Idiopathic Dyserythropoietic Jaundice

By SIEGFRIED BERENDSOHN, JAMES LOWMAN, DOROTHY SUNDBERG AND C. J. WATSON

IT IS well established that there is ineffective erythropoiesis in variable degree in a number of disease states, including pernicious anemia, thalassemia, erythropoietic porphyria, sideroblastic and refractory normoblastic anemia, erythremic myelosis, erythroleukemia, and certain other conditions, as mentioned in the following.

The normal fecal urobilinogen usually ranges from 100–250 mg./day. Of this amount 85–90 per cent is derived from destruction of mature circulating erythrocytes having a lifespan of 120 days, the remaining 10–15 per cent from other sources. This is easily demonstrated by administration of N15- or C14-labeled glycine, according to the method of London and co-workers.1 In the normal individual, a large peak in N15 concentration of the fecal stercobilin occurs at the time of destruction of mature circulating erythrocytes, coinciding with a rapid decline of N15 of the hemoglobin protoporphyrin, in the neighborhood of 120 days.1-3 A smaller, much earlier peak of the stercobilin N15 between 6–10 days represents the 10–15 per cent of the fecal urobilinogen which cannot be related to destruction of mature circulating red cells. Incorporation of N15 in the fecal mesobilifuscin or dipyrrylmethene moiety results in a peak N15 concentration slightly in advance of that in stercobilin. This is believed to be mainly anabolic in origin, possibly related to an excess of pyrrolic compounds formed at a relatively early stage of heme synthesis. In contrast to the urobilinogen, there is very little increase in labeling of mesobilifuscin at the time of destruction of mature circulating red cells.2

In the above-mentioned states of ineffective erythropoiesis, there is often excessive fecal urobilinogen excretion and the early labeling after N15 or C14 glycine is generally increased, often to a marked degree. This is commonly, though not invariably true of the fecal mesobilifuscin as well, as will be discussed in the following.

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In straightforward hemolytic anemias, as typified by familial hemolytic jaundice, the rapid destruction of red blood cells causes merging of the early and late peaks of the fecal urobilinogen label and this single peak roughly coincides with the rapid increase and decrease of the hemoglobin protoporphyrin N', the normal plateau being abolished because of the rapid destruction of circulating red cells. We have recently had opportunity to make a detailed study of an unusual case of jaundice, first believed to have hemolytic anemia, in which, however, it was found that the great excesses of bilirubin being formed and excreted as urobilinogen were not derived from the hemoglobin of mature circulating red cells but rather in relation to a dyserythropoiesis and disturbed hemoglobin synthesis in the bone marrow.

CASE REPORT

Case C. O., a Peruvian male civil engineer of 35, was admitted to the University of Minnesota Hospital on 11-30-61 with a tentative diagnosis of hemolytic anemia.* There was a history of mild anemia and jaundice for the past 3½ years, never previously. In 1956 he had an attack of thrombophlebitis in the left leg characterized by pain and swelling and responding readily to treatment with antibiotics, bed rest and anticoagulants. This recurred in 1957 and responded to the same treatment. In 1958 he had an attack of right upper quadrant pain with some nausea, and at this time jaundice was first observed. The jaundice persisted after the subsidence of the attack and has continued up to the present. In 1959 there was another attack of right upper quadrant pain. In 1960 there was a sharp attack of chest pain of pleural type with a cough, followed by hemoptysis. This was treated with antibiotics, anticoagulants and bed rest, and a left external iliac vein ligation was carried out. Since October, 1960, the patient has had recurrent brief attacks of right upper quadrant pain. In February, 1961, he received Meticortin, 45 mg./day, for 3 months without any apparent effect on his jaundice or anemia. There was no family history of jaundice, anemia, or splenomegaly. He had not received transfusions.

On physical examination there was a definite pallor and jaundice, a typical icter-anemic color. The spleen and liver were not palpable. There was mild swelling of the left leg with slight pitting edema of the ankle. The examination was entirely normal in other respects. X-ray of the chest was normal. Cholecystogram revealed a functioning gallbladder with multiple radiolucent gallstones. There was no evidence of splenic enlargement on x-ray.

On 5-2-62 there was recurrent right upper quadrant pain and mild fever. Because of continuing symptoms, cholecystostomy was carried out by Dr. H. D. Root on 5-7-62. Ten small dark green gallstones were removed (further study of these will be mentioned below). Bile was obtained from the catheter left in the gallbladder, and crystalline bilirubin was isolated by the method of Ostrow, Hammaker and Schmid. One of the gallstones was ground repeatedly in a mortar with dilute NaOH, and filtered. The filtrate was used for determination of bilirubin by the diazo reaction and combined bilirubin and biliverdin by means of the Evelyn-Malloy method. On 6-12-62, cholecystectomy was carried out following a single blood transfusion. Liver biopsy was also done at this time. This showed normal hepatic cells, moderate hemosiderosis with mild focal lymphocytic infiltration and no myeloid metaplasia. The gallbladder showed histologic changes of chronic cholecystitis.

Preliminary laboratory studies are shown in table 1. There was a mild anemia of normochromic, normocytic type with slight aniso- and poikilocytosis, no spheroidocytes.

*We thank Dr. Ernesto Oelgado, Lima, Peru, for reference to this patient.
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Osmotic fragility was normal. Coomb’s tests, direct and indirect, were negative as were also the 2-hour L.E. clot and the antinuclear antibody† tests. Paper electrophoresis revealed hemoglobin A. Fetal hemoglobin was 0.9 per cent by alkaline denaturation, this being a normal value for this laboratory. The amount of A₂ hemoglobin was not determined but there was no morphologic evidence of thalassemia. The results of the bile pigment and porphyrin studies, and references to the methods used, are given in table 2. The Cr⁵¹ T ½ of the red cells was first determined from 12-7-61, being 25–28 days. One month later it was repeated and found to be 16 days. This may have been related to the considerable amounts of blood withdrawn for N¹⁵ studies during this interval. There was no correlation with variations in reticulocytes or serum bilirubin in the two Cr⁵¹ studies. The erythrocyte life span as determined with N¹⁵ glycine was approximately 91 days, indicating a mild random destruction of red cells. It is possible that this was intermittent in character, thus perhaps contributing to the variation in Cr⁵¹ results in the two periods mentioned. Using the method of McCurdy and Bath¹¹ for the estimation of splenic sequestration of Cr²⁺⁺⁺-labeled erythrocytes, the spleen-precordial ratio was less than 0.43 in both periods. According to these criteria, there was no evidence of sequestration.

Two aspiration biopsies of sternal bone marrow were almost identical. The marrow was remarkably hyperplastic (fig. 1); only very occasional fat cells were found. The greatly increased cellularity was caused by hyperplasia of normoblasts and phagocytic reticular cells (figs. 1–6). Megakaryocytes were normal in number and morphology. There was neither myelofibrosis nor myelosclerosis. Differential counts (1000 cells) of normoblasts revealed the following:

```
<table>
<thead>
<tr>
<th>Types of Normoblasts per 100</th>
<th>12-7-61</th>
<th>1-8-62</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total normoblasts</td>
<td>65.0</td>
<td>69.0</td>
</tr>
<tr>
<td>Pronormoblasts</td>
<td>1.8</td>
<td>2.5</td>
</tr>
<tr>
<td>Basophilic normoblasts</td>
<td>3.0</td>
<td>2.1</td>
</tr>
<tr>
<td>Polychromatic normoblasts</td>
<td>32.1</td>
<td>27.2</td>
</tr>
<tr>
<td>mitoses</td>
<td>2.1</td>
<td>2.6</td>
</tr>
<tr>
<td>Orthochromatic normoblasts</td>
<td>14.8</td>
<td>25.4</td>
</tr>
<tr>
<td>mitoses</td>
<td>1.6</td>
<td>1.1</td>
</tr>
<tr>
<td>Late (pyknotic) orthochromatic normoblasts</td>
<td>9.6</td>
<td>8.1</td>
</tr>
</tbody>
</table>
```

The orthochromatic normoblasts which still showed greatly increased numbers of mitoses were cells which seemed to contain a full complement of hemoglobin and showed only a hint of grey (RNA) in their cytoplasm. Many of the pyknotic orthochromatic normoblasts seemed to be in the process of extruding their nuclei. In the first specimen, 40 per cent of the normoblasts were early or late orthochromatic forms; in the second, 50 per cent were in this stage of development. The shift towards orthochromatic forms resembled that seen in hemolytic anemia.

Mitoses were numerous in all cells of the normoblastic series, but unusually prominent in normoblasts which seemed to contain a full complement of hemoglobin. Almost all of the mitoses were in the meta-, ana- or telophase stages. Many of the mitoses appeared abnormal: reconstruction of nuclei occurred with aberrant chromosomes left in the cytoplasm; chromosomes appeared fused with one another in metaphase; and chromosomes were found in the mid-piece when telophase seemed otherwise complete.

Judging abnormalities in erythropoiesis is admittedly subjective to some extent. In brief, the normoblasts here were not megaloblastoid, and they were less abnormal in appearance than those of other sideroachrestic or refractory anemias, thalassemia major, erythropoietic porphyria, or erythroleukemia.

†We thank Dr. M. Göçen for carrying out this test.
Table 1.—Preliminary Laboratory Studies

<table>
<thead>
<tr>
<th>Test</th>
<th>Normal Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red blood cells (10^6/cu. mm.)</td>
<td>3.12–4.4</td>
</tr>
<tr>
<td>Hemoglobin (Gm./100 ml.)</td>
<td>9.8–11.3</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>27.0–30.0</td>
</tr>
<tr>
<td>Mean corpuscular volume (cu.μ)</td>
<td>87.0</td>
</tr>
<tr>
<td>Mean corpuscular hemoglobin (μg.)</td>
<td>31.0</td>
</tr>
<tr>
<td>Mean corpuscular hemoglobin concentration (%)</td>
<td>36.0</td>
</tr>
<tr>
<td>Reticulocytes (%)</td>
<td>2.6–10.1</td>
</tr>
<tr>
<td>Leukocytes (cu. mm.)</td>
<td>3600–6500</td>
</tr>
<tr>
<td>Platelets (cu. mm.)</td>
<td>82000–106000</td>
</tr>
<tr>
<td>B.S.P. (% retention 45 min. after 5 mg./Kg.)</td>
<td>2</td>
</tr>
<tr>
<td>Lactic dehydrogenase (stable, hepatic type*)</td>
<td>218</td>
</tr>
<tr>
<td>S.G.O.T.</td>
<td>16</td>
</tr>
<tr>
<td>S.G.P.T.</td>
<td>6</td>
</tr>
<tr>
<td>Cephalin cholesterol</td>
<td>2+</td>
</tr>
<tr>
<td>Alkaline phosphatase, K.A. units</td>
<td>9.0</td>
</tr>
<tr>
<td>Serum uric acid (mg.%)</td>
<td>6.4</td>
</tr>
<tr>
<td>Urine uric acid (mg./24 hr.)</td>
<td>540</td>
</tr>
</tbody>
</table>

*Carried out through the courtesy of Dr. Paul Strandjord.

Table 2.—Bile Pigment and Porphyrin Studies

<table>
<thead>
<tr>
<th>Test</th>
<th>(6a, 7)</th>
<th>Normal Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fractional serum bilirubin (mg.%)</td>
<td>0.5–0.6; total 6.2–7.0</td>
<td>&lt;0.25</td>
</tr>
<tr>
<td>prompt direct (1 min.)</td>
<td>&gt;1.5</td>
<td></td>
</tr>
<tr>
<td>Fecal urobilinogen (mg./day)</td>
<td>500–2200</td>
<td>40–230</td>
</tr>
<tr>
<td>Urine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>urobilinogen (mg./day)</td>
<td>8.6</td>
<td>&lt;3.0</td>
</tr>
<tr>
<td>coproporphyrin (μg./day)</td>
<td>340</td>
<td>100–300</td>
</tr>
<tr>
<td>uroporphyrin (μg./day)</td>
<td>21</td>
<td>5–40</td>
</tr>
<tr>
<td>porphobilinogen (mg./day)</td>
<td>2.0</td>
<td>&lt;4.0*</td>
</tr>
<tr>
<td>δ-aminolevulinic acid (mg./day)</td>
<td>6.0</td>
<td>&lt;4.0**</td>
</tr>
<tr>
<td>Fecal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>coproporphyrin (μg./day)</td>
<td>1193</td>
<td>322–1468</td>
</tr>
<tr>
<td>uroporphyrin (μg./day)</td>
<td>24</td>
<td>20–60</td>
</tr>
<tr>
<td>protoporphyrin (μg./day)</td>
<td>728</td>
<td>110–950</td>
</tr>
<tr>
<td>Erythrocyte</td>
<td></td>
<td></td>
</tr>
<tr>
<td>protoporphyrin (μg.%)</td>
<td>26.0</td>
<td>20–65</td>
</tr>
<tr>
<td>coproporphyrin (μg.%)</td>
<td>0.4</td>
<td>0–1.0</td>
</tr>
<tr>
<td>T-tube bile protoporphyrin (μg.%)</td>
<td>21.6</td>
<td>47–78</td>
</tr>
<tr>
<td>T-tube bile protoporphyrin (μg.%)</td>
<td>377.4</td>
<td>372–503</td>
</tr>
</tbody>
</table>

*Mauzerall and Granick10 give 1 and 2.5, respectively, our use of their method has given somewhat higher normal values as shown. There is evidence that a majority of the Ehrlich reactors responsible for these values in normal urine represent compounds other than PBG or ALA.10

†Adaptation of method for feces* according to Aziz.14 The normal values given relate to six patients, five with T-tubes following cholecystectomy for calculi, one with total external biliary fistula and carcinoma of the head of the pancreas.
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Sideroblasts and siderocytes, counted per 1000 late polychromatophilic and orthochromatophilic normoblasts and erythroblasts, respectively, on concentrated and direct films of marrow, stained as described previously, showed the following percentages:

<table>
<thead>
<tr>
<th></th>
<th>12-7-61 Concentrate</th>
<th>12-7-61 Direct</th>
<th>1-8-62 Concentrate</th>
<th>1-8-62 Direct</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sideroblasts</td>
<td>31.5</td>
<td>36.8</td>
<td>32.9</td>
<td>45.0</td>
</tr>
<tr>
<td>Siderocytes</td>
<td>36.0</td>
<td>7.4</td>
<td>10.4</td>
<td>8.0</td>
</tr>
</tbody>
</table>

Occasional siderocytes (less than 0.1 per cent) were found in the blood film from 12-7-61, and 0.1 per cent siderocytes were found in the blood from 1-8-62. The elevated siderocyte percentage (checked on 2 slides) on the concentrated marrow films from 12-7-61 is probably an artifact which reflects admixture of unusual numbers of siderocytes (from the reticulocyte- and siderocyte-rich layer immediately beneath the buffy coat in the centrifuged marrow) with the nucleated cells of the marrow in this specimen. (Ordinarily, siderocytes will be slightly more numerous in concentrated than in direct films of marrow, but the result for 12-7 is most unusual.) The other three ratios of approximately 5:1, 3.2:1, and 5.6:1 are not distinctive except that they are more comparable to those found with ineffective erythropoiesis than with familial hemolytic anemia.

Only a very few ringed sideroblasts (common in the sideroblastic and sideroachrestic anemias) were found, and the siderotic granules were generally small.

Neither bilirubin nor biliverdin was observed in the normoblasts before or after application of Fouchet’s reagent to Zenker-fixed sections. It is recognized, however, that their presence may have been masked by hemoglobin.

After extensive searching, only one PAS-positive normoblast was found. This is taken as a point of difference from our cases of erythroleukemia and thalassemia major and those reported in the literature where PAS positivity of normoblasts and reticulocytes is generally prominent. Because the possibility of a myeloproliferative syndrome was considered, and because a normoblast in mitosis was found in one blood film, Buffy coat films of venous blood (5-18-62) were examined in detail. These did not show pro- or basophilic normoblasts and only very occasional immature neutrophils could be found. In these Buffy coat films, endothelial cells were found singly and in masses, and phagocytic reticuloendothelial cells containing erythroblasts and iron (but no normoblasts) were also easily found. This latter finding is not explained. Possibly the vein had been thrombosed, so that subendothelial as well as endothelial tissue contaminated the specimen. No circulating macrophages were seen at any other time in capillary blood; nor were any found in the L.E. clot test.

Macrophages were remarkably increased in the marrow. These contained erythroblasts, normoblasts, mitotic normoblasts, occasional granulocytes and remarkably increased iron in granular and diffuse form (figs. 2, 3, 5, 6). Cytoplasmic fragments from macrophages were uniquely numerous in direct and concentrated films of marrows (figs. 3, 6). Possibly their remarkable content of phagocytosed material caused them to be liberated or torn away from the cell body. None of these fragments was found in the blood. Minute pseudopodia from macrophages closely invested many of the normoblasts. Some of these pseudopodia seem to extend as far as the nuclear membrane. (See blue green areas of cytoplasm of some of the normoblasts in figure 2. These represent iron-laden cytoplasmic fragments of macrophages.) Whether this phenomenon is a manifestation of rupheocytosis or incipient phagocytosis cannot be determined in light microscopy.

Plasma cells containing iron comparable to those observed in primary hemochromatosis and in refractory anemias with (secondary) hemochromatosis were also found in the marrow.
Figs. 1–6.—(See figure legends on facing page).
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A leukocyte alkaline phosphatase test showed an average score of 27 with a control of 58, this value being within the normal range for our laboratory.

The peripheral blood showed a normochromic, normocytic (M. C. D. 7.2–7.5 μ) anemia in which the amount of polychromasia always seemed less than that indicated by the reticulocyte percentage. There was no minor population of hypochromic erythrocytes comparable to that found in many sideroachrestic anemias. The most unusual feature was the presence of teardrop poikilocytes. In hanging drop preparations, one could see erythrocytes develop a diverticulum and, occasionally, a sub-diverticulum ending in a hair- or tail-like process. Hanging drop preparations of marrow were not studied. Platelets were moderately decreased but appeared to be normal in morphology. The differential leukocyte counts showed minimal neutropenia, very occasional neutrophil promyelocytes, minimal relative lymphocytosis, and on one occasion, eosinophilia.

Data related to the iron kinetics in this case are given in table 3. This study was carried out 3 months after the second Cr51 study. It is seen that the serum iron was generally elevated with an average of 237 μg. per cent. The red cell iron uptake was remarkably low. The total iron binding capacity was slightly increased and the transferrin was 61 per cent saturated, a considerably increased value. The plasma iron

Fig. 1.—Section of sternal marrow. H & E stain. X400. Note remarkable hyperplasia and normoblastosis.

Fig. 2.—Dry film, marrow concentrate. Wright’s stain, counterstained with Prussian blue. X800. Note two macrophages and one cytoplasmic fragment from a macrophage to the right of the lower macrophage. The top macrophage contains an erythrocyte, a mitotic figure and a pyknotic nucleus. The bottom macrophage contains four, and the cytoplasmic fragment three pyknotic nuclei. The blue-green color of the cytoplasm of the macrophages and of some of the granules is due to the presence of ferric iron (ferritin and hemosiderin) in the cytoplasm. Note the small blue band of cytoplasm “in” the orthochromatic normoblast at about 2 o’clock; this is interpreted as a Prussian-positive pseudopod from a phagocyte. Note also normal neutrophils and many orthochromatic normoblasts.

Fig. 3.—Dry film, marrow concentrate. Wright’s stain, counterstained with Prussian blue. X800. Note two cytoplasmic fragments from macrophages. The one on the right contains three pyknotic nuclei, one siderocyte and excessive iron. That on the left contains a virtually lysed erythrocyte and two pyknotic nuclei. Note also the probable abnormal mitosis in an orthochromatic normoblast.

Fig. 4.—Dry film, marrow concentrate. Wright’s stain. X430. Note normoblasts in all stages of development.

Fig. 5.—Dry film, marrow concentrate. Wright’s stain, counterstained with Prussian blue. X1000. Note macrophages containing erythrocytes, pyknotic nuclei and excessive iron. In the top center there is a polychromatic sideroblast. Several siderocytes may be discernible.

Fig. 6.—Dry film, marrow concentrate. Prussian blue reaction, no stain. X125. The blue-green color is ferric iron. The larger rings and clusters of rings are macrophages with iron-laden cytoplasm. Most of the small blue-green structures are cytoplasmic fragments comparable to those in figures 2 and 3, although some are undoubtedly small macrophages with narrow cytoplasmic bodies. The prominent small Prussian-positive structures are very small fragments from macrophages. The siderotic granules of sideroblasts and siderocytes are barely identifiable at this magnification.

The colored plate is made possible in part by the Hal Downey Memorial Fund for Research on Blood and Blood Diseases. We wish to acknowledge the photomicrography of Miss Dolores C. Breen, Senior Medical Photographer.
### Table 3.—Data Relating to Iron Kinetics

<table>
<thead>
<tr>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum iron</td>
<td>20% of injected dose (N = 85–105%)</td>
</tr>
<tr>
<td>Red cell utilization</td>
<td>392 mg.% (N = 250–375)</td>
</tr>
<tr>
<td>Total iron binding capacity</td>
<td>61.0% saturated (N = 30–35%)</td>
</tr>
<tr>
<td>Serum transferrin</td>
<td>44 min. (N = 55–135 min.)</td>
</tr>
<tr>
<td>Plasma clearance Fe59 T1/2</td>
<td>160.9 mg./24 hr. (N = 27–45 mg. Fe/24 hr.)</td>
</tr>
<tr>
<td>Plasma Fe59 turnover</td>
<td>55-135 mm.)</td>
</tr>
</tbody>
</table>

Clearance was distinctly increased in rate and the plasma iron turnover was markedly increased. The very slow uptake of Fe59 in the red cells is demonstrated graphically in figure 7. It is seen that the uptake is also low in degree, yet the reticulocytes during this period of study ranged between 3–8 per cent. The slow low uptake would not accord with any ordinary hemolytic event, for which there was no evidence in other respects. Differences in distribution of Fe59 over a period of time as compared with the normal are shown in figure 8. Of particular importance here is the high content at a late date in the bone marrow (sacrum) and liver. A tryptophane load test showed a normal increase of xanthurenic acid from 3.0 mg. to 18.9 mg. in 24 hours, and of kynurenic acid from 2.8 to 43.8 mg./24 hours.

### The N15 Glycine Study

Glycine containing 61.5 atom per cent excess N15 was fed in a total quantity of 2.75 Gm. This was given at the rate of 250 mg./hr. over a 10-hour period. Blood samples were drawn every day for the first 30 days and every 4th day thereafter up to 150 days. Feces were collected every day for the first 4 weeks and the entire amount for each 4-day period thereafter, up to 140 days, after the glycine was administered. The hemoglobin protoporphyrin was prepared and crystallized from each blood sample according to the method of Grinstein. The fecal urobilin was crystallized by the method of Watson et al., with employment of the ferrous hydroxide filtrate modification. The term urobilin, rather than stercobilin, is used advisedly, as in this case most or all of the fecal urobilinogen group was represented by a hitherto undescribed type of urobilin, indistinguishable from d-urobilin on the basis of absorption spectrum and behavior toward FeCl3. Although optically inactive at 589 mλ this compound exhibits activity at other wave lengths and has been distinguished from (dl) urobilin (H10). It will be described in detail in a separate communication. The fecal mesobilifuscin was isolated by the method of Siedel, modified slightly as previously described. The N15 percentages were determined in the mass spectrometer and preliminary digestion, in accordance with the method of Rittenberg. The data are plotted graphically in figure 9. It is at once evident that there was a marked early labeling of the fecal urobilin with an essentially normal hemoglobin porphyrin plateau and negligible increase in the urobilin label at the time of destruction of mature circulating red cells. The latter may be ascribed to the great dilution of N15 by N14 incident to the large uptake of glycine N15 in the early period of subsequent red cell generations, together

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*Carried out through the courtesy of Dr. Wm. Krivit, Department of Pediatrics.
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Fig. 7.—Fe$^{59}$ uptake by the circulating red blood cells.

Fig. 8.—Body surface counting following the injection if Fe$^{59}$. 
We thank Dr. Robert L. Evans, Department of Physiology, University of Minnesota, for valuable advice and assistance in this calculation.

Fig. 9.—N₁⁵ concentration in circulating hemoglobin protoporphyrin, fecal stercobilin and fecal mesobilifuscin.

with the relatively slow rate of red cell destruction. The striking disproportion between the early peak labeling of the urobilin and the maximal labeling of the hemoglobin protoporphyrin is expressed in the ratio

\[
P \left( \text{maximal labeling of hemoglobin protoporphyrin} \right) \over U \left( \text{early peak labeling of urobilin} \right)
\]

In the present case, this ratio is 0.06. Calculated for the data given by Israels and Zipursky,³³ it is 0.10. From the normal data given by London,¹ Gilbertsen and Watson,² Gray and co-workers,³ the values calculated for this ratio are 0.80, 1.30 and 0.60, respectively. In a case of familial hemolytic anemia studied with Hagen in this laboratory,⁴ the data provide a ratio of 0.70. The significance of these observations is discussed in the following.

Measurement of the red cell life span in the present case was attended with considerable difficulty. The hemoglobin protoporphyrin label plateaued up to 60 days after which there was a gradual slope to the last measurement at 132 days. Application of the mathematical formula of Shemin and Rittenberg³⁴ provided a value of 91 days* as contrasted with 120 days in the

*We thank Dr. Robert L. Evans, Department of Physiology, University of Minnesota, for valuable advice and assistance in this calculation.
usual normal individual. This would indicate that there was a minor hemolytic component, quite inadequate, however, to account for the greatly excessive bilirubin production, jaundice and urobilinogen excretion.

From figure 9 it is noted that there was a marked early peak labeling of the fecal mesobilifuscin preceding that of the urobilinogen by 24 hours. The significance of this will be discussed below.

The crystalline bilirubin from the bile (see above) had essentially the same N\(^15\) content as the urobilin isolated from the feces for the same period (5-9 to 5-12), i.e., 0.018 atom per cent excess, that for the urobilin being 0.012. The gallstones appeared to be almost entirely composed of bile pigment, mainly bilirubin. A small aliquot of the extract of one of the stones (see above) contained 3.1 mg. bilirubin and biliverdin by the Evelyn-Malloy method,\(^6b\) 2.60 mg. of bilirubin by the diazo method.

**DISCUSSION**

It is difficult to interpret the results which have been described in terms of a single abnormal basic mechanism. Israels and associates\(^5\) have observed four cases, three in one family, similar in many respects to the present case but differing in that they had enlarged spleens, spheroïdocytosis and increased fragility. Their observations after labeled glycine were quite similar to those in the present case, especially the marked early peak in the stercobilin without subsequent rise after a long, rather low plateau in the hemoglobin protoporphyrin. Israels has designated these cases as “primary shunt hyperbilirubinemia” to express the concept of an overproduction of bilirubin from precursors, presumably without formation of hemoglobin. The term “shunt” suggests a different pathway and a different enzyme system leading from precursors directly to bilirubin rather than to heme and hemoglobin. Recently, Israels and co-workers\(^3\) have described experimental evidence believed to support the existence of such a “shunt” in the normal human. After administering glycine-2-C\(^14\), they observed two early peaks in the serum bilirubin, the first at 24 hours, the second at 4 days. Even more remarkable was their finding of a single peak at 90 minutes after administration of \(\delta\)-aminolevulinic acid-4-C\(^14\), without a subsequent peak and without evidence of significant incorporation in the circulating heme. Regardless of the rather difficult interpretation of these findings, it is believed unnecessary to postulate a true “shunt” from precursors to bilirubin, bypassing heme, in order to explain the exaggerated early peak of N\(^15\) in the fecal urobilin of the present case. It is quite conceivable that the large amount of bile pigment is derived from intramedullary destruction of hemoglobin or heme, either in or after release from normoblasts. The question might arise whether heme was produced in excess of globin as it is widely accepted that the two molecules are synthesized independently, forming hemoglobin at a late stage.\(^37-39\) There is also evidence, however, that globin formation is highly significant in controlling the rate of heme synthesis.\(^38\) It is possible that serial N\(^15\) determinations on the globin of the circulating hemoglobin might have provided information as to a possible imbalance in heme-globin ratio; however, these
were not carried out. It is possible that hemoglobin was being formed at an excessive rate, a part escaping from the intact normoblast to be converted to bilirubin by virtue of the enzymatic activity of mesenchymal cells, especially those of reticuloendothelial type. It is also conceivable that excessive heme or hemoglobin in the normoblast might be converted to bilirubin intracellularly, to be released to the circulation. There is adequate evidence both from earlier and more recent studies that heme or hematin is readily converted to bilirubin in man and dog.

Nakajima and co-workers have recently reported the partial purification of the enzyme controlling this conversion and have reported that it is inactive with protoporphyrin alone, active only when iron is complexly bound, as in heme. They have also observed that haptoglobin, as well as TPNH and ferrous iron appear to be essential co-factors. The enzyme has been designated by Takemura as "heme α-methenyl oxygenase." The term α-methenase is believed to be a suitable abbreviation, and will be used below. Their observation that this enzyme is at very low levels in spleen and bone marrow, as contrasted with liver and kidney, is indeed difficult to reconcile with various earlier evidence of bilirubin formation from hemoglobin in both spleen and bone marrow.

The intracellular formation of bilirubin would presumably require the presence in the normoblast of the α-methenase-coenzyme activity postulated by Nakajima, although the possibility of non-enzymatic conversion is not excluded. With conversion of excessive heme to bilirubin, at least some of the iron might remain in the cell. This would accord well with the frequency of siderocytes and the decreased per cent of injected Fe recovered in circulating red cells. The possibility of a damaging effect of free bilirubin on cellular enzyme systems may deserve consideration. There is reason to believe that "kernicterus" is due to an uncoupling of oxidative phosphorylation and an inhibitory effect on oxygen consumption of brain tissue, perhaps due to the lipophilic nature of this non-polar compound. Also, adverse effects on bacterial enzyme systems have been recorded. If free bilirubin were formed in or in the immediate vicinity of the normoblasts, it is conceivable that it might be delayed in its transit across the lipid membrane of the red cell, or in any event cause sufficient damage to provoke phagocytosis; in other words, the phagocytosis might be a secondary rather than a primary abnormality. The failure to observe bile pigment in the normoblasts is believed not to exclude this possibility.

Arias has described "intramedullary hemolysis" as the pathogenesis of a somewhat similar case. Any abnormality of the normoblasts might, of course, be sufficient to provoke phagocytosis. The possibility of an abnormal immune mechanism in the present case is not excluded, but the results of the Coombs test and the antinuclear antibody test fail to indicate its presence.

Future studies must determine whether developing red cells contain the Nakajima enzyme essential for the conversion of heme to bilirubin. There is at least considerable evidence in previous literature suggesting that choleglobin, intermediate between heme and bilirubin, may be formed within red cells in small amount.
The increased plasma iron turnover and sharply diminished red cell utilization of Fe\textsuperscript{59} do not serve to distinguish between the various possible disturbances which have been discussed. It will be remembered that the curve of uptake of Fe\textsuperscript{59} in the sacrum first described a sharp rise, then a marked decline comparable to what is seen normally, followed, however, by a secondary rise and plateau at a high level. This brief exodus of Fe\textsuperscript{59} followed by the increase and high plateau is theoretically compatible with any of the basic disturbances just considered, but the exact explanation of the phenomenon is not clear, nor to our knowledge has it been previously described.

It is of much interest that the circulating erythrocyte porphyrin concentrations were entirely normal despite a consistent increase in reticulocyte percentage which under other circumstances of disease is almost invariably associated with elevated porphyrin concentrations, particularly of the coproporphyrin.\textsuperscript{13a} The erythrocyte coproporphyrin concentration usually correlates well with the reticulocyte percentage, although in certain states of disturbed hemoglobin synthesis, particularly lead poisoning, the increase of coproporphyrin is much greater than would be anticipated from the reticulocyte percentage. In this case the opposite is true. The possibility cannot be excluded that the circulating reticulocytes in this case lost their ribonucleic acid more slowly than in the normal state, thus accounting for the paradoxical increase of percentage despite an only slightly shortened red cell life span.

Although a profound disturbance of hemoglobin synthesis was quite likely it is evident that one must assume either a diminished red cell porphyrin formation or a highly efficient utilization. With diminished formation one would anticipate a hypochromic anemia with diminished hemoglobin concentration of the circulating red cells as in pyridoxine deficiency anemia.\textsuperscript{49} This, however, was consistently within the normal range, and the tryptophane load test was normal, although it is fully recognized that this does not exclude pyridoxine deficiency. A diminished hemoglobin concentration might also be anticipated if one were to assume a true shunt; in other words, bilirubin formation from porphyrin precursors. The fact that the hemoglobin concentration was normal is much more in accord with an efficient synthesis of heme, as postulated in either of the above suggested pathways. In fact, the lack of increase of copro- or protoporphyrin in the red cells is perhaps one of the strongest points in favor of a disturbance of this type. It seems unlikely that this discrepancy would be explained by a simple intramedullary destruction of red cells or normoblasts, as in this event one might reasonably expect an increase of red cell porphyrins as regularly observed in ordinary hemolytic anemias.

The remarkably high percentage of sideroblasts might raise the question as to whether this is a "sideroblastic anemia," sui generis, in the sense of Heilmeyer.\textsuperscript{50} We believe the conditions are quite distinct. Heilmeyer's cases were not jaundiced and did not show appreciable excesses of urobilinogen excretion; the reticulocytes were not significantly increased and erythrophago-
cytosis in the bone marrow was not mentioned. The erythrocyte coproporphyrin was usually increased to a considerable degree and there was often excessive urinary coproporphyrin. Principally because of this evidence of disturbed porphyrin metabolism in his cases, Heilmeyer believes there is diminished heme formation, due to partial blocks in the biosynthetic pathway between coproporphyrinogen and protoporphyrin, and between the latter and heme.

The cases of Dacie and co-workers, designated as refractory normoblastic anemia, are believed to be essentially similar to the "sideroachrestic-sideroblastic" anemia group, and dissimilar to the present case. The striking decrease in the $\frac{P}{U}$ ratio (see above) in this case, and as calculated from the data of Israels and Zipursky in their case, compared with the normal ratio and that in a case of hemolytic jaundice indicate that this value is a convenient expression of ineffective erythropoiesis, at least in most situations. It is fully recognized, however, that under some conditions, perhaps normally, there may be a significant contribution to $U$ (the early urobilin peak labeling) from sources unrelated to erythropoiesis or hemoglobin synthesis, as, for example, the hemes of the liver. The 90-minute peak observed by Israels, after giving C$^{14}$-ALA (see above), might well be so explained, for this compound readily enters intact cells. James and Abbott have observed highly significant early peak labeling of fecal stercobilin in cases of erythroid aplasia which might be on the same basis, as previously suggested.

As noted in figure 9, a marked labeling of the fecal mesobilifuscin was observed with a peak occurring 24 hours before the peak labeling of the fecal urobilin. Although this cannot be interpreted with certainty, it is compatible with an increased production of pyrrolic precursors of heme, possibly at the stage between porphobilinogen and uroporphyrinogen, in the biosynthetic pathway. However, it should not be assumed to indicate diminished heme synthesis, because in ordinary hemolytic jaundice a similar increase is observed. This observation in the present case may be contrasted with an unexplained lack of labeling of fecal mesobilifuscin in a similar case recently described by Robinson and co-workers, thought by them to represent an instance of highly ineffective erythropoiesis in thalassemia minor.

**Summary and Conclusions**

1. The term "dyserythropoietic jaundice" is used to designate a peculiar abnormality characterized by marked overproduction of bilirubin and associated with unconjugated hyperbilirubinemia and great excesses of fecal urobinginogen. Labeling with N$^{15}$ glycine revealed that these excesses were mainly unrelated to destruction of mature circulating red cells, which had only a slightly shortened life span.

2. The hyperplastic, normoblastic bone marrow exhibited considerable phagocytosis of red cells and normoblasts and marked hemosiderosis with many iron-laden phagocytes. Siderocytes were relatively rare in the peripheral blood, which also exhibited consistent reticulocytosis, 3–10 per cent, but
normal erythrocyte porphyrin concentrations. There was increased plasma iron turnover but decreased appearance of Fe^{59} in the circulating red cells.

3. The possibility is considered that there is a basic abnormality in hemoglobin synthesis in the normoblasts, with excessive production of hemoglobin or heme which is then converted to bilirubin either within the normoblast or after excretion into the blood. The observed phagocytosis of normoblasts and erythrocytes may be a secondary rather than basic disturbance of the disease.

4. This case appears to represent a unique form of chronic jaundice due to a remarkable overproduction of bilirubin without evidence of hemolysis in the ordinary sense. The extent to which this is related to intramedullary destruction of young red cells or to a basic abnormality in hemoglobin synthesis in the normoblasts, as above, cannot be determined. It is considered less likely that the overproduction is due to a true shunt—that is, bilirubin formation from porphyrin precursors. The marked dyserythropoiesis suggests that the overproduction of bilirubin was medullary rather than hepatic, although the latter is not excluded.

**Summario in Interlingua**

1. Le termino "jalnessa dyserythropoietico" es usate pro designar un peculiar anormalitate, caracterisate per marcate grados de hyperproduction de bilirubina e associate con non-conjugate hyperbilirubinemia e grande excessos de urobilinogeno fecal. Marcation con glycina a N^{15} revelava que iste excessos eseva essencialmente non-relationate con le destruction de matur erythrocytos circulante, viste que le durata vital de istos eseva reducitate solo levemente.

2. Le hyperplastic, normoblastic medulla ossee exhibiva considerable grados de phagocytosis erythrocytic e normoblastic e marcate hemosiderosis con numerose phagocytos cargate de ferro. Siderocytos eseva relativemente rar in le sanguine peripheric que etiam exhibiva reticulocytosis continue amontante a 3 a 10 pro cento sed normal concentrationes de porphyrina erythrocytic. Esseva constatate un augmento del metabolismo de ferro in le plasma sed un declino del apparititon de Fe^{59} in le erythrocytos circulante.

3. Es considerate le possibilitate que il existe un anormalitate fundamental del synthese de hemoglobina in le normoblastos, con excessos del production de hemoglobina o de hem le qual es convertite alora in bilirubina intra le normoblasto o post le excertion ad in le sanguine. Le observate phagocytosis del normoblastos e erythrocytos es possibilemente secundari plus tosto que un disturbation fundamental del morbo.

4. Iste caso pare representar un forma unic de jalnessa chronic causate per un remarcaible hyperproduction de bilirubina sin evidentia de hemolysis in le senso ordinari. Le mesura in que isto es relationate con le destruction intramedullari de juvenile erythrocytos o con un anormalitate basic del synthese de hemoglobina in le normoblastos non pote esser determinate. Es reguardate como minus probable que le hyperproduction es causate per un ver shunting, i.e., le formation de bilirubina ex precursores de por-
bilirubina esseva medullari plus tosto que hepatic, sed iste ultime non es phyrina. Le marcate dyserythropoiese suggere que le hyperproduction de excludite.

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Idiopathic Dyserythropoietic Jaundice
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