Ferritin and Ferruginous Micelles in Normal Erythroblasts and Hypochromic Hypersideremic Anemias

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In previous publications, we have shown that a part of the iron cycle may be studied by means of the electron microscope. Actually, in the bone marrow of both humans and mammals the reticulum cells may be observed in the process of digesting the aged cells. Digestion continues for approximately 5 to 15 minutes as revealed by microcinematography by which the red cells may be seen immediately after ingestion divided into two or more parts in the cytoplasm.

By electron microscopy, the different stages of digestion are easily followed. Generally, gradually extending vacuoles appear in the phagocytosed cells; sometimes hemolysis occurs in the cell cytoplasm where the remaining “ghost” is clearly seen. In both cases, the formation of a considerable quantity of small ferruginous granules around the red cell fragment is seen (fig. 1).

Under high-power magnification, these granules display the characteristic aspect of ferritin molecules. One of these molecules measures 50 Å and is seen as four black specks situated in each of the four corners of a square. It can thus be concluded that digestion of the red cells by reticulum cells in the marrow directly promotes the formation of ferritin molecules. These molecules are most frequently found in clusters visible in the optical microscope; they are known to histologists as hemosiderin. Among these clusters can be distinguished ferritin molecules and other substances not yet defined.

Sometimes the hemosiderin clusters are crystalline in type, yielding pure ferritin (fig. 2). Ferritin and hemosiderin observed in the reticular cells may originate from phagocytosis of the red cells or may simply be due to the storage, in ferritine form, of iron carried to the bone marrow by transferrin. Furthermore, the distribution of ferritin molecules to young erythroblasts by the reticulum cells can be studied. This occurs in the following manner: erythroblastic islands are present in the marrow bone, islands reported by the first cytologists but which now assume a new significance by means of electron microscopy. In the center of these islands are always found one or two reticulum cells, which, charged with ferritin molecules in either a dispersed state or in the form of hemosiderin clusters, give up these molecules to the erythroblasts by a phenomenon closely related to pinocytosis and which may be termed ropheocytosis. This phenomenon is quite constant in normal conditions and in numerous diseases. Nevertheless, it is not known whether all the iron required for hemoglobin formation enters by pinocytosis or whether a part of it reaches the red cell directly from the plasma transferrin. Otherwise, it may be surmised that several mechanisms play a simultaneous role:
Fig. 1.—(Left) Different stages during the digestion of a red cell by a reticular macrophage. The erythrocyte is trapped (black circle on the left) by the cell, then digested progressively. This is indicated by the appearance in the cell of vacuoles growing larger and larger, then by hemolysis and fragmentation of the stroma. Simultaneously, ferruginous granules appear and form small black groups.
(Right) Reticular cell carrying reserve iron (hemosiderin-ferritin). The cell contains clusters of ferruginous particles, some of which are crystallized. The cytoplasm contains numerous dispersed ferruginous granules.

Fig. 2.—(Left) Enlargement of the preceding plate. Top, a hemosiderin cluster; bottom, a ferritin crystal. The molecular arrangement is distinguished, its superstructure illustrated in the diagram on the right.
(Right) Ultra-structure of ferritin. Each grain of ferritin displays four ferruginous clusters.

1. Iron resulting from the destruction of old red cells goes directly to the erythroblasts which form the erythroblastic island.
2. Transferrin carries some iron to the reticulum cells of the bone marrow where it is transformed into ferritin and distributed to the island erythroblasts.
3. A part of the iron is presented directly to the erythroblasts by the transferrin. Several experiments in vitro have demonstrated that erythroblasts can actually extract the iron from transferrin.\textsuperscript{9,10}
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Fig. 3.—(Left) An erythroblast island; reticular cell surrounded by erythroblasts. The reticular cell, packed with ferruginous granules, is situated in the center of the island of erythroblasts which totally surround it.

(Right) Enlargement of the diagram on the left: penetration by ferritin molecules. Iron in the mitochondria. The ferritin molecules issuing from the reticular cell are seen penetrating the cytoplasm of the erythroblast (by pinocytosis) and thence the mitochondria. In the mitochondria on the right, the iron no longer exists as ferritin, but under a dispersed form. It disaggregates, liberating this iron into the cytoplasm.

This problem is not yet resolved; however, it is certain that ferritin molecules pass from the reticulum cells to the erythroblast and that the erythroblast is found to contain some iron in the ferritin form.

The purpose of this article is to describe in greater detail the aspect of intra-erythroblastic iron under normal conditions and in certain pathologic cases.

MATERIAL AND TECHNIC

Bone marrow was obtained from normal individuals or from patients (in the course of a surgical operation or by sternal puncture). Fragments of bone marrow were placed in Palade's or Dalton's fixative. Embedding was carried out in butyl-methacrylate. The Porter-Blum microtome was used for cutting sections which were examined in the electron microscope RCA EMU 3 B.

NORMAL ERYTHROBLASTS

In normal erythroblasts, it has been established that immediately following penetration of ferritin molecules by pinocytosis, the latter become frequently massed into clusters of a greater or lesser size. It is not known which mechanism sometimes causes the formation of important clusters visible in the optical microscope and gives sideroblasts. Pinocytosis, in fact, produces at first small vacuoles containing slightly more than 10 molecules per section. More often than not, these clusters measure $\frac{1}{2}$ to 1 micron in diameter, have a spherical shape and are enclosed in a simple membrane.

It is exceptionally rare to find iron in the mitochondria of normal marrow. Its occurrence is nevertheless quite definite. Several cases of thalassemia will later be described in which this fact is convincingly demonstrated.
Fig. 4.—Megaloblast (Addison-Biermer anemia). The cytoplasm is full of dispersed ferritin granules. Note that two clusters of these granules are already visible by low power magnification.

Erythroblasts in Thalassemia Major

In the bone marrow the iron content is distinctly increased. Hemosiderin in the reticular cells often exists in the crystallized state as a large deposit. The erythroblasts are packed with iron in several forms: as ferritin in a
Fig. 5.—Ferritin in the cytoplasm of an erythroblast. Inset: two of these granules display their particular tetradic ultra-structure. Each granule represents one ferritin molecule.

dispersed state; in the form of ferritin clusters; finally, in nearly all polychromatophilic and acidophilic erythroblasts, these ferritin molecules are seen in the mitochondria. The manner by which they penetrate and mass there is still unknown. Whatever the reason, a most singular transformation of the ferritin molecules is observed in the mitochondria: they lose their
"quadruplet" structure and assume a powder aspect. At this stage, we call them "ferruginous micelles."

In one case, the electron microscope revealed that all the erythroblasts contained such clusters of ferruginous micelles in their mitochondria. No ferritin molecule was discernible. Later, Pearl's reaction showed in the optical

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**Fig. 6**—(*Top*) Reticulocyte (Cooley's anemia). Two-egg-shaped clusters of black granules occupy the center of the reticulocyte.

(*Bottom*) Some smaller clusters and dispersed grains. At a higher magnification crystallization of ferritin is observed. One of these clusters illustrates perfectly the arrangement of the ferritin molecules.
Fig. 7.—Ferritin and ferruginous micelles in the mitochondria (reticulocyte), hypochromic hypersideremic anemia. Here iron is found in the mitochondria, easily recognized by its double membrane; a cluster of ferritin granules is observed (F). Below, two mitochondria show between their cristae clusters of ferruginous micelles (MF). Right, a ferritin cluster.

microscope about 10 blue granules in each erythroblast. Iron in this form thus reacts quite well with Prussian blue stain.

The mitochondria swell and finally burst, liberating their ferruginous
Fig. 8.—Ferritin and ferruginous micelles (hypochromic hypochromic hypersideremic anemia). The deposit of ferruginous micelles (MF) occurs between the mitochondrial cristae producing this ladder effect. The aspect of the mitochondria begins to change, the membranes forming the cristae tend to disappear. Bottom, two ferritin clusters (F).

micelles into the cytoplasm where one is given to believe they penetrate the hemoglobin molecule. The quantity of iron in the reticulocytes as well as in the erythrocytes is very high, whereas the amount of hemoglobin formed remains low as compared with normal state.
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ERYTHROBLASTS IN THE HYPOCROMIC HYPERSIDEREMIC ANEMIAS

These are the anemias which have been recently characterized and which include such cases as Heilmeyer's "sidero achrestic" anemia\textsuperscript{12} and that of Caroli et al.\textsuperscript{13} The anemia provoked by certain intoxications such as lead (cf. reference 14) can equally be classified under this heading. The electron microscope gives a picture closely resembling that in the major thalassemias. Although the erythrocytes may be very poor in hemoglobin, on the other hand, the number of ferritin molecules in either clustered or dispersed form is very great.

It is important to note that the bone marrow examination of patients suffering from simple hemachromatosis (without hypochromic anemia) offers a very different aspect. The accumulation of hemosiderin in the reticular cells is readily found, but the morphology of the erythroblasts remains perfectly normal; ferritin is present in normal amounts in the erythroblasts and disappears in mature erythrocytes.

SUMMARY AND CONCLUSIONS

1. All normal erythroblasts contain some iron in the ferritin form. It may be present in either a dispersed state or in compact clusters. When large enough, these clusters may be observed in the optical microscope: they are the granular particles of the siderocytes.

2. Iron can be found in the mitochondria. It may exist either in the form of ferritin granules or as ferruginous micelles.

3. In thalassemia, large quantities of iron accumulate in the erythroblasts and are even found in the erythrocytes as ferritin, in cluster formation or dispersed. Occasionally, iron is present in great quantities in the mitochondria as ferritin or micelles. It seems that the various disorders encountered in thalassemia may thus be ascertained; the disturbance in hemoglobin synthesis results in the accumulation of the unused iron in the hypochromic erythrocytes.

4. In diseases very similar to thalassemia and in which no fetal hemoglobin is found, i.e., the hypochromic-hypersideremic anemias (sidero-achestic anemia, hypochromic hypersideremic anemia, lead-poisoning), similar findings are observed.

5. Normally, it is probable that iron metabolism occurs in the mitochondria. In thalassemia and hypochromic hypersideremic anemias, on the other hand, iron metabolism often appears to be "blocked" in the same areas.

SUMMARIO IN INTERLINGUA

1. Omne erythroblastos normal contine un certe quantitate de ferro in le forma de ferritina. Illo pote esser presente in stato disperse o compactemente agglomerate. Quando illos es sufficientemente grande, tal agglomerationes pote esser observate in le microscopio optic: illos es le particulas granular del siderocytes.

2. In su stato normal, ferro es rar in mitochondrios. Illo pote exister in le forma de granulos de ferritina o como micellas ferruginose.

3. In thalassemia, grande quantitates de ferro se accumula in le erythroblastos e mesmo in le erythrocytos como ferritina, in forma agglomerate o
disperse. In certe occasiones ferro es presente in grande quantitates in le mitochondrios in le forma de micellas ferruginose. Il pare que le varie disordes in thalassemia pote esser verificate super iste base. Le disturbate synthese de hemoglobina resulta in le accumulation del non-usate ferro in le erythrocytos hypochromic.

4. In morbos que es simile a thalassemia sed in que nulle hemoglobina fetal es incontrate—i.e. le anemias hypochromico-hypersideremic (anemia sidero-achestic, anemia hypersideremic hypochromic, anemia per intoxication a plumbo)—simile observationes pote esser facite.

5. Sub conditiones normal il es probabile que metabolismo de ferro occurre in le mitochondrios. Del altere latere, in thalassemia e le anemias hypersideremic hypochromic, metabolismo de ferro pare frequentemente esser “blocate” in le mesme area.

REFERENCES
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