JCI The Journal of Clinical Investigation

THE ANEMIA OF INFECTION. IV. THE LACK OF RELATIONSHIP BETWEEN THE DIVERSION OF IRON FROM THE PLASMA AND THE ORIGIN OF THE ANEMIA

G. R. Greenberg, … , Marjorie Lauritsen, M. M. Wintrobe

J Clin Invest. 1947;26(1):114-120. https://doi.org/10.1172/JCI101783.

Research Article

THE ANEMIA OF INFECTION. IV. THE LACK OF RELATIONSHIP BETWEEN THE DIVERSION OF IRON FROM THE PLASMA AND THE ORIGIN OF THE ANEMIA [1]

By G. R. GREENBERG, HELEN ASHENBRUCKER, MARJORIE LAURITSEN, AND M. M. WINTROBE

(From the Department of Medicine, University of Utah Medical School, Salt Lake City)

(Received for publication September 21, 1946)

The pronounced and persistent hypoferremia which accompanies infection, and the rapidity with which intravenously injected iron is removed when given in such cases (1), have led to the suggestion that the anemia of chronic infection results from a local iron deficiency in the bone marrow. Since the quantity of free protoporphyrin in the erythrocytes has been found increased in association with the anemia of infection, the possibility exists that this anemia is the consequence of deficient formation of hemoglobin resulting from a lack of iron.

The studies to be described here were designed to test the validity of this hypothesis. Iron was infused continuously in patients with chronic infection in order to determine whether, by raising the iron level to the normal value, synthesis of hemoglobin could be induced. These observations have made it possible to study the rate and degree of diversion of iron from the plasma in infection. Several observations also have been made of the uptake of intravenously administered radioactive iron by the red cells of patients with acute and chronic infections. Evidence will be presented in this communication suggesting that while a rapid removal from the plasma of injected iron occurs in infection, another factor, rather than lack of iron, may be responsible for the development of anemia.

METHODS

Ferrous ascorbate was used for the continuous infusion studies. A 2 per cent aqueous solution was carried through a Seitz filter. With a syringe this was added to a bottle containing 500 ml. sterile 5 per cent glucose solution; thus, the final solution contained 4 mgm. of iron per 100 ml. Since ferrous ascorbate is a complex with varying amounts of iron (2), each lot had to be analyzed, and the amount used varied accordingly. To obtain quickly the elevated plasma iron level, a single dose of 10 mgm. of iron as the ascorbate was injected intravenously shortly after starting the infusion. Considerable difficulty was encountered because of the frequent development of phlebitis after prolonged infusion of iron. This may have been due to the fact that the solutions were too acid (the undiluted ferrous ascorbate solutions exhibited a pH of about 4), or it may have been caused by the continued exposure of the blood vessels to relatively high concentrations of iron. Later it was found that it is possible to raise the pH somewhat if an excess of ascorbic acid is added and the solution is titrated with alkali, as we have done in studies with radioactive iron (3). The radioactive iron for the uptake studies was prepared according to the procedure described in the preceding paper (3), but in patients pyrogen-free, water was used, and the final solution was heated at 70° C. for 3 hours.

The methods for preparing and electroplating Fe^{59} in the blood samples have been described elsewhere (4). In these studies, blood volume has been estimated by assuming a value of 80 ml. per kgm. body weight (5). A few determinations of hemin Fe^{59} have been made according to the technique described in the previous paper (3).

Plasma iron was estimated according to the method of Kitzes, Elvehjem and Shuette (6).

RESULTS

Diversion of intravenously injected iron:

In Figure 1 are presented the results of the continuous intravenous infusion of iron in patients with chronic infection and anemia. It will be seen that very great difficulty was experienced in maintaining an elevated plasma iron level in the presence of infection. In order to maintain the plasma concentration at the normal level, it was necessary to employ several times the quantity of iron that might have been expected on the basis of single intravenous injections of iron. When the quantity of iron infused per unit of time was doubled, the plasma iron level increased by much less than a factor of 2. On the other hand, it was relatively easy to produce a high plasma iron level in the iron-deficient patient shown in the same figure. It is known that the iron-deficient patient takes up iron rapidly for hemoglobin formation. Consequently the drain on the plasma iron in the pres-

[1] Aided by a grant for the study of the pathogenesis of the anemia of infection from the United States Public Health Service; and by grants for the study of hematology and nutrition from the Upjohn Company and Parke, Davis and Company.

ence of infection must be considerably greater than that associated with increased hemoglobin synthesis following the administration of iron in iron deficiency anemia.

That the forced elevation of the plasma iron level did not produce increased hemopoiesis is shown by the results in the case of C. S. (Figure 2). Although this patient's iron level was maintained at approximately the normal value for as much as 72 hours, and enough iron was infused in the 2 attempts to form 31 and 80 grams of hemoglobin, respectively, little increase in reticulocytes occurred, and no significant increase in hemoglobin per 100 ml. of blood took place.

Table I summarizes data presented in paper I of this series (1) on the rate of disappearance from the plasma of iron after a single intravenous injection. From these data it is possible to show that in both the normal subject and in the patient with infection, some mechanism acts to prevent the plasma iron level from rising to the expected values after the injection of iron. In the patient with

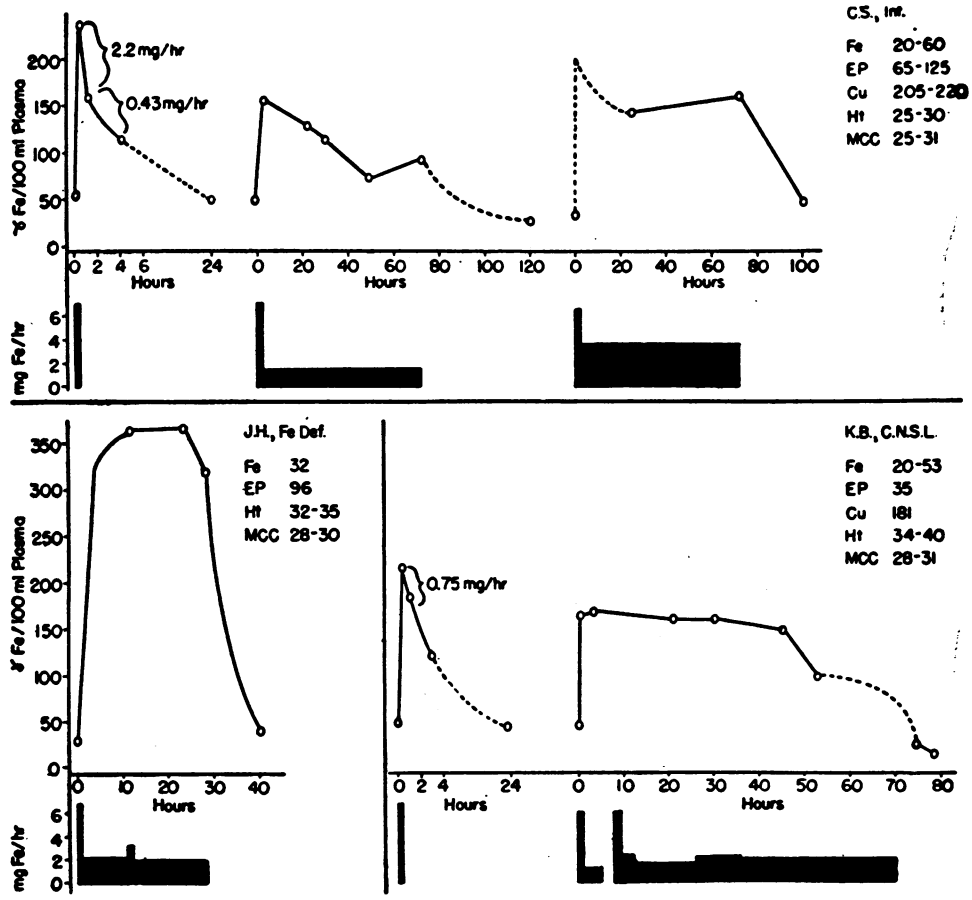

FIG. 1. THE EFFECT ON THE PLASMA IRON CONTENT OF SINGLE AND CONTINUOUS INJECTIONS OF IRON ASCORBATE IN A PATIENT WITH ANEMIA ASSOCIATED WITH INFECTION (C.S.), IN A PATIENT WITH IRON DEFICIENCY ANEMIA (J.H.), AND IN A PATIENT WITH CENTRAL NERVOUS SYSTEM SYPHILIS (K.B.)

Under each patient's initials are given the plasma iron (Fe), the erythrocyte protoporphyrin (E.P.), the serum copper (Cu), the volume of packed red cells (Ht.), and the mean corpuscular hemoglobin concentration (MCC). [For normal values see (1).]

Note that in the cases of infection the plasma iron content could not be maintained at a level higher than normal, even though much larger quantities of iron were being injected intravenously than would be expected to be necessary from the rate of disappearance of single injections of iron. This was not true in the case of iron deficiency anemia, even though the need for iron was great.

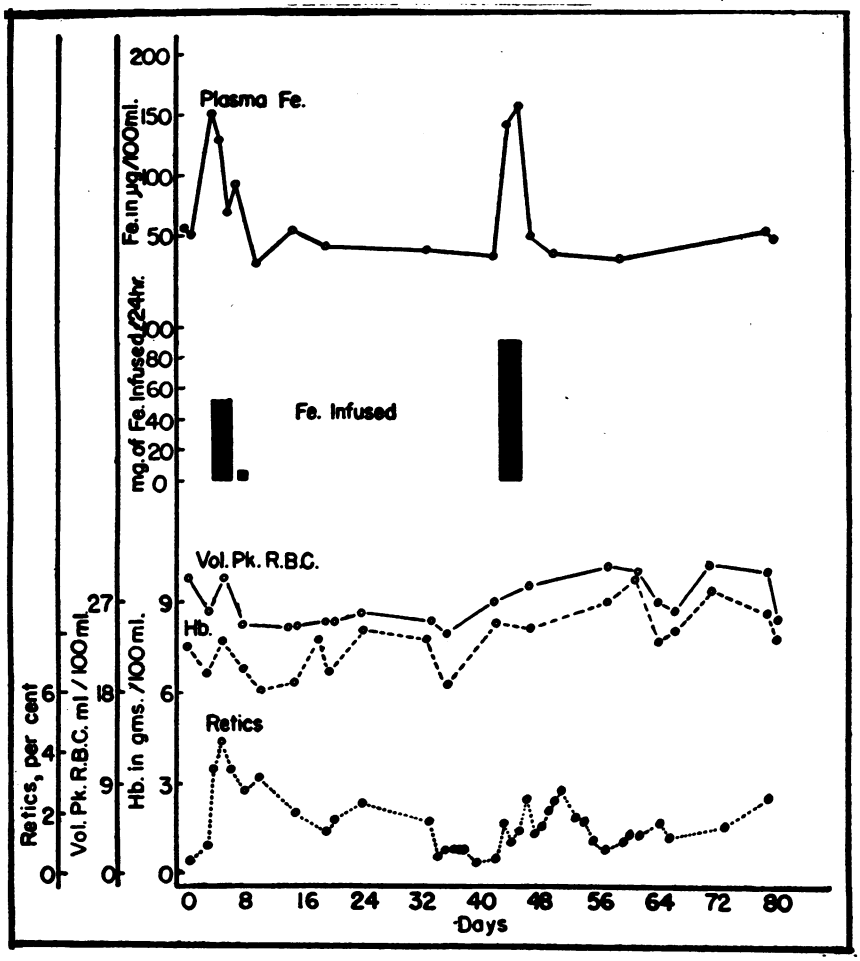

FIG. 2. FAILURE TO INDUCE BLOOD FORMATION OR TO PRODUCE A SIGNIFICANT RETICULOCYTE RESPONSE IN A PATIENT WITH A CHRONIC INFECTION, EVEN WHEN IRON WAS GIVEN CONTINUOUSLY BY VEIN AND THE PLASMA IRON WAS MAINTAINED AT A NORMAL LEVEL

Further details concerning this patient (C.S.) are presented in Figure 1.

infection this "brake" apparently acts at a lower level of iron. If it is assumed that the blood volume corresponds to 80 ml. per kgm. body weight, and that the patients and the normal subjects had average packed red cell volumes of 35 and 45 ml. per 100 ml., respectively, it is possible to calculate that, on the average, the plasma iron of the patients should have risen 250 μg. per 100 ml., and the plasma iron of the normal subjects should have increased 311 μg. per 100 ml. of plasma after the injection of 137 μg. and 130 μg. iron per kgm., respectively. Thus, in the patients, on the average, a maximum level of 43 + 250 = 293 μg. per 100 ml. should have been attained, and in the normal subjects a peak value of 126 + 311 = 437 μg. per 100 ml. should have been reached. The actual observed average levels were 196 and 315 μg., respectively. Failure to observe higher values was not due to a failure to obtain the true peak, since in one normal subject blood samples were drawn each minute or two for 10 minutes after the injection of iron; the peak rise was at approximately 5 minutes. Thus, the rate of loss of iron from the plasma in the first few minutes is approximately 100 times as rapid as in the succeeding hours. The data indicate that the rate of disappearance of iron from the plasma of a patient with infection, is about twice that seen in the normal subject in the time following the initial few minutes after the injection of iron (Table I).

TABLE I

Rate of disappearance of iron from the plasma following a single intravenous injection of iron in normal subjects and in patients with infection

	Fe injected	Initial plasma iron level	Maximum rise 5 minutes after injection	Iron lost per hour per kgm.		
				At hour 1	At hour 2.5	At level of 140 μg. iron per 100 ml. plasma
Normal subjects						
Number of subjects	13	13	13	13	13	4
	μg. per kgm.	*μg. per 100 ml.*	*μg. per 100 ml.*	*μg.*	*μg.*	*μg.*
Range	137	85–237	262–392	−38 to +11	−4 to −25	−11 to −13
Average	137	126	315	−10	−12	−12
Patients with infections						
Number of subjects	13	14	14	14	14	10
	μg. per kgm.	*μg. per 100 ml.*	*μg. per 100 ml.*	*μg.*	*μg.*	*μg.*
Range	130	16–90	137–246	0 to −38	0 to −39	−15 to −90
Average	130	43	196	−17	−27	−27

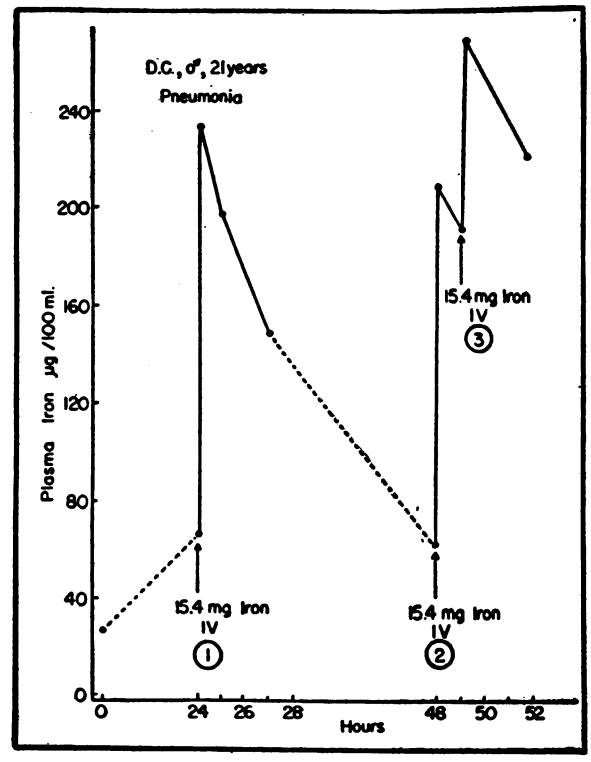

FIG. 3. THE EFFECT OF ATTEMPTS TO RAISE THE PLASMA IRON LEVEL BY THE INTRAVENOUS ADMINISTRATION OF REPEATED DOSES OF IRON IN THE PRESENCE OF INFECTION

It was difficult to maintain a high plasma iron level, even when a second injection was made before the plasma iron content had dropped. Note also that the rise following the third injection was relatively slight as compared with the rise following the previous injections. This suggests that a "braking" mechanism exists.

In order to determine how rapidly the mechanism operating in infection can readjust to prevent a further rise in the plasma iron level, 3 injections of iron, 2 in a period of 1 hour, were given to a patient suffering from pneumonia. The results of this experiment are shown in Figure 3. It will be observed that the rise obtained after the third injection was only slightly higher than that obtained after the second injection given 1 hour before. On the basis of the peak rise after the second injection it might be expected that the level following the third injection would have been 334 μg. iron per 100 ml. The observed rise was to 266 μg. per 100 ml. Apparently the mechanism preventing the rise in plasma iron is able to readjust itself quickly in infection.

The uptake of Fe^{59}:

Figure 4 illustrates the results of preliminary studies on the rate of incorporation of intravenously injected Fe^{59} into the erythrocytes as hemoglobin iron in patients with various infections, and in normal subjects. The dose of Fe^{59} employed in these studies ranged between 3 and 4 mgm., depending on the body weight. These data suggest that the degree of impairment of the uptake of Fe^{59} appears to be roughly proportional to the severity of the infection. It will be noticed that while there was an initial delay in the uptake of Fe^{59} by the patients with infection as compared to the normal subjects, in some of the patients after the first few days the rate of uptake approximated that of normal subjects, and the labelled iron was utilized almost completely. An example of this is seen in the case of T. G. who was suffering from a long-standing osteomyelitis. This pa-

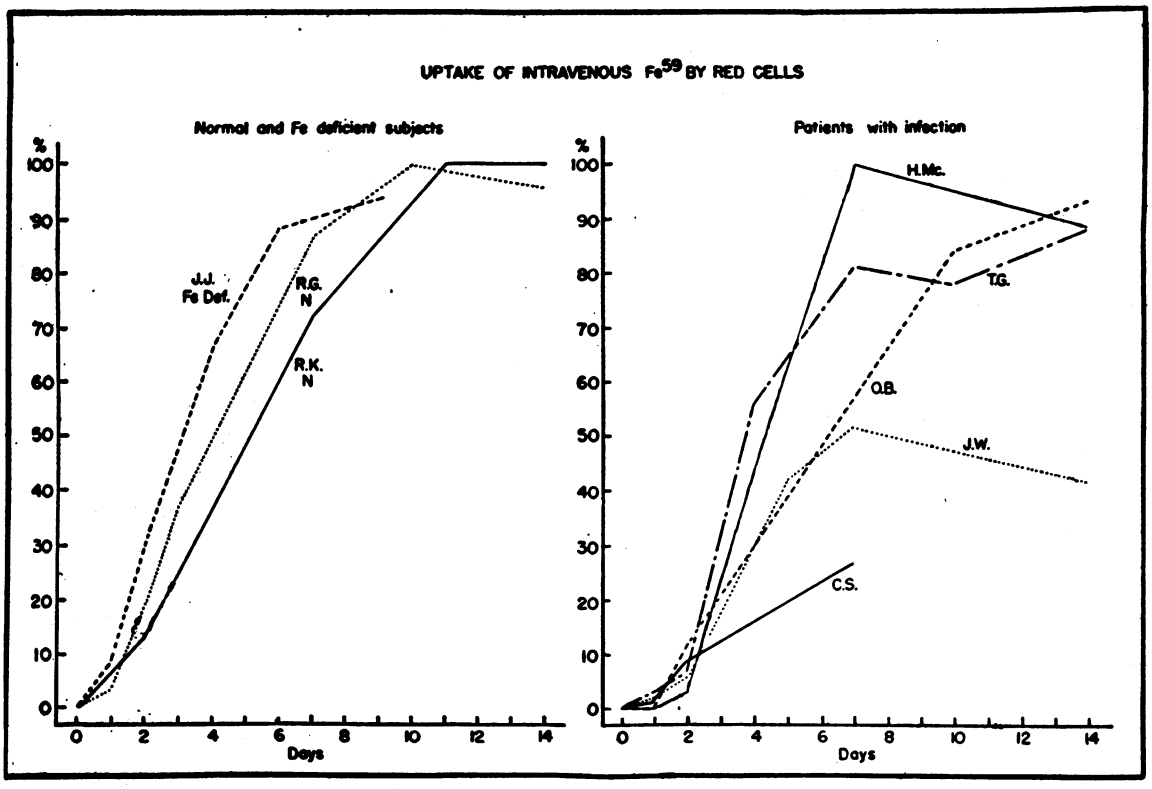

FIG. 4. UPTAKE OF 3 TO 5 MGM. INTRAVENOUSLY INJECTED RADIOACTIVE IRON (Fe^{59}) BY THE RED CELLS OF NORMAL (N) AND IRON-DEFICIENT (FE DEF.) PATIENTS, AND IN PATIENTS WITH VARIOUS INFECTIONS

In the patients with severe infections (C. S. and J. W.) there was not only a delay in uptake, but the uptake never was complete; whereas in those less ill, less difference was observed as compared with uptake in normal individuals. C. S. had chronic suppurative arthritis and chronic pyelonephritis. The volume of packed red cells (Ht.) was 28 ml. per 100 ml. blood. J. W. had subacute bacterial endocarditis, Ht. 30 ml. Both O. B. and T. G. had osteomyelitis and the volume of packed red cells was 39 and 40 ml., respectively. H. Mc. had rheumatic fever, Ht. 42 ml.

tient exhibited extremely low plasma iron concentration, rapid diversion from the plasma of intravenously injected iron, high erythrocyte protoporphyrin level and elevated serum copper, in addition to anemia. He was refractory to all forms of iron therapy (1). Yet approximately 100 per cent of the small dose of Fe^{59} which was given did enter his red cells. This was to be expected since, at a rate of hematopoiesis which maintains hemoglobin at 50 per cent of the normal, it can be calculated that about 12 mgm. of iron is being converted into hemoglobin in a man of average size (7).

Table II presents evidence that Fe^{59} appearing in the red cells after intravenous injection is present as hemoglobin iron. Hemin was isolated and its Fe^{59} content per mgm. of iron was compared with the Fe^{59} of the original blood per mgm. of hemoglobin iron. Such data have been presented for pigs in the preceding paper (3).

DISCUSSION

These observations indicate that in both normal subjects and in patients with infection, a mechanism operates which prevents the plasma iron level from increasing beyond certain limits. The major loss of injected iron occurs during the first few minutes when the rate of loss may be as much as 100 times as rapid as the loss in the ensuing hours. The initial loss of iron is approximately of the same absolute order of magnitude in both the normal subject and in the patient with infection, whereas the ensuing rate of loss is about twice as rapid in the presence of infection. This braking mechanism is so powerful that as more iron is given and the level is raised by repeated

TABLE II

Quantity of red cell Fe^{59} existing as hemoglobin iron

Subject	Status	Day following Fe^{59} injection	Percentage of RBC Fe^{59} present as hemoglobin
R.K.	Normal female	21	105*
R.G.	Normal male	16	107**
O.B.	Osteomyelitis	21	100*
A.G.	Pernicious anemia with iron deficiency	21	99*

* The actual quantity of isolated hemin analyzed for Fe^{59} was determined by measuring the iron content of an aliquot part (13) and calculating from the known percentage of Fe in hemin.

** The quantity of isolated hemin analyzed for Fe^{59} was determined by weighing after drying at 100° C.

injections or by continuous intravenous infusion of iron, it becomes increasingly difficult to maintain the plasma iron concentration. In a study of the use of intravenous iron tolerance curves for the differentiation of anemias, Waldenström (8) likewise concluded that a braking mechanism exists which prevents the attainment of calculated peak plasma iron values.

In spite of the existence of such a mechanism, it was possible to raise the plasma iron level temporarily to normal values in anemic patients suffering from chronic infections; yet hemoglobin formation did not take place. The strongest argument against the hypothesis that iron is the limiting factor in the anemia of chronic infection is the fact that both intensive and prolonged therapy with iron, administered orally and intravenously, will not bring about increased hemoglobin formation (1). Actually, iron therapy has been given the most severe test of any employed thus far. Schaefer (9) was able by intensive iron therapy to raise the serum iron level of children with infection to approximately normal values without effecting a change in the anemia. Brøchner-Mortensen and Stein (10) treated patients with tuberculosis with iron by mouth for several months at a time. These patients showed a greater and more constant rise in the serum iron than was observed in those given no iron, but the average change in hemoglobin before and after treatment was less than one per cent.

It cannot be maintained that a normal plasma iron level must exist before iron can be used for hematopoiesis, for in such a case, in the face of persistent hypoferremia, the anemia of infection should be progressive and ultimately should become very severe. Yet it is well known that the anemia tends to become fixed usually at a moderate and, often thereafter, at a rather stable level. Furthermore, in the presence of active hematopoiesis we have observed, like others (11), that in pernicious anemia after liver therapy as well as in iron deficiency anemia, the plasma iron level tends to remain low until the anemia has wholly or largely disappeared. Very active hematopoiesis can, thus, take place when the plasma iron content is low. Finally, as has been shown here by the use of radioactive iron, if small amounts of iron are given they can be utilized for the formation of hemoglobin in spite of the existence of infection. That this should be found to be the case is to be expected, since otherwise no red corpuscles containing hemoglobin would be available to replace those which become worn out in the course of time.

As a consequence, it must be concluded that although diversion of iron from the plasma occurs in infection, the anemia itself does not result from a lack of iron. Some other factor may be deficient, or some enzyme system may be interfered with by some process associated with infection. In agreement with Brøchner-Mortensen and Stein (10) it appears that there is a lack of capacity on the part of the bone marrow to utilize iron for hemoglobin synthesis rather than unavailability of iron.

In spite of the evidence that the lack of iron does not cause the anemia, it is clear that in acute and chronic infection a very marked diversion of iron from the plasma occurs. The system taking up intravenously injected iron in infection apparently withdraws it from the plasma with more avidity than an iron-deficient subject uses iron for hemoglobin formation. The site of diversion of the iron is the subject of the paper which follows (12).

SUMMARY

1. Patients with anemia of chronic infection have been subjected to continuous intravenous infusions of iron for periods as long as 72 hours.

2. Considerably more iron was required to maintain a normal plasma iron level than would be expected from the results of a single intravenous in-

jection of iron. The higher the plasma iron value desired, the more inefficient was the infusion.

3. In neither case did the infusion of iron result in increased hemoglobin formation.

4. Calculations have been made suggesting that a braking mechanism exists which prevents the plasma iron from rising above a certain level.

5. Evidence has been presented from radioactive iron studies and observations of the nature of the anemia, that some iron is capable of entering the erythrocytes as hemoglobin in the anemia of chronic infection.

6. Since even very intensive iron therapy is ineffective, it follows that iron is not the limiting factor in the production of the anemia, even though diversion of iron from the plasma does occur.

BIBLIOGRAPHY

1. Cartwright, G. E., Lauritsen, M. A., Humphreys, S. R., Jones, P. J., Merrill, I. M., and Wintrobe, M. M., The anemia of infection. I. Hypoferremia, hypercupremia and alterations in porphyrin metabolism in patients. J. Clin. Invest., 1946, **25**, 65.
2. Friend, D. G., Iron ascorbate in the treatment of anemia. New England J. Med., 1938, **219**, 910.
3. Wintrobe, M. M., Greenberg, G. R., Humphreys, S. R., Ashenbrucker, H., Worth, W., and Kramer, R., The anemia of infection. III. The uptake of radioactive iron in iron-deficient and in pyridoxine-deficient pigs before and after acute inflammation. J. Clin. Invest., 1947, **26**, 103.
4. Greenberg, G. R., Humphreys, S. R., Ashenbrucker, H., Lauritsen, M., and Wintrobe, M. M., A simple apparatus and procedure for preparing and electroplating radioactive iron. Blood, 1947, **2**, 94.
5. Moore, C. V., Dubach, R. Minnich, V., and Roberts, H. K., Absorption of ferrous and ferric radioactive iron by human subjects and by dogs. J. Clin. Invest., 1944, **23**, 755.
6. Kitzes, G., Elvehjem, C. A., and Schuette, H. A., The determination of blood plasma iron. J. Biol. Chem., 1944, **155**, 653.
7. Greenberg, G. R., and Wintrobe, M. M., A labile iron pool. J. Biol. Chem., 1946, **165**, 397.
8. Waldenström, J., Jarnbelastningar och vad de Lära oss om Järnomsättningen, Om Järn och Järnterapi, Utgiven av ab Ferrosan, 1944.
9. Schaefer, K. H., Untersuchungen uber den exogenen Eisenstoffwechsel bei fieberhaften Infekten im Kindesalter. Klin. Wchnschr., 1940, **19**, 979.
10. Brøchner-Mortensen, K., and Stein, K. S., Studies on the iron content of serum in patients with acute and chronic infections. Acta Tuberculosea Scandinav., **16**, 334.
11. Moore, C. V., Doan, C. A., and Arrowsmith, W. R., The mechanism of iron transportation: Its significance in iron utilization in anemic states of varied etiology. J. Clin. Invest., 1937, **16**, 627.
12. Greenberg, G. R., Ashenbrucker, H., Lauritsen, M., Worth, W., Humphreys, S. R., and Wintrobe, M. M., The anemia of infection. V. Fate of injected radioactive iron in the presence of inflammation. J. Clin. Invest., 1947, **26**, 121.
13. Delory, G. E., Preparation and analysis for iron of hemin and hemoglobin. Analyst, 1943, **68**, 5.