Electron Microscopy of the Red Cells in Erythropoietic Porphyria

By Samuel Gross, Melvin D. Schoenberg and Virgil R. Mumaw

In 1955 Schmid et al. noted the presence of intranuclear heme inclusions in the fluorescing normoblastic nuclei of several patients with (congenital) erythropoietic porphyria. With a routine Romanovsky stain they were able to demonstrate incompletely stained areas in the nuclei. In addition, these inclusions stained positively with benzidine and showed a strong absorption at 400 \( \mu \text{m} \), clearly establishing the presence of hemoglobin. Gross, in 1964, confirmed these findings in an additional case of erythropoietic porphyria with a severe hemolytic component, hyperferremia, and a bizarre peripheral smear characterized mainly by hypochromia and poikilocytosis. In the latter instance, a combination Wright's and benzidine-nitroprusside stain was employed which clearly distinguished the nonnucleated target forms from the abnormal nuclear inclusions. In all of the cases studied, erythropoietic cells without red fluorescence exhibited the usual nuclear structures. On the basis of the cumulative data supporting the existence of 2 red cell lines in erythropoietic porphyria, electron microscopic studies were carried out on the erythroid precursors in order to determine their morphologic characteristics and signify, if possible, differences relative to the porphyrin laden cells.

Materials and Methods

Between 5 and 10 ml. of heparinized marrow aspirates and venous blood were collected aseptically from a patient with erythropoietic porphyria. A detailed report of this case has been made by Gross. The marrow specimens and the buffy coat and the upper red cell layers of peripheral blood were fixed in phosphate buffered osmium tetroxide (pH 7.2-7.4) containing sucrose. The tissues were then dehydrated in ethanol and embedded in methacrylate. Sections from 500 \( \text{A} \) were cut with a glass knife on the Porter-Blimni microtome for examination with the RCA EMU-2D electron microscope.

Observations

Among the erythroid precursors and mature forms, two distinctly different red cell lines were noted. In the peripheral blood and marrow the ratio of fluorescing normoblasts (heme inclusions) to the normal counterparts was 3:1. A similar comparison could not be made with confidence from electron microscopic examination because of the limited sampling that is attendant to the method. However, from the sample size, it appeared that the same relationship existed.

Normoblasts

A representative example of the normal series is noted in figure 1 which...
Fig. 1.—A typical normoblast with the dense nucleus and somewhat less dense cytoplasm. Also noted are sections through two mature erythrocytes. X 27,000.

shows a normoblast with the typically dense nucleus and somewhat less dense cytoplasm. Adjacent to the normoblast are sections through 2 mature erythrocytes. In marked contrast to the normal cell line were many bizarre normoblasts with multiple intranuclear inclusions of electron density comparable to that of the surrounding cytoplasm. An example of such a cell is shown in figure 2. With the exception of an intact nuclear membrane, most of the remaining nuclear area is electron transparent. These, presumably are the porphyrin laden nuclei with intranuclear hemoglobin inclusions seen in the fluorescent studies.

Reticulocytes

Normal reticulocytes with their characteristic mitochondria were also visualized (fig. 3). At this stage in cellular maturation, iron micelles, located between the cristae, are not demonstrable. In the abnormal cell line many reticulocytes, loaded with both dispersed ferritin and masses of ferritin, were found. In addition many of the mitochondria appeared to be swollen and in various stages of degeneration. Some of these were massively loaded with finely dispersed ferritin (fig. 4).

DISCUSSION

In the present study two distinctly different red cell lines have been identified at the normoblastic stage. The cells that were submitted for analysis included both marrow aspirates and harvested normoblasts and reticulocytes from the peripheral circulation. In either situation the results were identical. By the use of absorption microscopy DeCarvalho supported previous stud-
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Fig. 2.—A normoblast with an electron transparent nuclear area containing several intranuclear inclusions of electron density comparable to those found in the surrounding cytoplasm. The nuclear membrane is intact. X 32,000.

ies7–10 concerning the presence of hemoglobin in the developing erythroblastic nuclei and indicated, in addition, its virtual absence in the proerythroblast, a maximum absorption in the basophilic erythroblast and a marked diminution in the pre-pyknotic normoblast. In light of Schmid's unusual finding of hemoglobin in the porphyric normoblastic nuclei, it was felt that studies on harvested normoblasts would be more germane and eliminate the question of interpreting the presence of intranuclear hemoglobin in the earlier erythroid precursors. The abnormal normoblastic nuclei were noted to contain material corresponding in appearance to the cytoplasm. This finding was fortuitously emphasized by the surrounding, relatively clear, nuclear zone, which most likely is the result of nuclear alteration secondary to the photodynamic action of light and the photosensitizing porphyrin compounds.11 It is interesting to note, in this regard, that this finding substantiates the previous observation12 that the abnormal porphyrins are contained predominately in the nucleus and appear only in small concentration in the cytoplasm of normoblasts and in the reticulocyte following pyknosis.

In the immature, nonnucleated cells further abnormalities were consistently noted. The degenerating mitochondria were swollen and contained ferritin in the intercristal spaces. In addition, there were masses of ferritin outside the mitochondria. In the normal state the mitochondria and their cristae are clearly recognizable, and ferritin is not found. These observations are in complete agreement with the findings of Bessis and Breton-Gorius13,14 and others15,16 who have described identically appearing cells in certain pathologic states, including the thalassemia syndromes and the hypochromic hypersid-
Fig. 3.—A normal reticulocyte containing several mitochondria. There is no demonstrable cytoplasmic or mitochondrial ferritin. X 42,000.

eremic anemias. It is also noteworthy in this respect that the characteristic findings in the porphyric individual from whom these tissues were obtained included both hypochromic cells and a marked hyperferremia.

On the cumulative data from isotopic studies and electron microscopy, Bes-sis and Breton-Gorius have suggested that the excessive nonhemic iron in the hypochromic hypersideremic anemias is due to a metabolic block, according to the disease in question, resulting in a marked accumulation of cytoplasmic and mitochondrial iron. This observation is especially noteworthy since it has been pointed out that mitochondria are important in certain phases of porphyrin synthesis, most likely the incorporation of iron into the porphyrin ring. It has been alternatively suggested that large excesses of iron interfere with the normal dynamics of hemoglobin formation, or that certain deficiencies, viz., folic acid or pyridoxine, might retard normal hemoglobin production leading to the excessive deposition of unusable iron.

None of these interpretations, however, adequately explains the presence of hemoglobin in the fluorescing normoblastic nuclei. The knowledge that hemoglobin is present in the erythroblastic nuclei is immutable, and a relationship between an abnormality in porphyrin metabolism in certain patients with sideroblastic anemia appears to be established. It is tempting to speculate, therefore, that in erythropoietic porphyria the excessive amounts of non-utilizable porphyrins may inhibit or retard normal heme synthesis, thus accounting both for its intranuclear presence late in the normoblastic series and the resultant hypochromic cell line.

As a result of Schmid's work and the present findings relative to electron microscopy, the genetic consideration in this disorder might well be revised.
Fig. 4.—A reticulocyte showing both masses of ferritin and dispersed ferritin. Several of the mitochondria are swollen and in various stages of degeneration. The mitochondria contain large amounts of finely dispersed ferritin. X 38,000.

In the previous report it was noted that chromosomal studies were normal. Because of its infrequent occurrence, the lack of any good evidence indicating any familial relationship, and the obvious evidence of two red cell lines, one must seriously consider this defect on a mosaic basis.

SUMMARY

With the aid of electron microscopy two different red cell lines have been identified in erythropoietic porphyria. A normal red cell series has been found in association with hemoglobin containing normoblastic nuclei and ferritin laden reticulocytes. The abnormal line presumably represents the porphyrin containing cells. A possible explanation to account for the abnormality in heme synthesis has been proposed.

SUMMARIO IN INTERLINGUA

Con le adjuta de microscopia electronic, duo differente lineas cellular esseva identificate in porphyria erythropoietic. Un linea normal de erythrocytos esseva trovate in association con hemoglobina a contento de nucleos normoblastic e ferritina cargate de reticulocytes. Le linea anormal representa presumitemente le cellulas a contento de porphyrina. Es presentate un explication possibile del anormalitate in le synthese de hem.

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