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A mathematical model of erythropoiesis in mice and rats

Part 2: Stimulated erythropoiesis

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Abstract. A mathematical model of erythropoietic cell production and its regulation process has been proposed in a preceding paper. It is primarily based on the assumption that the number of cell divisions taking place in the CFU-E and erythropoietic precursor stages is regulated depending on the oxygen supply of the tissue. Quantitative dose-response relationships for *in vivo* erythropoiesis are suggested. Here, we demonstrate that this model adequately reproduces data obtained in situations of stimulated erythropoiesis in mice and rats. In detail, this implies a quantitative description of the following processes: (1) Changes in tissue oxygen tension (Pto_2) following removal of red cells (bleeding, haemolytic anaemia) or increase in plasma volume (dilution anaemia) or decrease in atmospheric oxygen pressure (hypoxia). (2) Pto_2 dependent erythropoietin (EPO) production. (3) Dose-response of EPO on erythropoietic amplification (up to two to four additional mitoses). (4) The changes of the marrow transit time.

Model simulations are compared with experimental data for changes of erythropoiesis during hypoxia, EPO-injection, and different forms of anaemia. A satisfactory agreement suggests that the model adequately describes and correlates different direct and indirect ways to stimulate erythropoiesis. It quantifies the role and relative contribution of the haematocrit, haemoglobin concentration, atmospheric oxygen pressure, tissue oxygen pressure, and plasma volume as triggers in erythropoietic stimulation under various conditions. Furthermore, the model may allow to optimize the scheme of EPO-administration and to find the maximum increase of erythropoiesis for a given amount of erythropoietin.

The basic assumptions of the mathematical model of erythropoiesis in mice and rats and its mathematical techniques have been described in the first (Loeffler *et al.*, 1989) of a series of three papers. Now the validity of the model is investigated by simulating different situations of stimulated erythropoiesis as hypoxia, bleeding, treatment with phenylhydrazine, plasma

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dilution, injection of erythropoietin and comparing the model results with the experimental data. This will demonstrate, whether the assumptions made about the feedback mechanisms of erythropoiesis are able to reproduce the principal behaviour of this complex biological system.

MODEL SIMULATIONS

For the simulation of experiments the model parameters as described in Part 1 are used and kept unchanged in all subsequent *scenarios*. In addition, it is necessary to define the characteristics of each experimental manipulation in terms of the model. The subsequent descriptions are summarized in Table 1.

Bleeding anaemia

According to the loss of blood caused by acute bleeding, the cell number of the reticulocyte (RETI) and erythrocyte (ERY) compartments have to be reduced. In the model, both numbers are reduced by the same fraction at time zero. Thus, the calculations are performed as an initial value problem.

As known experimentally, the total blood volume returns to normal shortly after bleeding (Tribukait, 1960; Aoki & Tavassoli, 1981; Hanna, 1968). Since the mass of erythrocytes is reduced, the plasma volume increases compensating for the loss of volume. In the model, it is assumed that the total blood volume (BV) is constant. Thus, the actual plasma volume (PV) is

Table 1. Model simulation of experiments on stimulated erythropoiesis

Bleeding anaemia
- reduction of the initial values for reticulocytes and erythrocytes according to the experimental blood loss
- constant blood volume

Dilution anaemia
- the plasma volume is elevated according to the measurements after infusion of the plasma expander

Haemolytic anaemia induced by phenylhydrazine
- reduction of the initial values for reticulocytes and erythrocytes according to the haemolysis induced
- constant blood volume
- increase of the maximum proliferation of CFU-E to simulate (a) the large amount of heme and iron available due to haemolysis and (b) the migration of stem cells and progenitors from the bone marrow to the spleen where they find a better erythropoietic microenvironment

Injection of erythropoietin
- elevation of the plasma concentration of EPO according to the quantity administrated (distribution volume = plasma volume)
- prolonged activity of the injected EPO

Hypoxia
- reduction of the arterial oxygen pressure proportional to the atmospheric pressure (use of the barometric formula to simulate different altitudes)
- reduction of the plasma volume according to the measurements
- no shift of the oxygen dissociation curve
- simulation of the steep initial increase of erythropoietin experimentally observed in two different ways:
  Hypothesis 1: adaptational phenomena are responsible for excess production of EPO during the first 3–4 days (simulated by a high initial value of EPO which disappears exponentially)

  Hypothesis 2: erythropoietin is utilized by the EPO-sensitive progenitors and precursors (simulated by a turnover time which is inversely related to the number of EPO-sensitive cells)

the difference of BV and the actual volume of erythrocytes (EV) which is proportional to the total haemoglobin mass (Hb_{mass}).

Dilution anaemia

Dilution of the blood by plasma expander leads to a reduced haematocrit (Hct) and, thus, to anaemia. The experimental curve of the plasma volume during this treatment is used as input for the model calculation and no further assumptions are made. Thus, the calculations are performed as a boundary-condition problem.

Haemolytic anaemia induced by phenylhydrazine

Similar to bleeding anaemia the initial values of reticulocytes and erythrocytes are reduced in the model according to the degree of haemolysis induced. The blood volume is kept constant in the simulations.

The increase of erythropoietic proliferation is known to be more pronounced after phenylhydrazine than after bleeding (Rencricca *et al.*, 1970; Hara & Ogawa, 1976). Two reasons may be involved: (a) the catabolites of the destroyed red cells (especially heme and iron) are not lost but are immediately available for the formation of new erythropoietic cells: (b) there is a massive migration of cells from the marrow to the spleen where they find a more favourable environment for erythropoiesis. In the model simulation this is taken into account by using a maximum amplification rate of 10 times normal (instead of four times normal) for the CFU-E compartment of mice (max $f_{CFU\text{-}E} = 320$).

Erythropoietin (EPO) injection

Single or repeated injections of EPO stimulate the erythropoietic system. The total EPO content in the animal's body may be estimated by the product: EPO content = EPO-concentration·distribution-volume.

As there is a lack of data, we assume that the distribution-volume is identical with the plasma volume which is 0·8 ml in mice and 15 ml in rats (Tribukait, 1963a–d; Reissmann *et al.*, 1965; Alexianian, 1969). Thus, an EPO concentration of 20 mU/ml (which is assumed to be the normal value) represents a total EPO content of 16 mU for mice and 300 mU for rats. Therefore, the administration of 1 unit EPO increases the EPO content in mice to 62·5 times normal and in rats to 3·3 times normal.

In the model calculations, it is assumed that the EPO level remains elevated for 2 days after the injection. This corresponds to a prolonged effect of the rapidly eliminated hormone ($\tau_{EPO} = 3$ h, see Part 1).

Hypoxia

The atmospheric oxygen pressure ($P\text{atm}O_2$) decreases with higher altitude or during experimental hypoxia. This causes a corresponding decrease of the arterial oxygen tension (PaO_2) in the experimental animals exposed to hypoxia. For rats, the PaO_2 at sea level is 95 mmHg, for mice it is 80 mmHg (Altland *et al.*, 1967; Pepelko & Dixon, 1975; Cohen *et al.*, 1981).

In the model, $P\text{atm}O_2$ (in mmHg) is calculated from the altitude 'a' (in kilometres) by the barometric formula:

$$P\text{atm}O_2(a) = P_0 \cdot \exp\,(-0{\cdot}12515 \cdot (a - a_0)) \tag{1a}$$

with P_0 and a_0 as the values at sea level ($P_0 = 760$ mmHg, $a_0 = 0$ km) and

$$PaO_2 = 80 \cdot P\text{atm}O_2(a)/P_0 \text{ (for mice)} \tag{1b}$$

$$PaO_2 = 95 \cdot P\text{atm}O_2(a)/P_0 \text{ (for rats)} \tag{1c}$$

As has been shown by Mylrea & Abbrecht (1970), the plasma volume (PV) in mice decreases exponentially during the first days of hypoxia and reaches a new steady state within 3–4 d. These data can be approximated by the empirical formula

$$PV(t) = PV_0 \cdot ((PV_{loss} - (PV_{loss} - 1) \cdot \exp(-t/36\ \text{h})) \quad \text{for } 0\ \text{h} \leq t \leq 96\ \text{h} \tag{2a}$$

with

$$PV_{loss}(a) = a^2/1{\cdot}8 \tag{2b}$$

where $PV_{loss}(a)$ is the loss of plasma volume (in % of total PV). It has been found experimentally that the oxygen dissociation curve (ODC) is shifted to the left at the beginning of hypoxia as a result of hyperventilation which is followed by a shift to the right as a result of the increasing production of 2,3-diphosphoglycerate (Miller *et al.*, 1973). Since this shift only leads to a minor compensation of the hypoxic oxygen desaturation, it is neglected in the model calculations presented.

The quantitative effects of hypoxia (decrease of Pao_2, plasma loss, $P50$-shift) determined experimentally were found to be insufficient to reproduce the excessive EPO peak measured at the beginning of hypoxia. Therefore, further influences have to be discussed:

Hypothesis 1: Early excess production of erythropoietin

This assumption might be interpreted as an adaptational phenomena. Renal vasoconstriction which is found experimentally after a severe hypoxic stimulus (Deetjen, 1979) might enlarge haemoglobin desaturation in the kidney and, thus, stimulate EPO production. If this effect is only transient reversal of the vasoconstriction during adaptation may partially reduce hypoxaemia and lead to decreasing EPO levels. However, no quantitative knowledge is available and other adaptational processes might also be effective.

Due to this hypothesis in the model an initial stimulus on EPO production is assumed which vanishes exponentially within 3–4 d. The theoretical curves are calculated by the empirical term

$$p_h(t) = p_{h0} \cdot \exp(-k_h \cdot t) \tag{3}$$

with the initial maximum p_{0h} and the half-life $k_h = 18$ h for the initial peak according to the data from Abbrecht & Littell (1972). This term is added to the normal production rate p_{EPO} (see Appendix of Part 1).

Hypothesis 2: Consumption of erythropoietin

This hypothesis assumes that EPO is utilized by its target cells. These are the EPO-sensitive progenitor and precursor cells which quantitatively correspond to the precursors (PEP). Under this hypothesis, the loss of EPO should increase with the number of PEP. For simplicity, one can assume a proportional relation

$$\dot{Y}_{EPO} = P_{EPO}\ Y_{EPO}/\tau_{EPO}(PEP)$$

with

$$\tau_{EPO}(PEP) = 1/\text{const}_1 \cdot PEP + \text{const}_2),$$

where P_{EPO} denotes the production rate and τ_{EPO} the turnover time which is now inversely related to the number of precursors. From this assumption, it follows that the normal turnover time may be shortened to approximately one third in severe hypoxia.

RESULTS: MODEL CURVES VERSUS EXPERIMENTAL DATA

Bleeding anaemia

During anaemia, fewer oxygen carriers are available which lead to a higher desaturation of haemoglobin and a reduced tissue oxygen tension Pto_2. Figure 1a shows the oxygen tension in skin or peritoneal pouches obtained from different experiments on anaemia and polycythaemia in mice (a, b) and rats (c, d). They are compared with the corresponding *steady state* model curves which have been calculated for the average Pto_2 of the whole body. It is interesting to note that no experimental data are available for Hct values below 0·33 to 0·4 times normal, where the theoretical curves for Pto_2 reach zero. Figure 1b shows how peak values of EPO correlate with Hct. Approximately one finds an exponential increase with decreasing haematocrit. In the model curves, this mainly reflects the exponential dependence of EPO production on Pto_2 and, thus, on the degree of anaemia.

Shortly after acute blood loss, the Hct is unchanged because both blood cells and plasma volume have been lost. Within several hours, however, fluid enters from extravascular sites to fill up the blood volume to its normal value. For simplicity, we assume that the plasma volume is immediately substituted, thus, allowing to start the simulations with a reduced Hct from the beginning.

The reaction of erythropoiesis on acute loss of about 30% of RBC in rats is shown in Fig. 2. In the model EPO increases to 10 times normal and stimulates the proliferation of erythropoietic progenitors and precursors. The number of proliferative erythroblasts increases to 2·5 times normal and non-proliferative blasts to 3·5 times normal (the higher increase of non-proliferative blasts follows because their transit time is unchanged while the transit time of the

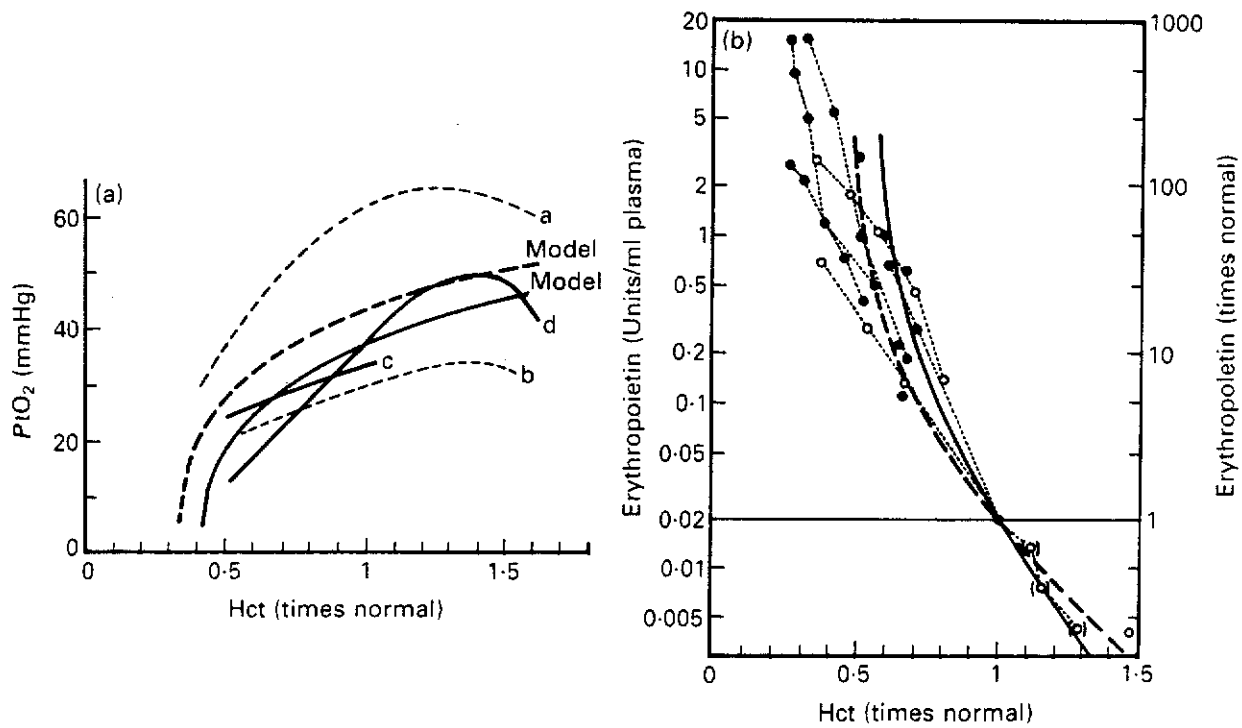


Fig. 1. (a) Relation of tissue oxygen pressure to haematocrit in skin pouches. Curves a, b: mice (Thorling & Erslev, 1968; Guidi & Scaro, 1970), curves c, d: rats (Thorling & Erslev, 1968; Bartlett & Tenney, 1963). Model curves for rats (—) and mice (---) refer to the average Pto_2 of the whole body. (b) Maxima of erythropoietin depending on hematocrit from different experiments and from model analysis for rats (●, —) and mice (○, ---).

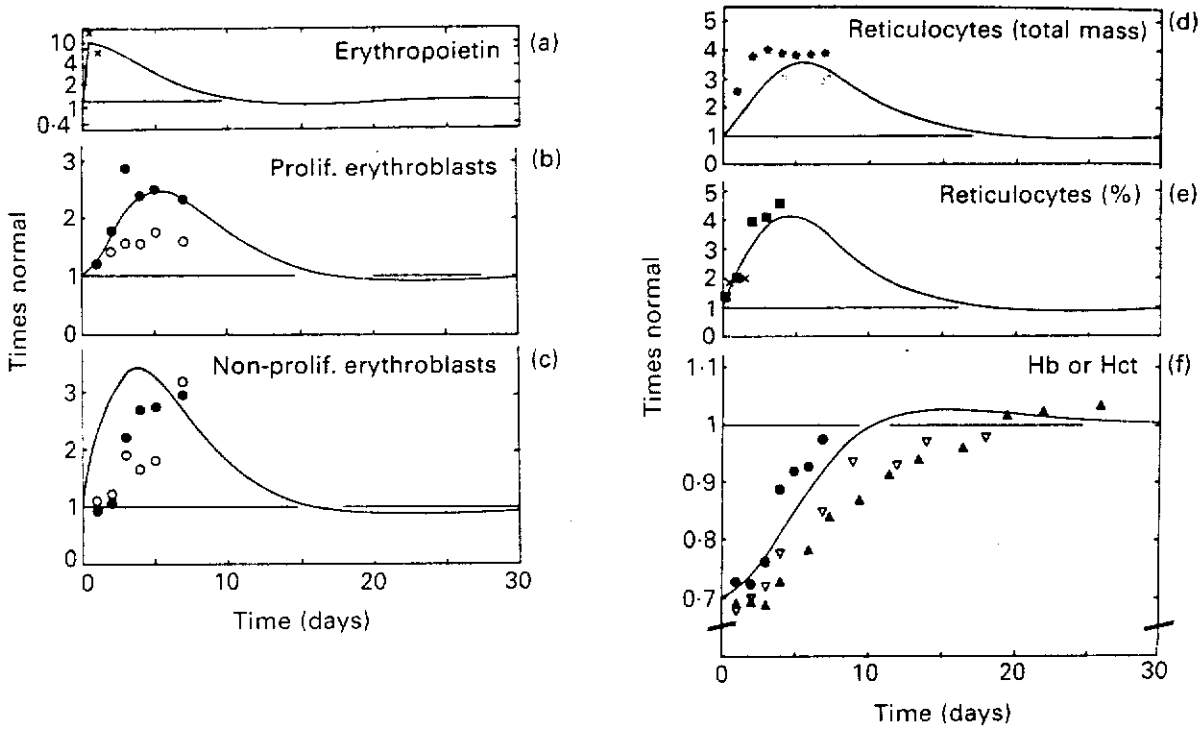
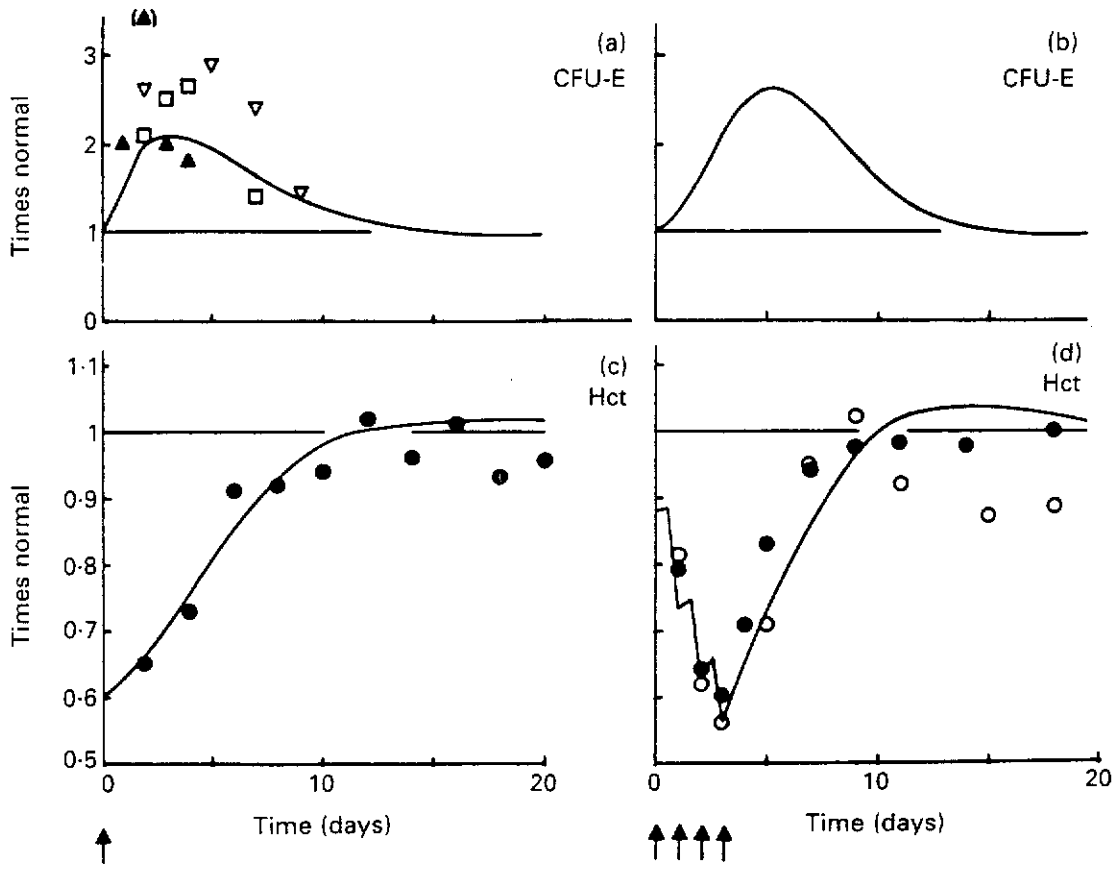


Fig. 2. Changes in erythropoiesis after an acute blood loss of 30% in rats. Data from: Miller (1980), X; Lord (1967), ●: bone marrow and spleen, ○: bone marrow; Tribukait (1960), ▲: total Hb_{mass}; Lamerton (1966), ▽: Hb-concentration; Yamato (1967), ■; Reissmann *et al.* (1960) ★. Model curves (—).



proliferative erythroblasts is reduced from 54 to 27 h, see Part 1). The number of reticulocytes in the blood also increases to 3·5–4 times normal followed by the recovery of Hct within 10 d.

Similar bleeding experiments have also been performed for mice. Recovery after an acute blood loss of 40% (left) and a repeated loss of 4 times 12% (right) is shown in Fig. 3. Both, for mice and rats, the model simulations fit the changes in blood parameters well. Some minor discrepancies can be seen for the marrow cell stages.

Dilution anaemia

The expansion of the plasma volume by eight repeated infusions of dextran is considered in Fig. 4. Since only data from rabbits are available, these are compared with the model for rats.

The plasma expander dextran reduces Hct to about 0·7 times normal which corresponds approximately to a doubling of the plasma volume. This dilution of the blood apparently stimulates the bone marrow production and leads to an increase of the reticulocytes from 1% to 4·8% on day 10. While the plasma volume returns to normal the Hct increases and reaches supranormal levels due to the enlarged proliferation during the first days. The enlarged number of red cells suppresses EPO production and subsequently the proliferation of the precursors which leads to subnormal numbers of reticulocytes. The system has normalized after about 60 d.

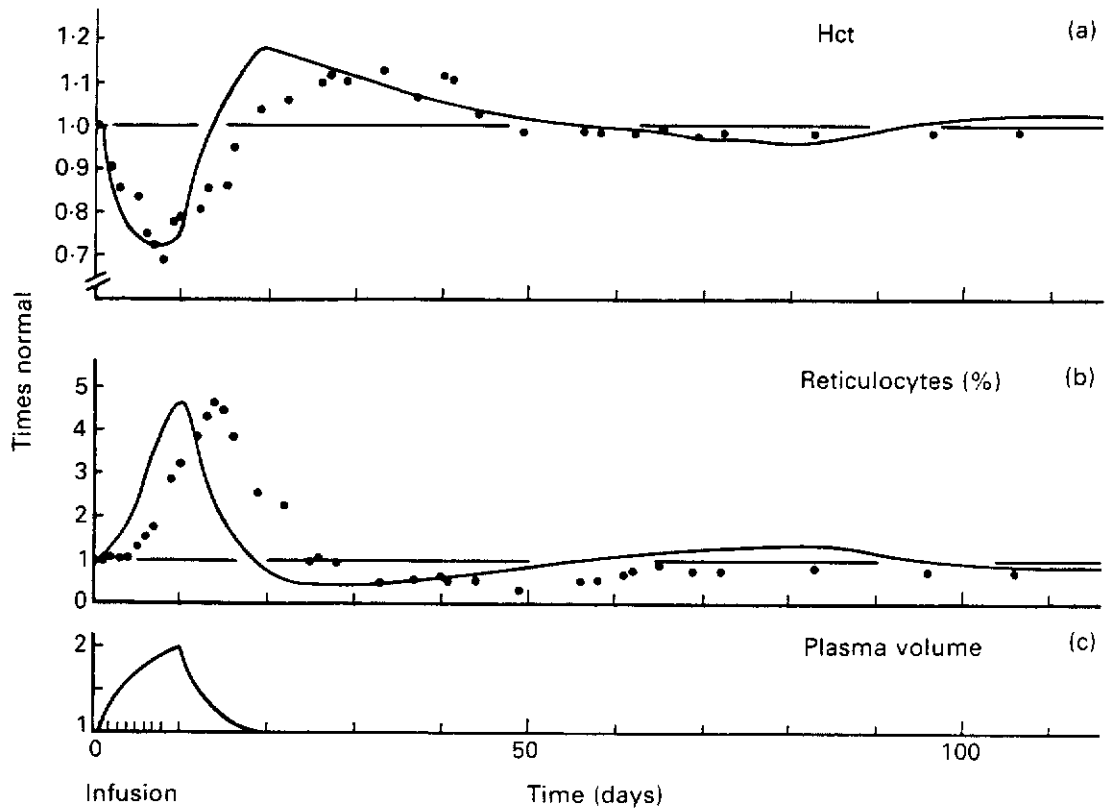


Fig. 4. Changes in erythropoiesis during and after increasing the plasma volume up to 200% of normal ('dilution anaemia'). Data from rabbits (Becker & Spengler, 1966; ●). Model curves (—) for rats.

Fig. 3. Changes in erythropoiesis mice after a single blood loss of 40% (left) and a repeated blood loss of 4·12% (right). (Adamson & Finch, 1975; ▲); (Hara, 1980; ▽); (Iscove, 1977; □); (Boggs *et al.*, 1969; ●, ○). Model curves (—).

Comparing the model curves with the experimental data (Fig. 4) one finds the same behaviour. Only the maxima of reticulocytes and Hct are too early in the model. This most likely follows from a longer marrow transit time in rabbits compared with rats.

Recovery from haemolytic anaemia induced by phenylhydrazine

Phenylhydrazine is a haemolytic agent which induces severe anaemia. In addition, it leads to a massive migration of bone marrow cells to the spleen where they find a more favourable milieu for erythropoietic amplification. This is taken into account in the model simulations. Repeated injections of phenylhydrazine are considered in Fig. 5. Since the minimum of Hct is reached on day 2 the model calculations start on this day. One observes a strong increase of the total concentrations of CFU-E which on day 4 is much higher in the data than in the model (the total cell number is calculated from the measurements in bone marrow and spleen according to Loeffler & Wichmann (1985)). Although the reticulocytes confirm this difference between model calculations and experiments the recovery of erythroblasts and Hct is sufficiently reproduced by the model.

Erythropoietin (EPO) injection

The changes of the percentage of reticulocytes in Fig. 6a reflect the increase of erythroid proliferation in hypertransfused rats after five injections of EPO. The theoretical curve (full line) reproduces the data points satisfactorily. Interestingly, this figure demonstrates the effect of the

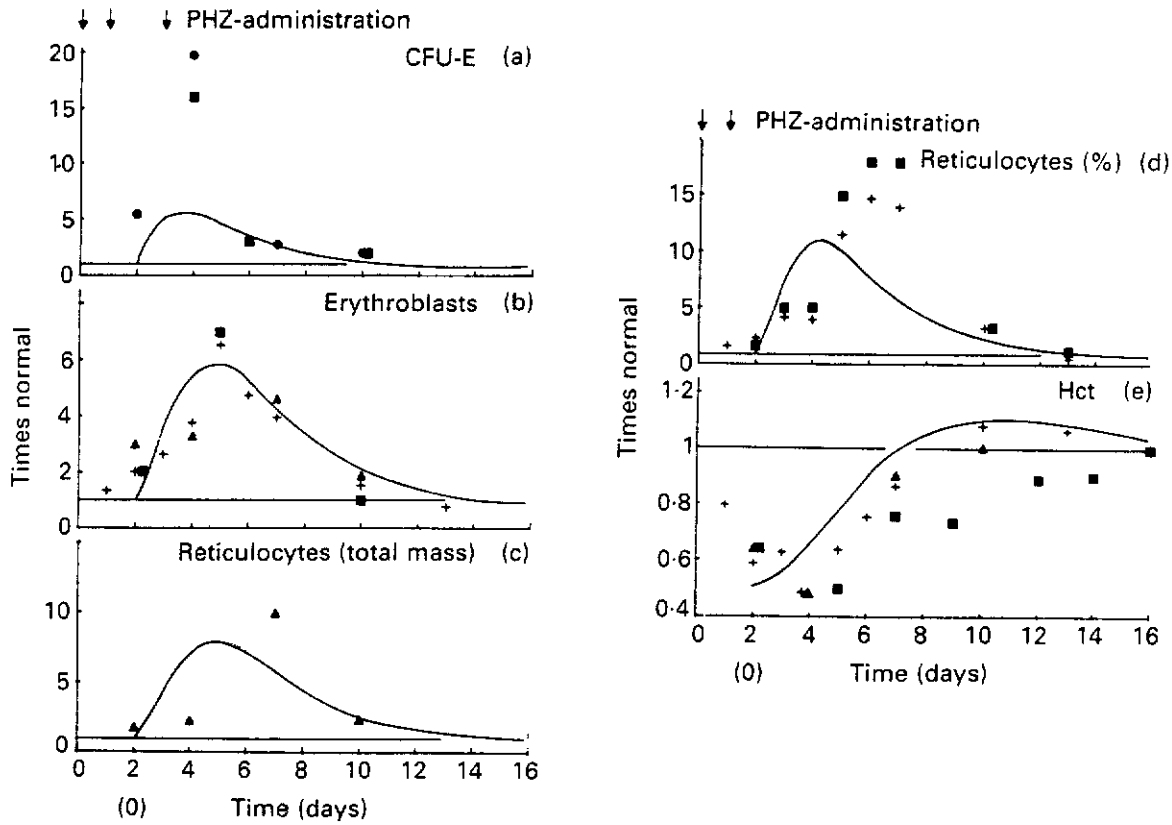


Fig. 5. Changes in erythropoiesis after treatment with phenylhydrazine for mice. Data are expressed as total cell counts (bone marrow plus spleen) (Hara & Ogawa, 1976; ●); (Rickard *et al.*, 1971; ▲); (Rencricca *et al.*, 1970; +); (Hodgson & Eskuche, 1966; Hodgson, 1982, ■). Model curves (—).

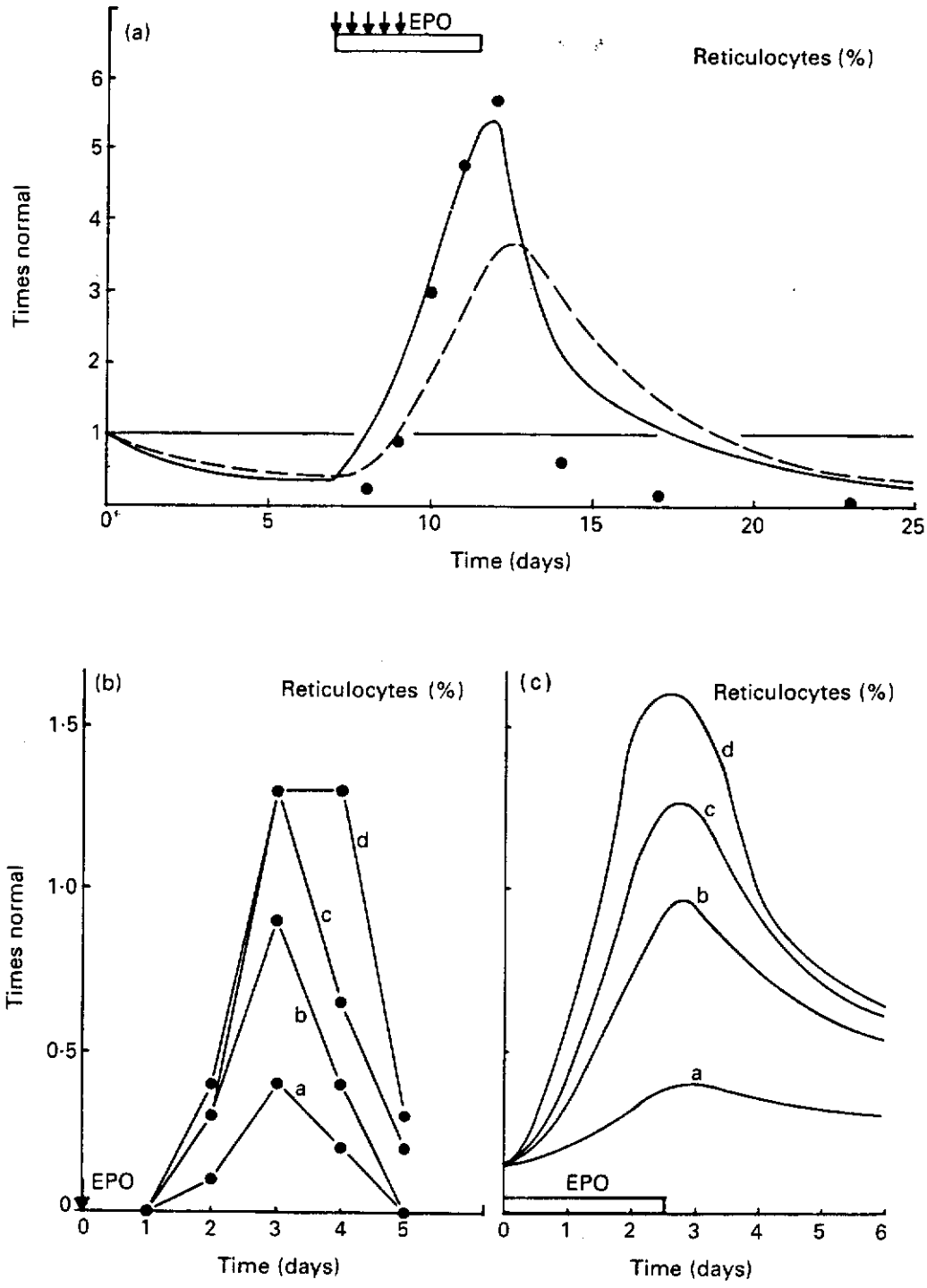


Fig. 6. Changes in erythropoiesis after injection of erythropoietin (EPO) into hypertransfused animals. (The injections are indicated by arrows, the corresponding elevation of EPO levels in the model by boxes.) (a) Repeated injections in rats. Data from: Stohlman *et al.* (1962), ●. Model calculations with (—) or without (---) shift of reticulocytes from bone marrow to blood. (b, c) Single injection in mice. Left: data from Filmanowicz & Gurney (1961), ●. Right: model curves (—) for corresponding EPO-concentrations.

shift of reticulocytes from the bone marrow into the circulation, which is found experimentally (Reiff *et al.*, 1958; Griffiths *et al.*, 1970) and assumed in the model. Without this shift (dashed line), the maximum would occur later and would be smaller.

In Figure 6b,c, the influence of a single injection of different doses of EPO to hypertransfused mice is presented. The model curves reproduce the dose-dependent maxima found in the data. Again, the initial increases of the model values are caused by the shift of reticulocytes into the blood.

Hypoxia

Figure 7 shows how the partial oxygen tension in the tissue *during steady state* depends on the atmospheric pressure. Only few data are available from skin or peritoneal gas pouches. However, the characteristics are similiar in the model and experiment with a steep decrease of Pto_2 below 500 mmHg (corresponding to an altitude of 3·5 km). The curves do not continue beyond 200–300 mmHg (corresponding to altitudes above 8 km) where, in the model, haemoglobin is completely desaturated in the tissues.

The stimulating influence of the reduced Pto_2 on erythropoiesis during chronic hypoxia is shown in Fig. 8. The model values for the steady state of EPO do not reach more than 10 times normal and are higher for mice than for rats (Fig. 8a). The model curves for erythroblasts (Fig. 8b) and for the total marrow production of erythropoietic cells including CFU-E and all erythroblasts (Fig. 8c) show a high amplification.

The steady state levels of haematocrit or haemoglobin concentration for different altitudes are presented in Fig. 8d–f. Hct increases to about 1·5 times normal for high altitudes both in experiment and in model. The fact that the increase in the total Hb-mass is higher than in the

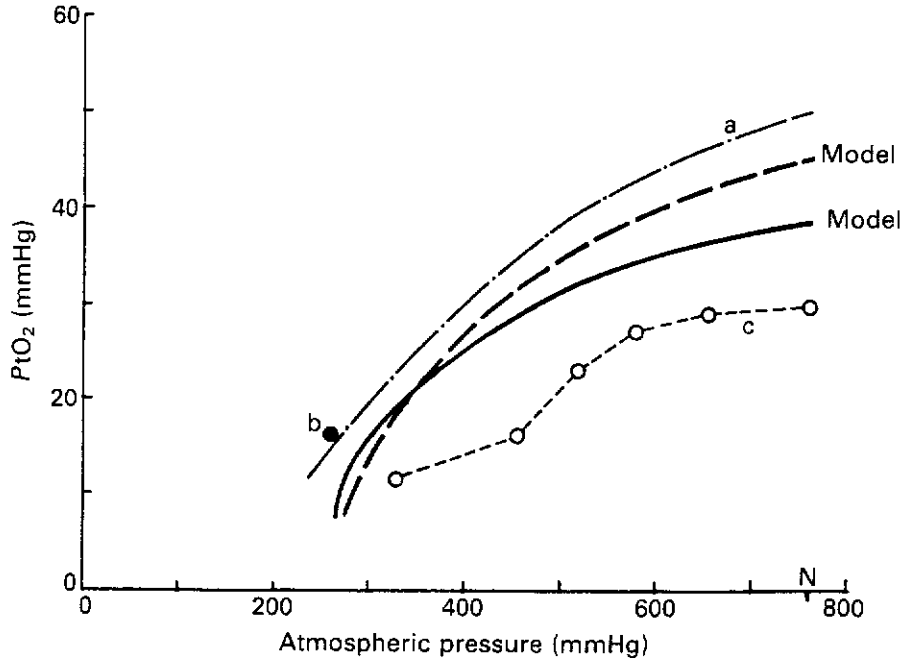


Fig. 7. Tissue oxygen pressure during hypoxia (steady state values) Curve (a): peritoneal gas pouches in rats (Necas *et al.*, 1972). Point (b): skin pouches in rats (Scaro *et al.*, 1975). Curve (c): skin pouches in mice (Guidi & Scaro, 1970). Model curves for rats (—) and mice (---).

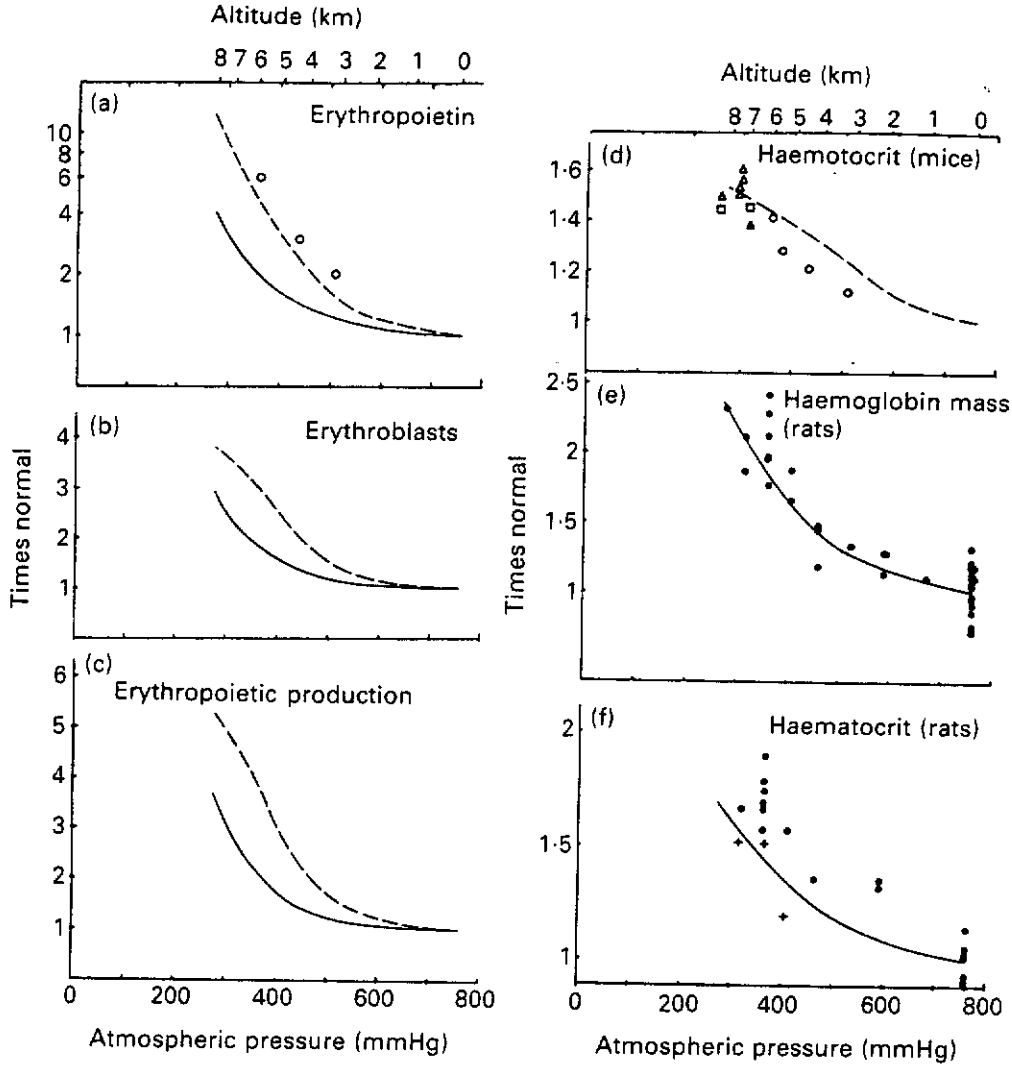


Fig. 8. Steady state curves during chronic hypoxia. Data for mice: (Mylrea & Abbrecht, 1970; Abbrecht & Littell, 1972; Russell & Keighley, 1972; Lord & Murphy, 1973; Huff *et al.*, 1975; Bozzini, 1965; McDonald *et al.*, 1970; Lail *et al.*, 1970; Markoe *et al.*, 1973; Zanjani, 1976); measurements less than 10 days (□), after 10 to 20 days (△) and after 20 to 30 days (○). Data for rats from: Tribukait (1963), ●: rats; Ou *et al.* (1980), +. Model curves for rats (—) and mice (---).

haematocrit is a consequence of the way Hct is calculated ($Hct = Hb_{mass}/(Hb_{mass} + \text{plasma volume})$). The difference between the increases of Hb_{mass} (2·3 times normal) and total marrow production (3·5 times normal), both for 8 km, follows from the shortened lifespan of erythrocytes produced under these circumstances.

During the first 3–4 days after *acute hypoxia* one observes extreme EPO concentrations with maxima which are 10–30 times higher than for the subsequent days. Within the framework of the model it is not possible to understand the origin of this EPO peak without further assumptions. If one uses the measured peak values of EPO as additional (unexplained) external input into the model one finds the model behaviour shown in Figs 9 (mice) and 10 (rats). Figure 9 represents hypoxia for mice at an altitude of 3·2 km (dashed lines) and 6 km (full lines). The initial EPO peaks reach about 100 times normal (6 km) followed by a decrease to less than 10 times normal within 10 d. Following this stimulation the number of erythroblasts increases to 3·5

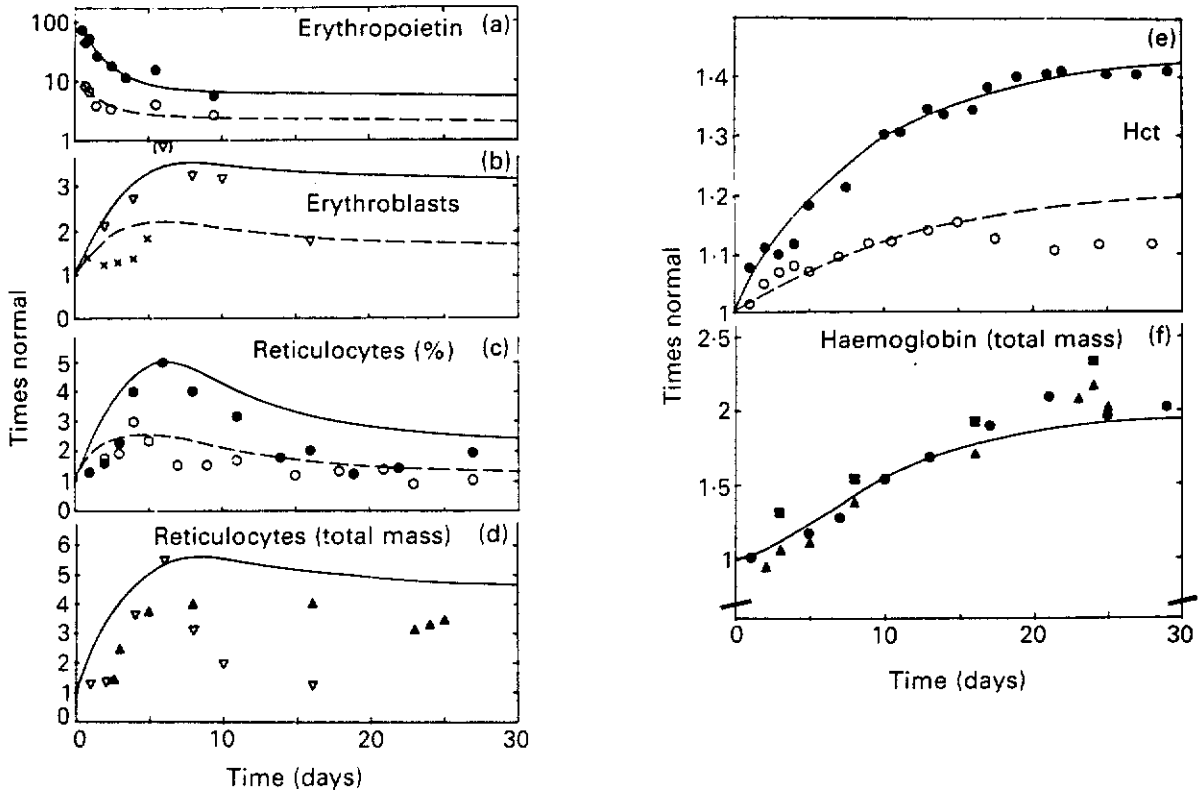


Fig. 9. Changes in erythropoiesis during chronic hypoxia for mice. Data from: Mylrea & Abbrecht (1970), ○: 3·2 km, ●: 6 km; Turner *et al.* (1967), X: 5·5 km, only bone marrow; Richard *et al.* (1971), ▽: 7 km, bone marrow and spleen; Kubanek *et al.* (1968), ▲: 7·5 km. Model calculations according to an altitude of 3·2 km (---) and 6 km (—).

times normal within 7 d followed by a reticulocyte peak of approximately five times normal. Hct (or Hb_{mass}) increases more slowly and reaches 1·4 times normal (1·8 times normal) after 20 d. Due to the increasing Hct, EPO, erythroblasts and reticulocytes decrease slightly. After 30 d, they have not yet reached their hypoxic steady state values. The model curves fit the experimental data sufficiently except for the decline of reticulocytes (Figs 9c,d) which is more pronounced in the experiments.

The results for rats are shown in Fig. 10 (for an altitude of 6 km). The proliferation rate (measured by the daily production of haemoglobin) increases to about 5·5 times normal in the experiment and 4·3 times normal in the model and decreases to a steady state value of two times normal after 60 d similar to the experimental data. The Hct (Hb_{mass}) increases to 1·4 (1·8) times normal within 60 d in theory and to similar values in the experiments.

DISCUSSION

The mathematical model of erythropoiesis in mice and rats described in Part 1 of this series (Loeffler *et al.*, 1989) has been applied to various erythropoietic stimulations. Comparing the model calculations with the experimental data, it is shown that the feedback system of the model is able to reproduce several experiments qualitatively and quantitatively in an acceptable manner although the data have been collected from a broad variety of animal strains and laboratories.

In the model, changes of the EPO concentration influence the amplification factors of CFU-E and the proliferating erythropoietic precursors and, furthermore, the marrow transit

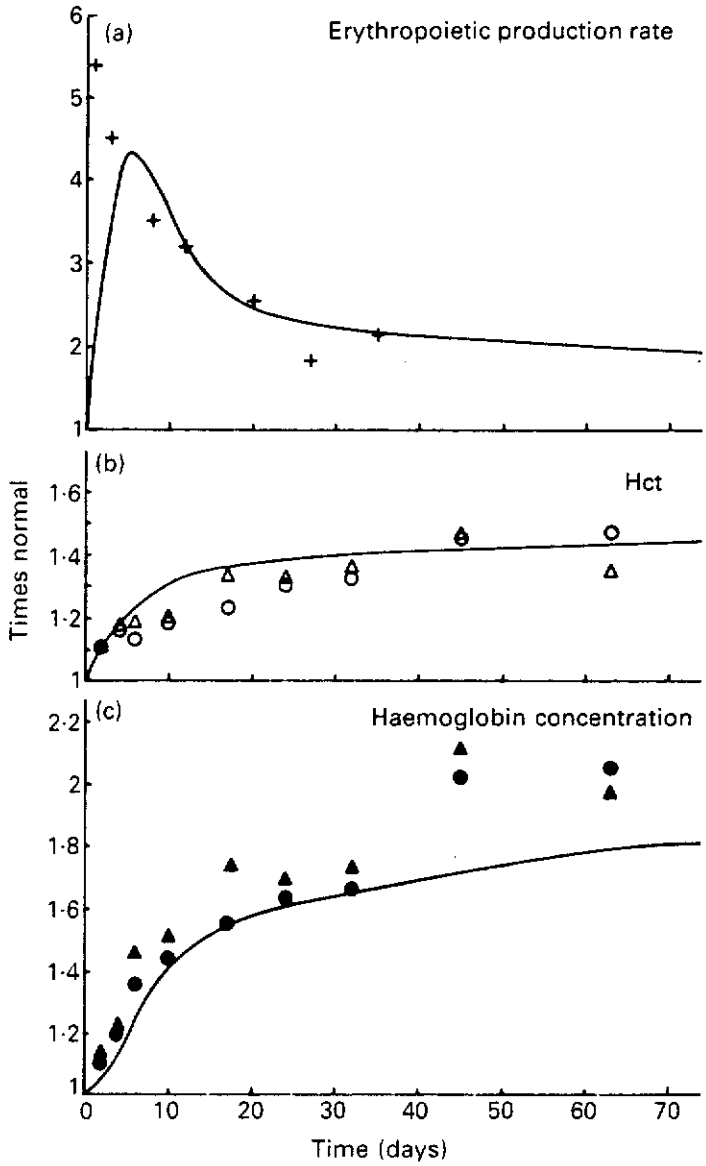


Fig. 10. Changes in erythropoiesis during chronic hypoxia for rats. Data from: Tribukait (1963), +: Hb_{mass} per day, ○: Hb concentration, △: Haematocrit, ●: Hb mass, ▲: RBC mass. Model calculations (—) according to an altitude of 6 km.

time (MTT). During the period of recovery from an erythropoietic stress, the first effect is more important while the accelerated shift of cells from the bone marrow into the blood leads only initially to a minor increase of the circulating red cell mass.

Except for the direct administration of the stimulating hormone, the experimental perturbations analysed here lead to erythropoietic stimulation by the reduction of the oxygen supply. This is realized by decreasing the concentration of the red blood cells (bleeding, plasma dilution, haemolysis) or by reducing the atmospheric oxygen pressure (hypoxia). The basic model assumptions and their limitations can be discussed as follows.

Anaemia

The loss of red cells in anaemia leads to a deficit in the oxygen supply. The decline of the tissue oxygen pressure of the whole body (kidney) is related to an increased production (or activation) of EPO. This hormone stimulates additional mitoses in the EPO-sensitive cells and the deficit in the oxygen supply is compensated by an increase of the red cell number.

After bleeding, the loss of cellular blood volume is rapidly compensated by an increase of the plasma volume such that the total blood volume is kept constant. It is concluded that not only the number of red cells but also the plasma volume is involved in the feedback mechanism. This effect can especially be seen in dilution anaemia caused by the injection of substances with high molecular weight like Dextran where the increased plasma volume stimulates erythropoiesis. In model terms, this is consistent with the view that Hct is responsible for the feedback although it cannot be excluded that red cells and plasma play separate roles. Whatever the detailed mechanism may be, it is important to look carefully at possible changes of plasma volume if one wants to understand the feedback loop of erythropoiesis. (Unfortunately, the model prediction for increasing the plasma volume could only be compared to data obtained from rabbits because no appropriate information was available for mice or rats. However, the general response pattern seems to be similar to the one predicted for rats.)

In terms of regulation one has to distinguish between haemolytic anaemia and other forms of anaemia. After haemolysis, a large amount of metabolites like heme or iron are immediately available for the formation of new cells. After the application of phenylhydrazine, this may lead to 1–3 additional mitoses in mice (Rencricca *et al.*, 1970; Hodgson *et al.*, 1972; Tambourin *et al.*, 1973) according to the situation in man where 1–2 additional mitoses occur in haemolytic anaemia beyond those already observed in comparable bleeding anaemia (Wichmann *et al.*, 1976). In addition, the shift of erythropoiesis from the bone marrow to the spleen plays an important role after phenylhydrazine in mice; under these circumstances 60–80% of the erythropoietic proliferation takes place in the spleen compared with 10–20% in normal steady state (Loeffler & Wichmann, 1985). Since previous results have shown that the spleen provides a better environment for CFU-E proliferation than the bone marrow this could explain at least partially the increase in total CFU-E observed.

In the model, an exponential dose-response curve between the tissue oxygen pressure in the renal sites of EPO production and the production rate of erythropoietin is assumed. Recalculating this relation to parameters which are experimentally easier available, it corresponds to a (nearly) exponential dose-response curve between Hct and EPO concentration. This type of relationship assumed in the model is supported by data of rats and mice as well as humans. However, it is hampered by the fact that indirect assays (*in vivo, in vitro*) have been used for measuring EPO.

There is a physiological limit to the degree of anaemia which the organism can survive during reduced oxygen supply. This borderline is reached for Hct between 0·3 and 0·4 times normal in experiments as well as in the model.

Injection of erythropoietin

Different from anaemia, the injection of EPO is an isolated manipulation of one parameter in the feedback loop which can be quantified by the injected amount of the hormone. Unfortunately, little is known about the pharmacokinetics of exogeneous EPO (half life time, distribution volume, degradation) in the experiments referred to.

A steep increase of the reticulocytes is found after injection of erythropoietin. The model analysis suggests that the shift of cells from the bone marrow into the blood may be responsible for this observation. Thus, EPO seems to be the substance which regulates the shift of primitive cells into the functional cell pool being observed in further experimental situations.

The model simulation of EPO injection is only successful if one assumes that the injected erythropoietin has some prolonged effect. Since, on the one hand, the number of progenitors and precursors is small in the hypertransfused animals used in these experiments and, on the other hand, EPO is available in excess, this finding might support the 'consumption hypothesis' that EPO is utilized proportional to the number of EPO-sensitive cells. This would implicate that half-life determinations of EPO undertaken in situations of high and low erythropoietic stimulation could yield different results. In this context, one might think of the binding of EPO to specific receptors at the surface of the EPO-sensitive erythropoietic cells. These bindings may last for a certain time leading to a prolonged erythropoietic stimulation although the plasma level of EPO might already have declined.

The application scheme of EPO is important for the maintenance of a stimulatory effect. Due to the short half-life of EPO, a single injection will be much less efficient in terms of erythrocyte production than a continuous low dose application. To reach the same increase of the haematocrit, further model calculations lead us to predict that one needs only 5–10% of the total amount of EPO if a daily fractionated or continuous application is used compared to a single injection. This high efficiency of low doses of EPO given over a long time period follows because the regulatory system tends to integrate the demand over a longer interval. The clinical relevance of this effect is obvious. In treatment of EPO-deficient uraemic patients for example it may be useful to deliver (recombinant) EPO in frequent intervals not longer than two times the half-life time of the hormone.

Hypoxia

The lower atmospheric pressure in hypoxic chambers or at high altitude reduces the arterial oxygen tension in the body. In this situation, the normal desaturation of haemoglobin leads to a reduced tissue oxygen pressure and consequently the production of EPO is stimulated. In turn, more red cells are formed until a new steady state is reached. This happens in a dose-dependent way, i.e. the lower the atmospheric pressure, the more red cells are produced in excess. The dose response curve is, however, fairly flat up to an altitude of 3 km and becomes considerably steeper below about 500 mmHg.

There is a limit below which the oxygen supply of the body cannot be maintained. In the model, this limit is reached at an atmospheric pressure of approximately 250 mmHg which corresponds to an altitude of 8 km. A similar borderline is found experimentally.

The threshold of 250 mmHg refers only to the initial phase of hypoxia where the red cell number is normal. In sustained hypoxia, the cell number is enlarged and reaches a new steady state after more than 60 days in mice and rats. In man, the steady state is reached after 100 days (Wichmann *et al.*, 1976).

However, it has to be kept in mind, that for higher Hct the blood viscosity increases steeply (for Hct of 1·5 times normal it is nearly doubled). In consequence, the blood flow is reduced which counteracts the purpose of the additional production of red cells, namely to improve the oxygen supply. The result of this mechanism is shown in Fig. 8 where for atmospheric pressures below 350 mmHg the discrepancy between model curves (without consideration of blood viscosity) and the measurements of Hct and haemoglobin mass becomes apparent. The steep increase of Hct may reflect the higher erythropoietic proliferation by which the body tries to compensate the impaired oxygen supply due to the reduced blood velocity. A similar result has been found in man where at altitudes above 4·5 km a steep increase of Hct is observed. The influence of viscosity on the oxygen tension can also be observed in Fig. 1 where Po_2 decreases in the measurements for Hct above 1·3 times normal while it continues to increase with increasing Hct in the model (without viscosity).

In sustained severe hypoxia, the erythropoietic proliferation remains enlarged to 3·5 and

5·5 times normal in the model calculations for rats and mice, respectively. The red cell mass, however, increases only to two times normal. This difference may be understood by the model prediction that a large amount (90%) of 'stress erythrocytes' is produced in this situation which reduces the average lifespan of the erythrocytes from 42 to 18 days in mice and from 56 to 24 days in rats.

Hypoxia dehydrates the body and reduces the plasma volume (Mylrea & Abbrecht, 1970). The hypoxic stimulus for a reduced plasma volume is lower than it would be for a normal one. On the other hand, after sustained hypoxia the reduced plasma volume worsens the problem of blood viscosity.

During the first days of hypoxia a high peak is found for the concentration of erythropoietin which disappears within 3–4 days. In terms of the model, this peak can only be understood by additional assumptions. Two hypothesis have been investigated. The first one assumes adaptational mechanisms. Renal vasoconstriction has been observed after a severe hypoxic stimulus (Deetjen, 1979) which might enlarge oxygen desaturation in the kidney and, thus, stimulate EPO production. However, no quantitative knowledge is available and other adaptational changes (cardiac output, blood pressure) also might be effective. The second hypothesis corresponds to the controversial contention that EPO might be utilized by EPO-sensitive erythropoietic bone marrow cells. In the model, this can be realized by a variable turnover time of the compartment for EPO which depends on the number of available precursors. Both hypotheses allow the quantitative reproduction of the experimental findings during the first days of hypoxia and the question which hypothesis is more appropriate cannot be answered on the basis of the available data.

In hypoxia of mice, up to 50% of the erythropoietic cells may be formed in the spleen (Loeffler & Wichmann, 1985). This has been taken into account by calculating (from the measurements in bone marrow and spleen) the total cell numbers, which are compared with the model curves.

There are animals which do not respond with an appropriate cell proliferation to severe hypoxia (Loeffler *et al.*, 1984; Wichmann & Loeffler, 1985). These animals either have insufficient production of EPO and/or the erythropoietic progenitors and precursors do not respond sufficiently to EPO (Lord & Murphy, 1985). Experiments with these animals have been omitted here.

The present model of erythropoiesis has two limitations: splenic erythropoiesis is only considered in a simplified way and the stem cells are neglected. One can expect a better understanding of the role of the spleen in mice if this organ is considered separately; this refinement of the model is in progress. Preliminary calculations show that the CFU-E of the spleen have a higher proliferative reserve (three to four additional mitoses) than those of the bone marrow (one to two additional mitoses). This is especially important for severe anaemia and to a certain extent also in severe hypoxia.

The neglection of the stem level may lead to an underestimation of the proliferative reserve of the CFU-E in the present analysis. As the comprehensive investigations of stem cell regulation suggest (Wichmann & Loeffler, 1985), the stimulated erythropoietic system may lead to a reduced output from the stem cell level into the erythropoietic lineage. At most the cell flux into CFU-E may be reduced to 50% in severe anaemia or hypoxia. This effect is small compared with an erythropoietic amplification of 6–10 times normal (corresponding to 2·5–3·5 additional mitoses) and may be compensated by one further mitosis in the CFU-E compartment during strong stimulation.

Finally, it should be emphasized that the dose-response curves of CFU-E and proliferative erythroblasts depending on EPO are the most important hypotheses of this mathematical model.

Therefore, the satisfactory reproduction of the various experimental treatments by using always the same dose-response curves has mainly supported the quantification of the erythropoietic feedback system. It has been demonstrated that the model is a good tool to estimate quantitative *in vivo* relationships which cannot, at present, be measured directly. It will be of interest to validate the model in the future for the mouse system and extend it to the human situation. The availability of recombinant murine and human EPO may allow to test specifically the *in vivo* dose-response relationship of EPO versus cell production as proposed here. For this purpose, the new steady states obtained after long term application of EPO should be examined. Such an exercise may also allow to quantify defects of the erythropoietic regulation processes such as a lack of responsiveness due to metabolic or genetic defects. We assume that the dose-response relation of EPO has a saturation characteristic. Thus, increasing the titres beyond a certain threshold will have no further beneficial effects in terms of cell production. In contrast, very long lasting high doses may even have counterproductive effects on the quality of erythrocytes produced (shortened lifespan) and perhaps also on the number of stem cells.

A more comprehensive discussion will follow at the end of Part 3 of this series (Wulff *et al.*, 1989) where the reaction of the erythropoietic system on suppressing influences (i.e. experimental treatments which elevate the oxygen supply) will be examined.

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