

# Study of Factors Causing Excess Protoporphyrin Accumulation in Cultured Skin Fibroblasts from Patients with Protoporphyrin

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**ABSTRACT** The activity of heme synthetase, which catalyzes the chelation of ferrous iron to protoporphyrin to form heme, is deficient in sonicates of skin fibroblasts cultured from patients with protoporphyrin. During culture in Eagle's medium supplemented with fetal calf serum, these cells do not accumulate protoporphyrin, however. This may be due to a minimal requirement for heme synthesis, since glycine is incorporated into heme at a low rate which is similar to that in normal fibroblasts. In addition, the activity of  $\delta$ -aminolevulinic acid (ALA) synthetase, the first and rate-limiting enzyme of heme biosynthesis which catalyzes the formation of ALA from glycine, is normal in lysates of the fibroblasts.

Cultured fibroblasts were therefore incubated with ALA in order to bypass the rate-limiting step of heme biosynthesis. In the presence of 25  $\mu$ M iron, protoporphyrin was detected in protoporphyrin cell lines when the concentration of ALA in the medium reached 50  $\mu$ M, but not in normal lines. As the concentration of ALA was increased above 50  $\mu$ M, all lines accumulated protoporphyrin. However, the amount was 2-3 times more in cultured fibroblasts from patients with protoporphyrin, reflecting their deficiency of heme synthetase activity. When iron was not added to the medium, protoporphyrin accumulated to a similar degree in normal and protoporphyrin fibroblasts; this was significantly more than that in the presence of iron.

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These studies indicate that excessive protoporphyrin accumulation in protoporphyrin, which is due principally to deficient heme synthetase activity, may be modified by the rate of ALA formation in heme-producing tissues, and by the availability of iron.

## INTRODUCTION

Protoporphyrin is a genetic disorder of porphyrin metabolism in man in which there is excessive accumulation and excretion of protoporphyrin, resulting in elevated levels of this compound in erythrocytes, plasma, and feces. Photosensitivity is the major symptom (1). Hepatobiliary disease is a rarer complication which has occasionally caused death from hepatic failure (2). Both of these clinical manifestations appear to be related to the protoporphyrin accumulation which characterizes this disorder (1, 2). As occurs in the other hereditary porphyrias, there is a marked variability in the severity of the disease among patients with the trait.

Ferrous iron is chelated to protoporphyrin to form heme by the activity of the intramitochondrial enzyme heme synthetase (protohaem ferro-lyase, EC 4.99.1.1). Recent studies have demonstrated a partial deficiency of heme synthetase activity in bone marrow (3), peripheral blood reticulocytes (3, 4), liver (5), and cultured skin fibroblasts (5, 6) of patients with protoporphyrin. In addition, deficient heme synthetase activity has been found in cultured skin fibroblasts from one parent in each of three families in which the children have protoporphyrin (6), a finding which is consistent with the postulated autosomal dominant mode of inheritance (7). These findings indicate that decreased activity of heme synthetase is the basic defect in protoporphyrin.

In this report, we show that cultured skin fibroblasts from individuals with protoporphyrin accumulate excessive amounts of protoporphyrin under cer-

tain conditions of incubation, reflecting the deficiency in heme synthetase activity.

## METHODS

**Fibroblast culture.** Fibroblast cultures were initiated from the skin of six patients in whom the diagnosis of protoporphyria was made on the basis of photosensitivity, a family history of the disease, and elevated erythrocyte protoporphyrin concentrations (range in the patients was 272–1428  $\mu\text{g}/100$  ml cells; normal is less than 30 in our laboratory). Additional skin biopsies were available from six age-matched normal individuals.

For determining enzyme activities, cells in the 5th to 10th passage were grown in 690-cm<sup>2</sup> roller bottles (Bellco Glass, Inc., Vineland, N. J.), using Eagle's minimum essential medium supplemented with 1% nonessential amino acids and 100  $\mu\text{g}/\text{ml}$  Kanamycin (MEM)<sup>1</sup> and 10% fetal calf serum (FCS) (Flow Laboratories Inc., Rockville, Md.). This is the routine culture medium used in our laboratory. They were harvested at confluence (7–10 days after plating) into Ca, Mg-free phosphate-buffered saline, pH 7.4, with 0.25% trypsin (Grand Island Biological Co., Grand Island, N. Y.), centrifuged, and suspended in 0.25 M sucrose–0.05 M Tris-Cl, pH 7.5. Cell count and viability were determined in a hemacytometer by trypan blue exclusion.

To compare protoporphyrin accumulation among various lines, the cells were cultured to confluence (6 days) in 9.6-cm<sup>2</sup> wells of FB-6-TC plates (Linbro Chemical Co., Hamden, Conn.), using the routine culture medium. The confluent monolayer was rinsed with phosphate-buffered saline, and fresh MEM supplemented with ferrous sulfate (Fe) and  $\delta$ -aminolevulinic acid (ALA) was added. In the routine culture medium, FCS is the principal source of iron. The concentration of iron in the FCS used in these studies was 60  $\mu\text{M}$  (concentration in the routine culture medium was therefore 6  $\mu\text{M}$ ). Iron is not an added component of MEM and is present only as a contaminant (concentration less than 1  $\mu\text{M}$ ). Incubation was carried out at 37°C in the dark in a 5% CO<sub>2</sub>/95% air atmosphere for 24 h. Under these conditions, protoporphyrin accumulation in the cells proceeded at a nearly linear rate.

**Enzyme assays.** A portion (15–20  $\times 10^6$  cells) of the cell suspension obtained from roller bottle cultures was centrifuged, and the cells were resuspended in 0.15 M NaCl–0.04 M Tris-Cl, pH 7.4, and stored at –70°C until assay of ALA synthetase activity (5, 8). The remainder of the cell suspension was used for measuring porphyrin content and heme synthetase activity. Heme synthetase activity was assayed in sonicates of the fibroblasts, using a radiochemical method (5). The concentrations of protoporphyrin and Fe used in the enzyme assay were both 25  $\mu\text{M}$ .

**Porphyrin measurement and characterization.** To determine the porphyrin content of cells cultured to confluence in roller bottles, a portion of the harvested cell suspension was extracted with ethyl acetate:glacial acetic acid (4:1), and the organic solution was partitioned with 1.5 N HCl (9). The medium in which the cells were grown was similarly processed. Porphyrins were extracted from fibroblasts grown in wells of FB-6-TC plates by rinsing the cell monolayer with phosphate-buffered saline and then adding two portions of

<sup>1</sup> Abbreviations used in this paper: ALA,  $\delta$ -aminolevulinic acid; FCS, fetal calf serum; Fe, ferrous sulfate; MEM, Eagle's minimum essential medium supplemented with 1% nonessential amino acids and 100  $\mu\text{g}/\text{ml}$  Kanamycin (routine culture medium minus fetal calf serum).

1 N perchloric acid–methanol (2.5 ml of a 1:1 vol/vol solution) in succession to the well.

The fluorescent spectrum of the porphyrin extract was examined with a Farrand spectrofluorometer (4818 photomultiplier tube; RCA Distributor & Special Products Div., Camden, N. J.) and compared with those of free porphyrin standards (Porphyrin Products, Logan, Utah) dissolved in the appropriate solvents. The concentration of porphyrin in the sample solution was determined from this comparison.

The porphyrins were also characterized by thin-layer chromatography, both as the free compounds and after conversion to the zinc chelates of their methyl ester derivatives (10, 11). The chromatograms were visualized with an ultraviolet light (Burton Div., Cavitron Corp., Van Nuys, Calif.), and the  $R_f$ s of the fluorescent spots in the sample were compared with those of porphyrin standards.

Intact cells were examined directly for fluorescence with a Zeiss binocular microscope fitted with a Zeiss fluorescence illuminator (HBO 200-W high-pressure mercury bulb), Zeiss BG 38/2.5 and BG 12/4 exciting filters, and a Zeiss 54 barrier filter (Carl Zeiss, Inc., New York).

The cells which had been incubated with ALA in FB-6-TC plates were also examined for the presence of zinc-protoporphyrin. After 24 h of incubation, they were rinsed free of medium, trypsinized, and centrifuged. They were resuspended in either absolute ethanol or in 0.1 g/100 ml human albumin in phosphate-buffered saline, sonicated, and the fluorescent spectrum of the solution examined with a Perkin-Elmer fluorescence spectrophotometer (R 446 photomultiplier tube) (Perkin-Elmer Corp., Instrument Div., Norwalk, Conn.). The excitation wavelength used for examining the ethanol solutions was 415 nm, and was 424 nm for the albumin-buffered saline solution (12). Comparisons were made with porphyrin standards (Porphyrin Products) in the appropriate solvents. The fluorescent spectra were characterized for cells incubated in MEM with ALA alone, and for those in which ZnSO<sub>4</sub> was also added.

**Isotope studies.** Glycine incorporation into heme was studied after fibroblasts had been grown to confluence in roller bottles. 50  $\mu\text{Ci}$  of [2-<sup>14</sup>C]glycine (sp act 49.7 mCi/mmol; New England Nuclear, Boston, Mass.) was added to the medium (MEM supplemented with 10% FCS). The concentration of glycine in the medium was 0.1 mM. After incubation for 48 h, the cells were rinsed thoroughly with phosphate-buffered saline, harvested with trypsin, centrifuged, resuspended in distilled water, and freeze-thawed. Human hemoglobin (0.25 ml of a 17 g/100 ml solution) was added to the cell suspension, and heme was crystallized after extraction with acetone:acetic acid (4:1) (13). The heme crystals were dissolved in pyridine, and heme recovery was determined by pyridine hemochromagen measurement (14). An aliquot of the pyridine solution was bleached in H<sub>2</sub>O<sub>2</sub>, and <sup>14</sup>C radioactivity in heme was measured by dissolving the solution in Aquasol (New England Nuclear) and counting in a liquid scintillation counter. Counting efficiency, determined after the addition of [<sup>14</sup>C]toluene internal standard (New England Nuclear), was 25–50%.

Iron uptake by cells grown to confluence in FB-6-TC plates was studied during their incubation in MEM supplemented with 25  $\mu\text{M}$  Fe, 1–2  $\mu\text{Ci}$  [<sup>59</sup>Fe]SO<sub>4</sub> (sp act 628 mCi/mmol; New England Nuclear), and 250  $\mu\text{M}$  ALA. The cells were harvested with trypsin after 4 and 24 h of incubation, and <sup>59</sup>Fe uptake was measured by counting the cells in a gamma scintillation counter. The incorporation of <sup>59</sup>Fe into heme was also determined by isolating heme after adding human hemoglobin to the cell suspension (5).

**Other members.** Protein concentrations were determined by the method of Lowry et al. (15), with crystalline bovine

serum albumin as a standard. The measurements were made on aliquots of the cell suspensions which had been harvested from roller bottles, and on digests of cells made in the wells of FB-6-TC plates with 0.5 N NaOH.

Data from normal cell lines were compared with those from protoporphyria cell lines by using Student's *t* test.

## RESULTS

Although heme synthetase activity was deficient in sonicates of skin fibroblasts cultured from patients with protoporphyria (Table I), protoporphyrin did not accumulate in the cells during their culture in Eagle's minimum essential medium supplemented with 10% FCS, 1% nonessential amino acids, and 100  $\mu\text{g/ml}$  Kanamycin (routine culture medium). This might occur if the heme requirements of the protoporphyria fibroblasts were met by such a low rate of synthesis of protoporphyrin precursors that the protoporphyrin formed could be completely converted to heme despite the deficient heme synthetase activity. Using [ $^{14}\text{C}$ ]glycine, we found that the rate of incorporation of glycine into heme by confluent protoporphyria cells in routine culture medium (Fig. 1) did occur at a low rate ( $69 \pm 10$  pmol glycine into heme per milligram protein in 48 h, mean  $\pm$  SEM) which was not significantly different than that in the normal lines ( $48 \pm 9$  pmol) ( $P > 0.1$ ). These findings are consistent with the observation that the activity of ALA synthetase, the rate-limiting enzyme which initiates the biosynthe-

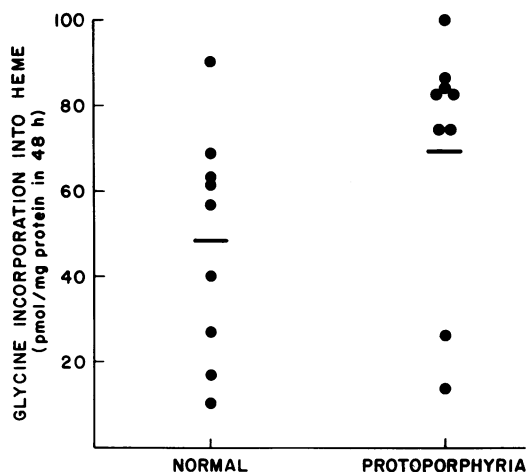


FIGURE 1 Glycine incorporation into heme for cultured skin fibroblasts from normal individuals and patients with protoporphyria. Confluent cells were incubated for 48 h in MEM (glycine concentration 0.1 mM) supplemented with 10% FCS and 50  $\mu\text{Ci}$  [ $^{14}\text{C}$ ]glycine. The horizontal lines are mean values of nine studies in six lines.

sis of heme (Fig. 2), was normal in lysates of protoporphyria cells (Table I).

We anticipated that excessive protoporphyrin might accumulate in protoporphyria fibroblasts when the culture medium was supplemented with ALA (Fig. 2), thereby bypassing the initial step in heme biosynthesis and increasing the rate of protoporphyrin formation. Protoporphyrin accumulation would occur under these experimental conditions if the activity of heme synthetase became rate-limiting for heme production. When ALA was added to the routine culture medium in a concentration of 500  $\mu\text{M}$ , both normal and protoporphyria fibroblasts developed red fluorescence that spared the nucleus and faded within 1 min, features that are characteristic of protoporphyrin in biological tissue. Additional evidence that protoporphyrin was the only porphyrin which accumulated during ALA supplementation was furnished by the observations that the fluorescent emission and excitation spectra of

TABLE I  
Properties of Skin Fibroblasts Cultured in Routine Medium\*

	Proto- porphyria	Normal	<i>P</i>
Yield ( $\times 10^6$ )	79 $\pm$ 16	57 $\pm$ 12	NS
Viability, %	80 $\pm$ 2	75 $\pm$ 2	NS
Protein content, mg/10 <sup>6</sup> cells	0.53 $\pm$ 0.07	0.53 $\pm$ 0.08	NS
Free porphyrin content, pmol/mg protein	ND	ND	—
ALA synthetase activity, † pmol ALA/mg protein per h	43.9 $\pm$ 7.2	35.3 $\pm$ 4.6	NS
Heme synthetase ac- tivity, † pmol protoheme/ mg protein per h	3.2 $\pm$ 0.5	25.5 $\pm$ 5.9	<0.01
Mean $\pm$ SEM (6 lines each)			

NS, not significant; ND, none detected.

\* Studies on fibroblasts grown to confluence in MEM supplemented with 10% FCS. Yield is from four 690-cm<sup>2</sup> roller bottles.

† Activities of ALA synthetase and heme synthetase were measured in lysates and sonicates, respectively, of the cultured fibroblasts.

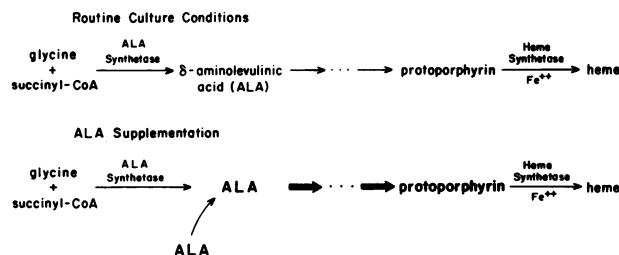


FIGURE 2 Pathway of heme biosynthesis for skin fibroblasts cultured in MEM supplemented with 10% FCS (routine culture medium), and during supplementation of the medium with ALA.

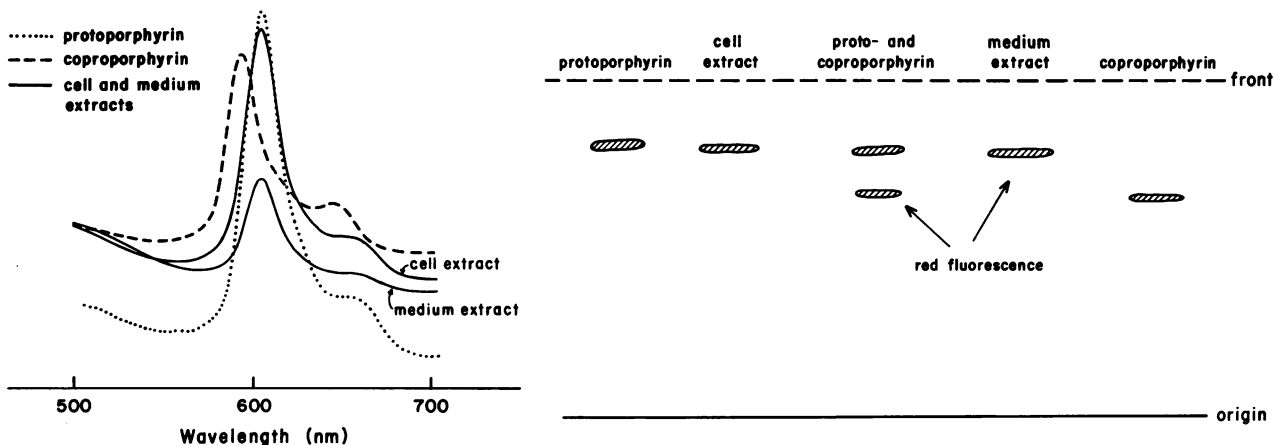


FIGURE 3 The porphyrin extracts from cultured skin fibroblasts and medium are compared with porphyrin standards. Confluent cells were incubated for 24 h in MEM supplemented with 10% FCS and 500  $\mu\text{M}$  ALA. *Left*: Fluorescent emission spectra of the porphyrin extracts in 1.5 N HCl (excitation wavelength 405 nm). *Right*: Thin-layer chromatography of the porphyrin extracts as the free porphyrins.

the cell extracts were identical with that of protoporphyrin (Fig. 3) and that the free porphyrin in the cell extract, as well as the zinc chelate of its methyl ester derivative, migrated at the same rate as the protoporphyrin standard in a thin-layer chromatogram (Fig. 3).

During the initial few hours after addition of ALA to the culture medium, protoporphyrin accumulated only in the cells. Subsequently, a significant amount was also measured in the medium (Fig. 3). When fetal calf serum was omitted, protoporphyrin was not detected in the medium at any time during incubation with ALA, indicating that protoporphyrin could move out of the cells and bind to compounds in fetal calf serum (presumably proteins). Protoporphyrin accumulation among different fibroblast lines was therefore compared by omitting fetal calf serum from the medium during ALA supplementation.

Initial studies were done with 25  $\mu\text{M}$  Fe in the medium. In the absence of added ALA, and at low ALA concentrations in the medium, protoporphyrin was not detected in either protoporphyria lines or normal lines (Fig. 4). When the concentration of ALA was increased to 50  $\mu\text{M}$ , protoporphyrin was detected in each of the protoporphyria lines, but in none of the normal lines (Fig. 4). With further increase in the concentration, protoporphyrin accumulated in all lines. However, the amount in the protoporphyria fibroblasts was two- to threefold greater at each concentration of ALA ( $P < 0.01$ ).

Since iron is the cosubstrate with protoporphyrin in the reaction catalyzed by heme synthetase, the effect of varying the Fe concentration in the medium was examined. As the concentration was lowered, protoporphyrin accumulation increased in both normal and

protoporphyria lines, most markedly in the normal lines (Fig. 5). Maximal accumulation occurred as the concentration fell below 1  $\mu\text{M}$ . At all concentrations of ALA, the amount of protoporphyrin in normal and protoporphyria lines was similar when Fe was not added to the medium (Fig. 6). This was significantly greater than that observed in the presence of 25  $\mu\text{M}$  Fe (Fig. 6). Normal and protoporphyria cells took up Fe from the medium at an equal rate (Table II), indicating there was no gross difference in Fe transport across the cell membrane.

Notably, protoporphyrin accumulated in the protoporphyria cells during supplementation with 25  $\mu\text{M}$  ALA when Fe was omitted from the medium ( $32 \pm 13$  pmol/mg protein), whereas none was detected in the presence of 25  $\mu\text{M}$  Fe (Fig. 6). In the absence of

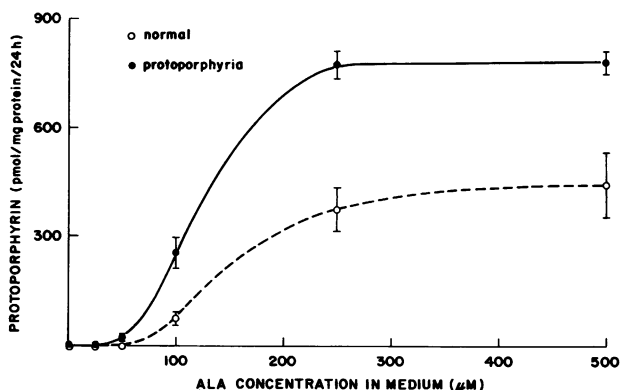


FIGURE 4 Protoporphyrin accumulation in cultured skin fibroblasts from patients with protoporphyria is compared with that in normal fibroblasts (mean  $\pm$  SEM of six lines each). Confluent cells were incubated for 24 h in MEM supplemented with 25  $\mu\text{M}$  Fe and ALA at the concentration shown.

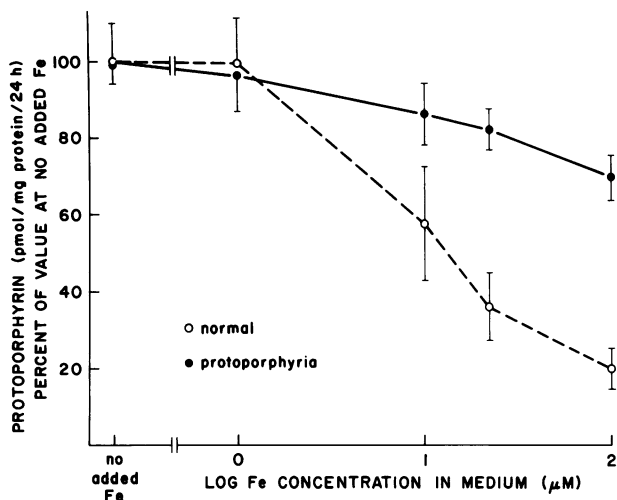


FIGURE 5. Effect of Fe on protoporphyrin accumulation in cultured skin fibroblasts from normal individuals and patients with protoporphyria (mean  $\pm$ SEM of six lines each). Confluent cells were incubated for 24 h in MEM supplemented with 250  $\mu$ M ALA and Fe at the concentration shown.

supplemental ALA, however, protoporphyrin accumulation was not observed even when Fe was omitted from the medium. This was probably due to the fact that protoporphyrin formation from glycine was so low that it could not be detected in the cell extract. Our studies with [ $^{14}$ C]glycine indicated that the fibroblasts formed only 0.6–6.0 pmol of protoporphyrin from glycine per milligram protein in a 24-h period

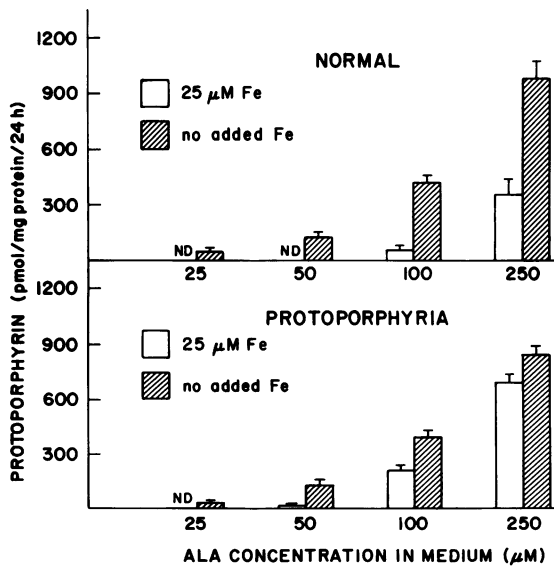


FIGURE 6. Protoporphyrin accumulation in confluent skin fibroblasts incubated for 24 h in MEM supplemented with ALA and 25  $\mu$ M Fe is compared with that when no Fe was added (mean  $\pm$ SEM of six lines each).

TABLE II  
Iron Uptake by Cultured Skin Fibroblasts\*

Incubation time	Iron uptake	
	Protoporphyria	Normal
<i>h</i>	<i>nmol/mg protein</i>	
4	172 $\pm$ 26	153 $\pm$ 5
24	402 $\pm$ 75	420 $\pm$ 58
Mean $\pm$ SEM (six lines each)		

\* Studies with confluent fibroblasts incubated in MEM supplemented with 25  $\mu$ M Fe, 1–2  $\mu$ Ci  $^{59}$ Fe, and 250  $\mu$ M ALA.

(1 pmol of protoporphyrin requires 8 pmol of glycine). The lower limit of sensitivity of our assay (10–20 pmol protoporphyrin/mg protein) exceeds this amount.

The effect of Fe appeared to be localized to the heme synthetase step, since protoporphyrin was the only porphyrin which accumulated in the cells at all ALA and Fe concentrations examined (Fig. 7). Zinc-protoporphyrin was not detected unless zinc had been added to the medium (Fig. 8). The difference in the amount of protoporphyrin which accumulated in the presence, versus absence, of added Fe presumably reflected the utilization of protoporphyrin in heme synthesis. This difference was remarkably similar to the activity of heme synthetase in sonicates of the fibroblasts (Tables I and III). However, there was poor agreement when

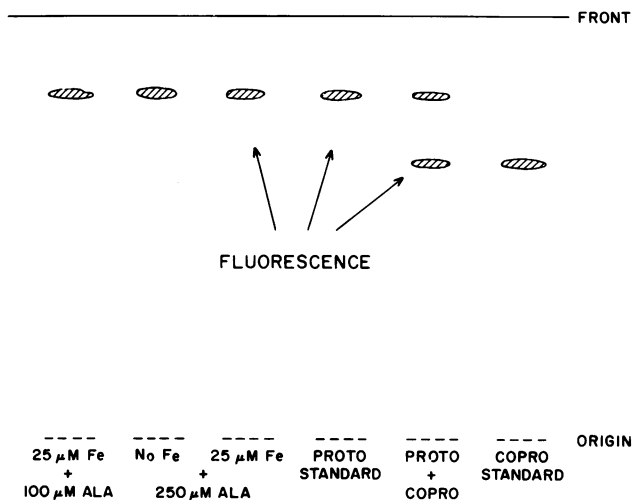


FIGURE 7. The porphyrin extracted from cultured normal and protoporphyria skin fibroblasts under different experimental conditions is compared with porphyrin standards. The porphyrin was converted to the zinc chelate after esterification and subjected to thin-layer chromatography (proto = protoporphyrin; copro = coproporphyrin).

heme synthesis was measured in the intact confluent fibroblasts with  $^{59}\text{Fe}$ . In normal fibroblasts, labeled heme recovery after 24 h accounted for only 16% of the protoporphyrin which was presumably used for this purpose. This may reflect simultaneous heme degradation, since the heme formed during ALA supplementation was excess heme. Heme degradation was not quantitated during ALA supplementation. However,  $^{14}\text{C}$  label was recovered in bilirubin crystallized from the medium of a few cell lines which had been incubated in routine culture medium supplemented with 250  $\mu\text{M}$  ALA and [ $^{14}\text{C}$ ]ALA, providing qualitative evidence that the fibroblasts degraded heme (at least excess heme) by some mechanism.

## DISCUSSION

Studies with cultured skin fibroblasts have helped to define the enzymatic abnormalities which underlie genetic disorders of porphyrin metabolism. Several groups have demonstrated that uroporphyrinogen I synthetase is deficient in cultured skin fibroblasts from patients with acute intermittent porphyria (16–18). We have shown that heme synthetase activity is deficient in sonicates of skin fibroblasts cultured from patients with protoporphyria (5, 6; Table I). In the present study, we have demonstrated that protoporphyria fibroblasts in culture accumulate excessive amounts of protoporphyrin under certain conditions of

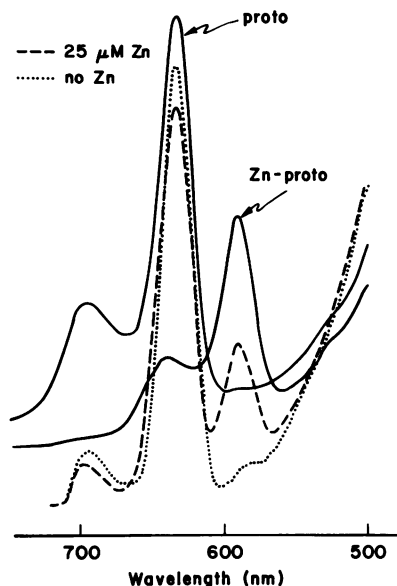


FIGURE 8 The fluorescence emission spectra of a normal fibroblast line which had been incubated for 24 h in MEM supplemented with 250  $\mu\text{M}$  ALA  $\pm$  25  $\mu\text{M}$   $\text{ZnSO}_4$  and then sonicated in ethanol, are compared with those of protoporphyrin and zinc-protoporphyrin (Zn-proto) standards in ethanol (excitation wavelength 415 nm).

TABLE III  
Effect of Iron on Protoporphyrin Accumulation  
in Cultured Skin Fibroblasts

ALA	Fe	$\Delta$ Protoporphyrin*	
		Protoporphyria	Normal
$\mu\text{M}$	$\mu\text{M}$	<i>pmol/mg protein per h</i>	
250	10	5.2 $\pm$ 1.8	16.0 $\pm$ 5.2
250	25	6.2 $\pm$ 0.5	27.2 $\pm$ 3.4
250	100	12.3 $\pm$ 2.2	34.0 $\pm$ 2.8
Mean $\pm$ SEM (six lines each)			

\* Difference in the amount of protoporphyrin which accumulated in confluent fibroblasts incubated with MEM and ALA in the absence of added Fe, vs. that at the Fe concentration indicated. This is converted to an hourly basis.

incubation, providing evidence that heme synthetase activity is also deficient in the intact cell. Sassa and co-workers have used similar experiments to show there is defective conversion of ALA to protoporphyrin in intact cultured skin fibroblasts of patients with acute intermittent porphyria, which reflects the enzymatic deficiency in that disease (18).

Nevertheless, the present study indicates that the enzyme deficiency in protoporphyria by itself may not cause excess protoporphyrin accumulation. Unless the culture medium had been supplemented with ALA, protoporphyrin was not detected in these cells. This appears to be due to the fact that fibroblasts have minimal requirements for heme synthesis when they are at confluence in routine culture medium (Fig. 1). ALA supplementation simulated increased activity of the heme pathway by bypassing the first and rate-limiting step. As the concentration of ALA in the medium was increased, the amount of protoporphyrin which accumulated in the protoporphyria cells similarly increased (Fig. 4) and protoporphyrin accumulated in normal cells. This also happens in vivo, since increased fecal protoporphyrin excretion has occurred in man and experimental animals after administration of ALA (19, 20).

These findings may explain certain features of protoporphyrin metabolism in protoporphyria. Red fluorescence is observed primarily when erythroid cells from patients with protoporphyria reach the late normoblast or early reticulocyte phase (21, 22). It is possible that at this stage of erythrocyte development the relative activities of ALA synthetase and heme synthetase predispose to protoporphyrin accumulation. In circulating cells, the excess protoporphyrin appears to leak rapidly into the plasma (23).

The rate of ALA production in heme-forming tissues should influence the degree of protoporphyrin accumu-

lation which occurs. This may in part explain why there is variable protoporphyrin accumulation and excretion in family members with protoporphyria, and in the same patient from time to time. Although the bone marrow probably accounts for the major fraction of protoporphyrin that is overproduced in these patients (23, 24), the liver may contribute a variable amount (25, 26). Several conditions which affect the rate of ALA formation in liver have been defined (27, 28). The demonstration that a high carbohydrate diet decreased fecal excretion of protoporphyrin in one patient with protoporphyria suggests that alteration in hepatic ALA synthetase activity does affect protoporphyrin production in some cases (29). ALA synthetase activity has been found by in vitro assay to be increased in bone marrow and liver of some, but not all, patients with protoporphyria (3, 30–32). The in vivo rate of ALA formation has not been quantitated in patients, however.

When iron was not added to the medium, protoporphyrin accumulation increased significantly in both protoporphyria and normal fibroblasts during ALA supplementation (Figs. 5 and 6). This was most pronounced in the normal cells, and protoporphyrin accumulation was similar to that in the protoporphyria lines. It presumably reflected the fact that protoporphyrin was not utilized for heme formation because this cosubstrate was lacking. There was no evidence that the presence of iron inhibited an earlier step of heme synthesis, since protoporphyrin was the only porphyrin which could be detected under all the experimental conditions examined (Fig. 7). In mitochondria-free extracts of pig liver, iron in ferrous state inhibits the formation of coproporphyrinogen from uroporphyrinogen (33).

The effect of iron on protoporphyrin accumulation in fibroblasts suggests that iron deficiency may increase the biochemical abnormality in patients with protoporphyria. There has been one report in which iron deficiency may have exacerbated symptoms in a 17-yr-old girl with protoporphyria (34). When oral iron was given along with cholestyramine, red cell and fecal protoporphyrin levels decreased, and photosensitivity abated.

The studies also suggest that increased red cell protoporphyrin levels in iron-deficient, but otherwise normal, individuals (35) reflect underutilization of protoporphyrin because of the lack of heme formation. However, since zinc-protoporphyrin is found in red cells of iron-deficient patients (12), this does not provide a complete explanation. Zinc-protoporphyrin was detected in fibroblasts grown with ALA in the absence of iron, provided that zinc was added to the medium (Fig. 8); this indicates that this aspect of porphyrin metabolism may also be examined in this system.

Although the present studies lend further support

to the premise that heme synthetase activity is deficient in protoporphyria, they do not clarify the nature of this deficiency. The in vitro activity of the enzyme in heme-forming tissues of protoporphyria patients has been 10–25% of normal (3–6), much lower than might be anticipated in an autosomal dominant disorder. Further study is required to characterize the nature of this deficient activity.

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