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Body Iron Loss in Animals (40343)

C. A. FINCH, H. A. RAGAN, I. A. DYER, AND J. D. COOK

Division of Hematology and the Regional Primate Research Center, University of Washington; Department of Animals Sciences, Washington State University; and Battelle, Pacific Northwest Laboratories

Quantitative information concerning body iron turnover in mammalian species is limited but suggests that turnover rates may differ by an order of magnitude (1, 2). Man appears to have the most restricted exchange, about 10%/yr (2), and this may be important in explaining both the high prevalence of iron deficiency (limited absorption) and the occurrence of parenchymal iron overload (limited excretion). The present study was undertaken to examine body iron turnover in a number of animal species, since with the exception of the mouse (1) and rat (3), little is known about iron exchange in these other species. It was hoped that these studies would not only be of general biologic interest but would also serve to guide efforts in establishing an animal model of parenchymal iron overload.

Materials and methods. The species studied included the rat, guinea pig, rabbit, dog, monkey, sheep, and cow. The first three were housed at the University of Washington, dogs were kept at the Battelle Northwest facility at Richland, monkeys were kept in the Primate Field Station at Medical Lake, and the sheep and cow populations were kept at Washington State University. All animals were male except for sheep and cows. Weights of animals were recorded during the experimental period. New Zealand white rabbits were fed Albers Rabbit Breeder Pak which contained by analysis 219 mg of iron/kg; Hartley guinea pigs were fed on Guinea Pig Chow by Purina, the iron content of which was 449 mg/kg and were also given 1 mg of ascorbic acid/100 g body wt/day in their drinking water. Sprague-Dawley rats were fed Laboratory Chow by Purina with an iron content of 373 mg/kg. Dogs were fed Wayne's Dog Chow with an iron content of 289 mg iron/kg. Pigtail and Fusicularis monkeys were fed Purina Monkey Chow containing 237 mg iron/kg. Sheep (Columbia ewes) of about 8 years and Hereford cows of about 12 years were fed alfalfa hay which had an iron content of 490 mg/kg and a salt mixture which together had an iron content of 512 mg/kg. Weekly food consumption was estimated and its iron content determined by wet ashing (4) and colorimetric analysis. Hematologic measurements including hemoglobin by the cyanmethemoglobin method and hematocrit by the micro technique were made at the beginning and termination of the study. Blood volumes of animals corresponding in weight to those studied were carried out at the beginning of the experiment and at the end in the rat, guinea pig, and rabbit, employing the Evan's blue dye method (5); the blood volume of other species was taken from previous reports in the literature (6–9).

Radioiron (^{55}Fe) in dosage of 1 μCi/kg (specific activity about 15 μCi/μg) was injected intravenously as the citrate salt (20 moles citrate/mole iron) at the beginning of the study, and blood samples were drawn at intervals until the radioactivity had fallen to <30% of the initial level. The interval of sampling was so adjusted that there would be about six samples before this level was reached. At that time an aliquot of all samples was wet ashed, prepared as described by Eakins and Brown (10) and counted in a liquid scintillation counter.

In previous studies in man there had been a rapid initial fall, presumably reflecting the mixing of radioiron with nonerythron iron (2, 11). In rats, guinea pigs, and rabbits the eleventh day sample was used as the first point in the turnover curve since that point and those following appear to fall on a single exponential clearance line. In the other species there was a more rapid initial fall, presumably related to mixing with nonerythron body iron. To avoid this mixing phase the start of the turnover slope (called 0 day) was taken after a single exponential rate of decrease in radioactivity was established. In dogs this began at 224 days, in Pigtail and Fusicularis monkeys 196 days, and in sheep and cows 168 days. Initial and subsequent values for

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each animal were plotted, and the best exponential rate of decrease in specific activity was derived by analysis of least squares. The point of intersection with the zero ordinate at the time of the first sample was taken at 100%. Mean $t_{1/2}$ was established by averaging the individual $t_{1/2}$ values (Table I) and also by employing the average values at each time interval for each species so as to give a composite turnover curve (Fig. 1). The mean $t_{1/2}$ was corrected for blood volume changes which occurred as a result of growth, and a further correction was made for blood withdrawn during the experimental period. Total blood removed from rat, guinea pig, rabbit, dog, monkeys, sheep, and cow was 1.2, 2.2, 10, 50, 30, 100, 100 ml, respectively. None of the female sheep or cows became pregnant during the study.

Estimated total body iron (TBI) was calculated from the following formula:

$$\text{TBI (mg)} = (\text{mg Fe/ml whole blood}) \times (\text{ml whole blood}) \times 3/2$$

where the factor 3/2 represents an estimate of the relation of total body iron to red cell iron.

The turnover of body iron (BIT) was calculated according to the formula:

$$\text{BIT (\%/d)} = 0.693 \times 100/t_{1/2}$$

$$\text{BIT (mg/kg/d)} = \text{mg Fe/kg} \times \text{turnover (\%/d)}$$

The daily iron intake was calculated from the amount of food consumed and its iron content, as determined by wet ashing and colorimetric analysis. In small animals food intake was monitored over a week; in large animals the food supply over a month's period was estimated. It was assumed that food iron intake/kg remained constant through the study. The % absorption was calculated from the daily turnover of iron plus growth requirements divided by iron uptake.

Results. Results of this study are summarized in Table I. The rate of isotope disappearance from circulating red cells in rats, guinea pigs, and rabbits required considerable correction for growth, whereas in the other five species blood volume change was relatively small. Average data points for the corrected rates of isotope turnover in the circulating erythrocytes are shown in Fig. 1. The half-time turnover varied from 138 days in the guinea pig to 761 days in the cow. Corrections were made for weight changes in all species showing increases (dog and cow excluded). Based on estimates of body iron content which varied in different species between 32 and 58 mg/kg, the actual turnover of body iron/kg varied from 220 μg/kg/d in the guinea pig to 41 μg/kg/d in the cow.

The balance sheet for iron requirements in each species, based on change in estimated body iron associated with growth and iron losses is displayed in Table II. These requirements are matched against food iron ingested which varied from about 3 mg/kg/d in the

TABLE I. MEASUREMENTS OF BODY IRON LOSS.[a]

Species	Rat (14)	Guinea pig (7)	Rabbit (7)	Dog (6)	Pigtail (7)	Fusicularis (3)	Sheep (7)	Cow (7)
Sex	M	M	M	M	M	M	F	F
Beginning days+	11	11	11	92	9	9	21	21
Hb (g/dl)	16 ± 0.8**	15 ± 0.3	13 ± 0.7	17 ± 0.5	13 ± 1.8	12 ± 1.1	11 ± 1.7	14 ± 1.5
Wt (kg)	0.40 ± 0.02	0.57 ± 0.01	3.2 ± 0.08	13 ± 0.8	9.6 ± 1.0	6.4 ± 1.1	80 ± 12.4	476 ± 65
BV (ml/kg)	60 ± 1	60 ± 4	52 ± 8	66	61	61	58	57
Ending days+	234	234	459	826	812	812	966	966
Hb (g/dl)	16 ± 0.7	15 ± 0.5	14 ± 1.1	17 ± 1.0	13 ± 1.6	13 ± 0.4	14 ± 1.7	16 ± 1.8
Wt (kg)	0.67 ± 0.08	1.2 ± 0.15	4.5 ± 0.3	13 ± 1	11 ± 2	7 ± 0.7	85 ± 11.9	460 ± 64
BV (ml/kg)	51 ± 3	54 ± 3	40 ± 3	66	61	61	58	57
^{55}*Fe loss* ($t½$)								
days (uncorr.)	129 ± 15	91 ± 8	273 ± 32	552 ± 92	383 ± 146	483 ± 131	663 ± 127	761 ± 205
days (corr.)***	182 ± 25	138 ± 15	288 ± 33		404 ± 174	452 ± 246	681 ± 171	
Estimated miscible body iron (mg/kg)	45	44	32	58	42	42	42	46
Body Iron Turnover								
(%/d)	0.38	0.50	0.24	0.13	0.17	0.15	0.10	0.09
(μg/kg/d)	171	220	77	75	71	63	42	41

[a] Number of animals studied; **SD; *** corrected for blood volume and hemoglobin change (the underlined value has been used for calculating-iron turnover rate); + days after injection of radioiron.

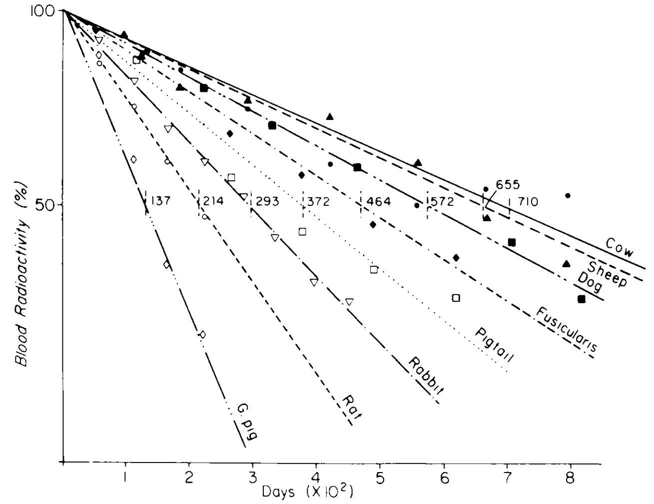

FIG. 1. Red cell radioactivity as a function of time. Symbols used in these studies are as follows: ◇—...—... guinea pig; ○----- rat; ▽ —.—.— rabbit; □ ..... pigtail; ◆ - -.- -.- - fusicularis; ■ —--—-- dog; ▲ — sheep; ● — cow. Each point represents the average activity of the individual groups of animals summarized in Table I. The lines are derived by least squares. The $t_{1/2}$ in days is indicated for each species.

TABLE II. CALCULATIONS OF IRON ABSORPTION.

Species	Rats	Guinea pig	Rabbit	Dog	Pigtail	Fusicularis	Sheep	Cow
Iron requirements (mg/d)								
growth	0.06	0.19	0.14	0	0.11	0.06	0.31	0
loss[a]	0.08	0.19	0.29	0.94	0.72	0.43	3.5	19
total	0.14	0.38	0.43	0.94	0.83	0.49	3.8	19
Food intake (g/d)	15	100	170	345	150	150	3200	8200
Fe intake (mg/d)	5.6	44	37	99	36	36	1600	4200
Food iron Absorption (%)	2.5	0.9	1.2	0.9	0.2	1.4	0.2	0.4

[a] Medium weights used in calculation.

monkey to as much as 50 mg/kg/d in the guinea pig. Iron intake by all species was far in excess of iron requirements, so that estimated absorption of food iron ranged between 0.2 to 2.5%.

Discussion. In these studies body iron turnover was estimated from the specific activity of radioiron in circulating red cells. Previous studies involving the injection of radioiron intravenously into small animals (3, 12) have shown an initial excessive loss of radioiron through the gastrointestinal tract. In man a more rapid initial fall in red cell activity is seen over the first 300 days due to mixing with body iron stores (2, 11). In order to avoid both of these, turnover was estimated only after a single exponential decrease in red cell activity was observed. At this time a steady state of iron exchange within the animal and between the animal and the external environment was presumed to exist. It was also necessary when following red cell activity to make some assumption concerning the miscible pool in which the isotope was diluted. Studies of iron distribution (12) and stores (3, 13) in the rat and of the miscible pool in man (2, 11) suggest the nonerythron portion to be about one-third of the total. Thus, values for red cell iron were increased by 50% to reflect total body turnover. In addition, adjustments were made for changes in blood volume and total body mass and also for the amount of blood removed for isotope measurements.

In the smaller animals, turnover rates ranged from 0.24 to 0.61%/day. In dogs and monkeys fractional turnover was from 0.15 to 0.19%/day. Sheep and cows showed rates of 0.10 and 0.08%/day. These differences appear to have an inverse relationship to body weight. Man, however, falls outside of this relationship since daily excretion is 0.03%/day (2, 11). The major difference between man and small animals appears to be in the much greater capacity for excretion of iron through the intestinal mucosal cells in the latter (3, 12, 13). Possibly a variation in this degree of intestinal excretion explains the difference observed in other species.

The data on iron losses also permit estimates of iron absorption. While it might seem more direct to measure absorption itself, this is not practical. Balance studies (food iron ingested minus fecal loss) are not meaningful since the amount of iron ingested is within 1 or 2% fecal iron and contains most of the iron excreted as well as that not absorbed. Isotope studies of absorption must assume similar absorption of isotope and of food iron and are affected by a number of factors which make results highly variable (14). An alternate means of calculating absorption is from the sum of iron requirements for growth and iron losses. The highly favorable ratio between dietary iron and absorption required to maintain iron balance is evident. The estimated absorption range from 0.2 to 2.3% may be contrasted with iron balance in the human. While iron intake in this country is about 150 to 200 μg/kg/d, absorption in man is about 12 μg/kg and in menstruating women about 24 μg/kg (15, 16). This represents an absorption in the male of about 7% and in the female of about 14% of dietary iron. Obviously, requirements will vary depending on the amount of growth during the period of study. There is also some adjustment of loss in relation to the amount of iron in the diet (1). Thus, both absorption and excretion may be modified somewhat depending on the amount of iron provided. However, the much greater iron intake of all animal species is evident.

Summary. Measurements have been made of the decrease in specific activity of radioiron in circulating red cells of eight animal species. From these data calculations of body iron turnover have been made and establish a general inverse relationship between body size and rate of external iron exchange. A comparison of iron requirements and iron intake in these animals indicate extremely low absorption requirements, ranging from 0.2 to 2.3% of their dietary iron intake.

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