Effect of Transfused Reticulocytes on Iron Exchange

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An animal model was developed whereby reticulocyte-rich blood was introduced into normal rats by exchange transfusion. Measurements of plasma iron turnover were made at similar plasma iron concentrations before and after exchange transfusions. High reticulocyte blood obtained from animals rendered iron deficient by diet or by treatment with phenylhydrazine resulted in a mean increase of 86% in internal iron exchange, while the plasma iron turnover was unaffected by exchange with normal red cells. Since iron input from reticuloendothelial cells could have increased due to breakdown of transfused cells, iron absorption was also measured. Within 1 hr and for at least 6 hr after exchange with high reticulocyte blood, mean absorption in six groups of animals was increased over control animals by 50%–130%. The increased plasma iron turnover and absorption was not mediated by a decrease in plasma iron or an increase in unsaturated iron-binding capacity. Indeed, a higher plasma iron and transferrin saturation augmented the movement of iron into the plasma from iron-donating tissues. It is proposed that the donation of iron by transferrin in some way immediately facilitates the procurement of more iron by transferrin.

IN MAMMALIAN SPECIES, internal iron exchange is mediated by the plasma transferrin molecule. The behavior of this protein in loading iron from donor tissues and in releasing iron to membrane receptors has been a topic of considerable discussion, particularly in respect to the individual behavior of the two iron-binding sites.[1] Iron loading has been recently demonstrated to be essentially random and the release of iron an "all-or-none" phenomenon.[2-4] While this aspect of the molecular behavior of transferrin constitutes a mechanism for minimizing fluctuations in the plasma iron level, such studies do not explain how the amount of iron entering the plasma is regulated. Nevertheless, some sort of regulation exists, since the widely varying requirements of the erythron are met with little or no change in plasma iron concentration.[5] It has been suggested that internal iron exchange might be automatically regulated by the number of unsaturated iron binding sites on the transferrin molecule.[6-7] According to the proposal, as more iron is removed from transferrin by tissues, the number of transferrin sites capable of binding iron increases, and this leads to an increased rate of entry of iron into the plasma. One of the important supplier tissues is the gut. A regulation of absorption related to iron stores and erythropoiesis is well recognized,[8] although again, the way the message is transmitted is speculative. Most studies of absorption suggest that several days are required to effect a change,[9,11] although exposure

to hypoxia has been reported to effect change within 24 hr.[12-14]

The purpose of the present study was to produce abrupt changes in tissue iron requirements and to examine the immediate effects of this on plasma iron turnover and absorption. It was hoped that such studies might provide useful clues as to the nature of internal iron regulation.

MATERIALS AND METHODS

Normal male Sprague-Dawley rats, 9–11 wk old, weighing 180–230 g were used. They were fed Purina Rat Chow containing about 360 mg/kg of iron. Venous access was established by the insertion of a PE-50 polyethylene tube through the right jugular vein into the superior vena cava under Innovar or ether anesthesia.[15] Operative blood losses were replaced. The animal was then used within 3 hr in one of two types of studies.

In the first of these, the protocol consisted of an initial plasma iron turnover study followed in most instances by an exchange transfusion of approximately two blood volumes (120 ml/kg body weight) requiring about 12 min, followed by a second plasma iron turnover. Exchange transfusion was carried out by alternately removing and injecting 2-ml aliquots of blood through the caval catheter. The plasma iron turnover was performed as described elsewhere,[16] except that ether anesthesia was used in these studies.* Circulating transferrin was labeled by the injection of 0.75 μCi of ^{59}Fe as $FeSO_4$ (specific activities about 10 μCi/μg iron) over 3 min. Five blood samples of 0.2 ml each were then drawn from the caval catheter for radioactive determinations over the expected $T\frac{1}{2}$ clearance time. In addition, three blood samples of 0.8 ml were drawn for plasma iron determination at intervals to bracket the $T\frac{1}{2}$, and a final 2-ml sample was then taken for determination of total iron-binding capacity. Blood removed during the first plasma iron turnover was replaced by establishing a positive balance during the exchange transfusion of a similar amount. In these studies the plasma iron concentration at the time of the $T\frac{1}{4}$ radioiron disappearance was

*Innovar was used in previous studies[16] but was subsequently observed to give lower values for plasma iron turnover than did ether. For example, in a group of 6 animals subjected to sequential plasma iron turnover, the mean value for the first plasma iron turnover carried out under ether at a plasma iron of 117 ± 31 μg/dl was 2.03 ± .22 mg/dl whole blood/day, while that of the second turnover carried out under Innovar at a plasma iron of 114 was 1.45 ± 0.27 mg/dl whole blood/day.

selected as the most representative value to use in the iron turnover calculation.[16] When ^{55}Fe was used for the first plasma iron turnover, 0.75 μCi of ^{59}FeSO$_4$ was used for the second. When ^{59}Fe was employed initially, the same isotope at 3 times the original dosage was employed in the second study.

The three samples taken for determination of plasma iron permitted an evaluation of the changes during the plasma iron turnover. The rate of plasma iron change extrapolated from these values was converted to mg/dl whole blood/day. This value, subtracted from the total plasma iron turnover, also expressed as mg/dl whole blood/day, provided an estimate of the rate of iron mobilization from tissues during the turnover study.

High reticulocyte blood was obtained either through the administration of 50 mg/kg acetylphenylhydrazine (Amend Drug and Chemical Co., New York, N.Y.) intraperitoneally 6 days previously, or by obtaining blood from animals rendered iron deficient by low iron diet.[15] In the latter instance, the hypoferremic plasma was removed and replaced by normal plasma. In addition, it was necessary in certain studies to adjust the plasma iron of the blood exchanged to the level present in the experimental animals by the addition of ferrous ammonium sulfate dissolved in pH 2 saline. The high reticulocyte blood was used for exchange transfusion within 3 hr of the time donor animals were bled. In one study, apotransferrin purified from rat plasma was injected before the second turnover. Techniques employed for the preparation of this material have been described elsewhere.[2,3]

In a second set of studies, the effect of exchange transfusion on intestinal iron absorption was evaluated. The animals were anesthetized with ether and the abdomen opened by a 3–4-cm cut along the linea alba. Ten centimeters of gut (small loop), or in some studies a 20-cm segment of gut (large loop), beginning at the duodenal jejunal ligament was isolated. One end was ligated and the other end was ligated after a solution of ferrous ammonium sulfate alone at pH <2 or stabilized with 20 mg human serum albumin at pH 7.4 had been introduced. The amount of iron employed varied from 15 to 112 μg and was labeled with 1 μCi of ^{59}Fe^{++}. The wound was then closed by clips and the animal kept in a controlled temperature environment. Immediately preceding this operative procedure, an exchange transfusion with normal or high reticulocyte heparinized blood was carried out as previously described. At the end of the exchange transfusion 2.5 mg Protamine sulfate (Upjohn Company, Kalamazoo, Mich.) was injected to prevent bleeding. At the end of an hour, the animal was sacrificed by bleeding from the inferior vena cava. The gut loop was removed, opened, and perfused with 50 ml of ice-cold saline. Activity of the washed gut loop and of the remaining carcass, along with an appropriate standard of the isotope administered, were determined by counting in a small animal

counter. Results were expressed as (1) total uptake, i.e., activity found in the washed gut segment and carcass; (2) mucosal activity, i.e., activity present in the washed gut loop; and (3) iron absorbed, i.e., activity that had entered the plasma and was distributed in the carcass.

Plasma iron was determined according to a scaled down procedure requiring only 0.3–0.5 ml plasma but was carried out otherwise as described by the International Standardization Committee.[17] Total iron binding capacity was performed by the method of Cook et al.[18] Reticulocyte counts were carried out employing new methylene blue for staining, and the number of reticulocytes in 1000 red cells were determined. Hematocrits were determined by the microhematocrit technique and hemoglobin by the cyanomethemoglobin method.

Statistics were carried out according to standard methods, sequential studies of plasma iron turnover being examined by the paired t test, and absorption studies by the single-tail t test. Significance was assumed at $p \leq 0.05$.

RESULTS

The Effect of Transfused Reticulocytes on Internal Iron Exchange

The first three studies in Table 1 were carried out to evaluate the effects of different red cell populations on plasma iron turnover. In each animal an initial baseline turnover was carried out followed by a second turnover after an experimental procedure was performed. In group 1, exchange transfusion with normal blood resulted in no significant change in the plasma iron turnover of a group of 6 animals ($p = 0.5$). On the other hand, exchange transfusion with phenylhydrazine reticulocytes (group 2) resulted in an increase from 1.65 before exchange to 3.55 mg/dl whole blood/day immediately afterward ($p \leq 0.001$). It seemed possible in this study that a portion of the phenylhydrazine reticulocytes that visibly enlarged the spleen of transfused animals might be rapidly destroyed and the iron returned to the plasma, thereby contributing to the increased iron turnover. In order to avoid red cell breakdown caused by phenylhydrazine, reticulocytes from iron-deficient rats were used in the third group of animals. This time there was

Table 1. Effects of Exchange Transfusion and Other Manipulations on Plasma Iron Turnover

Group	Conditions	Hematocrit (%)	Retic (%)	Plasma Iron (μg/dl)	Total Iron Bind Capac (μg/dl pl)	Radioiron Clearance (T½ in min)	Plasma Iron Turnover (mg/dl Whole Blood/day)	Plasma Iron Change (μg/dl Plasma/hr)	Tissue Iron Supply (mg&dl Whole Blood/Day)
1	(a) Before exchange	40 ± 2	5 ± 1	136 ± 61	372 ± 55	42.2 ± 8.6	2.00 ± 0.50	+2.8	2.04
(n – 6)	(b) After exchange with normal blood	42 ± 1	4 ± 1	150 ± 27	420 ± 43	47.6 ± 6.7	1.96 ± 0.28	+3.5	2.01
2	(a) Before exchange	34 ± 1	7 ± 1	120 ± 65	466 ± 57	46.8 ± 9.4	1.68 ± 0.69	– 1.1	1.65
(n – 8)	(b) After exchange with blood from phenylhyd-treated rats	43 ± 1	31 ± 4	110 ± 46	457 ± 34	19.1 ± 3.6	3.54 ± 1.45*	+.7	3.55*
3	(a) After exchange w/normal blood	37	5 ± 1	189 ± 61	380 ± 43	43.4 ± 4.1	2.88 ± 0.80	+15.6	3.13
(n – 8)	(b) After exchange w/iron defic retics in normal plasma	41	21 ± 3	172 ± 41	303 ± 39	25.6 ± 2.6	4.18 ± 0.77*	–38.6	3.60*
4	(a) Before	38 ± 1	6 ± 1	180 ± 34	442 ± 39	44.7 ± 5.2	2.66 ± 0.36	+.3	2.66
(n – 8)	(b) After apotransferrin injection	39 ± 1	5 ± 1	192 ± 41	686 ± 43	50.0 ± 6.3	2.54 ± 0.60	– 7.7	2.42
5	(a) Before exchange	40 ± 2	5 ± 1	145 ± 75	347 ± 34	37.2 ± 7.8	2.38 ± 0.74	– 34.3	1.84
(n – 7)	(b) After exchange w/iron defic rbc, normal buthypo-ferremic plasma	41 ± 3	25 ± 5	80 ± 17	462 ± 27	18.1 ± 8.0	2.49 ± 0.42	– 51.7	1.70

*Indicates significance ($p < 0.05$).

no change in spleen size, but a significant increase in plasma iron turnover was again observed from the basal value of 2.88 after an exchange with normal cells to 4.11 mg/dl whole blood/day after exchange with iron-deficient reticulocytes ($p \leq 0.0025$). In these combined studies of groups 2b and 3b, 15 of 16 animals showed an increase in plasma iron turnover after receiving reticulocytes. The increase averaged 86% (range −4% to +276%).

Calculations of the amount of iron mobilized from tissues in these turnover studies were also made. The change in plasma iron turnover was adjusted for the change in the plasma iron compartment (Table 1). Exchange transfusion with normal red cells did not affect tissue iron donation ($p = 0.3$), whereas reticulocyte transfusion in the animals of groups 2 and 3 showed significant increases ($p \leq 0.001$ and $p \leq 0.02$).

In these first three study groups, the mean plasma iron at the onset of the two turnovers varied by no more than ±10%, i.e., +16, −10, and −17 μg/dl whole blood. In animals of group 4 (Table 1), the unsaturated iron-binding capacity was increased from 262 to 498 μg/dl plasma by an injection of apotransferrin. This increase did not affect the plasma iron or plasma iron turnover ($p = 0.3$). Animals in group 5 were given iron-deficient red cells suspended in hypoferremic plasma so that the second turnover study had a decrease in plasma iron of 45% and an increase in unsaturated iron-binding capacity of 89%. Despite the increase in circulating reticulocytes, the plasma iron turnover in this group of animals did not increase significantly ($p = 0.37$).

In the 29 turnover studies (groups 1a, 2a, 4a, and 5a of Table 1) performed before transfusion, initial plasma iron values ranged from 38 to 280, with a direct relationship between plasma iron concentration and plasma iron turnover (PIT = $0.7 + 0.01 \times$ PI, $r = 0.91$). When normal red cells were transfused (groups 1b and 3a), there was also some relationship between the change in plasma iron produced by the transfusion and the change in plasma iron turnover ($r = 0.75$). This relationship was not evident in the reticulocyte transfusion studies of groups 2b and 3b ($r = 0.36$), perhaps hidden in the more important effect of the reticulocytes.

Three plasma iron measurements were made during each iron turnover study so that it was possible to evaluate the effect of transfused reticulocytes on plasma iron concentration. In the 29 pretransfusion studies of iron turnover, the change in plasma iron averaged −6% during the first 30 min and +7% during the second 30 min (Table 2). The 14 animals (groups 1b and 3a) transfused with normal red cells showed average changes of −2% and −3%. Animals receiving

Table 2. Percent Change in Plasma Iron During Plasma Iron Turnover Measurements

	Plasma Iron Change (%)*	
Control		
	0–30 min	30–60 min
Group 1a (n = 6)	+4.8 ± 13.6	−9.1 ± 19.0
Group 2a (n = 8)	−5 ± 6.8	+23.4 ± 43.2
Group 4a (n = 8)	−6.1 ± 4.6	+12.1 ± 18.1
Group 5a (n = 7)	−18.1 ± 16.8	+0.6 ± 9.7
Average	−6.1	+6.7
Exchange with normal red cells		
	0–30 min	30–60 min
Group 1b (n = 6)	+8.0 ± 20.9	−2.4 ± 4.3
Group 3a (n = 8)	−11.4 ± 8.2	−3.3 ± 12.6
Average	−1.7	−2.9
Exchange with high reticulocyte blood		
	0–20 min	20–40 min
Group 2b	−11.0 ± 19.2	+28.8 ± 54.8
Group 3b	−11.4 ± 8.2	−3.3 ± 12.6
Group 5b	−15.8 ± 15.6	−12.1 ± 8.0
Average	−12.7	+9.2

*The percent change in plasma iron concentration of individual animals over 30 min is averaged.

reticulocyte transfusions (groups 2b and 3b) showed an initial change of −13% over the first 20 min and +9% during the next 20 min. The greatest temporary fall in plasma iron was seen in animals exchange transfused with iron-deficient reticulocytes (groups 3b and 5b). In the first 20 min during the plasma iron turnover, there was a 12% and 16% fall in plasma iron, and it was calculated that about 80% of the iron delivered to tissues came from the plasma compartment; in contrast, in the next 20 min, >100% came from the tissues. None of the changes in mean plasma iron values in all of these studies including the reticulocyte exchanged animals were significant when compared to the much greater spontaneous fluctuations in plasma iron in individual animals ($p = 0.3$–0.5).

Absorption Studies

In order to evaluate more directly the effect of reticulocytes on tissue iron supply to transferrin, absorption studies were carried out (Table 3). The exchange transfusion of normal blood had no effect on iron absorption. While in that study there was an increased mucosal uptake, fractionation of the mucosa showed the increased activity to be in the particulate fraction, assumed to represent adsorbed rather than absorbed iron. Both phenylhydrazine and iron-deficient reticulocytes caused a significant increase in absorption. The effect appeared maximal immediately after the exchange transfusion, although it persisted at 6 hr. The enhancing effect of reticulocytes on iron

Table 3. Effect of Reticulocyte Transfusion on Iron Absorption

Experimental Conditions	Average Plasma Iron at Sacrifice (μg/dl)	Hematocrit (%)	Retic (%)	Percent of Radioactivity		
				Total Uptake	Mucosal Activity	Absorbed†
Small loop, 15 μg Fe						
I(a) No Tx (n = 6)	148 ± 62	40 ± 1	4.5 ± 1.4	58 ± 16	37 ± 9	21 ± 11
(b) Tx normal cells absorp at 1 hr (n = 6)	135 ± 15	41 ± 2	4.1 ± 1.1	69 ± 10	52 ± 12	17 ± 9
II(a) No Tx (n = 9)	174 ± 36	41 ± 1	—	49 ± 11	30 ± 6	19 ± 7
(b) Phenylhydr reticulocytes absorp at 1 hr (n = 6)	209 ± 43	37 ± 4	31.7 ± 1.4	75 ± 18*	36 ± 8	39 ± 12*
III(a) No Tx (n = 9)	149 ± 60	41 ± 2	—	70 ± 12	46 ± 12	24 ± 8
(b) Phenylhydr reticulocytes absorp at 1 hr (n = 8)	158 ± 28	41 ± 1	31.1 ± 1.5	70 ± 11	34 ± 7	36 ± 11*
IV(a) No Tx (n = 4)	172 ± 15	42 ± 2	—	45 ± 10	25 ± 9	20 ± 6*
(b) Iron Def reticulocytes absorp at 1 hr (n = 5)	86 ± 24	40 ± 2	21.4 ± 3.1	72 ± 14*	42 ± 12*	30 ± 6*
Large loop, 25 μg Fe						
V(a) No Tx (n = 5)	131 ± 34	41 ± 1	—	50 ± 6	43 ± 5	7 ± 3
(b) Iron Def reticulocytes absorp at 1 hr (n = 6)	111 ± 24	43 ± 1	19.5 ± 1.6	65 ± 7*	49 ± 10	16 ± 7*
Large loop, 112 μg Fe						
VI(a) No Tx (n = 4)	292 ± 37	42 ± 1	—	42 ± 13	27 ± 3	15 ± 4
(b) Normal blood absorp 6 hr (n = 5)	321 ± 42	42 ± 1	3.5 ± .8	44 ± 8	29 ± 5	15 ± 6
(c) Phenylhydr reticulocytes (n = 5)	358 ± 54		19.8 ± 1.5	58 ± 8*	32 ± 2	26 ± 8*

*Indicates significance (p < 0.05).

†Activity in carcass minus activity in gut loop.

absorption was repeatedly demonstrated employing varying dosages of iron and varying sizes of gut loops.

Further absorption studies were carried out to clarify the mechanism by which these rapid changes in absorption were accomplished. Additional apotransferrin was injected and absorption was measured 4 hr later (group 1, Table 4). There was no effect of the increased iron-binding capacity on absorption. In a further study, exchange transfusion was carried out with iron-deficient blood in the control group and with the same blood to which iron had been added to saturate iron-binding capacity. These animals with the higher initial plasma iron (group 2b) showed a greater absorption (p < 0.005) than the animals receiving blood with a low plasma iron (group 2a). The change in plasma iron during the absorption test of −207 in group 2b versus +18 in group 2a suggested a greater iron delivery in the hyperferremic group, as would be expected.

DISCUSSION

Internal iron kinetics are predominantly concerned with the erythron. About 80% of transferrin-bound iron is delivered to the erythroid marrow in the normal 200-g rat.[19] This amount can be decreased or increased by suppressing or stimulating erythropoiesis, but such changes take several days to occur. An abrupt change in iron requirements was achieved in about 12 min by the exchange transfusion of high reticulocyte blood. This permitted observations of early changes in plasma iron and tissue iron mobilization. By performing an initial basal turnover and repeating immedi-

Table 4. Effect of UIBC and Plasma Iron on Absorption

Experimental Conditions	Plasma Iron/TIBC (mg/dl)		Absorption
	After Exchange Transfusion	After Absorption	
I. Small loop, 28 μg Fe and 20 mg albumin, absorption 4 hr after injection			
A. Normal, saline injection (n = 6)		244/304	49 ± 18
B. Injection with apotransferrin (n = 6)		237/662	45 ± 18
II. Small loop, 17.5 μg Fe			
A. Absorption immediately after exchange with iron deficient blood (n = 7)	57/676	75/427	26 ± 7
B. Absorption immediately after exchange with iron-saturated iron deficient blood (n = 7)	516/666	309/442	36 ± 7

ately after the exchange transfusion, each animal served as its own control.

The process of internal iron exchange may be divided into (A) iron procurement by transferrin and (B) the donation of this iron to tissues according to their iron requirements. Prior studies in rat and man have demonstrated that iron donation from transferrin to tissues in normal animals bears a direct relationship to plasma iron concentration.[16,20–22] These studies were concerned with the effect of increased iron requirement and therefore the plasma iron was held constant (mean value ± 10%) at the beginning of the two turnover measurements. A mean increase of 86% in iron delivery was observed as an immediate response to the presence of reticulocytes. The interplay between the reticulocyte effect and that of the plasma iron concentration was shown by group 5 animals (Table 1, group 5), where the increased iron requirements resulting from the reticulocyte transfusion and the decrease in plasma iron that would be expected to decrease turnover resulted in virtually no change in the plasma iron turnover. The amount of iron delivered appeared to depend on both tissue iron requirements and plasma iron concentration.

The iron supply used to meet the additional iron requirements could be derived from the plasma iron pool itself or from tissues such as the gut, reticuloendothelial cells, or hepatocyte, known to be major providers.[19] When phenylhydrazine reticulocytes were employed, no change in the plasma iron occurred, and thus the increment in iron delivered was derived entirely from tissues. Iron-deficient reticulocytes produced a mean decrease of 12% in the plasma iron concentration over the first 20 min but little change thereafter. If these differences between the two types of reticulocytes have significance, they would suggest that a more ample iron supply was provided by the increased catabolism of phenylhydrazine reticulocytes and that iron not immediately available from tissues would be obtained at the expense of the plasma iron pool. This phenomenon has been described in patients with vitamin B_{12} deficiency responding to the injection of vitamin B_{12} where a reduction in the supply of iron from the breakdown of red cell precursors is associated with a fall in plasma iron.[23] The fall in plasma iron in turn reduces plasma iron turnover until a new equilibrium is reached.

Iron mobilization could be more directly examined by monitoring iron absorption, which in 200-g normal rats provides about 50% of the iron supply for erythropoiesis.[19] Absorption after exchange transfusion with phenylhydrazine and iron-deficient reticulocytes was consistently increased, with mean values of +50% to 130% over controls in the various groups studied. The

increase observed at 1 hr was continued for at least 6 hr. It should also be noted that the use of phenylhydrazine reticulocytes, which appeared to be associated with more red cell destruction, and the more ample supply of iron for transferrin from internal tissues, did not decrease the absorptive response despite some evidence that this might occur.[24,25]

The extremely rapid regulation of iron flow through the plasma and across the gut demonstrated by these studies would be consistent with the suggestion that internal iron exchange might be automatically regulated by the number of unsaturated iron binding sites on the circulating transferrin molecules.[6,7] According to the "open site" proposal, as more iron is removed from transferrin by tissues, the number of transferrin sites capable of binding iron increases, and this leads to an increased rate of entry of iron into the plasma. Measurements of plasma iron change in the present study did not support this hypothesis. While an early decrease of −11% in animals exchanged with high reticulocyte blood was somewhat more than the −6% in animals exchanged with normal blood, there was no significant correlation between plasma iron fall and changes in turnover. A better test of the hypothesis was carried out by markedly increasing the unsaturated iron binding capacity in three studies (groups 4 and 5 of Table 1 and group 1 of Table 4). These additional studies along with other prior reports[26,27] also failed to support the "open site" hypothesis in respect to either plasma iron turnover or absorption.

There are two different effects that influence internal iron exchange. The first is the direct relationship between the amount of diferric transferrin and the plasma iron turnover.[2,4] Thus, iron delivery to tissues from transferrin can be retarded by a lowering of plasma iron concentration or increased by an increase in plasma iron concentration. This documented effect is quite the opposite of the "open site" hypothesis referred to above. The second effect is the dominant role of tissue iron requirements as mediated by the number of membrane receptors for the transferrin iron complex. With an increase in requirements (or tissue transferrin receptors), iron procurement is increased as long as there is iron to mobilize. If available iron supply from tissues is not adequate, the plasma iron concentration will fall, reducing the amount of diferric transferrin and decreasing turnover until a new equilibrium point is reached.

Little can be said at this time about the nature of the remarkable coupling between iron donation and iron procurement except that it is not mediated by the unsaturated iron-binding capacity. Although it is tempting to postulate that some change in the transferrin molecule is responsible, no such alteration has

yet been detected. The presence of such a regulating mechanism, however, raises the possibility that a disorder at this point might explain the iron block of inflammation and the uncontrolled parenchymal iron loading in idiopathic hemochromatosis. In such disorders the abnormality involves the release of iron from both the reticuloendothelial cell and intestinal mucosa,[8,28] one blocking and the other augmenting tissue iron release.

REFERENCES

1. Aisen P: The transferrins, in Jacobs A, Worwood M (eds): Iron in Biochemistry and Medicine, II. London, Academic, 1980, pp 87–129

2. Huebers H, Huebers E, Csiba E, Finch CA: Iron uptake from rat plasma transferrin by rat reticulocytes. J Clin Invest 62:944–951, 1978

3. Huebers H, Csiba E, Josephson B, Huebers E, Finch C: Interaction of human diferric transferrin with reticulocytes. Proc Natl Acad Sci USA 78:621–625, 1981

4. Huebers H, Finch CA, Eng M, Miller L: Uptake and release of iron from human transferrin. Proc Natl Acad Sci USA 78:2572–2576, 1981

5. Finch CA, Deubelbeiss K, Cook JD, Eschbach JW, Harker LA, Funk DD, Marsaglia G, Hillman RS, Slichter S, Adamson, JW, Ganzoni A, Giblett ER: Ferrokinetics in man. Medicine 49:17–53, 1970

6. Cavill I, Worwood, M, Jacobs A: Internal regulation of iron absorption. Nature 256:328, 1975

7. Hershko C: Storage iron regulation, in Brown EB (ed): Progress in Hematology, vol X. New York, Grune & Stratton, 1977, pp 105–148

8. Bothwell TH, Charlton RW, Cook JD, Finch CA: Iron Metabolism in Man. Oxford, Blackwell Scientific, 1979, p 276

9. Weintraub LR, Conrad ME, Crosby WH: The significance of iron turnover in the control of iron absorption. Blood 24:19–24, 1964

10. Hahn PF, Bale WF, Ross JF, Balfour WM, Whipple GH: Radioactive iron absorption by gastrointestinal tract. Influence of anemia, anoxia and antecedent feeding. Distribution in growing dogs. J Exp Med 51:24, 1943

11. Bothwell TH, Pirzio-Biroli G, Finch CA: Iron absorption. I. Factors influencing absorption. J Lab Clin Med 51:24, 1958

12. Brittin GM, Haley J, Brecher G: Enhancement of intestinal iron absorption by a humoral effect of hypoxia in parabiotic rats. Proc Soc Exp Biol Med 128:178–184, 1968

13. Weintraub LR, Conrad ME, Crosby WH: Regulation of the intestinal absorption of iron by the rate of erythropoiesis. Br J Haematol 11:432–438, 1965

14. Hathorn M: The absorption and plasma clearance of iron in the hypoxic rat. J Physiol 191:133–134, 1967

15. Finch CA, Miller LR, Inamdar AR, Person R, Seiler K, Mackler B: Iron deficiency in the rat. Physiological and biochemical studies of muscle dysfunction. J Clin Invest 58:447–453, 1976

16. Bauer W, Stray S, Huebers H, Finch CA: The relationship between plasma iron and plasma iron turnover in the rat. Blood 57:239–242, 1981

17. The International Committee for Standardization in Hematology: Proposed recommendations for measurement of serum iron in human blood. Blood 37:598, 1971

18. Cook JD: An evaluation of absorption methods for measurement of plasma iron binding capacity. J Lab Clin Med 76:497, 1970

19. Cook JD, Hershko C, Finch CA: Storage iron kinetics. V. Iron exchange in the rat. Br J Haematol 25:695–706, 1973

20. Cook JD, Marsaglia G, Eschbach JW, Funk DD, Finch CA: Ferrokinetics: A biologic model for plasma iron exchange in man. J Clin Invest 49:197–205, 1970

21. Skarberg K, Eng M, Huebers H, Marsaglia G, Finch C: Plasma radioiron kinetics in man: Explanation for the effect of plasma iron concentration. Proc Natl Acad Sci USA 75:1559–1561, 1978

22. Finch CA: Iron kinetics and erythropoiesis, in Ricketts C, Cavill I (eds): Iron Metabolism (CIBA). Amsterdam, Elsevier, 1977, pp 161–166

23. Finch CA, Coleman DH, Motulsky AG, Donohue DM, Reiff RH: Erythrokinetics in pernicious anemia. Blood 11:807–820, 1956

24. Rosenmund A, Gerber S, Huebers H, Finch C: Regulation of iron absorption and storage iron turnover. Blood 56:30–37, 1980

25. Worwood M, Jacobs A, Cavill I: Iron absorption: Regulation by internal iron exchange, in Crichton RR (ed): Proteins of Iron Storage and Transport in Biochemistry and Medicine. Amsterdam, North-Holland, 1975, pp 401–404

26. Schade SG, Bernier GM, Conrad ME: Normal iron absorption in hypertransferrinaemic rats. Br J Haematol 17:187, 1969

27. Levine PH, Levine AJ, Weintraub L: The role of transferrin in the control of iron absorption: Studies on a cellular level. J Lab Clin Med 80:333, 1972

28. Fillet G: Etude radioisotopique de la reutilisation du fer chez l'animal et chez l'homme. Institut de Medecine, Universite de Liege, Thesis, 1977