The Effect of Chronic Iron Deficiency on Some Biochemical Functions of the Human Hemopoietic Tissue

By Ch. Hershko, A. Karsai, L. Eylon and G. Izak

In view of the erythroid hyperplasia found in the bone marrow of subjects with chronic iron deficiency anemia, the delayed appearance of reticulocytosis following iron therapy is unexplained. Profound disturbances were observed in some biochemical functions of the bone marrow cells isolated from such patients. There was a substantial decrease in the cellular nucleic acid content, associated with a marked drop in the rate of $^3$H-thymidine incorporation into DNA. The utilization of $^{59}$iron and of glycine-$2^-14$C for heme production, and of the latter compound for protein synthesis was also reduced, as compared to the findings in bone marrow cells from normal subjects. These metabolic alterations returned to the normal pattern in the bone marrow cell suspensions obtained from the patients following recovery after iron therapy.

The possible implications of these findings are discussed in the light of available information.

The response of iron deficient anemic patients to treatment has been thoroughly described.1-4 It has been shown that when iron is supplied orally the reticulocyte response occurs 3–4 days later, and usually precedes the rise in hemoglobin concentration by 24–48 hours. The response can be somewhat enhanced if the iron is administered intravenously,4-6 but, even then, several days elapse between institution of therapy and the reticulocyte response. In view of what is now known of the erythroid cell cycle in human bone marrow, and considering the reported erythroid hyperplasia in patients with chronic iron deficiency anemia, the cause for this delay in the appearance of reticulocytosis, and in the subsequent rise in hemoglobin, is not clear. A substantial portion of the erythroid elements in the marrow of these patients has been found to consist of late orthochromatic normoblasts.7 It would be reasonable to expect, therefore, that once iron reaches the hemopoietic tissue, these late normoblasts would mature into reticulocytes which should be released into the peripheral blood within 24–48 hours after the administration of iron. This has not, however, been the experience of clinicians.

In an attempt to elucidate this problem, the rate of incorporation of labeled...
precursors into heme, protein and nucleic acids by bone marrow cell suspensions obtained from patients with chronic iron deficiency anemia was studied. Similar experiments were carried out with bone marrow suspensions from normal subjects and the comparative results are presented in this communication.

**MATERIALS AND METHODS**

The diagnosis of iron deficiency was established when hypochromic microcytic anemia with low serum iron\(^34\) and low per cent saturation of the plasma iron binding protein were found in association with the absence of stainable iron in the bone marrow. In all patients, the cause of the anemia was chronic blood loss, either from a known gastrointestinal lesion, i.e. duodenal ulcer, or menometrorrhagia of long duration.

Reticulocytes for ribonucleic acid (RNA) determination\(^13\) were concentrated\(^9\) from the peripheral blood samples of four of the iron deficient patients before therapy and, again, 7 days after the institution of iron treatment during the ascending phase of reticulocytosis. Reticulocytes from six normal subjects served as controls.

Bone marrow studies were performed in four of the iron deficient patients before treatment, and were repeated 6–10 weeks after parenteral iron therapy had raised their hemoglobin concentration to, or near the normal values. Bone marrow suspensions prepared from five normal subjects served as controls. Bone marrow tissue was aspirated from the sternum or iliac crest through repeated punctures and was collected in cold, buffered Krebs-Ringer solution. A homogenous cell suspension was obtained by repeatedly passing the cells through a 26 gauge needle. The cell suspensions were washed three times in cold Krebs-Ringer solution and counted in a counting chamber. Smears made from the suspensions were stained with supravital and May-Grünwald-Giemsa stains, for viability and for differential counts, respectively.\(^11\) The suspension was adjusted to contain 10–15 × 10\(^6\) nucleated cells per cubic millimeter.

The following determinations were performed on the bone marrow cell suspensions: hemoglobin (cyanmethemoglobin), total protein,\(^8\) deoxyribonucleic acid (DNA),\(^12\) RNA, adenosinetriphosphate (ATP),\(^14\) nonheme iron content\(^35\) and hemosiderin.\(^36\) Portions of 0.2 ml. of the suspensions were incubated at 37\(^\circ\)C for 1 and 3 hours in the following media: pooled normal human serum (obtained from donors of group AB), 0.4 ml.; glucose, 0.4 per cent; penicillin, 10,000 U; and one of the following labeled precursors: 0.1–0.14 \(\mu\)Ci of \(^{59}\)FeSO\(_4\),\(^*\) 0.2 \(\mu\)M (2 \(\mu\)Ci/\(\mu\)M) glycine-2-\(^14\)C,\(^1\) 3–6 \(\mu\)Ci \(^{32}\)P,\(^1\) 0.1–0.3 \(\mu\)Ci uridine-5-\(^3\)H,\(^\$\) or 0.1–0.3 \(\mu\)Ci thymidine-3\(^3\)H.\(^\|\)

The incubation was terminated by washing the cells three times in cold, buffered saline. The radioiron incorporation into the whole cells and into the hemin moiety, isolated according to Thunell,\(^15\) was estimated using a well type scintillation counter (Elron-Haifa, Model: NIS-17-P). The incorporation of glycine into isolated hemin and into the remaining protein precipitates was also determined, after repeated extraction of the latter with alcohol-ether (1:3), using a gas flow-thin window beta counter at infinite thickness (Nuclear-Chicago, Model. 181B). DNA and RNA were isolated from the cells as described before,\(^27\) and the radioactivity was determined in a \(\beta\)-liquid scintillation counter (Packard.

\(^*\)\(^{59}\)FeSO\(_4\), supplied by Abbott Labs., North Chicago, Ill., specific activity, 20 mCi./mg. Fe.
\(^1\)Glycine-2-\(^14\)C, supplied by the Radiochemical Centre, Amersham, England, specific activity, 21 mCi./mM.
\(^\$\)Uridine-5-\(^3\)H, supplied by the Radiochemical Centre, Amersham, England, specific activity, 30–50 Ci./\(\mu\)g. P.
\(||\)Thymidine-3\(^3\)H, supplied by the Radiochemical Centre, Amersham, England, specific activity, 23 mCi./\(\mu\)M.
CHRONIC IRON DEFICIENCY

Table 1A.—The Distributions of the Erythroid Cell Population

<table>
<thead>
<tr>
<th></th>
<th>Pro- and Basophilic Normoblast Per Cent</th>
<th>Polychromatic Normoblast Per Cent</th>
<th>Orthochromatic Normoblast Per Cent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Range</td>
<td>Mean</td>
</tr>
<tr>
<td>Normal controls</td>
<td>8.2</td>
<td>3.0–15.0</td>
<td>6.4</td>
</tr>
<tr>
<td>Iron-deficient before treatment</td>
<td>2.5</td>
<td>0.6–3.9</td>
<td>37.0</td>
</tr>
<tr>
<td>Iron-deficient after therapy</td>
<td>11.6</td>
<td>6.5–14.3</td>
<td>9.1</td>
</tr>
</tbody>
</table>

* Per cent of nucleated bone marrow cells.

Five normal subjects and four iron-deficient patients were studied, the latter group both before and 6–10 weeks after iron therapy.

Table 1B.—Nucleic Acid, ATP and Iron Content of Bone Marrow Cells

<table>
<thead>
<tr>
<th></th>
<th>Normal Control</th>
<th>Before Treatment</th>
<th>After Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Range</td>
<td>Mean</td>
</tr>
<tr>
<td>DNA mg./10^9 nucleated cells</td>
<td>17.5</td>
<td>11.3–22.4</td>
<td>11.5</td>
</tr>
<tr>
<td>RNA mg./10^9 nucleated cells</td>
<td>5.18</td>
<td>3.70–6.95</td>
<td>2.4</td>
</tr>
<tr>
<td>ATP μM/10^9 nucleated cells</td>
<td>4.6</td>
<td>4.10–6.60</td>
<td>6.3</td>
</tr>
</tbody>
</table>

Bone marrow iron:

<table>
<thead>
<tr>
<th></th>
<th>Normal Control</th>
<th>Before Treatment</th>
<th>After Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μg./10^9 nucleated cells</td>
<td>1335</td>
<td>955–1850</td>
</tr>
<tr>
<td>Hemosiderin 1–4 +</td>
<td>+ +</td>
<td>1–2 +</td>
<td>0 – ±</td>
</tr>
</tbody>
</table>

Five normal subjects and four iron-deficient patients were studied, the latter group both before and 6–10 weeks after iron therapy.

Tricarb Scintillation Spectrometer, Model: 3375. The results were expressed as substrate incorporated in mmoles per 10^9 nucleated cells, or as c.p.m. per 10^9 nucleated cells.

Siliconized, sterile glassware was used throughout.

RESULTS

Bone Marrow Suspensions

The differences observed between the cell suspensions obtained from normal subjects, and those from patients with iron-deficiency anemia, both before and after parenteral iron therapy are illustrated in Tables 1A, and 1B. It can be seen that bone marrow cells of iron-deficient patients contained substantially less RNA and DNA, but slightly more ATP than normal marrow cell populations. The cellular DNA and RNA content returned to normal during the therapeutic response to iron (Table 1B).

The rate of incorporation of labeled thymidine into DNA in the iron-deficient marrow was about a third of that in the normal subjects, and returned to the normal range after treatment with iron (Table 2). On the other hand, no significant difference was found in the rate of incorporation of labeled uridine into RNA in normal regenerating bone marrow cell populations, as compared to the iron-deficient and the therapeutically-induced populations (Table 2).
Radioiron Incorporation. The rate of iron incorporation by the iron-deficient bone marrow cell suspensions was about the same as that found in the normal marrow cells (Table 3). This is of particular interest since more than half of the former and only one-fifth of the latter cell populations consisted of erythroid cells. It should also be stressed, that the cellular iron concentration in the iron-deficient marrow was less than half of that found in the hemopoietic tissue obtained from normal controls. The iron incorporation activity in the regenerating bone marrow cell populations was found to be within the normal range. An even more remarkable difference was found between the iron-deficient and normal marrow cells when the utilization of the incorporated iron for heme production was compared. While in the normal controls and in the regenerating bone marrow obtained after iron therapy more than 50 per cent of the iron incorporated by the cells was utilized for heme production, only 20–30 per cent was used for heme synthesis in the iron-deficient cell population.

Glycine-2-14C utilization. The rate of incorporation of glycine into heme by the marrow cells from iron deficient patients was much slower than that of the control bone marrow suspensions; this activity returned to the normal range in the samples obtained during the regenerative phase after iron therapy (Table 4).
Table 5.—Reticulocyte RNA Content

<table>
<thead>
<tr>
<th></th>
<th>Per Cent Reticulocytes After Concentration</th>
<th>RNA µg 1 ml Packed Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Range</td>
</tr>
<tr>
<td>Normal controls</td>
<td>4.1</td>
<td>3.5-5.5</td>
</tr>
<tr>
<td>Iron-deficient</td>
<td>5.1</td>
<td>4.2-6.8</td>
</tr>
<tr>
<td>After 7 days of intramuscular iron therapy</td>
<td>4.8</td>
<td>4.0-5.7</td>
</tr>
</tbody>
</table>

Five normal subjects and four iron-deficient patients were studied, the latter group both before and after 6-10 weeks after iron therapy.

The rate of incorporation of this amino-acid into protein was also slow in the iron deficient marrow, as compared to the normal, or to the regenerative marrow cell suspensions (Table 4).

Reticulocytes

The reticulocytes obtained from iron deficient subjects had a low RNA content, which returned to normal levels 6-7 days after the initiation of iron therapy (Table 5).

Discussion

It has been shown repeatedly, that the time required for a stem cell to give rise to mature erythroid elements is about 15 days. The bone marrow in chronic iron-deficiency anemia, as described here, has been shown previously by several investigators to be hyperplastic with a preponderance of poly- and orthochromatic normoblasts. The clinical experience of a delayed reticulocyte response to iron therapy in these patients may suggest that the erythroid cells developing in iron deficient subjects are defective. There is substantial evidence to support this assumption. It has been shown repeatedly by Stohlman and his associates that, as a result of iron therapy, a macrocytic red cell population emerges from the bone marrow in iron-deficient animals and human subjects. This phenomenon was found to be proportional to the amount of iron administered and occurred simultaneously with the reticulocytosis. The type of the newly emerging red cells and the delay in their appearance support the contention that they were produced by an erythropoietic tissue different from the one which delivered hypocromic microcytes to the peripheral blood before iron therapy.

Further evidence to support the defective biological functions of the bone marrow cells of iron-deficient patients was supplied by most of the biochemical parameters studied here. Thus, the rate of production of heme as measured by the incorporation of ⁵⁹Fe or glycine-²⁴C, the rate of protein synthesis and deoxyribonucleic acid synthesis were all considerably less in the iron-deficient marrow as compared to normal marrow.
In view of the erythroid hyperplasia with a preponderance of poly- and orthochromatic normoblasts found in the bone marrow of iron deficient patients, the diminished rate of heme production was rather surprising. Polychromatic normoblasts, as studied by us in cohorts of erythroid cell populations produced in rabbits with Actinomycin D, revealed a high rate of in vitro incorporation of labeled precursors into heme. Lajtha et al. made similar observations on human bone marrow culture. Since in a mixed bone marrow population the bulk of iron is incorporated by the erythroid precursors, the relatively low iron uptake by the marrow of iron-deficient patients, in the face of erythroid preponderance and a low intracellular iron pool, is unexpected from the observations made in normal erythroid precursors and denotes a fundamental difference between iron deficient and normal normoblasts. The decreased incorporation of both glycine-2-14C and 59Fe into heme points to a defect in porphyrin synthesis. Our observations are in line with those of Lichtman et al. and Prado et al., who found diminished porphyrin synthesis in iron-deficient human erythrocytes. An alternative explanation for an apparent reduction of heme synthesis might be the mechanism of ineffective erythropoiesis. That such a mechanism is a feature of severe iron-deficiency anemia was supported by the in vivo studies of Brunström et al.

The low DNA and RNA content of bone marrow from iron-deficient patients and the diminished rate of thymidine incorporation might reflect a change in the cellular constituents of a mixed bone marrow population and does not necessarily imply a fundamental difference in the properties of these cells. Thus, it could be expected that in the presence of large numbers of poly- and orthochromatic normoblasts which are no longer capable of deoxyribonucleic acid synthesis, the incorporation of thymidine into the whole group would be depressed. However, despite a diminished ribonucleic acid content of iron-deficient marrow, the incorporation of uridine-5-3H into RNA was identical in both the normal and iron-deficient bone marrow samples. It might be postulated, therefore, that the cause of diminished cellular RNA content in iron deficient marrow is not a suppressed synthetic activity but an enhanced intracellular RNA breakdown. This assumption is in line with the reported observations of Rabinovitz and Waxman who found that iron prevented the disaggregation of polyribosomes in rabbit reticulocytes. Since it has been shown that the early stage of ribosomal RNA breakdown is characterized by the disaggregation of polysomes, which is probably the case in iron-deficient subjects, the augmented RNA breakdown may be accepted as the cause for the drop in the cellular RNA content in the erythroid precursors of the iron-deficient patients. A further expression of this same defect is the lower RNA content of the reticulocytes of untreated patients when compared to that of reticulocytes after iron therapy or of normal subjects. In addition to these profound alterations in the nucleic acid metabolism of these cells, and perhaps as a result of them, their ability to synthesize protein was also markedly diminished. Deranged protein synthesis in iron-deficient bone marrow is reflected in the inadequate enzymatic conversion of delta-amino levulinic acid into porphyrin in human red cells, and this defect is not repairable by the in vitro addition of iron.
The restitution of normal bone marrow function upon addition of iron is, therefore, dependent on the correction of defects induced by iron-deficiency at a very early stage of erythroid differentiation and maturation. It seems plausible that the delay in emergence of reticulocytes from the marrow in response to iron therapy is due to the establishment of an entirely new "normal" erythroid cell population, a process which requires 4-6 days. It should be stressed, however, that these anomalies were found only in patients with chronic, longstanding iron-deficiency. Acute blood loss produces the opposite effects on all the parameters reported here, and the increased metabolic activity persists for as long as sufficient amount of iron can be mobilized for blood production, even when the hemorrhage is repeated or continuous.

The exact nature of the biochemical anomalies brought about by chronic iron-deficiency in the erythroid precursors is at present under investigation. It seems justified to conclude, however, that they are not the result of a deficiency of the energy generating system, as suggested by the normal glucose utilization and ATP contents of these cell suspensions. The anomalies described here added to the observations of Beutler et al. and others on the effects of chronic iron-deficiency on tissues other than the bone marrow and on some heme-containing enzymes serve to emphasize the widespread nature of the lesions associated with the lack of this metal.

REFERENCES

15. Thunnell, S.: Determination of incorporation of Fe59 in hemin of peripheral


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