The Effect of Inflammation on the Utilization of Erythrocyte and Transferrin Bound Radioiron for Red Cell Production

By EMIL J. FREI ReICH, AARON MILLER, CHARLES P. EMERSON AND JOSEPH F. ROSS

INFECTION AND INFLAMMATION have been shown to impair the ability of the bone marrow to respond to increased demands for red cell production. Following acute or chronic blood loss, inflammation slows the rate of hemoglobin production;4 in pernicious anemia patients undergoing adequate vitamin B-12 therapy, infection slows the rate of recovery;5 in patients with hemolytic anemias, inflammation can depress hemoglobin production and result in hypoplastic crises.3, 4 While inflammation does depress hemoglobin production, the mechanism by which this occurs is not known.

Inflammation also produces marked changes in iron metabolism. A decrease in serum iron concentration is a constant finding.5 The mechanism of the hypoferremia and its role in the bone marrow depression is not clearly understood. Previous studies utilizing intravenously injected radioiron have indicated that the incorporation of radioiron into red cells is reduced in the presence of infection or inflammation.5, 8 These findings suggested that inflammation interferes with the incorporation of iron into protoporphyrin for heme synthesis in the bone marrow.5 However more recent studies utilizing trace quantities of high specific activity radioiron have shown no impairment of iron incorporation into red cells in the presence of chronic infection and inflammation.9-11 This disparity suggested that inflammation may impair the release of iron from the tissues to the plasma, since the earlier studies showing impaired red cell uptake of injected radioiron utilized relatively large quantities of unbound iron which is cleared rapidly from the plasma by the tissues.

In normal animals the iron released from destroyed red cells is rapidly reutilized for red cell production in preference to storage iron.12 The present study demonstrates that in the presence of inflammation, the rate of reutilization of radioiron from senescent red cells is markedly impaired while the utilization of plasma transferrin bound iron is unimpaired. These changes result in hypoferremia and could contribute to the impaired hemoglobin production which is so consistently found in the presence of inflammation and infection. A preliminary report of this work has been previously published.13

MATERIALS AND METHODS

Ten mongrel dogs were studied (table 1). These animals were inoculated against distemper, de-wormed and subjected to a three week observation period before study. No anesthesia was used in this study. All the animals were apparently normal with one exception (Dog H). Dog A served as a plasma donor. This plasma was used as the source of...
transferrin in all experiments. Dog K served as a red cell donor for the radioactive iron labeled red cells used in all experiments.

Iron Heavy Animals. Two animals (Dogs E and F) were given intravenous injections of Feojectin three times a week over a 60 day period. Individual doses of 100 to 200 mg. of iron were given for a total dose of 2,700 mg. of iron for each animal.

Iron Deficient Animal. Dog H was fortuitously found to have a microcytic hypochromic anemia, with hematocrits in the range of 30 to 33% and reticulocytes of 1.5%. This animal's red cell indices (mean of five determinations) were: M.C.H.C. = 30.0, M.C.V. = 58.3, M.C.H. = 17.9. For comparison, the mean red cell indices in the normal animals studied were: M.C.H.C. = 31.1, M.C.V. = 72.7, M.C.H. = 22.1. At the end of the experiment, Dog H was sacrificed and at autopsy no stainable iron was found in any of this animal's tissues, confirming the diagnosis of iron deficiency. The etiology of the iron deficiency was not determined.

Radioiron Preparation and Detection. Fe²⁺ of high specific activity was obtained as ferric chloride from the Oak Ridge National Laboratory, Oak Ridge, Tennessee. This was converted chemically to ferric ammonium citrate, its pH adjusted to 6.8 to 7.0, and the product sterilized by autoclaving. Radioactivity assays were performed with gamma scintillation counting equipment using thallium activated sodium iodide crystals. Liquid samples were counted under conditions of constant geometry in a well type crystal. Counting efficiency was 4-5% and sufficient counts were recorded to give a counting error of less than 2%.

Preparation of Senescent, Non-viable Radioiron Labelled Red Cells. Dog K, a 10 Kg. female was used as a red cell donor on three separate occasions. For each donation, the animal was bled approximately 100 ml. daily for 5 to 8 days. This caused marked anemia (Hgb. of 5 to 7 Gm.%) and hypochromia (M.C.H.C. of 25 to 28%). At the end of this period of bleeding, a 200 to 300 microcurie dose of Fe²⁺ was injected intravenously. Seven to 10 days later, blood was drawn aseptically into 2.5% trisodium citrate. This blood was stored at 37 C. for 7 to 9 hours and at room temperature for an additional 36 hours. At

Table 1

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Animal</th>
<th>Sex</th>
<th>Weight (Kg.)</th>
<th>Condition</th>
<th>Total Hemoglobin Injected (mg.)</th>
<th>Total Iron Injected (mg.)</th>
<th>% Of Circulating Red Cell Mass</th>
<th>Fe²⁺ µc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>B</td>
<td>F</td>
<td>18</td>
<td>Abscessed</td>
<td>822</td>
<td>2.80</td>
<td>0.43</td>
<td>5.0</td>
</tr>
<tr>
<td>C</td>
<td>M</td>
<td>19</td>
<td>Abscessed</td>
<td>707</td>
<td>2.40</td>
<td>0.34</td>
<td>5.3</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>J</td>
<td>F</td>
<td>18</td>
<td>Abscessed</td>
<td>337</td>
<td>1.14</td>
<td>0.17</td>
<td>3.0</td>
</tr>
<tr>
<td>D</td>
<td>M</td>
<td>17</td>
<td>Control</td>
<td>337</td>
<td>1.14</td>
<td>0.16</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>F</td>
<td>M</td>
<td>19</td>
<td>Abscessed</td>
<td>668</td>
<td>2.26</td>
<td>0.36</td>
<td>6.0</td>
</tr>
<tr>
<td>E</td>
<td>M</td>
<td>21</td>
<td>Control</td>
<td>663</td>
<td>2.25</td>
<td>0.33</td>
<td>6.0</td>
<td></td>
</tr>
<tr>
<td>V₁</td>
<td>H</td>
<td>M</td>
<td>18</td>
<td>Control</td>
<td>706</td>
<td>2.39</td>
<td>0.48</td>
<td>5.3</td>
</tr>
<tr>
<td>V₂</td>
<td>H</td>
<td>M</td>
<td>18</td>
<td>Abscessed</td>
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<td>2.25</td>
<td>0.45</td>
<td>6.0</td>
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<tr>
<td>VI</td>
<td>G</td>
<td>F</td>
<td>16</td>
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<td>702</td>
<td>2.38</td>
<td>0.42</td>
<td>5.3</td>
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</table>
the end of this period there was no visible hemolysis, and assay of the supernatant plasma revealed a hemoglobin concentration of less than 65 mg%. Less than 2% of the total radioactivity of the whole blood was present in the supernatant plasma. The specific activity of this blood was approximately 1 microcurie per 500 to 600 micrograms of iron. After storage, the blood was injected into study animals in a 10 ml. volume containing a total of 335 to 825 mg of hemoglobin which represented 0.1 to 0.5% of the recipient animals circulating hemoglobin (table 1). The total radioactivity injected ranged from 3 to 6 microcuries.

**Preparation of Transferrin Bound Radioiron.** Radioactive iron was added to plasma from Dog A in a ratio of less than one microgram of iron to each ml. of plasma, and incubated at 37 C. for 30 minutes to insure complete binding of the iron to transferrin. A total of 3 to 10 micrograms of iron and 7 to 10 microcuries of radioactivity was injected into each study animal.

**Blood Volume Measurement.** After injection of transferrin bound Fe\(^{59}\), the activity per ml. of plasma in the 10, 20 and 30 minute samples was plotted on semi-log paper and extrapolated linearly to zero time. The activity present at zero time was used for computation of plasma volume. Total blood volume and red cell volume were computed from plasma volume and venous hematocrit in accord with the data of Reeve et al.\(^4\) Venous hematocrit was determined in Wintrobe type hematocrit tubes spun at 2500 G for 30 minutes. The mean value obtained from three separate samples was multiplied by a factor of 0.96 to correct for trapped plasma; and since normal, unsplenectomized, unanesthetized dogs were found to have a mean total body hematocrit/venous hematocrit ratio of 0.981,\(^4\) no further correction factor was utilized.

**Red Cell Uptake of Radioiron.** Samples of whole blood were hemolyzed with saponin and radioactivity assayed. Activity was expressed per ml. of red cells, which multiplied by the red cell mass gave the total activity in the red cell mass. This value was expressed as a percentage of the total activity injected.

**Sterile Inflammation.** One ml. of U.S.P. turpentine was injected subcutaneously in the sub-scapular region. The animals promptly developed a local cellulitis and subsequently a large abscess which drained spontaneously 6 to 8 days after injection. This lesion was associated with fever, anorexia and marked lethargy. These symptoms subsided rapidly following drainage of the abscess.

**Plan of the Experiment.** Experiments I through IV, and IA through IVA were performed in pairs. Both animals of a pair were handled in the same fashion, receiving injection of radioiron and having samples removed at the same time under identical conditions. One animal of each pair was infected and the partner served as a control. Equal aliquots of the same preparation of radioiron were injected into each member of a pair of animals for all experiments. Senescent radioiron labelled red cells were first injected into each pair of animals (Exps. I, II, III, IV). One hour following injection of these cells, only 3 to 12% of the injected radioactivity was found in the recipients plasma, while less than 0.5% of the injected activity was found in the circulating red cells. 24 hours following injection, less than 0.1% of the injected activity was present in the plasma. Less than 2% of the injected radioactivity was recovered in the urine and stools during the four days following injection. These findings indicate that the injected red cells were rapidly and completely removed from the circulation of the recipient animals with minimal hemoglobinemia.

Samples of venous blood were taken over the next 3 to 6 weeks for measurement of red cell uptake of Fe\(^{59}\). After blood levels of Fe\(^{59}\) had become stabilized, transferrin bound Fe\(^{59}\) was injected (Exps. IA, IIA, IIIA, IVA) and samples of venous blood removed over the next 2 to 3 weeks for measurement of red cell uptake of Fe\(^{59}\).

Experiments V and VI were each performed in a single animal. The senescent radioiron labelled red cells utilized for these experiments were aliquots of the blood used in Exp. II, and these three experiments were performed simultaneously. The senescent radioiron labelled red cells injected in Exp. V were an aliquot of the blood used in Exps. III and IV, and these three experiments were carried out simultaneously.
Results

Animals With Normal Iron Stores (fig. 1)

Twelve studies were performed in four normal animals. In Exps. I and IA, Dog B was abscessed while Dog C served as control. The infected animal (Dog B) showed distinct impairment of reutilization of radioiron from senescent red cells (Exp. I). The control animal (Dog C) utilized 3 to 4 times more radioiron for red cell production than did the abscessed animal (Dog B), during the 10 days following the injection of radioiron labelled red cells. On the other hand when plasma transferrin bound iron was injected (Exp. IA), the rate of incorporation of radioiron was similar in both the abscessed animal (Dog B) and the normal animal (Dog C). These experiments were then repeated in these same two animals reversing their roles. In Exps. II and IIA animal B now served as the control while animal C was abscessed. Again the abscessed animal showed marked impairment in reutilization of radioiron from injected senescent red cells (Exp. II) and minimal to no impairment of utilization of plasma transferrin bound iron.

![Diagram of red cell uptake of Fe59 in animals with normal iron stores.](image_url)

Fig. 1.—Red cell uptake of Fe59 in animals with normal iron stores.
Control Animals

Abscessed

Senescent RBC Fe$^{59}$

Transferrin Bound Plasma Fe$^{59}$

Experiments I, II & III

Experiments Ia, IIa & IIIa

% RBC UPTAKE

EFFECT OF ACTH THERAPY (Dog G)

CONTROL ANIMALS

ABSCESSED ANIMALS

Exp. VI

Exp. VIa

Days after injection of Fe$^{59}$

ACTH (12 days)

Fig. 2.—Red cell uptake of Fe$^{59}$ in animals with normal iron stores.

(Exp. IIA). In both Exps. I and II, the abscessed animals' reutilization of senescent red cell Fe$^{59}$ was least during the period of active inflammation. When the inflammation subsided, the radioiron was more rapidly incorporated into red cells. In Exp. II where active inflammation was maintained for 18 to 20 days, marked impairment of radioiron utilization persisted during this period, followed by prompt utilization of radioiron in the 13 days after the subsidence of the inflammation.

In Exps. I and II the total amount of hemoglobin injected in the form of senescent red cells represented 0.33 to 0.43 per cent of the animals circulating hemoglobin. In Exp. III approximately half this dose (0.16 to 0.17 %) was given to two animals with normal iron stores. Again the abscessed animal (Dog J) showed impaired reutilization of senescent red cell Fe$^{59}$ (Exp. III) with minimal
to no impairment of utilization of transferrin bound radioiron (Exp. IIIA), when compared with the control animal (Dog D).

The 12 studies performed in animals with normal iron stores are shown together in the upper half of figure 2. The abscessed animals show a consistent decrease in the rate of red cell uptake of Fe59 after injection of radioiron tagged senescent red cells when compared to control non-abscessed animals. Whereas, no consistent difference in red cell uptake of Fe59 is noted between abscessed and control animals following injection of transferrin bound Fe59.

In one animal (Dog G) the effect of ACTH therapy on the reutilization of senescent red cell Fe59 in the presence of inflammation was studied (Exp. VI, fig. 2). This animal received two 80 unit doses of ACTH gel intramuscularly, daily, for 11 days. On the third day of therapy sterile inflammation was produced, and radioiron tagged senescent red cells were injected on the fourth day of therapy. This animal showed slow development of an abscess and active inflammation persisted for over 12 days following a single turpentine injection; whereas in non-ACTH treated dogs active inflammation persisted only for 5 to 8 days. During the period of active inflammation the animal showed marked impairment of reutilization of radioiron from senescent red cells, with more rapid uptake occurring after the inflammation had subsided at 12 to 14 days. In contrast this animal showed rapid unimpaired utilization of plasma transferrin bound radioiron despite the presence of active inflammation.

Animals With Abnormal Iron Stores (fig. 3)

Two iron heavy animals were studied, Dogs E and F. In Exp. IV, the abscessed iron heavy animal (Dog F) showed a definite decrease in red cell uptake of Fe59 following injection of radioiron labelled senescent red cells when compared to the control iron heavy animal (Dog E). During the 14 days of active inflammation the abscessed animal utilized as little as 1/5 the amount of Fe59 utilized by the control animal during the same period. Again the abscessed animal showed a prompt increase in Fe59 utilization following subsidence of the active inflammation. Both iron heavy animals showed a slower rate of reutilization of senescent red cell Fe59 than did animals with normal body iron stores. In contrast to these findings were the results of Exp. IVA. Following the injection of transferrin bound Fe59, the abscessed iron heavy animal (Dog F) showed a rapid red cell uptake of Fe59 which was similar to the rate of red cell uptake in the control animal (Dog E). In both of the iron heavy animals the rate of red cell uptake of Fe59 following injection of transferrin bound Fe59 was similar to the rate of Fe59 red cell uptake in animals with normal body iron stores.

One animal with iron deficiency was studied (Dog H). In Exp. V1, the non-abscessed iron deficient animal showed very rapid reutilization of the injected senescent red cell Fe59. Virtually complete utilization of the injected red cell Fe59 occurred within 5 days after injection. In contrast when the animal was abscessed (Exp. V2) reutilization of senescent red cell Fe59 was slower, 1/3 to 1/2 as much radioiron being incorporated into red cells in the 7 days after injection, when compared to the control experiment (Exp. V1). The total quantity of radioiron
tagged red cells injected was similar for both Exps. V1 and V2, being respectively 0.48 and 0.45 per cent of the animals total circulating red cell mass (table 1). When transferrin bound Fe$^{59}$ was injected the iron deficient animal showed rapid, unimpaired red cell uptake of Fe$^{59}$ despite the presence of active inflammation (Exp. VA).

**Plasma Radioiron Clearance Rate** (table 2)

The rate of clearance of transferrin bound Fe$^{59}$ was much more rapid in all the animals with inflammation than in controls. The mean half time of plasma Fe$^{59}$ clearance in abscessed animals was 0.56 hours, while in controls the mean was 1.50 hours.
TABLE 2

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Abscessed</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal</td>
<td>1/2 Time (hours)</td>
<td>Animal</td>
</tr>
<tr>
<td>IA</td>
<td>B 0.58</td>
<td>C 1.73</td>
</tr>
<tr>
<td>II</td>
<td>C 0.48</td>
<td>B 1.70</td>
</tr>
<tr>
<td>IIIA</td>
<td>J 0.50</td>
<td>D 1.62</td>
</tr>
<tr>
<td>IVA</td>
<td>F 0.65</td>
<td>E 0.94</td>
</tr>
<tr>
<td>VA</td>
<td>H 0.47</td>
<td></td>
</tr>
<tr>
<td>VIA</td>
<td>G 0.68</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.56</td>
<td>1.50</td>
</tr>
</tbody>
</table>

DISCUSSION

The animals with sterile inflammation showed definite impairment of the rate of reutilization of iron from senescent red cells, while the rate of utilization of transferrin bound plasma iron was unimpaired. These findings indicate that inflammation results in a delay in the release of iron from senescent red cells to the plasma transferrin iron pool. Inflammation does not impair the bone marrow utilization of plasma transferrin bound iron for red cell production. These observations account for the discrepancy between earlier investigations and more recent investigations of the effect of infection and inflammation on the incorporation of radioiron into red cells. In the earlier studies the quantity of iron injected was large relative to the plasma iron pool. Therefore a significant portion of the injected iron would probably be deposited in the tissues with a distribution very similar to that of non-viable red cells. This would result in impaired red cell uptake of the injected iron. In contrast more recent studies utilizing trace quantities of transferrin bound iron have shown prompt red cell incorporation of injected iron in the presence of infection and inflammation. The diminished reutilization of radioiron from senescent red cells probably does not result from a defect in red cell breakdown or hemoglobin catabolism since the injection of relatively large quantities of iron in other chemical forms has resulted in findings similar to those of the present study. It is possible that the impaired release of iron from the tissue cells to the plasma transferrin is the result of a defect in the transport mechanism required for transferring iron from the cell to transferrin.

Sterile inflammation was found to impair the reutilization of senescent red cell Fe in an animal with iron deficiency and an animal with greatly increased iron stores (over four times normal), as well as in the animals with normal body iron stores. This indicates that the effect of inflammation in impeding the release of iron from the cell to the transferrin pool is not dependent on the amount of storage iron available. It is therefore unlikely that in the abscessed animals a greatly enhanced tissue requirement for iron could result in a relative deficiency of iron and limit the iron available to the plasma transferrin pool. For if this were the case then the iron deficient animal should show a more definite impairment of reutilization, while the iron heavy animal should show minimal to no impairment.
of reutilization of iron for red cell production. However the iron deficient animal showed minimal reduction of iron reutilization while the iron heavy animal showed a definitely diminished reutilization in the presence of inflammation. It is also unlikely that the impaired reutilization of hemoglobin iron could have resulted from dilution of injected iron with increased tissue iron stores which have been described in the presence of chronic inflammation; because the animal with enormously increased iron stores still showed impaired reutilization and the iron deficient animal with no storage iron showed impaired reutilization of injected red cell iron in the presence of sterile inflammation.

The amount of iron in the body iron stores did effect the rate of reutilization of radioiron from injected senescent red cells in both the non-abscessed (control) animals and the abscessed animals (fig. 4). The iron deficient animal showed much more rapid and more complete reutilization of radioiron and the iron heavy animal showed much slower and less complete reutilization than did the animals with normal iron stores, in both the control and abscessed state. In contrast, the rate of utilization of radioiron following injection of transferrin
bound radioiron was not consistently influenced by the total body iron stores of the animal. It is possible that measurement of the rate of reutilization of radioiron from senescent red cells (tissue iron turnover rate) may prove to be a useful technique for estimation of body iron stores. It should be emphasized that while body iron stores do affect the rate of reutilization of radioiron from senescent red cells they do not affect the impaired reutilization of radioiron which occurs in the abscessed animal when compared to a control animal with similar body iron stores.

Infection and inflammation may be accompanied by increased red cell destruction.\textsuperscript{5-9,11,17} It is possible that the relatively reduced uptake of radioiron results from introducing the tagged hemoglobin into a larger pool of hemoglobin which is constantly destroyed in the abscessed animal. However the amount of hemoglobin injected was usually from 0.33 to 0.48% of the recipients' circulating hemoglobin, while in the animals receiving only 0.16 to 0.17% of their circulating hemoglobin the impaired reutilization was still apparent. Moreover, the increased red cell destruction which has been described is only moderate in degree, while the animals studied showed \textsuperscript{1.3 to 1.5 the rate of reutilization of control animals, which would require rates of cell destruction of 3 to 5 times normal. Finally the prompt reutilization of the radioiron following cessation of inflammation indicates impaired release during active inflammation rather than increased dilution of the injected radioiron.

ACTH treatment did not correct the impaired reutilization of senescent red cell radioiron in the abscessed animal studied. In this animal however the sterile inflammation was not suppressed but was more prolonged and severe than in non-ACTH treated animals. In those situations where ACTH or steroids are capable of suppressing the inflammation, this observed delay in tissue iron release may be corrected.\textsuperscript{18}

A defect in the release of iron from destroyed red cells to the plasma transferrin pool coupled with the unimpaired removal of iron from the plasma transferrin pool by the hematopoietic tissues for red cell production would result in a fall in plasma iron concentration. The consistent occurrence of hypoferremia in all forms of acute and chronic infection and inflammation, as well as in other secondary anemias associated with malignancy, uremia, cirrhosis, etc., suggests that this defect is also present in these disease states. It is probable that this impaired reutilization of hemoglobin iron results in an inadequate supply of iron for red cell production and plays an important role in the genesis of these secondary anemias. Large quantities of parenteral iron do not increase the amount of iron available for red cell production, since the major portion of the injected iron is deposited in the tissues and the hypoferremia is corrected for only short periods of time.\textsuperscript{19} If the hypoferremia could be corrected for periods of months, these hypoferremic anemias would probably improve.

**Conclusions**

Sterile inflammation results in a diminished reutilization of iron from senescent, nonviable erythrocytes for red cell production. The utilization of transferrin bound plasma iron for red cell production is not impaired. The results of this
study indicate that there is a defect in the release of iron from the tissues to the plasma transferrin pool in the presence of inflammation. This defect was exhibited by abscessed animals with normal iron stores, with iron deficiency, with greatly increased iron stores and in an animal receiving ACTH therapy. The defect in release of iron from senescent red cells to the plasma coupled with unimpaired utilization of plasma iron by the marrow for red cell production results in hypoferremia which is so consistently found in association with inflammatory states, and contributes to the anemia which is associated with infection and inflammation.

**SUMMARIO IN INTERLINGUA**

Inflammationes sterile resulta in un diminution del reutilisation erythropoietic de ferro ab senescente e non-viabile erythrocytos. Le utilisation erythropoietic de ferro plasmatic ligate a transferrina remane intacte. Le resultatos del presente studio indica que il existe in le presentia de inflammation un defecto in le liberation de ferro ab le tessutos a in le reserva de transferrina plasmatic. Iste defecto esseva exhibite per animales con abscessos quando le reservas de ferro esseva normal, quando le reservas de ferro esseva basse, e quando le reservas de ferro esseva grandemente augmentate. Le mesme defecto esseva etiam demonstrate in un animal que recipeva ACTH therapeutic. Le defecto del liberation de ferro ab senescente erythrocytos a in le plasma in combination con le intacte utilisation de ferro plasmatic per le activitate erythropoietic del medulla resulta in le hypoferremia que es si uniformemente constatate in le presentia de statos inflammatori. Le mesme combination contribue al disveloppament.o del anemia que es associate con infection e inflammation.

**REFERENCES**

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