Iron Incorporation Into Ferritin and Hemoglobin During Differentiation of Murine Erythroleukemia Cells


Hemoglobin and ferritin iron content have been followed during differentiation in tissue cultures of murine erythroleukemia cells (MELC) using the techniques of Mössbauer spectroscopy and electron microscopy. In undifferentiated cells grown without DMSO, only iron stored in ferritin was detected. The amount of iron in a cell grown in the presence of iron citrate is \( \sim 1.2 \times 10^{-14} \) g, whereas in a cell grown in the presence of transferrin the amount is \( \sim 0.28 \times 10^{-14} \) g. These quantities do not depend on the iron concentration in the nutrition medium in a range from 0.3 to 2.0 \( \mu \)g Fe/ml and are the same for growth times between 8 hr and 7 days. Cells grown with DMSO contain, in addition to ferritin, increasing concentrations of hemoglobin. Chase experiments prove that ferritin iron participates in hemoglobin synthesis. The amount of ferritin iron reaches saturation within less than 8 hr in MELC grown with or without DMSO. In differentiating cells grown with iron citrate there is a decrease with time in ferritin iron content concomitant with the increase in hemoglobin. Cells grown with transferrin incorporate additional amounts of iron, which are approximately equal to the amounts used for hemoglobin synthesis maintaining a constant ferritin iron level. In the electron microscope, iron is seen only as ferritin within lysosomes. The density of the ferritin in lysosomes correlates with the ferritin iron concentrations determined by Mössbauer spectroscopy.

The dynamics of hemoglobin synthesis in erythroid cells and the possible role played by the iron storage protein ferritin in hemoglobin synthesis is a subject of current interest. Among recent studies, Speyer and Fielding \(^1\) have separated out the major iron-bearing cytosol components of human reticulocytes after in vitro incubation with \(^{57}\)Fe and have identified hemoglobin and ferritin. From the results of these experiments, they suggested that ferritin is an obligatory intermediate in hemoglobin synthesis, since, with time, there was a fall of ferritin label and a corresponding increase in hemoglobin iron incorporation. Other reports \(^2\) presented evidence suggesting that ferritin is not always an intermediate required for hemoglobin synthesis. Most of these studies have been carried out using reticulocytes that represent the penultimate stage of maturation. Heme synthesis, however, is initiated and becomes maximal in earlier erythroid precursors. The characterization of the relationship between iron metabolism and cellular maturation has been hampered by the technical difficulty of obtaining uniform populations of erythrocyte cells at early stages of maturation. Solutions to this problem have been proposed by Konijn et al. \(^3\), who synchronized rabbit bone marrow cells following treatment with actinomycin-D, and by Nunez et al. \(^4\), who utilized the different rate of sedimentation under unit gravity of cells at various stages of differentiation. The results of both studies showed that iron uptake and ferritin synthesis are maximal in early differentiating cells and precede the phase of maximal hemoglobin synthesis.

In the present study, hemoglobin and ferritin content have been followed during differentiation in tissue cultures of the murine erythroleukemia cell line using the techniques of recoil-free resonance spectroscopy (Mössbauer effect) and electron microscopy. MELC, virus-transformed cells established by Friend et al. \(^5\) are maintained in continuous culture as proerythroblasts, with less than 0.1% of the cells exhibiting overt erythroid differentiation. The addition of certain chemicals, including dimethylsulfoxide (DMSO), \(^6\) to the culture medium results in expression of a program of erythroid differentiation in the majority of the cells. This includes characteristic morphological changes, \(^7\) synthesis of globin mRNA, \(^8\) synthesis of \( \alpha \) and \( \beta \) globin chains indistinguishable from those of the adult mouse, \(^9\) increased heme synthesis, \(^10\) appearance of erythrocyte-specific membrane antigens, \(^11\) and commitment to terminal differentiation and terminal cell division. \(^12\) These cells provide a model system to study the uptake of iron from the extracellular medium and the successive synthesis of the major iron-containing components during differentiation.

The technique of recoil-free absorption (Mössbauer effect) in \(^{57}\)Fe as a quantitative probe for the detection of iron has by now been successfully applied in a variety of biologic systems. In particular, Bauminger et al. \(^13\) have shown that the Mössbauer spectroscopy of frozen red blood cells can be used to quantitatively determine the major iron-bearing components, namely hemoglobin and ferritin, in red blood cells of patients.
with thalassemia, sickle-cell anemia, and unstable hemoglobin Hammersmith.

In the present study, recoil-free absorption measurements were carried out on cells grown in nutrition media containing $^{57}$Fe either in the form of citrate or bound to transferrin. In the presence of DMSO, both ferritin and hemoglobin iron could be quantitatively discerned in samples of packed frozen cells prepared at various stages of differentiation. Without DMSO, only ferritin iron was identified, and its quantity during cell growth was measured.

Although iron citrate, unlike transferrin, is not the source for intracellular iron uptake under normal physiologic conditions it was used in the present studies for the following reason. Preliminary studies showed that the amount of iron taken up by MELC in the presence of iron citrate is significantly larger than the amount taken up in the presence of transferrin. The large quantities of iron taken up by the MELC in the presence of iron citrate enabled the detailed ultrastructural studies described below and the comparison of the intracellular iron kinetics in MELC with and without iron overload.

Ultrastuctural studies of the cells were also carried out and the results correlated with those of Mössbauer spectroscopy.

MATERIALS AND METHODS

Cells

MELC, clone 745, were maintained in suspension cultures in minimal essential alpha medium (Gibco, Grand Island, N.Y.) supplemented with 15% (v/v) fetal bovine serum (Biolab, Jerusalem, Israel). Cultures of 50 ml of the medium and serum in 250-ml flasks (Lux, Newbury Park, Calif.) were inoculated with $10^4$ cells/ml from 1-day-old cultures with or without 2% DMSO. The cells were incubated at 37°C in a humidified atmosphere of 5% CO$_2$ in air. The pattern of cell growth under these conditions has been described and did not change when iron was added either in the form of citrate or bound to transferrin.

Chemicals

$^{57}$Fe-citrate was prepared as follows. Metallic iron enriched to 95% in $^{57}$Fe was dissolved in concentrated HC1. The pH was adjusted to ~2 by adding NaOH. Sodium citrate was added in excess and the pH adjusted to 7 with NaOH. The final iron concentration was 0.5 mg/ml. $^{57}$Fe-bound transferrin was prepared as follows. Metallic $^{57}$Fe dissolved in HC1 (300 $\mu$g/ml) was added dropwise to a solution of human iron-free transferrin (Sigma, St. Louis, Mo.) in 0.02M NaHCO$_3$. The pH was adjusted to 7.0 after every drop by the addition of 0.5M NaHCO$_3$. One microgram $^{57}$Fe was added per 1 mg of transferrin. The solution was left at room temperature for 1 hr and then dialysed for 24 hr against 3 changes of double distilled water. Finally, the solution was sterilized by filtration through a 0.4 $\mu$m Millipore filter (Bedford, Mass.).

Mössbauer Spectroscopy

The recoil-free resonance absorption in $^{57}$Fe was measured in the frozen cells using a conventional Mössbauer spectrometer. A 100-mCi $^{57}$Co-in-rhodium radioactive source that gives a narrow unsplit emission line was used. The 14.4-keV gamma rays were detected using a Harwell proportional counter operating at about 5 x 10$^4$ cpm.

The samples used for Mössbauer absorption spectroscopy usually contained about $10^6$ cells in 1 ml phosphate-buffered saline. They were sealed in lucite containers and stored in liquid nitrogen before measurement. The samples were placed in cryostats where absorption measurements at any temperature between 4.1°K and 273°K could be carried out. Most of the measurements were made at 85°K. At this temperature the spectra of oxyhemoglobin, deoxyhemoglobin, and ferritin are well resolved and distinct. Each of the latter components yields a quadrupole split hyperfine interaction doublet, characterized by different parameters (quadrupole splitting and isomer shift). Least-square computer fits to the experimental spectra were performed, taking the hyperfine interaction parameters corresponding to each sub spectrum and the relative intensity of each sub spectrum as free parameters. Lorentzian line shapes were assumed. Since earlier work on Mössbauer absorption in thalassemic red blood cells has shown that the recoil-free efficiencies of ferritin, oxyhemoglobin, and deoxyhemoglobin are approximately equal in a frozen aqueous solution at 85°K, the relative concentrations of ferritin and hemoglobin can be directly obtained by comparing the areas under the respective sub spectra. The absolute amounts of hemoglobin $^{57}$Fe and ferritin $^{57}$Fe in each sample were derived from the areas of the sub spectra corresponding to hemoglobin and to ferritin using their previously determined recoil-free fractions. In all the measurements carried out in the present work there was no evidence for the existence of sub spectra in addition to those corresponding to hemoglobin iron and ferritin iron. It is estimated that the limit of detection of $^{57}$Fe in any additional nonhemoglobin nonferritin compound was less than 10% of the total $^{57}$Fe in the sample. Therefore, the sum of the amounts of hemoglobin $^{57}$Fe and ferritin $^{57}$Fe determined in each sample represents quite accurately the total amount of $^{57}$Fe.

The methodolgy is illustrated by displaying some typical Mössbauer spectra obtained under specific conditions.

Figure 1 shows the recoil-free spectra obtained at 85°K in 3 x 10$^6$ cells grown for 6 days in 100 ml nutrition medium containing a total of 100 $\mu$g $^{57}$Fe in the form of iron citrate. The medium was supplemented with 15% serum containing a total amount of about 15 $\mu$g of unenriched iron in the form of iron transferrin. However, since the isotopic abundance of $^{57}$Fe in ordinary iron is only 2%, Mössbauer spectroscopy will detect almost exclusively the $^{57}$Fe, which originates from the citrate. Figure 1A shows the Mössbauer spectrum obtained from cells grown without DMSO, i.e., undifferentiated cells. The spectrum consists solely of a quadrupole hyperfine doublet with parameters exactly like those of the storage protein ferritin at 85°K. Moreover, the Mössbauer spectrum taken at 4.1°K shows a sharp six-line spectrum displaying the full dipole magnetic hyperfine interaction characteristic of ferritin at low temperatures. From the integrated area under the quadrupole doublet one can estimate the concentration of ferritin $^{57}$Fe to be about 1.2 $\mu$g/10$^6$ cells.

Figure 1B shows the spectrum obtained in 3 x 10$^6$ cells after 6 days of growth under conditions similar to those of the previous experiment, except for the addition of 2% DMSO in order to induce cell differentiation. The resulting spectrum is very different and shows the presence of the characteristic quadrupole doublets of oxyhemoglobin (sub spectrum a), deoxyhemoglobin (sub spectrum b), and ferritin (sub spectrum c). The decomposition into subspectra is
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Fig. 1. (A) Mössbauer spectrum at 85°K of 3 x 10⁶ cells grown for 6 days without DMSO in the presence of iron citrate. Only one quadrupole doublet corresponding to ferritin iron is observed. (B) Mössbauer spectrum at 85°K of 3 x 10⁶ cells grown for 6 days with DMSO in the presence of iron citrate. The solid lines are least-square computer fits to the spectra. The upper spectrum is a sum of 3 subspectra. Subspectrum a corresponds to oxyhemoglobin; subspectrum b corresponds to deoxyhemoglobin, and subspectrum c corresponds to ferritin iron.

Electron Microscopy

A suspension of cells at a concentration of about 2 x 10⁶ cells/ml was washed 3 times by centrifugation in 0.1 M saline buffer, pH 7.2, and fixed in 2% glutaraldehyde in saline buffer for 1 hr on ice. Cells were washed once in buffered saline and allowed to remain overnight at 4°C. Cells were postfixed in 1% OsO₄ in saline buffer followed by washing with double distilled water and en bloc staining with 0.25% uranyl acetate in 50% alcohol for 1 hr. In certain preparations this staining step was omitted. After staining, cells were dehydrated in a graded series of alcohols and embedded in Spurrs low viscosity embedding medium.¹⁵

Ultrathin sections were cut with a diamond knife on an LKB ultratome III. In order to obtain a clearer visualization of the electron-dense iron clusters in the cell cytoplasm, sections were not poststained with either uranyl or lead salts. Preparations were viewed with a Philips EM400 operating at 60 kV.

RESULTS

Iron Uptake in Differentiated and Undifferentiated Cells in the Presence of ⁵⁷Fe-Citrate

The results showing the total uptake of iron during 6 days of cell growth in the presence or absence of DMSO are presented in Fig. 2. In these experiments, the ⁵⁷Fe concentration in the culture medium was 1 µg ⁵⁷Fe/ml. The results were independent of this concentration over a wide range from about 0.3 to 2.0 µg ⁵⁷Fe/ml. The total ⁵⁷Fe content in both types of cells reaches saturation at a level of about 1.2 µg ⁵⁷Fe/10⁶ cells within 8 hr and remains constant thereafter. The amount of ⁵⁷Fe in the form of citrate added to the nutrition medium is about 10 times larger than the amount of the nonenriched transferrin iron. In addition, as will be shown later, the intracellular iron uptake from iron citrate, is about 5 times more than the uptake of iron bound to transferrin. Therefore, one may conclude that the total amount of intracellular iron (not only ⁵⁷Fe) at saturation is close to 1.2 µg/10⁶ cells, and thus about 3% of the total iron in the culture medium was incorporated in the cells.

Whereas only ferritin iron was identified in the undifferentiated cells, both hemoglobin iron and ferritin iron were present in differentiated cells. Figure 3 shows the ratios of hemoglobin ⁵⁷Fe and ferritin ⁵⁷Fe to total ⁵⁷Fe in the differentiated cells as a function of time. The ratio of hemoglobin iron to total iron rises from 7% after 2 days to about 64% after 6 days. The amount of hemoglobin iron per cell increases approximately linearly between 2 and 6 days, whereas the amount of ferritin iron decreases correspondingly. After 6 days, the amount of hemoglobin iron was about 0.75 µg/10⁶ cells, and the amount of ferritin iron was about 0.45 µg/10⁶ cells, the total iron remaining constant in accordance with the data displayed in Fig. 2.

Iron Uptake From ⁵⁷Fe-Transferrin in Differentiated and Undifferentiated Cells

From the analysis of the Mössbauer spectra obtained with MELC grown in the presence of ⁵⁷Fe-transferrin, the amounts of intracellular ferritin ⁵⁷Fe
1. Ratio of hemoglobin $^{57}$Fe to total $^{56}$Fe and ratio of ferritin $^{57}$Fe to total $^{56}$Fe, as a function of growth time, in cells grown in the presence of DMSO and iron citrate.

and hemoglobin $^{57}$Fe were measured. Figure 4 displays the total amounts of intracellular ferritin iron and hemoglobin iron as calculated, assuming that the iron uptake into the MELC was the same whether it originated from $^{57}$Fe-transferrin or from transferrin that was present in the serum. As described previously for cells grown in a medium containing $^{57}$Fe-citrate, the total amount of ferritin iron in cells grown with transferrin was independent of the $^{57}$Fe concentration in the range between 0.15 and 1.0 $\mu$g/ml. There are, however, significant differences in the uptake and in the intracellular distribution of iron in cells grown in the two media.

In the case of undifferentiated cells, the amount of iron taken up from iron transferrin in growth times between 8 hr and 6 days was found to be only about 25% of that taken up from iron citrate during the same period of growth (0.28 $\mu$g/10$^8$ cells as compared to 1.2 $\mu$g/10$^8$ cells). In these cells only ferritin iron could be identified. In the differentiated cells, both hemoglobin iron and ferritin iron were identified. The amount of hemoglobin synthesized in the presence of transferrin is similar to that synthesized in the presence of iron citrate (~ 0.3 $\mu$g hemoglobin iron/10$^8$ cells after 3 days and ~ 0.65 $\mu$g after 6 days). But, unlike the results obtained for cells grown in the presence of iron citrate, the amount of ferritin iron in differentiating cells grown in the presence of $^{57}$Fe-transferrin did not change as a function of growth time and increasing quantities of hemoglobin in the cells and was equal to the amount found in nondifferentiated cells. Consequently, the total iron content increased from 0.6 $\mu$g/10$^8$ cells after 3 days to 0.96 $\mu$g/10$^8$ cells after 6 days.

Changes in Hemoglobin $^{57}$Fe and Ferritin $^{57}$Fe Content Following Chase Experiments

Chase experiments were performed in order to find out unambiguously whether the ferritin iron of the cells is used for the synthesis of hemoglobin. For these experiments, the cells were first grown for 3 days in a nutrient medium containing $^{57}$Fe (1 $\mu$g $^{57}$Fe/ml), and then growth was continued in a nutrient medium containing transferrin iron unenriched in $^{57}$Fe. The chase is thus performed on the $^{57}$Fe isotope, to which the Mössbauer technique is sensitive.

Figure 5 shows the Mössbauer spectra obtained during the chase experiment using $^{57}$Fe-citrate. Figure 5A shows the spectrum obtained in a sample consisting of a portion of the cells grown for 3 days in the presence of DMSO and $^{57}$Fe-citrate. In accordance with the results described above, most of the iron is in the form of ferritin (the central doublet), but low intensity subspectra corresponding to oxyhemoglobin and deoxyhemoglobin are evident in the wings. From this spectrum we obtain a ratio of 0.29 ± 0.04 for
hemoglobin $^{57}\text{Fe}$ to ferritin $^{57}\text{Fe}$. The remaining portion of the original culture was washed and growth continued in a fresh medium containing DMSO, without any enriched $^{57}\text{Fe}$, with the only source of iron being unenriched iron present in the added serum. Figure 5B shows the spectrum obtained after 3 additional days of growth. The amount of hemoglobin $^{57}\text{Fe}$ per cell has increased appreciably, accompanied by a corresponding decrease of ferritin $^{57}\text{Fe}$. The ratio of hemoglobin $^{57}\text{Fe}$ to ferritin $^{57}\text{Fe}$ is now 0.65 ± 0.05.

Chase experiments were also performed on MELC grown in the presence of $^{57}\text{Fe}$-transferrin. Cells were first grown for 3 days in the presence of DMSO and $^{57}\text{Fe}$-transferrin ($\sim 1 \ \mu\text{g} \ ^{57}\text{Fe}/1 \ \text{ml of nutrition medium}$). The ratio of hemoglobin $^{57}\text{Fe}$ to ferritin $^{57}\text{Fe}$ was found to be 0.95 ± 0.09. Part of the original culture was washed and the growth continued for 3 additional days in a fresh medium containing DMSO and transferrin not enriched in $^{57}\text{Fe}$ ($\sim 1 \ \mu\text{g Fe/ml}$). The ratio of hemoglobin $^{57}\text{Fe}$ to ferritin $^{57}\text{Fe}$ was now 1.60 ± 0.15. From both sets of chase experiments we conclude that iron that had been stored in ferritin during the first phase of growth was used for the synthesis of hemoglobin in the second phase of growth.

**Electron Microscopy**

In the present study, MELC grown for 6 days in the presence of DMSO show the typical ultrastructural changes associated with erythroid differentiation, as described by Sato et al. Our description will be limited to the electron microscope appearance of iron in MELC grown either with iron citrate or transferrin for 6 days with and without DMSO. In differentiated and undifferentiated cells grown under these conditions, the only ultrastructural indication of iron is that found in the form of ferritin-like particles located within membrane bound cytoplasmic vesicles. These vesicles that fill the morphological criteria of lysosomes contain electron-dense particles of the order of 50–60 Å, corresponding to the size of the ferritin iron core.

When grown in the presence of iron citrate, comparison of the iron-containing lysosomes in differentiated and nondifferentiated cells shows a clear distinction in their appearance.

In nondifferentiated cells, the lysosomes containing the electron-dense particles appear to be bound by a single membrane with the ferritin exhibiting a close packed appearance (Fig. 6). In some areas of the lysosomes, the ferritin shows a quasicrystalline appearance that may be evidence for a transition between the close packing and an observed crystalline state. The lysosomes measured in a number of micrographs are of variable size between 0.2 and 0.5 μm. Both in the densely packed and crystalline forms, the electron-dense particles are bound by an electron-transparent region, presumably corresponding to the protein shell of the ferritin molecule. A fully crystalline lysosome from a nondifferentiated cell is seen in Fig. 7. Optical diffraction of this crystalline array shows the ferritin to be packed in a hexagonal lattice with a center-to-center spacing of 75 Å; the width of the apoprotein shell has been reduced from 25 Å to 10–15 Å. This observation is in agreement with studies of the appearance of ferritin crystals in liver cells as seen in the electron microscope.

In the differentiated cells grown in the presence of DMSO, the lysosomes are characterized by loosely dispersed ferritin and irregularly ordered intravesicular membranes. (Fig. 8). This appearance is indicative of a lower concentration of ferritin in the lysosomes from differentiated cells.

The above differences found when cells are grown with Fe-citrate do not exist when MELC are grown in the presence of transferrin with the ferritin in the
Figs. 6, 7, 8. See legends on facing page.
ly, an increasing proportion of the iron content of the cells is found in hemoglobin. After 6 days, the amount of hemoglobin iron in cells grown either in the presence of iron citrate or transferrin is $\sim 0.7 \times 10^{-14}$ g/cell. The results of chase experiments demonstrated, in agreement with earlier observations, that ferritin iron participates in the synthesis of hemoglobin and showed that most of the hemoglobin iron originates from iron that had been stored in ferritin during the early stages of erythroid maturation. However, the possibility that part of the transferrin or citrate iron is utilized directly for hemoglobin synthesis without going through the intermediate state of storage in ferritin cannot be excluded.

The kinetics of ferritin iron uptake during differentiation of MELC was found to be dependent on the presence of iron citrate or transferrin as the major source of iron in the culture medium. In cells undergoing differentiation in the presence of iron citrate, the total iron content remains constant (Fig. 2), while the amount of ferritin iron decreases concomitantly with an increase in hemoglobin iron (Fig. 3). The results imply that during the first 3 days of growth, when the cells are still multiplying, iron from the nutrition medium is incorporated into ferritin, reaching a saturation level within less than 8 hr after each cell division. At later stages of differentiation, when cells cease to multiply, the iron stored in ferritin is used for hemoglobin synthesis and no additional iron is incorporated into ferritin.

In cells differentiating in the presence of transferrin, the initial amount of iron stored in ferritin ($\sim 0.28 \times 10^{-14}$ g/cell) is not sufficient for the total cellular synthesis of hemoglobin. Additional iron is continuously taken up by the cell from the nutrition medium and stored in ferritin. The level of ferritin iron in these cells, therefore, kept approximately constant as a function of time, while the total iron content increases with the increase of the amount of hemoglobin (Fig. 4).

Electron microscope observations show the existence of ferritin-like particles located within lysosomes of differentiating and nondifferentiating cells. This observation is confirmed by the detection of ferritin by Mössbauer spectroscopy and in agreement with the

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**Fig. 6.** Typical lysosomes from undifferentiated MELC grown for 6 days in the presence of 1 $\mu$g/ml iron citrate. The lysosomes show a close packed homogeneous distribution of ferritin. At certain locations (arrows), the ferritin has a quasicrystalline appearance. N, nucleus. Bar equals 0.1 km. (x 122,000)

**Fig. 7.** (A) A lysosome from an undifferentiated cell grown in the presence of 1 $\mu$g/ml iron citrate, showing a fully crystalline arrangement of ferritin (x 120,000). Bar equals 0.1 km. (B) Optical diffraction pattern of 7A showing the hexagonal symmetry of the arrangement of the ferritin iron cores. The lattice spacing is 75 A.

**Fig. 8.** Lysosomes from differentiated MELC grown for 6 days in the presence of 1 $\mu$g/ml Fe-citrate and DMSO. In contrast to undifferentiated cells, ferritin is loosely dispersed within the lysosomes and intralysosomal membranes are evident. N, nucleus; CM, cell membrane. Bar equals 0.1 km. (x 125,000)
description of iron overload in the liver cells of patients with \( \beta \)-thalassemia major, where the iron accumulates as ferritin in lysosomes. Clear differences exist in the appearance of the lysosomes in undifferentiated and DMSO-induced differentiating cells grown in the presence of iron citrate. The high ferritin concentration in undifferentiated cells is reflected in a dense packing of the ferritin particles within the lysosome. The above difference does not appear to exist in cells grown in the presence of transferrin, since the ferritin in the lysosomes is seen only in low concentrations in both differentiated and undifferentiated cells. Thus, the differences in ferritin concentration, as seen in the electron microscope, correlate well with the findings of the Mössbauer spectroscopy.

The methodology employed in the present study can by utilized for the investigation of normal and pathologic red blood cells obtained from patients with intracellular iron overload, such as congenital hemolytic anemias and particularly the thalassemia syndrome. In red blood cells of thalassemic patients, large quantities of intracellular ferritin-like iron have been found, and it is not yet clear whether the excess is a consequence of the high rate of intracellular denaturation of hemoglobin during the accelerated and ineffective erythropoiesis or follows abnormal iron uptake from the extracellular compartments.

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Iron incorporation into ferritin and hemoglobin during differentiation of murine erythroleukemia cells

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