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IRON KINETICS IN INDIVIDUAL BONE MARROW REGIONS (BONES) IN MICE

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Abstract

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The course of concentration of labelled iron (^{59}Fe) after its intravenous administration was observed experimentally in plasma, erythrocytes, spleen and individual bones (here in haem and non-haem fraction separately). The optimum number of iron compartments in individual bones and the constants characterizing the flow through these compartments was then sought by means of mathematical modelling. It was possible to type the compartments by comparing flows into the bones of various types and to try to find their physiological interpretation. The following compartments were determined (apart from the circulating plasma and erythrocytes): 1. An iron pool with a half-time of efflux of several tens of minutes to several hours, probably corresponding to the iron contained in the interstitial marrow and splenic spaces, in a rapidly assumed equilibrium with plasma; 2. An iron pool with a half-time of efflux about nine hours, corresponding to the iron incorporating into haem or deposited in the pre-haem pool in erythroblasts; 3. An iron pool with a half-time of efflux of about 1–3 days, having the character of stores, probably deposited both in erythroblasts (half-time 3.15 days) and in cellular elements of the haematopoietic microenvironment (half-time 1.65 days); 4. An iron pool bound in the long-term in the teeth. The bones could be divided according to the representation of compartments into three groups: 1. Long bones and spine, in which intensive erythropoiesis takes place, exhausting the interstitial pool and consuming most of the storage iron pool; 2. Acral bones and the skull, with minimal erythropoiesis, in which the dynamics of the interstitial and storage iron pool localized beyond erythroblasts predominantly occurs; 3. Thoracic bones, representing a group intermediate between the two former ones in several aspects. — The model is also in accordance with the independently (chemically) ascertained iron content in the bones. The results represent a considerable refinement of our general model of ferrokinetics in mice (Vácha et al. 1982) and provide a firmer basis for the study of ferrokinetics in bone marrow under pathological states of erythropoiesis. Studies of this type are an inevitable prerequisite of correct interpretation of the labelled iron incorporation method as an indicator of the intensity of erythropoiesis.

Ferrokinetics, iron compartments in bones of various types, haem and non-haem iron, ^{59}Fe-incorporation method.

Knowledge of the internal exchange of iron in an organism is a prerequisite for the correct interpretation of the commonly used method of labelled iron incorporation into the erythropoietic organs. The internal iron kinetics in mice are, however, known only incompletely as yet. In our previous paper (Vácha et al. 1982) we proposed a model of the ferrokinetics of mice encompassing the sizes of basic iron compartments in the erythropoietic organs, the blood and the liver and the extent of flows between them. The employment of a model of ferrokinetics for the study of erythropoiesis presupposes, however, an analysis of iron compartments in the bone marrow more sophisticated than that which can be made in such a general model. The basic idea of the present paper

is to analyse experimental data separately in individual types of bones; a more refined division of iron compartments and a higher degree of conformity of the model with the experimental data could be achieved by comparing or correlating the ferrokinetic characteristics of fifteen different bones.

The method employed was again compartmental analysis. The plot of changes in concentration of tracer (^{59}Fe) in individual bones after its administration into the circulation of an animal can be decomposed into components by means of mathematical modelling, the components corresponding to the dynamically differing pools (compartments) of iron in bones. Indices (constants) characterizing the intensity of the compartment influx or efflux can be found by an optimization method, the fluxes germane to individual bones can be mutually compared and possibly physiologically interpreted. A knowledge of marrow iron compartments in the undisturbed state is a prerequisite of the proposed study of their changes under conditions where erythropoiesis is displaced from the steady state by a pathogenic stimulus. It is also a prerequisite for the employment of labelled iron incorporation into the erythropoietic organs as an indicator of the intensity of erythropoiesis.

Materials and Methods

The experimental techniques used are covered in the previous work and will only be briefly summarized here (for details see Vácha et al. 1982). Most data used in the present article were obtained in the identical animals as in the previous work; mathematical methods are, however, totally different. Where data from our previous works were used for correction and interpretation of the results, they always concern animals of the same strain, sex, age and breeding conditions as in the present article.

1. The Animals. All experiments were performed on males of the strain C57BL/10ScSnPh bred in our institute, 11.5–14 weeks old, mean body mass 28.8 g. The mice were kept at a temperature of 22 °C, diet Larsen (Velaz, CSSR) with an iron content of 6.3–7.5 mmol/kg, water ad libitum.

2. ^{59}Fe Administration. The form of administration used was ferrous citrate labelled with ^{59}Fe, mfr. Rotop (GDR), supplied in an isotonic buffer containing ascorbic and citric acids, supplemented with 0.9 % benzylalcohol, pH 4–6, specific activity 10.5–34.3 GBq/mmol Fe. The preparation was diluted immediately prior to administration with saline to an equivalent of 1.8 or 5.4 nmol elementary iron per mouse, corresponding to a radioactivity of 5.6–18.5 10^4Bq per mouse (in 0.1 ml). However, it follows from the discussion contained in Vácha et al. (1982) that any proportion of iron not bound to transferrin is entirely negligible in our experimental regimen, since it should above all be apparent in a very rapid initial decline in the plasma and the fast depositing of this iron in the liver, which was not the case. The proportion of unbound iron can be estimated at less than 1 % of administered activity.

3. ^{59}Fe Clearance from Plasma. At selected intervals following ^{59}Fe administration groups of 10–35 mice with ^{59}Fe administered were investigated. The animals were sacrificed at 0900 h in all cases by bleeding from the axillar blood vessels under ether narcosis. The blood was transferred using a heparinized pipette to a test-tube containing powdered heparin and after 20 min centrifugation at 1400 g 0.25 ml plasma was taken from each sample, 0.75 ml distilled water added, and this mixture was thoroughly shaken and the extinction of oxyhaemoglobin from any haemolysed erythrocytes present was spectrophotometrically measured at a wavelength of 540 nm. Using a calibration curve the proportion of haemolysed erythrocytes in each plasma sample was then calculated. Following the measurement of extinction 2 ml trichloroacetic acid (c = 1.22 mol/l) was added to each sample, the sample was stirred, centrifuged, and the radioactivity of the supernatant measured. On precipitation of the haemoglobin released by haemolysis using trichloroacetic acid a certain unprecipitable fraction remains; according to our previous results (Holá et al. 1975) the amount of this depends on the time elapsed from ^{59}Fe administration. This dependence was utilized in the correction of the influence of haemolysis on plasma radioactivity. The corrections amounted to up to 35 % of the original value after the longer intervals following ^{59}Fe application, some other corrections made (for the natural radioactivity of plasma and the body mass of animals) being almost negligible.

The radioactivity of the plasma samples was finally calculated in respect of the total plasma volume determined in turn from the degree of dilution of ^{59}Fe at the time to (at the moment of ^{59}Fe application). The conversion factor for 0.25 ml plasma was 7.73.

4. The Appearance of ^{59}Fe in Circulating Erythrocytes. The erythrocytes sedimented on centrifugation of blood samples were washed three times in an excess of saline, and following the final centrifugation (20 min at 1400 g) were pipetted in amounts of 0.1 ml, haemolysed with distilled water, and their radioactivity measured. The values obtained were corrected for washing solution retained (by a factor of 0.965), for the natural radioactivity of the erythrocytes, and for

body mass of animals. The radioactivity of the volume of erythrocytes withdrawn was calculated in respect of the total volume of circulating erythrocytes, determined using erythrocytes labelled with ^{59}Fe in a parallel experiment (Vácha 1975). The conversion factor for samples of 0.1 ml was 7.78 or 7.68 according to the body mass of animals.

5. Organ Measurements. Following the bleeding of the animal the spleen was removed and weighed and its radioactivity measured. The complete skeleton, free of all soft tissue, was obtained by placing the carcasses of the animals in a colony of the beetles *Dermestes vulpinus* Fbr. (kept according to Grulich 1977); prior to measuring it was divided into individual bones or groups of bones. The teeth were loosened from the mandible and maxilla by trypsin. All organ samples were corrected for natural tissue radioactivity. The mass of bones was determined by weighing in dry state (values were taken from Vácha et al. 1981).

6. ^{59}Fe Incorporation into the Haem and non-Haem Fractions of Iron in Organs. In another group of animals, in addition to the overall radioactivity of the spleen and skeleton, the radioactivity of the haem fraction in these organs was also measured at selected intervals after ^{59}Fe administration. The organs were mechanically homogenized after freezing with liquid nitrogen, the cells haemolyzed with distilled water, and the haem fraction extracted using acid ethylacetate (for details see Vácha et al. 1978). In another group of animals, the absolute quantum of non-haem iron in bone marrow and haem iron in marrow erythroblasts were determined by extraction and mineralization technique (values were taken on from Vácha et al. 1983).

7. Measurement of ^{59}Fe Activity. This was measured using an Automatic Gamma Well Counting System (Nuclear Chicago) in the range 0.5–1.4 MeV, efficiency 19 %. All samples were formed into approximately similar geometrical shapes and measured together with a standard formed by a known fraction of administered radioactivity. The length of measuring was chosen for the given background so as to give a dispersion of less than 5 % of the measured value even in the least radioactive samples.

8. Mathematical Analysis. The pool of iron present in a bone can be divided roughly into two parts: iron contained in the blood circulating in blood vessels of the marrow (as iron-transferrin complex in plasma and as erythrocyte iron) and iron contained in all other marrow components (erythroblasts, cells of the hematopoietic microenvironment, interstitial fluid etc.). The model count can be based primarily on the iron contained in circulating blood. The values of the radioactivity of plasmatic and erythrocyte iron were measured as a function of time after ^{59}Fe administration and these values could be very well approximated by means of the least squares method with a curve. In the case of plasmatic ^{59}Fe the approximation was performed by the sum of three exponentials, in the case of erythrocyte ^{59}Fe it was performed according to the following equation:

$$E(t) = (1 - \sum_{j=1}^{2} a_j \exp(-k_j t))\, a_3 \exp(-k_3 t), \qquad (1)$$

with the constants a_j, k_j ($j = 1, 2, 3$).

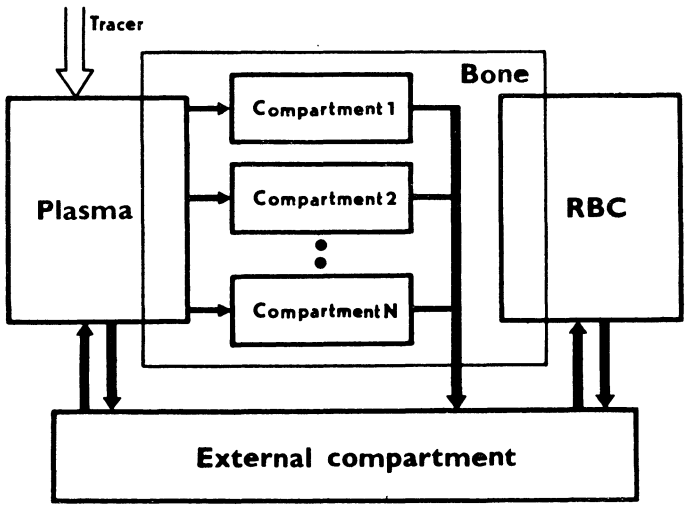


Fig. 1. Scheme of model compartments.

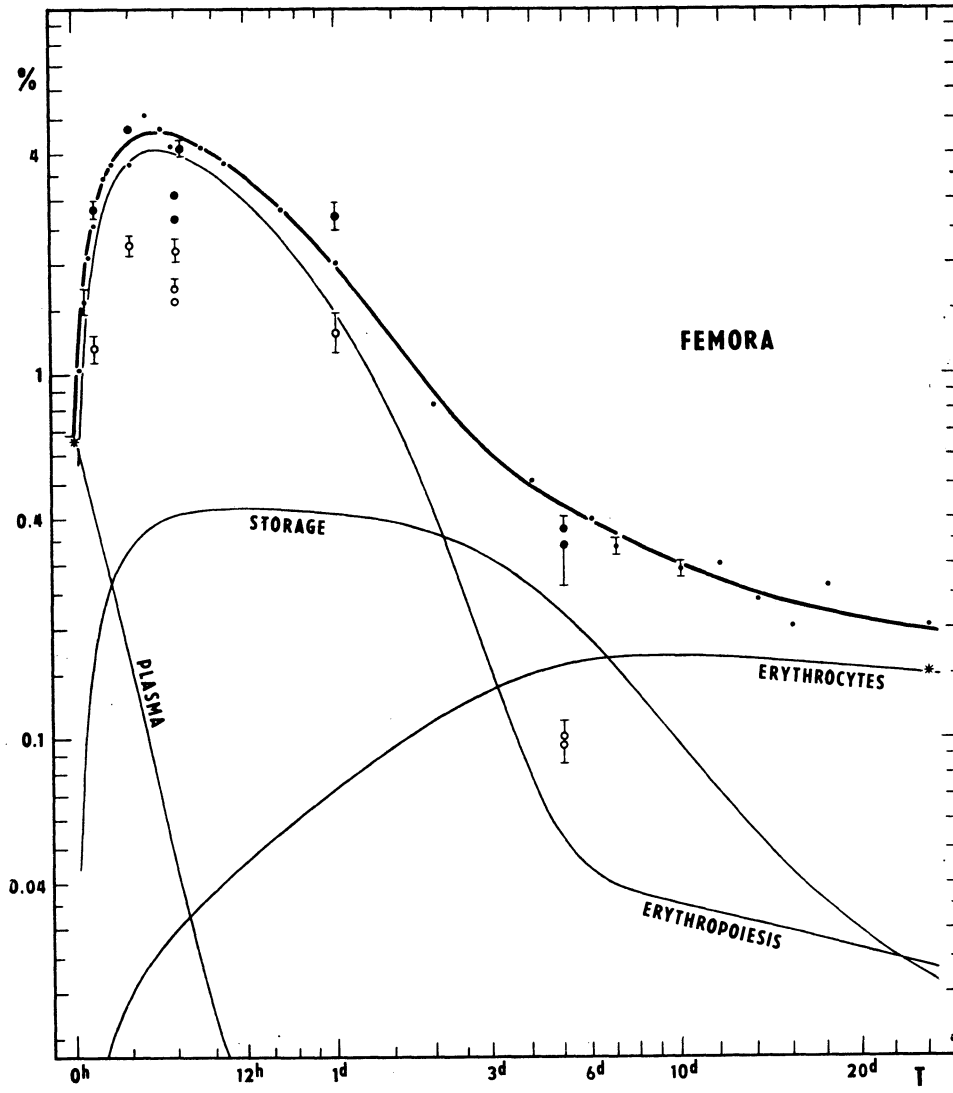


Fig. 2. Course of ^{59}Fe radioactivity in femurs as dependent on time after tracer administration. Full circles — total ^{59}Fe quantity in the bone in % of the quantity administered, empty circles — ^{59}Fe bound into the haem fraction in % of the quantity administered. Bigger circles — Experiment No. 6 (see Methods), smaller circles — Experiment No. 5. Stars — ^{59}Fe content in plasma (in time $T = 0$) and in erythrocytes (24 days after the tracer administration) on the basis of the direct measurement of the plasma and erythrocyte volume in a bone (Vácha et al. 1981) and in the case of erythrocytes also on the basis of their unitary volume. Each point usually represents 10 to 25 experimental animals, bars indicated S.E. (if greater than 5 % of the mean). All curves derived from a model calculation; light ones represent individual iron compartments, the thick one being a summation of all of them. x-axis — time in hours and days, y-axis — % of radioactivity administered.

The scheme of the model is in Fig. 1. The tracer moves from plasma into N parallel iron compartments in the bone, directly proportionally to the tracer concentration in plasma. The influx rate of labelled iron (at time t) into a compartment is given by the formula $A \,.\, c\,(t)/100$, where A is a proportionality constant ("compartment affinity of plasmatic iron" as a percentage of the iron present in plasma, per day) and $c(t)$ is the tracer concentration in plasma as a percentage of the ^{59}Fe administered (it is the same for all compartments and bones). The efflux rate of labelled iron at time T from a compartment equals the product $Kq(T)$, where K is the rate constant of efflux and $q(T)$ is the quantum of labelled iron in a compartment as a percentage of the ^{59}Fe administered. Then

$$q(T) = \int_0^T \frac{Ac(t)}{100}\, e^{-K(T-t)}\, dt. \qquad (2)$$

The total amount of iron Q in a compartment (determinable by chemical analysis) is given by the equation

$$Q = \frac{A}{K}. \qquad (3)$$

The rate constant of efflux K is equivalent to the half-time of efflux according to the formula: $T\,1/2 = \ln 2/K$ (hereafter we shall for clarity's sake mostly use instead of K vaues the $T\,1/2$ values).

The model calculation set out from the entered curves of the ^{59}Fe content in circulating plasma and erythrocytes and from the required number of compartments N, which was selected as minimal. The model enabled us to choose the rate constants A and K of individual compartments by the least squares method, and the quantity of plasma and erythrocytes contained in a bone. As we had plasma and erythrocyte volumes at our disposal, measured independently by means of ^{131}I-albumin and ^{51}Cr-labelled erythrocytes (Vácha et al. 1981), we could evaluate the adequacy of the results thus obtained. Because the model calculated plasmatic volume by mere extrapolation to the time $T = 0$, the results ensuing from the model turned out to be less reliable than directly measured values and we used the latter for further calculations. As regards erythrocytes, more reliable values were obtained from the model calculation in most cases than from direct measurement (burdened with a considerable experimental error). An exception was the skull and the mandible, where the iron contained in erythrocytes interfered with the iron of a very slowly disappearing compartment (dental tissue) during the calculation. Therefore the experimentally ascertained quantity of erythrocytes was entered for the model calculation in the case of the skull and the mandible.

The curves of the tracer content in all individual compartments as a function of time after ^{59}Fe administration were calculated, on the basis of the determined values A and K and the ^{59}Fe content in the circulating blood. The calculation was done separately for fifteen bones (or bone groups), for the skeleton as a whole, and for the spleen. Examples of these results for some typical bones are given in Fig. 2–5.

The possibility to compare the iron quantity as between individual compartments and therefore between individual bones follows from equation (3). These results could be compared with the results of chemical analysis of the iron content and the model can be tested in a manner independent of others. The sum of the A/K values for all compartments 2 to 4 (representing the iron content in a bone in relative units) was calculated and converted to the absolute content (by means of an coefficient derived from a comparison of iron flows in plasma and circulating erythrocytes compartments with the elementary iron content in these compartments). The iron content in bones and spleen deduced from the model was then compared with the chemically determined iron quantity (Tab. 2 in Vácha et al. 1983).

Results

A synopsis of calculated constants A and half-times (T1/2) for individual compartments and bones is given in Tab. 1. It follows from the table that it is possible to distinguish three main components of the total radioactivity of a bone according to the half-time of efflux: (i) A very heterogenous compartment 2 with a half-time of about 0.2 to 7.3 hours; (ii) Compartment 3 with a relatively homogenous half-time with the mean value 9.2 hours; (iii) Compartment 4 with a half-

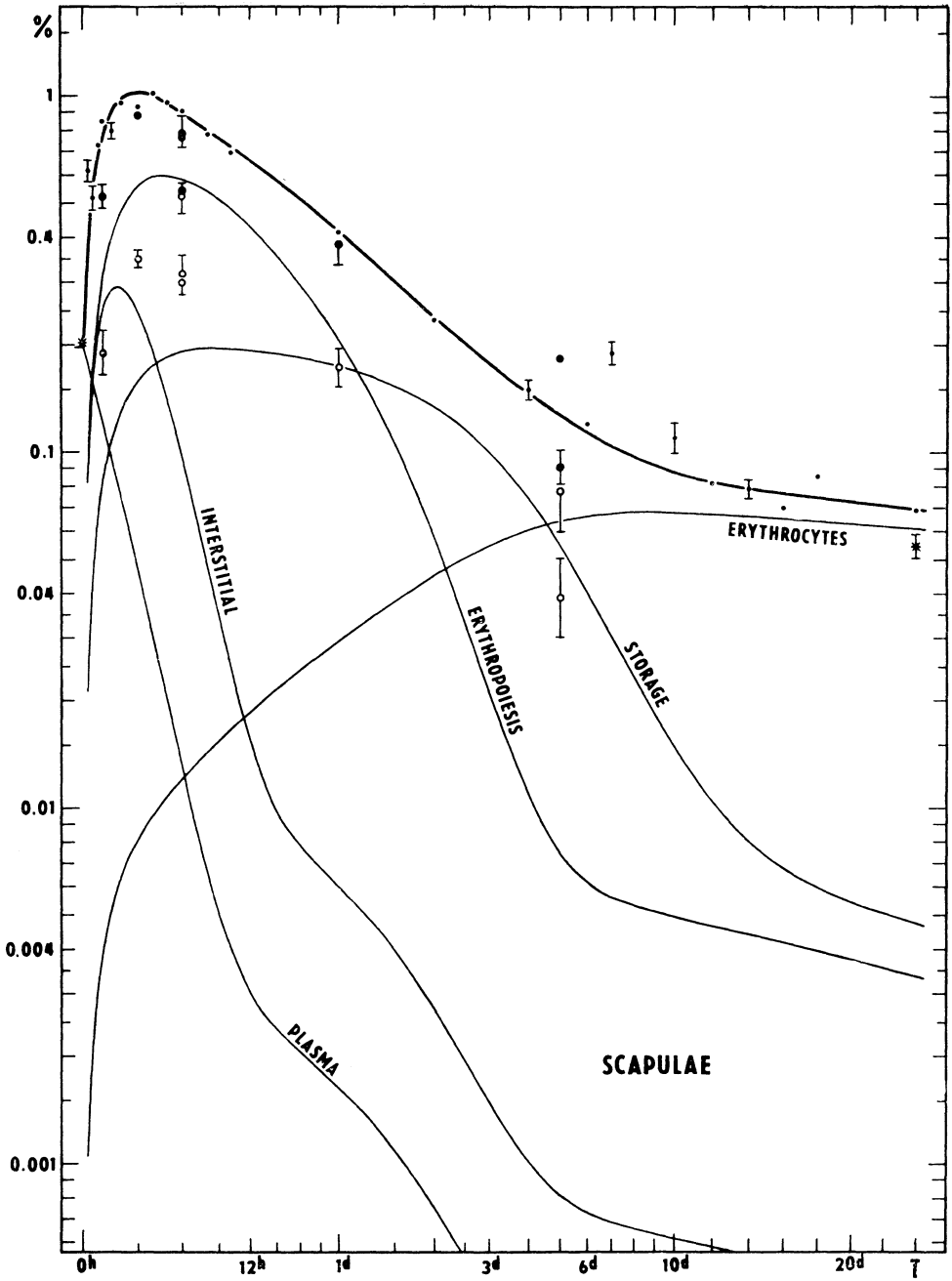
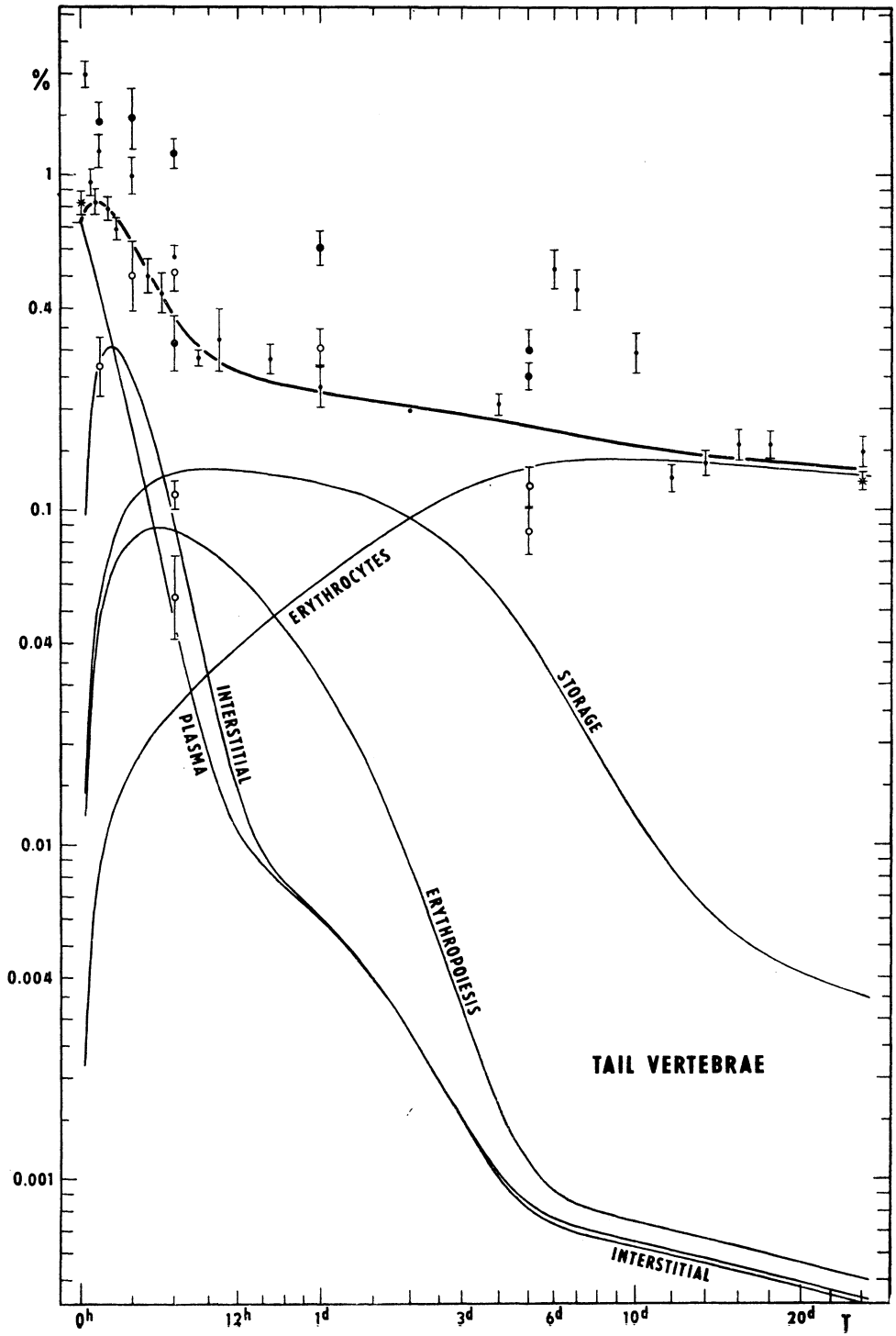


Fig. 3. Course of ^{59}Fe radioactivity in scapulae as dependent on time after the tracer administration. Notes as in Fig. 2.

Fig. 4. Course of ^{59}Fe radioactivity in tail vertebrae as dependent on time after the tracer administration. Notes as in Fig. 2.


%
1
0.4
0.1
0.04
0.01
0.004
0.001
ERYTHROCYTES
PLASMA
INTERSTITIAL
STORAGE
ERYTHROPOIESIS
TAIL VERTEBRAE
INTERSTITIAL
0h
12h
1d
3d
6d
10d
20d
T

-time of about 1 to 3.4 days. Besides these compartments occurring in most bones, it was possible to distinguish the compartment 5 in the skull and the mandible, with an extremely long half-time of 108 to 221 days. (Numbering of compartments begins here with No. 2 to accord with our general model of ferrokinetics.)

Compartment 2. The compartment cannot be demonstrated in most long bones of extremities. It is clearly differentiated from the experimentally determined course of radioactivity in the haem compartment by a high efflux rate (Figs. 3—5, compare the curve designated as „interstitial" with the empty rings).

Compartment 3. This compartment is the biggest of all compartments in most bones (it has the highest constant A). Its half-time is the same in all bones within the framework of accuracy of the experiment, in spite of considerable differences in A3 values. This compartment is most prominent in long bones, its value is lower in thoracic bones on average, and it is near zero in acral bones,

Table 1

Characteristics of iron compartments in the erythropoietic organs in mice

a	b	c	Long bones and spine					Thoracic bones		
			humeri	pelvis	femurs	tibiae, fibulae	spine[1]	sternum	ribs	scapulae
1	1 circulating plasma	per cent[3]	0.346	0.773	0.682	0.478	2.438	0.325	0.567	0.198
2[4]	2 interstitial space in bones	A2 A2/M T1/2 2[5]	4.030 0.0948 3.217	0.0 0.0 —	0.0 0.0 —	0.0 0.0 —	31.73 0.1183 1.124	0.0 0.0 —	103.3 1.611 0.168	10.96 0.400 1.038
2[4] + 3	3 pre-haem and haem pool in erythroblasts	A3 A3/M T1/2 3[5]	30.09 0.708 10.35	61.29 0.829 9.481	66.57 0.719 9.498	42.58 0.497 9.480	133.0 0.496 9.676	16.68 0.854 10.46	24.46 0.381 6.712	9.744 0.356 9.205
4[4]	4 storage iron	A4 A4/M T1/2 4[6]	2.386 0.0561 3.393	5.459 0.0739 2.624	5.182 0.0560 3.423	4.100 0.0478 2.740	12.031 0.0449 2.564	1.232 0.0631 2.864	5.731 0.0894 1.780	2.491 0.0910 1.950
4[4]	5 teeth	A5 A5/M T1/2 5[6]								
8	8 circulating erythrocytes	per cent[3]	0.148	0.289	0.274	0.226	1.293	0.129	0.270	0.110
	4a storage iron in haemopoietic microenvironment	A4a[8]	2	12	0	19	14	0	67	69
	4b storage iron in erythroblasts	A4b[8]	98	88	100	81	86	100	33	31

Column *a* — designation of the compartment in our general model of ferrokinetics (Vácha et al. 1982), column b — number and designation of the compartment, column *c* — characteristics of compartments ascertained by a model calculation. The constants A2 to A5 express the proportionality between the plasmatic iron concentration and the iron flow into compartments ("affinity of the compartment"), A2/M to A5/M — the same constants divided by bone mass in mg (data regarding the bone mass taken from Vácha et al. 1981). T1/2 — half-time of the efflux from compatments (T1/2 =

after the conversion of the A3 values to unit mass of bones (taken from Vácha et al. 1981). It was not possible to distinguish this compartment from compartment 4 in the skull as a result of similarity of T1/2 values; it could be assessed only approximately that the A3—4 value is evenly divided between A3 and A4 values in the skull (without the mandible) and in a ratio of 1 : 3 in the mandible (T1/2 values are the same for compartment 3 and 4 in both bones). The calculated course of radioactivity of compartment 3 coincides very satisfactorily with the experimentally determined course of ^{59}Fe incorporated into the haem of the erythropoietic tissue, especially in the period of maximum radioactivity, when the disturbing effect of labelled erythrocytes is not yet too strong (Fig. 2—4, compare the curve designated "erythropoiesis" with the empty circles).

Compartment 4. Although the values T1/2 of compartment 4 are distinctly different from the T1/2 value of compartments 3 and 5, they differ considerably

Acral bones					Skull		Whole skeleton	Spleen
fore feet	hind feet	tail vertebr.	clavicles	radii, ulnae	skull[3]	mandible		
0.152	0.305	0.718	0.053	0.127	1.906	0.381	9.572	0.524
0.0	0.525	12.23	7.571	4.215	21.31	10.67	276.2	53.07
0.0	0.0067	0.0781	1.314	0.127	0.0907	0.124	0.215	
—	7.245	0.938	0.210	2.415	1.544	1.337	0.430	1.528
0.0	0.0	1.434	1.830	2.561	25.69[7]	2.183[7]	398.4	25.39
0.0	0.0	0.0092	0.318	0.0769	0.109[7]	0.0255[7]	0.310	
—	—	9.384	7.689	9.855	0.717[7]	1.438[7]	10.809	8.342
0.299	0.554	1.667	0.316	1.127			30.963	3.352
0.0134	0.0071	0.0106	0.0548	0.0339			0.0241	
0.959	2.273	2.136	2.017	2.004			5.428	1.684
					6.262	2.190	5.257	
					0.0266	0.0255	0.0041	
					222	108	1386	
0.0393	0.0973	0.230	0.0117	0.0554	1.013	0.306	4.689	0.715
100	100	93	55	82				
0	0	7	45	18				

= ln 2/K). Circulating plasma — plasma volume in % of the total plasma volume in the body after Vácha et al. 1981 circulating erythrocytes — calculated erythrocyte volume in % of the total erythrocyte volume in the body. Notes: 1 — without tail vertebrae, 2 — without mandible, 3 — % of the whole body plasma or erythrocyte content, 4 — a part only, 5 — in hours, 6 — in days, 7 — values shared by comparments 3 and 4, 8 — % of the A4 flow.

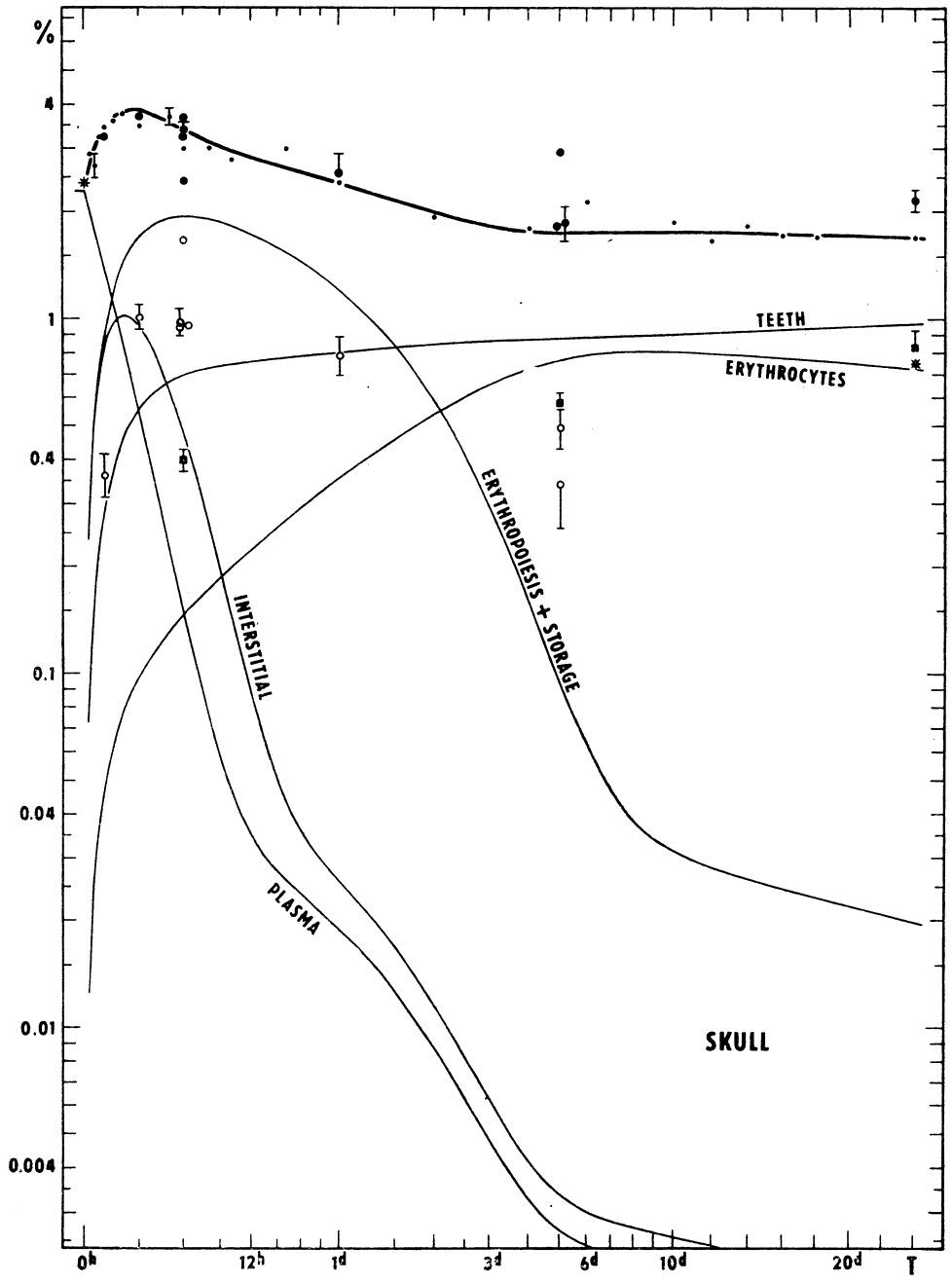


Fig. 5. Course of ^{59}Fe radioactivity in the skull (incl. the mandible) as dependent on time after the tracer administration. Rectangles — ^{59}Fe content in the teeth, other notes as in Fig. 2.

from each other in individual bones, viz. they are significantly higher in the strongly erythropoietic bones (i. e. in the bones with a high A3/M value) than in bones with minimal erythropoiesis (with low values of A3/M). It further follows from Tab. 1 that there is a positive correlation between A3/M and A4/M values

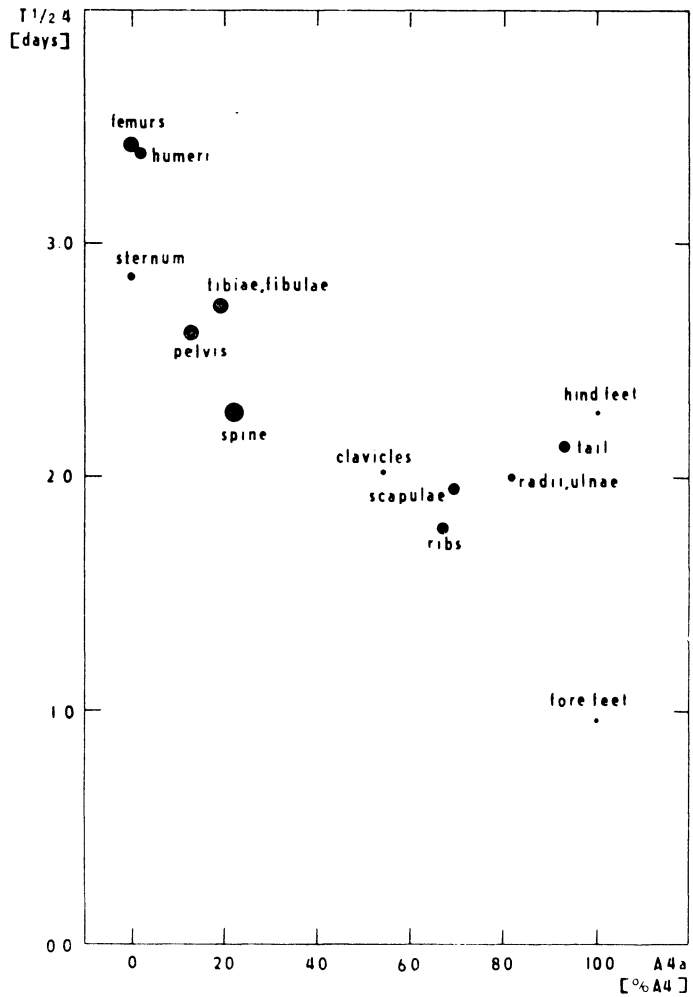


Fig. 6. Dependency between the value of the hypothetical subcompartment A4a (x-axis) and the T1/2 value of the compartment 4 (y-axis). Diameters of circles indicate the magnitude of the compartment 4 in the bone (i. e. the reliability of the results).

($r = 0.842$, $n = 13$, $P < 0.001$, weighted values of the constants), which is especially marked in strongly erythropoietic bones. In these bones the A4/M values constantly represent 7.8 to 9.6 % of the A3/M value. In contrast to this the magnitude of the A4 component depends mainly on the mass of bones in bones with little erythropoiesis.

In order to obtain more homogenous subcompartments we presupposed accordingly that comparment 4 is a heterogenous one, that its more slowly changing component is closely tied with erythropoiesis, while the more rapidly changing component is not. Under the assumption that the *A* constant of the slowly changing compartment represents 7.8 % of the A3 value in *all* bones, it was possible to divide the flow 4 into two components in all bones, designated as A4a and A4b in the two bottom rows of Table 1. The relative magnitude of the two components is given in these rows calculated according to the relations: the relative magnitude A4a = (A4 − 0.078 × A3) × 100/A4 %; the relative magnitude A4b = (100 − − A4a) %. The half-time of the components 4a and 4b is about 1.65 and 3.15 days, respectively.

The justification of this dividing of the flow 4 follows a. o. (see Discussion) from Fig. 6, from which is apparent a high degree of dependence of the half-time of outflow 4 on the relative magnitude of the A4a component.

Compartment 5. During the calculation of the variations in time of the tracer concentration in the circulating erythrocyte compartment, a certain excess of radioactivity occurred in the skull and the mandible in comparison with experimentally determined values. It was possible on this basis to separate an extremely slow component in these two bones, almost without an efflux. The absolute magnitude of this compartment is not negligible even from the viewpoint of the whole skeleton, and the large incorporation into it suggests that after some time (evidently longer than the duration of the experiment) a sudden efflux must occur.

The behaviour of all compartments mentioned as a function of the time after tracer administration in some typical bones is depicted in Figs. 2—5. Both "external" compartments (circulating plasma and erythrocytes in the marrow vessel system) change mutually inversely, as the tracer passes gradually from plasma to erythrocytes. The maximum of the "internal" (fixed) compartments occurs in the order of compartments 2, 3 and 4. It is possible to read from these figures which components the sum ^{59}Fe incorporation is composed of at any moment after the tracer administration, which is of great interest in using the ^{59}Fe incorporation as an indicator of the intensity of erythropoiesis, i. e. of compartment 3.

The values given in Tab. 1 referring to the skeleton as a whole must be regarded as less reliable than the values pertaining to the individual bones. Wherever there

Table 2

Comparison of iron quantity in bones calculated from the parameters ascertained by the model with the result of the direct chemical determination

Organ	Real value	Model value
Spine	370.6 ± 55.4	334.8
Sternum	61.3 ± 16.6	40.0
Ribs	78.4 ± 10.9	65.7
Scapulae	32.7 ± 30.0	33.5
Clavicles	14.5 ± 5.2	4.8
Humeri	86.6 ± 71.6	80.0
Radii, ulnae	29.0 ± 4.7	13.8
Fore feet	7.2 ± 2.6	1.1
Pelvis	160.2 ± 16.4	142.5
Femurs	149.4 ± 14.4	163.0
Tibiae, fibulae	115.9 ± 10.0	103.7
Hind feet	14.3 ± 3.9	5.2
Spleen	240[1]	66[1]

Arithmetical means ± S. E., values in nmol Fe. 1 − approximative value. Chemically determined iron quantities ("real values") taken from Vácha et al. 1983.

are numerous subcompartments these become superimposed, which slightly impairs the reliability of the identification of compartments and of the estimation of their dynamic characteristics, together with the effect of measurement errors. The differences appear predominantly in small compartments (4 and 5).

The results of the model calculation of the absolute iron quantities in bone marrow are, along with the results of a direct measurements (Vácha et al. 1983), given in Tab. 2.

Discussion

It is possible to deduce some hypothetical conclusions about the physiological meaning of the compartments from a comparison of the behaviour of iron compartments in the bones of various types.

Compartment 2. It follows from the discrepancy between the calculated course of tracer concentration in this compartment and experimentally derived values of radioactivity in the haem fraction (Figs. 3 to 5) that iron incorporated into haem is not in question here. Neither, apparently, is a fraction destined for short-term incorporation into the haem (pre-haem pool), because this compartment is absent in most strongly erythropoietic bones (in pelvis, femurs, tibiae and fibulae). We isolated a large pool of non-haem iron in rapidly assumed equilibrium with the plasma compartment in our general model of ferrokinetics (Vácha et al. 1982), which we interpreted as a pool consisting of iron-transferrin complex in the interstitial ("extravascular") space of various tissues and organs. The dynamics of the present compartment 2 best correspond to this pool in the bone marrow and spleen; the half-time of the efflux from the interstitial pool took 2.29 hours in our previous model, which is a value roughly comparable to the T1/2 values of our present compartment 2 (Tab. 1). Accordingly, compartment 2 could most probably be identified for the most part with the iron in the interstitial space of the bone marrow and the spleen, exchanging rapidly with plasma with a half-time of about one hour. The impossibility demonstrating this compartment in strongly erythropoietic bones by means of a tracer apparently derives from its fast consumption for erythropoiesis, so that its presence is not manifested in the ^{59}Fe incorporation curve.

Compartment 3. The close resemblance of the curves calculated for the A3 flow to experimentally determined ^{59}Fe values in the haem fraction of bone marrow implies that this flow is directly connected with haem production, i. e. with erythropoiesis. A slight shift of the calculated curve in front of that of the experimental data then shows that compartment 3 encompasses not only the iron incorporating immediately into the haem, but also the labile pool of pre-haem iron destined for short-term incorporation, whose existence in maturing red cells is amply documented by biochemical methods (for summary see Romslo 1980). The scheme of our model does not allow differentiation of pre-haem and haem compartments by calculation; it would be possible, however, to assess the magnitude of the haem fraction at various points of time from the mentioned shift of the curves in front of the experimentally determined values. But, apart from this differentiation, it appears beyond doubt that the compartment 3 represents an iron pool connected immediately with erythropoiesis localized in maturing red cells. An approximate idea can be formed from the A3/M line in Tab. 1 of the erythropoiesis intensity per unit volume of the marrow of various bones (it must, of course, be assumed that the marrow represents

a fixed fraction of the total bone mass). The situation is graphically illustrated in Fig. 7; the most intensive erythropoiesis takes place in long bones of the extremities and in the spine (without tail vertebrae), while the tail, fore and hind feet and skull participate only very slightly in erythropoiesis (4.3 % of the erythropoietic activity of the whole skeleton, in spite of their mass representing 45 % of the whole skeletal mass).

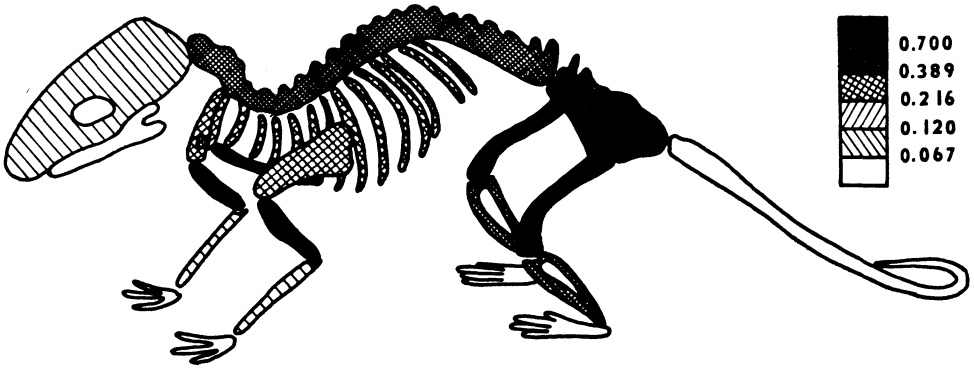


Fig. 7. Distribution of the erythropoiesis in the skeleton of mice (A3/M values).

Compartment 4. There are several reasons why we must suppose this compartment to consist of at least two components. It is represented both in bones with intensive erythropoiesis (with a high A3/M value) and in bones with a weak one, though the constant A4/M has lower values in these bones; also the T1/2 values differ in the two types of bones. From the correlation between the A3/M and A4/M constants it can be deduced that a substantial part of the A4 flow is directed into the same structures (cells) as the flow destined for the production of haem immediately, i. e. into the erythroblasts. As the A4 value is dependent on the bone mass in bones with low erythropoiesis while it is more dependent on the intensity of erythropoiesis in the strongly erythropoietic bones, we drew the tentative inference that the behaviour of the compartment 4 as a whole could best be explained by its division into two parts, A4a (which is not connected with erythropoiesis immediately and prevails in acra) and A4b (which displays a close connection with erythropoiesis and prevails in erythropoietic bones).

A prerequisite for the quantitative division of compartment 4 was that the influx into the subcompartment 4b equals 7.8 % of the flow employed for erythropoiesis immediately (i. e. of the flow A3). It is possible to verify whether a flow amounting to $0.078 \times$ A3 corresponds quantitatively to the need of the storage iron in erythroblasts, or whether it perhaps surpasses their capacity for consuming iron. The mass of iron consumed by a tissue is proportional to the A/K ratio (see equation 3). As the K4 value of the strongly erythropoietic bones roughly equals 0.22, the fraction of iron incorporated into maturing red cells as storage iron equals $7.8/0.22 = 35.4$; the fraction of iron consumed for haem production immediately then equals $100.0/1.745 = 57.1$ % (the K3 value of strongly erythropoietic bones is about 1.754). So the ratio between the quantity of the storage iron deposited in the marrow and supplied as the flow A4b to the iron brought as A3 flow and incorporated in hemoglobin equals 35 : 57. This ratio approaches the ratio between the

quantity of iron deposited in pronormoblasts and early erythroblasts on the one hand and the late erythroblasts on the other (37 : 63) reported on the basis of autoradiographic studies by Harriss (1958). As the haem synthesis in the early phases of the erythroblast development is small, the flow of iron into them could be considered a storage flow and in this case the two data agree quantitatively very well. It could therefore reasonably be supposed that a great part of the A4 flow is directed to the early erythroblasts.

The appropriateness of dividing the A4 flow into these two components also follows from the dependence shown in Fig. 6. It is apparent that the fraction of storage iron directed outside erythropoiesis (A4a) is in a close relation to the half--time of iron vanishing from compartment 4. This means that the greater the part of flow 4 consumed for erythropoiesis immediately (the smaller A4a is) the slower the tracer disappears from compartment 4 in the bone; physiologically this apparently means that the bulk of the storage iron is destined for incorporation into the

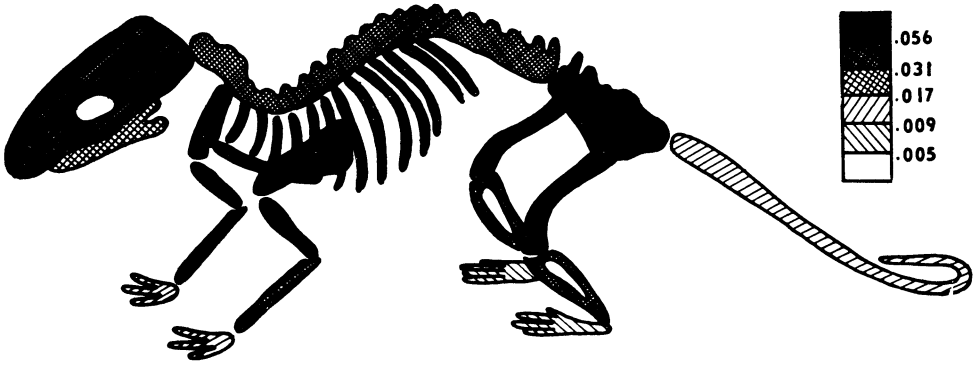


Fig. 8. Distribution of the storage iron in the skeleton of mice (A4/M values).

haem of (early) erythroblasts, with which it leaves the bone after their transit time has elapsed. The T1/2 4b value equal to 3.15 days is in a reasonable agreement with the transit time of red blood cell from the proerythroblast stage, reported to be equal to 2.7 days in mice (Mary et al. 1980).

It follows also from the above considerations that the slow non-haem flow A4 is composed of two different fluxes, the faster one (T1/2 = 1.65 days) detectable in bones with a low intensity of erythropoiesis per unit mass, and the slower one (T1/2 = 3.15 days) localised in strongly erythropoietic bones. While the slower flow of storage iron into erythroblasts prevails in the long bones and in the spine, the storage iron in the skull and acra is directed into the further marrow constituents (i. e. into reticular cells, endothelial cells and RES); the thoracic bones represent an intermediate group in this respect (Fig. 6). The distribution of the storage pool 4 (as a whole) in the skeleton is evident from the row denoted as A4/M in Tab. 1 and from Fig. 8. This picture does not differ from Fig. 7 too much, as there is a strong correlation between fast (A3/M) and slow (A4/M) reflux components as mentioned above.

Compartment 5. The calculated course of the tracer concentration in this compartment coincides very well with the experimentally determined radioactivity in the teeth (the curve designated "teeth" and rectangles in Fig. 5).

The extremely long half-time of this compartment could be explained by deposition of iron into a certain anatomical zone of a tooth; the iron would persist here till the time determined by abrasion of the tooth, when the sudden loss probably occurs (at a time beyond the scope of our experiment).

The adequacy of our model of ferrokinetics in bones could also be assessed independently by comparing an experimentally determined quantity of iron in the erythropoietic tissue with values deduced from the model, Tab. 2. The quantity of iron in stores could not be calculated quite reliably, as the distribution of iron into various types of stores is no doubt determined well with direct incorporation from plasma, but it is not guaranteed that the transfer of iron from the phagocyted elderly erythrocytes in RES is distributed among bones in an identical manner. The iron content in the spleen was experimentally determined only approximately, as the iron in circulating erythrocytes could not be distinguished from the iron contained in erythroblasts in this organ. Subject to these limitations we can, however, consider the agreement of results obtained from the model (i. e. on the basis of dynamic measurement of ^{59}Fe incorporation) with independent chemical determination of absolute quantities of iron to be very satisfactory; the justification of our scheme of compartments and their dynamic characteristics was thus verified independently.

The present model of ferrokinetics enabled us to refine our previous analysis (Vácha et al. 1982). The interstitial pool of iron in erythropoietic organs was differentiated from the pre-haem pool in the erythroblasts. At the same time the parameters of iron kinetics in the interstitial space were more precisely determined; considerable differences were found between them in various bones. The flow of iron destined immediately for erythropoiesis showed roughly identical dynamic characteristics in all bones. The flow into the teeth was differentiated from the flow into stores and the latter was divided into a part destined immediately for erythropoiesis and a part destined for storage outside the erythroblasts. It is important from the practical point of view that the components participating in the radioactivity of the erythropoietic tissue at various time intervals after administration of labelled iron are now known with great certainty, at least in the rest state of erythropoiesis. It is a basic presupposition of employing of the common method of labelled iron incorporation as an indicator of the intensity of erythropoiesis.

Kinetika železa v jednotlivých okrscích kostní dřeně (kostech) u myši

Experimentálně byl sledován průběh koncentrace značeného železa (^{59}Fe) po jeho nitrožilní aplikaci v plasmě, erytrocytech a v jednotlivých kostech (zde zvlášť v hemové a nehemové frakci). Pomocí matematického modelu se pak hledal optimální počet kompartmentů železa v jednotlivých kostech a konstanty charakterizující průtok kompartmenty. Srovnáním toků v kostech různého typu bylo možno typizovat i kompartmenty a pokusit se o jejich fyziologickou interpretaci. Kromě cirkulující plasmy a erytrocytů byly stanoveny tyto kompartmenty: 1. Fond ("pool") Fe s poločasem odtoku několika desítek minut až několika hodin, odpovídající pravděpodobně železu obsaženému v intersticiálním prostoru dřeně a sleziny, v rychle se ustavující rovnováze s plasmou; 2. Fond Fe s poločasem odtoku okolo 9 hodin, odpovídající železu vbudovanému do hemu nebo uloženému v prehemovém fondu erytroblastů; 3. Fond Fe s poločasem odtoku 1—3 dny, charakteru zásob uložených pravděpodobně jak v erytroblastech (poločas 3,15 dne);

tak v buněčných elementech hematopoetického mikroprostředí (poločas 1,65 dne) 4. Fond Fe dlouhodobě vázaného v zubech. Podle zastoupení Fe kompartmentů bylo možno rozdělit kosti do tří skupin: 1. Dlouhé kosti a páteř, v nichž probíhá intenzívní erytropoesa, vyčerpávající intersticiální fond a obracející na sebe většinu toku zásobního Fe; 2. Akrální kosti a lebka s minimální erythropoesou, u nichž se projevuje hlavně dynamika intersticiálního fondu a zásobního fondu Fe lokalizovaného mimo erythroblasty; 3. Kosti hrudníku představující v mnoha směrech přechod mezi oběma skupinami. — Model je v souhlase i s nezávisle (chemicky) zjišťovaným obsahem železa v kostech. Popsané výsledky znamenají podstatné zpřesnění našeho obecného modelu ferrokinetiky u myši (Vácha a spol. 1982) a poskytují pevnější základ ke studiu metabolismu železa v kostní dřeni za patologických stavů erytropoesy. Studie tohoto typu jsou nezbytným předpokladem správné interpretace metody inkorporace značeného železa jako indikátoru intenzity erytropoesy.

Кинетика железа в отдельных участках костного мозга (костях) у мышей

Экспериментально исследовалась временная зависимость концентрации меченного железа (^{59}Fe) после его внутривенного введения в плазме, эритроцитах и в отдельных костях (в последнем случае отдельно в гемовой и негемовой фракции). При помощи математической модели потом производились поиски установления оптимального количества компартментов железа в отдельных костях и констант, характеризирующих прохождение компартментами. В результате сравнения токов в костях различного типа было возможно типизировать и компартменты и попытаться произвести их физиологическую интерпретацию. Кроме циркулирующей плазмы и эритроцитов были установлены следующие компартменты: 1. Пул Fe с периодом полуоттока от нескольких минут до нескольких часов, соответствующий по всей вероятности железу, содержащемуся в интерстициальном пространстве костного мозга и селезенки, быстро достигающий равновесия с плазмой; 2. Пул Fe с периодом полуоттока около 9 часов, соответствующий железу, включенному в геме или находящемуся в предгемовом пуле эритробластов; 3. Пул Fe с периодом полуоттока 1—3 дня, имеющий характер запасов, уложенных по всей вероятности как в эритробластах (период полуоттока 3,15 дня), так и в клеточных элементах гемопоэтического микроокружения (период полуоттока 1,65 дня); 4. Пул Fe длительно уложенного в зубах. На основании представления компартментов железа было возможно кости разделить на три группы: 1. Длинные кости и позвоночник, в которых происходит интенсивный эритропоэз, исчерпывающий интерстициальный пул и берущий на себя большинство тока запасного железа; 2. Акральные кости и череп с минимальным эритропоэзом, в которых проявляется в основном динамика интерстициальяного пула и запасного пула Fe, локализированного вне эритробластов; 3. Кости грудной клетки, представляющие собой в многих направлениях переход между обеими группами. Модель находится в соотвествии и с независимо (химически) определяемым содержанием железа в костях. Описанные результаты означают существенное уточнение нашей общей модели ферроκинетики (Вахa и др. 1982) у мышей, и представляют собой более прочную основу для изучения метаболизма железа в костном мозге при патоло-

гических состояниях эритропоэза. Изучения такого типа являются необходимым предположением правильной интерпретации метода меченного железа в качестве индикатора интенсивности эритропоэза.

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