Evidence that in chick embryos destruction of hepatic microsomal cytochrome *P*-450 haem is a general mechanism of induction of δ -aminolaevulinate synthase by porphyria-causing drugs

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A variety of porphyrinogenic compounds were tested for their effect *in ovo* on chick-embryo liver microsomal cytochrome *P*-450 haem concentration and mitochondrial δ -aminolaevulinate synthase activity. With all drugs tested, there was a 30–50% decrease in cytochrome *P*-450 haem concentration within 1 h of treatment, and this was closely followed by an increase in δ -aminolaevulinate synthase activity. The relationship was independent of the extent of enzyme induction and is consistent with the proposal that drug-mediated destruction of cytochrome *P*-450 haem is the primary mechanism of induction of δ -aminolaevulinate synthase. After induction, synthesis of δ -aminolaevulinate synthase could be maintained by inhibiting further haem synthesis. These studies suggest that induction of porphyria is a combination of two distinct processes: (a) induction of δ -aminolaevulinate synthase synthesis by destruction of cytochrome *P*-450 haem and consequent depletion of cellular free haem; (b) maintenance of continued δ -aminolaevulinate synthase synthesis by preventing replenishment of cellular haem either by inhibiting haem synthesis and/or by promoting continuous removal of newly synthesized haem.

Hepatic porphyrias are a group of hereditary diseases that typically produce neuropsychiatric symptoms and in which the common biochemical feature is a loss of control of the haem-biosynthetic pathway (Schmid, 1960). Normally this pathway is strictly regulated; δ -aminolaevulinate synthase (EC 2.3.1.37), the first and rate-limiting enzyme, has a short half-life (Tyrrell & Marks, 1972) and is present in mitochondria in very small amounts (Granick & Urata, 1963). In hepatic porphyrias, the activity of this enzyme is greatly increased (Granick, 1966), resulting in the production of excess δ -aminolaevulinate, elevated concentrations of which have been shown to inhibit release of the neurotransmitter γ -aminobutyrate (Brennin & Cantrill, 1979). Usually this disease is latent, but patients are abnormally sensitive to a number of drugs that can precipitate or exacerbate attacks of porphyria (Granick & Urata, 1963).

In experimental animals a wide variety of xenobiotics have been shown to induce hepatic δ -aminolaevulinate synthase rapidly (Granick, 1963, 1966; Sassa & Kappas, 1977), and this feature links the experimental porphyrias with the genetic diseases. Elucidation of the mechanism by which drugs induce δ -aminolaevulinate synthase is there-

fore an important step in understanding the molecular cause of porphyria.

Immunological studies have shown that, in experimental porphyria, increased δ -aminolaevulinate synthase activity is due to enzyme synthesis *de novo* (Whiting & Granick, 1976). Haem, the end product of the pathway, prevents this response (Granick, 1966; Tyrrell & Marks, 1972; Tomita *et al.*, 1974; Sassa & Kappas, 1977; Srivastava *et al.*, 1980). Intracellular haem is therefore believed to control induction of δ -aminolaevulinate synthase (Granick, 1966; Tyrrell & Marks, 1972; Tomita *et al.*, 1974), probably at the level of transcription (Srivastava *et al.*, 1979), but considerable confusion exists as to the mechanism by which drugs and chemicals override this control and induce enzyme synthesis.

A number of theories have been proposed in which the various inducers compete with, or bind to, putative regulators of the transcription (Granick, 1966) or translation (Sassa & Granick, 1970; Tyrrell & Marks, 1972) of δ -aminolaevulinate synthase, and we have recently demonstrated increased translation of δ -aminolaevulinate synthase from polyadenylated RNA of induced chick-embryo livers (Brooker *et al.*, 1980). However, the diversity of chemical inducers with few common structural

features is not easy to reconcile with a direct effect on either transcription or translation.

Some of the compounds that induce δ -aminolaevulinate synthase are also known to promote a fall in the concentration of intracellular haem. 2-Allyl-2-isopropylacetamide, a potent porphyrinogenic agent, caused destruction of the haem prosthetic group of rat hepatic microsomal cytochrome *P*-450, but left the apoprotein intact (De Matteis, 1970). Reconstitution of the haemoprotein *in vivo* was suggested to occur, utilizing unconjugated haem, thereby lowering the intracellular concentration of free haem and inducing δ -aminolaevulinate synthase. Farrell *et al.* (1979) have demonstrated reconstitution of cytochrome *P*-450 *in vivo* from exogenous [^{14}C]haem. De Matteis (1970) therefore proposed that, for 2-allyl-2-isopropylacetamide, destruction of cytochrome *P*-450 haem is the primary mechanism by which this chemical induces δ -aminolaevulinate synthase. Moreover, White & Muller-Eberhard (1977) have shown that contraceptive steroids, which have been suggested to induce porphyria in humans, caused destruction of rat liver cytochrome *P*-450 haem and induction of δ -aminolaevulinate synthase activity.

However, despite the implications of these results, the haem-destruction theory has not been proposed as a general mechanism of drug induction. This is largely because two potent inducers of δ -aminolaevulinate synthase were believed not to cause destruction of cytochrome *P*-450 haem. 2-Isopropyl-2-propylacetamide, an analogue of 2-allyl-2-isopropylacetamide, had been reported not to affect the concentration of chick-embryo liver cytochrome *P*-450 haem over a 3 h period (Krupa *et al.*, 1974), and phenobarbitone, a barbiturate known to precipitate attacks of acute intermittent porphyria in man, caused an increase in the concentration of rat liver cytochrome *P*-450 (Levin *et al.*, 1972). Furthermore inconsistencies in the effects of different inducers in a variety of experimental systems has led to considerable confusion as to the mode of action of these chemicals.

We have therefore re-investigated the effect of a variety of porphyrinogenic compounds on chick-embryo hepatic microsomal cytochrome *P*-450 haem and mitochondrial δ -aminolaevulinate synthase. These experiments considered only the first 3 h of treatment, and in all cases destruction of cytochrome *P*-450 haem can be postulated to be the mechanism of induction of the enzyme. Furthermore, studies on the relative effectiveness of various drugs to induce δ -aminolaevulinate synthase indicate that the induction of porphyria is a combination of two distinct processes: (a) induction of δ -aminolaevulinate synthase synthesis by cytochrome *P*-450-mediated destruction of intracellular haem; (b) maintenance of continued δ -amino-

laevulinate synthase synthesis by inhibition of haem synthesis and/or continual removal of newly synthesized haem.

Experimental

Materials

Chick embryos were obtained from the Parafield Poultry Research Farm, Adelaide, S. Austral., Australia. The following compounds were gratefully received as gifts: 2-allyl-2-isopropylacetamide from Hoffman-La Roche, Nutley, NJ, U.S.A.; deferoxamine methanesulphonate from CIBA-GEIGY (Australia), N.S.W., Australia, compound SKF-525A (2-diethylaminoethyl 2,2-diphenylvalerate) from Smith, Klein and French (Australia), N.S.W., Australia, and Emulgen 913 from ICI (Australia), Vic., Australia. Phenobarbitone was purchased from May and Baker, Dagenham, Essex, U.K., and 3,5-diethoxycarbonyl-1,4-dihydrocollidine from Eastman Organic Chemicals, Rochester, NY, U.S.A.

Preparation of chemicals

3,5-Diethoxycarbonylcollidine was prepared from 3,5-diethoxycarbonyl-1,4-dihydrocollidine by the method of Racz & Marks (1972) and was determined to be pure by m.p. analysis (107–115°C) and i.r. spectroscopy. 2-Isopropyl-2-propylacetamide was prepared from 2-allyl-2-isopropylacetamide by the procedure of Marks *et al.* (1973) and was determined to be pure by m.p. analysis (134.5–135°C) and n.m.r. spectroscopy.

Treatment of chick embryos

The various compounds were dissolved in an appropriate solvent and were injected through the air sac into the fluid surrounding the 17-day chick embryo. The eggs were returned to the incubator and the embryos were harvested at various times after treatment. Livers were quickly removed on to ice.

Preparation of mitochondria

Each treated or untreated liver was homogenized separately in 5 ml of homogenizing solution containing (final concentrations) 0.01 mM-pyridoxal 5'-phosphate, 0.1 mM-EDTA, 0.25 M-sucrose and 5 mM-Tris/HCl buffer, pH 7.4 (PEST buffer). Cell debris was removed by centrifuging twice at 2000g for 5 min, and mitochondria were isolated from the supernatant by centrifugation at 10000g for 15 min. Pelleted mitochondria were resuspended in PEST buffer and ruptured by ultrasonication for four 5 s periods. δ -Aminolaevulinate synthase activity in the ruptured mitochondrial suspension was determined by the colorimetric assay method of Poland &

Glover (1973) and was expressed as nmol of δ -aminolaevulinate formed/h per mg of mitochondrial protein.

Preparation of microsomal fraction

Total microsomal fraction was obtained from the post-mitochondrial supernatant by centrifugation at 105 000 *g* for 1 h. The pellet was resuspended in a solution of 0.1 M-sodium phosphate buffer, pH 7.4, containing 1 mM-EDTA, 1 mM-dithiothreitol, 0.2% (v/v) Emulgen 913 and 20% (v/v) glycerol, to a concentration of 120 mg wet wt. of liver/ml.

Cytochrome *P*-450 was measured in this suspension by the procedure of Omura & Sato (1964), with a CO bubbling time of 45 s. CO-treated and untreated samples were incubated at room temperature (20°C) with 2 mg of Na₂S₂O₄/ml for 45 min before measurement of cytochrome *P*-450. The concentration of cytochrome *P*-450 was expressed as nmol/mg of microsomal protein. Protein determinations were by the procedure of Lowry *et al.* (1951), with bovine serum albumin as standard.

Preparation of liver-cell suspension

A suspension of isolated hepatocytes was prepared from 17-day chick-embryo livers, and total protoporphyrin and mitochondrial δ -aminolaevulinate synthase were determined as described by Srivastava *et al.* (1979).

Results

Effects of the xenobiotics 2-allyl-2-isopropylacetamide, 2-isopropyl-2-propylacetamide and 3,3-diethoxycarbonyl-1,4-dihydrocollidine on cytochrome *P*-450 haem concentration and δ -aminolaevulinate synthase activity

Chick embryos were treated with three porphyrinogenic agents, 2-allyl-2-isopropylacetamide, 2-isopropyl-2-propylacetamide and 3,3-diethoxycarbonyl-1,4-dihydrocollidine, and the hepatic microsomal cytochrome *P*-450 haem concentration and mitochondrial δ -aminolaevulinate synthase activity were measured. Each of these compounds caused a rapid fall in cytochrome *P*-450 haem concentration within 30 min of treatment (Figs. 1 and 3b), with the final value being 35–50% of the original value. The decrease preceded an increase in δ -aminolaevulinate synthase activity, which by 3 h was 8-fold higher than the basal value with 2-allyl-2-isopropylacetamide and 2-isopropyl-2-propylacetamide, and 16-fold higher with 3,3-diethoxycarbonyl-1,4-dihydrocollidine. For each drug tested there was a reciprocal correlation between the drug-induced decline in cytochrome *P*-450 haem concentration and the induction of δ -aminolaevulinate synthase.

Fig. 1. Effects of 2-allyl-2-isopropylacetamide and 2-isopropyl-2-propylacetamide on cytochrome *P*-450 haem concentration and δ -aminolaevulinate synthase activity in chick-embryo livers *in ovo*

The 17–18-day chick embryos were injected with 2 mg of 2-isopropyl-2-propylacetamide (a) or 2 mg of 2-allyl-2-isopropylacetamide (b) in 0.1 ml of dimethyl sulphoxide. Controls were given 0.1 ml of dimethyl sulphoxide. At various times chick-embryo livers were removed, and cytochrome *P*-450 haem (●) and δ -aminolaevulinate synthase activity (■) were assayed. Each point is the mean for three to nine chick embryos (vertical bars show S.E.M.). The 100% value of cytochrome *P*-450 ranged from 0.15 to 0.19 nmol/mg of microsomal protein. The basal activity of δ -aminolaevulinate synthase generally ranged from 0.30 to 0.43 unit/mg of mitochondrial protein.

Effects of exogenous haemin on the 2-allyl-2-isopropylacetamide-mediated decline in cytochrome *P*-450 haem concentration and induction of δ -aminolaevulinate synthase

Intact embryos were treated with 2-allyl-2-isopropylacetamide, and exogenous haemin was then administered intravenously. Hepatic microsomal cytochrome *P*-450 haem concentration and mitochondrial δ -aminolaevulinate synthase activity were measured over a 2 h period. The results (Fig. 2) show

that, after haemin treatment, the decline in cytochrome *P*-450 haem concentration was delayed by approx. 1 h relative to the control without haemin treatment. A similar delay was observed before increased δ -aminolaevulinate synthase activity occurred.

This result shows that the temporal relationship between the 2-allyl-2-isopropylacetamide-mediated decline in cytochrome *P*-450 haem concentration and increased δ -aminolaevulinate synthase activity was still demonstrable even though the presence of exogenous haemin had delayed the responses. However, these data do not establish that the two responses are related.

Effects of phenobarbitone on cytochrome P-450 haem concentration and δ -aminolaevulinate synthase activity

The preceding results indicate a possible general mechanism by which drug-mediated induction of δ -aminolaevulinate synthase could occur: depletion of regulatory haem resulting in de-repression of

enzyme synthesis. However, phenobarbitone, a known porphyrinogenic agent in man, has been shown to cause an increase in the concentration of rat liver cytochrome *P*-450 after several hours (Levin *et al.*, 1972). Therefore, to determine whether phenobarbitone-mediated induction of δ -aminolaevulinate synthase was preceded by an initial fall in cytochrome *P*-450 haem concentration, chick embryos were treated with phenobarbitone; cytochrome *P*-450 haem concentration and δ -aminolaevulinate synthase activity were measured at various times after treatment. The results of this experiment (Fig. 3a) show that, within 1 h of treatment, the concentration of cytochrome *P*-450 haem had decreased by 30%; increased δ -aminolaevulinate synthase activity was detectable at 2 h,

Fig. 2. Effects of exogenous haemin on 2-allyl-2-isopropylacetamide-mediated changes in cytochrome *P*-450 haem concentration and δ -aminolaevulinate synthase activity in chick-embryo livers *in ovo*.

The 17–18-day chick embryos were injected with 2 mg of 2-allyl-2-isopropylacetamide in dimethyl sulphoxide (▲ and △) or intravenously injected with 0.1 ml of a neutral 5 mM-haemin solution followed by 2 mg of 2-allyl-2-isopropylacetamide (● and ○). At various times chick-embryo livers were removed, and cytochrome *P*-450 haem concentration (▲ and ●) and δ -aminolaevulinate synthase activity (△ and ○) were measured. Experimental conditions were the same as described in Fig. 1.

Fig. 3. Effects of phenobarbitone and 3,5-diethoxycarbonyl-1,4-dihydrocollidine on cytochrome *P*-450 concentration and δ -aminolaevulinate synthase activity in chick-embryo livers *in ovo*.

The 17–18-day chick embryos were injected with 4 mg of phenobarbitone (a) or 4 mg of 3,5-diethoxycarbonyl-1,4-dihydrocollidine (b) in 0.1 ml of dimethyl sulphoxide. At various times chick-embryo livers were removed, and cytochrome *P*-450 haem concentration (●) and δ -aminolaevulinate synthase activity (○) were assayed. Experimental conditions were the same as described in Fig. 1.

and by 3 h was 3–4-fold the basal value. The cytochrome *P*-450 haem concentration did not begin to return to the control value until 3 h after phenobarbitone treatment.

Effects of two relatively poor porphyrinogenic agents on cytochrome P-450 haem concentration and δ -aminolaevulinate synthase activity

A number of compounds have been reported to have variable inducing activity in different experimental systems. 3,5-Diethoxycarbonylcollidine, an analogue of 3,5-diethoxycarbonyl-1,4-dihydrocollidine, has been shown to cause induction of enzyme synthesis in cultured chick-embryo liver cells but to have little inducing activity in chick-embryo livers *in ovo* (Racz & Marks, 1969). Furthermore, compound SKF-525A, an inhibitor of cytochrome *P*-450-mediated mixed-function oxidase reactions, has been reported to inhibit 3,5-diethoxycarbonyl-1,4-dihydrocollidine-mediated induction of rat liver δ -aminolaevulinate synthase (De Matteis, 1978), but, by itself, to cause some enzyme induction in cultured chick-embryo liver cells and in chick-embryo livers *in ovo* (Marks, 1973). To test whether with these compounds there was any correlation between drug interaction with cytochrome *P*-450 and induction of δ -aminolaevulinate synthase, we treated intact chick embryos with 3,5-diethoxycarbonylcollidine or compound SKF-525A. Microsomal cytochrome *P*-450 haem concentration and mitochondrial δ -aminolaevulinate synthase activity were measured over the first 3 h of treatment.

Within 1 h of 3,5-diethoxycarbonylcollidine treatment the cytochrome *P*-450 haem concentration fell to 30% of the original value (Fig. 4a), and increased δ -aminolaevulinate synthase activity was detected at 2 h. However, within 5 h the cytochrome *P*-450 haem concentration had returned to its original value and did not change for at least a further 3 h. In concert with this, δ -aminolaevulinate synthase activity rapidly declined back to the non-induced value. A qualitatively similar effect was seen after administration of compound SKF-525A (Fig. 4b). Although continued synthesis of δ -aminolaevulinate synthase did not occur with these drugs, the initial response was identical with that of the more potent porphyrinogenic agents 2-allyl-2-isopropylacetamide, 2-isopropyl-2-propylacetamide and 3,5-diethoxycarbonyl-1,4-dihydrocollidine: a rapid decrease in cytochrome *P*-450 haem concentration closely followed by an increase in the δ -aminolaevulinate synthase activity. These data are therefore consistent with a model of de-repression of δ -aminolaevulinate synthase by drug-mediated degradation of intracellular haem.

We also compared the effects of 3,5-diethoxycarbonylcollidine and compound SKF-525A *in ovo*

Fig. 4. Effects of 3,5-diethoxycarbonylcollidine and compound SKF-525A on cytochrome *P*-450 haem concentration and δ -aminolaevulinate synthase activity in chick-embryo livers *in ovo*

The 17–18-day chick embryos were injected with 4 mg of 3,5-diethoxycarbonylcollidine (a) or 3 mg of compound SKF-525A (b) in 0.1 ml of dimethyl sulphoxide. At various times chick-embryo livers were removed, and cytochrome *P*-450 haem concentration (●) and δ -aminolaevulinate synthase activity (■) were assayed. Experimental conditions were the same as described in Fig. 1.

with that in isolated chick-embryo liver cells *in vitro*. The results (Table 1) show that *in vitro* the two drugs were powerful porphyrinogenic agents. Compound SKF-525A and 3,5-diethoxycarbonylcollidine promoted 66- and 113-fold increases respectively in δ -aminolaevulinate synthase activity over an 8 h period, and an 18–30-fold increase in protoporphyrin concentration. The induction was similar to that promoted by 2-allyl-2-isopropylacetamide in these cells. This result suggests that, in chick embryos *in ovo*, failure of 3,5-diethoxy-

Table 1. *Effects of 2-allyl-2-isopropylacetamide, compound SKF-525A and 3,5-diethoxycarbonylcollidine on protoporphyrin accumulation and δ -aminolaevulinate synthase synthesis in isolated chick-embryo liver cells in vitro* Isolated chick-embryo liver cells were incubated in Eagle's basal medium supplemented with dibutyryl cyclic AMP (50 μ M), insulin (1 μ g/ml) and cortisol (0.05 μ g/ml). Further additions were made at 0 h. After 8 h incubation, the cells were sonicated, mitochondria were isolated and the δ -aminolaevulinate synthase activity was determined. Total intracellular protoporphyrin was determined spectrophotofluorimetrically after 10 h of incubation. Results are the averages of four separate experiments.

Addition	δ -Aminolaevulinate synthase activity (nmol/h per mg of protein)	Protoporphyrin (pmol/10h per mg of protein)
None	0.10	6.4
2-Allyl-2-isopropylacetamide (500 μ g/ml)	7.51	115.2
Compound SKF-525A (500 μ g/ml)	6.60	102.1
3,5-Diethoxycarbonylcollidine (2.5 μ g/ml)	11.30	173.2

carbonylcollidine or compound SKF-525A to cause prolonged induction of δ -aminolaevulinate synthase may be due to drug removal.

Effect of an inhibitor of haem synthesis on prolonging compound SKF-525A-induced synthesis of δ -aminolaevulinate synthase

These data suggest that depletion of intracellular haem (via drug-mediated degradation of cytochrome *P*-450 haem) may be a necessary prerequisite for induction of δ -aminolaevulinate synthase, but, if the inducing drug is removed, haem concentrations build up and prevent further enzyme synthesis.

To test this hypothesis we treated intact chick embryos with compound SKF-525A, and then 1 h later (when the cytochrome *P*-450 haem concentration was lowest; Fig. 4b) with deferoxamine methanesulphonate, a potent iron chelator and inhibitor of haem synthesis (Granick *et al.*, 1975). Mitochondrial δ -aminolaevulinate synthase and microsomal cytochrome *P*-450 haem were measured at times up to 3 h. The results of this experiment (Fig. 5) show that, in the presence of deferoxamine methanesulphonate, continued synthesis of δ -aminolaevulinate synthase occurred and by 3 h the activity was 5-fold that in the untreated control. With compound SKF-525A alone δ -aminolaevulinate synthase activity had declined to control values by 3 h. Deferoxamine methanesulphonate alone did not induce δ -aminolaevulinate synthase, and after addition of deferoxamine methanesulphonate cytochrome *P*-450 haem concentrations did not increase at later times as rapidly as in the control without the inhibitor.

These results are therefore consistent with the proposal that induction of δ -aminolaevulinate synthase is a consequence of cytochrome *P*-450-mediated haem depletion and further suggest that continued synthesis of the enzyme is dependent on maintaining intracellular haem at a low concentration.

Fig. 5. *Effects of compound SKF-525A plus deferoxamine methanesulphonate on cytochrome P-450 haem concentration and δ -aminolaevulinate synthase activity in chick-embryo livers in ovo*

The 17–18-day chick embryos were injected with 3 mg of compound SKF-525A (■ and □), 10 mg of deferoxamine methanesulphonate (● and ○) or 3 mg of compound SKF-525A for 1 h followed by 10 mg of deferoxamine methanesulphonate (▲ and △), each injection being in 0.1 ml of dimethyl sulfoxide. At various times chick-embryo livers were removed, and cytochrome *P*-450 haem concentration (■, ● and ▲) and δ -aminolaevulinate synthase activity (□, ○ and △) were measured. Experimental conditions were the same as described in Fig. 1.

Discussion

The feature most difficult to reconcile in experimental porphyria is the diversity of compounds that can induce δ -aminolaevulinate synthase. The only consistent feature noted by Marks (1978) is that all inducers are of a lipophilic nature, and as such would probably interact with the cytochrome *P*-450-associated microsomal mixed-function oxidase

system. That this may be the common link between all porphyria-inducing drugs is suggested by the data in the present paper.

We have shown that, over the early time period when δ -aminolaevulinate synthase induction is initiated, all drugs tested that increased δ -aminolaevulinate synthase activity also caused a loss of cytochrome *P*-450 haem. Even where prolonged synthesis of δ -aminolaevulinate synthase did not occur, the initial induction was preceded by a loss of cytochrome *P*-450 haem. The hypothesis that drug-mediated induction of δ -aminolaevulinate synthase is a consequence of cytochrome *P*-450 haem depletion is therefore tenable.

We suggest that the mechanism of δ -aminolaevulinate synthase induction first proposed by De Matteis (1970) for 2-allyl-2-isopropylacetamide, namely depletion of microsomal cytochrome *P*-450 haem, may be a general mechanism by which all porphyria-inducing drugs act.

However, induction of porphyria is dependent, not only on drug-mediated initiation of induction of δ -aminolaevulinate synthase, but also on maintaining intracellular haem at a concentration sufficiently low that synthesis of δ -aminolaevulinate synthase continues. The effectiveness of a potential porphyrinogenic compound will therefore depend on (a) its ability to destroy cytochrome *P*-450 haem, thereby depleting the free haem pool and depressing δ -aminolaevulinate synthase synthesis, and (b) its effectiveness in keeping the haem pool depleted. This concept is demonstrated by the effect of compound SKF-525A on cytochrome *P*-450 haem and δ -aminolaevulinate synthase in chick embryos *in ovo*. Although cytochrome *P*-450 haem was initially depleted and δ -aminolaevulinate synthase was induced, this effect was only transient. However, enzyme synthesis was prolonged by the addition of an inhibitor of haem synthesis, deferoxamine methanesulphonate. This result is consistent with previous work in which we have shown that in isolated chick-embryo liver cells 2-allyl-2-isopropylacetamide-mediated (Srivastava *et al.*, 1979) or 3,5-diethoxycarbonyl-1,4-dihydrocollidine-mediated (G. Srivastava, unpublished work) induction of δ -aminolaevulinate synthase was maintained after complete removal of the inducing drug, provided that haem synthesis was prevented by addition of deferoxamine methanesulphonate. Inhibition of haem synthesis by itself, however, was not sufficient to initiate induction. This concept is supported by the data of Cole *et al.* (1979), who showed that induction by δ -aminolaevulinate synthase by compound SKF-525A was potentiated by a concentration of 3,5-diethoxycarbonyl-1,4-dihydrocollidine that was sufficient to inhibit ferrochelatase but not to induce porphyria.

These results confirm a report by Rifkind (1979)

that in chick embryos inhibition of ferrochelatase by low concentrations of 3,5-diethoxycarbonyl-1,4-dihydrocollidine was not sufficient to cause δ -aminolaevulinate synthase induction. However, in that work it was reported that a concentration of 3,5-diethoxycarbonyl-1,4-dihydrocollidine sufficient to induce δ -aminolaevulinate synthase did not affect the concentration of cytochrome *P*-450 haem; the conclusion was based on an initial time point of 4 h. In the present paper we have shown that the concentration of cytochrome *P*-450 haem falls within 1 h of treatment with 3,5-diethoxycarbonyl-1,4-dihydrocollidine and by 3 h was beginning to return to the control value. This result emphasizes the need for an investigation of the induction process at early times after drug treatment.

We propose therefore that induction of experimental porphyria is a combination of two distinct processes, namely (a) induction of δ -aminolaevulinate synthase synthesis by drug-mediated destruction of cytochrome *P*-450 haem and (b) maintenance of continued enzyme synthesis by inhibition of haem synthesis and/or continual removal of newly synthesized haem. This latter process may occur by several different mechanisms. 3,5-Diethoxycarbonyl-1,4-dihydrocollidine has been reported to cause the formation of a green porphyrin-like pigment that inhibits ferrochelatase activity (Tephly *et al.*, 1979) and therefore haem synthesis. Phenobarbitone, on the other hand, has been demonstrated to cause increased synthesis of cytochrome *P*-450 apoprotein (Levin *et al.*, 1972), thereby providing increased capacity to utilize newly synthesized haem. The fact that in our experiments increased concentrations of cytochrome *P*-450 were not detected until several hours after the initiation of δ -aminolaevulinate synthase synthesis demonstrates that this is not the primary mechanism of induction. However, a note of caution should be added. We have recently found that in phenobarbitone-treated isolated chick-embryo liver cells *in vitro* increased activities of δ -aminolaevulinate synthase did not occur for 10 h, in contrast with an almost immediate response in chick-embryo livers *in ovo*. Although we can only speculate as to the reasons for this anomalous behaviour, it may be related to the fact that isolated liver cells contain less cytochrome *P*-450 than do intact livers (Sinclair *et al.*, 1979). Nevertheless the rapid induction of δ -aminolaevulinate synthase *in ovo* correlates well with destruction of cytochrome *P*-450 haem and supports the concept that the primary mechanism of induction is haem depletion.

These concepts, formulated to explain chemically induced porphyria in experimental animals, could also be applicable to drug induction of porphyria in man. Induction of δ -aminolaevulinate synthase may be initiated by depletion of intracellular haem, and

this event is probably quite normal, occurring with a large number of drugs and steroids (Granick, 1966) that interact with the mixed-function oxidase system. In most cases the rate of haem synthesis is probably sufficient to reconstitute cytochrome *P*-450 and replete the haem pool rapidly, thereby repressing further synthesis of δ -aminolaevulinate synthase. In porphyria patients, however, the activity of the haem-biosynthetic pathway is lower than normal (Granick & Urata, 1963), and rapid reconstitution of cytochrome *P*-450 may not occur, leading to an accumulation of δ -aminolaevulinate and porphyrins, and the clinical symptoms of the disease. This interpretation is supported by the finding that barbiturates are porphyrinogenic in rats only when hepatic haem synthesis is partially inhibited (De Matteis, 1978).

An alternative is that the hepatic microsomal cytochrome *P*-450 of porphyric patients is more sensitive than normal and the haem moiety is more readily degraded by drugs. However, at present there is no direct evidence to support one or other of these proposals.

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