Characteristics of Iron Dextran Utilization in Man

By Perry A. Henderson and Robert S. Hillman

IRON DEXTRAN* THERAPY has received increasing attention as an alternative to orally administered iron in the treatment of some cases of iron deficiency. It may be administered either as a series of intramuscular injections or by intravenous infusion. In contrast to other parenteral iron preparations, it has been given intravenously in doses as high as 2–3 Gm. without apparent toxicity from the release of excessive free iron into circulation. As for its effectiveness, hemoglobin regeneration after administration is as good or better than that observed with orally administered iron. These observations do not, however, exclude the possibility of specific patterns of physiological behavior of the iron dextran which can influence the patient’s response to therapy.

In anticipation of the more frequent use of the intravenous route for iron dextran administration, it was considered important to characterize the metabolic fate of the material. Studies were carried out in a number of iron deficient subjects with special attention given to: 1) direct iron exchange between iron dextran complex and transferrin, 2) the clearance rate of iron dextran complex from plasma, 3) incorporation of the material into iron stores, 4) the characteristics of iron dextran breakdown and release from the reticuloendothelial cells and 5) iron dextran utilization in patients with complicating illnesses.

METHODS

I. Iron Dextran Exchange Studies

Iron exchange between iron dextran and transferrin was studied in vitro by direct addition of iron dextran solutions to fresh, human serum and assay of the unbound iron binding capacity (UIBC) after suitable incubation. Transfer measurements were performed both on unbuffered sera, pH 7.8–8.0 and serum buffered to pH 7.4 with 0.1 molar phosphate buffer, under varying conditions of time and temperature. In vivo exchange was studied by repeated measurements of UIBC saturation during intravenous infusions of 1–3 Gm. of iron dextran into iron deficient individuals. All subjects receiving iron dextran infusions were hospitalized on the Clinical Research Center of King County Hospital, Seattle, Washington.

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This work was supported by research funds from Lakeside Laboratories, American Heart Association, Grant #67-680, and United States Public Health Service Grant, AM-09950. A portion of this work was conducted through the University of Washington Clinical Research Center at Harborview Hospital supported by the National Institutes of Health (Grant FR-133).

First submitted January 14, 1969; accepted for publication May 8, 1969.

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*Imferon, Lakeside Laboratories

Blood, Vol. 34, No. 3 (Sept.) 1969
Table 1.

1. Accuracy of the TIBC Measurement

<table>
<thead>
<tr>
<th>Serum</th>
<th>Determinations</th>
<th>Mean TIBC (μg.%)</th>
<th>S.D. (μg.%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>12</td>
<td>290 ±15</td>
<td>±15</td>
</tr>
<tr>
<td>B</td>
<td>12</td>
<td>325 ±13</td>
<td>±13</td>
</tr>
<tr>
<td>C</td>
<td>18</td>
<td>330 ±14</td>
<td>±14</td>
</tr>
</tbody>
</table>

2. Comparison of Serum Iron, UIBC and TIBC Measurements for Chemical Determinations and Immunoassay

<table>
<thead>
<tr>
<th>Serum</th>
<th>Serum Iron (μg.%)</th>
<th>UIBC (μg.%)</th>
<th>TIBC (μg.%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>A</td>
<td>B</td>
<td>A</td>
</tr>
<tr>
<td>1</td>
<td>34</td>
<td>40</td>
<td>271</td>
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</tr>
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<td>179</td>
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<td>9</td>
<td>155</td>
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</tr>
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<td>10</td>
<td>122</td>
<td>72</td>
<td>247</td>
</tr>
<tr>
<td>Mean</td>
<td>130</td>
<td>136</td>
<td>214</td>
</tr>
<tr>
<td>S.D.</td>
<td>±41.5</td>
<td>±53.5</td>
<td>±41.9</td>
</tr>
</tbody>
</table>

A. Serum iron was performed by the method of Bothwell and Mallett. TIBC by the method of Morgan and Carter. UIBC equals the difference between the TIBC and serum iron.

B. TIBC and UIBC were measured by the immunoassay technic. Serum iron equals the differences between the TIBC and UIBC.

Washington. They were volunteers selected from the patient population because of an absence of marrow iron stores on Prussian blue stain and serum iron saturation of less than 20 per cent. A subject was not accepted if he had a previous history of iron dextran therapy. Iron dextran for intravenous infusions was diluted in 5 per cent dextrose solution to a final concentration of 5 mg./ml. and was only infused after initial sensitivity testing by infusion of 1 ml. of the solution. Measurements of the UIBC in the presence of large concentrations of iron dextran were performed using a radio-immunoassay. The transferrin concentration (TIBC) was assayed by quantitative immuno-precipitation with an antiserum developed in rabbits against human transferrin. The precipitin reaction was incubated for 30 minutes at 37 C. and 24 hours at 4 C. The precipitate was then washed three times with buffered saline and dissolved in 3 ml. of biuret reagent. The transferrin concentration was then determined using a standard precipitin curve for dilutions of control serum whose total iron binding capacity was measured by the colorimetric method of Morgan and Carter. The UIBC was simultaneously measured by determining the radioactivity of the washed transferrin precipitate after presaturating the UIBC with either 59Fe ferric chloride or ferrous citrate. Serum was incubated with 59Fe (4 μg./ml.) at 37 C. for 30 minutes to saturate the UIBC, diluted 1:10 with saline and the transferrin precipitated with antiserum. After the precipitate was washed free of unbound 59Fe, the UIBC was measured by counting aliquots of precipitate-biuret solution and the saline dilution of 59Fe labelled serum. Transferrin-bound iron was calculated as the difference between the TIBC and UIBC. Total serum iron (transferrin iron plus iron dextran) was measured by the method of Bothwell and Mallett after total digestion in concentrated sulphuric acid.

*Purified human transferrin, Behringwerke, Marburg-Lahn, Germany.
Fig. 1.—The transferrin of normal human serum was progressively saturated with increments of ferric chloride (solid line). Subsequent measurements of the UIBC with 59Fe ferric chloride (open circles) demonstrated the accuracy of the 59Fe immunoassay over a wide range of UIBC saturations. The discrepancy at full saturation is related to a falsely high measurement of the TIBC by the method of Morgan and Carter.\textsuperscript{14,15}

The accuracy of representative TIBC and UIBC measurements by the anti-transferrin immunoassay technic is summarized in Table 1. Moreover, the 59Fe UIBC immunoassay technic accurately measured the UIBC over a full range of saturations experimentally produced by adding increments of ferric chloride to normal human serum. (Fig. 1). The measurement was also shown to be valid in the presence of high concentrations of iron dextran. First, the stability of the UIBC measurement with radioiron was studied by presaturating the UIBC with 59Fe (4 µg./ml. serum) before exposure to increasing concentrations of iron dextran. (Fig. 2 A). Despite concentrations of iron dextran as high as 50,000 µg. per cent, there was no significant exchange between the iron dextran and the 59Fe bound to transferrin, the UIBC remained saturated with radioiron. Second, trapping of the 59Fe by iron dextran was excluded by examining the effect of the UIBC measurement of varying the concentration of the 59Fe solution in the assay procedure over a forty-fold range (Fig. 2 B). Once the serum UIBC was saturated to 85–90 per cent of its binding capacity with iron dextran iron, increasing concentrations of 59Fe ferric chloride (from 4–136 µg./ml.) failed to change the UIBC measurement.

Finally, to confirm true saturation of the UIBC with exchangeable iron in the in vivo situation, the T3i clearance of radioiron was measured just before and immediately after iron dextran infusion in four individuals. Five-ten µc. of citrated 59Fe was incubated with normal plasma and injected intravenously. Venous samples were obtained at frequent intervals over the next three-five hours and the centrifuged plasma counted in a gamma well counter. The T3i clearance was obtained from the semilog plot of the count rates.

\textbf{II. Iron Dextran Clearance Rates} 

In order to measure the clearance rate of iron dextran from the plasma compartment
Fig. 2 A.—Once the UIBC had been saturated with $^{59}$Fe, exposure to high concentrations of iron dextran from 5,000 to 50,000 μg. per cent failed to displace the isotopic iron from the transferrin. The UIBC saturation with radioiron is expressed here as counts/min./ml. of antitransferrin precipitated serum after washing × 3 to remove the contaminating iron dextran and unbound radioiron (mean of 5 experiments).

Fig. 2 B.—To exclude the possibility of apparent saturation of the UIBC because of binding of $^{59}$Fe to iron dextran, the UIBC was measured with increasing concentrations of $^{59}$Fe in the assay system once the UIBC had been saturated to 85–90 per cent of its capacity with iron dextran iron. At the most, only 5–7 per cent of the iron dextran iron was displaced by the higher concentrations of $^{59}$Fe FeCl₃.

into the reticuloendothelial cells, infusion doses of 100–2000 mg. were prelabelled with 5–10 μc. of $^{59}$Fe iron dextran.* After intravenous infusion, frequent blood samples were obtained over a 5–10 day period. Two ml. of plasma was counted in a gamma well counter to 10,000 counts above background for ± 1 per cent accuracy. According to the clearance characteristics, the clearance rates were expressed as either a Cartesian or semilog plot of the count rates minus background.

III. Iron Store Accumulation

Twenty-five patients with absent iron stores on Prussian blue stained smears of marrow stroma were selected for this determination. Each subject received an infusion of 100–2000 mg. of iron dextran containing 5–10 μc. $^{59}$Fe labelled iron dextran. Between the second and fifth day following infusion, a second marrow aspirate was obtained and stained for

*Prepared by Fisons Pharmaceutical Ltd., Holmes Chapel, Cheshire, England: identical to the material prepared for Woods, Milner and Pathak.¹⁰
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iron. Frequent blood samples were drawn during the 10–14 days post-infusion and both plasma and whole blood aliquots were counted for radioactivity.

The appearance of iron in marrow reticuloendothelial stores was graded histologically and compared to the amount of iron dextran cleared into the reticuloendothelial cell by the time of the second marrow examination. The latter was calculated from the amount infused, the amount still in plasma at the time of the marrow and the amount incorporated into red cells as determined from whole blood minus plasma count rate 2–3 days after the marrow. Whole blood activity was measured 2–3 days after the marrow in order to permit labelled normoblasts to mature to adult red cells and enter circulation. The calculation was as follows:

\[
\text{Reticuloendothelial cell iron (mg.) = Infusion dose (mg.) - } \left( \frac{\text{Amount in plasma at the time of the marrow (mg.)}}{\text{Amount in red cells 2–3 days after the marrow (mg.)}} \right)
\]

For histologic grading of iron stores, bone marrow was aspirated either from the sternum or iliac crest with a 16 gauge Rosenthal needle. Stroma and bony spicules were harvested after ejection onto watchglass and thick coverslip smears made. These were fixed with methanol and stained with 2 per cent potassium ferrous cyanide. The amount of iron present within the reticuloendothelial cells was graded by three observers according to the following classification:

- 0 = no visible reticuloendothelial cell iron
- 1+ = small quantities
- 2+ = moderate
- 3+ = heavy
- 4+ = massive amounts

IV. MARROW PRODUCTION STUDIES

The rate of iron delivery to the marrow from the reticuloendothelial iron dextran stores was measured in terms of the erythroid marrow’s production response at a standard level of anemic stimulus. Three iron deficient patients with hematocrit levels of 25–30 per cent were studied for periods of 2–10 weeks after receiving intravenous iron dextran. The level of anemia was kept constant by daily graded phlebotomy, adjusted so as to maintain the hematocrit between 25–30 per cent despite changes in marrow production. The marrow production response was measured by repeated determinations of the plasma iron turnover, twice daily reticulocyte counts and the level of phlebotomy required to maintain the hematocrit at a constant level.

1) Plasma iron turnover measurements. All determinations of the plasma iron turnover were performed at 10:00 a.m. One to five mc. of ferrous citrate was incubated with five to ten ml. of the patient’s or normal plasma for 30 minutes prior to injection. The injected dose and standard dilutions were determined gravimetrically. Accurately timed venous samples were obtained at frequent intervals over the next two hours for plasma counting. The plasma iron turnover was calculated according to Bothwell and Finch using the T3i clearance and the mean of two serum irons drawn at zero time and 30 minutes after injection.

2) Phlebotomy production measurements. In individuals maintained at a constant level of anemia, with graded daily phlebotomy, marrow production could be estimated from the rate of cell loss, i.e. the amount of cells removed each day by phlebotomy plus those lost from senescence. The latter was obtained by daily calculation of the red cell age distribution as continuously modified by the previous day’s cell death, the actual removal by phlebotomy, and the change in production pattern. The derivation of this calculation has been previously described.
3) Reticulocyte production measurement. Marrow production was also estimated from
the reticulocyte count. Assuming a mean normal reticulocyte count of 1.0 ± 0.5 per cent a
production index was calculated as follows:

\[
\text{Reticulocyte Production} = \frac{\text{observed reticulocyte count} \times \text{observed hematocrit}}{1.0 \text{ (normal reticulocyte count)} \times 45 \times \text{maturation time}}
\]

\[
\text{Normal Production Index} = 1.0
\]

This calculation of a reticulocyte production index provides corrections both for hematocrit
variation and prolonged reticulocyte maturation times within circulation. These three
measurements of production have been shown to be in close agreement in normal individu-
als over a range of hematocrits from 25 to 45 per cent and marrow production levels
of 1 to 7 times normal.

V. Iron Dextran Utilization Studies

Sixteen patients with iron deficiency were studied to determine their per cent utilization
of iron dextran for hemoglobin production. After intravenous injection of a 200 mg. dose
labelled with 5 µc. \(^{59}\text{Fe}\) iron dextran, blood samples were obtained immediately for cal-
culation of the total blood volume and over the next 10-14 days to determine percent
utilization:

\[
\text{Iron dextran utilization (\%)} = \frac{\text{Iron dextran Count rate/ml. whole blood} \times \text{blood volume} \times 0.91}{\text{at 10-14 days (ml.)}} \times \frac{\text{Total counts injected}}{\text{(ml.)}}
\]

RESULTS

I. Iron Exchange Studies

In vitro direct iron exchange from iron dextran to transferrin was evident
from the progressive saturation of the UIBC with increasing concentrations of
iron dextran (0-50,000 µg. per cent) (Fig. 3). Exchange was similar with
incubations at either pH 7.8-8.0 or pH 7.4. For these exchange experiments,
the sera and iron dextran were incubated for 30 minutes at 37 C. before
measurement of the UIBC. These conditions were selected because in vitro
iron exchange was found to be both temperature and time dependent (Table
2). At 4 C., exchange was incomplete after 30 minutes and with incubation for
less than 2 minutes only a small portion of the iron transfer occurred. How-
ever, by 30 minutes, exchange was complete; further saturation did not occur
by 60 minutes or even after storage for as long as 7 days at -20 C.

From Figure 3 it was possible to estimate the exchangeable iron pool of
iron dextran as approximately 1.0-1.4 per cent of the total iron present, that
is, full saturation of the UIBC (200 µg. per cent) occurred at an iron dextran
concentration of 15,000-20,000 µg. per cent. The behavior of this iron pool was
further studied by measuring the ability of increasing concentrations of iron
dextran iron to compete with a constant amount of \(^{59}\text{Fe}\) ferric chloride for
transferrin binding. An equal saturation of the UIBC, 50 per cent exchange-
able iron dextran iron and 50 per cent \(^{59}\text{Fe}\), occurred at an iron dextran
concentration of approximately 33,000 µg. per cent (Fig. 4). This supported a
1.2 per cent exchangeable iron pool with binding characteristics similar to
the \(^{59}\text{Fe}\) ferric chloride. However, when the same experiment was per-
formed at 4 C. the behavior of the iron dextran iron pool and the \(^{59}\text{Fe}\) ferric
chloride were not the same. As shown in Figure 4, the iron dextran iron dem-
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Fig. 3.—In vitro iron exchange between iron dextran and transferrin was evident from the progressive saturation of the UIBC with increasing concentrations of iron dextran. Exchange was studied both at pH 7.4 (open circles) and pH 7.8–8.0 (solid dots).

Table 2.—Per cent Saturation of the UIBC

<table>
<thead>
<tr>
<th>Sample Concentration of Iron dextran (μg.)</th>
<th>1–2 mins.</th>
<th>30 mins.</th>
<th>60 mins.</th>
<th>5–7 days</th>
<th>30 mins.</th>
</tr>
</thead>
<tbody>
<tr>
<td>5,000</td>
<td>40</td>
<td>44</td>
<td>40</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>10,000</td>
<td>60</td>
<td>69</td>
<td>68</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>15,000</td>
<td>85</td>
<td>82</td>
<td>91</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>20,000</td>
<td>89</td>
<td>89</td>
<td>92</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>25,000</td>
<td>92</td>
<td>—</td>
<td>—</td>
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</tr>
<tr>
<td>30,000</td>
<td>92</td>
<td>91</td>
<td>93</td>
<td>36</td>
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</tr>
<tr>
<td>40,000</td>
<td>93</td>
<td>92</td>
<td>92</td>
<td>49</td>
<td></td>
</tr>
</tbody>
</table>

*These samples were incubated for 60 minutes at 37 C. and then stored at −20 C. for 5–7 days before UIBC measurement.

...onstrated a lower facility for exchange, suggesting a more complex relationship between the exchangeable iron pool and the iron dextran than mere excessive free iron contamination.

Iron exchange was studied in vivo in five individuals (Table 3). UIBC measurements made at frequent intervals during infusion of 1–3 Gm. of iron dextran demonstrated a progressive saturation of the transferrin UIBC with higher concentrations of iron dextran. The quantity of iron transferred was comparable to the amounts exchanged in vitro. (The amount of iron exchanged is compared rather than the per cent saturation of the UIBC because of the wide variation in the initial UIBC value among these individuals).
Table 3.—Iron Transferred to UIBC (μg.%)

| Iron Dextran Concentration (μg.%) | In Vitro (5 patients) | In Vitro (Fig. 2) |
|--------------------------------||----------------------|-----------------|
| 2000–4000                      | A 75 B 30 C — D 62 E 70 | 30–50 |
| 5000–7000                      | A 119 B 90 C 90 — E 105 | 70–100 |
| 10,000–15,000                  | A 290 B 250 C 170 D 120 E — | 140–180 |
| 20,000–25,000                  | A — B — C 205 D 210 E — | 180–190 |
| 40,000                         | A 297 B 437 C 170 D 220 E 203 | 180–190 |
| Maximum UIBC (μg.%) before infusion of iron dextran | 312 460 190 240 225 | 190–220 |

Fig. 4.—Increasing concentrations of iron dextran mixed with 400 μg. per cent of 59Fe, ferric chloride solution were incubated with human serum. Equal saturation of the UIBC by isotope and iron dextran (line A—incubation at 37 C.) occurred at an iron dextran concentration of about 33,000 μg. per cent, compatible with a 1.2 per cent exchangeable iron dextran iron pool. When the incubation was performed at 4 C. (line B), the iron demonstrated a lower facility for exchange so that only 30 per cent of the UIBC was saturated with iron dextran iron at the 40,000 μg. per cent concentration.

In four individuals, the radioiron clearance was determined just before and immediately after infusion of 2 Gm. of iron dextran. Since the plasma iron turnover can be expected to remain essentially unchanged during the 4–5 hours of the study, a true elevation of the serum iron by direct iron exchange from iron dextran would be reflected in a prolongation of radioiron T½ clearance. As shown in Table 4, the serum irons were elevated and the T½ clearance was significantly prolonged immediately after infusion. By five days after infusion the serum iron had returned towards normal and the T½ clearance shortened with removal of the iron dextran material from circulation by the reticuloendothelial cells. This occurred despite tissue iron stores of 1.5–2 Gm. of the infused iron dextran.

II. Iron Dextran Clearance Rates

Twenty-one patients received a single intravenous infusion of 100–2000 mg. of iron dextran labelled with 5–10 μc. of 59Fe iron dextran. For doses less than 500 mg., the initial clearance rate was exponential (Fig. 5). For doses
Table 4.—Radioiron Clearance Studies After Infusion of Iron Dextran

<table>
<thead>
<tr>
<th>Patient</th>
<th>Hct. (%)</th>
<th>T% Clearance Radioiron* (min.)</th>
<th>Transferrin Bound Iron (ug.%)* 5 days</th>
<th>Post-infusion</th>
<th>Post-infusion 5 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>42</td>
<td>90</td>
<td>182</td>
<td>255</td>
<td>160</td>
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<tr>
<td>2</td>
<td>31</td>
<td>74</td>
<td>136</td>
<td>11</td>
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</tr>
<tr>
<td>3</td>
<td>28</td>
<td>29</td>
<td>180</td>
<td>437</td>
<td>—</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td>54</td>
<td>52</td>
<td>337</td>
<td>135</td>
</tr>
</tbody>
</table>

*Post-infusion studies were performed immediately after completion of the iron dextran infusion, except for patient #4 when the post-infusion study was done 18 hours after infusion.

†These studies were performed 5 days after infusion when the majority of the iron dextran had been removed by the reticuloendothelial cells.

Fig. 5.—Doses of 100, 200, 300, 400 and 500 mg. of $^{59}$Fe labelled iron dextran were infused into two subjects and the clearance rate determined by frequent venous sampling. The mean and range of values of the percent remaining in circulation are shown for all 10 patients. For doses up to 500 mg. clearance was exponential.
Fig. 6.—After infusion of 1600 mg. of iron dextran, the initial disappearance from circulation was a linear function (plot A), indicating a constant maximum uptake by the reticuloendothelial system. Only when approximately 1/3 of the original dose remained (less than 500 mg.) did the clearance become exponential (plot B).

III. Iron Store Accumulation

In order to examine the distribution of iron dextran stores, the appearance of visible marrow iron stores after iron dextran infusion was studied in twenty-five patients. The histologic appearance of marrow iron as compared to the calculated amount of iron dextran present in the reticuloendothelial cells is shown in Figure 7. Iron stores of less than 100 mg. could not be seen on marrow iron stains. Iron stores greater than 100 mg. were readily apparent and could be graded as indicated from 1 to 4+.

IV. Marrow Production Studies

The marrow production response of iron deficient patients was studied after a single intravenous dose of 1700–2100 mg. of iron dextran. It was immediately apparent that the post-infusion increase in red cell production could be roughly divided into an acute and chronic response phase. In the acute phase, Table 5, the serum iron rapidly increased to near saturation and marrow production rose to peak levels of 4-6 times normal by the eighth day. However, this level of marrow production was not maintained. In associ-
Fig. 7.—The appearance of Prussian blue stained iron stores were graded histologically on a scale of 0–4+: (0 - no visible iron, 1+ - small amounts, 2+ - moderate amounts, 3+ - heavy amounts, 4+ - massive amounts) in marrow stroma obtained from 25 patients who had received 100–200 mg. of iron dextran 2–5 days previously. The amount of iron dextran iron stores was calculated from the amount infused, the amount still in plasma and the amount incorporated into hemoglobin (see methods).

The chronic phase of the marrow production response to intravenous iron dextran is illustrated in Figure 8. Here, a single patient was maintained at a constant level of anemia by repeated phlebotomy and the marrow production response measured for seven weeks after infusion of 1700 mg. of iron dextran. Immediately after infusion production rose to approximately 4–5 times normal, the acute phase response. Subsequently, production declined despite the presence of significant amounts of iron in the reticuloendothelial system. By five weeks following the iron dextran infusion, production was only 1.5 times normal and the serum iron had fallen to 29 μg. per cent with a 9 per cent transferrin saturation. This was at a time when at least 200 mg. of iron dextran
Table 5.—Acute Response of Marrow Production to Intravenous Iron Dextran

<table>
<thead>
<tr>
<th>Subject</th>
<th>Hct.</th>
<th>Iron Dextran Infusion (mg.)</th>
<th>Serum Iron (µg./100 ml.)</th>
<th>Marrow Productions (x's normal)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>24-27</td>
<td>1800</td>
<td>64§</td>
<td>276</td>
</tr>
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<td>2</td>
<td>24-28</td>
<td>1900</td>
<td>160§</td>
<td>292</td>
</tr>
<tr>
<td>3</td>
<td>38-42I</td>
<td>1700</td>
<td>41</td>
<td>356</td>
</tr>
<tr>
<td>4</td>
<td>28-32</td>
<td>2100</td>
<td>11</td>
<td>437</td>
</tr>
</tbody>
</table>

* True transferrin bound serum iron by antittransferrin assay.12
† Determined from reticuloocyte production index, measurements of plasma iron turnover and the level of daily phlebotomy.
‡ A patient with severe obstructive lung disease and anoxia, hematocrit 58-65 per cent when not iron deficient.
§ On oral iron up to the time of infusion.
iron remained in the reticuloendothelial cell system and a Prussian blue stain of marrow stroma revealed 1–2+ iron stores. To exclude a marrow defect other than restriction of production by inadequate iron delivery, the patient was then given a second infusion of 300 mg of iron dextran. This was followed by a prompt increase in marrow production to 3 times normal.

Studies of three other patients who were followed for 6–9 months after intravenous iron dextran therapy demonstrated a similar impairment of iron dextran release from the reticuloendothelial cell (Table 6). Patient A received a total of 3500 mg of intravenous iron dextran as therapy for chronic gastrointestinal hemorrhage secondary to hereditary telangectasia. His initial iron deficiency anemia cleared within 2 months of therapy. However, six months after the iron dextran infusion, his hematocrit once again fell to 31 per cent with serum iron values of less than 77 µg per cent. A bone marrow examination revealed defective hemoglobinization of the developing normoblasts and confirmed the absence of erythroid marrow proliferation. A Prussian blue stain of marrow stroma revealed a decreased number of sideroblasts but 1–2+ iron stores. Once again, a primary marrow defect was excluded by a second infusion of iron dextran. Following 1000 mg of intravenous iron dextran,
Table 6.—Patients Followed for 6-9 Months After Iron Dextran Therapy

<table>
<thead>
<tr>
<th>Before Therapy</th>
<th>Hematocrit (%)</th>
<th>Peripheral Smear</th>
<th>Serum Iron (μg./100 ml)</th>
<th>TIBC (μg./100 ml)</th>
<th>Iron Stores</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient A</td>
<td>34</td>
<td>Microcytic</td>
<td>42</td>
<td>420</td>
<td>absent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hypochromic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>26</td>
<td>Microcytic</td>
<td>5-15</td>
<td>210-265</td>
<td>absent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hypochromic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>38-42</td>
<td>Normocytic</td>
<td>36-44</td>
<td>500-520</td>
<td>absent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Normochromic</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

After Therapy: 2-3 months (1200-3500 mg. intravenously)

| Patient A      | 44             | Normocytic       | 100-150                | 330-370          | 3-4+        |
|                |                | Normochromic     |                        |                  |             |
| B              | 35-37          | Microcytic       | 130-170                | 220-245          | 3-4+        |
|                |                | Normochromic     |                        |                  |             |
| C              | 48             | Normocytic       | 113-127                | 305-340          | 3+          |
|                |                | Normochromic     |                        |                  |             |

After Therapy: 6-9 months

| Patient A      | 31             | Microcytic       | 50-77                  | 225-300          | 1-2+        |
|                |                | Normochromic     |                        |                  |             |
| B              | 22-25          | Microcytic       | 6-10                   | 475-510          | 1-2+        |
|                |                | Hypochromic      |                        |                  |             |
| C              | 30             | Microcytic       | 24-40                  | 415-495          | 1+          |
|                |                | Hypochromic      |                        |                  |             |

marrow production immediately increased to 2-3 times normal and the hematocrit returned to a normal value.

Patient B demonstrated a similar course after treatment with 2100 mg. iron dextran intravenously for a severe iron deficiency anemia secondary to recurrent gastrointestinal bleeding. In response to this therapy, his hematocrit rose to 35-37 per cent, and the serum iron returned to a normal value. However, over the next nine months, gastrointestinal blood loss continued and the hematocrit again fell to 25-22 per cent. Although blood studies indicated a return of his iron deficiency anemia, repeated bone marrow examinations over the next two months revealed persistent 1-2+ iron stores. Finally, a normal individual, Patient C, was subjected to controlled phlebotomy 6-7 months after the creation of artificial iron dextran stores. A single intravenous infusion of 1200 mg. of iron dextran returned his serum iron to normal and produced 3+ iron stores on a marrow aspirate. Seven months later when he was phlebotomized to a hematocrit of 30 per cent by the removal of 3500 ml. of whole blood over a 4 week period, iron deficient erythropoiesis appeared as evident from his smear, mean cell constants and serum iron values. Despite the apparent iron lack, repeated marrow examinations over the next two months revealed easily visible iron stores.

V. Iron Dextran Utilization In Iron Deficient Subjects

The utilization of small doses of intravenous iron dextran was studied in 16 iron deficient patients with or without a complicating illness. Employing a 200 mg. dose of 59Fe labelled iron dextran, the per cent incorporation into
IRON DEXTRAN UTILIZATION

Table 7.—Iron Dextran Utilization (200 mg. dose)

<table>
<thead>
<tr>
<th>Patients</th>
<th>Hematocrit (%)</th>
<th>Serum Iron/TPIC (µg./100 ml)</th>
<th>% saturation</th>
<th>% utilization (10–14 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood loss (9) alone</td>
<td>20–31</td>
<td>15–45/310–530</td>
<td>3–12.6</td>
<td>89–96</td>
</tr>
<tr>
<td>Blood loss plus: emphysema (1)</td>
<td>36</td>
<td>44/270</td>
<td>16.3</td>
<td>62</td>
</tr>
<tr>
<td>Ca. of lung (1)</td>
<td>35</td>
<td>40/248</td>
<td>16.1</td>
<td>86</td>
</tr>
<tr>
<td>Ca. of bowel (1)</td>
<td>33</td>
<td>35/266</td>
<td>13.2</td>
<td>40</td>
</tr>
<tr>
<td>Hodgkin’s disease (1) (Stage IV)</td>
<td>31</td>
<td>31/265</td>
<td>11.7</td>
<td>26</td>
</tr>
<tr>
<td>Ca. of prostate (2)</td>
<td>30</td>
<td>56/450</td>
<td>12.4</td>
<td>30</td>
</tr>
<tr>
<td>Ca. of prostate (2)</td>
<td>22</td>
<td>34/375</td>
<td>9.0</td>
<td>85</td>
</tr>
<tr>
<td>Allergic purpura (1)</td>
<td>31</td>
<td>49/310</td>
<td>15.8</td>
<td>39</td>
</tr>
</tbody>
</table>

circulating red cells was determined at 10–14 days after intravenous infusion. The results of these studies are shown in Table 7. Despite significant anemia, the absence of iron stores and serum iron percent saturations of less than 20 per cent in all patients, 4 of these individuals demonstrated iron dextran utilizations of less than 50 per cent at 14 days. In each instance these patients demonstrated a complicating illness characterized by tissue inflammation.

DISCUSSION

Studies of the metabolic fate of iron dextran in animals and man have shown that the material follows a distinct pathway after intravenous or intramuscular injection.10,11,16,18 Iron dextran complex is first cleared into the reticuloendothelial system, where ionic iron is liberated for later return to circulation and transport to the erythroid marrow as transferrin bound iron. Only a small fraction of the complexed iron bypasses this route by direct exchange with transferrin. Iron exchange was studied by in vitro and in vivo measurement of the transferrin UIBC after exposure to high concentrations of iron dextran, 5,000–50,000 µg. per cent. Approximately 1–1.2 per cent of the iron dextran iron was found to be available for direct exchange to transferrin. Full saturation of the UIBC occurred at iron dextran concentrations of 15,000–20,000 µg. per cent, well below plasma concentrations attained by infusion of 2–3 Gm. of iron dextran in vivo. This was shown to be an actual transfer of iron from dextran to transferrin and not merely the formation of a transferrin-iron dextran complex by way of attachment of transferrin to the surface of the iron dextran molecules. The latter situation should have little effect on the rate of radioiron clearance of 59Fe labelled transferrin since the dextran iron of the transferrin iron complex would not be expected to participate in the normal transferrin iron delivery system. The observed prolongation of the radioiron clearance immediately after infusion implied a complete removal of some iron from the dextran complex and delivery of that iron to marrow normoblast receptor sites.

The exchangeable iron fraction of iron dextran does not appear to be merely a contaminating pool of unbound ionic iron. Intravenous infusion of 2–3 Gm. of iron dextran results in post-infusion plasma concentrations of 40,000–50,000 µg. per cent without evidence of iron toxicity from the release of sufficient ionic iron to exceed the transferrin UIBC. Moreover, iron dextran iron
exchange is impeded by lower temperatures to a greater extent than $^{59}$Fe ferric chloride binding to transferrin. This would suggest a loosely bound iron dextran iron fraction which can be removed by transferrin but is otherwise in a stable complex with dextran and will not dissociate to free ionic iron. These findings are similar to those of Cox et al. By polarographic and electrophoretic technics they were unable to detect any significant free ionic iron in dextran solutions but did estimate that 1–2 per cent of the total iron may be present as a loosely bound ferrous iron which is in equilibrium with the ferric iron dextran complex. Their estimate of a 1–2 per cent iron pool is in close agreement with the 1–1.2 per cent exchangeable iron fraction we identified.

The reticuloendothelial system plays a key role in the utilization of the remaining portion of the iron dextran complex. After an intravenous administration of large doses, 2,000–3,000 mg. of the iron dextran complex, complete removal of the material from plasma takes as long as 2–3 weeks. With infusions of up to 500 mg. of the material at one time, clearance into the reticuloendothelial system is exponential. When doses in excess of 500 mg. are administered, the initial clearance rate does not exceed 10–20 mg./hr., the maximum removal rate of the reticuloendothelial system. Once cleared, the material is readily visible as iron stores on Prussian blue stain of marrow stroma. The pattern of deposition in the marrow reticuloendothelial system is apparently similar to that of normal stores of hemosiderin iron. The relative amounts of visible iron stores generated by the administration of 100–2,000 mg. of the complex were essentially the same as that observed by Rath and Finch and others for normal stores. In contrast to studies in rabbits, the distribution of iron dextran in man appears to be limited to the reticuloendothelial system; hepatic parenchymal cell uptake is minimal.

Theoretically, these characteristics of iron dextran removal and storage should permit a level of iron supply to the marrow of better than 10 times normal as long as reticuloendothelial cell release of ionic iron is not rate limiting. However, the present studies of iron delivery to the erythroid marrow indicated that soon after administration, reticuloendothelial cell release falls far below this potential level. Two characteristics of reticuloendothelial cell function appeared to be of prime importance in determining the rate of iron delivery: 1) The length of time the iron dextran iron was stored within the reticuloendothelial system and, 2) The additional inhibition of iron release which occurred when a complicating illness of inflammatory nature was present. In the uncomplicated iron deficient patient, hemoglobin synthesis increased dramatically immediately after infusion. In the first two weeks, iron supply was sufficient to permit marrow production levels of 4–6 times normal. This was associated with a higher than normal serum iron level, the result of both direct exchange of iron dextran iron to transferrin and an early, rapid release of iron from the reticuloendothelial cells. However, after 10–14 days, the serum iron fell and iron delivery decreased as indicated by a fall in marrow production to approximately one-half of the level observed initially. With the passage of time a further decrease in the rate of iron
delivery was observed so that, by the fifth to seventh week after infusion marrow production fell to less than twice normal despite adequate iron dextran stores. This was accompanied by a fall in the serum iron to iron deficient levels, a phenomenon which has been previously reported by Wood et al.10 This tendency for reticuloendothelial cell iron dextran stores to become progressively unavailable with time was further demonstrated by studies of individuals given large amounts of iron dextran to create artificial stores.

It should be recognized that a progressive unavailability of the iron dextran iron may not necessarily be related only to storage time or reticuloendothelial cell metabolism. Inasmuch as iron dextran complex is composed of particles of widely disparate size, up to 3 micra in diameter, it is quite possible that the rate of iron mobilization is in part or entirely the result of the physical characteristics of the material. Similar to the experience with large accretions of hemosiderin, the larger particles of iron dextran may either require prolonged periods of time for dissolution or even be totally unavailable for iron release. The reticuloendothelial cell could therefore be an innocent victim, maintaining a level of iron supply to the marrow which was predetermined by the spectrum of iron dextran particle site. Moreover, if a portion of these particles were completely unavailable, a gradual deposition of unusable iron stores would be expected with repeated or large doses of the material.

These facts do not completely exclude a specific regulatory role of the reticuloendothelial cell on iron release, however. It was possible to demonstrate that the presence of a complicating illness of inflammatory nature may further interfere with the release of iron dextran from the reticuloendothelial system. The effect is similar to the relative block in iron release from the reticuloendothelial cell which occurs in individuals with normal iron stores and an inflammatory state.26

In conclusion, it is apparent that the major portion of iron dextran is available for hemoglobin synthesis only after processing by the reticuloendothelial system, where, by nature of its physical characteristics and/or iron transport mechanisms, the final rate of iron delivery to the marrow falls far below potential requirements. This problem cannot be circumvented by the injection of large amounts of iron dextran intravenously. Although iron supply to the marrow immediately after the injection of 1500–2500 mg. is sufficient to support a marrow production level of 4–6 times normal, this rate of delivery is not sustained. The last 500–1000 mg. is released at a rate which permits no better than twice normal red cell production. This is hardly an improvement over the level of hemoglobin synthesis generated by oral iron therapy. Moreover, large doses of iron dextran complex given for the purpose of creating artificial iron stores may with time be totally unavailable for hemoglobin production. This in effect can destroy the value of the marrow iron stain in the diagnosis of iron deficiency anemia.

**SUMMARY**

The major portion of iron dextran iron becomes available to the erythroid marrow only after uptake and release of ionic iron by the reticuloendothelial system. With large intravenous infusions, (1000–2000 mg.), the rate of removal
of the iron dextran complex from plasma is enough to supply the marrow with more than 200 mg of iron/day. However, in the present studies the observed rate of iron delivery to the erythroid marrow was far below this level. Although iron supply immediately after infusion was sufficient to permit marrow production to rise to 4–6 times normal, this was maintained for less than two weeks. With time, reticuloendothelial iron dextran stores became less available, the serum iron level fell and marrow production was restricted to levels below twice normal. In fact, large infusion doses and prolonged storage may have made a portion of the iron dextran stores completely unavailable. Thus, an iron deficient type of erythropoiesis appeared in some patients while iron dextran stores were still readily visible on examination of marrow stroma.

**SUMMARIO IN INTERLINGUA**

Le plus grande portion del ferro de dextrano a ferro deveni disponihile pro le medulla erytliroide solo post le acceptation e le liberation de ferro ionic per le systema reticuloendothelial. Post forte infusions intravenose (1000–2000 mg), le prorata del elimination del complexo de dextrano a ferro ab le plasma suffice pro provider le medulla con plus que 200 mg ferro per die. Tamen, in le presente studios le observate prorata del provision de ferro al medulla erytliroide esseva marcatemente infra iste nivello. Ben que le provision de ferro immediatemente post le infusion sufficeva pro permitter un augmento del production in le medulla per un factor de 4 o 6 in supra del norma, iste augmento esseva mantenite durante minus que duo septimanas. In le curso del tempore, le reservas de dextrano a ferro reticuloendothelial deveniva minus disponibile, le concentration seral de ferro declinava, e le production medullari esseva restringite a nivellos infra duo vices le norma. De facto, alte doses de infusion e prolongate periodos de thesaurisation ha possibilemente rendite completely nondisponibile un portion del dextrano a ferro. Assi, un typo de erythropoiese a carentia de ferro appareva in certe patientes durante que reservas de dextrano a ferro esseva ancora prestemente visible in le examine del stroma medullari.

**ACKNOWLEDGMENTS**

We wish to express our appreciation to Dr. William Janssen and Dr. J.S.G. Cox for their assistance in this project and willing preparation of the $^{59}$Fe labelled iron dextran.

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Characteristics of Iron Dextran Utilization in Man

PERRY A. HENDERSON and ROBERT S. HILLMAN

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