The biochemical defect in erythropoietic protoporphyrin is an impaired ferrochelatase activity, and protoporphyrin, but not iron accumulates in large amounts in erythrocytes, liver, and skin. In sideroblastic anemia, large amounts of iron accumulate in erythroblast mitochondria although in the majority but not all cases reduced bone marrow ferrochelatase activity has also been observed. It remains unclear why patients with erythropoietic protoporphyrin do not accumulate mitochondrial iron nor develop significant anemia and why patients with sideroblastic anemia do not accumulate much intracellular protoporphyrin nor develop photosensitivity. In only two reports was a possible relationship between sideroblastic anemia and erythropoietic protoporphyrin suggested. Rothstein et al. described an elderly man with the triad of photosensitivity, sideroblastic anemia, and greatly increased erythrocyte protoporphyrin. Scott et al. reported a middle aged woman with erythropoietic protoporphyrin who developed features of sideroblastic anemia and fatal liver disease later in life.

We describe a patient with acquired sideroblastic anemia and greatly increased erythrocyte protoporphyrin but who lacked photosensitivity and evidence of liver disease.

CASE REPORT

A 73 yr old fisherman had been in good health all of his life. From 1949 to 1954, while working in a shipyard, he experienced occasional headaches, joint pains, and malaise. He attributed these symptoms to "lead intoxication" being exposed to gases from heated steel plates coated with read lead. However, no examinations were carried out to test this assumption. The hemograms were normal in 1953 and 1960 when he was hospitalized for removal of epididymal cysts. On occasional visits to physicians for minor complaints during 1975-1976 he was found to have moderate anemia which was unresponsive to iron administration. He remained asymptomatic although in 1979, when a benign prostatic adenoma was removed, the hemoglobin was 9.8 g/dl. In March, 1980, he was referred for evaluation of persistent anemia (Hb, 9.5 g/dl). He had been out in the sea for decades and often worked in full sunshine for many hours without experiencing symptoms or signs of photosensitivity. There was no family history of anemia or photosensitivity.

Physical examination was unremarkable except for mild pallor. The hemogram revealed hemolytic anemia and mild leukopenia; the platelet and reticulocyte values were normal (Table 1). The serum iron and transferrin saturation were raised as was serum ferritin. The bone marrow aspirate was normocellular but showed marked erythroid hyperplasia. With Prussian blue stain the majority (95%) of erythroblasts were typical ring sideroblast (Fig. 1a); reticuloendothelial iron was moderately increased. Liver function tests and serum protein electrophoresis were normal.

MATERIALS AND METHODS

Electron Microscopy

Spicules of the bone marrow aspirate were fixed in buffered 10% formalin—1% glutaraldehyde (4 CF–1C), postfixed in 1% osmium tetroxide, washed and en bloc stained with 2% uranyl acetate. The stained specimen was next embedded in Polybed/812 and ultra thin sections were examined with an IEO100CX electron microscope.

Porphyrin Analyses

Erythrocyte- and plasma (heparin)—protoporphyrin were quantified following solvent extraction. Urine and fecal porphyrins were measured by HPLC after esterification. Fluorescence spectra of whole blood, plasma and blister fluid were recorded after appropriate dilutions as described by Poh–Fitzpatrick and Lamola. Blister fluid (~0.5 ml) was obtained by creation of suction blisters on the volar forearm with the suction device Dermovac of Dr. U. Kiistala (Oy Instrumentarium, Helsinki, Finland) employing a negative pressure of 220–240 mm Hg.

Erythrocyte Photohemolysis

Erythrocytes were washed three times in 0.15 M NaCl and suspended to a concentration of 8 × 10⁹ cells/L. Aliquots of 3 ml were irradiated in 3 mm light path cuvettes with the photochemotherapy unit PUVA 200 (H. Haldemann, D 722, Schweningen, German Free Republic) containing 14 fluorescence tubes (FT85/BL, PUVA Sylvania) in a bank. Approximately 10% of the emission energy of these lamps is between 340–380 nm. The light intensity was 66 W/m², as measured at sample level with a UDT model 80X.
optometer equipped with a UDT-1116 filter optic probe (United Detector Technology, Inc., Santa Monica, Ca.). Following irradiation for up to 60 min, the cell suspension was left in the dark for 30 min before the degree of hemolysis was measured.

**Protoporphyrin Release from Erythrocytes**

Erythrocytes were washed as above, resuspended in ABO compatible normal heparin plasma (Hct 30%) and stored in the dark at +4°C. At timed intervals aliquots were removed, centrifuged and fluorescence spectra and the protoporphyrin content examined in the supernatants.

**Skin Irradiation**

Skin irradiation studies were performed on the patient’s low back region using an Osram high pressure Xenon arc lamp (XBO 150 W) equipped with a UVL 215 liquid light guide, active diameter 5 mm, length 1500 mm with blue coloring, and a thermal protective filter, Schott 114. The irradiation dose given was 2500 W/m² as measured with a UDT model 80 X optometer, equipped with a UDT-1116 filter optic probe (United Detector Technology, Inc., Santa Monica, Ca.). Results were assessed 2,3 and 18 hr after exposure.

**Bone Marrow Metal Chelatase Determination**

Bone marrow cells were washed three times with 0.15 M NaCl, centrifuged at 2500 x g for 10 min and suspended to a concentration of 70 x 10⁶ nucleated cells/l. Cell lysate was prepared by sonication at 4°C for 30 s x 3 using an MSE 150 W ultrasonic disintegrator (Model MK 2), operated with a 9.5 mm diameter end probe and an amplitude reading of 18 μm. Following centrifugation the pellet was taken up in 0.25 M sucrose, 10 μM ⁵¹CoCl₂, and 10 mM HEPES buffer, pH 7.4. The reaction was initiated by adding 10 μM deuteroporphyrin. After 60 min duplicate aliquots of incubation mixture were removed and ⁵¹Co-deuteroporphyrin formed was quantified following extraction into cyclohexanone as described previously. To account for nonenzymatic metalloporphyrin formation an identical incubation with 50 μM Pb²⁺ was included.
RESULTS

Electron microscopy of bone marrow sections showed dense, granular iron containing material in numerous mitochondria and in lysosomes of nearly all erythroblasts (Fig. 1b). Protoporphyrin crystals were not identified.

Erythrocyte protoporphyrin was markedly increased to over 100 fold of normal but erythrocyte coproporphyrin was not detectable. Plasma protoporphyrin was also greatly increased. Urine and stool porphyrins were normal (Table 2).

Fluorescence spectra of whole blood, plasma and blister fluid revealed an emission peak at 627 nm and 635 nm respectively (Fig. 2). These spectra correspond closely to those seen in patients with iron deficiency and lead intoxication (Fig. 3). The fluorescence peak at 627 nm implies binding of protoporphyrin to (hemo–) globin and the peak at 635 nm binding to albumin. The fluorescence spectrum of whole blood remained quantitatively and qualitatively unchanged for at least 1 hr when left in the fluorometer under anaerobic conditions.

Photohemolysis of the erythrocytes was markedly increased as would be expected from their very high free protoporphyrin content (Table 2). However, after a 4 day incubation of the erythrocytes in normal plasma in the dark, less than 0.2% of total red cell protoporphyrin leaked out into the plasma. This figure corresponds closely to the per cent hemolysis during the same incubation. However, whereas the fluorescence peak of the patient’s plasma was at 635 nm, the fluorescence peak of the protoporphyrin that had leaked from his erythrocytes into the normal plasma was at 627 nm.

In contrast to patients with erythropoietic protoporphyria who have very high erythrocyte and serum protoporphyrin, this individual had no evidence of an increased photosensitivity. On phototesting no reaction was observed with test doses up to 1000 kJ/m².

Bone marrow metal chelatase activity was 582 pmoles Co–deuteroporphyrin/10⁶ erythroblasts/30 min which was within the range of normal values (200–1000).

DISCUSSION

Sideroblastic anemia is recognized by a hypochromic erythrocyte population in the peripheral blood and the associated hallmark ring sideroblasts in the bone marrow, reflecting pathologic iron deposits in mitochondria. Because this hematologic picture occurs under a variety of circumstances, e.g., from
several drugs and ethanol, as an inherited and as an acquired abnormality, multiple mechanisms must be involved in producing the defective hemoglobin synthesis.7 Enzymatic defects in erythroid heme biosynthesis have been described at the level of ALA-synthase,8-10,19,20 coproporphyrinogen oxidase21 and ferrochelatase.8-11 However, the basic defect in this group of disorders remains obscure.7

The patient reported herein has been anemic for at least 5 yr and an underlying cause for the sideroblastic anemia is not evident. The current data also exclude lead intoxication as blood lead and ALA-dehydratase analyses were normal (Table I). Hence he would be considered to have idiopathic sideroblastic anemia. However, the biochemical findings with respect to heme biosynthesis differ from those of most other reported cases with this disorder in that he has normal erythroid metal chelatase activity and greatly increased erythrocyte and plasma protoporphyrin concentrations. This individual's features also differ from those of two previous atypical cases: a) a patient with idiopathic sideroblastic anemia who had an erythrocyte protoporphyrin concentration of 1700 µg/dl, markedly reduced reticulocyte ferrochelatase activity and dermal photosensitivity11 and b) a patient with lifelong protoporphyria who acquired sideroblastic anemia.12

Detailed studies in sideroblastic anemias have not been carried out to determine whether the often slightly to moderately raised erythrocyte protoporphyrin is of the “free type,” as in erythropoietic protoporphyria, or is metal chelated (i.e. with zinc), as in iron deficiency and lead intoxication.22,23 The “free type” protoporphyrin has been considered the hallmark of erythropoietic protoporphyria and thought responsible for the photosensitivity in this disease.23 Our patient lacks photosensitivity in the face of very high concentrations of erythrocyte and plasma protoporphyrin whose fluorescence spectra are identical to the “free type” protoporphyrin observed in erythropoietic protoporphyria. The fluorescence maximum of his plasma suggests that the protoporphyrin is bound to albumin.23 On the other hand, the fluorescence spectrum of the normal plasma incubated with the patient's erythrocytes suggests that the protoporphyrin released into the plasma is bound to hemoglobin. Apparently, in situ protoporphyrin is released from the erythrocytes to be taken up by albumin, whereas during prolonged incubation in vitro protoporphyrin is released with hemoglobin in parallel with the degree of hemolysis.

Accumulation of “free type” protoporphyrin in erythropoietic protoporphyria has been ascribed to a defective ferrochelatase, and accumulation of zinc protoporphyrin in iron deficiency to iron substrate depletion, zinc serving as the alternate metal substrate.23 In vitro, high concentrations of lead (>1 x 10 -6M) inhibit ferrochelatase,24 yet zinc protoporphyrin is the species which accumulates in lead intoxication22 (Fig. 3), perhaps because ferrochelatase activity is not sufficiently suppressed but ferrous iron is less available.7 Since our patient had normal metal chelatase activity and no evidence of zinc depletion, the erythrocyte protoporphyrin may accumulate because of a reduced availability of ferrous iron as well as zinc for the enzyme. In contrast to cobalt, the insertion of iron into protoporphyrin by mitochondrial ferrochelatase requires the reduction of ferric iron (mediated by the respiratory chain) followed by an energy-dependent transport of ferrous iron to within the mitochondria.16,25 Thus, a primary mitochondrial lesion may impair the supply of ferrous iron (and zinc) to the metal chelatase.

Other features which distinguish this patient from the majority of patients with erythropoietic protoporphyria are the normal fecal porphyrin values and the absence of affected family numbers. These findings are similar to those described by Rothstein et al. whose patient also had normal fecal porphyrins.11 Fecal por-
phyrins are generally considered to arise in the liver, in part from hepatocytes and in part from erythrocyte catabolism in Kupffer cells. The data in our patient could be interpreted to mean that hepatic heme synthesis as well as the capacity of the Kupffer cells to handle the abnormal erythrocytes is normal. One could speculate that the photosensitivity in patients with erythropoietic protoporphyria originating mainly in the liver,26 where it accumulates in part due to reduced ferrochelatase activity.27 It also accumulates after release from circulating erythrocytes28 and from catabolism of senescent protoporphyrin loaded erythrocytes. Although the protoporphyrin content in our patient’s erythrocytes with respect to cell age was not determined, the very minimal release of protoporphyrin from the erythrocytes observed in vitro suggests intracellular binding of protoporphyrin which differs from that in erythropoietic protoporphyria.21,28 Another explanation for the absence of dermal photosensitivity in this case may be that, in contrast to patients with erythropoietic protoporphyria,29 heme synthesis in the skin is normal so that any excess protoporphyrin reaching the dermis is readily converted to heme. Lastly, impaired protoporphyrinogen oxidase activity in erythroid tissue could conceivably produce the clinical and biochemical features observed. However, our findings do not support the presence of excess red cell protoporphyrinogen.

**ADDENDUM**

*Nov. 1981.* The patient developed acute erythroleukemia and died within 2 wk. Two days before he died erythrocyte protoporphyrin was 212 μmol/l. However, neither liver nor skin removed at autopsy showed increased concentrations of porphyrins. The results are compatible with a defect limited to the erythroid tissue.

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Sideroblastic anemia with markedly increased free erythrocyte protoporphyrin without dermal photosensitivity

I Romslo, A Brun, S Sandberg, SS Bottomley, G Hovding and I Talstad