Phototoxicity of protoporphyrin as related to its subcellular localization in mice livers after short-term feeding with griseofulvin

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In mice, feeding with griseofulvin leads to the rapid accumulation of protoporphyrin in liver mitochondria. When liver mitochondria from mice fed with griseofulvin for 2 days are exposed to irradiation (320–400 nm), uncoupling of oxidative phosphorylation followed by inhibition of respiration occurs at light doses above 3–5 kJ/m². When combined preparations of mitochondria and lysosomes are irradiated, inactivation of enzymes occurs in the following order: succinate dehydrogenase > glutamate dehydrogenase > acid phosphatase > β -glucuronidase. Qualitatively, the photodamaging effect of endogenously produced protoporphyrin is indistinguishable from that of externally added protoporphyrin. Quantitatively, however, when protoporphyrin is added externally, more protoporphyrin is taken up by lysosomes, and photoinactivation of the lysosomal enzymes is correspondingly more severe. The results are further evidence that porphyrin-induced photodamage is largely determined by the solubility properties of the porphyrins and the target structures [Sandberg & Romslo (1980) *Biochim. Biophys. Acta* 593, 187–195], and also that protoporphyrin-induced photodamage is essentially similar whether protoporphyrin is generated endogenously or added exogenously.

In erythropoietic protoporphyria, protoporphyrin accumulates in large amounts, particularly in the liver (Barnes *et al.*, 1968) and the erythrocytes (Magnus *et al.*, 1961), but also in the skin (van Gog & Schothorst, 1973; Miura *et al.*, 1980). The most serious complication is fulminant liver failure (Barnes *et al.*, 1968; Cripps *et al.*, 1977). The most frequently occurring symptom, however, is increased cutaneous photosensitivity. This results from interaction of cellular structures with singlet oxygen generated by energy transfer from photoexcited triplet-state protoporphyrin of the skin (Cannistraro *et al.*, 1978; Bodaness & Chan, 1977).

Protoporphyrin accumulates primarily in lipophilic environments (Reese & Rimington, 1964). The resulting photodamage, as in erythropoietic protoporphyria, would therefore be expected to affect lipid-containing structures. By analogy, accumulation of uroporphyrin, as in porphyria cutanea tarda (Malina *et al.*, 1978), would also result in photo-

damage to hydrophilic structures. Experimental evidence to support these suggestions has recently been reported (Sandberg & Romslo, 1981). Experiments have also been performed showing that photodamage to plasma membranes and mitochondria occurs earlier than photodamage to lysosomes (Sandberg, 1981). These results were obtained with externally added protoporphyrin. It is not known, however, whether protoporphyrin of the skin in erythropoietic protoporphyria is generated locally (Bickers *et al.*, 1977) or is accumulated from protoporphyrin of the plasma (Gschnait *et al.*, 1975a). Hence, it would be worthwhile to study photodamage in systems where excessive protoporphyrin production occurs endogenously, since exogenous protoporphyrin may distribute differently from that produced endogenously.

Griseofulvin-induced murine protoporphyria has many similarities to human protoporphyria, with increased cutaneous photosensitivity (Konrad *et al.*, 1975), liver damage (Janousek, 1970) and accumulation of large amounts of protoporphyrin in the liver (De Matteis & Rimington, 1963; Gschnait *et al.*, 1975b). Recently it has been shown that the

Abbreviations used: h.p.l.c., high-pressure liquid chromatography; Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid.

porphyrinogenic effect of griseofulvin results from the accumulation of *N*-methylporphyrins with powerful ferrochelatase-inhibitory properties (De Matteis & Gibbs, 1980; De Matteis *et al.*, 1980a,b,c). The fundamental defect(s) resulting in decreased ferrochelatase activity in erythropoietic protoporphyria is not known. Griseofulvin-induced murine protoporphyria should, however, nevertheless be well-suited to assess the susceptibility of subcellular structures to photodamage from endogenous protoporphyrin.

The present paper deals with photodamage to subcellular organelles of mouse liver after short-term feeding with griseofulvin. Data are also reported on the subcellular localization of the porphyrins that accumulate.

Materials and methods

Animals

Male mice, 15–25 g, were given a powdered diet containing 2.5% (w/w) griseofulvin. Control mice were fed the same diet without griseofulvin. Most animals were killed after 3 days of griseofulvin feeding.

Preparation of subcellular and submitochondrial fractions

Livers from three mice were cut into small pieces and rinsed in 10 vol. of 0.25 M-sucrose/10 mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid] buffer, pH 7.40. The tissue was homogenized in a Potter-Elvehjem homogenizer at 720 rev./min with two strokes of a loose-fitting Teflon pestle. Subcellular fractions were then prepared as described by Berge & Farstad (1979). The purity of the fractions was assessed by marker enzymes (see below).

Mitochondrial subfractions were prepared by digitonin treatment, essentially as described by Berge & Farstad (1979).

Irradiation

Mitochondria (approx. 0.5 mg of protein/ml) or combined preparations of mitochondria and lysosomes (approx. 1.5 mg of protein/ml) were incubated at 4°C in 3 ml of 50 mM-glucose/175 mM-sucrose/5 mM-MgCl₂/10 mM-KCl/5 mM-P_i/10 mM-Hepes buffer, pH 7.4. Further additions were as indicated in the legends to the Figures. The suspension, in a test tube of diameter 10 mm, was irradiated with a PUVA photochemotherapy unit (H. Haldmann, D 722, Schwenningen, Germany) containing 14 fluorescent tubes (F 8 T5/BL PUVA Sylvania) in a bank. About 70% of the emission energy of these lamps is between 340 and 380 nm. The light intensity was 66 W/m² as measured at sample level with an UDT-1116 filter optic probe (United Detector Technology, Santa Monica, CA,

U.S.A.). The irradiation dose was from 0 to 80 kJ/m², corresponding to irradiation of from 0 to 20 min. After irradiation, the suspension was transferred to cuvettes for measuring the enzymic activities, safranine wavelength shift and oxygen consumption.

Other analytical procedures

Separation of porphyrins was performed by h.p.l.c. as described by Husby & Romslo (1980).

Determination of protoporphyrin in the various fractions was done by extracting the fractions twice with ethyl acetate/acetic acid (3:1, v/v). The porphyrins were thereafter transferred to 3 M-HCl and determined as described by Piomelli *et al.* (1976).

Mitochondrial respiration rates were determined in the suspension of the irradiated mitochondria with a Clark oxygen electrode (Yellow Springs Instruments biological oxygen monitor) with appropriate polarization.

Assays

The spectral shift of safranine was determined by adding 20 µM-safranine to the irradiated mitochondrial suspension. Further additions and experimental conditions were as described in Fig. 4 (below). The spectra were recorded on an Aminco DW-2 UV/VIS spectrophotometer by using the wavelength pair 524–554 nm at 22°C (Åkermann & Wikström, 1976).

Griseofulvin was determined by extracting mitochondria (200 µg of protein) or combined preparations of mitochondria and lysosomes (200 µg of protein) with 3.0 ml of toluene. Toluene was evaporated and the sample was dissolved in 100 µl of toluene with desmethyldiazepam (600 ng/ml) as internal standard. The concentration of griseofulvin was then measured on a Hewlett-Packard gas chromatograph [model 5730 A, equipped with an EC detector (⁶³Ni)]. The concentration of griseofulvin was also measured fluorometrically [see Fig. 1 below (insert)].

The release of enzymes from lysosomes was determined from the enzymic activities of a supernatant obtained by centrifugation (24 000 g, 30 min).

Glutamate dehydrogenase (Schmid, 1974), succinate:phenazine methosulphate oxidoreductase (Arrigoni & Singer, 1962), adenylate kinase (Szasz *et al.*, 1976), amine oxidase (Aas, 1971), acid phosphatase (Walter & Schütt, 1974), β-glucuronidase (Fishman, 1974), urate oxidase (Beaufay *et al.*, 1959), rotenone-insensitive NADPH:cytochrome *c* oxidoreductase (Sottocasa *et al.*, 1967) and lactate dehydrogenase (Bergmeyer & Bernt, 1974) were assayed as described in the cited references. Protein was determined by the Folin-Ciocalteu reagent (Flatmark *et al.*, 1971).

Chemicals

Griseofulvin was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Safranin was obtained from Merck A.G. (Darmstadt, Germany), and protoporphyrin (crystalline dichloride) was from Porphyrin Products (Logan, UT, U.S.A.). The purity of protoporphyrin was better than 95% as assessed by h.p.l.c. (Husby & Romslo, 1980). Other chemicals were of highest purity commercially available. Double-quartz-distilled water was used throughout.

Results

Griseofulvin is itself a phototoxic compound (Ljunggren & Möller, 1978). As Fig. 1 shows, when normal mouse liver mitochondria were irradiated in the presence of griseofulvin, a progressive decline in succinate dehydrogenase activity was seen at concentrations of griseofulvin in excess of 10 nmol/mg of protein. In the present study, however, photodamage by griseofulvin is thought to be of no importance, since the concentration of griseofulvin of the mitochondria did not exceed 0.1 nmol/mg of protein (determined by g.l.c.) (see also the insert to Fig. 1).

In the present experiments we wanted to study the joint effect of light and protoporphyrin. Therefore

Fig. 1. Effect of irradiation of 40 kJ/m^2 (corresponding to 10 min) on the activity of succinate dehydrogenase of isolated mitochondria at increasing concentrations of griseofulvin

The mitochondria were incubated and irradiated as described in the Materials and methods section. Griseofulvin added to mitochondria in the dark had no effect on the succinate dehydrogenase (results not shown). Insert: fluorescence emission spectra of mitochondria from normal mouse liver, with added griseofulvin: (a) 20 nmol of griseofulvin/mg of protein; (b) 8 nmol of griseofulvin/mg of protein; (c) 3 nmol of griseofulvin/mg of protein; (d) control without griseofulvin (indistinguishable from mitochondria from griseofulvin-treated mice). Protein concentration was $200 \mu\text{g/ml}$. Excitation wavelength 300 nm.

Table 1. Intracellular distribution of protoporphyrin, enzyme activity and protein, in mice livers after feeding with griseofulvin

Protoporphyrin, enzymes and protein were determined as described in the Materials and methods section. Abbreviations used: N, nuclear fraction; M, mitochondrial fraction; L, lysosomal fraction; P, microsomal fraction, and S, soluble fraction. The results are given as percentages of the total homogenate in each fraction. The relative specific content represents the percentage recovered in the fraction divided by the percentage of protein in the fraction, taking the cumulative values of fractions N, M, L, P, S as 100%. The results are the means from four separate experiments. Protein and protoporphyrin of the total homogenate were 285 mg and 191 nmol respectively.

Fraction ...	Distribution (%) in fractions and (in parentheses) relative specific content					Recovery (%)
	N	M	L	P	S	
Protoporphyrin	19.4 (1.5)	24.0 (2.2)	7.0 (0.9)	4.4 (0.6)	17.3 (0.5)	72
Amine oxidase	18.5 (1.5)	25.8 (2.3)	12.0 (1.6)	2.7 (0.4)	12.6 (0.4)	72
Glutamate dehydrogenase	13.6 (1.1)	42.9 (3.9)	6.8 (0.9)	1.2 (0.1)	6.2 (0.2)	71
Succinate dehydrogenase	20.6 (1.6)	37.8 (3.3)	16.0 (2.0)	1.6 (0.2)	0.0 —	76
Acid phosphatase	10.3 (1.0)	9.1 (1.0)	16.8 (2.7)	6.0 (0.9)	20.0 (0.7)	62
Urate oxidase	10.2 (0.8)	29 (2.5)	27 (3.5)	3.4 (0.4)	0.0 —	70
Rotenone-insensitive NADPH : cytochrome c oxidoreductase	10.0 (0.9)	5.0 (0.5)	14.1 (2.0)	19.1 (2.6)	19.2 (0.6)	67
Lactate dehydrogenase	3.6 (0.2)	0.9 (0.1)	1.5 (0.2)	1.1 (0.1)	76.3 (2.0)	83
Protein	14.1	12.5	8.5	9.0	38.7	83

griseofulvin was given only for a period of time sufficient to increase liver protoporphyrin to concentrations that did not affect the respiratory-control rate of the mitochondria (Koller & Romslo, 1980) and that were similar to those used in our previous studies with externally added protoporphyrin (Sandberg & Romslo, 1980).

Subcellular localization of protoporphyrin in livers from mice fed with griseofulvin

Administration of griseofulvin to mice leads to increased liver weight and accumulation of large amounts of protoporphyrin in the liver (Janousek, 1970; Wessely *et al.*, 1979). In the present experiments the amount of protoporphyrin increased from about 1.5 nmol/g wet weight to an average of 260 nmol/g wet weight (range 181–309 nmol/g wet weight) after 3 days administration of griseofulvin.

The subcellular distribution of protoporphyrin paralleled that of mitochondrial marker enzymes (Table 1), with about 25% of the total amount of protoporphyrin in the mitochondrial fraction. On subfractionation of the mitochondria, protoporphyrin recovery was found to be 58, 15, 24 and 2% in the outer membrane, the inter membrane space, the

inner membrane and the matrix space respectively (Fig. 2). On a protein basis, the highest concentration of protoporphyrin was found in the outer membrane and was approx. four times that in the inner membrane (Fig. 2).

On h.p.l.c. the porphyrins of the mitochondria were eluted as two closely adjacent peaks (Fig. 3), with the major peak corresponding to protoporphyrin. The nature of the smaller peak has not been further explored.

A few mice were given griseofulvin for 3 weeks. In these animals the liver mitochondria contained 10–14 nmol of protoporphyrin/mg of protein and the mitochondrial preparations were all largely uncoupled.

Irradiation of liver mitochondria and lysosomes from griseofulvin-fed mice

Irradiation of mitochondria from griseofulvin-fed mice led to the same pattern of destruction as found when protoporphyrin was added to normal rat liver

Fig. 2. Distribution of protoporphyrin in relation to some marker enzymes in subfractions of mouse liver mitochondria (see the Materials and methods section)

Abbreviations used: Ma, matrix fraction; In, inner-membrane fraction; Sp, intermembrane-space fraction; Ou, outer-membrane fraction. The abscissa shows the percentage of total protein in each subfraction presented as cumulative values. The ordinate shows the relative specific activity (or content) on a protein basis of the various subfractions, taking the specific activity of the cumulative fractions Ma, In, Sp and Ou as 1. (a) Amine oxidase; (b) adenylate kinase; (c) succinate dehydrogenase; (d) glutamate dehydrogenase; (e) protoporphyrin.

Fig. 3. H.p.l.c. ester profiles of (a) mitochondrial porphyrin from griseofulvin-treated mice and (b) parent protoporphyrin

Mitochondria or parent protoporphyrin was taken up in methanolic 10% (v/v) H_2SO_4 . After esterification at 20°C for 24 h in the dark, the porphyrin esters were extracted into chloroform. A μ Porasil (300 mm $\times$ 4 mm) column was used. The elution solvent was n-heptane/methyl acetate (3:2, v/v) at a flow rate of 1.5 ml/min and a pressure drop of 500 lbf/in² (3.45 MPa). Abbreviations used: Proto., protoporphyrin; Solv., solvent; Inj., injection.

Fig. 4. Effect of irradiation on (a) the respiration rates and (b) the energy-dependent wavelength shift of safranine of liver mitochondria from mice treated with griseofulvin (●, ○, □) or from normal mice (▲, △, ◆). Mitochondria were incubated and irradiated as described in the Materials and methods section. The concentration of protoporphyrin of the mitochondria after griseofulvin feeding (2 days) was 0.7 nmol/mg of protein. After irradiation, one portion of the mitochondrial suspension was removed for determination of the energy-dependent wavelength shift of safranine and one portion was removed for determination of State-4 (●, ▲) and State-3 (○, △) respiration rate. The State-4 and State-3 respiration rates were initiated by adding 5 mM-succinate and 1 mM-ADP respectively. The energy-dependent wavelength shift of safranine was determined by adding 3 mM-succinate and 20 μ M-safranine and de-energizing with 1 μ M-carbonyl cyanide *m*-chlorophenylhydrazine.

mitochondria (Sandberg & Romslo, 1980). With increasing light doses, State-4 respiration transiently increased and concomitantly State-3 respiration declined (Fig. 4a). At irradiation of about 5 kJ/m², the mitochondria were uncoupled. At higher light doses, succinate oxidation rapidly declined, and at the same time the membrane potential, as measured by the safranine-wavelength-shift method, collapsed (Fig. 4b).

Release of lysosomal enzymes has been claimed to be an early event in, and of decisive importance to, the subcellular lesions in some of the porphyrias (Allison *et al.*, 1966; Slater & Riley, 1966; Torinuki *et al.*, 1980). Recently, however, it has been shown that after the addition of protoporphyrin to preparations of mitochondria and lysosomes, irradiation results in severe damage to the mitochondria before there is any significant damage to the lysosomes (Sandberg, 1981). With endogenously generated protoporphyrin, this difference was even more pronounced (Fig. 5). Thus, in griseofulvin-fed mice, acid phosphatase and β -glucuronidase were less inactivated than glutamate dehydrogenase and succinate dehydrogenase, and there was only negligible release of enzymes from the lysosomes. By comparison, when similar experiments were run with protoporphyrin added to normal mitochondria and lysosomes, the susceptibility of the lysosomal enzymes apparently increased relative to the mito-

Fig. 5. Effect of increasing light doses on the activity of enzymes from combined preparations of lysosomes (a) and mitochondria (b).

Mitochondria and lysosomes were incubated and irradiated (as described in the Materials and methods section) in the presence of about 0.45 nmol of protoporphyrin added exogenously (open symbols) or generated endogenously (2 days griseofulvin feeding) (closed symbols). (□, ■) β -glucuronidase; (○, ●) acid phosphatase; (◇, ◆) glutamate dehydrogenase; (△, ▲) succinate dehydrogenase. The concentrations of protoporphyrin of the mitochondria and the lysosomes were as indicated in Table 2. The irradiation (80 kJ/m²) corresponds to an irradiation time of 20 min.

Table 2. *Protoporphyrin in mitochondria and lysosomes from griseofulvin-fed mice (a) or after adding an equivalent amount of protoporphyrin to a combined preparation of mitochondria and lysosomes from normal mice liver (b)*

The results are the means from three separate experiments.

	Protoporphyrin (nmol/mg of protein)	
	(a)	(b)
Mitochondria	0.49	0.46
Lysosomes	0.23	0.44

chondrial enzymes (Figs. 5a and 5b). The most obvious reason for this difference was that protoporphyrin added externally distributed more evenly between lysosomes and mitochondria than when generated endogenously [where it concentrated preferentially in the mitochondria, at least during short-term treatment with griseofulvin (Table 2)].

Discussion

The increase in protoporphyrin of the liver as found here is similar to that reported by Janousek (1970) and Gschnait *et al.* (1975b). According to Janousek (1970), accumulation of protoporphyrin in the liver starts within a few hours after administration of griseofulvin, and by feeding griseofulvin beyond 2–3 months, liver protoporphyrin may reach values above 8000 nmol/g (Gschnait *et al.*, 1975b). By comparison, in patients dying from liver failure of erythropoietic protoporphyria, the concentration of protoporphyrin of the liver varies from 370 nmol/g (Donaldson *et al.*, 1971) to more than 100 000 nmol/g (Cripps *et al.*, 1977). In the present study we found protoporphyrin concentrations in the liver of about 1300 nmol/g wet weight after 3 weeks of administration of griseofulvin and with uncoupling of oxidative phosphorylation in the liver mitochondria of these animals even without exposure to light. This finding supports our recent suggestion that liver failure in erythropoietic protoporphyria may result from mitochondrial dysfunction secondarily to the accumulation of excessive amounts of protoporphyrin (Koller & Romslo, 1980).

After 3 days administration of griseofulvin, the subcellular distribution of liver protoporphyrin paralleled that of the mitochondrial marker enzymes, with the greatest concentration (per mg of protein) in the mitochondrial fraction (Table 1). Ultrastructurally, early alterations in griseofulvin-induced murine protoporphyria and in human erythropoietic protoporphyria are swelling of the mitochondria followed at a later stage by the appearance of distorted and bizarrely shaped mitochondria with needle-like crystals of protoporphyrin (Matilla & Molland, 1974; Gschnait *et al.*, 1975b; Cripps *et al.*,

1977; Shapiro *et al.*, 1980). The hepatocyte content of NADH also declines (Shapiro *et al.*, 1980). Accumulation of protoporphyrin within, or adjacent to, the mitochondria is also to be expected from the localization of coproporphyrinogen oxidase to the inner membrane space (Elder & Evans, 1978), protoporphyrinogen oxidase associated with the mitochondrial inner membrane (Poulson, 1976) and ferrochelatase firmly bound within the inner membrane (Jones & Jones, 1969). With time, however, protoporphyrin within the mitochondria may redistribute to other organelles, be excreted or be metabolized.

From electron-microscopic studies, evidence has been presented that protoporphyrin in griseofulvin-induced protoporphyria accumulates in a close relationship with the mitochondrial outer membrane (Matilla & Molland, 1974; Shapiro *et al.*, 1980). In agreement with this, we found about 60% of protoporphyrin of the mitochondria associated with the outer membrane. However, if assessed relative to lipid content of the membranes (Palmer & Hall, 1972), protoporphyrin distributed at approximately the same concentrations between the outer and the inner membrane, i.e. as to be expected by passive partition in membrane lipids. The concentrations of protoporphyrin in the outer membrane most likely represent maximum values, because contaminating lysosomes and peroxisomes of the mitochondria would lead to a disproportional enrichment of contaminants in the outer-membrane fraction after subfractionation.

The porphyrins of the mitochondria eluted as double peaks with retention times identical with (the major peak of Fig. 3a), or slightly shorter than, that of the pure protoporphyrin methyl ester. According to Malina *et al.* (1978), double peaks may result from the formation of Cu–porphyrin complexes with shorter retention times on h.p.l.c. Another possibility could be ethanol impurities in the methanol/H₂SO₄ solution with formation of ethyl esters. This explanation is also unlikely, at least in the present system, because double peaks were not found on esterification of parent protoporphyrin (Fig. 3). A third possibility is accumulation of the ferrochelatase inhibitor *N*-methylprotoporphyrin (Tephly *et al.*, 1979, 1980; De Matteis *et al.*, 1980a,b,c). However *N*-methylprotoporphyrin is less mobile than parent protoporphyrin in the development system used in our h.p.l.c. experiments, and it is therefore unlikely that the additional peak of Fig. 3(a) is *N*-methylprotoporphyrin. As yet, however, the nature of this peak has not been further explored.

Uncoupling of oxidative phosphorylation, inhibition of respiration and dissipation of the transmembrane electrochemical potential as seen in mitochondria from griseofulvin-fed mice after irradiation (Fig. 4) are similar to the findings in

normal mitochondria with external protoporphyrin (Sandberg & Romslo, 1980). Thus, with rat liver mitochondria, uncoupling of oxidative phosphorylation was obtained with 240 pmol of protoporphyrin/mg of protein and an irradiation of 10 kJ/m² (Sandberg & Romslo, 1980). Moreover, in combined preparations of lysosomes and mitochondria, mitochondria were more susceptible than lysosomes to protoporphyrin-induced photodamage, irrespective of whether protoporphyrin was added externally or produced endogenously (Fig. 5).

What then are the relevance of the present findings to the photodamage in erythropoietic protoporphyria? Gschnait *et al.* (1975a) have shown that, in erythropoietic protoporphyria and in griseofulvin-induced protoporphyria, endothelial cells of the dermis are damaged shortly after irradiation. Damage to epidermal cells is also observed (Kint & De Weert, 1978). The amount of protoporphyrin of the skin in erythropoietic protoporphyria is far below that of the liver; e.g., in patients with severe skin symptoms, the skin protoporphyrin concentration has been found to be 0.2–8.0 nmol/g wet weight (van Gog & Schothorst, 1973; Miura *et al.*, 1980). Photodamage, however, is a function both of irradiation and of the protoporphyrin concentration linked with the target structure (Sandberg & Romslo, 1980), and skin symptoms may therefore develop even with very low porphyrin concentrations provided the skin is exposed to sufficiently high light doses.

The cellular target for the porphyrin-induced photodamage of the skin in erythropoietic protoporphyria is largely unknown. According to Schothorst *et al.* (1977), damage to the plasma membrane and lysosomes are early lesions to cultured human skin fibroblasts after exposure to protoporphyrin and irradiation. Wakulchik *et al.* (1980), however, did not find release of lysosomal enzymes when human fibroblasts were exposed to protoporphyrin and irradiation. They concluded that photolysis of the fibroblasts was similar to that which occurs in protoporphyrin-sensitized erythrocytes. Damage to the cell membrane has also recently been emphasized by Malik & Djaldetti (1980). Our results (Figs. 4 and 5) suggest that, rather than lysosomes, the mitochondria and the plasma membrane should be considered possible cellular targets for the porphyrin-induced photodamage in erythropoietic protoporphyria. Evidence favouring this assumption is also (1) the recent finding that rat liver mitochondria are more susceptible to porphyrin-induced photodamage by external porphyrins than are lysosomes (Sandberg, 1981), and (2) damage to leucocyte mitochondria and plasma membranes in advance of leucocyte lysosomes after irradiation in the presence of protoporphyrin (Sandberg *et al.*, 1981).

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