Photohemolysis. The Comparative Behavior of Erythrocytes from Patients with Different Types of Porphyria[1] (34274)

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Porphyrins have long been recognized as being involved in light-induced tissue damage. Fischer (1) was probably the first to describe their role in photohemolysis. Other early studies were carried out by Günther (2) and by Kammerer and Weisbecker (3) who varied several parameters as well as comparing the effectiveness of more than one porphyrin. Later Cook and Blum (4) found that the photohemolysis of normal red cells required oxygen and suggested that the hemolysis was attributable to their protoporphyrin content. Interest in this phenomenon was revived when Harber *et al.* (5) noted the unusual susceptibility to photohemolysis of erythrocytes from patients with erythropoietic protoporphyria. This is a recently recognized disease of porphyrin metabolism in which there is an abnormal skin sensitivity to sunlight and greatly increased levels of protoporphyrin in erythrocytes and feces. Subsequent investigations led Fleischer *et al.* (6) to conclude that the photohemolysis of these protoporphyrin-containing cells involves membrane damage, then increased permeability, and finally colloid osmotic lysis.

The occurrence of skin symptoms in certain other types of porphyria, as well as a high erythrocyte porphyrin concentration in one, posed the question of possible photohemolysis in these other diseases of porphyrin metabolism. Therefore, the present investigation was undertaken as a possible aid in understanding the photohemolytic process. Compared are the behavior toward irradiation of normal erythrocytes and those from patients with four different types of porphyria that are characterized by cutaneous manifestations: erythropoietic protoporphyria (EPP), congenital erythropoietic porphyria (CEP), South African porphyria (porphyria variegata), and porphyria cutanea tarda. Also compared are the photohemolytic properties of uro-, copro-, and protoporphyrins when added to erythrocytes.

Methods. Erythrocytes from citrated or heparinized human blood were washed three times with 0.9% sodium chloride, then routinely suspended in Hanks' solution at a dilution of 1:2000. This dilution afforded uniform illumination and further dilution of the cells changed neither the lag time nor the rate of hemolysis. In no case was light exposure of whole blood studied.

Twenty-ml aliquots of cell suspensions were placed in 90-mm uncovered petri dishes on a rotary shaker. The studies were done at 4° in order to limit the errors introduced by evaporation. Irradiation was obtained from a Blak-Ray fixture fitted with two shielded long wave ultraviolet tubes of 15 W each. These gave an intensity at the surface of the cell suspension of 4 lm/ft^2 as measured with a Weston Ranger 9 cadmium sulfide meter having 24% sensitivity at 400 $m\mu$, the region of maximum porphyrin absorption. Two-ml samples of irradiated cell suspension were withdrawn at various time intervals and centrifuged. Released hemoglobin was measured at 540 $m\mu$. The percentage hemolysis was

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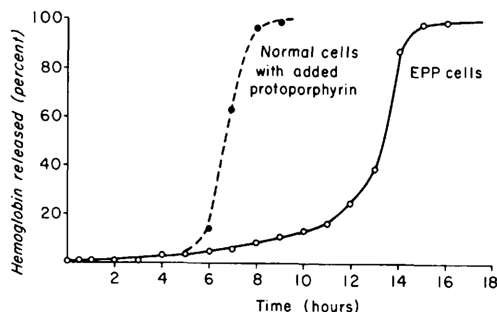

FIG. 1. Photohemolysis under long wave ultraviolet irradiation of protoporphyrin-containing erythrocytes (from erythropoietic protoporphyria patient) and normal erythrocytes to which had been added an equivalent amount of protoporphyrin. The results represent an experiment in which a relatively concentrated cell suspension (1:400) was used.

determined by comparison with a sample in which 100% hemolysis was induced by freezing. The initial experiment reported (Fig. 1) was unique in that 200 ml of suspension at 1:400 dilution was used and 7-ml samples were taken for hemoglobin determination.

Uroporphyrin and coproporphyrin were isolated from pathological urine by solvent extraction and purification. Purity was checked by thin-layer chromatography (7). Protoporphyrin was prepared by reducing hemin in formic acid with powdered iron according to the method of Fischer and Putzer as described by Schwartz *et al.* (8). Erythrocyte porphyrin concentrations were determined essentially by the method of Schwartz *et al.* (9). The porphyrin content of intact erythrocytes was observed in dried smears by fluorescence microscopy.

Results. Approximately 20% of the erythrocytes from the patient with erythropoietic protoporphyria showed the orange-red fluorescence of porphyrins when observed microscopically with long wave ultraviolet light. The initial experiment, in which conditions were slightly different from those employed routinely, demonstrated clearly that hemolysis occurred in two phases (Fig. 1). Following about a 5-hr delay, there was an initial phase with a slow rate of hemolysis at the end of which none of the remaining red cells showed microscopically observable fluorescence (9 hr). The second phase was characterized by a very rapid rate of hemolysis that proceeded to completion. Under similar conditions, normal red cells showed insignificant hemolysis. However, when an approximately equivalent amount of protoporphyrin was added to normal erythrocytes, these lysed in a single rapid phase that began after a shorter period of irradiation.

In the subsequent experiments where a 1:2000 dilution was used, the two phases of hemolysis of erythropoietic protoporphyric red cells occurred with a shorter lag period and at a very rapid rate (Fig. 2A). These erythrocytes had a protoporphyrin concentration that averaged about 1000 μg/100 ml or 40 times normal (Table I). Cells from normal individuals, a patient with congential erythropoietic porphyria (Table I), and from two patients with forms of cutaneous hepatic porphyria in which the red cell porphyrins

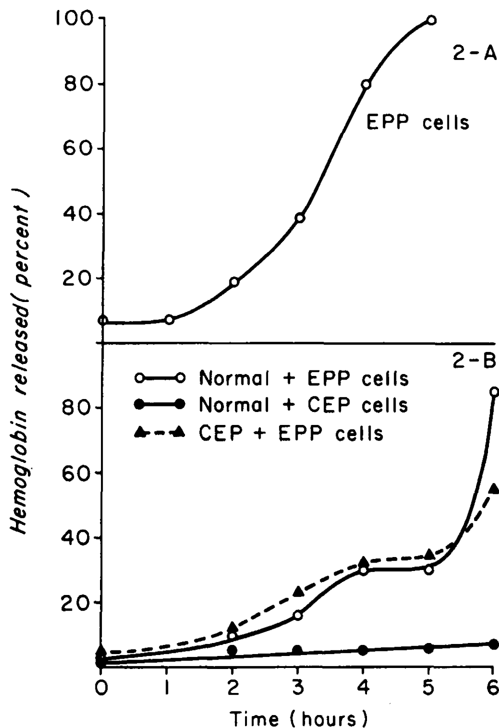

FIG. 2A. Photohemolysis of protoporphyrin-containing erythrocytes from a patient with erythropoietic protoporphyria. The cells were in dilute suspension (1:2000). (B) Photohemolysis occurring in mixtures of erythrocytes. Combinations of cells were diluted 1:2000 in each case.

TABLE I. Erythrocyte Porphyrin Concentrations of Individuals Studied.

Clinical status	Erythrocyte porphyrin concentrations[a] (μg/100 ml of packed cells)		
	Uro	Copro	Proto
Normal	Trace	1–3	20–40
Erythropoietic protoporphyria	3	30	1000
Congenital erythropoietic porphyria	1000	30	450

[a] Typical values or ranges only are given since many different blood specimens were used in the course of the experiments.

are normal (porphyria variegata and porphyria cutanea tarda) were similarly irradiated. Each of these latter cell suspensions showed no significant hemolysis during a 6-hr period of irradiation.

In order to observe possible effects of one cell type on another, combinations of three cell types (normal, CEP, and EPP) were prepared by adding together equal proportions of cells in pairs to give three separate cell mixtures (Fig. 2B). Hemolysis was seen only in those mixtures containing protoporphyric erythrocytes. In each instance there was a biphasic hemolysis that was separated by a lag period of about 1 hr. Since the experiment was terminated at 6 hr, complete hemolysis was not always achieved, but it is clear that the course of hemolysis was approaching 100%. The decreased rates of hemolysis in the cell mixtures can be ascribed to the greater dilution of the protoporphyrin-containing cells; this cell concentration effect was determined in experiments not reported here.

The fact that protoporphyrin-containing red cells led to the hemolysis of normal cells and the time course required for total hemolysis suggested that the porphyrin was released to attack these latter cells. This possibility was examined by adding protoporphyrin individually to three types of cells: normal, CEP, and EPP. At a protoporphyrin concentration of 10^{-7} M in the suspension medium, all cells behaved alike, hemolyzing completely in 2 hr (Fig. 3A). At a porphyrin concentration of 10^{-8} M, complete hemolysis still occurred, but with a longer lag period and at a slower rate.

In contrast to protoporphyrin, uro- and coproporphyrins at concentrations up to 10^{-6} M exhibited no photohemolytic effect, and in the presence of these porphyrins all three types of cells behaved on irradiation the same as in their absence (Fig. 3B). Therefore, uro- and coproporphyrins appeared to be ineffective whether located either intracellularly (as in the CEP cells) or extracellularly.

Discussion. Direct comparison of our findings with those of the early investigators (1–3) is difficult due to the many differences in techniques. Nevertheless, the general conclusions reached have been confirmed. Recently the hemolysis by long wave ultraviolet irradiation of protoporphyrin-containing erythrocytes from patients with erythropoietic protoporphyria has been studied. Our experi-

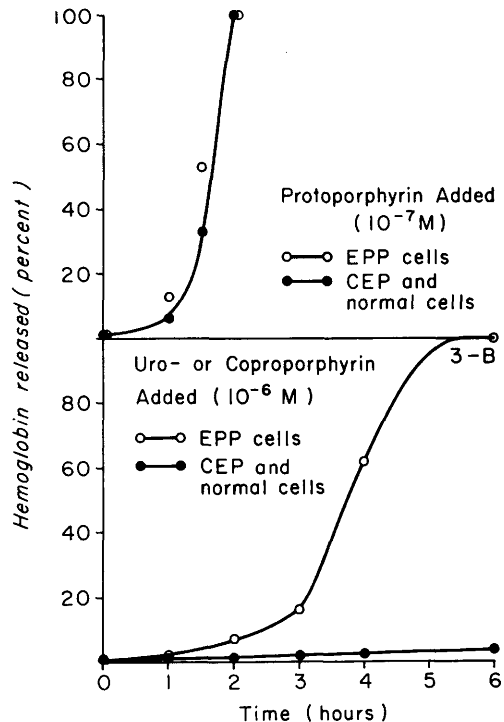

FIG. 3A. Effects of added protoporphyrin on the photohemolysis of different cell types. (B) Effects of added uro- and coproporphyrins on the photohemolysis of different cell types.

ments further indicate that added extracellular protoporphyrin will promote photohemolysis of normal cells. The fact that uroporphyrin and coproporphyrin whether intra- or extracellular did not catalyze photohemolysis suggests that the nearly identical spectral properties (absorption and fluorescence) of these three porphyrins are not the only important factors in this process. There are, however, marked solubility differences between these porphyrins. Protoporphyrin is highly lipophilic, whereas coproporphyrin and especially uroporphyrin are more hydrophilic. The importance of these solubility properties is supported by other studies (to be reported elsewhere) which have shown that a variety of other lipophilic porphyrins can induce photohemolysis, in some cases even more rapidly than protoporphyrin. This specificity implies that solubility of the porphyrin in the lipid milieu of the red cell membrane is a prerequisite for the initiation of photohemolysis.

The lack of photohemolysis in erythrocytes from lead poisoning (10), in which the protoporphyrin concentration is in the range found in EPP (11), and in those cells from our patient with CEP (which contained unusually high concentrations of protoporphyrin for this disease) indicates that additional factors are involved in the phenomenon of photohemolysis. Possibly in these instances the protoporphyrin is bound intracellularly in such a manner that it cannot deposit in the cell membrane.

The possible relationships between red cell porphyrins and photosensitivity of skin are of interest. Even though lead poisoning and iron deficiency are commonly accompanied by elevated erythrocyte protoporphyrin concentrations, we are unaware of skin photosensitivity of the type seen in EPP ever having been described in these conditions. Other effects of light in lead poisoning have been noted. Acutely poisoned rabbits excreted even greater amounts of coproporphyrin following exposure to ultraviolet light (12), perhaps related is the higher incidence of clinical lead poisoning appearing in the summer months (13). Other diseases of porphyrin metabolism, porphyria cutanea tarda and South African porphyria, are characterized by light-induced skin symptoms with normal red cell porphyrins. This indicates that elevated concentrations of free red cell porphyrins *per se* are not the responsible agent for the skin manifestations seen in these diseases. It is believed that when the plasma porphyrin level is elevated, porphyrins deposit in the skin in sufficient quantities to allow photosensitive reactions to occur.

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