Iron Metabolism in the Bone Marrow as Seen by Electron Microscopy: A Critical Review

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THE ELECTRON MICROSCOPE has enabled us to visualize with relative ease objects only 10 Å in diameter. At this resolution, practically all organic molecules should be visible. However, present-day preparatory technics do not yet permit recognition of macromolecules within cells. The sole exception is ferritin, thanks to its unique configuration.

The ferritin molecule contains about 23 per cent iron, i.e., about 2,000 iron atoms. This large number of atoms of high atomic weight gives a very contrasting image in the electron microscope. This is not all, however, for in the ferritin molecule the iron is arranged in a characteristic pattern, which permits its easy identification at a resolving power of about 12 Å. Other iron-containing substances can be seen under the electron microscope provided the iron atoms are sufficiently tightly packed to give an image of adequate contrast. For example, under particular circumstances iron is present in mitochondria in a form not yet chemically identified and can be seen in the electron microscope as masses of black granulations. The iron content of the larger masses with dimensions exceeding 0.2 μ is readily verified under the optical microscope by the Prussian blue reaction. The other iron-containing substances in the organism have not been characterized so far. Molecules, as important in iron metabolism as hemoglobin, siderophilin (transferrin) and cytochromes cannot be identified within cells at this time. The quantity of iron they contain is too small to be visible.

This study deals primarily with the part of iron metabolism which can be followed under the electron microscope. Although morphology represents but one aspect of iron metabolism, it is important to recognize the significance of the ultramicroscopic observations and to integrate them with the results of biochemical and isotopic studies. Electron microscopy has enabled us to visualize ferritin and has contributed to a better understanding of the composition of hemosiderin, the stages of erythrophagocytosis and the nature of the inclusions in sideroblasts and siderocytes. The new findings also pose new questions. The most important of these problems concerns the part played by the ferritin contained in erythroblasts during hemoglobin formation in normal and pathologic states.

TECHNICS

Peripheral blood cells, bone marrow, liver and occasionally other tissues were fixed immediately in Palade's fixative (1 per cent osmic acid buffered with veronal-acetate at 635}
pH 7.4) or Dalton's fixative (equal parts of 2 per cent potassium dichromate buffered with KOH at pH 7.2 and 2 per cent osmic acid with NaCl added to a final concentration of 0.85 per cent). Specimens were embedded, sectioned and examined by standard technics. A sample of each preparation was also examined by phase contrast microscopy or the ordinary optical microscope after Giemsa or Prussian blue staining. In certain cases, the same section has been examined by the optical microscope and by the electron microscope to localize with certainty the areas of positive Prussian blue staining.

Most of the studies have been done on specimens from healthy individuals or patients affected by some derangement of iron metabolism. A large number of control experiments have also been done on laboratory animals (rats, mice, guinea pigs), using strains at different ages and under different experimental conditions, such as iron overloading, lead poisoning, inflammation, certain vitamin deficiencies, and others.

It is necessary to emphasize here the importance of using magnifications of about 200,000 and a perfectly centered microscope, the resolving power of which must be at least 15 Å. Many authors have not seen any granulations when they were present in finely dispersed form because they were using too low a magnification or had a poorly centered microscope.

**The Ferritin Molecule**

Ferritin is a protein which was isolated in crystalline form in 1937 by Laufberger. It has a molecular weight of 580,000 and contains about 23 per cent iron, in the form of ferric-hydroxy-phosphate, which can be removed without denaturation of the protein by reducing agents such as cystein. The protein thus freed of its iron is called apoferritin. The viscosity and electrophoretic mobility of ferritin and apoferritin are identical. In 1954, Farrant examined with the electron microscope a solution of pure ferritin prepared from horse spleen homogenate by use of the Granick technic. Farrant observed that the ferritin iron was gathered in the four corners of a square whose sides measure 55 Å. It then became easy to identify the ferritin molecule, even within cells where the protein envelope is no longer visible. Ferritin was identified in the interior of cells as a constituent of hemosiderin, and in dispersed form or aggregates in erythroblasts, reticular cells and intestinal epithelium.

Later investigations, using higher resolving power and newer technics, have added further details of the morphology of the ferritin molecule.

**Shape of the molecule.** The negative staining of Brenner and Horne (either with phosphotungstic acid or with sodium and uranyl ethylene diacetate) permits the visualization of the shape of the ferritin and apoferritin molecules. They are generally polygonal, usually a regular hexagon with a diameter of 100 to 110 Å, the sides measuring 70 to 80 Å. Occasionally, the shape appears to be pentagonal. Ferritin contains a central mass or core of iron. In apoferritin, the central part is clear when the phosphotungstic acid is allowed to act only briefly. If the staining process is allowed to go on for a longer period, the tungsten of phosphotungstic acid is apparently able to occupy the central part of apoferritin, which then becomes almost as dense as in ferritin.

The polygonal shape of the apoferritin, and the central masses of iron of ferritin, can also be demonstrated by carbon shadowing.

**Spatial arrangement of the iron.** Recent investigations have shown that the
Iron within the ferritin core is not arranged in the four corners of a small square as the first observations led one to believe, but rather at the vertices of an octahedron, and that the different appearances of ferritin observed with the electron microscope coincide with the images given by an octahedron when viewed from every possible angle (fig. 1). It should be noted that one occasionally observes ferritin molecules which contain only a single aggregate of iron.

*Ferritin crystals.* When one examines with the electron microscope sections of ferritin crystals obtained by chemical methods, one sees very well their orderly molecular arrangement and can readily measure the dimensions of the iron micelles as well as the intermolecular spacings which are about 93 Å. In certain cases of iron overload, one finds, both in the liver and the
reticular cells, the crystalline appearance characteristic of ferritin obtained by chemical methods.\textsuperscript{20,29} In the same specimens, one may also observe mixed crystals, containing some apoferritin intermingled with ferritin molecules.\textsuperscript{20,29}

\textit{Localization of ferritin in cells.} Ferritin is normally found in varying quantities in reticular cells, in hepatic cells, in erythroblasts and in intestinal cells. It may also be found in kidney cells and in many other parenchymatous cells, in case of an iron overload. The ferritin molecules may be scattered in the cytoplasm. In other cases, ferritin may be found in aggregates. Such masses may be large or small; they may or may not be surrounded by a simple or double membrane; and they may be associated with other substances, which may or may not contain iron.

\textit{Hemosiderin}

To the histologist, hemosiderin signifies an “ochre pigment” which appears as yellow or brown granules containing iron in the form of ferric hydrate and which gives a positive Prussian blue reaction.

To the chemist, hemosiderin is a complex of protein, iron and other substances (lipids, sugars, copper, calcium). Estimates of the relative proportions of the different constituents vary from author to author. The iron content of “purified” hemosiderin has been reported as 1.5 to 5 per cent,\textsuperscript{72} 8.3 per cent,\textsuperscript{54,55} 25–29 per cent\textsuperscript{103} and 37 per cent.\textsuperscript{114} It is germane here to mention the general procedure by which the chemists prepare hemosiderin. Splenic or hepatic tissue is homogenized and the “soluble” iron removed by saline extraction. The insoluble fraction that is sedimented and contains iron is considered to represent hemosiderin. The electron microscope has shown that ferritin is a constant component of hemosiderin\textsuperscript{18,20,93,94} and permits, tentatively, at least four types of hemosiderin to be distinguished.

\textit{1st form:} Pure ferritin crystals or ferritin mixed with apoferritin (fig. 2).

\textit{2nd form:} Masses of ferritin, surrounded by a simple or double membrane, sometimes showing clear zones.

\textit{3rd form:} Masses made of large amounts of heterogenous substances. These include spheres which are relatively dark and correspond to PAS positive bodies; starlike or irregular masses, often containing myelin figures, corresponding to Sudan black positive bodies; and a large quantity of irregularly arranged ferritin molecules (fig. 3).

\textit{4th form:} Aggregates of similar composition, but containing some extremely fine and very dense granules, which seem to represent iron in a nonferritin form. This last form is seldom observed.

In view of these findings it is clearly erroneous to consider hemosiderin a uniquely defined compound. Hemosiderin constitutes an extremely variable mixture of substances and in any chemical studies the degree of homogeneity of the preparations should be checked at each step of extraction and purification by electron microscopy.

\textit{Erythropagocytosis}

It is well known that normal destruction of old erythrocytes takes place through phagocytosis by reticular cells and histiocytes. One may follow all
stages of phagocytosis and of intracytoplasmic digestion of red cells by phase contrast microscopy, aided by microcinematography. One may observe the capture of the red cells, generally preceded by their division into two parts. After their introduction into the cytoplasm, the fragments are first divided
Hemosiderin forms 2 and 3. Ferritin molecules dispersed in the cytoplasm and masses of ferritin irregularly distributed within cytoplasmic structures (108,000 X).

Fig. 3.—Hemosiderin forms 2 and 3. Ferritin molecules dispersed in the cytoplasm and masses of ferritin irregularly distributed within cytoplasmic structures (108,000 X).

into smaller, spherical particles, still fully hemoglobinized. Only then does hemolysis take place. Subsequently, the remaining stroma is divided into smaller and smaller particles. Such observations made in the living state have shown that the entire process of phagocytosis and digestion takes only about 10 minutes. The rapidity of the digestion and the fragmentation into minute
particles explains why the different steps of the phenomenon escape the ordinary optical microscope, where hemoglobin, when present in small amounts, is not readily observed.

With the electron microscope, one notes around the fragments of the phagocytized red cell, a large amount of small, dense granulations which, under high magnification, prove to be ferritin molecules. The ferritin is seen at the edge or inside the partially lysed and phagocytized stroma. One may, therefore, suggest that the iron resulting from the catabolism of hemoglobin is concentrated into ferritin molecules. These molecules of ferritin, grouped in larger or smaller masses, often attain sufficient size to be demonstrable with the optical microscope after staining with Prussian blue. In these masses, one sees a mixture of ferritin molecules and different substances which we have already described in the preceding section as the third form of hemosiderin. Usually these masses contain myelin figures derived from lipids of the digested red cell stroma.92,106

Location of erythrophagocytosis. One finds in the bone marrow of normal individuals a large number of reticular cells in the process of digesting red cells. They are also found in the spleen, though in lesser numbers. Normally, the Kupffer cells of the liver will only rarely be seen to digest red blood cells. This is in agreement with recent investigations which indicate that normally the greater part of erythroclasia takes place in the bone marrow.43,78 This is quite different from pathologic states. For example, in hemolytic anemias the greatest part of erythrophagocytosis takes place in the spleen and frequently also in the Kupffer cells of the liver. The observations of Noyes et al.83 which suggest destruction of normal cells in the spleen with subsequent rapid release of iron and transport by siderophilin to the liver and marrow were actually made on red cells damaged by storage.

Experimental Iron Overloading

Different authors have injected organic iron salts intramuscularly or subcutaneously in order to follow, with the electron microscope, the fate of iron thus introduced into the organism.20,53,69,82,96,113 The results of all such studies are quite similar and can be summarized as follows.

The injection of different iron compounds is immediately followed by their phagocytosis; later one finds in the cytoplasm of the phagocytic cells and adjacent to the ingested material, typical ferritin molecules. They appear quite rapidly; two hours after injection one can already see some of them, but it is usually necessary to wait for three to six days before the bulk of the phagocytized iron compounds is transformed into ferritin.

Iron transport. The iron thus injected, transformed locally into ferritin, is found after several days or weeks in other cells of the body and especially in the bone marrow and spleen. Theoretically, one may conceive of three different mechanisms for this observed transport of iron:

1. Transport by siderophilin which takes up iron from the ferritin-loaded cells and carries it to other reticular cells which store it again in the form of ferritin.

2. Transport by ferritin which travels either via the lymph or in the general
circulation. In certain pathologic cases, such as acute hepatitis and intoxication by carbon tetrachloride,\textsuperscript{115} it is easy to demonstrate that some ferritin is present in the blood. It is possible that it is also present normally but in too small an amount to be seen with our present technics.*

3. Transport by reticular cells or histiocytes. Such cells, loaded with iron, could travel either via the blood or the lymph, as seen in hemochromatosis, and thus be transported from the place where the iron has been injected to the bone marrow, the spleen, or other organs.

It would be very important to perform some accurate studies on the subject. So far, the isotopic studies have been restricted to siderophilin ( transferrin), neglecting completely ferritin and hemosiderin.

\textit{The Erythroblastic Island}\

The existence of “erythroblastic islands” in the bone marrow was known to the earliest histologists. In the preparations obtained by sternal puncture, these islands are difficult to recognize. The sudden suction exerted during sternal puncture and the preparation of smears disrupts the normal contiguity of cells, and one very seldom finds a complete island in the smear. It is, however, easy to see its various constituents with phase contrast or fluorescent microscopy,\textsuperscript{76,77} using somewhat thicker preparations.

With the electron microscope, the erythroblastic island may be seen very clearly as a constant anatomic feature of the marrow,\textsuperscript{10,21} of the spleen of rats and mice, and of the yolk sac.\textsuperscript{105} The island consists of one or two central reticular cells, surrounded by a ring of erythroblasts (figs. 4 and 5A). Sometimes there are two concentric rings, the one nearer the reticular cell made up of basophilic erythroblasts, the other of more mature erythroblasts.\textsuperscript{†} The reticular cell extends pseudopodia which occasionally surround the erythroblast entirely. Very often, the central cell of the island does not have a rounded or oval form but is star-shaped, with very thin cytoplasmic extensions, sometimes in the form of veils.\textsuperscript{21} It is along the surface of these veils that the young erythroblasts are located. Presumably the thinness of these extensions prevents their being seen by light microscopy. The varying appearances under the electron microscope lead one to expect that the extensions of the central

\*It should be noted that one siderophilin molecule, the weight of which is 90,000, carries two atoms of iron while a ferritin molecule, the molecular weight of which is 560,000, carries about 2,000 atoms of iron, or a factor of 1,000 per molecule.

\†In order to avoid the awkward repetition of “erythroblast, pronormoblasts and normoblasts in all stages of maturation” on each page, “erythroblast” is used throughout to denote the entire series of nucleated red cell precursors.

\‡The examination of fluochromed supravital preparations by fluorescent microscopy has enabled Marmont to demonstrate a third crown of reticulocytes all around the erythroblastic island. These newly generated red cells possess a great quantity of so-called granulofilamentous substance, and are still adherent to the erythroblasts. It has been hypothesized by Marmont that one factor in the still obscure “cytodiabatic” mechanism governing the release of the red cells from the bone marrow might reside in the opposing forces of adhesion of the “sticky” immature reticulocytes on the one hand, and of their intrinsic mobility on the other.\textsuperscript{76,77}
Fig. 4.—An erythroblastic island. The erythroblasts surround the central nurse cell which contains a number of phagocytized inclusions (3,100 X, reduced).

cell are continually changing in the living state, and that they attach themselves or actually surround now one erythroblast, now another.

Frequently, sections may not pass through the center of the island, but cut more or less tangentially. In these instances, careful observation shows that the erythroblasts always have at their periphery a fragment of the cytoplasm of the reticular cell. These bits of cytoplasm may sometimes also be seen with the light microscope since they remain attached to erythroblasts when the central reticular cell is fragmented during the preparation of smears (fig. 5B). Inconspicuous and usually overlooked in Giemsa stains, the shreds of cytoplasm occasionally stand out after Perl's staining. At times they appear as bluish spheres included in the cytoplasm of an erythroblast, corresponding to the larger invaginations of rhopheocytosis. Other fragments lie free and represent the so-called extracellular iron of marrow smears.

The studies of the erythropoietic island have raised a number of questions which we can only enumerate here, for they go beyond the scope of this review.

1. What is the number of erythroblastic cells forming the island? Examination by phase contrast and electron microscopy suggests that the islands are quite variable in size. Occasionally only one row surrounds the central cell; at other times an island contains three or four rows of cells. In such instances, the internal row is composed of basophilic erythroblasts and the most external one of reticulocytes, while the middle one is made up of polychromatophilic erythroblasts.
Fig. 5A.—Schematic representation of erythroblastic island. The reticular cell shown has a single dendritic extension. Others have several. Upper right corner: A transverse cut through the dendritic process, an aspect frequently seen at the electron microscope. With the optical microscope, both in sections and smears, the processes are not seen except when they contain ferritin and Perls' staining is used.

Fig. 5B.—Schematic representation of bone marrow smear from a case of acute rheumatoid arthritis, Perls' staining. The hatched areas appear blue in the smear. They are the fragments of reticular cells which were pulled apart in the preparation of the smears. Note that these fragments frequently remain attached to erythroblasts.

2. How many successive generations of erythroblasts are produced before an island disappears?
3. Is the central reticular cell the stem cell of the proerythroblasts?
4. Does the island interfere with the liberation of reticulocytes into the gen-
eral circulation. The arrangement of the cells in the islands suggests that erythroblasts migrate toward the periphery as they mature. The acidophilic erythroblasts and reticulocytes acquire a distinct motility which permits them to free themselves from the grasp of the reticular cell and to pass by diapedesis into the sinusoids of the bone marrow.

**Rhopheocytosis**

A close examination with the high magnification of the electron microscope reveals a peculiar phenomenon at the surface of contact between the reticular cell and the erythroblast. The surface of erythroblasts shows small invaginations which become vacuoles and penetrate into the cytoplasm of the erythroblasts. This phenomenon is reminiscent of the process of incorporating droplets from the "milieu extérieur," a process well known for more than 20 years from observations with the optical microscope as pinocytosis. The cell sends into the surrounding medium, the "milieu ambiant," a cytoplasmic veil which, coming back toward the cellular surface, imprisons a droplet from the medium and incorporates it into the cytoplasm. The electron microscope has shown the existence of an identical phenomenon at a much lower order of magnitude, the liquid droplets having a diameter of only a few hundred angstroms. Such micropinocytosis is observed in different cells. In the erythroblastic islands, however, one sees a slightly different phenomenon. The cell does not extend any veils as is characteristic of micropinocytosis, but appears to "aspirate" the external material. This process, therefore, appeared to deserve a special designation, and coined for it the term "rhopheocytosis," from the Greek, "I aspire."

The stages of this phenomenon are quite specific and are found at every examination of all preparations of human or animal, pathologic or normal bone marrow. At first, one sees ferritin molecules adhere to the surface of the membrane of the erythroblast; these molecules come without any doubt from the central reticular cell, whose cytoplasmic membrane is partially or completely dissolved. In some sections, one sees the reticular cell membrane broken up in spots and some free ferritin adhering to the surface of the erythroblasts. In the second stage, small invaginations form which contain ferritin molecules at their base. In the third stage, the invaginations are pinched off at their narrow neck, thus forming intracytoplasmic vacuoles, (fig. 6). The lower part of the vacuole contains the ferritin molecules, located a few hundred Angstroms from the wall, as if there was a protein sheath between them and the edge of the vacuole. Usually the vacuoles contain between 5 and 30 molecules in each section. On occasion, the process of rhopheocytosis is seen to take place in erythroblasts in the absence of ferritin molecules.

**Direction of rhopheocytosis.** The pictures given by the electron microscope evidently represent only particular moments in the evolution of rhopheocytosis.

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*Phagocytosis, pinocytosis, micropinocytosis, phagotrophy* and rhopheocytosis probably involve identical general mechanisms. However, in morphologic details, these processes are certainly different and it seems practical to give them separate names.
Fig. 6.—Rhopheocytosis ("micropinocytosis") of an erythroblast. Ferritin molecules are seen on the surface of the erythroblasts and at the site of an invagination of the cell membrane. Two cytoplasmic vacuoles are lined by ferritin molecules (70,000 X).

With the help of photographic records, one must attempt to reconstruct the entire sequence of events. One may ask, however, whether the static images of rhopheocytosis suffice to establish that the ferritin molecules indeed pass from the reticular cell to the erythroblasts. Could they perhaps move in the opposite direction? The arguments in favor of our interpretation are as follows.

Appearances identical to rhopheocytosis by erythroblasts have been observed many times with the electron microscope during the absorption of particles of colloidal gold or ferritin injected into the circulation, i.e., in circumstances in which the direction of the process is predetermined. Furthermore, as the erythroblast matures, it contains more and more ferritin which is grouped increasingly in aggregates and not in vacuoles near the periphery, suggesting a progressive accumulation of iron during the earlier stages of maturation.

However, there is no absolute proof that the phenomenon does not take place in the opposite direction, that is to say, that the erythroblast imparts the ferritin to the reticular cell.

Jandl has suggested this possibility, which appeared also to be in accord with the experiments of Crosby, who transfused siderocytes into normal and splenectomized recipients. The siderocytes disappeared rapidly from the circulation of normals. Simultaneous survival measurements by differential agglutination or Cr indicated that the donor cells had actually survived but had lost their iron granules. The "degranulation" presumably took place in the spleen, since siderocytes did not disappear from the blood of splenectomized recipients. We shall discuss alternative explanations below. We only wish to recall here that rhopheocytosis is a phenomenon which involves the sidero-
blasts and not the siderocytes and that our observations refer to the marrow, not the spleen. Crosby's experiment can, therefore, have no direct bearing on the direction of rphoecytosis.

**Sideroblasts and Siderocytes**

Those names are given to erythroblasts and erythrocytes which contain small, iron-positive granules as demonstrated with the Prussian blue reaction. Under the electron microscope, these granulations are seen to consist of masses of ferritin molecules, often embedded in a substrate which may or may not be surrounded by a membrane. In fact, erythroblasts of all stages of maturation contain some ferritin molecules, either scattered or grouped in larger or smaller aggregates, although only cells with masses larger than 0.2 \( \mu \) in diameter can be identified in the optical microscope as sideroblasts. It is particularly interesting to note that erythroblasts in the first stages of maturation are full of ferritin molecules scattered throughout the cytoplasm. Roughly 500 to 1,000 ferritin molecules are present in individual sections of proerythroblasts in normal man. In contrast, in the most fully matured normoblasts, the scattered ferritin molecules are less numerous and instead one observes a greater and greater number of aggregates.

As already noted, there are some reasons to think that ferritin enters the erythroblasts by the process of rphoeocytosis. This phenomenon accounts for the scattered molecules within the cytoplasm. The presence of masses of ferritin, reaching sometimes a diameter of 0.5 to 1 \( \mu \) remains to be explained. If the scattered ferritin molecules were to be used immediately, they would presumably remain in a dispersed state until they were utilized and disappeared from view. If, however, ferritin were to remain for some time in the cytoplasm, it may be expected to follow the general pattern of behavior of all foreign bodies in a cell, i.e., to form larger aggregates. The existence of such aggregates indicates that the entrance or formation of ferritin molecules much exceeds their consumption at this particular stage of development.

We must add here yet another localization of iron seen only in the proerythroblasts: ferritin may be included in cytosomes located within the Golgi bodies.

**Iron and mitochondria.** In certain instances, very rare in normal man, but frequent in the normal guinea pig and constant in certain human pathologic states such as refractory hypochromic anemias, thalassemia and others, one sees that the granules which stain with Prussian blue correspond to mitochondria filled with very dark granules. High magnification shows that occasionally these granules are molecules of ferritin, but more often one sees no structure in them at all (fig. 7).

The perinuclear distribution of mitochondria is responsible for the appearance of "ringed" sideroblasts in those pathologic conditions in which iron granules fill the spaces between the mitochondrial cristae.
Fig. 7.—Mitochondria and iron aggregates. Left: A mitochondrion from a patient with hypochromic, hypochromic anemia. The mitochondrion is somewhat swollen and the cristae are indistinct. The iron micelles are crowded into the space between the crista and fill it entirely, resulting in a "step-ladder" appearance. In the cytoplasm above the mitochondrion one notes a mass of ferritin molecules.
How does ferritin enter the mitochondria? We do not know whether the iron granules arise from a transformation of the ferritin in situ, or from the accumulation of iron which entered the mitochondria in some other chemical form.

Siderocytes. As a rule, red blood cells do not contain iron granules visible under the optical microscope. With the electron microscope, one may note in very rare red cells some ferritin molecules in the sections. One sees them fairly well in the stroma after hemolysis, as was shown in the early investigations of Hoffman and Hillier, though at that time the authors did not know that they were dealing with ferritin.

These rare siderocytes are actually reticulocytes. Electron microscopic examinations show that almost all reticulocytes contain aggregated or dispersed ferritin, while older red blood cells have none. The reticulocytes of the bone marrow may be distinguished by their larger masses of ferritin from the older reticulocytes of the peripheral blood.

Pathology of Iron Metabolism

The total quantity of iron in man is about five Gm.: three Gm. in hemoglobin, one Gm. in myoglobin and certain enzymes, and one Gm. in the form of ferritin, hemosiderin and nonhemic iron. This distribution of iron is readily altered. Blood loss of more than one-third of the red cell mass, if uncompensated by increased intestinal iron absorption, suffices to produce a state of iron deficiency. Conversely, some disturbances of intestinal absorption can augment the iron stores to enormous proportions; in hemochromatosis, the body may contain more than 30 Gm. of iron.

In certain cases, in spite of the presence of iron in large quantities in the body, a hypochromic anemia is present. In such instances, the iron stores in the spleen, the liver or the lungs may be unavailable for hemoglobin synthesis, iron may fail to enter red blood cells, or finally iron utilization may be blocked in the interior of the red cells. The following paragraphs will be devoted to a discussion of these pathologic states as they affect the appearance and distribution of iron seen with the electron microscope.

Hypochromic iron-deficiency anemia. With the optical microscope, neither sideroblasts, nor siderocytes nor hemosiderin are seen after Perls' staining. With the electron microscope, one observes that the digestion of old red cells proceeds normally, but no ferritin molecules are seen around the phagocytized fragments. In contrast, the red cells undergoing digestion are surrounded by round dark masses which resemble the PAS positive particles described by Astaldi, and by myelin figures in larger numbers than normal. The erythroblasts, whether basophilic, polychromatophilic or acidophilic are devoid of either massed or isolated molecules of ferritin. The erythroblastic islands, however, show a normal configuration. Rhopheocytosis is very intense, although no ferritin molecules are present.

Several interpretations of these observations are possible; ferritin is available and passes into the erythroblast but its quantity is too small and its life too brief for its detection by present technics; ferritin is not available at all
and rhopheocytosis is ineffective; finally, rhopheocytosis may transfer other substances than ferritin necessary for the erythroblast but invisible with the electron microscope. In this case, one may wonder whether, in the normal state, the transfer of ferritin may simply be superimposed on other more basic processes. We shall return to this hypothesis later.

*Idiopathic hemochromatosis.* With the optical microscope, one observes an over-abundance of hemosiderin in all organs, but particularly in the liver, spleen and bone marrow. Sideroblasts are numerous in the bone marrow but siderocytes are generally absent.

With the electron microscope, reticular cells are seen gorged with iron in the form of hemosiderin and accumulations of ferritin, as well as very large numbers of dispersed ferritin molecules. The mitochondria of erythroblasts contain small aggregates of iron micelles comparable to those found in normal guinea pigs. In addition, the stroma of the mature erythroblasts (normoblasts) contains a number of ferritin molecules. In other words, there is an excess of ferritin and iron micelles, but it is slight compared to that seen in hypochromic hypersideremic anemias. Dispersed ferritin is also found in plasmocytes and lymphocytes.

*Hypersideremic anemias.* This syndrome was identified only a short time ago. Without doubt, it includes a variety of diseases. In all of them, a considerable increase of nonhemoglobin iron in circulating red blood cells has been found by biochemical methods. At present, it appears convenient to distinguish the following entities in this group:

1. thalassemias;
2. hypochromic hypersideremic non-thalassemic anemias;
3. normochromic sideroblastic anemias; and the
4. anemia of lead poisoning.

**Thalassemias.** The electron microscope shows the following: In the liver, spleen and bone marrow, an increase of hemosiderin and of dispersed ferritin molecules, and the presence of crystalline ferritin.

In the erythroblasts, one also finds very characteristic changes. In all stages of maturation, there are large quantities of iron, either ferritin molecules or individual micelles, and occasionally masses of ferritin. Occasionally the ferritin molecules are aligned in a fashion which suggests interposition of apoferritin molecules. Quite frequently, one sees some ferritin and iron micelles in the mitochondria. The large masses of ferritin disappear almost completely in the circulating erythrocytes. However, they retain a large amount of dispersed ferritin molecules.

In addition, one finds regions with irregular or “geographic” outlines, which are of a darker grey than the rest of the partially hemoglobinized cytoplasm. These areas are of varying size, between 0.1 to 5 μ, and sometimes contain a few molecules of ferritin which may be aligned in a lattice. It is possible that these regions of amorphous substances represent apoferritin.

One also sees very dense spherules from 0.1 to 0.3 μ in diameter, sharply outlined and without a membrane. It is possible that these correspond to the PAS positive particles described by Astaldi and colleagues.
Finally there are some vacuoles, sometimes in large numbers, usually empty but containing in rare cases fine vesicles or a cloudy looking substance, the nature of which is unknown.

The findings in thalassemia minor are similar, though quantitatively less pronounced.47,48

HYPOCHROMIC HYPERSONEREMIC NON-THALASSEMIC ANEMIAS. All these anemias present the same findings under the electron microscope.7,10,33,74

In the spleen, the liver and the bone marrow, the reticular cells are completely loaded with hemosiderin. The erythroblasts present a very intense rhopheocytosis; they are full of dispersed ferritin or masses of ferritin. All erythroblasts have some mitochondria completely filled with iron-containing micelles. Reticulocytes contain some more or less degenerated mitochondria which are loaded with iron (fig. 8). The mature cells have a large quantity of dispersed ferritin molecules.

REFRACTORY SIDEROBLASTIC ANEMIAS. Certain of these anemias closely resemble the preceding group though they are not hypochromic. In certain cases, they comprise a double population of normochromic and hypochromic red cells.41,74 The findings in the electron microscope are identical to those of hypersideremic hypochromic anemias. Some forms of these anemias precede myeloid leukemias, erythremias or cancers.41,58,59

Until now, we have seen only one case of this kind. The pathologic iron localization was the same as in hypochromic hypersideremic anemias.

LEAD POISONING. With the optical microscope, one notes the presence of erythrocytes with punctate basophilia and of numerous bone marrow sideroblasts which sometimes pass into the blood.73 There is an overload of iron in all reticular cells. Hypochromic erythroblasts and erythrocytes present numerous dispersed ferritin molecules or masses of ferritin molecules. The most characteristic finding in this anemia is the considerable alteration of mitochondria in erythroblasts. While the mitochondria of other cells are not altered, the mitochondria of erythroblasts are all enormous and their crests are empty.15,16 It is probable that the altered mitochondria correspond to the "basophilic granulations" often seen in plumbism. We have not seen any iron inside the mitochondria.

Inflammation.* It is well known that inflammation interferes considerably with iron metabolism.34,51,52,61,65,66,68 The morphologic findings are similar in all types of inflammation as well as in malignancies. With the optical microscope, one sees large quantities of iron in the reticular cells, and, classically, sideroblasts are not seen.65,80 One might therefore suspect a block in the transport of ferritin. The electron microscope,27 however, shows a rhopheocytosis of ferritin of extreme intensity, perhaps greater than in cases of hypochromic hypersideremic anemia where it is already excessive. This ferritin does not

*Under this heading, we include chronic infections, rheumatoid arthritis, and certain other inflammatory processes, including sterile turpentine abscesses, known to produce similar disturbances of iron metabolism.
Fig. 8.—Reticulocyte from a case of hypochromic hypersideremic anemia. In the mitochondria the spaces between the cristae are filled with iron micelles. Masses of ferritin are present outside the mitochondria (44,000 X).

form masses, and therefore escapes visualization by the optical microscope. The ferritin disappears as the cells mature, so that there is none in the mature red cells.
IRON METABOLISM AS SEEN BY ELECTRON MICROSCOPY

DISCUSSION

We would like to discuss briefly three of the problems raised by observations with the electron microscope: 1) the role of erythroblastic ferritin; 2) the role of rhopheocytosis; and 3) the significance of the non-hemoglobin iron in the erythroblasts of hypochromic hypersideremic anemias.

Role of ferritin in erythroblasts. Two hypotheses have been advanced: According to the first hypothesis, the erythroblastic ferritin represents an iron reserve for the synthesis of hemoglobin. The granules of the sideroblasts represent a temporary excess of iron. The arguments supporting this hypothesis are as follows:

a. As the erythroblasts mature, the number of ferritin molecules decreases; in the reticulocytes they are still present in numbers but are absent from mature red cells. One may assume that this iron has been used for hemoglobin formation. This is in accord with the observations of Alpen and Lajtha that the erythroblasts incorporate considerably more iron than young reticulocytes, and with the microspectrophotometric observations of Sondhaus and Thorell who found that non-hemic iron, probably ferritin, decreases as the total amount of hemoglobin increases.

b. In sideropenias, sideroblasts are not seen. In general, shifts in plasma iron and sideroblasts parallel each other in the development and treatment of iron deficiency.

c. One finds occasionally ferritin or iron micelles in the mitochondria, especially in pathologic states. It is known that mitochondria play an important part in the incorporation of iron into protophorphyrin. One might, therefore, assume that iron passes into mitochondria at some stage in the process of transforming nonhemic iron into hemoglobin iron, although we do not know if the iron micelles of mitochondria come from ferritin or from another iron-containing substance.

In hypochromic hypersideremic anemias, one might consider that the mitochondrial mechanism is disturbed so that the mitochondria store iron. In plumbism, the iron apparently cannot enter the abnormal mitochondria.

d. In inflammation and in hemolysis, one observes dispersed ferritin molecules but no masses of ferritin are visible under the optical microscope. This may be explained by a greater speed of the ferritin metabolism so that there is no time to pile up larger masses of ferritin.

The second hypothesis asserts that the erythroblastic ferritin represents an excess of iron unusable for hemoglobin formation. When the red cell matures, ferritin accumulates in masses resulting in siderocytes. These siderocytic granules are then removed by the spleen. The concept is supported by experiments in which transfusion of siderocytes from pathologic cases into normal recipients was followed by the disappearance of siderocytic granules. In splenectomized recipients, the siderocyte granules persisted.

As an alternative explanation for the experiments on which Crosby has based his theory, one may suggest that the spleen possesses a maturation factor. In fact, when the spleen is removed, one observes the appearance in the peripheral blood of large numbers of reticulocytes, occasional siderocytes,
and a few red cells with Howell-Jolly bodies. All three changes indicate a delay in maturation of the young red cells.

The hypothesis that the function of the spleen is to promote the maturation of reticulocytes rather than to remove siderocyte granules from them is supported by observations in hemachromatosis. In this disease, with an overabundance of iron in the whole body, but without red cell abnormality, there are large numbers of sideroblasts, but no siderocytes in the marrow. Thus, increased amounts of ferritin can disappear during normal red cell maturation in the marrow, the ferritin probably being used for hemoglobin synthesis. In contrast, in plumbism and Cooley’s anemia, where there are abnormalities of red cell development, siderocytes have been observed in the circulation in the presence of a normal spleen.

Role of rhopheocytosis. It has been suggested that rhopheocytosis enables ferritin iron to penetrate into the interior of the erythroblast. Thus, it enables part of the iron necessary for the synthesis of hemoglobin to enter the cell.

Unfortunately, it is impossible to evaluate this thesis quantitatively, since we are not at present able to state the number of ferritin molecules contained in the erythron. The quantity of ferritin molecules contained in a normal erythroblast section varies. On the average, it contains about 800 molecules per section. This gives us roughly 80,000 molecules for the whole cell. We do not know the speed of entry of the molecules, nor the speed of their utilization. From the fact that much of the ferritin disappears by the time the reticulocyte stage is reached, one may infer that a large portion of ferritin is metabolized as it enters the cells. However, we do not know whether the degree of rhopheocytosis is constant at all stages, or whether the successive cellular divisions interfere with the phenomenon.

Alternatively, rhopheocytosis may be essential for the transfer of other, yet undetermined, substances necessary for the maturation of the erythroblast.

The thesis is supported by the fact that in iron deficiency anemias, rhopheocytosis is present in the absence of ferritin. Further support is given by the fact that in essential hemochromatosis and in all diseases where the reticular cell is full of iron, the passage of iron is very intense. In such instances, an excess of ferritin would be transferred which may or may not be used for hemoglobin synthesis.

This thesis agrees with a fundamental fact: Isotopic studies have demonstrated that almost all of the iron which is used for hemoglobin formation is carried by the plasma siderophilin4,6,8,5,92 (fig. 9). Moreover, in vitro studies have demonstrated that reticulocytes and erythroblasts entirely free of reticular “nurse cells” are able to incorporate the siderophilin iron directly into hemoglobin4,6,8,5. It is possible that both mechanisms coexist and siderophilin imparts iron to both the erythroblast and the reticular cell. In the reticular cell the iron forms ferritin, which is transferred to the erythroblast by rhopheocytosis9. The alternative—that ferritin is manufactured within

* Occasionally, one sees the same reticular cell which has phagocytized old red cells and formed hemosiderin transfer ferritin to the erythroblasts which surround it. However, this is evidently a special case. The reticular cells of the entire body and particularly of the spleen are able to phagocytize red blood cells, and iron is then transported by siderophilin to the marrow and the central reticular cells of the erythroblastic islands.
Fig. 9.—Schematic representation of iron transfer by erythrophagocytosis, siderophilin and rhopheocytosis.

crythroblasts—seems unlikely, although the ability to synthesize ferritin is widespread and has been demonstrated by Richter for liver cells in vivo and Hela cells in vitro.98

Significance of excess non-hemic iron in hypochromic hypersideremic anemias. The data presented may be interpreted as follows: the metabolism of hemoglobin is blocked at a place which varies with the disease in question and the immobilized iron accumulates in the cytoplasm and in the mitochondria. In our total ignorance of the regulation of entrance of iron into the cells, one might assume that iron continues to enter and that this continued accumulation results in much more iron than necessary for the formation of hemoglobin. The calculations are very difficult but it seems that in certain cases there is more non-hemic iron than necessary to produce the hemoglobin content of a normal red cell. This thesis is in accordance with the observations of Bannerman and colleagues.3

Alternatively, it has been suggested that iron enters the erythroblasts in excess and this excess inhibits hemoglobin formation. Anemia and malformations of erythrocytes would then be the result of an excess of iron in the erythroblasts rather than an excess of iron resulting from the abnormalities in hemoglobin formation.31,78 Sundberg et al.107 assume the existence of multiple superimposed deficiencies (pyridoxine, folic acid, etc.) which would slow down the hemoglobin formation and thus permit the entrance of a greater quantity of unusable iron. Further experiments are needed to decide between these hypotheses.

SUMMARY AND CONCLUSIONS

High resolution electron microscopy has made possible the visualization of transport and storage iron in the form of ferritin, both in dispersed form and
in aggregates and in the form of “iron micelles” in mitochondria. Hemosiderin was found to consist either of pure ferritin in crystalline clusters or, more frequently, of ferritin associated with other substances, including a lipid component in the form of myelinic figures and PAS positive material.

In the following paragraphs we have summarized the new morphologic findings and what appears to us the most likely interpretation in the light of known biochemical and isotopic studies. Alternative interpretations have been discussed in the body of the paper.

Electron microscopy has established the erythroblastic island as a morphologic and functional unit of the bone marrow. A central reticular “nurse cell” appears to impart nutrients to surrounding rows of erythroblasts by the process of rhopheocytosis. Transfer of ferritin by this process is probably a passive phenomenon, since the amount transferred parallels the amount of iron present in the central reticular cell. Ferritin is increased both in the reticular cell and in erythroblasts in hemochromatosis. It is absent in iron deficiency, although rhopheocytosis remains prominent. Normally all erythroblasts (proerythroblasts and normoblasts) and reticulocytes contain ferritin. Only the larger aggregates can be visualized by the Prussian blue reaction in sideroblasts and siderocytes.

Ferritin generally disappears when reticulocytes mature, even in hemochromatosis and infections, two conditions in which there is an excess of ferritin in erythroblasts. Interestingly, the increase in infections is entirely in form of dispersed ferritin and cannot be visualized by the Prussian blue reaction; i.e., sideroblasts are absent, in contrast to hemochromatosis where they are normal or increased.

It appears most likely that ferritin disappears from normal maturing reticulocytes because it is utilized for hemoglobin formation. It persists in mature red cells in Cooley's anemia, hypersideremia, hypochromic anemia and lead poisoning where hemoglobin formation is disturbed.

The origin of the ferritin in the nurse cells and the extent to which ferritin rather than siderophilin contributes to hemoglobin synthesis are unsolved problems. Isotopic studies indicate that almost all of the iron used for hemoglobin synthesis is derived from siderophilin and hemoglobin synthesis can proceed without any visible ferritin, as in iron deficiency anemia. These facts must be reconciled with the electron microscopic observations which suggest that normally some iron reutilization within the marrow proceeds by way of erythrophagocytosis, fragmentation, intracellular hemolysis of red cells, formation of ferritin and rhopheocytosis. Iron derived from erythrophagocytosis elsewhere in the body probably reaches the marrow bound to siderophilin. Such iron can be incorporated into ferritin of reticular cells as may be seen in hyperferremia and following injection of iron compounds. The process of rhopheocytosis would then lead to utilization of at least part of this ferritin iron for hemoglobin synthesis.

In certain pathologic states, accumulation of ferritin and related visible dispersed or conglomerated iron micelles may point to the sites where hemoglobin synthesis or iron transport is blocked. In Cooley's anemia and the hypersideremic hypochromic (non-thalassemic) anemias, iron accumulates in the
mitochondria, which are known to be involved in hemoglobin synthesis. In lead poisoning, the mitochondria are markedly abnormal, and probably correspond to the areas of punctate basophilia. However, the iron accumulates in other areas of the cell, suggesting a different type of block.

**SUMMARIO IN INTERLINGUA**

Microscopia electronic a alte resolution ha facite possibile le visualisation de ferro in transporto e thesaurage in le forma de ferritina, in forma disperse e etiam in aggregatos, e in le forma de “micellas de ferro” in mitochondrios. Il esseva trovate que hemosiderina consiste de pur ferritina in gruppamentos crystallin o, plus frequentemente, de ferritina in association con altere substantias, incluse un component de lipido in le forma de figuras myelinic e material positive a acido-periodic-Schiff.

In le sequente paragraphos nos ha summarisate le nove constatationes morphologic e nos ha presentate le interpretation que pare a nos le plus probable in le lumine de cognoscite studios biochimic e isotopic. Alternative interpretationes es discutite.

Le microscopia electronic ha establite le insula erythroblastic como un unitate morphologic e functional del medulla ossee. Un central trophocyto reticular apparentemente imparti nutrientes a circumjacente series de erythroblastos per le processo del rhopheocytose. Le transferentia de ferritina per iste processo es probablemente un phenomeno passive, viste que le quantitate transferite es parallel al quantitate de ferro presente in le central cellula reticular. Ferritina es augmentate in le cellula reticular e etiam in le erythroblastos in hemochromatosis. Illo es absent in casos de carentia de ferro, ben que le rhopheocytose remane prominente. Normalmente omne erythroblastos (proerythroblastos e normoblastos) e reticulocytos contine ferritina. Solmente le plus grande aggregatos in le sideroblastos e siderocytos pote esser visualisate per medio del reaction a blau de Prussia. Ferritina generalmente dispare con le maturation del reticulocytos, mesmo in hemochromatosis e infectiones, le quales es duo conditiones in que il existe un excesso de ferritina in le erythroblastos. Il es interessante que in infectiones le augmento es complete in le forma de ferritina disperse e non pote esser visualisate per le reaction a blau de Prussia; i.e., sideroblastos es absent, in contrasto con hemochromatosis in le qual lor numero es normal o augmentate.

Il pare le plus probable que ferritina dispare ab normal reticulocytos maturante proque illo es utilizate pro le formation de hemoglobina. Illo persiste in matur erythrocytos in anemia de Cooley, hypersideremia, anemia hypochromic, e plumbismo, in le quales le formation de hemoglobin es disturbate.

Le origine del ferritina in le trophocytos e le grado a que ferritina plus tosto que siderophylina contribue al synthese de hemoglobina representa problemas non ancora resolvite. Studios isotopic indica que quasi omne le ferro utilizate pro le synthese de hemoglobina es derive ab siderophylina e que le syntheses de hemoglobin es proceder in le absentia de visible ferritina, come in anemia a carentia de ferro. Iste factos debe esser reconcile con le observationes electronomicroscopic que suggere que normalmente un
cette grado de reutilisation de ferro intra le medulla occurre per erythrophagocytes, fragmentation, hemolyse intracellular de erythrocytos, formation de ferritina, e rhopheocyte. Ferro derivate ab erythrophagocytes alterubi in le corpore probabilmente arriva al medulla ligate a siderophyllina. Tal ferro pote esser incorporate in ferritina de cellulara reticular como pote esser visite in hyperferremia e post injectiones de compositos de ferro. Le processo de rhopheocyte alora resulteria in le utilisation de al minus un parte de iste ferro in ferritina pro le synthese de hemoglobina.

In certe statos pathologic, accumulation de ferritina e de connexe visible micellas de ferro disperse o conglomerate indica possibilemente le situs ubi le synthese de hemoglobina o le transporto de ferro es blocate. In anemia de Cooley e le anemias hypersideremic hypochromic (nonthalassemic), ferro se accumula in le mitochondrios, le quales es cognoscitemente interessate in le synthese de hemoglobina. In plumbismo le mitochondrios es marcatemente anormal, e illos probabilmente corrisponde a areas de basophilia punctate. Nonobstante, le ferro se accumula in altere areas del cellula, lo que suggere un differente typo de blocage.

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