

## **An inherited enzymatic defect in porphyria cutanea tarda: decreased uroporphyrinogen decarboxylase activity.**

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### Research Article

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# An Inherited Enzymatic Defect in Porphyria Cutanea Tarda

## DECREASED UROPORPHYRINOGEN DECARBOXYLASE ACTIVITY

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**ABSTRACT** Uroporphyrinogen decarboxylase activity was measured in liver and erythrocytes of normal subjects and in patients with porphyria cutanea tarda and their relatives. In patients with porphyria cutanea tarda, hepatic uroporphyrinogen decarboxylase activity was significantly reduced (mean 0.43 U/mg protein; range 0.25–0.99) as compared to normal subjects (mean 1.61 U/mg protein; range 1.27–2.42). Erythrocyte uroporphyrinogen decarboxylase was also decreased in patients with porphyria cutanea tarda. The mean erythrocyte enzymatic activity in male patients was 0.23 U/mg Hb (range 0.16–0.30) and in female patients was 0.17 U/mg Hb (range 0.15–0.18) as compared with mean values in normal subjects of 0.38 U/mg Hb (range 0.33–0.45) in men and 0.26 U/mg Hb (range 0.18–0.36) in women.

With the erythrocyte assay, multiple examples of decreased uroporphyrinogen decarboxylase activity were detected in members of three families of patients with porphyria cutanea tarda. In two of these families subclinical porphyria was also recognized. The inheritance pattern was consistent with an autosomal dominant trait.

The difference in erythrocyte enzymatic activity between men and women was not explained but could have been due to estrogens. This possibility was supported by the observation that men under therapy with estrogens for carcinoma of the prostate had values in the normal female range.

It is proposed that porphyria cutanea tarda results from the combination of an inherited defect in uroporphyrinogen decarboxylase and an acquired factor, usually siderosis associated with alcoholic liver disease.

## INTRODUCTION

Specific enzymatic defects in the porphyrin biosynthetic pathway have been defined in several types of inherited porphyria (acute intermittent porphyria, congenital erythropoietic porphyria, and protoporphyria) (1–5). Assays for these enzymes have made family studies possible and the genetic characteristics of the diseases have been documented (6, 7).

In contrast, a specific enzymatic defect has not been defined in porphyria cutanea tarda (PCT).<sup>1</sup> This disease is usually considered to be an acquired disorder of porphyrin metabolism because of its onset late in life and common association with alcoholic liver disease (8, 9).

Biochemically PCT is characterized by hepatic synthesis and urinary excretion of excessive amounts of porphyrins, particularly uroporphyrin I and smaller amounts of 7-carboxyl porphyrin (10–12). The preponderance of uroporphyrin I and 7-carboxyl porphyrin in both the liver and urine suggests that the enzymatic decarboxylation of the reduced forms of these compounds (porphyrinogens) to coproporphyrinogen I is impaired. This decarboxylation is catalyzed by the enzyme uroporphyrinogen decarboxylase (URODECARB) (13) (Fig. 1).

The finding of hepatic siderosis in virtually all patients with PCT and the clinical benefits of phlebotomy therapy (14–16) have raised the possibility that iron might play an important part in the etiology of the disease. In support of this hypothesis it has been shown, in an in vitro model system, that iron

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<sup>1</sup>Abbreviations used in this paper: ALA,  $\delta$ -aminolevulinic acid; PCT, porphyria cutanea tarda; URODECARB, uroporphyrinogen decarboxylase.

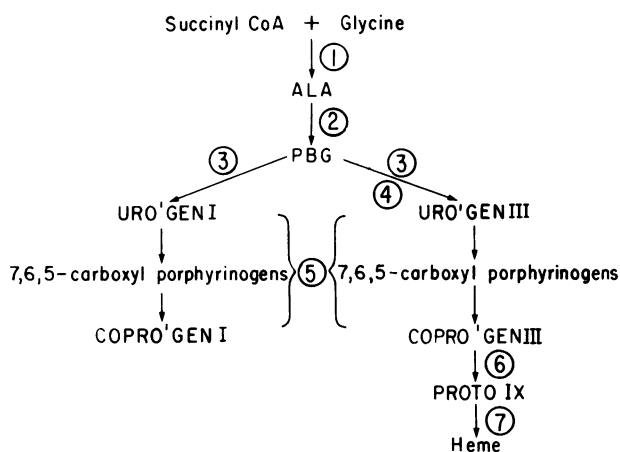


FIGURE 1 The enzymatic pathway for the biosynthesis of heme. (1) = ALA synthetase, (2) = ALA dehydratase, (3) = uroporphyrinogen I synthetase, (4) = uroporphyrinogen III cosynthetase, (5) = uroporphyrinogen decarboxylase, (6) = coproporphyrinogen oxidase, (7) = heme synthetase, ALA =  $\delta$ -aminolevulinic acid, PBG = porphobilinogen; URO'GEN = uroporphyrinogen, COPRO'GEN = coproporphyrinogen, PROTO = protoporphyrin.

alters hepatic porphyrin metabolism in a way that parallels the abnormalities observed in PCT (17).

However, the vast majority of patients with alcoholic liver disease and hepatic siderosis do not develop PCT. This clinical observation has led to speculation that patients with PCT have an occult genetic abnormality expressed only when the liver cell is injured in a specific way (18). The concept that there may be a genetically determined contribution to the pathogenesis of PCT has been strengthened by occasional reports describing a familial occurrence of the disease (19, 20). In addition, several reports have described abnormalities of porphyrin metabolism in apparently unaffected relatives of patients with PCT (21, 22).<sup>2</sup>

This study was designed to evaluate the hypothesis that PCT is associated with an inherited defect in the enzyme URODECARB. To evaluate the hypothesis, a method for assaying URODECARB activity was applied to both crude liver extracts and erythrocyte hemolysates of six patients with PCT. Three family studies were done in which erythrocyte URODECARB was assayed in clinically normal relatives of patients with PCT.

## METHODS

Normal human liver was obtained at laparotomy from nonalcoholic patients undergoing surgery for a variety of benign intra-abdominal disorders. None had received

prior transfusion therapy. Liver was obtained by needle biopsy from one nonporphyric patient with hemochromatosis. Porphyric human liver was obtained by open biopsy in two instances and by needle biopsy in the remaining cases. In all cases the informed consent of the patient was obtained. Fresh or frozen liver was first carefully rinsed in saline and then homogenized in 10 vol of 0.1 M Tris-HCl buffer, pH 6.8, in a 2-ml TenBroeck glass-glass homogenizer. The homogenate was centrifuged at 37,000 g for 30 min at 4°C yielding a mitochondria-free supernate. Aliquots of 0.2 ml were then utilized as the source of URODECARB in the assay system. Protein determinations were performed by the method of Lowry et al. (23). A portion of each liver biopsy sample was processed by routine histologic methods and stained for iron by the usual Perls' Prussian blue method. The stainable iron content of the parenchymal cells was graded according to the criteria of Scheuer et al. (24). These criteria assign a value of grade 0 to normal hepatic iron content and increasingly severe hepatic siderosis is graded 1 through 4.

Venous blood samples were processed by centrifuging the blood, removing the buffy coat, and washing the packed erythrocytes twice with chilled saline. The washed cells were then resuspended in an equal volume of 0.1 M Tris pH 6.8. Cell lysis was accomplished by sonication followed by freeze-thawing twice. The lysate was then centrifuged at 32,000 g for 30 min at 4°C and aliquots of 0.4 ml of the supernate were utilized as the source of URODECARB in the assay system. The hemoglobin content of the supernate was determined with a Coulter counter model S (Coulter Electronics Inc., Hialeah, Fla.).

URODECARB was assayed by a previously published method by utilizing enzymatically generated, tritiated uroporphyrinogen as the substrate and thin layer chromatographic identification and liquid scintillation quantitation of the reaction products (25).

Hepatic porphyrin content was quantitated by analysis of the pellet and the remaining supernate from the centrifuged homogenates. These were both extracted with 2.0-ml aliquots of 3.0 N HCl until no fluorescence was observed in the eluates. The HCl concentration was then adjusted to 1.5 N, and the porphyrin content was measured spectrophotometrically by scanning the spectrum from 370 to 440 nm with a Cary spectrophotometer (Cary Instruments, Fairfield, N. J.) and applying the correction factors of With (26). The porphyrins were then adsorbed on talc and esterified in  $\text{H}_2\text{SO}_4$ -methanol (27). Porphyrins were then identified chromatographically by the method of Doss (28).

Urine porphyrin concentrations were determined by a previously published method (29). Chromatographic identification of the urinary porphyrins was done as described above for hepatic porphyrins. Isomers of uroporphyrin were identified by decarboxylation to coproporphyrin (30) and lutidine  $\times$   $\text{H}_2\text{O}$  chromatography (31).

## RESULTS

1 U of URODECARB activity, in the assay system used, is defined as the amount needed to catalyze the formation of 1 nmol of 7, 6, 5, and 4-carboxyl porphyrinogens per hour at 37°C. The majority of the experiments were done by using enzymatically generated uroporphyrinogen I as the substrate in the URODECARB assay system. Reference in the text to URODECARB activity will indicate the I isomer

<sup>2</sup> Redeker, A. G. 1975. Personal communication.

TABLE I  
*Erythrocyte Uroporphyrinogen Decarboxylase Activity\**  
*in Normal Subjects*

|                         | Males     | Females   |
|-------------------------|-----------|-----------|
| Number of subjects      | 29        | 27        |
| Mean enzymatic activity | 0.38      | 0.26      |
| Standard deviation      | 0.05      | 0.05      |
| Determined range        | 0.33–0.45 | 0.18–0.36 |

\* Units/milligram Hb, uroporphyrinogen I substrate.

was used as the substrate. Data referring to experiments in which enzymatically generated uroporphyrinogen III was used as the substrate will be specifically identified.

*Determination of normal values.* Hepatic URODECARB activity was measured in seven normal subjects, five males and two females. The hepatic histology was normal in all cases and there was no evidence of parenchymal siderosis (grade 0). The mean enzymatic activity was 1.61 U/mg protein (range 1.27–2.42).

Erythrocyte URODECARB activity was measured in 56 normal subjects, 29 males and 27 females. In all the subjects, the hemoglobin concentration was normal, as was the volume of packed erythrocytes, and the reticulocyte count. The enzymatic activity in erythrocytes appeared to differ according to sex. The mean enzymatic activity in male subjects was 0.38 U/mg Hb (range 0.33–0.45) and in the female subjects was 0.26 U/mg Hb (range 0.18–0.36) (Table I). Enzymatic activity did not increase by freeze thawing the erythrocytes more than twice after sonication.

In 12 normal subjects, erythrocyte URODECARB activity was determined with uroporphyrinogen III as the substrate in the assay system. Results did not differ appreciably from those obtained from simultaneously performed assays with uroporphyrinogen I as the substrate (Table II).

*Studies in family 1.* The index patient T. E., a 55 yr old white male, was admitted to the Salt Lake V. A. Hospital in July 1973. He was a heavy drinker and had noted a bullous photosensitive dermatitis on his hands and face for approximately 1 yr. The clinical diagnosis of porphyria cutanea tarda was confirmed by the finding of a urinary uroporphyrin output of 15,600  $\mu\text{g/day}$  (normal = < 50  $\mu\text{g/day}$ ). Isomer analysis of the urinary uroporphyrin by chemical decarboxylation and lutidine  $\times$  H<sub>2</sub>O chromatography revealed that virtually all was isomer I. At biopsy, liver tissue was obtained which fluoresced brilliantly under ultraviolet light. Histologic examination re-

vealed moderate fatty metamorphosis and extensive periportal fibrosis. Grade I parenchymal siderosis was present. Hepatic URODECARB activity was 0.38 U/mg protein, substantially below the lowest value in the normal series.

The patient was instructed to abstain from alcohol and was treated with six phlebotomies of 500 ml each. The urinary excretion of uroporphyrin decreased towards normal and the skin lesions cleared entirely (Fig. 2). Some 30 wk after the initial studies were done, a liver biopsy was repeated. The specimen again fluoresced brilliantly under ultraviolet light and contained 68  $\mu\text{g}$  of porphyrins per gram of liver (wet weight). Normal liver contains only trace amounts of porphyrins (0.5  $\mu\text{g}$  per gram). At this time there was no evidence of parenchymal siderosis (Grade 0). Chromatographic identification of the hepatic porphyrins as their methyl esters revealed the predominant porphyrin present to be uroporphyrin and the only other component detected represented a small amount of 7-carboxyl porphyrin. The hepatic URODECARB activity was 0.41 U/mg protein, a value not appreciably different from the pretreatment value of 0.38 U/mg protein. Erythrocyte URODECARB activity was determined on four separate blood samples drawn between February and September, 1974. The mean erythrocyte enzyme activity was 0.21 U/mg Hb (range 0.20–0.24), about half the normal mean and substantially below the

TABLE II  
*Erythrocyte Uroporphyrinogen Decarboxylase Activity\**  
*Determined in Normal Subjects with Both Isomers*  
*I and III of Uroporphyrinogen As Substrate*

| Subject | Sex | Enzymatic activity<br>isomer I substrate | Enzymatic activity<br>isomer III substrate |
|---------|-----|------------------------------------------|--------------------------------------------|
| 1       | M   | 0.37                                     | 0.39                                       |
| 2       | M   | 0.33                                     | 0.32                                       |
| 3       | M   | 0.40                                     | 0.38                                       |
| 4       | M   | 0.40                                     | 0.45                                       |
| 5       | M   | 0.41                                     | 0.39                                       |
| 6       | M   | 0.38                                     | 0.37                                       |
| 7       | M   | 0.38                                     | 0.38                                       |
| 8       | M   | 0.35                                     | 0.39                                       |
| 9       | M   | 0.35                                     | 0.41                                       |
| 10      | M   | 0.39                                     | 0.39                                       |
| 11      | F   | 0.36                                     | 0.27                                       |
| 12      | F   | 0.26                                     | 0.21                                       |

\* Units/milligram Hb.

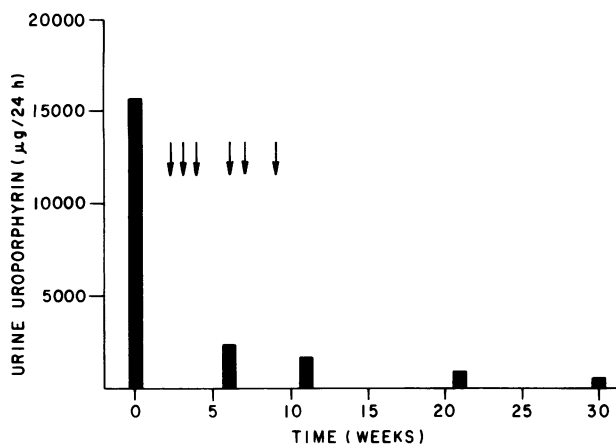


FIGURE 2 The clinical course of patient T. E. Each arrow represents phlebotomy of 500 ml of whole blood. At 30 wk, the urine uroporphyrin excretion was near normal and all clinical symptoms of PCT had cleared, but a liver specimen fluoresced brilliantly. Hepatic uroporphyrinogen decarboxylase values were low both before (0.38 U/mg protein) and after (0.41 U/mg protein) therapy.

lower limits of normal. Erythrocyte URODECARB activity was assayed on a single blood sample by using uroporphyrinogen III as the substrate. The enzymatic activity with the III isomer substrate was 0.18 U/mg Hb.

T. E.'s parents were deceased but he had two living siblings, a sister, M. C., and a brother, E. E. M. C., a 64-yr-old woman, was in good health but had noticed increasing amounts of facial hair in recent years. She consumed two martinis daily. Urinary excretion of uroporphyrin ranged from 950 to 1,410 µg/day, which was predominantly uroporphyrin I. Hepatic tissue obtained by needle biopsy fluoresced brilliantly under ultraviolet light and contained 12 µg porphyrin per gm (wet weight) (68% uroporphyrin, 32% 7-carboxyl porphyrin). Moderate fatty metamorphosis, minimal periportal fibrosis, and grade 2 parenchymal siderosis were observed. The hepatic URODECARB and erythrocyte URODECARB activity were both distinctly subnormal (Table III). With uroporphyrinogen III as the substrate the erythrocyte URODECARB activity was 0.13 U/mg Hb. She was treated with phlebotomy therapy by her private physician in another state and no further follow-up was obtained.

E. E., a 61-yr-old man was in good health and had no complaints related to the skin. Urinary excretion of uroporphyrin, measured on two occasions, was minimally increased (87 and 90 µg/24 h). He was a regular blood donor and had donated approximately 15 U of whole blood in the 5 yr before evaluation. No hepatic tissue was obtained for study, but erythrocyte URODECARB was determined on three sepa-

TABLE III  
*Findings in the Families of Patients with Porphyria Cutanea Tarda*

|           |   | Sex | Clinical manifestations of PCT | Alcohol use | Urine                     | Hepatic                      | Erythrocyte                                          |
|-----------|---|-----|--------------------------------|-------------|---------------------------|------------------------------|------------------------------------------------------|
|           |   |     |                                |             | uroporphyrin normal = <50 | URODECARB normal = 1.27–2.42 | URODECARB normal = 0.33–0.45 males 0.18–0.36 females |
| Family 1  |   |     |                                |             | <i>μg/24 h</i>            | <i>U/mg protein</i>          | <i>U/mg Hb</i>                                       |
| T.E.      | M | +   | +                              | 15,600      | 0.38*                     | 0.21*                        |                                                      |
| M.C.      | F | +   | +                              | 950         | 0.99*                     | 0.15*                        |                                                      |
| E.E.      | M | 0   | +                              | 87          | —                         | 0.25*                        |                                                      |
| Family 2  |   |     |                                |             |                           |                              |                                                      |
| Ia        | M | 0   | 0                              | Normal      | —                         | 0.27*                        |                                                      |
| Id        | F | 0   | 0                              | Normal      | —                         | 0.27                         |                                                      |
| IIa       | F | +   | +                              | 2,000       | 0.35*                     | 0.15*                        |                                                      |
| IIb       | M | 0   | 0                              | Normal      | —                         | 0.28*                        |                                                      |
| IIc       | F | 0   | 0                              | Normal      | —                         | 0.23                         |                                                      |
| IIIa      | M | 0   | +                              | 350         | —                         | 0.17*                        |                                                      |
| IVa       | M | 0   | 0                              | Normal      | —                         | 0.20*                        |                                                      |
| IVb       | M | 0   | 0                              | Normal      | —                         | 0.17*                        |                                                      |
| IVc       | F | 0   | 0                              | Normal      | —                         | 0.22                         |                                                      |
| Family 3  |   |     |                                |             |                           |                              |                                                      |
| G.H.      | M | +   | +                              | 2,660       | 0.66*                     | 0.16*                        |                                                      |
| Sibling 1 | F | 0   | 0                              | Normal      | —                         | 0.24                         |                                                      |
| Sibling 2 | F | 0   | 0                              | Normal      | —                         | 0.21                         |                                                      |
| Sibling 3 | F | 0   | 0                              | Normal      | —                         | 0.14*                        |                                                      |

\* Subnormal value.

rate samples drawn over a 5-mo period. The enzymatic activities were 0.21, 0.25, and 0.29 U/mg Hb, all subnormal values (Table III).

Thus in this family, of the three siblings studied, two had clinically detectable PCT with decreased hepatic and erythrocyte URODECARB activity (Table III). The third sibling, a regular blood donor, did not have clinical symptoms of PCT but erythrocyte URODECARB activity was reduced (Table III) and there was a minimal increase in urinary excretion of uroporphyrin. No parents or offspring of these three individuals were available for study.

**Studies in family 2.** The index patient R. D. (IIa in Fig. 3, and Table III), a 57-yr-old white woman, was referred to the University of Utah Hematology Clinic by her dermatologist in May, 1974. She related a 2-yr history of a bullous, photosensitive dermatitis, skin fragility, and increasing amounts of facial hair. She admitted to drinking six cocktails daily. The clinical diagnosis of PCT was established by the finding of an increased value for 24 h urinary excretion of uroporphyrin (Table III). Hepatic tissue was obtained by needle biopsy. The specimen fluoresced brilliantly under ultraviolet light and contained 65  $\mu$ g of porphyrins per gram (wet weight) (74% uroporphyrin, 26% 7-carboxyl porphyrin). Moderate fatty metamorphosis and grade 3 parenchymal siderosis were observed. Hepatic and erythrocyte URODECARB activities were subnormal (Table III). With uroporphyrinogen III as the substrate the erythrocyte URODECARB activity was 0.14 U/mg Hb. A remission was obtained after phlebotomy therapy with clearing of the skin lesions and return of the urinary porphyrin excretion towards normal. A follow-up liver biopsy was not carried out but subsequent determinations of erythrocyte URODECARB activity showed no change in the enzymatic activity.

The patient's parents were deceased but a paternal uncle (Ia, Fig. 3 and Table III), a maternal aunt (Id), two of her four siblings (IIb and IIc), her only child, a son (IIIa), and three grandchildren (IVa, IVb and IVc) were studied. None had clinical manifestations of porphyria, and only the son, a heavy drinker, had an increased urine uroporphyrin level. Values for erythrocyte URODECARB were decreased in six of the nine family members, including representatives in each of four generations.

**Studies in family 3.** The index patient, G. H., a 25-yr-old white man, was a heavy drinker. He was initially evaluated in November, 1974, at the Salt Lake V. A. Hospital because of a bullous, photosensitive dermatitis. The clinical diagnosis of PCT was confirmed by finding an increased value for 24 h urinary uroporphyrin excretion (Table III). A liver biopsy yielded tissue that fluoresced brilliantly under ultraviolet light. The total porphyrin content

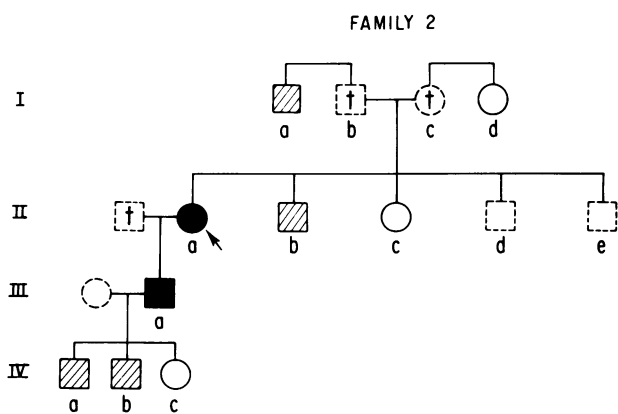


FIGURE 3 Pedigree of family 2

- ↑ = proband
- † = deceased
- = clinical PCT with increased urine uroporphyrin content
- ▨ = clinically nonporphyric. Erythrocyte URODECARB subnormal
- = clinically nonporphyric. Erythrocyte URODECARB normal
- = not studied

was 300  $\mu$ g/g (wet weight). Minimal focal fatty metamorphosis and grade 2 parenchymal siderosis were found.

The predominant hepatic porphyrin was found to be uroporphyrin; a small amount of 7-carboxyl porphyrin was also noted. Hepatic and erythrocyte URODECARB activities were distinctly subnormal (Table III). With uroporphyrinogen III as the substrate the erythrocyte URODECARB activity was also subnormal, 0.18 U/mg Hb.

The patient's parents were both deceased but three siblings, all females (ages 25–32 yr) were studied. All admitted to occasional social use of alcohol. None used oral contraceptives. None had skin disease, and all had normal urinary porphyrin determinations. Two of the sisters had normal erythrocyte uroporphyrinogen decarboxylase activity but the third had a subnormal value. The findings in this family are summarized in Table III.

**Additional studies.** In addition to the four porphyric livers studied above, hepatic URODECARB activity was determined on needle biopsy specimens from two other unrelated patients with porphyria cutanea tarda. Grade I parenchymal siderosis was present in one specimen and grade 2 in the other. Subnormal URODECARB activity was found in both. The results of the six porphyric hepatic URODECARB assays are compared with the results in seven normal individuals in Fig. 4. The mean enzymatic activity in the porphyric livers was 0.43 U/mg protein as compared to a mean of 1.61 U/mg protein in the normal specimens. Furthermore, even the

highest value from PCT liver was appreciably less than the lowest value from normal livers.

Hepatic URODECARB was found to be normal (1.70 U/mg protein) in a needle biopsy specimen obtained from a single patient with hemochromatosis. Grade 4 parenchymal siderosis was present. Urine porphyrin excretion in this patient was normal.

Erythrocyte URODECARB activity was found to be normal (0.33–0.37) in four alcoholic patients with histologically documented Laënnec's cirrhosis. Hepatic iron content ranged from grade 0 to grade 1. None were anemic and in all the reticulocyte count was normal. None of these patients excreted abnormal amounts of uroporphyrin in the urine.

Erythrocyte URODECARB activity was also measured in four men with metastatic carcinoma of the prostate. In all four men the reticulocyte count was normal. All four men were being treated with diethylstilbesterol in a daily oral dose of 5 mg. None had increased urinary uroporphyrin excretion. The eryth-

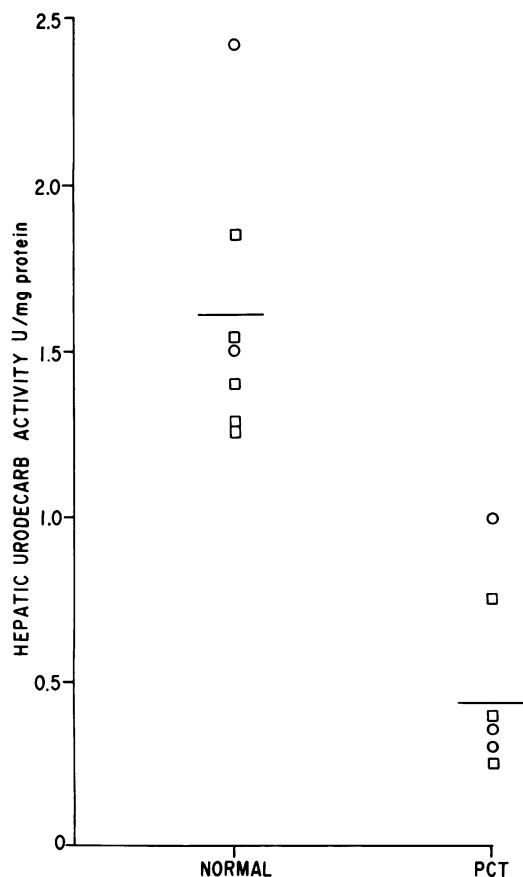


FIGURE 4 URODECARB activity in crude liver extracts. The mean activity in normal subjects = 1.61 U/mg protein. In patients with PCT the mean activity = 0.43 U/mg protein  
□ = males  
○ = females

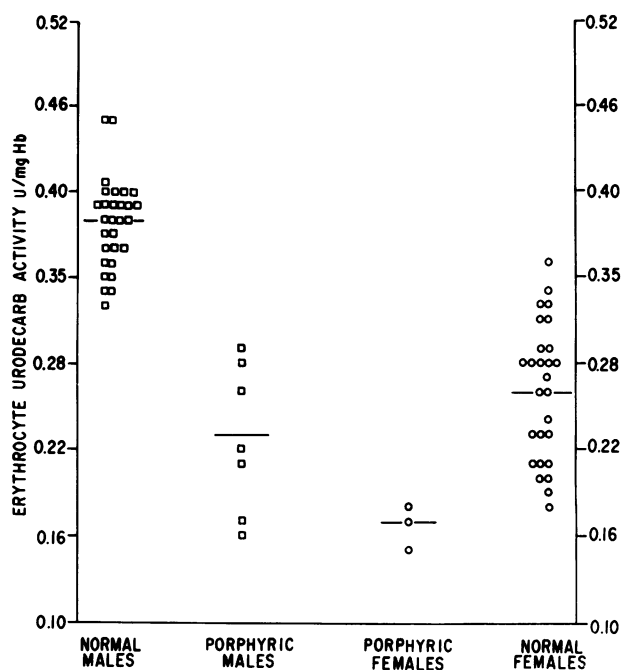


FIGURE 5 Erythrocyte URODECARB activity in normal subjects and patients with PCT. The mean enzymatic activity in normal males was 0.38 U/mg Hb whereas in porphyric males it was 0.23 U/mg Hb. The mean enzymatic activity in normal females was 0.26 U/mg Hb whereas in porphyric females it was 0.17 U/mg Hb.

rocyte URODECARB activity in these individuals ranged from 0.23 to 0.31 U/mg protein. These are subnormal values for males but fall in the range observed in normal female subjects.

Erythrocyte URODECARB was determined in an additional five unrelated patients with porphyria cutanea tarda, four males and one female. All had subnormal enzyme activity. The results of erythrocyte URODECARB assays in all porphyric patients studied are compared with control values in Fig. 5. The mean erythrocyte enzyme activity in porphyric males was 0.23 U/mg Hb as compared to a mean of 0.38 U/mg Hb in normal males. The mean erythrocyte enzymatic activity in porphyric females was 0.17 U/mg Hb as compared to a mean of 0.26 U/mg Hb in normal females.

## DISCUSSION

These studies demonstrate diminished activity of hepatic URODECARB in patients with PCT. Hepatic URODECARB activity in such patients was approximately 25% of the normally observed enzymatic activity. This partial block in the heme biosynthetic pathway is consistent with the observed accumulation of uroporphyrin and 7-carboxyl porphyrin in

porphyric liver, a pattern previously reported by others (12).

URODECARB activity was decreased not only in hepatic tissue but also in the erythrocytes of patients with PCT. Decreased URODECARB activity in two heme-forming tissues suggests that an inherited, generalized metabolic defect is present. The erythrocyte assay provided a convenient tool for studying URODECARB activity in family members of patients with PCT. By means of this assay, in the first family studied (family 1), a clinically apparent but unrecognized case of PCT was discovered in the sister of the propositus. Similarly in family 2, the son of the propositus was found to have subclinical PCT as manifested by increased urinary excretion of uroporphyrin. Thus, this finding may serve to alert carriers to their increased risk of acquiring porphyria on exposure to alcohol and other hepatic toxins.

The deficiency in erythrocyte URODECARB appeared to be inherited as an autosomal dominant trait. In family 2, decreased erythrocyte URODECARB activity was discovered in four generations (Fig. 3, Table III). Clinically apparent PCT, or a detectable increase in the urinary excretion of uroporphyrin, was found only in individuals who consumed alcoholic beverages in large amounts.

The reduction of URODECARB activity to about 60% of normal in erythrocytes (Fig. 5) is not inconsistent with a heterozygous state. However, the reason for the apparently greater reduction in hepatic URODECARB activity is not clear from these studies. Perhaps acquired or environmental factors affecting only the liver are additive to the genetic defect. Iron overload alone probably does not account for this finding, since removal of iron by phlebotomy did not change hepatic URODECARB activity in the propositus in family 1. The assay, however, is performed with highly diluted homogenates, a procedure which may eliminate the iron effect.

A single enzyme is probably responsible for the decarboxylation of the four acetic acid side chains of uroporphyrinogen, but the first decarboxylation proceeds more readily than the next three (13). We have reported that iron, an inhibitor of URODECARB, exerts its major inhibitory effect upon the removal of the second carboxyl group, leading to the accumulation of uroporphyrin and 7-carboxyl porphyrin in an *in vitro* hepatic model of PCT (25). Histologic evidence of excessive hepatocellular iron was found in specimens from all of the patients studied here, a finding that is virtually uniform in PCT (16). However, the absence of PCT in most patients with either primary or secondary hemochromatosis makes it unlikely that iron is the sole etiologic factor in the pathogenesis of PCT. The finding of normal

hepatic URODECARB activity in the single patient with hemochromatosis studied lends support to this hypothesis. An underlying biochemical defect in patients with PCT, that results in unusual sensitivity to the effects of iron (and perhaps other toxic agents), seems a more plausible possibility. Such a biochemical defect was found in the liver and erythrocytes of all PCT patients studied. Thus, the concordance of an inherited defect (decreased URODECARB activity) and an acquired factor (hepatic siderosis) seems to be required for the clinical expression of PCT. Of interest in this regard was the finding that in the brother of the propositus in family 1, a very minimal increase in urinary uroporphyrin excretion was detected, even though his alcohol consumption was large, and URODECARB activity was decreased in his erythrocytes. This individual was a regular blood donor and thus may have had "prophylactic" phlebotomy therapy.

In normal subjects the erythrocyte URODECARB activity differed according to sex, with females demonstrating lower activities than males. In addition, four males with prostatic cancer, who were ingesting estrogens were found to have erythrocyte URODECARB activities in the normal female range. Thus, estrogens appear to lower URODECARB activity. This finding is of interest as PCT has been reported in individuals receiving estrogen therapy (32-34). Also relevant is the observation that chronic male alcoholics, who represent the largest group of patients with PCT, frequently have testicular atrophy (35) and increased estrogen levels. However the relatively rare occurrence of PCT in individuals receiving estrogens and in chronic alcoholics suggests that estrogens alone do not reduce URODECARB activity enough to cause a clinically recognizable alteration in porphyrin metabolism. As in the case with iron, it is likely that estrogen-induced PCT occurs only in individuals with an underlying biochemical defect.

The hepatic URODECARB assays reported herein were all performed by using uroporphyrinogen I as the substrate in the assay system. URODECARB in erythrocyte hemolysates from nonporphyric individuals was active in decarboxylating uroporphyrinogen I and III to an equivalent degree (Table II). Hemolysates from the patients with PCT demonstrated decreased URODECARB activity with either substrate and, as with the controls, there was no difference in the activity of the erythrocyte enzyme in decarboxylating uroporphyrinogen I or III. The equivalent decarboxylation of either substrate is in agreement with the findings of Romeo and Levin for mouse spleen URODECARB (36) and our own finding for porcine hepatic URODECARB (25). Although older data suggest that the rate of decarboxylation is higher with the type III isomer (37-39),

the current studies support the validity of assaying human hepatic URODECARB with uroporphyrinogen I as the substrate.

If URODECARB deficiency is the underlying defect in heme biosynthesis in PCT, then the question arises as to why no heme deficiency is observed. Ordinarily an enzymatic defect would be expected to result in a deficiency of the end product of the metabolic pathway involved. However, this generalization does not hold true for most porphyrias. Hepatic heme synthesis in PCT has not been extensively studied. However, the lack of anemia in most patients with PCT speaks against a decrease in the synthesis of heme by the erythrocyte.

Classically, the  $\delta$ -aminolevulinic acid (ALA) synthetase reaction is considered to be the rate controlling step in the porphyrin biosynthetic pathway (13). Increased ALA synthesis could compensate for a partial block later in the pathway, and such a compensation occurs in acute intermittent porphyria and certain other porphyrias (40). However, the hepatic synthesis of ALA is probably not increased in PCT. Studies with techniques which discriminate ALA from other aminoketones have revealed normal ALA synthetase activity in liver biopsy specimens from patients with PCT (1, 40, 41). An increase in the synthesis of uroporphyrinogen despite normal ALA synthetase activity suggests a regulatory mechanism later in the heme biosynthetic pathway. We previously reported that, under conditions which inhibit both URODECARB and uroporphyrinogen III cosynthetase (heating or the addition of ferrous iron) uroporphyrin synthesis from porphobilinogen in crude liver extracts was greatly increased (17). These studies suggest a regulatory effect on uroporphyrinogen I synthetase activity by URODECARB and(or) uroporphyrinogen III cosynthetase. Such a regulatory effect might explain the increased activity of hepatic uroporphyrinogen I synthetase that has been reported by others in a small number of patients with PCT (1, 2). The mechanism of this regulatory effect is unknown at present.

The predominance of the I series isomer in the hepatic and urinary porphyrins in PCT suggests that uroporphyrinogen III cosynthetase might also be impaired. This enzyme, like URODECARB, is inhibited by ferrous iron (17). Furthermore, uroporphyrin III and uroporphyrinogen III are potent inhibitors of the cosynthetase (42) and the accumulation of these compounds resulting from impaired activity of URODECARB could lead to the synthesis and accumulation of uroporphyrin I, by its secondary effects on cosynthetase. The detection of decreased cosynthetase activity may be difficult in vitro because of the dilution of the proposed inhibitors (uroporphyrin and iron) inherent in the assay system. De-

creased activity of uroporphyrinogen III cosynthetase is not incompatible with an overall increase in heme synthesis as has been discussed by Levin (43).

The studies reported herein suggest that PCT occurs in patients with a genetically determined abnormality leading to reduced URODECARB activity. The decreased enzymatic activity appears to be inherited as an autosomal dominant trait. Family studies suggest that the disease remains latent until excessive hepatic iron stores accumulate.

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