CFU\text{-}E}$, f_{PEP} and BR) and the parameter dso_2. The relevance of these parameters for understanding of the *in vivo* regulation of erythropoiesis is an interesting result of our model analysis.

DISCUSSION

Two types of models on erythropoiesis have been developed in the last years: models dealing with steady state kinetics and regulatory models (see review by Wichmann, 1983). The first type intends to quantify the absolute values of cell counts or transition rates in steady state. These models have been applied to the analysis of mitotic indices, ^{59}Fe-labelling indices and [^{3}H]TdR or [^{14}C]TdR labelling curves. In contrast, regulatory models are primarily interested in relative changes during perturbation from steady state. This means that the parameters assumed

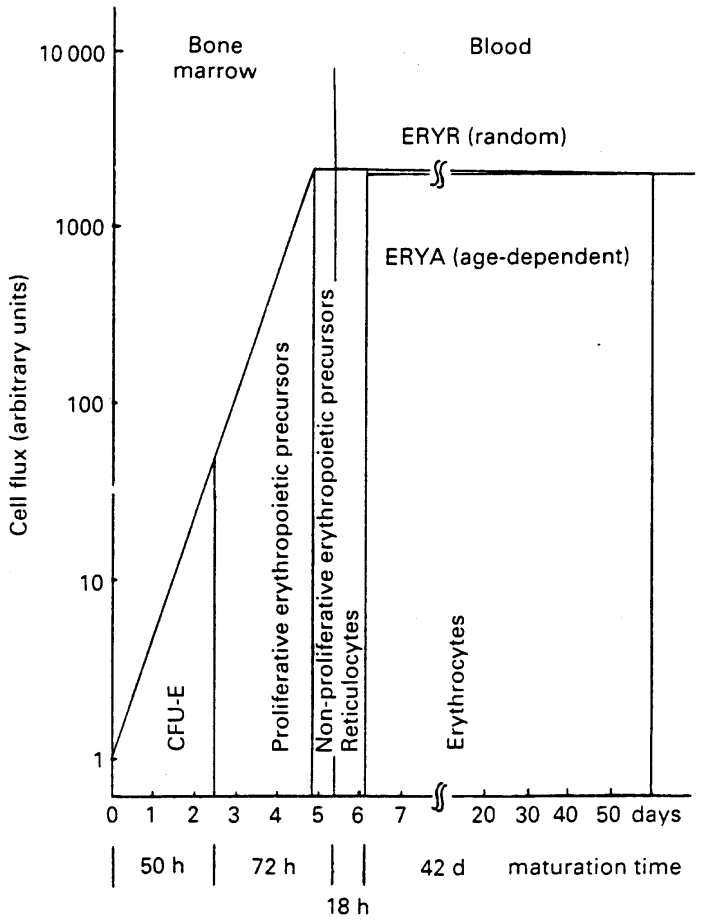

Fig. 4. (a) Steady state erythropoiesis in normal mice.

for steady state are only important in their relation to the actual compartment values which are changing after different perturbations depending on the regulating mechanisms of the model. For the simulation of erythropoietic stresses, the actual content of each compartment is divided by the steady state value and changes of these ratios (expressed as fraction of normal of the particular compartment) are calculated over a certain period. These changes can be easily compared with experimental data without being influenced by differences in absolute numbers, plating efficiencies etc. Data obtained in different laboratories and for different strains of mice can thereby largely be standardized on a compartmental basis. However, it also remains clear that this standardization procedure cannot eliminate differences in the relative reactivity of comparable cell stages in different mouse strains. Part of the heterogeneity found in the data expressed as a fraction of normal has to be attributed to such effects.

The model presented is a regulatory model as especially the applications on stimulated or suppressed erythropoiesis will show (Part 2 and 3). Therefore, the absolute model parameters for steady state situations as given in Table 1 or Fig. 4 are less important than the ratio of these parameters and the dose-response relations between the stimulus and the reaction on the stimulus. In the model, the stimulus is given by a demand for oxygen which is mediated by erythropoietin to the sites of erythropoietic cell production in the bone marrow.

The representation of each cell population by only one ordinary differential equation with a random output term implies the assumption of a large variance of the transit time within such a

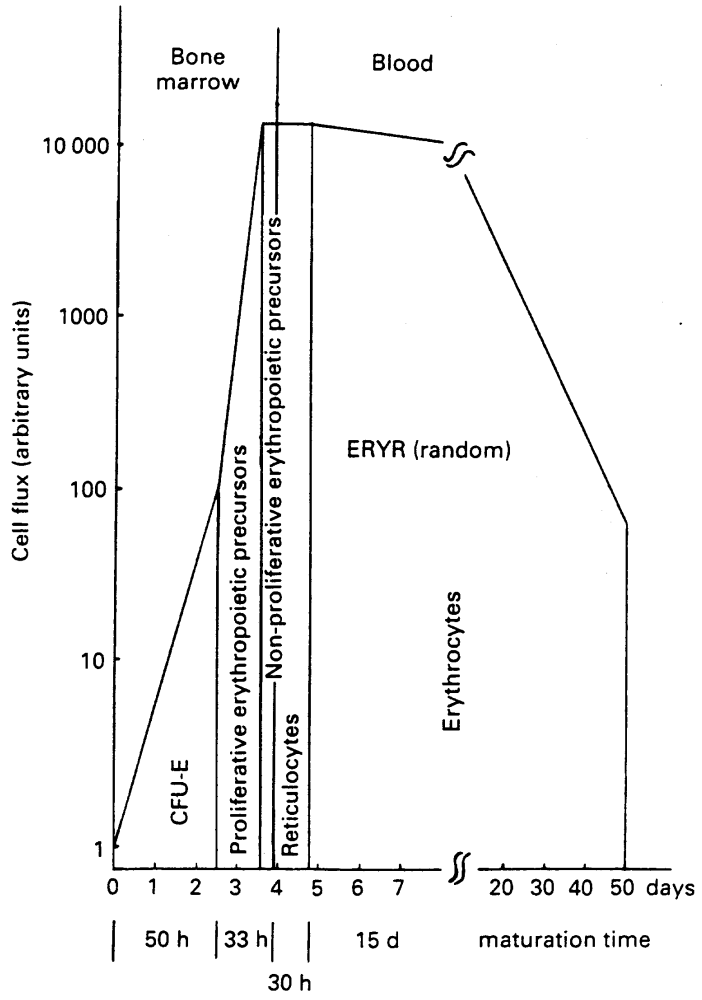

Fig. 4. (b) Steady state erythropoiesis in mice under maximum stimulation.

compartment. Biologically, the implication that some cells should be able to leave a compartment already very soon after entry with a full differentiation program performed seems unrealistic. One would postulate that every cell of a specific population requires a certain minimum maturation time. Therefore, a certain delay should be included in a more refined model version. However, other model simulations have shown that the assumption of a maturation time with only a small coefficient of variation (e.g. 10%) leads to oscillations of the immature and mature cell pools (Wichmann, Loeffler & Schmitz, 1988). Thus, we believe that the presence of a large variance in maturation time is a fairly important characteristic of erythropoiesis. For numerical simplifications we chose random transition terms in the model differential equations. Despite the practical usefulness of this simplification future investigations should focus on a more realistic description of the processes that generate such variances that is on the relation between cell proliferation and morphology changes.

Two further assumptions are made to simplify the model: (1) A constant influx from the stem level into the CFU-E compartment is assumed. This may be justified under circumstances where direct manipulations of the stem cells is avoided. Such situations (e.g. irradiation or cytotoxic drugs) will not be considered subsequently. Furthermore, it is known that even strong

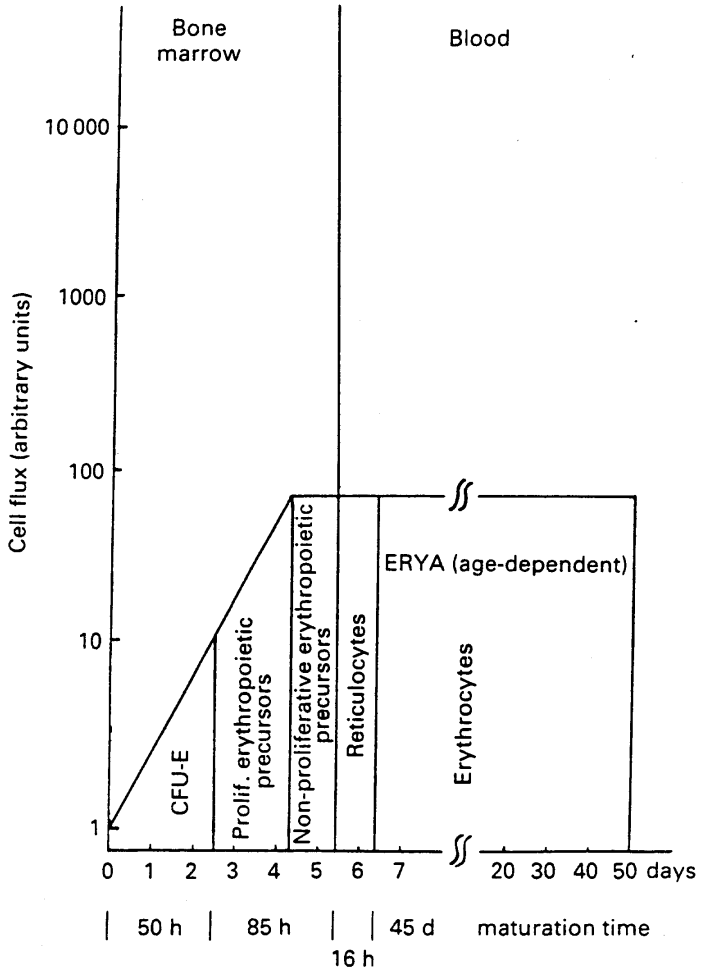

Fig. 4. (c) Steady state erythropoiesis in mice under minimum stimulation.

erythropoietic stimulation has only small effects on CFU-S and BFU-E numbers (reviewed, e.g. in Wichmann & Loeffler, 1985). According to the experimental data we estimate that the input into erythropoietic differentiation varies at maximum by $\pm 50\%$ (Wichmann & Loeffler, 1985) which is small compared to the effects produced by EPO on the CFU-E and the precursor cells. Therefore, we assume that the release of regulating factors affecting the proliferating CFU-S and BFU-E is overruled by EPO under conditions which mainly stimulate erythropoiesis. Preliminary model calculations combining the models of stem cell regulation (Loeffler & Wichmann, 1980; Wichmann & Loeffler, 1985) with this model of erythropoiesis show consistency with this point of view. The following conclusions and simulations will only be affected to a negligible degree if stem cell regulation is considered, additionally. (2) The model comprehends erythropoiesis in the bone marrow and the spleen. For normal or suppressed proliferation it represents mainly the bone marrow since the amount of splenic erythropoiesis is 5–10% in these situations. However, under severe stimulation the spleen may contribute a third or more to erythropoietic production in mice. A more detailed analysis of splenic erythropoiesis investigating the major difference between marrow and spleen is presently in preparation.

The model assumptions about how the production of erythropoietin depends on the partial oxygen pressure in the kidney (Pto_2) and the effects of varying EPO-levels on the erythropoietic production had to be derived indirectly due to lack of experimental data on these dose-response curves *in vivo*. It is partly the purpose of mathematical modelling to demonstrate that a particular set of such dose-response curves can be selected that enables a satisfying description of recovery data following a variety of different experimental data.

The Pto_2 is assumed to depend on the oxygen desaturation at the locus of the EPO production in the kidney. Biologically, this locus is probably situated in the cortex of the kidney (Demopoulos *et al.*, 1965; Dunn *et al.*, 1976; Gruber *et al.*, 1977) which shows a higher desaturation than the whole organ (Carriere *et al.*, 1966; Aperia *et al.*, 1968; Deetjen, 1979) or the whole body (Dunn *et al.*, 1981). These experimental results are controversial because other data of bleeding or hypoxia in rats (Caro & Erslev, 1984) show a stronger increase of EPO in the tubular fraction of the kidney compared with the glomerular fraction. One main problem in identifying an appropriate Pto_2 trigger experimentally is the high blood perfusion of the kidney. However, although formulated as a particular process in the kidney this process represents the oxygen supply of the whole body. It is certainly reasonable to assume that the body oxygen supply depends on the haematocrit or the haemoglobin concentration, i.e. on both the red cell mass and the plasma volume, and that the assumptions made in the Appendix about O_2 utilization and blood flow hold true for the whole body.

Erythropoietin influences the proliferation of the erythropoietic progenitors and precursors. In the model, the determination of the amplification factors is based on the normal, maximum and minimum values of these functions. Using these three values and assuming a sigmoidal dependence on EPO, one can express all model parameters by measurements. Thus, for the feedback function which was at first totally unknown only the shape and its steepness remains hypothetical. This steepness factor determines the width of the 'regulatory window' in which the erythropoietic system is able to react on perturbations of its steady state. The choice of this window is an important assumption for the dose-response curves.

The validity of the model assumptions and the parameters cannot be judged from steady state considerations alone but requires a comprehensive description of the reactions measured after various perturbations. This will be presented in the subsequent two papers (Wichmann *et al.*, 1989; Wulff *et al.*, 1989).

APPENDIX

The following notation is used for the model compartments: Y = number of cells in a compartment; f = amplification factor ($f = 2^m$, m = number of mitoses), and τ = average transit time.

Amplification factor

In each proliferative compartment m cell divisions may take place such that the number of cells leaving the compartment is amplified by the factor 2^m compared with the number of cells entering. This amplification factor may, however, change with the status of EPO-stimulation.

Average transition time

In proliferative compartments (CFU-E, proliferative erythropoietic precursors), the average transit time τ is identical to the product of the number of cell divisions and the average cell cycle time. In non-proliferating compartments τ represents the average maturation time (non-proliferating erythroid precursors, blood reticulocytes) or the average lifespan (erythrocytes).

The actual cell counts are calculated by differential equations of the influx-efflux type

$$\dot{Y} = \text{INFLUX} - \text{EFFLUX}$$

In proliferative compartments the influx term is multiplied by the amplification factor f.

Model equations of the compartments

The compartment of the late erythropoietic progenitors (CFU-E) is described by

$$\dot{Y}_{CFU\text{-}E} = f_{CFUE} \cdot A - Y_{CFU\text{-}E}/\tau_{CFU\text{-}E} \tag{1}$$

A is the efflux from the early erythropoietic progenitors (BFU-E) which are not explicitly considered in the model. While A is constant, the amplification of this influx, $f_{CFU\text{-}E}$, depends on EPO. In contrast to $f_{CFU\text{-}E}$, $\tau_{CFU\text{-}E}$ is assumed as constant.

The cells enter the second compartment, which represents the proliferative erythropoietic precursors (PEP):

$$\dot{Y}_{PEP} = f_{PEP} \cdot Y_{CFU\text{-}E}/\tau_{CFU\text{-}E} - Y_{PEP}/\tau_{PEP} \tag{2}$$

Both the amplification factor f_{PEP} and the transit time τ_{PEP} depends on EPO.

The erythroblasts lose their ability to proliferate and enter the compartment of the non-proliferative erythropoietic precursors (NPEP = non-proliferative erythroblasts and bone marrow reticulocytes) described by

$$\dot{Y}_{NPEP} = Y_{PEP}/\tau_{PEP} - Y_{NPEP}/\tau_{NPEP} \tag{3}$$

with the transit time τ_{NPEP} depending on EPO.

The blood reticulocytes (RETI) are described by

$$\dot{Y}_{RETI} = Y_{NPEP}/\tau_{NPEP} - Y_{RETI}/\tau_{RETI} \tag{4a}$$

Considering that the reticulocytes shifted from the bone marrow to the blood need a certain time to mature, we assume that

$$\tau_{NPEP} + \tau_{RETI} = \tau_{NPEP}^{norm} + \tau_{RETI}^{norm} = \text{constant} \tag{4b}$$

Thus,

$$\tau_{RETI} = \tau_{NPEP}^{norm} + \tau_{RETI}^{norm} - \tau_{NPEP} \tag{4c}$$

where τ_{NPEP}^{norm} and τ_{RETI}^{norm} are the transit times of compartment NPEP and RETI in normal steady state. Formula (4c) implies that any regulation shift (shortage of τ_{NPEP}) is associated with a corresponding prolongation of τ_{RETI}.

The compartment of erythrocytes (ERY) is divided into two parallel parts: the first one, Y_{ERYA}, obeys an almost age-dependent destruction law (rectangular age structure) and represents the majority of cells produced at normal proliferation. The second part, Y_{ERYR}, has an exponential age structure, suggesting a random destruction process. It represents stress erythrocytes and is important during severe stimulation.

The distribution of the influx into the subcompartments Y_{ERYA} and Y_{ERYR} is governed by a function $\alpha(h(t))$. It depends on the actual influx rate $\alpha(h(t))$ into compartment ERY representing approximately the state of erythropoietic stimulation. Hence,

$$\dot{Y}_{ERYA} = \alpha(h(t)) \cdot h(t) - \alpha(h(t\text{-}\tau_{ERYA})) \cdot h(t\text{-}\tau_{ERYA}) \tag{5a}$$

with

$$h(t) = Y_{RETI}(t)/\tau_{RETI}$$

$$\dot{Y}_{ERYR} = (1\text{-}\alpha(h)(t)) \cdot h(t) - Y_{ERYR}/\tau_{ERYR} \tag{5b}$$

and

$$\dot{Y}_{ERY} = Y_{ERYA} + Y_{ERYR} \tag{5c}$$

with

$$\alpha(h(t)) = \exp\,(-0{\cdot}1 \cdot (h(t)/h_{norm})^2)$$

*h*norm denotes the normal efflux from the RETI-compartment and alpha(t-τ_{ERYA})·h(t-τ_{ERYA}) is the influx into subcompartment Y_{ERYA} delayed by τ_{ERYA}.

Finally, compartment EPO describes the feedback hormone erythropoietin. The model equation is:

$$\dot{Y}_{EPO} = p_{EPO} - Y_{EPO}/\tau_{EPO} \tag{6}$$

with p_{EPO} as the production rate depending on the oxygen-supply and τ_{EPO} as the constant turnover time.

Feedback influences of erythropoietin

In the model it is assumed that EPO influences directly the proliferation rates $f_{CFU\text{-}E}$ and f_{PEP} as well as the transit times τ_{PEP} and τ_{NPEP}.

For the regulatory functions which describe $f_{CFU\text{-}E}$, f_{PEP}, τ_{PEP} and τ_{NPEP} a sigmoid form is assumed:

$$f(Y_{EPO}/Y_{EPO}^{norm}) = A - B \cdot \exp\,(-C \cdot (Y_{EPO}/Y_{EPO}^{norm})^{BR}) \tag{7a}$$

and

$$\tau(Y_{EPO}/Y_{EPO}^{norm}) = A + B \cdot \exp\,(-C \cdot Y_{EPO}/Y_{EPO}^{norm})^{BR}) \tag{7b}$$

The constants A, B, C and A′, B′, C′ are positive and can be determined from measurements of the minimum, maximum and normal values of the proliferation rates and transit times (Table 1). The parameter BR influences the slope of the dose response curve. BR is chosen arbitrarily as 0·7.

Feedback influences on erythropoietin production

It is assumed that the production rate of erythropoietin p_{EPO} depends exponentially on the tissue oxygen pressure of the kidney Pto_2:

$$p_{EPO}(Pto_2/Pto_2^{norm}) = D \cdot \exp\,(-E \cdot Pto_2/Pto_2^{norm}) \tag{8}$$

The constants represent the maximum production rate (D) and the sensitivity (E) of EPO production to changes of Pto_2. Obviously Pto_2 depends on several factors like atmospheric pressure, renal blood flow, the form of the oxyhaemoglobin dissociation curve, oxygen utilization and red blood cell count. The details of this regulation are described in detail elsewhere (Wulff, 1983; Wichmann, 1983) whose essentials are summarized subsequently:
(1) Pto_2 is determined by the tissue oxygen saturation (Sto_2) according to the Hill equation

$$Pto_2 = P50 \cdot (Sto_2/(100\text{-}Sto_2))^{1/n} \tag{9}$$

The parameters $P50$ and n characterize the oxyhaemoglobin dissociation curve.

(2) Sto_2 depends on the arterial saturation (Sao_2) and the oxygen desaturation in the kidney (dSo_2) by

$$Sto_2 = Sao_2 - dSo_2 \tag{10}$$

Sao_2 is determined by the arterial oxygen tension (Pao_2) via the Hill Equation.

(3) The oxygen desaturation dSO_2 in the kidney is determined by the oxygen utilization (O_2util), the renal blood flow (RBF) and the haematocrit (HCT) via

$$dSO_2 = (\text{constant} \cdot O_2\text{util})/(\text{HCT} \cdot \text{RBF}). \quad (11)$$

(4) It is assumed that the oxygen utilization in the kidney is proportional to BRF and the plasma fraction (1-HCT) in the renal blood:

$$O_2\text{util} = \text{constant} \cdot (1\text{-HCT}) \cdot \text{RBF}. \quad (12)$$

This means that the energy consumption necessary for elimination of salt from the plasma and reabsorption of water at the sites of erythropoietin production increases proportional to the renal blood flow and the plasma fraction. If, furthermore, the ratio of plasma volume and haemoglobin mass is the same in the kidney and the total body one finds

$$dSO_2 = \text{const.} \cdot \text{PLVOL}/\text{Hb}_{\text{mass}} \quad (13)$$

with PLVOL as the total plasma volume and $\text{Hb}_{\text{mass}} = Y_{\text{RETI}} + Y_{\text{ERY}}$ as the total haemoglobin mass.

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