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Research Article

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Storage Iron Kinetics

VII. A BIOLOGIC MODEL FOR RETICULOENDOTHELIAL IRON TRANSPORT

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ABSTRACT The processing of erythrocyte iron by the reticuloendothelial cell has been characterized by kinetic measurements of blood radioactivity made after the intravenous injection of heat-damaged erythrocytes labeled with ^{59}Fe and of transferrin-bound ^{55}Fe. The early reticuloendothelial release of iron, a matter of hours, was calculated from the plasma turnover rate of ^{55}Fe and the plasma reappearance of ^{59}Fe. Late release was calculated from the ratio of the cumulative incorporation of both tracers into the circulating red cell mass over a period of 2 wk. There was an initial processing period within the reticuloendothelial cell, after which radioiron either rapidly returned to circulation ($t_{\frac{1}{2}}$ 34 min) or was transferred to a slowly exchanging pool of storage iron within the reticuloendothelial cell ($t_{\frac{1}{2}}$ release to plasma of 7 days). These pathways were of equal magnitude in the normal dog. Reticuloendothelial release of iron was largely independent of the pre-existing plasma iron level or transferrin saturation. Diurnal fluctuations in the plasma iron level were shown to be the result of a variable partitioning of iron between the early and late release phases. Acute inflammation resulted in a prompt and marked increase in the fraction of iron stored (late phase), whereas depletion of iron stores resulted in a marked increase in early release.

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INTRODUCTION

Recircuiting of iron from senescent red cells by the reticuloendothelial cells (RE)[1] is an essential link in internal iron exchange. Previous studies (1, 2) have shown that part of the red cell iron processed by the RE is rapidly returned to the plasma and another portion exchanges with RE stores and is slowly reutilized. The kinetics of those components has not yet been characterized. A number of studies purporting to characterize RE transport of iron have employed hemoglobin (3, 4), without the knowledge that plasma hemoglobin is cleared by the hepatocyte (5, 6). Other studies dealing with RE uptake of nonviable red cells have used so large an amount as to be unphysiologic (1, 2, 7). In addition, no firm relationship has been established between the plasma iron level and RE iron release, although it is generally acknowledged that plasma transferrin is primarily dependent on the RE for its supply of iron. It has been suggested on the one hand, that diurnal variations in plasma iron concentration are due to variations in RE iron supply (3) and on the other hand, that RE iron release is affected by the plasma iron level (2). The present study is an attempt to quantify iron transport by the RE "in vivo" and to evaluate the role of the RE in the regulation of the plasma iron level. The dog was selected as an experimental model because of the similarity between internal iron metabolism of dog and man (8).

METHODS

Adult dogs of both sexes weighing 15–22 kg were studied. The animals were inoculated against distemper, dewormed, treated for ectoparasites, and then observed for a 2–3-wk

[1] *Abbreviations used in this paper:* ACD, citric acid, sodium citrate, and dextrose; ^{59}DRBC, ^{59}Fe-damaged red cells; PIT, plasma iron turnover; RE, reticuloendothelial cell; TIBC, total iron-binding capacity; TT, mean transit time.

period. They were housed in individual cages and fed with Purina Chow (Ralston Purina Co., St. Louis, Mo.). 13 normal dogs were studied under basal conditions and a repeat study was performed in two of these. Special studies were conducted on an additional 13 dogs that were pretreated as described below so as to modify internal iron kinetics. All animals had normal hematologic values with a mean hemoglobin of 15.6±1.3 g/100 ml (±1 SD), a mean fasting plasma iron (9) of 151±45 μg/100 ml (range 85–239) with a total iron-binding capacity (TIBC) measured with $MgCO_3$ (10) of 336±46 μg/100 ml, and a leukocyte count below 12,000/mm³. After an overnight fast, these animals were injected simultaneously with ^{55}Fe transferrin and ^{59}Fe-damaged red cells (^{59}DRBC) through an ongoing i.v. infusion between 11 a.m. and and 12 noon unless otherwise noted.

To determine the effect of increasing the plasma iron on RE iron kinetics, eight dogs were given an infusion of ferric ammonium citrate to saturate their circulating transferrin. Saturation of the TIBC (325±34 μg/100 ml) was achieved by the initial injection of 370 μg Fe/kg equal to four times the unsaturated iron-binding capacity of their plasma; this was followed by a continuous infusion at a rate of 140 μg Fe/kg/h. The infusion was stopped after 6 h and radioiron was injected as described above. By bleeding 300 ml iron, depletion without anemia was produced in two dogs placed on an iron-deficient diet. Iron deficiency was established by measurements of spleen nonheme iron (11) which were 77 and 192 μg iron/kg body weight in these two animals as compared with a mean of 1,518 μg (range, 464–3,712) in 13 normal dogs. Three other dogs were injected i.v. with 1 μg endotoxin/kg either 4 or 18 h before injection of radioiron.[2]

Labeled compounds used for injection were prepared in the following manner. Radioiron was bound to transferrin by incubating fresh dog plasma with 0.8 μCi/kg $^{55}FeCl_3$ (sp act 9 μCi/μg) which had been mixed with sufficient 4% sodium citrate to ensure a molar ratio of citrate to iron of 50 to 1. The iron-binding capacity of the plasma was at least three times the quantity of radioiron added. Nonviable red cells containing hemoglobin labeled with ^{59}Fe were prepared in dogs as follows. A dog was injected repeatedly over a 3-mo interval with intravenous ^{59}Fe citrate in amounts sufficient to maintain a circulating activity between 8 and 10 μCi/ml packed cells. Erythrocytes obtained on the morning of each study were washed three times in saline, resuspended in four times their volume of ACD (4.4 g citric acid, 13.2 g sodium citrate, and 14.7 g dextrose) and heated to 40.1°C for 11 min. After three additional washings in ACD at room temperature, the DRBC were injected within 30 min. Their disappearance from the circulation of the recipient animal occurred as a single exponential with a $t_{\frac{1}{2}}$ of 5.6±2.1 min. Intravascular hemolysis was monitored after the infusion of these cells by analyzing the 15 min sample of plasma for ^{59}Fe activity. Only 0.75±0.28% of the activity injected as DRBC was found in the plasma over all iron loads employed. The plasma volume was measured by extrapolated zero time of ^{55}Fe plasma activity.

Early RE release was studied according to the following procedure. Each animal was kept quiet by administering the smallest possible amount of tranquilizer (acepromazine maleate) and sodium pentobarbital through an ongoing infusion of isotonic dextrose. The total amount of these drugs was 1.3 and 35 mg/kg, respectively over an 8-h period. The dogs were also heparinized by injecting 800 IU/kg initially and every 3 h thereafter. After injection of ^{55}Fe transferrin and ^{59}DRBC, the plasma disappearance rate of ^{55}Fe transferrin was determined from the activity in 1-ml blood samples obtained at 15, 30, 45, 60, 120, 180, 300, and 480 min after injection. Radioactivity of ^{59}Fe and ^{55}Fe was measured simultaneously by a modification of the liquid scintillation method of Eakins and Brown (12). Blood ^{59}Fe activity was continuously monitored during the first 6–8 h by extracorporeal circulation of blood through a 15-ft long siliconized polyethylene tube[3] (ID 0.034 inch) inserted into the femoral veins through a 19-gauge needle and coiled within the well of an NaI (T1) scintillation counter. Circulation was maintained by a pump[4] with a flow adjusted to 1 ml/min. The overall capacity of this system was 6 ml of blood two-thirds of which was contained within the scintillation well. Activity was recorded at 10-min intervals giving a total of 36–48 points in each experiment. Blood ^{59}Fe activity determined by this extracorporeal circulation technique was indistinguishable with that obtained by multiple blood sampling. Initial plasma iron levels and TIBC were determined immediately before the injection of the labels. Plasma iron was subsequently measured at 1, 2, 3, 5, and 8 h. The rate of change of the plasma iron during the first 3 h of the study was determined by the slope of the regression line fitted through plasma iron values at 0, 1, 2, and 3 h. Loads of DRBC iron ranging from 10–25 μg Fe/kg did not affect the plasma iron level. Higher doses injected into three dogs did increase the plasma iron level. The rate of change of plasma iron in these animals was calculated from observed values at each time corrected for the amount of iron derived from DRBC (micrograms DRBC Fe injected times percent ^{59}Fe plasma activity). To determine if the experimental procedure in itself affected the plasma iron concentration during the 8-h experimental period, measurements over a similar period were made on 12 unanesthetized dogs housed in cages. The degree of variation was similar in the two groups of animals. Previous studies have shown that anesthesia does not affect the plasma iron level as compared with the injection of saline (13).

The calculation of early RE release of iron was based on the rate of clearance of ^{55}Fe transferrin and the reappearance curve of transferrin ^{59}Fe derived from DRBC (Fig. 1). The fractional plasma iron turnover rate (α, min^{-1}) was determined by fitting a least-squares regression line to the log of ^{55}Fe counts plotted against time. If X represented the amount of ^{59}Fe at any time which reappeared in the plasma (percent of dose injected):

$$\frac{dX}{dt} = F(t) - \alpha X, \qquad (1)$$

where $F(t)$ = rate of entry to the plasma of ^{59}Fe released by the RE (percent of dose injected per minute). Values of the reappearance curve of ^{59}Fe, X, and the slope of this curve, dX/dt, were determined at 10-min intervals throughout the observation period after fitting the observed ^{59}Fe counts by a least-squares spline routine and a digital computer (14). $F(t)$ was calculated from Eq. 1 and integration of $F(t)$ with respect to time then gave a hypothetical plasma ^{59}Fe radioactivity time curve $P(t)$ which represented the pattern of accumulation of ^{59}Fe in the plasma

[2] *Salmonella typhosa,* Difco Laboratories, Detroit, Mich.

[3] Intramedic, Clay-Adams, Inc., Persippany, N. J.

[4] Model RL 175, The Holter Company, Division of Extracorporeal Medical Specialties, Inc., King of Prussia, Pa.

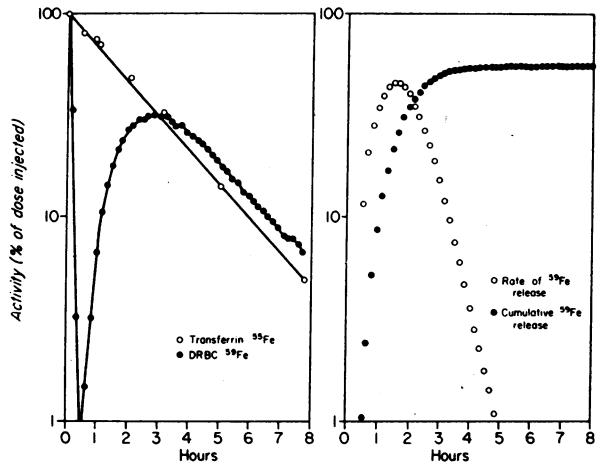

FIGURE 1 Measurement of the early phase of RE release in a normal dog. On the left is shown the disappearance curve of ^{55}Fe transferrin (open circles) and the curve of ^{59}DRBC activity (closed circles) measured at 10-min intervals over an 8-h period. The initial rapid fall in ^{59}Fe represents the clearance of DRBC while the subsequent reappearance represents ^{59}Fe which has been released to transferrin by the RE cell. On the right is shown the calculated parameters of RE release of ^{59}Fe as described in the text. The open circles depict the rate of ^{59}Fe entry into the plasma, $F(t)$, expressed as percent of dose per minute and multiplied by 100. The closed circles depict the cumulative ^{59}Fe release, $P(t)$, obtained by integrating $F(t)$ with respect to time. The accumulative early release was stable after 4 h and represented 50% of the injected dose in this study.

that would have occurred in the absence of uptake by tissues. In all studies, $F(t)$ had declined to virtually 0 by 360 min resulting in a plateau of $P(t)$ at that time. The value of $P(360)$ was therefore considered equal to $P(\infty)$ and taken as the proportion of iron released in the early phase. After 180 min $F(t)$ could be described by an exponential curve, the $t_{\frac{1}{2}}$ of which was used to characterize the speed of early release (Fig. 1). The *mean transit time* (TT) of the early radioiron release was defined as the mean time of exit of tracer from the RE minus the mean time of entrance of tracer into the RE (15), or:

$$\mathrm{TT} = \frac{\int_0^\infty tF(t)\mathrm{d}t}{\int_0^\infty F(t)\mathrm{d}t} - \frac{\int_0^\infty te\mathrm{d}t}{\int_0^\infty e\mathrm{d}t},$$

where e is the rate of input of radioactivity in the RE. As DRBC disappear from the circulation according to a single exponential of slope r, the second term at the right hand of the equation becomes $1/r$. The *lag period* was estimated by subtracting the mean release time (release $t_{\frac{1}{2}} \times 1.44$) from the TT.

The *late release* was studied by removing 8–10 samples of 1 ml blood during the 2 wk period after isotope administration to determine the cumulative incorporation of ^{59}Fe and ^{55}Fe in the circulating red cell mass (Fig. 2). The late release of ^{59}Fe by the RE was calculated at any time by the ratio of ^{59}Fe to ^{55}Fe activity (2, 16). Formal proof of this method of calculation is outlined in the Appendix. When the complement of this ratio (expressed as a percent), was plotted on semilogarithmic paper, late release appeared to be a single exponential curve. After fitting a regression line by least squares, the calculated value of the exponential at the time of reappearance of one-half the final erythrocyte, ^{55}Fe incorporation gave the proportion of radioiron in the late release, whereas the $t_{\frac{1}{2}}$ of the exponential was used to characterize the speed of late release.

RESULTS

Ferrokinetic and RE cell kinetic parameters observed in normal dogs are listed in Table I. Initial plasma iron levels ranged from 96 to 228 μg/100 ml with a composite mean in 15 studies of 146 μg/100 ml. During the first 3 h of study, the plasma iron was increasing in seven animals at a rate as high as 14.5 μg/100 ml/h whereas in the remaining eight studies, the plasma iron was falling at a rate between 1.2 and 12.3 μg/100 ml/h. The transferrin-bound ^{55}Fe disappearance $t_{\frac{1}{2}}$ averaged 102 min with a range of 62–169. The plasma iron turnover ranged between 0.56 and 1.00 mg Fe/kg/day with a composite

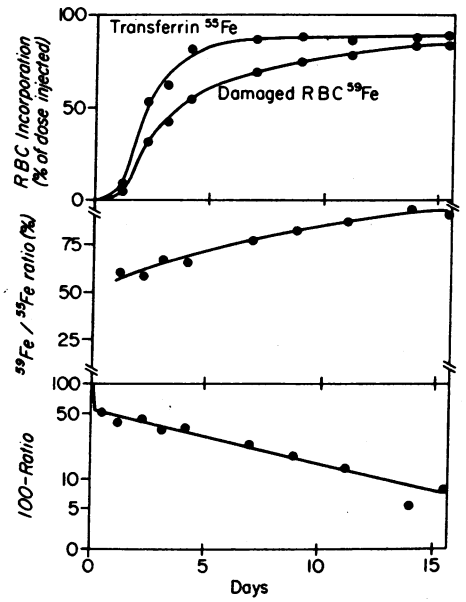

FIGURE 2 Measurement of the late phase of RE release in a normal dog. In the upper portion of the figure is plotted the incorporation curves of transferrin ^{55}Fe and ^{59}DRBC into circulating red cells over a 2-wk period. The lower incorporation curve of ^{59}Fe reflects the delay of radioiron within RE cells. When the ratio of ^{59}Fe to ^{55}Fe (middle portion of the figure) is subtracted from 100 and plotted on semilogarithmic coordinates (lower portion of the figure), late release is represented by a single exponential curve. The proportion of the injected dose released in the late phase is obtained by extrapolation of this curve to the half reappearance time of RBC ^{55}Fe while the half time of the curve represents the speed of late release.

TABLE I

RE Cell Kinetic Measurements in Normal Dogs

Dog no.	DRBC iron load	Initial plasma iron	Plasma iron change	Plasma ^{55}Fe $t_{\frac{1}{2}}$	PIT	Early release					Late release				Sum of early and late release
						TT	Lag phase	Peak plasma ^{59}Fe activity	Proportion early release	$t_{\frac{1}{2}}$	RBC ^{55}Fe incorporation*	RBC ^{59}Fe incorporation*	Proportion late release	$t_{\frac{1}{2}}$	
	μg/kg	*μg/100 ml*	*μg/100 ml/h*	*min*	*mg/kg/day*	*min*		*% of dose*		*min*	*% of dose*			*days*	*% of dose*
Low dose															
1	10.7	119	−12.3	79	0.76	109	60	16.4	34	34	75	58	58	8.3	92
2	10.8	107	−3.3	62	0.77	80	40	20.8	45	28	88	80	43	5.5	88
3	11.2	181	+2.3	168	0.56	99	47	32.5	52	36	71	65	51	4.2	103
4*a*	11.4	90	+6.1	85	0.57	108	62	32.3	60	32	—	—	—	—	—
4b‡	15.6	191	+14.5	144	0.56	121	79	43.6	66	29	—	—	—	—	—
5	14.6	228	−7.6	126	0.89	88	60	27.9	50	20	96	78	47	9.5	97
6	15.7	138	−3.8	91	0.80	101	55	17.2	42	32	87	83	54	9.4	96
7	15.9	133	−7.8	81	0.91	94	40	15.9	31	38	—	—	—	—	—
Mean	13.2	148	−1.5	105	0.73	100	55	25.8	48	31	83	73	51	7.4	95
SEM	0.9	17	3.1	13	0.05	5	5	3.5	4	2	5	5	3	1.1	3
Intermediate dose															
8	20.8	110	−7.5	66	0.95	100	45	15.1	36	38	69	59	53	7.0	89
9	21.0	130	−9.5	90	0.86	126	61	12.9	33	45	85	72	62	8.5	95
10*a*	22.1	96	−1.2	72	0.69	120	68	18.2	47	36	—	—	—	—	—
10*b*‡	22.7	119	+10.8	76	0.74	112	43	28.0	66	48	—	—	—	—	—
11	30.0	191	+6.7	108	1.00	105	65	30.9	56	28	87	83	38	6.0	94
Mean	23.3	129	−0.1	82	0.85	113	56	21.0	48	39	80	71	51	7.2	93
SEM	1.7	16	3.9	8	0.06	5	5	3.6	6	4	6	7	7	0.7	2
High dose															
12	86.0	176	+0.9	108	0.73	108	58	29.6	55	35	82	75	42	4.6	97
13	116.0	185	+6.2	169	0.60	113	73	40.6	65	28	67	65	36	7.3	101
Composite															
Mean	28.3	146	−0.4	102	0.76	106	57	26.1	49	34	81	72	48	7.0	95
SEM	7.9	11	2.1	9	0.04	3	3	2.6	3	2	3	3	3	0.6	2

* At 15 days.

‡ Repeat study performed at 4 a.m. 12 h after first study.

mean of 0.76. The red cell incorporation of ^{55}Fe at 2 wk averaged 81%, a value almost identical to that observed in normal man (17).

RE kinetics was measured in eight studies after a minimal dose of damaged cells ranging from 11 to 16 μg Fe/kg body weight (Table I). Analysis of early release curves indicated a cell TT of 100 min ranging from 80 to 121. This was composed of a lag phase of 55 min followed by an exponential discharge of radioactivity with a $t_{\frac{1}{2}}$ of 31 min. Early release represented from 31 to 66% of the ^{59}DRBC activity with a mean of 48%. Analysis of the erythrocyte incorporation curves of ^{55}Fe and ^{59}Fe for late release indicated a mean appearance $t_{\frac{1}{2}}$ of 7.4 days in this same group of dogs. In each study, good agreement was observed between early release of radioiron calculated from the immediate plasma reappearance of ^{59}Fe and late release determined from the accumulative incorporation of radioiron into the circulating red cell mass. Thus, addition of the early and late releases calculated independently accounted for an average of 95% (range 88–103) of activity injected as DRBC. At the end of 2 wk, an average of 12% of the administered ^{59}Fe activity was still present in the RE cells as determined by the ratio of RBC incorporation ^{59}Fe/^{55}Fe (73/83 = 88% released). Experiments carried out over longer periods of time showed that virtually all of the iron had left the RE cell after 3–4 wk.

Additional studies were carried out to determine the pattern of RE kinetics with higher loads of DRBC iron. Five studies were performed with an intermediate dose (20–30 μg Fe/kg) while two studies were performed with loads of 86 and 116 μg Fe/kg (Table I). No changes in any of the parameters of early or late phases of RE iron release were observed with higher dosage. Thus the kinetics pattern of RE cell iron transport in respect to DRBC did not appear dose dependent within a 10-fold range.

To examine the relationship between RE release and plasma iron, the correlation of plasma iron concentration with the lag phase, TT, and the $t_{\frac{1}{2}}$ of early and late re-

lease was examined and in no instance was a significant correlation observed ($P < 0.1$). While no correlation was observed between the proportion of early release and the initial plasma iron level ($r = 0.41$, $P > 0.1$), a significant positive correlation was observed with the plasma iron level measured 3 h after the injection of the labels (80–230 μg/100 ml) (Fig. 3). In these same animals, when the rate of change of plasma iron during the first 3 h of study was used, an even better correlation with the proportion of early release was observed (Fig. 3). This relationship was further examined in two dogs by repeating the initial study 12 h later when an appreciable change had occurred in the plasma iron level. The alteration in early release relative to the rate of change of plasma iron in these serial studies was consistent with the relationship observed in randomly selected normal dogs. It would therefore appear that diurnal variations in plasma iron concentration are explained by differences in the partitioning of iron between early and late phases of RE iron release.

To determine whether the degree of transferrin saturation influenced iron transport by the RE cell, eight dogs were infused with ferric ammonium citrate before study to saturate plasma transferrin. Proportioning of iron between the early and late release phases was determined from the RBC incorporation curves of ^{55}Fe and ^{59}Fe after injection of 12±3 μg/kg DRBC and was compared with that obtained in eight control dogs given the same load of DRBC. The mean plasma iron at the time of injection was 148±47 μg/100 ml in controls as compared to 309±35 μg/100 ml in the iron-loaded animals. In the latter group 3 h after injection, the mean plasma iron was 297±32 μg/100 ml and the transferrin saturation greater than 90%. In the animals with saturated transferrin, the proportion of late RE release was 62.8±9.2% as compared with 50.7±7.8% in control animals. Thus, despite a twofold increase in plasma iron, there was only a 12% increase in the late release fraction ($t = 2.84$, $P < 0.02$). The $t_{\frac{1}{2}}$ of late release in the study was similar in the treated and control animals.

The effect of acute inflammation on RE iron transport was studied in three dogs. Endotoxin injected i.v. resulted in a rapid fall in the plasma iron to less than 50 μg/100 ml within a few hours and with a gradual return to normal by the following day. In two animals, ^{55}Fe transferrin and ^{59}DRBC were injected 4 h after endotoxin administration at which time the plasma iron was decreasing at a rate of 16 and 20 μg/100 ml/h. The $t_{\frac{1}{2}}$ clearance of the ^{59}DRBC was increased to 23 and 35 min. No plasma ^{59}Fe reappearance curve was detectable. The proportion of late release as determined by erythrocyte incorporation curves was 91 and 92% with late release $t_{\frac{1}{2}}$ of 11.2 and 5 days, respectively. In a third dog, DRBC were given 18 h after endotoxin at a time when the

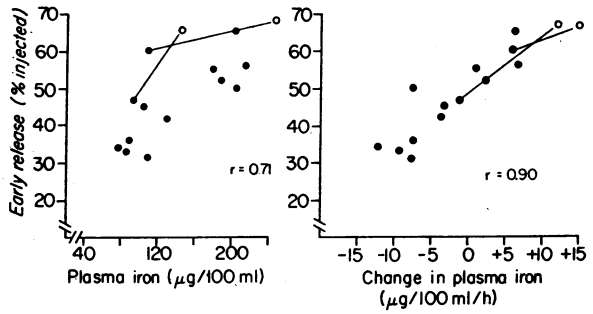

FIGURE 3 Relationship between the percent of the injected dose of ^{59}DRBC released in the early phase and either the plasma iron level 3 h after injection (left) or the rate of change in plasma iron during the first 3 h of study (right). A significantly better correlation was observed when early release is related to the rate of change in plasma iron ($P < 0.001$) than to the level of plasma iron ($P < 0.01$). The open circles represent studies repeated in the same dog 12 h after the initial study.

plasma iron was increased towards normal at a rate of 10 μg/100 ml/h. The late release phase in this study represented only 40% of the injected activity.

A further two studies were performed in dogs with iron stores reduced by prior phlebotomy. The plasma iron at the time of the study was 70 and 80 μg/100 ml. Late release in these animals accounted for only 7 and 10% of the injected activity respectively.

DISCUSSION

In the present study, small amounts of heat-damaged erythrocytes have been used to evaluate iron processing by the RE. The lowest doses of injected cells provided in the range of 10 μg Fe/kg. Because the mean clearance $t_{\frac{1}{2}}$ of the DRBC was 6 min, the rate of iron input into the RE was about four times the normal rate of senescent red cell destruction in dogs based on a mean life span of 100 days (18, 19) and a blood volume of 90 ml/kg (20). Despite the fact that the normal rate of erythrocyte iron turnover was exceeded, a more than 10-fold increase of the iron load to above 100 μg/kg produced no apparent difference in the pattern of early or late release of iron by the RE. It may therefore be assumed that the kinetic pattern obtained in this study does not significantly differ from those obtained under conditions of normal physiologic loading of the RE.

The experimental approach employed provides a relatively simple and yet precise means of characterizing iron transport by the RE. From analysis of kinetic data, a model of iron transport by the RE may be constructed along the following lines. There is an initial processing period or lag phase, presumably required for red cell phagocytosis and heme catabolism. Iron freed from hemoglobin then enters a labile pool from which it is either promptly returned to circulating transferrin with

a $t_{\frac{1}{2}}$ of 34 min or is transferred to a more slowly exchanging pool within the RE which releases its iron with a $t_{\frac{1}{2}}$ of 7 days. These two distinct phases of release account for the entire exchange of iron between the RE and circulating plasma. A sudden increase in the input of red cell iron to the RE in amounts ranging from 4 to 40 times basal input produces no change in the proportion of iron released in the early or late phases. This does mean, however, that the immediate output of iron from the RE is a function of the amount of iron entering the RE over a relatively wide range.

The study reported here has also demonstrated an intrinsic mechanism within the RE for modifying plasma iron supply by modifying the relative amount of iron entering the early and late release phases. The high degree of correlation between rate of plasma iron change and early release as compared with the negligible effect of change in plasma iron level by iron injection indicated that the RE cell was responsible for changes in plasma iron rather than the converse. Thus diurnal variation in plasma iron could be attributed to variations in the proportion of iron immediately released by the RE cell. Considering that about 80% of the iron leaving the plasma re-enters this pool through the RE (21), it can be calculated that two-thirds of the fluctuations in the plasma iron level under normal conditions are due to variations in early RE output.[5]

It has also been demonstrated that there exists little or no immediate feedback control of RE output by the level of the plasma iron. Thus increasing the plasma iron by continuous perfusion of ferric ammonium citrate had little effect on the proportion of iron between the early and late release phases. Only 12% more iron was retained in RE stores despite near complete saturation of transferrin during 6 h. This finding is at variance with a previous study in rats (2) where a single injection of 100 μg nitrilotriacetic acid iron 3 h before injection of ^{59}DRBC was found to markedly depress the immediate release of RE iron and to proportionately increase the radioiron incorporation into ferritin. However, a direct tissue uptake of nitrilotriacetic acid may have influenced the results of these studies.

In addition to these physiologic responses, the RE cell handling of iron appears to be markedly altered in certain pathologic states. After reduction in body iron stores by prior phlebotomy, almost all the iron entering the RE cell was released in the early phase. The reverse phenomenon was observed after endotoxin-induced acute inflammation. These observations indicate that the RE system may change its early output from 10 to 90% of the iron entering the cell and in that way determine the availability of iron for erythropoiesis.

It is clear that alternate pathways exist within the RE cell and that changes between them occur which result in alterations in the flow of iron. Such changes may occur independent of erythron needs, and both diurnal changes and those with inflammation seem to be examples of this. However, there are other reasons to believe that flow is regulated according to marrow needs. The phlebotomized animal responds by the early release of virtually all catabolized iron, and in hemolytic anemia the amount of iron passing through the plasma is increased to four to five times normal with very little change in plasma iron concentration. Whereas the present study casts little light on how this adaptation occurs, the method described of quantitating RE behavior should permit further characterization of this phenomenon.

APPENDIX

Let $R_i(t)$, $P_i(t)$, and $E_i(t)$ be respectively the amount of radioiron in the RE, the plasma and the erythron, i representing either ^{55}Fe or ^{59}Fe. In the simplest case, consider the time course of activity in the RE and the plasma as a single exponential curve:

$$R_{59}(t) = e^{-\beta t}, \tag{1}$$

$$P_{55}(t) = e^{-\alpha t}. \tag{2}$$

Assuming that all the iron leaving the RE enters the plasma, the exchange of ^{59}Fe between the RE and the plasma is characterized by the differential equation:

$$\frac{dP_{59}(t)}{dt} = \beta R_{59}(t) - \alpha P_{59}(t). \tag{3}$$

Solution of Eq. 3 for the case where $P_{59}(0) = 0$ gives:

$$P_{59}(t) = \frac{\beta}{\beta - \alpha}(e^{-\alpha t} - e^{-\beta t}). \tag{4}$$

The time course of radioiron activity in the erythron can be described by the following equation (22):

$$E_i(t) = K\int_0^t P_i(t)dt, \tag{5}$$

where K is the rate constant from the plasma to the erythron. If RA (t) represents the ratio of ^{59}Fe to ^{55}Fe activities in the erythron, substituting Eqs. 4 and 2 in Eq.

[5] In two dogs on whom two studies were carried out at different times of the day, the total iron inflow into the plasma at each time was calculated by adding the plasma iron change (micrograms per hour) to the plasma iron turnover (PIT) (micrograms per hour). The iron flow through the early RE release phase was obtained by multiplying 80% of the PIT by the proportion of radioiron early released. In those two dogs, the change in RE early outflow amounted respectively to 61 and 67% of the change in total inflow into the plasma.

In 13 randomly selected dogs having a mean PIT of 62 μg/100 ml plasma/h, a mean change in plasma iron of 10 μg/100 ml/h was associated with a mean change in the early RE release of 15.3% (Fig. 3). Estimating a total flow through the RE of 50 μg/100 ml plasma/h, 75% of the fluctuations in the plasma iron level are due to variations in the early RE output.

5 gives:

$$RA(t) = \frac{E_{59}(t)}{E_{55}(t)} = \frac{\beta}{\beta-\alpha}\left(1 - \frac{\alpha(1 - e^{-\beta t})}{\beta(1 - e^{-\alpha t})}\right). \quad (6)$$

Consider now that the order of magnitude of α and β are respectively 10 and 10^{-1} day^{-1}. In Eq. 6 we have:

$$\frac{\beta}{\beta-\alpha} \simeq -\frac{\beta}{\alpha},$$

and

$$1 - e^{-\alpha t} \simeq 1 \quad (\text{for } t = 1,\ e^{-10} \simeq 10^{-5})$$

Eq. 6 may be written:

$$RA(t) \simeq -\frac{\beta}{\alpha} + (1 - e^{-\beta t}),$$

β/α being negligible compared with $(1 - e^{-\beta t})$, then:

$$RA(t) \simeq 1 - e^{-\beta t} = 1 - R_{59}(t).$$

Computations of Eq. 6 for various values of α and β have shown the validity of the above approximations. After 24 h, the ratio is practically independent of the plasma radioiron disappearance rate for $t_{\frac{1}{2}}$ ranging from 40 to 400 min, and it provides a measurement of the amount of ^{59}Fe released by the RE with an error less than 1%.

In a more general case, when $R_{59}(t) = \Sigma_n A_n e^{-\lambda_n t}$ (where $\Sigma_n A_n = 1$), it can be similarly demonstrated that: RA (t) $= 1 - R_{59}$ (t) $(t > 1$ day).

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