Defective Globin Synthesis in Hypochromic Hypersideremic Anemia (α-Thalassemia?)

By Paul Heller, John Van Stone, David Apple and Richard D. Coleman

A RARE FORM of hereditary anemia characterized by hypochromia of the erythrocytes in the presence of increased iron stores has been designated by Heilmeyer1,2 as “hereditary hypochromic sideroachrestic anemia.” Garby and his co-workers3 and Heilmeyer1,2 have suggested that in this disorder heme synthesis is disturbed possibly because of a genetically determined defect in the enzymatic conversion from coproporphyrin to protoporphyrin. This pathogenic mechanism has been considered responsible for the elevated erythrocyte coproporphyrin levels in patients with this disorder. The similarity of the clinical and laboratory features with those of thalassemia minor has been recognized, but the absence of any stigmata of increased hemolysis, the normal proportion of fetal and A2 hemoglobin and the marked increase of sideroblasts in the bone marrow have been considered distinctive characteristics of “hereditary hypochromic sideroachrestic anemia.”2 Verloop and his co-workers4 have recently emphasized the importance of the presence of a large number of “ringed” sideroblasts5 in bone marrow smears as a diagnostic criterion of this disorder for which they use the designation “hereditary hypochromic hypersideremic anemia.” In these cells the perinuclear region of the cytoplasm is densely filled with coarse iron granules. These investigators have also observed patients with hypochromic hypersideremic anemia without ringed sideroblasts and they have raised the question whether the abnormality in such patients might not be a form of α-thalassemia.

The patient, described in the following report, was found to have an erythropoietic disorder which had several characteristics of hypersideremic anemia with the postulated defect in heme synthesis, including an abundance of ringed sideroblasts in the bone marrow. Studies of heme and globin production with glycine-2-C14 indicated that it was the globin moiety which was synthesized in an abnormal pattern suggesting the effect of a thalassemia gene.

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CASE REPORT

The patient, a Negro, born in 1928, was found to be anemic for the first time in 1952 when he was admitted to a hospital because of pain and swelling of the right tibia which had gradually developed following a minor accident in 1950. X-ray diagnosis of chronic osteomyelitis was made and the involved area excised. The histologic diagnosis was sclerosing osteitis and no further therapy was advised. The anemia (hemoglobin 8–9 Gm./100 ml.) was diagnosed as being due to iron deficiency; iron medication was prescribed for several months, but its results were not evaluated.

The patient remained well until 1959, when he was admitted to the Veterans Administration West Side Hospital because of a febrile upper respiratory infection which subsided rapidly. Abnormal findings consisted only of slight pallor of the mucous membranes and the well healed operative scar of the leg. Liver and spleen were not palpable. The level of hemoglobin was 8.5 Gm./100 ml., the hematocrit was 30 and the erythrocyte count was 3.2 X 10^6/mm.³ (MCH 26.5 µg, MCHC 28 per cent, MCV 94 µ³). The erythrocytes showed marked anisocytosis, poikilocytosis and hypochromia. Ring and pencil cells were numerous, target cells less frequent (fig. 1). The reticulocyte count was 0.5 per cent. Leukocytes and platelets were normal, the former had a normal content of alkaline phosphatase. The bone marrow showed erythroid hyperplasia with a left shift and a scarcity of eosinophilic normoblasts. The ratio of erythroid to nonerythroid nucleated cells was approximately 1:1. Prussian Blue stainable material was markedly increased. Many sideroblasts were present, most of which were of the "ringed" type (fig. 2), but there were no polyploid, megaloblastic or megloblastoid cells. The peripheral erythrocytes had increased osmotic resistance. Staining with Brilliant Cresyl Blue did not reveal any inclusion bodies.
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Fig. 2.—Sideroblasts from patient's bone marrow, stained only with Prussian blue.

indicating the absence of denatured hemoglobin H. The serum iron was elevated to 237 μg. per cent and 90 per cent of the total iron binding capacity was saturated. Biopsy specimens of the liver showed marked hemosiderosis with only minimal fibrosis. The values in the serum for bromsulphalein retention, bilirubin, cephalin flocculation, thynnol turbidity, albumin, globulin, glutamic-oxaloacetic transaminase, lactic dehydrogenase, and alkaline phosphatase were within normal limits. Roentgenograms of the chest, upper and lower gastrointestinal tract, and skeletal system were normal, except for an area of increased translucency in the proximal third of the right tibia related to the previous surgical procedure. The results of a glucose tolerance test were within normal limits. The urine was normal, including 24-hour urobinogen excretion.

The patient was an only child; the father died in 1950 of a "heart attack" at the approximate age of 45, and his mother, 54 years old, was said to be in good health. No other surviving relatives were known to the patient.

From 1959 until the present, the patient remained well except for occasional colds and periods of increased fatigue, but he continued to work as a bricklayer's aide and since 1963 as a hospital aide. He was followed as an outpatient at irregular intervals and several diagnostic and therapeutic studies were performed. The physical findings remained unchanged except for a palpable liver which had been felt 2–3 cm. below the costal margin since 1962.

The results of the erythrokinetic and porphyrin studies are listed in table 1. Most of these tests were performed several times. Hemoglobin electrophoresis on starch gel and agar gel at alkaline and neutral pH revealed no abnormal fractions. The proportions of hemoglobins A2 and F were normal. Tryptic digests of hemoglobin and chymotryptic digests of the trypsin-resistant core were prepared and "fingerprinted." The peptide patterns, including stains for histidine, arginine, tryptophane, tyrosine, and methionine were entirely normal. The activity of erythrocyte catalase was determined because of the possibility that in case of defective synthesis of heme this enzyme might have diminished activity. It was found to be at the lower limit of normal (table 1).

STUDIES WITH GLYCINE-2-C14

With the morphologic, biochemical and ferrokinetic studies having suggested the presence of an abnormality in hemoglobin production, it appeared to be important to determine whether the limiting factor was in the synthesis of heme or of globin. For
Table 1

<table>
<thead>
<tr>
<th></th>
<th>Patient</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cr$^{51}$-erythrocyte half-life (days)</td>
<td>24</td>
<td>24-28</td>
</tr>
<tr>
<td>Serum iron (µg. %)</td>
<td>207-274</td>
<td>50-120</td>
</tr>
<tr>
<td>Serum iron binding capacity (µg. %)</td>
<td>240-320</td>
<td>300-350</td>
</tr>
<tr>
<td>Plasma-iron (Fe$^{59}$) turnover (mg./day)</td>
<td>208</td>
<td>~30</td>
</tr>
<tr>
<td>Fe$^{59}$-incorporation into erythrocytes (% of administered amount in 10 days)</td>
<td>20.4</td>
<td>70-90</td>
</tr>
<tr>
<td>Fetal hemoglobin (%) (6)</td>
<td>0.7-0.9</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Hemoglobin A$_2$ (%) (7)</td>
<td>1.5-2.1</td>
<td>1.6-2.5</td>
</tr>
<tr>
<td>Erythrocyte protoporphyrin (8)</td>
<td>12.8-19.5</td>
<td>15-21</td>
</tr>
<tr>
<td>Erythrocyte coproporphyrin (8)</td>
<td>0.2-3.5</td>
<td>0-2</td>
</tr>
<tr>
<td>Urinary porphobilinogen (10)</td>
<td>204</td>
<td>80-280</td>
</tr>
<tr>
<td>Urinary delta-aminolevulinic acid (10) (mg./24 hrs.)</td>
<td>1.4</td>
<td>&lt;2.5</td>
</tr>
<tr>
<td>Urinary porphobilinogen (10)</td>
<td>0.3</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Erythrocyte catalase (11)</td>
<td>39.2</td>
<td>39.6-49.5</td>
</tr>
<tr>
<td>(Units)*</td>
<td>(1.4)</td>
<td>(1.2-1.5)</td>
</tr>
</tbody>
</table>

*1 Unit = 1 mEq. H$_2$O$_2$ reduced per ml. of packed red cells in 10 seconds. The values in brackets represent units of enzyme activity per 10 mg. of hemoglobin in 10 seconds.

For this purpose the temporal relationship of maximal heme and globin synthesis following the injection of glycine-2-C$^{14}$ was studied according to the technic suggested by Nathan, Piomelli and Gardner with a slight modification in the calculations. One hundred µc. of glycine-2-C$^{14}$ were injected intravenously into the patient and two 22-year-old male medical students, who served as controls. From these individuals, 10-15 ml. of blood were withdrawn twice daily for the subsequent 7 days and then once daily for 5 days. Heme was prepared from washed packed cells and globin from stroma-free hemolysates. A second aliquot of hemolysate was left unaltered. The samples were combusted and the radioactivity was determined in a liquid scintillation counter to a counting error of less than 1 per cent. The theoretic specific activity of hemoglobin in each blood sample was calculated by adding 3.8 per cent of the specific activity of heme to 96.2 per cent of the specific activity of globin. This value was then compared to the actual specific activity of hemoglobin. The values agreed within 5 per cent. From the glycine-2-C$^{14}$ incorporation curves, the 12 hour increments of specific activity of heme and globin were obtained and plotted as percentages of the maximal specific activities (fig. 3). These maxima were reached 7 to 8 days after the injection of the labeled glycine in the normal subjects (fig. 3A and B) and approximately 24 hours earlier in the patient (fig. 3C). The assumption was made that the fraction of glycine-2-C$^{14}$ which was utilized for hemoglobin synthesis was available to the erythropoietic cells only on the first day of injection, and after that day the amount of labeled glycine which remained was insignificant. Thus, the increase of specific activity in the peripheral erythrocytes can be considered to reflect hemoglobin synthesis by the erythropoietic cells in sequential stages of maturation. The radioactivity determined in heme and globin on the first day after the injection was utilized by the most mature erythropoietic cells, the reticulocytes, and the increments from the sixth to eighth day reflect hemoglobin synthesis by the most immature hemoglobin producing cells, probably the pronormoblasts. In the interpretation of these
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Fig. 3.—Daily increments of specific activity (S.A.) of heme and globin, expressed as per cent of the highest value of this specific activity, achieved on the fifth to eighth day after the injection of 100 μc. of glycine-2-C14. (A and B) Normal, (C) “sideroachrestic” anemia, and (D) sickle cell anemia.

increment curves it must be emphasized that they reflect only the relative rate of synthesis of heme and globin, especially the timing of their maximal synthesis during erythropoiesis, but not their total production.

The normal curves resembled those obtained by Nathan, Piomelli and Gardner and suggest that in normal man the production of globin slightly precedes that of heme in the early stages of erythropoiesis (fig. 3A and B). The bulk (70–80 per cent) of their production occurs during the intermediate stages of maturation, presumably in the polychromatic and eosinophilic normoblasts and bone marrow reticulocytes (fig. 3A and B, days 2–5). In the patient the pattern of heme production resembled the normal one, but that of globin production was markedly abnormal (fig. 3C). In the early erythropoietic cells (fig. 3C, days 6 and 7) globin and heme were apparently synthesized according to the normal pattern but during the more mature stages of erythroid maturation, when normally 70–80 per cent of the total hemoglobin production occurs, globin synthesis was markedly decreased. It is, therefore, possible that during this phase of erythropoiesis there was in the cells an excess of “free” heme or heme precursors. This “free” heme might stimulate the synthesis of globin in the more mature cells and might be responsible for the observed high increments of the specific activity in the globin samples obtained during the first 2 days after the administration of the labeled glycine. On the other hand, the possibility was considered that the apparent discrepancy between the amounts of newly synthesized globin and heme during this phase might be indicative of the presence of free globin in the cells or of a nonheme protein. Free globin was determined according to a chromatographic technic suggested by Winterhalter and
Huehns,21 in which the Fe59-heme binding capacity of hemolysates is measured. Only an infinitesimal amount (less than 0.03 Gm./100 ml. of packed red cells) was found in the patient's erythrocytes which was not greater than that found in the normal subject.

**Therapy**

Therapeutic attempts consisted in periodic use of pyridoxine, which, when 400-600 mg. daily were given, resulted in an increase in the hemoglobin values from an average of 8.5 to 10.5 Gm./100 ml. Larger amounts of pyridoxine caused nausea and vomiting. In an attempt to decrease the body iron stores periodic phlebotomies were performed, but, unfortunately, this regimen could not be continued for a sufficiently long time to permit an estimate of the amount of mobilizable iron and to evaluate the possibility of improved hemoglobin synthesis following diminution of the stored iron. In March, 1962, an attempt was made to increase urinary excretion of iron by daily intravenous injection of 1 Gm. of trisodium calcium diethylenetriaminepentacetic acid (Calcium Chel 330, kindly supplied by Geigy Pharmaceuticals, Ardsley, New York) in 500 ml. of 5 per cent dextrose. This was abandoned after 5 days because of the small increase of iron excretion (37 mg. in 5 days) and the inconvenience to the patient. In March, 1964, 20 daily intramuscular injections of 500 mg. of desferrioxamine B (Desferal, kindly supplied by Ciba, Summit, New Jersey) caused the daily urinary excretion of iron to increase from 0.5 to 5-8 mg. during the period of treatment. The treatment had no effect on the level of hemoglobin, the hematocrit, and the erythrocyte and reticulocyte count.

**Family Studies**

Family studies could not be performed in a satisfactory manner. The patient's only known living relative was his mother who lived several hundred miles from Chicago and could not be persuaded to submit to examinations. Nevertheless, a blood smear and a blood sample were obtained and sent to our laboratory. The hematocrit was 37 per cent, the hemoglobin 11.5 Gm./100 ml. The blood smear showed very mild anisocytosis and hypochromia with a small number (approximately 2-3 per cent) of more severely hypochromic cells and rare pencil cells. Osmotic fragility was normal. Hemoglobin electrophoresis on starch gel and agar gel showed no abnormality. The proportion of hemoglobin A2 was 1.5 per cent. Fetal hemoglobin was slightly increased to 1.5 per cent (normal < 1 per cent). The serum iron concentration was markedly increased to 340 μg. per cent and the iron binding capacity was saturated.

**Discussion**

The abnormal pattern of the utilization of glycine-2-C14 for globin production in the described patient with hypochromic hypersideremic anemia raises the question whether the marked relative diminution of synthesis of globin as compared to that of heme during the mid-stages of erythropoiesis is indicative of a specific defect in globin synthesis or merely the result of
the erythroid hyperplasia and the predominance of the primitive over the more mature erythroid precursor cells. The second possibility seems unlikely since it would necessitate the assumption that pronormoblasts and basophilic normoblasts, which were increased in the patient's bone marrow, normally produce less globin than heme. The opposite, however, appears to occur. The production of globin by the immature cells is ahead of that of heme (fig. 3A and B). One might, therefore, expect that accelerated erythropoiesis with a left shift in the distribution of erythroid precursor cells is associated with greater than normal globin synthesis during the early phases of erythropoiesis. This phenomenon was observed by Nathan and Gardner in a patient with megaloblastic anemia who received glycine-2-C\textsuperscript{14} 1 hour after the injection of 1000 µg of vitamin B\textsubscript{12}. This situation provided ideal experimental conditions for the study of hemoglobin synthesis by the most immature erythroid precursor cells because their maturation and functional activity had presumably been freed from inhibition a few minutes prior to the administration of the pulse of glycine-2-C\textsuperscript{14}. Five days later the largest increment of newly synthesized globin and heme, appearing synchronously, was measured in the peripheral blood.

We recently studied hemoglobin synthesis in a patient with iron depletion due to hereditary telangiectasia associated with recurrent gastrointestinal hemorrhages. There was, however, no evidence of bleeding during the time of the study. Because of the markedly increased erythropoietic activity maximal hemoglobin synthesis was achieved on the fourth day after the administration of glycine-2-C\textsuperscript{14}, but the asynchronism between the peak increments of the specific activities of heme and globin was similar to that in the normal. These findings in megaloblastic and iron deficiency anemia suggest that erythroid hyperplasia per se is not likely to lead to a shift of maximal globin synthesis from the primitive erythroid precursor cells to the more mature ones as has been observed in the patient of this report. On the other hand, Nathan and Gardner reported in an abstract that in several patients with increased erythropoiesis due to unusual circumstances (e.g., testosterone-treated hypoplastic anemia) maximal specific activity of globin occurred 1–2 days rather than 5 days after the administration of labeled glycine. This problem, therefore, requires further clarification by the study of more patients with accelerated erythropoiesis.

In a patient with sickle cell anemia we recently found that maximal globin synthesis preceded the peak increment of heme synthesis (fig. 3D). No further increment of specific activity of heme occurred after the fourth day following the injection of glycine-2-C\textsuperscript{14} while synthesis of globin continued for 2 more days. This altered pattern of globin synthesis is possibly an effect of the abnormal gene. If the synthetic rate of hemoglobin S is measured in the absence of hemolysis, as is feasible in individuals with the sickle cell trait, it is found to be lower than that of hemoglobin A. It is, therefore, likely that the retardation of globin synthesis which was found to be severest during the normally most productive stages of erythropoiesis, is part of the genetic abnormality.

A similar consideration seems justified in the patient of the present study
There was marked asynchronism in the synthesis of heme and globin and the daily increments of newly synthesized globin decreased almost linearly during the intermediate stages of erythroid maturation which normally provide for 70–80 per cent of the output of hemoglobin. The relative rate of globin production during this phase of erythropoiesis appears to be even lower in this disease than in sickle cell anemia, probably because of the absence of the stimulating effect of hemolysis. As in sickle cell anemia, the effects of the genetic abnormality appeared to be most pronounced in those cells which normally are functionally most active.

The demonstration of a defect in globin synthesis seems to justify the inclusion of the described hypersideremic hypochromic anemia among the group of thalassemia syndromes. While the basic mechanism of diminished hemo- globin synthesis in thalassemia is not known, the available evidence is strongly in favor of the hypothesis that thalassemia is caused by an abnormality in the genetic factors which govern the rate of synthesis of the α- and β-chains of normal adult hemoglobin. The "classical" type of thalassemia is considered to be caused by diminished production of β-chains. Here the synthesis of α-, γ-, and δ-chains is not disturbed and, therefore, the relative or absolute amount of hemoglobin F (α₂γ₂) and A₂ (α₂δ₂) is increased. On the other hand, if the production of α-chains is diminished, that of hemoglobins A₂ and F is also impaired. The excessive β-chains may tetramerize to form hemoglobin H (β₄), an unstable hemoglobin which precipitates. Alpha-thalassemia appears to be the genetic prerequisite for the occurrence of hemoglobin H, but the latter is not always present. The findings in the patient of this report appear to be compatible with the diagnosis of α-thalassemia without hemoglobin H. Unfortunately, no satisfactory family studies could be performed. Only a blood sample from the mother was available for examination which revealed features compatible with the diagnosis of thalassemia trait. It is noteworthy that only a few erythrocytes in her blood smear were severely hypochromic, a phenomenon similar to that observed in the sex-linked hypochromic anemia described by Rundles and Falls. Unfortunately, the absence of other family members precluded the exploration of the possibility of sex-linkage.

The pathogenic mechanism of the sex-linked hypochromic anemia has to our knowledge not yet been clarified. It is possible that there is an abnormality of globin synthesis. In this respect it is of great interest that an individual suffering from this disease, who was treated by Bishop and Bethell with large doses of pyridoxine, had an incomplete response, similar to the patient of this report. The slight increase of the hemoglobin level is most likely due to a pharmacodynamic action of the vitamin. Since pyridoxal-5-phosphate is essential for the synthesis of heme one might speculate that the administration of large amounts of the vitamin leads to an increase of the concentration of free heme in the cells which in turn could stimulate globin production to its genetically determined limit. We have postulated such a mechanism as a possible explanation for the marked asynchronism of heme- and globin production during the late stages of erythropoiesis in our patient (fig. 3C, days 1 and 2).
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One of the main reasons for the hypothesis that hereditary “sideroachrestic” anemia is caused by a defect in heme synthesis is the observed increase in the coproporphyrin content of erythrocytes,13 suggesting a block in the enzymatic conversion of coproporphyrin to protoporphyrin. The question must be raised whether this finding is a sufficiently distinct feature to establish this anemia as a genetic disorder of heme synthesis. Multiple porphyrin abnormalities have been described in acquired anemias associated with disturbances in erythroid maturation34 where genetically determined disturbances in porphyrin synthesis could hardly be assumed to be present. The possibility must be considered that the elevated erythrocyte coproporphyrin level in “hereditary hypochromic sideroachrestic anemia” also is a secondary phenomenon which is not necessarily always present. The same might be the case with the reported elevations of erythrocyte protoporphyrin in patients with thalassemia minor.34

The finding of an abnormal pattern of globin synthesis in the patient of this report does not justify the generalization that such an abnormality is the biochemical basis of all hereditary hypochromic hypersideremic anemias. The cause of this group of anemias is probably not uniform. Unfortunately, the very popular term “sideroachrestic” has the connotation of a genetic abnormality of iron incorporation into protoporphyrin in a more specific sense3 than is justified on the basis of the available evidence. For this reason the more descriptive and less committal designation “hereditary hypochromic hypersideremic anemia” is to be preferred until it becomes possible to classify this group of diseases in a more meaningful way on the basis of the biochemical pathogenic mechanism. It is possible that kinetic studies as described in this report might aid in such a classification.

Summary

1. The clinical, hematologic and biochemical findings in a patient with hereditary hypochromic hypersideremic anemia are described. The pattern of heme and globin production, measured by glycine-2-C14 incorporation, suggests an abnormality of globin synthesis.

2. The hypothesis that this disorder might represent a variant of thalassemia, probably α-thalassemia, is discussed.

3. The partial therapeutic response to pyridoxine is considered to be due to pharmacodynamic stimulation of heme synthesis. The hemoglobin level was not restored to normal, probably because of the limited capacity for globin synthesis.

4. It is suggested that kinetic studies of hemoglobin synthesis may aid in the classification of hereditary hypochromic hypersideremic anemias.

Summario in Interlingua

1. Le constatationes clinic, hematologic, e biochimic in un patiente con hereditari anemia hypersideremic hypochromic es describite. Le configuration del production de hemo e globina, mesurate per studios del incorporation de glycina-2-C14, suggere un anormalitate del synthese de globina.
2. Es discutite l’hypothese que iste disordine representa un variante de thalassemia, probablemente α-thalassemia.

3. Es opinate que le partial responsa therapeutic a pyroxidina reflecte un stimulation pharmacodynamic del synthese de hemo. Le nivello de hemo-globina non esseva restaurate a nivellos normal, probablemente a causa del limitate capacitate pro le synthese de globina.

4. Es proponite que studios kinetic del synthese de hemoglobina pote esser de adjuta in le classification de hereditari anemias hypersideremnic hypochromic.

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