Factors Involved in the Regulation of Iron Transport through Reticuloendothelial Cells

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Abstract. **The effects of various maneuvers on the handling of ^{59}Fe-labeled heat-damaged red cells (^{59}Fe HDRC) by the reticuloendothelial system were studied in rats. Raising the saturation of transferrin with oral carbonyl iron had little effect on splenic release of ^{59}Fe but markedly inhibited hepatic release. Splenic ^{59}Fe release was, however, inhibited by the prior administration of unlabeled HDRC or by the combination of carbonyl iron and unlabeled HDRC. When carbonyl iron was administered with unlabeled free hemoglobin, the pattern of ^{59}Fe distribution was the same as that observed when carbonyl iron was given alone. ^{59}Fe ferritin was identified in the serum after the administration of ^{59}Fe HDRC but the size of the fraction was not affected by raising the saturation of transferrin. Sizing column analyses of tissue extracts from the spleen at various times after the administration of ^{59}Fe HDRC revealed a progressive shift from hemoglobin to ferritin, with only small amounts present in a small molecular weight fraction. The small molecular weight fraction was greater in hepatic extracts, with the difference being marked in animals that had received prior carbonyl iron. The increased hepatic retention of ^{59}Fe associated with a raised saturation of transferrin was reduced by a hydrophobic ferrous chelator (2,2′-bipyridine), a hydrophilic ferric chelator (desferrioxamine), and an extracellular hydrophilic ferric chelator (diethylenetriaminepentacetic acid). Transmembrane iron transport did not seem to be a rate-limiting factor in iron release, since no differences in ^{59}Fe membrane fractions were noted in the different experimental settings. These findings are consistent with a model in which RE cells release iron from catabolized red cells at a relatively constant rate. When the saturation of transferrin is raised, a significant proportion of the iron is transported from the spleen to the liver either in small molecular weight complexes or in ferritin. Although a saturated transferrin has no effect on the release of iron from reticuloendothelial cells, prior loading with HDRC conditions them to release less iron.** [P.S.E.B.M. 1990, Vol 193]

Internal iron exchange involves a circuit in which iron is released from cells of iron procurement (gut mucosal cells, reticuloendothelial (RE) cells, and hepatic parenchymal cells), bound to the transport protein transferrin, and delivered to erythroid precursors and other actively growing cells. Senescent red blood cells are taken up by RE cells and the iron so released recycled (1–3). The processes involved in the delivery of transferrin iron to erythroid and other cells have been well characterized (2, 3). In contrast, little is known of the factors involved in the transport of iron through RE cells, although there is some evidence that release is inhibited when circulating transferrin is saturated with iron (4, 5). The aim of this study was to obtain further insight into the factors involved in RE handling of iron derived from heat-damaged red cells (HDRC).

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Materials and Methods

Male Sprague-Dawley rats (250–300 g) were used and maintained on a standard commercial cube diet (Epol, Johannesburg, South Africa). A prolonged raised saturation of transferrin was achieved physiologically by oral administration of SF grade carbonyl iron (3- to 4-μm particle size; GAF Corp.) (6). A dose of 400 mg, suspended in 1 ml of acidified saline (pH 2), was introduced using an olive-tipped feeding cannula, fol-

lowed by 200 mg 24 hr later. The animals had free access to water alone during this period. At various times after the first and second doses of carbonyl iron, two animals were exsanguinated under anesthesia and the serum iron, and unsaturated iron-binding capacity and percentage of saturation were measured (7, 8). In this way it was possible to determine the time period during which there was near or complete steady-state saturation of transferrin. Similar time-dependent studies examining the effects of HDRC on the serum iron were carried out by transfusing either a single dose of 3 ml of HDRC/kg (20% hematocrit) at time 0, or three repeated doses of 6 ml of HDRC/kg at time 0, 4, and 8 hr. Unless otherwise stated, groups of at least three rats were studied in each experiment.

The hemoglobin of rat red cells was uniformly labeled *in vivo* by repeated injections of ^{59}Fe. They were then heat damaged (4) and injected into the tail veins of recipient rats within 1 hr of preparation. The dose used in most experiments (3 ml of HDRC/kg) had an iron content of approximately 0.8 mg/kg. At various time intervals after the injection of ^{59}Fe HDRC, rats were anesthetized, exsanguinated by cardiac puncture, and washed out using normal saline perfusion. ^{59}Fe activity in 1 ml of blood, both kidneys, one femur, the liver, and spleen was counted in a Packard Autogamma scintillation spectrometer model 5650 (Packard Instruments Autogamma Co., Downers Grove, IL) and the mean ± SE organ distribution of ^{59}Fe was expressed as percentages of recovered counts. Total marrow ^{59}Fe was taken as 13-fold greater than ^{59}Fe present in one femur (9). Total blood ^{59}Fe assumed a blood volume of 65 ml/kg. Similar experiments were also performed in rats in which the saturation of transferrin had been raised with carbonyl iron, in those with prior infusion of unlabeled HDRC (6 ml/kg), in those with enhanced (reticulocytes >20%) erythropoiesis (by removal of 4 ml of blood on the sixth and fifth days prior to ^{59}Fe HDRC injection), and in those injected with an equivalent amount of unlabeled free hemoglobin (10) 1 hr before ^{59}Fe HDRC. In those experiments in which prior carbonyl iron had been given, the ^{59}Fe HDRC were administered 4 hr after the last dose.

In some experiments hepatic and splenic tissues were prepared for sizing column chromatography. The buffer and column procedures were those of Pollack *et al.* (11). Chelators with various modes of action were tested (12). The hydrophobic ferrous chelator 2,2′-bipyridine (Merck, Darmstadt, FDR) and the hydrophilic ferric chelator desferrioxamine (Ciba Pharmaceuticals, Basel, Switzerland) were included in tissue preparations to characterize the intracellular chelatable pools of ^{59}Fe. At each time interval studied, tissue samples were coarsely homogenized in running buffer (0.05 *M* NaCl, 0.02 *M* Hepes, pH 7) containing soybean trypsin inhibitor (100 μg/ml) and either desferrioxamine (5 mg/ml) or 2,2′-bipyridine (1.39 mg/ml). The homogenized sample was centrifuged at 1000 rpm for 10 min, the supernatant (containing near single-cell suspensions) sonicated with three 30-sec bursts of medium intensity ultrasound using an MSE ultrasonicator (Crawley, Sussex, UK), and ultracentrifuged at 25,000 rpm for 40 min at 4°C. The resultant supernatant was fractionated on AcA 44 ultrogel chromatography and ^{59}Fe activity in the collected fractions was counted. Between each sample application, the column was washed with 1% Triton X-100 and 0.1 *M* EDTA and at least 3 column volumes of buffer. The column had been calibrated with radiolabeled ferritin, transferrin, and hemoglobin. The ferritin peak was confirmed by immunoprecipitation (13, 14) and by sizing on an AcA 22 ultrogel column. The colors of the various fractions were of further use in confirming the nature of the peaks. Detergent-solubilized preparations of hepatic and splenic tissue were also subjected to sizing chromatography. The tissues were coarsely homogenized in running buffer containing soybean trypsin inhibitor but no chelators, and centrifuged at 1000 rpm for 10 min. Running buffer containing 2% Triton X-100 was added to the resultant pellet and, after agitation at 4°C for 2 hr, the sample representing a membrane-enriched fraction was subjected to ultracentrifugation at 20,000 rpm for 1 hr at 4°C. The supernatant was fractionated on an AcA 44 ultrogel chromatography column and the fractions collected counted for ^{59}Fe.

Chelators were also used in studies *in vivo*. Desferrioxamine or 2,2′-bipyridine (50 mg/kg) was given intramuscularly at 1, 3, 5, and 7 hr after the intravenous injection of ^{59}Fe HDRC to normal rats and to rats with carbonyl iron-induced raised transferrin saturations. The hydrophilic extracellular ferric chelator diethylenetriaminepentacetic acid (DTPA) (12) (Merck, Darmstadt, FDR) was given intravenously (50 mg/kg) to enhance the extracellular iron-binding capacity of the plasma and interstitial fluid. After 8 hr, the rats were killed and the percentage of distribution of ^{59}Fe in the liver, spleen, blood, kidneys, and marrow was determined.

Finally, in an attempt to define the nature of iron released from RE cells, sera obtained after ^{59}Fe HDRC infusion into animals with normal and raised transferrin saturations were fractionated on AcA 44 column chromatography.

Statistical analyses utilized Student's *t* test with Bonferroni corrections applied when more than two groups were compared. When analysis of variance was applied, an *F* value was generated. Statistical significance was taken to be $P < 0.05$.

All experiments had been approved by the Animal Ethics Committee of the Univeristy of the Witwatersrand.

Results

The oral administration of 400 mg of carbonyl iron resulted in a transient increase in the percentage of saturation of transferrin, while an additional later dose

caused a more protracted rise (Fig. 1). The fact that the saturation never exceeded 100% indicated that carbonyl iron was being absorbed physiologically and was not causing supersaturation of transferrin at any time (3). HDRC infusion caused a less striking rise in transferrin saturation which may itself have modified later results. A single infusion of 3 ml of HDRC/kg caused a transient rise in transferrin saturation, maximal at 3-hr postinfusion. The first of three 6-ml/kg infusions given four hourly was followed by a similar increase, with the subsequent two infusions having no effect.

Raising the saturation of transferrin with two doses

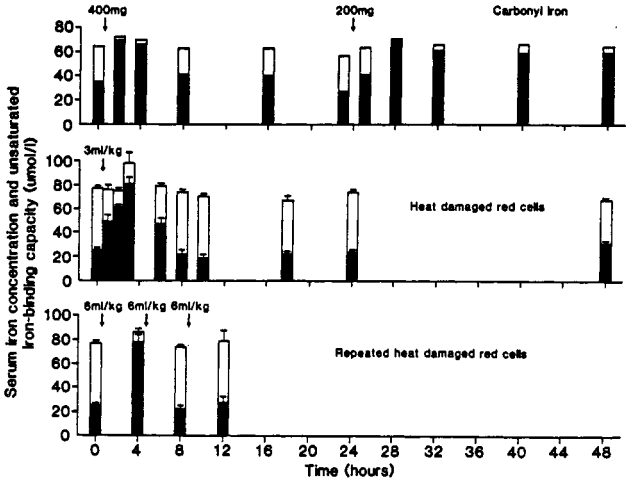

Figure 1. Time-dependent effect on the serum iron (shaded bar) and the unsaturated iron-binding capacity (open bar) of two doses of oral carbonyl iron, a single infusion of 3 ml of HDRC/kg, or three repeated infusions of 6 ml of HDRC/kg into rats. Results are shown as mean (±SE) values, except for the carbonyl iron group where the number of animals (two) at each time was too small for statistical analyses.

of carbonyl iron affected the fate of intravenously administered ^{59}Fe HDRC, with a reduction in blood and marrow ^{59}Fe and an increase in hepatic ^{59}Fe (Table I). The splenic ^{59}Fe content was essentially similar to control rats. In contrast, the previous intravenous injection of unlabeled HDRC was associated with a hold up of ^{59}Fe in the spleen and a consequent reduction of blood and marrow ^{59}Fe uptake. The fact that less than 1.5% of the injected ^{59}Fe was present in the kidney indicated that there was very little intravascular hemolysis of the HDRC. In a further experiment only one dose of carbonyl iron was given 1 hr before the labeled HDRC and the animals were sacrificed 4 hr later. The organ distribution was similar to that noted when a high percentage of saturation was maintained for >24 hr with two doses of carbonyl iron. Significantly more ^{59}Fe was present in the liver.

Enhanced erythropoiesis was associated with markedly increased ^{59}Fe in the blood and marrow, predominantly at the expense of splenic counts (Table II). These effects were not present in the animals that had received carbonyl iron.

The organ distribution of ^{59}Fe HDRC at 8 hr was tested in several groups of animals, *viz.* a control group, one that had received prior carbonyl iron, one that had received prior unlabeled HDRC, one that had received both, and one that had received prior carbonyl iron and unlabeled free hemoglobin (Table III). The prior administration of both carbonyl iron and unlabeled HDRC was associated with increased splenic ^{59}Fe which is the pattern obtained in a previous experiment when HDRC were given alone (Table I). Although hepatic retention was also increased, the rise did not reach statistical significance. When prior carbonyl iron and

Table I. Comparison of the Fate of ^{59}Fe HDRC in Four Experimental Settings

	Time postinfusion (hr)	Marrow	Liver	Spleen	Blood
Control	0.5	3.2 (0.1)	16.6 (0.9)	45.1 (9.7)	35.2 (10.5)
	1.5	6.3 (0.7)	26.0 (1.8)	47.8 (3.5)	19.9 (3.0)
	4.0	16.4 (1.2)	22.9 (2.1)	37.8 (1.7)	22.9 (1.7)[a]
	8.0	26.7 (1.2)	29.0 (3.2)	29.3 (2.7)	13.4 (1.4)
	24.0	26.9 (1.8)	14.6 (3.3)	33.9 (1.5)	24.7 (0.1)
Carbonyl iron (2 doses)	0.5	3.8 (2.2)	19.7 (4.2)	30.1 (2.7)	46.5 (0.7)
	1.5	6.7 (0.8)	39.0 (2.2)[a]	40.9 (3.3)	11.1 (2.9)
	4.0	10.4 (1.3)[b]	44.0 (3.9)[b]	36.3 (2.5)	9.3 (2.4)
	8.0	15.7 (1.2)[b]	48.1 (1.8)[a]	28.6 (1.9)	7.6 (0.7)[b]
	24.0	14.7 (1.0)[c]	43.7 (4.3)[c]	33.2 (5.2)	8.6 (0.1)[c]
Carbonyl iron (1 dose)	4.0	5.9 (0.7)[d]	59.8 (3.8)[d]	28.1 (4.6)	6.2 (0.1)[d]
HDRC	0.5	5.8 (1.0)	26.6 (6.8)	56.7 (2.8)	11.1 (4.9)
	1.5	7.0 (0.4)	25.6 (0.4)	63.6 (0.6)[e]	3.8 (0.2)
	4.0	11.3 (3.0)	29.5 (2.2)	53.5 (5.4)[a]	11.3 (0.3)
	8.0	18.0 (0.8)	21.6 (3.9)	52.2 (2.6)[a]	8.3 (0.6)

[a] Statistically different ($P < 0.05$) from the other three groups which are statistically similar.
[b] Statistically different from control group ($P < 0.05$).
[c] First 2 groups different at 24 hr ($P < 0.05$).
[d] Statistically different from control group ($P < 0.0005$).
[e] Statistically different from group given two doses of carbonyl iron ($P < 0.05$).

free hemoglobin were administered together, the pattern of increased hepatic ^{59}Fe was similar to that obtained with carbonyl iron alone. Marrow and blood ^{59}Fe were reduced by all the maneuvers and the administration of free hemoglobin was associated with a nonstatistically significant increase in the percentage of injected ^{59}Fe found in the kidney (5.5 (±0.2)%) as compared with figures varying between 1.2 (±0.9)% and 3.3 (±1.4)% in the other groups.

The results of sizing chromatographic analysis on splenic and hepatic cytosolic extracts, obtained between 30 and 480 min after the administration of ^{59}Fe HDRC, are shown in Tables IV and V, respectively. The relative sizes of the peaks were calculated as a percentage of the

Table II. Comparison of the Fate of ^{59}Fe HDRC Cells 4 hr after Infusion into Control Rats and Animals with Enhanced Erythropoiesis

	Marrow	Liver	Spleen	Blood
Control	16.4 (1.2)	22.9 (2.1)	37.8 (1.7)	22.9 (1.7)
Control + 2 doses of carbonyl iron	10.4 (1.3)	44.0 (3.9)	36.3 (2.5)	9.3 (2.4)
Venesection	27.3 (1.1)[a]	18.6 (6.8)	23.8 (3.0)[a]	30.4 (2.7)
Venesection + 2 doses of carbonyl iron	11.4 (1.8)	44.6 (1.9)	34.2 (3.6)	10.0 (0.2)

[a] Significantly different from the control group ($P < 0.01$).

Table III. Effects of Prior Carbonyl Iron or Pretransfusion with Unlabeled HDRC or a Combination of Both, or of Prior Oral Carbonyl Iron Plus an Infusion of Free Hemoglobin on the Organ Distribution of ^{59}Fe 8 hr after the Injection of ^{59}Fe HDRC

	Marrow	Spleen	Liver	Blood	% Saturation transferrin
Control	35.4 (3.9)[a]	20.6 (2.3)	35.0 (5.0)[b]	9.0 (0.2)[c]	17.7 (2.7)
Carbonyl iron	18.0 (0.6)	23.5 (1.0)	52.1 (0.7)	6.4 (0.7)	77.3 (6.2)
HDRC	15.7 (0.7)	39.1 (1.1)[d]	40.9 (2.4)[b]	4.3 (1.0)	22.0 (2.3)
Carbonyl iron + HDRC	15.9 (1.6)	34.2 (7.0)[d]	45.2 (6.3)	4.7 (0.3)	79.0 (4.5)
Carbonyl iron + free hemoglobin	20.7 (1.4)	20.6 (1.0)	54.4 (2.1)	4.3 (0.5)	71.3 (7.1)

[a] Statistically different from the other groups which were statistically equivalent ($F = 61.7$, $P < 0.0001$).
[b] Statistically similar but different from the other groups ($F = 12.7$, $P < 0.006$).
[c] Statistically different from the other groups which are statistically equivalent ($F = 34.9$, $P < 0.0001$).
[d] Statistically similar but different from the other groups which were also similar ($F = 24.0$, $P < 0.0006$).

Table IV. Time-Dependent Fractionation of Splenic Extracts

	Time postinfusion (min)	Ferritin	Hemoglobin	Small molecular weight	
				Ferric	Ferrous
Control		1.6 (0.5)	88.1 (0.5)	6.0 (0.2)	—
Carbonyl iron	30	1.9 (0.2)	86.9 (0.9)	4.0 (0.4)	—
HDRC		4.0 (1.3)	87.9 (2.3)	6.1 —	2.4 (0.1)
Control		9.9 (1.1)	80.7 (1.3)	5.7 (1.5)	2.7 (0.2)
Carbonyl iron	90	11.9 (0.4)	75.0 (1.4)[a]	8.5 (0.7)	7.6 (2.1)
HDRC		12.5 (1.3)	79.7 (1.2)	3.7 (0.1)	2.3 (0.2)
Control		19.2 (2.8)[b]	62.7 (3.6)	4.8 (0.3)	5.2 (0.2)
Carbonyl iron	240	37.2 (3.3)	51.4 (3.1)	5.6 (0.8)	3.6 (0.4)
HDRC		42.0 (2.4)	51.4 (3.0)	3.4 (0.3)	2.1 (0.2)
Control		73.8 (3.7)	18.5 (3.5)	3.6 (0.3)	—
Carbonyl iron	480	86.0 (1.9)	9.5 (1.5)	2.5 (0.1)	—
HDRC		72.7 (0.9)	22.7 (1.2)	2.4 (1.5)	2.2 (0.9)

Note. Statistical comparisons refer only to ferritin and hemoglobin as the small molecular weight characterizations were performed on only small numbers of samples.
[a] Statistically different from control group ($P < 0.05$).
[b] Statistically different from carbonyl iron and HDRC ($P < 0.05$).

counts recovered from the column. In the spleen there was a steady drop in ^{59}Fe hemoglobin activity which was accompanied by a reciprocal rise in ^{59}Fe ferritin activity (Table IV). A small amount was consistently present in a small molecular weight fraction. There were no major differences among the percentages of distribution of the iron peaks in the three groups. Liver fractionation revealed somewhat different findings (Table V). At the times studied (90 and 240 min), the liver contained less ^{59}Fe hemoglobin but more ^{59}Fe ferritin and small molecular weight ^{59}Fe (both ferrous and ferric) than did the spleen. The carbonyl iron group had more hepatic small molecular weight ^{59}Fe (both in percentage and absolute terms) than the other two groups, while ^{59}Fe ferritin was increased in both the carbonyl iron and previously transfused groups.

In an attempt to find out how the raised transferrin saturation was exerting its effects on cellular iron release, detergent-solubilized extracts of hepatic and splenic cell and membrane pellets, obtained 90 min after the labeled HDRC infusion, were fractionated for ^{59}Fe activity on AcA 44 ultrogel sizing column chromatography. Three regions of ^{59}Fe were identified: a high molecular weight (void) fraction, a midrange fraction, and a hemoglobin fraction. No significant differences were noted between the total ^{59}Fe activity in the pellets or in the distribution between the peaks in controls as compared with rats with a high transferrin saturation induced by carbonyl iron (data not shown).

The effects of parenterally administered chelators on the distribution of ^{59}Fe HDRC are shown in Table VI. In rats with normal transferrin saturations, all three chelators caused a modest reduction in the hepatic ^{59}Fe pool. In rats given carbonyl iron, the hepatic ^{59}Fe pool was increased and the three chelators caused a significant reduction in its size. Marrow ^{59}Fe uptake was significantly decreased by 2,2′-bipyridine in the control group and there was a concomitant increase in blood ^{59}Fe. A similar, although less marked trend was noted in the carbonyl group. DTPA significantly reduced the splenic ^{59}Fe pool in the control group. A similar but less significant reduction was noted in the animals given prior carbonyl iron.

Serum obtained 30 min after the administration of the ^{59}Fe HDRC was fractionated on AcA 44 ultrogel chromatography. Organ distributions in the control and carbonyl iron groups were similar. The transferrin saturations were 51.0 (±1.5)% and 91.3 (±5.5)%, respectively. In the control animals there was an equal distribution of ^{59}Fe in the serum between ferritin and transferrin whereas in the animals that had received carbonyl iron the transferrin peak was very small (Fig. 2).

Table V. Time-Dependent Fractionation of Hepatic Extracts

	Time postinfusion (min)	Ferritin	Hemoglobin	Small molecular weight	
				Ferric	Ferrous
Control	90	5.0 (0.6)[a]	61.9 (3.5)[b]	17.3 (2.4)	17.9 (0.9)
Carbonyl iron		20.0 (1.9)[a]	42.2 (3.3)	28.3 (4.8)	24.9 (0.9)
HDRC		36.3 (1.8)[a]	38.8 (2.0)	15.9 (0.3)	16.0
Control	240	47.4 (2.7)	17.8 (3.6)	18.5 (5.6)	20.8 (3.7)
Carbonyl iron		51.4 (7.1)	10.8 (1.5)	22.0 (9.6)	28.4 (11.9)
HDRC		81.2 (8.2)[c]	7.1 (3.0)	6.9	4.3

Note. Statistical comparisons refer only to ferritin and hemoglobin peaks as the samll molecular weight comparisons were performed on small numbers of samples.

[a] Each statistically different from the other two groups ($P < 0.05$).

[b] Statistically different from the other two groups ($P < 0.05$).

[c] Statistically different from control group ($P < 0.05$).

Table VI. Effect of Various Chelators on the Distribution of ^{59}Fe in Organs 8 hr after ^{59}Fe HDRC Infusion

	Chelator	Marrow	Liver	Spleen	Blood	Nonrecovered
Control	None	20.0 (2.3)	20.8 (3.6)	24.3 (3.4)	9.7 (1.7)	25.3 (2.7)
	2,2′-bipyridine	5.7 (0.4)[b]	15.1 (1.7)	15.6 (0.3)	22.3 (3.4)[b]	41.4 (3.5)
	Desferrioxamine	20.8 (2.2)	14.6 (1.7)	22.0 (0.5)	10.7 (1.1)	31.6 (4.1)
	DTPA	14.0 (3.2)	17.4 (2.0)	14.5 (2.0)[b]	9.8 (1.3)	43.3 (8.3)
Prior carbonyl iron	None	13.3 (3.4)	34.0 (3.5)	18.2 (1.1)	6.4 (0.2)	28.2 (3.9)
	2,2′-bipyridine	6.4 (2.0)	16.9 (0.3)[a]	18.2 (0.9)	19.0 (1.1)[b]	39.6 (0.4)
	Desferrioxamine	9.8 (0.2)	17.4 (0.2)[a]	20.2 (0.5)	5.8 (1.2)	46.9 (1.7)[b]
	DTPA	7.5 (0.5)	21.0 (3.8)[a]	14.8 (3.3)	7.3 (1.0)	49.5 (0.0)[b]

[a] All three statistically equivalent but together significantly different from liver counts in the carbonyl group that did not receive any chelators ($F = 23.5$, $P < 0.005$).

[b] Significantly different from the appropriate group which did not receive any chelator ($P < 0.05$).

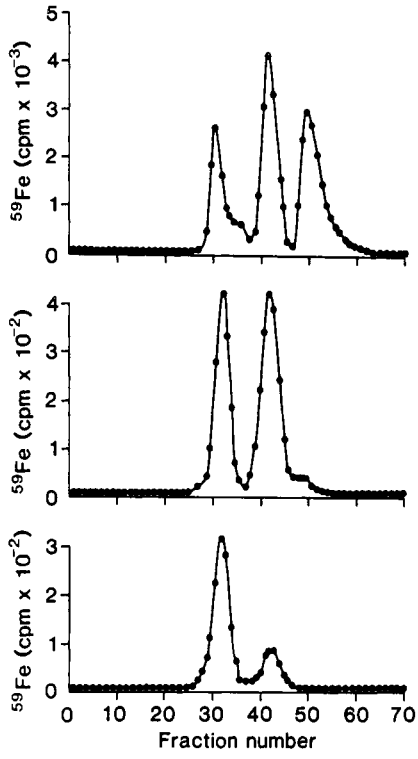

Figure 2. Fractionation on an AcA 44 ultrogel column of equal volumes of whole serum 30 min after ^{59}Fe HDRC infusion into normal rats (middle frame) and in rats given prior oral carbonyl iron (bottom frame). Calibration of the column for ferritin, transferrin, and hemoglobin is shown in the top frame.

Discussion

Previous work has shown that a raised transferrin saturation induced by infused iron markedly reduces iron release from the RE system (4, 5). However, the infused iron may have supersaturated the plasma and depleted the nontransferrin iron-binding capacity in such studies. In the present investigation, oral carbonyl iron administration was used to raise the transferrin saturation and to assess its effect on the RE handling of ^{59}Fe HDRC. Once solubilized by gastric acid, carbonyl iron absorption is the same as that of iron given as ferrous ammonium sulfate (6). The possibility that carbonyl iron might also be absorbed in particulate form has been raised by the finding of some small particles in the mucosa and liver of rats given 100 mg of carbonyl iron in one study (15). However, no parenchymal particulate iron has been found in another (16). Both acute and more sustained carbonyl iron-induced increases in transferrin saturation had no effect on the splenic retention of HDRC-derived ^{59}Fe but an increase in hepatic ^{59}Fe was noted. These findings are in agreement with our previous observations on the effect of polycythemia on organ ^{59}Fe distribution after ^{59}Fe HDRC infusion in rats (4). Splenic ^{59}Fe retention was, however, enhanced by the prior infusion of unlabeled HDRC which presumably conditioned RE cells to store the released iron rather than to deliver it back to the plasma. Stimulation of erythropoiesis in animals with normal transferrin saturation caused mobilization of iron from both liver and spleen and its transfer to red cell precursors; a raised saturation of transferrin induced by carbonyl iron prevented this enhanced cellular iron release.

The differential effects of a raised transferrin saturation on iron release from the spleen and liver, with a redistribution from spleen to liver, were studied further by paying particular attention to the nature of iron being transported. Neither a raised transferrin saturation nor the injection of free hemoglobin reduced splenohepatic iron transfer which suggested that neither transferrin nor a putative haptoglobin-hemoglobin mechanism (5) was playing a significant role. Other possible modes of transport include ferritin and/or small molecular weight complexes. In this context, it has been shown that isolated macrophages release ferritin which is then rapidly taken up by hepatocytes (17), presumably by a receptor-mediated pathway (18). Hepatocytes can also take up nontransferrin iron (19) by an electrogenic transport mechanism (20). ^{59}Fe ferritin was, in fact, identified in the serum after ^{59}Fe HDRC injection. Its possible role as a transporter of iron from spleen to liver could perhaps have been tested further by injecting large amounts of unlabeled ferritin into the circulation. However, it was not found possible to prepare material that was free of endotoxin, a substance which itself causes major perturbations of internal iron transport (21). Further insight into possible mechanisms of iron transport between the spleen and liver was obtained by fractionation studies after the infusion of ^{59}Fe HDRC. In both organs there was a progressive drop in ^{59}Fe hemoglobin with time which was accompanied by a reciprocal rise in the concentration of labeled ferritin. Initial concentrations of ^{59}Fe hemoglobin were lower in the liver, while ^{59}Fe ferritin was higher, especially in animals that had received prior carbonyl iron or unlabeled HDRC. The striking difference was, however, in the small molecular weight component. Very little ^{59}Fe was in this form in splenic extracts while it accounted for one third to one half of the activity in the liver, with an equal division between ferrous and ferric iron. The patterns were similar in control animals and in those given prior carbonyl iron or unlabeled HDRC. The *in vitro* findings were supplemented by *in vivo* experiments in which the effects of various iron chelators on the internal distribution of ^{59}Fe HDRC were studied. The extracellular chelator DTPA enhanced iron release from the spleen in animals with a normal transferrin saturation similar to that observed in animals with increased erythropoiesis. These combined findings suggest that RE iron release is promoted by an increase in the circulating iron-binding capacity. The reduction of marrow uptake effected by the membranous ferrous chelator 2,2′-bipyridine is compatible with the observation that it

decreases iron uptake from diferric transferrin by a number of cell types, including erythroid precursors (22–25). The increased hepatic retention of iron dervied from ^{59}Fe HDRC noted in animals with saturated transferrin was reduced by all three chelators. Each, however, must have obtained its iron from a different pool. 2,2′-Bipyridine presumably removed ferrous iron from splenic, or hepatic cellular membranes, or both; DTPA obtained ferric iron released by the spleen, while desferrioxamine procured its iron from a ferric pool predominantly in hepatocytes (10). Last, fractionation of solubilized membrane-enriched fractions from spleens and livers after the infusion of ^{59}Fe HDRC did not show any build-up of activity in the hepatic membranes of animals with a saturated transferrin, which suggests that membrane transport is not a rate-limiting step in iron release from the liver.

The results of these various experiments are compatible with a model of internal iron exchange in which the RE cells of the spleen release hemoglobin-derived iron derived at a constant rate. This is in agreement with previous data of Noyes *et al.* (26). After having been transported across the RE cell membrane in the ferrous state, the iron is oxidized to ferric iron which is then either taken up by transferrin or transported in a form which is available to a ferric chelator. Ferritin, as has been previously suggested (27), also contributes to the circuit between the spleen and the liver at least in settings associated with red cell breakdown but its quantitative significance is not clear. Splenic derived iron which is taken up by transferrin is distributed to tissues in proportion to the density of transferrin receptors on their surfaces, with the major recipient tissue being the erythroid marrow. Nontransferrin iron, either nonspecifically bound or in ferritin, is taken up by hepatocytes. This ferritin is then degraded (28) and the released iron along with any other iron entering the hepatocyte is incorporated into new ferritin. During this phase of ferritin formation, relatively large amounts of ferrous and ferric iron are available to chelators. Although some of the iron may be derived from ferritin itself, the very early appearance of chelatable iron suggests that it is derived from a more labile pool (10). The fact that splenic iron release is unaffected by a highly saturated transferrin while hepatic iron release is inhibited may possibly be due to the relative density of transferrin receptors on their surfaces. The much larger numbers of receptors on hepatocytes compared with splenic tissue macrophages (29–31) would be associated with increased uptake of iron in the presence of a saturated transferrin. The increased uptake, in its turn, would be expected to stimulate ferritin synthesis by triggering the so-called iron responsive element to shift cytoplasmic ferritin mRNA to the polyribosomes (32–37). In contrast, splenic macrophages can be conditioned to form increased amounts of ferritin by prior loading with HDRC. When this is done, subsequent injections are followed by a retention of the released iron and its incorporation into ferritin.

1. Bothwell TH, Charlton RW, Cook JD, Finch CA. Iron metabolism in man. Oxford: Blackwell Scientific Publications, 1979.
2. Morgan EH. Transferrin, biochemistry, physiology and clinical significance. Mol Asp Med **4**:1–123, 1981.
3. Huebers HA, Finch CA. The physiology of transferrin and transferrin receptors. Physiol Rev **67**:520–582, 1987.
4. Lipschitz DA, Simon MO, Lynch SR, Dugard J, Bothwell TH, Charlton RW. Some factors affecting the release of iron from reticuloendothelial cells. Br J Haematol **21**:289–303, 1971.
5. Bergamaschi G, Eng MJ, Huebers HA, Finch CA. The effect of transferrin saturation on internal iron exchange. Proc Soc Exp Biol Med **183**:66–73, 1986.
6. Huebers HA, Brittenham GM, Csiba E, Finch CA. Absorption of carbonyl iron. J Lab Clin Med **108**:473–478, 1986.
7. International Committee for Standardization in Haematology (Iron Panel). Recommendations for the measurement of serum iron in human blood. Br J Haematol **38**:291–294, 1978.
8. International Committee for Standardization in Haematology (Iron Panel). The measurement of total and unsaturated iron-binding capacity in serum. Br J Haematol **38**:281–290, 1978.
9. Hershko C, Cook JD, Finch CA. Storage iron kinetics. II. The uptake of hemoglobin iron by hepatic parenchymal cells. J Lab Clin Med **80**:624–634, 1972.
10. Pippard MJ, Johnson DK, Finch CA. Hepatocyte iron kinetics in the rat explored with an iron chelator. Br J Haematol **52**:211–224, 1982.
11. Pollack S, Campana T, Weaver J. Low molecular weight iron in guinea pig reticulocytes. Am J Hematol **19**:75–84, 1985.
12. Bridges KR, Cudkowicz A. Effect of iron chelators on the transferrin receptors in K562 cells. J Biol Chem **259**:12970–12977, 1984.
13. Morrow WJW, Wharton M, Stricker RB, Levy JA. Circulating immune complexes in patients with acquired immune deficiency syndrome contain the AIDS-associated retrovirus. Clin Immunol Immunopathol **40**:515–524, 1986.
14. Enler HH, Kern P, Löffler H, Dietrich M. Precipitable immune complexes in healthy homosexual men, acquired immune deficiency syndrome and the related lymphadenopathy syndrome. Clin Exp Immunol **59**:267–275, 1985.
15. Nakamura T, Mori M, Awai M. Process of iron absorption from rat duodenal mucosa. Acta Pathol Jpn **38**:1377–1390, 1988.
16. Bacon BR, Tavill AS, Brittenham GM, Park CH, Recknagel RO. Hepatic lipid peroxidation in vivo in rats with chronic iron overload. J Clin Invest **71**:429–439, 1983.
17. Sibille J-C, Kondo H, Aisen P. Interactions between isolated hepatocytes and Kupffer cells in iron metabolism: A possible role for ferritin as an iron carrier protein. Hepatology **8**:296–301, 1988.
18. Mack U, Powell LW, Halliday JW. Detection and isolation of a hepatic membrane receptor for ferritin. J Biol Chem **258**:4672–4675, 1983.
19. Wright TL, Brissot P, Ma W-L, Weisiger RA. Characterization of non-transferrin-bound iron clearance by rat liver. J Biol Chem **261**:10909–10914, 1986.
20. Wright TL, Fitz JG, Weisiger RA. Non-transferrin-bound iron uptake by rat liver. J Biol Chem **263**:1842–1847, 1988.
21. Torrance JD, Charlton RW, Simon MO, Lynch SR, Bothwell TH. The mechanism of endotoxin-induced hypoferraemia. Scand J Haematol **21**:403–410, 1978.
22. Morgan EH. Chelator mediated iron efflux from reticulocytes. Biochim Biophys Acta **733**:39–50, 1983.
23. Nunez M-T, Cole ES, Glass J. The reticulocyte plasma membrane pathway of iron uptake as determined by the mechanism

of dipyridyl inhibition. J Biol Chem **258**:1146–1151, 1983.
24. Baynes RD, Friedman BM, Bukofzer GT, Bothwell TH, Macfarlane BJ, Lamparelli RD. The effect of ferrous and ferric chelators on transferrin iron-macrophage interactions. Am J Hematol **29**:27–32, 1988.
25. Baynes R, Friedman B, McNamara L, Bothwell T, Bukofzer G, Macfarlane B. Transferrin iron interactions with cultured hepatocellular carcinoma cells. Eur J Cell Biol **46**:282–288, 1988.
26. Noyes WD, Bothwell TH, Finch CA. The role of the reticuloendothelial cell in iron metabolism. Br J Haematol **6**:43–55, 1960.
27. Siimes MA, Dallman PR. New kinetic role for serum ferritin in iron metabolism. Br J Haematol **28**:7–18, 1974.
28. Unger A, Hershko C. Hepatocellular uptake of ferritin in the rat. Br J Haematol **28**:169–179, 1974.
29. Page MA, Baker E, Morgan EH. Transferrin and iron uptake by rat hepatocytes in culture. Am J Physiol **246**:G26–G33, 1984.
30. Trinder D, Morgan E, Baker E. The mechanisms of iron uptake by fetal rat hepatocytes in culture. Hepatology **6**:852–858, 1986.
31. Hamilton TA, Weiel JE, Adams DO. Expression of the transferrin receptor in murine peritoneal macrophages is modulated in the different stages of activation. J Immunol **132**:2285–2290, 1984.
32. Aziz N, Munro HN. Both subunits of rat liver ferritin are regulated at a translational level by iron induction. Nucl Acids Res **14**:915–927, 1986.
33. Rogers J, Munro H. Translation of ferritin light and heavy subunit mRNAs is regulated by intracellular chelatable iron levels in rat hepatoma cells. Proc Natl Acad Sci USA **84**:2277–2281, 1987.
34. Hentzel MW, Caughman SW, Rouault TA, Barriocanal JG, Dancis A, Harford JB, Klausner RD. Identification of the iron-responsive element for the translational regulation of human ferritin mRNA. Science **238**:1570–1573, 1987.
35. Leibold EA, Munro HM. Cytoplasmic protein binds in vitro to a highly conserved sequence in the 5′-untranslated region of ferritin heavy- and light-subunit mRNAs. Proc Natl Acad Sci USA **85**:2171–2175, 1988.
36. Casey JL, Hentze MW, Koeller DM, Caughman SW, Rouault TA, Klausner RD, Harford JB. Iron-responsive elements: regulatory RNA sequence that control mRNA levels and translation. Science **240**:924–928, 1988.
37. Dickey LF, Wang YH, Shull GE, Wartman IA, Theil EC. The importance of the 3′-untranslated region in the translational control of ferritin mRNA. J Biol Chem **263**:3071–3074, 1988.