REPORTS

RATES OF PLASMA PORPHYRIN DISAPPEARANCE IN FLUORESCENT VS. RED INCANDESCENT LIGHT EXPOSURE

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The rates of porphyrin disappearance in plasma specimens were assessed during exposure to standard fluorescent room lighting. Protoporphyrin half-life in specimens from patients with erythropoietic protoporphyria appeared to be less than 30 min under these conditions. Uroporphyrin-coproporphyrin mixtures in plasmas of patients with porphyria cutanea tarda were more photostable, with half-lives measurable in terms of hours. All plasma porphyrins could be protected for several days from similar photodegradation by performing all blood drawing, processing, and assay procedures under ordinary red-incandescent illumination, and by storage in the dark.

Current concepts for the pathogenesis of phototoxict cutaneous reactions in patients with erythropoietic protoporphyria (EPP) imply circulation of excess protoporphyrin (PP) in blood plasma. Nevertheless, many plasma PP values within normal limits have been reported in blood from symptomatic patients with EPP [1,2]. Delay times and storage conditions prior to assay of plasma specimens for porphyrin content are often not stated in these reports. Instability of protoporphyrin in various solutions to light exposure is well known [3-5]. Porphyrins absorb light maximally in regions of the electromagnetic spectrum which pass through ordinary window glass and laboratory glassware. These wavelengths (near 400 nm) are abundant in natural sunlight and present in fluorescent room lighting. In one report noting that efforts were made to avoid such light exposure during assay procedures, PP values were uniformly abnormal, although often low (<5 μg/dl) [3]. Redeker, Brownow, and Sterling noted "high values" for plasma PP in all 4 patients with EPP they reported, but no details of light exposure, assay techniques, or numerical data were given [6]. Van Gog and Schothorst noted a >90% loss of measurable PP in plasma of an EPP patient kept for 2 hr without light protection, as well as PP decline in plasma kept refrigerated or frozen in the dark [3]. Schlenker, Davis, and Kitchell reported diminution of plasma uroporphyrins and coproporphyrins stored up to 96 hr at room temperature, but did not note light exposure conditions [7].

We have done studies to more closely determine rates of porphyrin disappearance in plasma specimens not rigorously protected from ambient light. We also wished to determine if spuriously low values possibly resulting from inadvertent light exposure of specimens during blood collection, transport, and storage procedures could be easily avoided.

A rapid quantitative fluorometric assay for porphyrins in minute quantities of red blood cells [8] recently adapted to use in blood of patients with EPP [9] was modified for use in quantitative assay of plasma porphyrins. This rapid micromethod allowed frequent determinations to closely follow porphyrin disappearance from porphyric plasma specimens exposed to our standard laboratory fluorescent lighting compared with specimens kept in the dark and completely processed under red-incandescent illumination. Effects of several storage temperatures on plasma porphyrin stability were also investigated.

METHODS

Blood was obtained by venipuncture from symptomatic patients with EPP or porphyria cutanea tarda (PCT) in tubes containing a trace of heparin. In plasmas from patients with EPP the porphyrin is spectrally

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Abbreviations:
EPP: erythropoietic protoporphyria
PCT: porphyria cutanea tarda
PP: protoporphyrin
identical with protoporphyrin; in plasmas from patients with PCT spectral studies suggest mixtures of uro- and coproporphyrins [10]. Plasma was immediately separated from cells by centrifugation for 5 min at 2700 rpm without cooling. Plasmas were removed and transferred to separate tubes to preclude hemolytic contamination by intracellular porphyrins. Each plasma specimen was divided into several aliquots for storage in glass tubes with tightly-sealing screw tops. Pairs of these sealed specimens were then maintained at 4°C, 28°C and 37°C. One of each pair was continuously exposed to the standard fluorescent lighting in the laboratory: 6 banks of 4 Durolite cool-white lamps behind plastic diffusion panels. Radiance intensity at 405 nm from these lamps was measured by a International Light Co. IL600A research photometer to be ~7.2 μW/cm² at bench level. The second paired aliquot was kept in the dark except during procedures. All blood drawing, processing, and porphyrin assay techniques were carried out in red incandescent illumination: 4, 25-w General Electric red incandescent bulbs. Bench level radiance at 405 nm from these red lamps measured only ~4.5 × 10⁻³ μW/cm². Sterile technique was used wherever possible, but was not unbroken.

Immediately after each plasma specimen was separated from the cells plasma porphyrin content was determined in triplicate. The average value was assumed to represent 100% porphyrin content at time zero. Minute quantities of each of the paired, sealed aliquots were subsequently removed for similar assay in duplicate at variable time intervals from 5 min to several days during storage varying as described above (see Figures).

Porphyrin assay was accomplished by a modification of previously described techniques [8,9]. Briefly, 100 μl of plasma was added to 0.2 μl of a mixture of 5% Celite in phosphate buffered saline (pH 7.4) in a test tube. Two ml of a 4:1 mixture (v:v) of ethyl acetate:acetic acid was added to the tube and the contents vortexed for 10 sec. After a 30 sec centrifugation at 2000 rpm, the clear supernatant was completely decanted into another test tube containing 2 ml of 1.5 N HCl. After a 10 sec vortexing, 2 phases separated with the bulk of the porphyrin in the lower acid layer. Fluorescence of the acid layer was measured as previously described, compared with the fluorescence of a standard solution of coproporphyrin I, 0.05 μg/ml in 1.5 N HCl. Subsequent calculations included appropriate correction factors to accommodate the different relative fluorescence intensities of equivalent μg quantities of proto- and uroporphyrin in ethyl acetate-saturated 1.5 N HCl compared to the coproporphyrin standard.

Exhaustive reextraction of 30 Celite sediments and of several ethyl acetate layers (both from plasmas of EPP and PCT) yielded 10 ± 2% additional porphyrin recovery. Therefore ~90% of plasma porphyrin extractable by this method appeared readily obtainable with 1 pass. Only 1 pass was performed in assays for this study; reported values are not corrected for incomplete recovery.

Calculations of quantitative plasma PP content were performed as previously described [9], modified for the larger original plasma volume assayed.

Calculations of quantitative plasma porphyrin content in specimens containing predominantly mixtures of 4 to 8 carboxyl porphyrins were performed assuming a 100% uroporphyrin content. The "total porphyrin" value thus obtained, although not rigorously quantitative, was adequate to the course of these experiments, since the "total porphyrin" value at time zero was assumed to be 100% and subsequent values were compared in terms of percentage of original value.

Plasma specimens from 20 patients with EPP were then drawn, processed, and assayed for PP using the red illumination only. Data thus obtained was compared with those of previously reported studies.

Values for plasma porphyrins determined in 60 specimens from patients with other dermatologic disorders and normal individuals ranged from 0.0 to 0.9 μg/dl (X = 0.2, SD = 0.09).

RESULTS

All plasma specimens exposed to fluorescent light showed marked changes in measurable porphyrin compared with the paired specimens stored in the dark. Specimens stored in the dark retained measurable porphyrin content near 100% of original values in all cases, whether from patients with EPP or PCT, and at all temperatures tested, for as long as 3 wk.

All plasma specimens from patients with EPP showed rapid decline in measurable PP under our ordinary laboratory fluorescent light (Fig 1). The half-life (T 1/2) was so short that it was impossible to discern any difference among the temperatures tested. In 4 specimens where frequent measurements were made during the first 2 hours of fluorescent light exposure, the T 1/2 was less than 30 min, and as little as 6 min (Fig 1).

Although 4 plasma specimens from patients with PCT showed a similar decline in measurable total porphyrin with continuing fluorescent light exposure, the rate of decline was much slower than that observed in plasmas containing protoporphyrin. The T 1/2 for these porphyrin mixtures

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**Fig 1.** Protoporphyrin disappearance from 4 different erythropoietic protoporphyria plasmas in dark storage vs. laboratory fluorescent lighting exposure, 28°C. Open symbols represent light-exposed specimens. Closed symbols represent paired, dark-stored specimens. Original values: □, 45 μg/dl; ⋄, 12.5 μg/dl; ○, 30 μg/dl; △, 19 μg/dl.
was measurable in terms of hours rather than minutes (Fig 2). We were not able to appreciate any significant difference in rate of decline of porphyrins in PCT plasmas with variable temperatures tested.

Quantitative data for PP concentration in plasma specimens from 20 symptomatic patients with EPP, obtained and assayed under red incandescent illumination as described, are shown in Fig 3. These data, uncorrected for incomplete recovery, are compared with data reported in other series (including data adjusted upward in one series for recovery of 60% [3]). Significant observations are the absence of any normal values (<1 µg/dl) and uniformly high values (X = 42, range 11–100 µg/dl) for plasma PP in this population.

DISCUSSION

The type of lighting present in our laboratory is similar to that in many clinical and laboratory areas. It is clear that the present data confirm and expand observations of prior investigators regarding photolability of plasma porphyrins and that even very brief exposures of plasma specimens containing increased porphyrins, especially those with PP, to ambient light may result in significant loss.

It appears advisable that plasma specimens from porphyrin patients be collected, transported, and otherwise processed with due caution against inadvertent and prolonged exposure to the fluorescent light usually abundant in patient care and laboratory facilities. Properly light-protected specimens will, even after several ‘days’ refrigerated storage, yield close to 100% of original measurable porphyrin content.

REFERENCES