Sideroblasts

A Study of Stainable Nonhemoglobin Iron in Marrow Normoblasts

By Eugene Kaplan, M.D., Wolf W. Zuelzer, M.D., and Claude Mouriquand, M.D.

Siderocytes are erythrocytes which contain iron granules stainable with the prussian blue reaction. The term was introduced in 1941 by Gruneberg who observed such cells in the blood of human fetuses and of newborn flexated mice. Later Doniach and Pearson in collaboration with Gruneberg, and Pappenheimer et al. independently reported the occurrence of siderocytes in the blood of adults, chiefly in splenectomized patients. Dacie and Doniach, in 1947, called attention to the presence of similar iron granules in the normoblasts of human bone marrow. In the same year, MacFadzean and Davis reported a comprehensive study of iron granules in both blood and bone marrow in a variety of conditions. These authors found abundant iron granules in the marrow normoblasts in lead poisoning and in certain atypical hemolytic anemias. In normal individuals and in patients with various hematologic disorders, they reported iron granules in fewer than 10 per cent of the normoblasts. They considered the presence of iron granules as the probable result of a disturbance in hemoglobin synthesis. Dacie and White, however, in their 1949 review article, expressed the opinion that such granules might represent a normal phase in the utilization of iron by normoblasts.

The study of visible nonhemoglobin iron deposits in erythrocytes and their precursors seemed to us a promising tool in the investigation of ferrokinetics and erythropoiesis. Our first concern was with the question whether the presence of iron granules in the marrow normoblasts is indeed a normal phenomenon. If this fact were established it would become of interest to re-examine the incidence of iron granules in various hematologic disorders and, in particular, to see whether a correlation could be made between iron granules in normoblasts and the hypochromic anemia of iron deficiency. Such correlation, apart from furnishing a method for the estimation of available iron, might provide information concerning the intracellular metabolism of iron and of hemoglobin synthesis. For this purpose a comparison of the anemia of iron deficiency with other hypochromic anemias seemed especially promising.
Material and Methods

Bone marrow specimens were obtained from approximately one hundred and fifty infants and children hospitalized for various hematologic disorders and other conditions. Apart from the diverse anemias, the study included hematomically normal subjects with minor orthopedic and surgical conditions, convulsive disorders, and the like. Also included in the normal group were children without anemia, reticulocytosis, or marrow hyperplasia, who had the sickle cell trait and others who were convalescing from purpura. Frequently more than one specimen was obtained from the same patient in the course of our study.

The bone marrow was aspirated as a rule from the spinous processes or from the iliac crest and, in younger infants, from the tibia. Direct smears of the first drop obtained were thinly drawn across a slide. The fixation and staining procedure was adapted from Mac-Fadzean and Davis. The slides were fixed for at least 30 minutes in formalin vapor by standing them upright in a Coplin staining jar covered with formalin saturated absorbent paper. They were stained for at least 1 hour in equal parts of 2 per cent aqueous potassium ferrocyanide and 2 per cent hydrochloric acid, washed in tap water, then counterstained briefly with dilute basic fuchsin. Excess counterstain was removed by rinsing in tap water and dipping in absolute ethyl alcohol. All solutions were filtered immediately before use. The potassium ferrocyanide gave best results when aged at least several days. A basic fuchsin stock solution was prepared by dissolving 1 Gm. basic fuchsin in 10 cc. absolute ethyl alcohol and adding 90 cc. 5 per cent aqueous phenol. Six cc. of freshly filtered stock solution was added to 100 cc. distilled water for use as the counterstain. Depending on the thickness of the blood or bone marrow film, the counterstain was applied for 30 to 60 seconds.

All studies of iron granules were performed on films so prepared. With oil immersion magnification no fewer than twenty and usually at least fifty hemoglobin-containing normoblasts were carefully examined. The percentage of such normoblasts containing distinct blue-staining cytoplasmic granules was recorded. For the purpose of this report these cells were designated as "sideroblasts" in analogy to the term siderocyte already generally accepted for erythrocytes with iron-staining inclusions.

The bone marrow pattern and cytology was routinely studied for comparison in duplicate films stained with May-Grünwald Giemsa.

For the in vitro incubation of bone marrow, several drops of sterile heparin-saline were added to 1 to 2 cc. whole bone marrow aspirate, the culture flask rotated slowly in a 37 C. incubator, and samples obtained for study at 24 hour intervals.

The peripheral blood of each patient was studied for hemoglobin level, erythrocyte and reticulocyte count, and erythrocyte morphology.

The serum iron was determined by a modification of the method of Barkan and Walker. The normal range for serum iron in our laboratory was between 60 and 150 g per cent.

Observations

Bone Marrow Studies

With the method described, iron granules were readily visible in the cytoplasm of hemoglobin-containing erythroblasts (fig. 1). The granules of the sideroblasts appeared as light to dark blue, discrete, round, rarely rod-shaped bodies of varying size. The smallest granules were barely visible at 970 X magnification, the largest ones were approximately 0.5 mm in diameter. They were usually multiple, ranging from 2 to 5 to 20 to 30 inclusions in a given cell. Their distribution in the cytoplasm was found to be highly variable, although frequently they were located in the perinuclear zone as described by Mac-Fadzean and Davis. Generally, the size, number, distribution, and staining intensity of the granules in a given cell were similar to those in other cells of the same bone marrow.

The identification of the cell type presented no great difficulty. The normo-
blasts were readily distinguishable by their chromatin pattern and their homogeneous cytoplasm resembling that of the surrounding erythrocytes. Occasionally the distribution of the cells was checked by comparable counts of parallel specimens stained with May-Grünwald Giemsa or Wright’s stain.

Sideroblasts were consistently present in hematologically normal subjects (group I, fig. 2). They varied considerably in number, ranging from 20 to 90 per cent, but in every normal marrow examined, sideroblasts were found without difficulty. As a rule, the iron granules in this group were small and lightly stained. No correlation could be established between the percentage of sideroblasts and erythroid activity of the marrows as judged by the M:E ratios or maturation patterns.

Group II consisted of a variety of anemias, some associated with infections, others with renal disease, still others apparently primary as, for example, two cases of anemia of the Fanconi type and two cases of aplastic anemia. In some of the marrows of this group, erythroid hypoplasia was present while others had a normocellular or even hypocellular marrow. The number and distribution of sideroblasts in this miscellaneous group did not differ significantly from the normal group. The size and staining intensity of the granules in a given cell was, however, often distinctly greater than in the normal controls. This was especially true for three out of four of the hypoplastic-aplastic anemia patients all of whom had received multiple blood transfusions over long periods of time.

Group III was composed of several types of hemolytic anemias, both acute and chronic. In the former category there were cases of erythroblastosis fetalis,
acute hemolytic anemia due to naphthalene poisoning, and acute hemoglobinurias of undetermined etiology. The chronic conditions included congenital spherocytosis, sickle cell anemia, sickle cell-hemoglobin C disease, and several unusual congenital hemolytic anemias.

The percentage of sideroblasts in the group as a whole was somewhat higher than in the normal group, ranging from 40 to 90 per cent. There was no noticeable difference between the acute and the chronic types of hemolytic anemias.

The size and staining intensity of the iron granules were on the whole distinctly greater than those seen in group I. There were several exceptions however. The marrows of the five patients with hemoglobin C-sickle disease, all having mild hemolytic anemia, were comparable as a group to the normal pattern. Even among the marrows from severe hemolytic anemias one could find an occasional specimen in which the granules were small and lightly stained.

Group IV included the marrows of twelve infants with megaloblastic anemia prior to therapy. In this group the incidence of sideroblasts with one exception was above 90 per cent. The iron granules moreover were consistently large, prominent, and heavily stained.

In striking contrast to the normal controls and the various anemias thus far considered, the marrows from fifty-five infants and children with hypochromic iron deficiency anemia showed a consistent and marked reduction of sideroblasts with a range from 0.5 to 15 per cent, the vast majority falling below 1 per cent (group V). Those sideroblasts that could be found usually contained few, small, faintly stained granules.

For comparison, the marrows of another hypochromic-microcytic type of anemia, six cases of thalassemia major, were studied (group VI) and here not
only were sideroblasts abundant but the granules were comparable in size and appearance to those seen in the majority of hemolytic anemias as described for group III. A similar picture was obtained in the marrows of eight patients with the hypochromic anemia of lead poisoning (group VII).

Serum Iron Studies

Figure 3 shows the correlation between serum iron and marrow sideroblasts. As might be expected, the serum iron values were low in the iron deficiency anemia group in which sideroblasts were also greatly decreased. Conversely, in thalassemia, serum iron values were consistently high and correlated well with the large numbers of sideroblasts in the bone marrow. Similarly, a good correlation was apparent between the high serum iron values of untreated megaloblastic anemia and the striking iron deposits in the megaloblasts. Apart from these extreme conditions of hypoferremia and hyperferremia the correlation of serum iron and sideroblasts was less distinct. Considering the inherent sources of error in measuring the serum iron and marrow sideroblasts, a considerable spread was to be expected, and it was for this reason that the extremes were chosen for illustration. However, taking the material as a whole it was apparent that a relationship between these measurements existed. With the exception of several cases of lead poisoning, hypoferremia was not associated with a high percentage of sideroblasts, nor were high serum iron levels accompanied by a decrease of sideroblasts to the iron deficiency range.

To test this correlation further, selected cases were studied with serial observations. Intravenous iron therapy was administered to six patients with iron deficiency anemia. In each case, the sideroblasts in the marrow increased dramatically and usually there was a concomitant rise of the serum iron level.
Fig. 4.—Sideroblasts in iron deficiency anemia. Serum iron, γ per cent; Hb. = hemoglobin, Gm. per cent; Retic. = reticulocytes, per cent.

Fig. 5. Sideroblasts in megaloblastic anemia. Serum iron, γ per cent; Megaloblasts, relative frequency in bone marrow films; Hb. = hemoglobin, Gm. per cent; Retic. = reticulocytes, per cent. Rₙ = therapy; Transf. = transfusion; C.F. = citrovorum factor; B₁₂ = vitamin B₁₂; F.A. = folic acid.
to the normal range. One patient was given an inadequate dosage of iron by
the intravenous route (fig. 4). Following a prompt initial rise of both sideroblasts
and serum iron, the values for both fell again to the iron deficiency range, only
to rise once more when a second course of iron was given. The response of the
marrow sideroblasts was always more impressive than was the rise in the serum
iron levels. While no systematic attempt was made to establish the time re-
quired for the appearance of iron granules in the normoblasts following intraven-
ous iron therapy, a major increase in marrow sideroblasts was observed
after 18 and 24 hours but not after 1 hour.

In contrast to iron deficiency states, the cases of megaloblastic anemia studied
by us had a consistently high percentage of sideroblasts which correlated well
with the serum iron values obtained. The effect of therapy and the ensuing
remission was followed in several patients. Following effective treatment with
citrovorum factor or folic acid, the numbers of sideroblasts rapidly decreased
while the serum iron showed a parallel fall. During the later stages of con-
valescence both iron granules and serum iron levels returned to normal values
(fig. 5).

**Bone Marrow Cultures**

In cultures of bone marrow from patients without anemia or with a variety of
hematologic disorders other than iron deficiency, the percentage of sideroblasts
remained relatively constant over a period of four days. The initial level was
either maintained or tended to fall slightly (fig. 6). In marrow cultures from
patients with iron deficiency anemia incubated in their own serum, the initial
sideroblast count was lower than in the other group, but within 24 to 48 hours
there was a rise to about 20 per cent, a percentage still significantly lower than
in the first group. After 24 to 48 hours there was no further appreciable change.
A specimen of marrow from one such patient was divided into two parts (fig. 7): one was maintained in its native serum without addition; to the other was added 1 drop of saccharated iron oxide solution. The first culture showed the expected rise to 20 per cent sideroblasts while in the iron-treated culture, the sideroblasts increased progressively, reaching 80 per cent by the fifth day.

**Discussion**

The occurrence of sideroblasts in every marrow studied by us indicates that the presence of stainable nonhemoglobin iron in normoblasts is a normal phenomenon. Sideroblasts were found in appreciable numbers in normal individuals and in all the various hematologic conditions studied, with the single exception of iron deficiency anemia in which they were consistently and markedly reduced. Our observations are in disagreement with those of MacFadzean and Davis whose report described the only comparable study thus far made concerning iron granules in red cell precursors. These authors found appreciable numbers of sideroblasts only in certain acquired hemolytic anemias and in lead poisoning. We are unable to account for the discrepancy between our results and those of the British authors. The staining methods were essentially the same. The reproducibility of our sideroblast counts, the clear-cut staining reactions obtained, and the circumscribed character of the cytoplasmic granules excludes to our satisfaction errors due to artefact in our preparations. It is possible that the previous investigators paid attention only to heavily stained granules of relatively large size or to those granules which were seen first in preliminary marrow films stained by the Romanowsky method. It is important to realize that with Romanowsky stains the majority of the iron granules demonstrable
by the prussian blue stain are not visible. On the basis of their findings, Mac-
Fadzean and Davis concluded that the iron granules were produced by abnormal
mechanisms of hemoglobin catabolism peculiar to certain hemolytic disturbances.
Our results lead us to believe that, on the contrary, the presence of visible iron
represents a normal phase of intracellular iron metabolism probably related to
hemoglobin synthesis rather than to breakdown. The fact that of all the condi-
tions under investigation only iron deficiency anemia was characterized by
marked reduction in sideroblasts indicates that the presence of iron granules is
dependent on the availability of iron to the normoblasts. This relationship is
also evident in the good correlation obtained between sideroblast counts and
serum iron levels. The prompt rise of the sideroblasts following the adminis-
tration of iron to subjects with iron deficiency furnishes additional support for
the assumption that the iron granules represent iron taken up from the available
stores, rather than a degradation product of hemoglobin. The experimental
evidence obtained with marrow cultures from iron deficient individuals when
iron was added to the medium parallels the experience in vivo. The estimation
of marrow sideroblasts can therefore be used as a simple and sensitive index of
available iron.

The prussian blue reaction has been utilized by Rath and Finch and others⁹ for
the demonstration of iron in the form of hemosiderin in particle preparations
and sections of bone marrow. This iron occurs in reticulum cells and in extra-
cellular clumps. It is readily visible in unstained preparations as golden yellow
refractile deposits and is obviously not identical with the granules within the
erthroblastic cells which form the subject of this study. The marrow hemosiderin
is generally regarded as a storage form of iron and has been used as an index
of iron available for hemoglobin synthesis. Although the hemosiderin iron is
demonstrable in particle preparations, it is not easily demonstrable in the
particle-poor bone marrow films of infants and children. While the significance
of the two types of stainable iron in the bone marrow in terms of available
stores seems to be the same, the ultimate role of the iron granules within the
normoblasts is not as yet clear. It may well represent direct visual evidence of
an essential step in hemoglobin synthesis.

The exact chemical state in which the stainable iron exists in the normoblasts
is not known. Pappenheimer and others⁵ have shown essential differences
between these granules and hemosiderin. An iron-porphyrin combination has
been considered but in studies by MacFadzean and Davis,⁶ material derived
from the iron granules produced no fluorescence. Attempts to identify the
granules as ferritin have likewise failed.⁵ Pending further investigations along
these lines, one may speculate about the functional significance of the non-
hemoglobin iron of the normoblasts. If one discounts the hypothesis that this
iron results from the degradation of hemoglobin, two possibilities may be con-
sidered. Either the iron granules represent an intracellular depot for heme
synthesis, or they indicate an excess of iron which has entered the cells but is
not destined for utilization. The sequence of events in treated iron deficiency
anemia, namely the appearance of iron granules in the normoblasts followed by
reticulocytosis and evidence of adequate hemoglobin synthesis might be inter-
preted as favoring the first of these hypotheses. The fact that the mature red
cells produced under these conditions do not contain stainable iron would suggest utilization of the iron granules in the process of maturation.

The presence of abundant iron granules in the normoblasts of patients producing hemoglobin-deficient erythrocytes on the basis of Mediterranean anemia or lead poisoning shows, however, that the accumulation of intracellular iron in marrows with active erythropoiesis does not in itself assure proper utilization. One might postulate that in these conditions an intracellular block to heme synthesis interferes with the utilization of iron. The mature red cells, however, even in these conditions do not contain iron granules except after splenectomy.12

The disappearance of stainable iron in the course of the transformation of normoblasts into mature but hemoglobin-deficient erythrocytes as in Mediterranean anemia cannot readily be accounted for in terms of faulty utilization. It is possible, therefore, that the iron granules are not utilized but are extruded or diffused, not only in these conditions but in the normal as well. The questions raised here obviously require further studies, particularly with reference to the relationship between sideroblasts in the bone marrow and siderocytes in the circulating blood. Studies bearing on this question and on the role of the spleen in this connection will form the subject of another publication.

**Summary**

Bone marrow specimens from a large group of infants and children were studied with the prussian blue stain. Iron-staining granules were present in the normoblasts of all the subjects studied, including normal individuals and those with a large variety of hematologic disorders. The term sideroblast was proposed for normoblasts with nonstaining inclusions. The profound decrease in sideroblasts in iron deficiency anemia and their prompt increase following both iron therapy in vivo and the addition of iron to marrow cultures in vitro indicated that the estimation of normoblast iron granules provides a sensitive index of the availability of iron. In thalassemia and in lead poisoning, hemoglobin-deficient erythrocytes were found despite the presence of abundant iron granules within the normoblasts.

The presence of stainable nonhemoglobin iron in red cell precursors is a normal phenomenon and provides a useful adjunct for the study of iron metabolism.

**Summario in Interlingua**

Specimenes de medulla ossee ab un gruppo de 150 babie e infantes esseva studiate in lor reactiones al tintutura blau de Prussia. Granulos ferritingente esseva presente in le normoblastos de omne le individuos studiate, inclusive individuos normal e individuos con un grande varietate de disordines hematologic. Le termino "sideroblasto" es proponite pro normoblastos con tìngible inclusiones ferric. Le marcate diminution de sideroblastos in anemia a carentia ferric e lor prompte augmentation tanto post therapia a ferro in vivo e post le addition de ferro a culturas de medulla in vitro indicava que le estimation del granulos ferric in le normoblastos provide un sensitive indice del disponibilitate de ferro. In thalassemia e in plumbismo, erythrocytas deficiente in hemoglobina esseva trovate nonobstante le presentia de abundante granulos ferric intra le normoblastos.
Le presentia de tingibile ferro nonhemoglobinisc in le precursos del erythrocytas es un phenomeno normal; illo provide un utile adjuncto in le studio del metabolismo ferric.

REFERENCES
12 Kaplan, E.: Unpublished observations.
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EUGENE KAPLAN, WOLF W. ZUELZER and CLAUDE MOURIQUAND