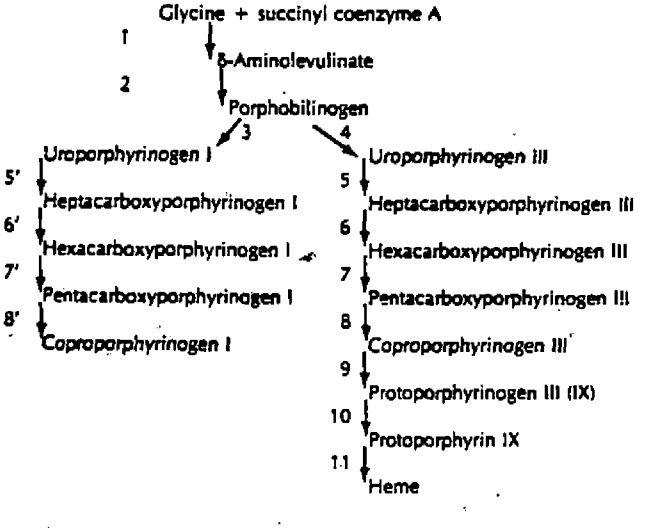
Laboratory Report

Porphyrinogens, Porphyrins, and the Porphyrias

The porphyrias are consequences of impairment either in the formation of the porphyrinogens from porphobilinogen or in the transformation of the porphyrinogens to heme.[1] Such an impairment causes a pathologic accumulation of porphobilinogen or porphyrins (or both) in tissues and body fluids.

The manifestations of the porphyrias include photosensitivity of the skin, persistent abdominal aching or pain, and neuropathy with widely variable expression that may affect the central, autonomic, and peripheral systems and include mental disturbance, motor dysfunction, and sensory loss. The cutaneous photosensitivity may range from very mild to very severe with mutilating lesions. The neuropathy of porphyria is especially significant in that it can progress to life-threatening respiratory paralysis (Table 1).

The formation of the porphyrinogens and heme from glycine and succinyl coenzyme A occurs in human cells of nearly all types and includes the following 15 major enzyme-catalyzed steps:



Only the porphyrinogens of the series III are precursors of heme, and the isomeric porphyrinogens of the series I and the porphyrins of both series I and III are by-products with no known physiologic functions; the porphyrins are formed from the porphyrinogens by nonenzymic oxidation.

As shown in Table 1, seven forms of porphyria are known to occur, and with one exception, each form is inheritable and is caused by or potentiated by an abnormally low activity of one of the several enzymes involved in the formation of the porphyrinogens and heme.

Two forms of porphyria are expressed primarily in the erythropoietic system. Congenital erythropoietic porphyria is a rare disorder that is readily recognizable in neonatal life by the voiding of dark or wine-red urine; uroporphyrin I is primarily responsible for the color of the urine. Congenital erythropoietic porphyria is characterized also by moderate to extreme photosensitivity, hypertrichosis, hemolysis, splenomegaly, and increased uroporphyrin and coproporphyrin in the erythrocytes and blood plasma. Examination of the bone marrow in violet light (400 to 410 nm) shows red fluorescence of excessive porphyrins in the cytoplasm of red cell precursors. In addition, in most known cases uroporphyrin is deposited in the teeth and can be demonstrated by red fluorescence when exposed to violet light. Protoporphyria is relatively mild and is expressed in both the erythropoietic system and the liver. It is characterized by photosensitivity resulting in acute solar urticaria and chronic solar eczema; the cutaneous lesions usually heal in hours or days without scarring. The chemical features include increased erythrocyte free protoporphyrin and increased fecal excretion of protoporphyrin. Urinary porphyrins and porphyrin precursors in most cases of protoporphyria have not been increased.

A third and very rare form of erythropoietic porphyria has clinical characteristics like those of congenital erythropoietic porphyria and is expressed chemically by increased protoporphyrin in erythrocytes, increased urinary excretion of uroporphyrin III, heptacarboxyl porphyrin III, and an unidentified porphyrin, and increased fecal excretion of coproporphyrin, tricarboxyl porphyrin, and protoporphyrin.[2]

A fourth form of erythropoietic porphyria has been observed but has not been characterized fully in chemical terms. In one reported case[3] and in one case of a neonate evaluated in the Mayo Medical Laboratories, extreme photosensitivity and porphyrinuria were observed, and erythrocytes

Table 1.—Acknowledged Forms of Porphyria

Condition	Mode of inheritance	Enzyme deficient*	Clinical expression
Erythropoietic porphyrias			
Congenital erythropoietic porphyria	Autosomal recessive	Uroporphyrinogen III cosynthase (step 4)	Photosensitivity, hemolytic anemia, hypertrichosis
Protoporphyria	Autosomal recessive	Ferrochelatase (step 11)	Photosensitivity
Hepatic porphyrias			
Acute intermittent porphyria	Autosomal dominant	Uroporphyrinogen I synthase (step 3)	Neuropathy, abdominal pain, psychosis
Hereditary coproporphyria	Autosomal dominant	Coproporphyrinogen oxidase (step 9)	Same as acute intermittent porphyria
Variegate porphyria	Autosomal dominant	Protoporphyrinogen oxidase or ferrochelatase (step 10 or 11)	Same as acute intermittent porphyria + photosensitivity
Porphyria cutanea tarda	?	Uroporphyrinogen decarboxylase (steps 5-8)	Photosensitivity
Intoxication porphyria	Acquired	Variable	Abdominal pain, neuropathy

*Numbered steps refer to the steps in the described reaction sequence for the formation of porphyrinogens.

Table 2.—Some Substances Reported to Induce Attacks of Acute Intermittent Porphyria[4]

Barbiturates
Sulfonamides
Phenytoin (Dilantin)
Meprobamates (Miltown and Soma)
Griseofulvin
Methsuximide
Dichloralphenazone
Pyrazolones (antipyrenes)
Methprylone
Imipramine
Eucalyptol
Ergot substances
Chlordiazepoxide (Librium)
Estrogens
Contraceptive steroids

contained increased coproporphyrin and normal concentrations of protoporphyrin.

Acute intermittent porphyria, coproporphyria, variegate porphyria, and porphyria cutanea tarda have been classified as "hepatic porphyrias" because it is assumed that errors in porphyrinogen synthesis are predominantly expressed in hepatocytes. Acute intermittent porphyria, coproporphyria, and variegate porphyria are characterized by acute attacks of abdominal pain and by hypertension, neuropathy, and mental disturbance that may last from several days to several months. Chronic photosensitivity and sensitivity of the skin to mechanical trauma are usual characteristics of variegate porphyria but not of acute intermittent porphyria; in coproporphyria, sensitivity of the skin to light and to mechanical trauma may be pronounced, mild, or nonexistent. In readily measurable chemical terms, acute intermittent porphyria is characterized by an increased urinary excretion of porphobilinogen during acute attacks and by low activity of uroporphyrinogen I synthase in erythrocytes. Coproporphyria may be expressed in part by increased coproporphyrin in erythrocytes and by increased urinary excretion of coproporphyrin; however, the pathognomonic finding is persistently increased fecal excretion of coproporphyrin III. Similarly, variegate porphyria may cause increased urinary excretion of coproporphyrin, but the distinguishing characteristic is increased fecal excretion of coproporphyrin and protoporphyrin. In addition, variegate porphyria may cause increases in the concentrations of porphyrins in the blood plasma. Acute attacks of acute intermittent porphyria, coproporphyria, and variegate porphyria may be provoked by medications, and a variety of other commonplace substances are suspect. Table 2[4] lists some of the medications reported to induce attacks. The sensitivity of affected persons to barbiturates is especially noteworthy. The use of pentothal as an anesthetic during surgery or during dental procedures would be especially risky, and the use of barbiturates to control psychiatric disturbances could cause exacerbations of the symptoms.

Porphyria cutanea tarda is characterized by photosensitivity and increased urinary excretion of uroporphyrinogen I, uroporphyrin I, and the heptacarboxyl porphyrinogen and porphyrin of series III. Sensory neuropathy may occur in association with this disorder, but episodic abdominal pain, motor dysfunction, and mental disturbance are not characteristics. Porphyria cutanea tarda appears to be potentiated by a partial deficiency of

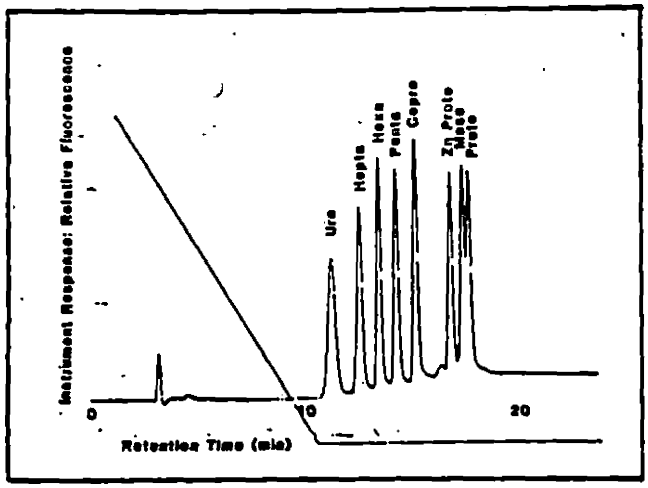


Fig. 1. Chromatogram of porphyrins standard. Injected sample consisted of 5 pmol of each standard porphyrin: octacarboxyl (Uro), heptacarboxyl (Hepta), hexacarboxyl (Hexa), pentacarboxyl (Penta), tetracarboxyl (Copro), and dicarboxyl (Meso, Proto, and Zn Proto) porphyrins.

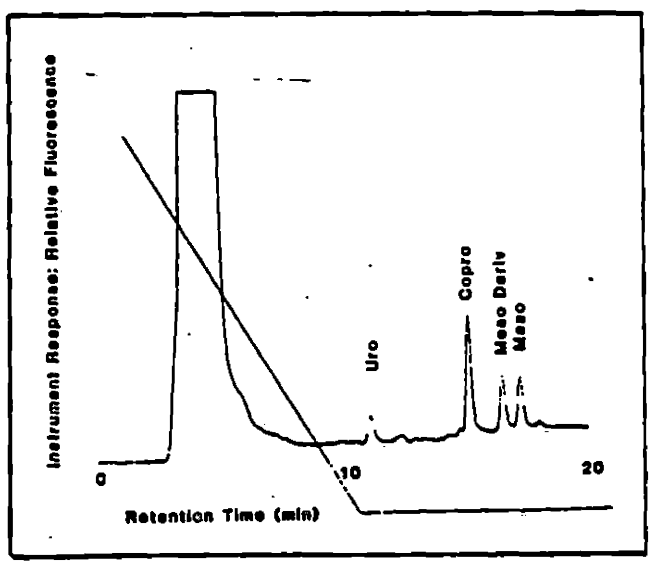


Fig. 2. Porphyrins in urine from a healthy nonporphyric male. Injected sample volume, 100 µl. Abbreviations are as in Figure 1.

uroporphyrinogen decarboxylase, and its clinical expression is provoked by factors that include iron overload,[5] chronic abuse of alcohol, and prolonged use of estrogens or contraceptive steroids. A porphyria cutanea tarda-like disorder has been caused in at least two cases by porphyrin-producing hepatic adenomas;[6] in one of those cases, the porphyria disappeared after removal of the tumor. In addition, postmortem

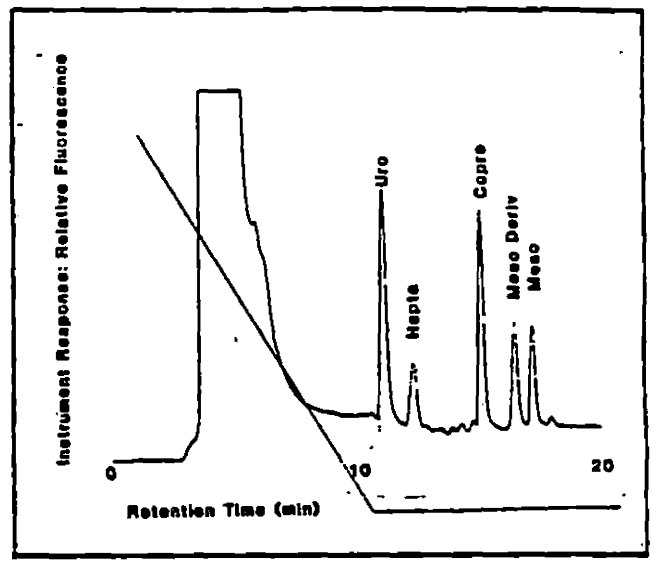


Fig. 3. Porphyrins in urine from a male patient with porphyria cutanea tarda. Injected sample volume, 200 µl. Abbreviations are as in Figure 1.

examinations of 36 persons with porphyria cutanea tarda revealed hepatocellular carcinomas in 17 cases (47%);[7] the significance of that observation is uncertain. Early signs of porphyria cutanea tarda include enhanced facial pigmentation and hypertrichosis of the forehead, malar region, and forearms.

Intoxication porphyria is characterized by increased erythrocyte protoporphyrin (especially the zinc-complexed form), increased urinary excretion of δ-aminolevulinate and porphobilinogen, and increased fecal excretion of protoporphyrin; the urinary excretion of porphyrinogens and porphyrins can be increased also. This condition can be caused by ingestion of or exposure to any of a variety of toxic substances; probably most of the recognized cases have been caused by ingestion of lead, hexachlorobenzene, or 2,3,7,8-tetrachlorodibenzo-p-dioxin. Heavy metals, halogenated aromatic hydrocarbons, and a variety of other chemicals can cause suppression of enzymes involved in porphyrinogen metabolism, with an accumulation of intermediates. Enzymes known to be suppressible by toxic substances include δ-aminolevulinate dehydratase, uroporphyrinogen I synthase, and ferrochelatase. Probably other porphyrinogen-related enzymes, also, are susceptible.

In the past, the separate quantitation of uroporphyrins, coproporphyrins, and protoporphyrins in patients' specimens involved tedious procedures for

Table 3.—Reference Values[7-9]

Blood (μg porphyrins/dl)	Protoporphyrin	Other porphyrins
Erythrocytes		
Men (n = 39)	17-52	<1
Women (n = 50)	16-65	<1
Plasma		
Men and women	<1	<1

Urine	Uro-porphyrin	Hepta-carboxylic	Hexa-carboxylic	Penta-carboxylic	Copro-porphyrin
(μg porphyrins/24 h)					
Men (n = 33)					
Median	16	7	3	2	43
Mean (SD)	20 (11)	7 (3)	3 (1)	2 (1)	46 (25)
Range	8-44	0-12	0-5	0-4	10-109
Women (n = 24)					
Median	11	4	2	1	29
Mean (SD)	11 (5)	5 (2)	2 (1)	1 (1)	29 (14)
Range	4-22	3-9	0-5	0-3	3-56
Men and women (n = 57)					
Median	13	6	2	1	33
Mean (SD)	17 (10)	6 (3)	2 (1)	1 (1)	39 (23)
Range	4-44	0-12	0-5	0-4	3-109
Porphyria cutanea tarda (n = 15)					
Range	104-5,177	43-1,508	0-161	0-305	7-263

Feces (μg porphyrins/24 h)	Uroporphyrins	Coproporphyrins	Protoporphyrins
	<1,000	<200	<1,500

differential extractions. Recently, the quantitative analysis has been improved greatly in terms of procedural convenience, accuracy, and specificity by the development of methods for simplified extractions of the total porphyrins and by applications of high-performance liquid chromatography to the analysis of buffered urine directly and of extracts of blood.[8-10] Figures 1 to 3 illustrate some features of porphyrins analysis by high-performance liquid chromatography in our laboratories. Figure 1 presents a chromatogram of the standard mixture of purified porphyrins used for calibration of the chromatography system and illustrates the separational power and sensitivity of the system. The system is calibrated in terms of the size of the instrument response (peak size on the chart) for a measured quantity of each porphyrin in the standard mixture. Quantities of the porphyrins in the patients' specimens are determined from the sizes of the porphyrin peaks in the charts (Fig. 2 and 3) and the instrument response data from the primary standard mixture. The test data are converted to and reported as quantities of porphyrins excreted per 24 hours, calculated by means of the volumes of the samples analyzed (usually 100 to 200 μl) and the total volumes of the timed collections. The various porphyrins appear in the eluate at different times and are identified, therefore, by the respective retention times (elution times). In cases in which the presence

Table 4.—Recommended Tests

Suspected porphyria	Recommended tests
Congenital erythropoietic porphyria	Urinary porphyrins
Protoporphyria	Urinary, fecal, erythrocytic porphyrins
Acute intermittent porphyria	Urinary porphobilinogen, porphyrins; erythrocyte uroporphyrinogen I synthase
Hereditary coproporphyria	Urinary porphobilinogen, porphyrins; fecal porphyrins; erythrocyte uroporphyrinogen I synthase
Variegate porphyria	Urinary porphobilinogen, porphyrins; fecal porphyrins; erythrocyte uroporphyrinogen I synthase
Porphyria cutanea tarda	Urinary porphyrins
Intoxication porphyria	Erythrocytic porphyrins; urinary δ-aminolevulinate, porphobilinogen, porphyrins; fecal porphyrins

or quantity of porphyrin in a chromatographic fraction (peak) is doubtful, the fraction is trapped in a collector and evaluated by scanning spectrofluorometry. In the figures, the intervals (minutes) required for elution of the porphyrins are shown on the abscissas. Uroporphyrin is shown to elute from the chromatographic column at 10 to 11 minutes after injection of the specimen sample, whereas elution of coproporphyrin requires 15 to 16 minutes and elution of protoporphyrin requires 18 to 19 minutes. Figure 3 presents an example of an analysis of a porphyric urine—in this case a porphyrins profile typical of porphyria cutanea tarda—showing large increases of uroporphyrin and heptacarboxyl porphyrin; those features are readily evident on comparison with the normal profile in Figure 2. The mesoporphyrin (a dicarboxylic porphyrin) and the mesoporphyrin derivative shown in the chromatograms were added to the specimens for use as internal standards; dicarboxylic porphyrins are usually not excreted in the urine.

Reference values for porphyrins in blood, urine, and feces are given in Table 3.[8,9,11]

Table 4 is offered as a guide to the selection of tests to establish the diagnosis of porphyria and to identify the specific form for any individual case.

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REFERENCES

1. Meyer UA, Schmid R: The porphyrias. *In* The Metabolic Basis of Inherited Disease. Fourth edition. Edited by JB Stanbury, JB Wyngaarden, DS Fredrickson. New York, McGraw-Hill Book Company, 1978, pp 1166-1220
2. Hofstad F, Seip M, Eriksen L: Congenital erythropoietic porphyria with a hitherto undescribed porphyrin pattern. Acta Paediatr Scand 62:380-384, 1973
3. Heilmeyer L, Clotten R: Die kongenitale erythropoetische Coproporphyrie: eine dritte erythropoetische Porphyrieform. Dtsch Med Wochenschr 89:649-654, 1964
4. Tschudy DP, Valsamis M, Magnussen CR: Acute intermittent porphyria: clinical and selected research aspects. Ann Intern Med 83:851-864, 1975
5. Pimstone NR: The hepatic aspects of the porphyrias. *In* Modern Trends in Gastroenterology. Vol 5. Edited by AE Read. Boston, Butterworths, 1975, pp 373-417
6. Tio TH, Leijnse B, Jarrett A, Rimington C: Acquired porphyria from a liver tumour. Clin Sci 16:517-527, 1957
7. Kordač V: Frequency of occurrence of hepatocellular carcinoma in patients with porphyria cutanea tarda in long-term followup. Neoplasma 19:135-139, 1972
8. Ford RE, Ellefson RD: Erythrocyte porphyrins. Clin Chem (in press)
9. Ford RE, Ou C-N, Ellefson RD: Liquid-chromatographic analysis for urinary porphyrins. Clin Chem 27:397-401, 1981
10. Piomelli S: Free erythrocyte porphyrins in the detection of undue absorption of Pb and of Fe deficiency. Clin Chem 23:264-269, 1977
11. Mason HL: Unpublished data