0031-3998/92/3102-0196$03.00/0
PEDIATRIC RESEARCH

Vol. 31, No. 2, 1992
Printed in U.S.A.

Comparative Effects of Tin- and Zinc-Protoporphyrin on Steroidogenesis: Tin-Protoporphyrin is a Potent Inhibitor of Cytochrome P-450-Dependent Activities in the Rat Adrenals

G. MICHAEL TRAKSHEL, PATRICK M. SLUSS, AND MAHIN D. MAINES

Department of Biophysics [G.M.T., M.D.M.] and Department of Urology [P.M.S.], University of Rochester School of Medicine, Rochester, New York 14642

ABSTRACT. Synthetic metalloporphyins inhibit formation of bilirubin by the heme oxygenase system, an ability that is of considerable experimental and clinical interest for suppression of jaundice in the newborn. The present investigation compares the consequences of treatment with Sn- and Zn-protoporphyrin on hemoprotein-dependent enzymes of the rat adrenals and corticosterone production and defines Sn-protoporphyrin as a potent toxin to adrenal functions. Treatment of rats with Sn-protoporphyrin (two doses of 50 μmol/kg, in 7 d) resulted in a marked reduction of 30–40% in cytochrome P-450-dependent adrenal microsomal 21α-hydroxylase and mitochondrial 11β-hydroxylase activities. In the serum, the levels of corticosterone were reduced to about 70% of the control value. In addition, the mitochondrial cytochrome P-450$_{SCC}$ activity was decreased by about 50%. This decrease, however, could not be attributed to a reduced total heme level or an accelerated heme degradatory activity. Disruption by Sn-protoporphyrin of adrenal hemoprotein-dependent functions was not restricted to steroidogenic activities and encompassed drug metabolism activity of the organ; benzo(a)pyrene hydroxylase activity of both the microsomal and the mitochondrial fractions, as well as the microsomal NADPH-cytochrome P-450 reductase activity, were significantly reduced. Zn-protoporphyrin did not cause significant alterations in the above measured parameters although it too was effective in inhibiting the hepatic microsomal heme oxygenase activity. In light of the presently defined adverse effects of Sn-protoporphyrin on adrenal steroidogenesis, we suggest Zn-protoporphyrin is the agent of choice for potential use in treatment of hyperbilirubinemia in humans. (*Pediatr Res* 31: 196–201, 1992)

Suppression of bilirubin production has been considered a desirable method for controlling severe cases of neonatal hyperbilirubinemia in premature babies or infants at high risk for severe postnatal jaundice. In this context, synthetic metalloporphyrins have recently become of interest to neonatologists because of their ability to suppress heme degradation by microsomal heme oxygenase (1–13). This in turn reflects the function of heme oxygenase isozymes, HO-1 and HO-2 (14), to serve as the first and rate-limiting step in the pathway of heme degradation and bilirubin production (15, 16). As is well recognized, after parturition human neonates have transient deficiencies in the mechanisms involved in the disposal of bilirubin, which result, in certain instances, in critically high serum levels of the bile pigment and the need for clinical intervention.

When the ability of synthetic metalloporphyrins to inhibit *in vivo* heme oxygenase activity was first described (2), based on consideration of the relative toxicity of the various metalloporphyrins including Zn and Sn complexes, the suggestion was made that Zn-protoporphyrin is a relatively safer compound with potential usefulness to suppress heme oxygenase activity. However, among the various metalloporphrins that are now known capable of inhibiting heme oxygenase activity, only the clinical use of Sn-protoporphyrin has been actively promoted. The promotion is based on the seemingly superior efficiency of the complex to suppress heme oxygenase activity (4) and the assumption that this metalloporphyrin is rather safe and competitively inhibits the enzyme activity (3, 17, 18). Indeed, recently the complex has been used on an experimental basis in humans, both infants and adults, with various disorders of heme metabolism (6, 9, 10, 12). The presumed safety, however, has been based on examining a relatively select spectrum of hepatic and renal hemoprotein-dependent drug metabolic activities and hematological parameters (17, 18).

Drug biotransformation activities, however, are not the only heme dependent cellular processes. Metabolism of a variety of endogenous compounds is also hemoprotein dependent. For example, a number of catalytic steps in the biosynthesis of steroids are mediated by the hemoproteins, cytochromes P-450. For adrenals this includes the mitochondrial 11β-hydroxylase, cytochrome P-450$_{SCC}$ (which catalyzes side-chain cleavage of cholesterol), and the microsomal 17α and 21α-hydroxylases (19–24). As could be predicted, there exists an intimate relationship between the pathways of steroidogenesis and heme metabolism, wherein changes in heme biosynthesis and degradation are reflected in the concentration of cytochromes P-450 and ultimately in the production of steroids (25–29). In fact, rather recently, catalysis of the heme moiety of different cytochrome P-450 species by both heme oxygenase isozymes has been demonstrated in reconstituted systems (30).

The intimate relationship between heme metabolic activity and functions that depend on cytochrome P-450 promoted the present investigation to examine and compare the effect of Sn- and Zn-protoporphyrin on hemoprotein-dependent adrenal steroidogenesis. We have found that Sn-protoporphyrin is a potent inhibitor of steroidogenesis in the adrenals and adversely affects

Received June 11, 1991; accepted August 29, 1991.
Correspondence and reprint requests: Mahin D. Maines, Ph.D., University of Rochester School of Medicine, Department of Biophysics, 601 Elmwood Ave., Rochester, NY 14642.
Supported by NIH Grants ES03968 and ES01247. M.D.M. is a Burroughs-Wellcome Toxicology Scholar.

hemoprotein-dependent functions of this organ. In contrast, Zn-protoporphyrin appears to be relatively innocuous in altering the assessed parameters. We suggest that Zn-protoporphyrin is the more desirable complex for suppression of bilirubin production in humans.

MATERIALS AND METHODS

Cofactors and steroids (progesterone, 17α-hydroxyprogesterone, 25-hydroxycholesterol, pregnenolone, 11-deoxycorticosterone, corticosterone) were obtained from Sigma Chemical Co. (St. Louis, MO). Sn-protoporphyrin-IX dichloride and Zn-protoporphyrin IX were obtained from Porphyrin Products (Logan, UT). Cyanoketone was a gift from the Sterling-Winthrop Laboratories (Rensselaer, NY). Pregnenolone antisera were purchased from Radioassay Systems Laboratories (Carson, CA). [7-^{3}H] Pregnenolone was purchased from New England Nuclear (Boston, MA). All animal experimentation described in this study was performed with the highest standards of humane care according to the guidelines set forth by the National Institutes of Health.

Animal and tissue preparation. Adult male Sprague-Dawley rats (200–250 g) were obtained from Harlan Industries (Madison, WI) and were allowed access to food and water *ad libitum*. Rats were injected with either Sn- or Zn-protoporphyrin (50.0 μmol/kg, s.c.) and 4 d later were given a second similar dose of the respective compound. These animals were killed 7 d after the first injection. In one series of experiments, rats were treated with the same dose of metalloprotoporphyrins and killed 6 h later. Control animals received saline. Shortly before use, 25.0-mM metalloprotoporphyrin solutions were prepared by dissolving the compounds in minimum volumes of 0.1 N NaOH and 95% ethanol. Subsequently, the solution was diluted with 0.9% (wt/vol) NaCl and adjusted to pH 7.2–7.4 by the addition of 1.0 N NaOH. The volume of injection was 200 μL/100 g body weight. Blood was obtained by cardiac puncture after the administration of 50.0 mg/kg sodium pentobarbital (intraperitoneally). Subsequently, the animals were decapitated and the organs were perfused *in situ* with saline. The adrenals were defatted and homogenized in 20 tissue volume of 10.0 mM Tris-HCl buffer, pH 7.4, that contained 0.25 M sucrose (27). The mitochondrial and microsomal fractions were prepared by differential centrifugation as described previously (27). The hepatic microsomal fraction was obtained as detailed elsewhere (14).

The serum was assayed for the estimation of corticosterone. The adrenal mitochondrial fraction was assayed for cholesterol side-chain cleavage and 11β-hydroxylase activities and cytochrome P-450 and heme contents. The mitochondrial and microsomal fractions were assayed for benzo(a)pyrene hydroxylase, and the microsomal fraction was used for assessment of NADPH-cytochrome P-450 reductase and 21α-hydroxylase activities. Heme oxygenase activity was measured in the liver and adrenal microsomal fractions. The experimental procedures described in the following sections were carried out under subdued lighting.

Enzyme assays on subcellular fractions. Benzo(a)pyrene hydroxylase activity was measured by the fluorometric procedure of Dehnen *et al.* (31). NADPH-cytochrome P-450 reductase activity was determined by measuring the increase in absorbance at 550 nm due to the reduction of cytochrome c in the presence of NADPH (32). The microsomal heme oxygenase activity was assessed as detailed previously (30). The activity of adrenal 21α-hydroxylase was determined by using a modification of the assay procedure described by Menard *et al.* (33). Briefly, an incubation mixture (1.0 mL) consisting of 200 μg of adrenal microsomal protein, 150 μM progesterone, and 0.2 mM NADPH was incubated for 15 min at 37°C. The hydroxylated products were extracted into methylene chloride, evaporated to dryness, and subsequently redissolved in methanol. The products were analyzed by HPLC using a 246-nm wavelength for detection. A Waters 6000 LC system (Waters, Milford, MA) equipped with a Rheodyne 7025 injector (Rheodyne, Cotati, CA) with a 20-μL loop was used. The separations were performed using a 5-μm Zorbax ODS column (Dupont, Wilmington, DE) (4.6 mm × 25 cm) with an isocratic solvent system that was composed of 75% methanol in water at a flow rate of 1 mL/min. The system was operated at ambient temperature. The steroids were quantitated by comparing the sample peak area with that of the external standards.

Adrenal mitochondrial 11β-hydroxylase activity was estimated by measuring the rate of conversion of the substrate, deoxycorticosterone, to corticosterone (34). The simultaneous additions of 10 mM D,L-isocitrate and 0.2 mM NADPH were used to initiate the reaction. The product was analyzed by HPLC using the same Zorbax column and an isocratic elution of 67.5% methanol in water at a flow rate of 1 mL/min. Detection was made spectrophotometrically at 246 nm and quantitated by use of the external standards.

Mitochondrial cholesterol side-chain cleavage activity was determined as described previously (27) using the modified procedure of Mason *et al.* (35). The assay system (1.0 mL) consisted of 0.1 mg of adrenal mitochondrial protein, 25-hydroxycholesterol (100 μM) and cyanoketone (6 μM). The reaction mixture was initiated by the simultaneous addition of 10 mM D,L-isocitrate and 0.2 mM NADPH. At 10 min, 500-μL aliquots were removed and used for extraction of the product, pregnenolone. Pregnenolone was subsequently measured by the RIA method of Abraham *et al.* (36) and quantitated using a standard curve for pregnenolone concentrations of 0.1–2.0 ng.

Spectral studies on subcellular fractions. The mitochondrial cytochrome P-450 was measured by the procedure previously described (28). The mitochondrial preparation (0.25 mg protein/mL) was suspended in 20 mM Tris-HCl buffer, pH 7.5, containing 1 mM EDTA, 20% glycerol, and 0.2% Emulgen 911 (KAO Atlas, Japan) and frozen/thawed one time. Sodium ascorbate and *N,N,N′,N′*-tetramethylphenylenediamine (16 and 0.24 mM, final concentrations, respectively) were added to the suspension as reducing agents, and the mixture was incubated for 5 min at 30°C. The suspension was equally divided between two cuvettes. The test cuvette was bubbled with CO and the baseline was recorded. Thereafter, sodium dithionite was added to both cuvettes and the CO difference spectrum was recorded. An extinction coefficient of 91 $cm^{-1} \cdot mM^{-1}$ was used for calculation of the hemoprotein content. Total heme concentration was measured by the pyridine hemochromogen method of Paul *et al.* (37).

The protein concentration was determined by the method of Lowry *et al.* (38) with BSA as the protein standard.

Determinations of serum corticosterone. The determination of serum concentration of corticosterone was performed by a modification of the procedure of Stoks and Benraad (39). Briefly, 1-mL aliquots of serum were extracted with 5.0 mL of methylene chloride. Subsequently, the organic phase was separated and evaporated to dryness, and the residue was reconstituted with methanol. The extracts were analyzed by the HPLC procedure that was described above for 11β-hydroxylase assay.

Data analysis. The data were analyzed using nonpaired *t* test with a two-tailed *t* distribution; a value of $p \leq 0.05$ was considered to denote significance. The data are the mean ± SD; the number of determinations are indicated in appropriate tables and figures. One or two rats were used for each determination.

RESULTS

Figure 1 depicts the comparative effects of Zn- and Sn-protoporphyrins on rat adrenal microsomal 21α-hydroxylase and mitochondrial 11β-hydroxylase activities. For this experiment, animals were treated with the synthetic metalloporphyrins (50 μmol/kg, s.c.) on d 1 and 4 and killed 3 d after the last injection. As shown, both hydroxylase activities were notably decreased in Sn-protoporphyrin-treated rats, with 21α-hydroxylase measuring only about 30% of the control and 11β-hydroxylase amounting

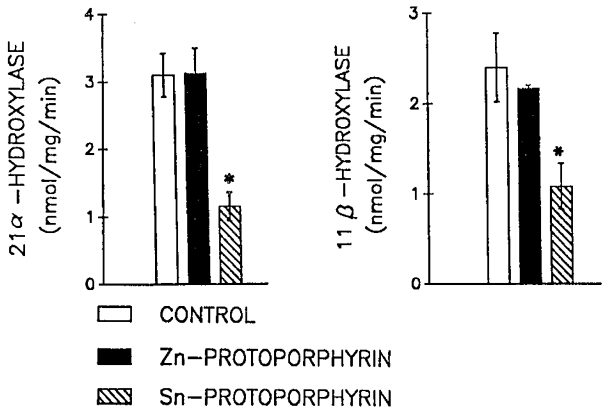

Fig. 1. Comparative effects of Zn- and Sn-protoporphyrin *in vivo* on adrenal microsomal 21α-hydroxylase and mitochondrial 11β-hydroxylase activities. Male Sprague-Dawley rats were treated with two doses of 50 μmol/kg Zn- or Sn-protoporphyrin as described in Materials and Methods. The adrenal microsomal fraction was used for measurement of 21α-hydroxylase, as assessed from the rate of 11-deoxycorticosterone formation. The mitochondrial fraction was used for measurement of 11β-hydroxylase activity, as determined by the rate of corticosterone production. Experimental details are provided in Materials and Methods. Data shown are the mean ± SD of four to five determinations per treatment group; the pooled adrenals of two rats were used for each determination. An *asterisk* denotes $p \leq 0.05$ when compared with the control group.

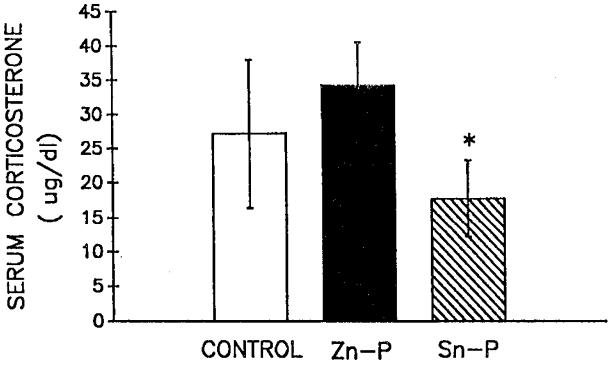

Fig. 2. Effect *in vivo* of Zn- and Sn-protoporphyrin treatments on serum corticosterone levels. Rats were treated as described in the legend to Figure 1 with metalloporphyrins. Blood was removed under pentobarbital anesthesia by cardiac puncture and serum was used for measurement of corticosterone levels by the HPLC method described in the text. Data shown are the mean ± SD of 11 determinations per treatment group. An *asterisk* denotes $p \leq 0.05$ when compared with the control group.

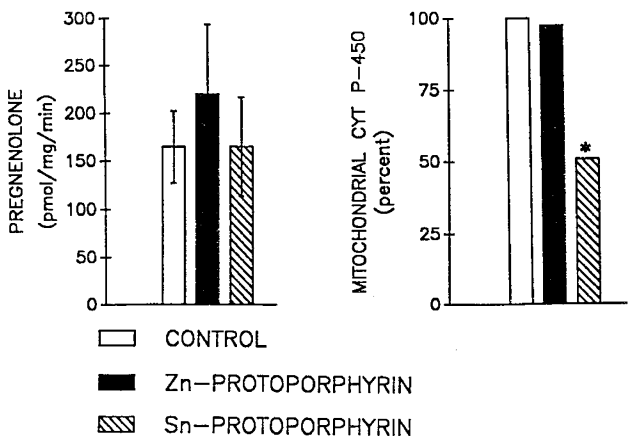

Fig. 3. Effect *in vivo* of Zn- and Sn-protoporphyrin on adrenal mitochondrial contents of cytochrome P-450 and side-chain cleavage activity. Rats were treated with two doses of metalloporphyrins as detailed in Materials and Methods. The adrenal mitochondrial fraction was prepared as detailed previously (27) and used for assessment of cytochrome P-450$_{SCC}$ content and the rate of side-chain cleavage activity. The control value for cytochrome P-450 was 817 ± 93 pmol/mg protein. Methods used for enzyme assays are described in detail in the text. The data shown are the mean ± SD of six to eight determinations per treatment group. The pooled adrenals from two rats were used for each determination. An *asterisk* indicates $p \leq 0.05$ when compared with the control group.

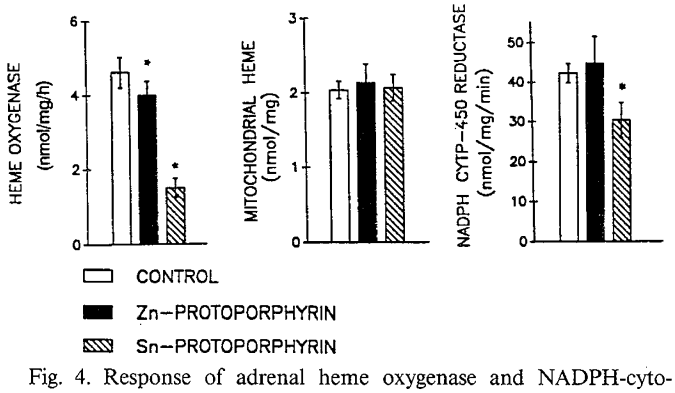

Fig. 4. Response of adrenal heme oxygenase and NADPH-cytochrome P-450 reductase activities and total mitochondrial heme content to Zn- and Sn-protoporphyrin treatments. Adrenals were obtained from rats treated with synthetic metalloporphyrins (50 μmol/kg, twice) and used for subcellular fractionation. The microsomal preparation was used for measurement of heme oxygenase and NADPH-cytochrome P-450 reductase activities and the mitochondrial fraction was used for assessment of total heme content by the pyridine hemochromogen method (37). Experimental details are provided in Materials and Methods. Data shown are the mean ± SD of five to six determinations per treatment group. An *asterisk* indicates $p \leq 0.05$ when compared with the control group.

to about 40% of the control value. In contrast, in Zn-protoporphyrin-treated animals neither activity was altered. To examine the ramification of these changes in steroid hydroxylase activities, serum corticosterone levels were measured. The findings that are shown in Figure 2 denote a significant reduction in serum corticosterone levels in Sn-protoporphyrin-treated rats. As could have been predicted, Zn-protoporphyrin did not exert an effect on serum glucocorticoid levels.

To further explore the effect of the metalloporphyrins on adrenal steroidogenesis, the mitochondrial cytochrome P-450 content and side-chain cleavage activity were assessed. Data are shown in Figure 3. As shown, a marked reduction of nearly 50% in the cytochrome P-450 content was detected in preparations obtained from Sn-protoporphyrin-treated animals. Again, Zn-protoporphyrin did not affect the hemoprotein level. The cholesterol side-chain cleavage activity as assessed by pregnenolone formation, however, was not significantly altered in Sn-protoporphyrin-treated animals.

The underlying basis for decrease in cytochrome P-450 was examined by measuring total mitochondrial heme concentration. As noted in Figure 4, a decrease in heme content was not detected, suggesting that modulation in the hemoprotein concentration was not a result of an overall decrease in cellular concentrations of the heme prosthetic moiety. Furthermore, the decrease in the cytochrome level did not reflect an accelerated rate of cellular heme degradation because the activity of heme oxygenase was severely decreased in Sn-protoporphyrin-treated animals (Fig. 4). Zn-protoporphyrin also caused a modest but significant decrease in heme oxygenase activity. The activity of heme oxygenase requires the concerted activity of the oxygenase and NADPH-cytochrome P-450 reductase; therefore, the possibility

was examined that the observed inhibition of bilirubin formation by metalloporphyrins may be due to an inhibited reductase activity. The results of this investigation are also shown in Figure 4. As shown, Sn-protoporphyrin significantly inhibited the reductase activity. The magnitude of inhibition, which measured about 30%, could contribute to the observed depression in bilirubin formation by the heme oxygenase system; Zn-protoporphyrin treatment did not affect the reductase activity.

To determine whether the observed perturbations were restricted to steroid hydroxylation processes or extended to other biotransformations that depend on cytochrome P-450, hydroxylation of the prototype drug, benzo(a)pyrene, by the adrenals of the metalloporphyrin-treated animals was examined. Data are shown in Table 1. As noted, drug hydroxylation by both the mitochondrial and the microsomal fractions of Sn-protoporphyrin-treated rats was significantly decreased. The decrease in the microsomal activity was particularly notable wherein activity was decreased by more than 45%. The activity of the mitochondrial fraction was decreased by about 20%. Because the microsomal drug hydroxylation also requires the concerted activity of NADPH-cytochrome P-450 reductase, the depression of the reductase activity (Fig. 4) could be, in part, responsible for this observation. In Zn-protoporphyrin-treated rat adrenals, significant alterations in benzo(a)pyrene hydroxylase activities of the microsomal or mitochondrial fraction were not detected.

To examine the acute effectiveness of the metalloporphyrins to inhibit bilirubin production by the adrenals as well as in other organs, the adrenal and hepatic heme oxygenase activity of animals treated once with the metalloporphyrins and killed 6 h later was examined (Table 2). In the adrenals of treated rats, the enzyme activity was not severely affected by the metalloporphyrins; however, in the liver, activity was essentially undetectable in Sn-protoporphyrin-treated rats, whereas it was inhibited by about 55% in the Zn-protoporphyrin-treated animals.

Table 1. *Effect in vivo of Sn- and Zn-protoporphyrin on adrenal mitochondrial and microsomal cytochrome P-450-dependent mixed-function oxidase activities**

	Benzo(a)pyrene hydroxylase	
Treatment	Mitochondria (pmol/mg/min)	Microsomes (pmol/mg/min)
Sn-protoporphyrin	100.8 ± 4.6†	241.4 ± 15.7†
Zn-protoporphyrin	130.0 ± 9.9	398.0 ± 57.2
Control	126.9 ± 15.1	425.4 ± 24.7

* Sprague-Dawley Rats were treated with two doses of Zn- or Sn-protoporphyrin in 7 d as described in Materials and Methods. Control rats received saline. The mitochondrial and the microsomal fractions were prepared and used for measurement of benzo(a)pyrene hydroxylase activity. Experimental details are provided in the text. Data show the mean ± SD of four to six determinations.

† $p \leq 0.05$ when compared with the control group.

Table 2. *Suppression in vivo of hepatic heme oxygenase activity by Zn- or Sn-protoporphyrin treatment**

	Heme oxygenase (nmol/mg/h)	
Metalloporphyrin	Adrenals	Liver
Control	4.99 ± 0.98	5.9 ± 0.89
Zn-protoporphyrin	3.16 ± 0.70†	2.6 ± 0.32†
Sn-protoporphyrin	3.85 ± 0.44	≈0†

* Rats were treated with Zn- or Sn-protoporphyrin (50 μmol/kg) and killed after 6 h. The adrenal and hepatic microsomal fractions were used for assessment of heme oxygenase activity. Activity was measured in the presence of purified biliverdin reductase as detailed previously (30). Data shown are the mean ± SD of four determinations.

† $p \leq 0.05$ when compared with the control value.

DISCUSSION

When the ability of metalloporphyrins to inhibit *in vivo* heme oxygenase activity was first discovered (2), although both Sn- and Zn-protoporphyrins were found to be effective, the potential use of the Zn-protoporphyrin but not of the tin complex was suggested as a possible means to obviate the postparturition surge in serum bilirubin levels. This suggestion was based on considerations of the molecular properties of the two metalloporphyrins, as well as the apparent selectivity of Zn-protoporphyrin to inhibit the hepatic heme oxygenase activity, albeit only a few drug metabolic parameters were examined at that time (2). The subsequent reports (3, 4) on the superior efficiency of Sn-protoporphyrin to inhibit heme oxygenase rapidly culminated in clinical testing of the tin complex in humans, including newborn infants, to suppress production of bilirubin (9, 10, 12); it was assumed that Sn-protoporphyrin is a safe and selective inhibitor of the enzyme. Again the assumption of safety was for the most part based on limited findings mainly obtained by assessing a narrow spectrum of hepatic cytochrome P-450-dependent drug metabolism and hematologic parameters (7, 9, 17, 18). The selected parameters apparently were nonresponsive to the Sn-protoporphyrin, because long-term administration of massive doses (100 μmol/kg/wk) of the metalloporphyrin for up to 32 wk or treatment of animals for five consecutive d with large doses of 100 μmol/kg of the metalloporphyrin complex was reported not to have appreciable adverse effects on activities of several enzymes of heme biosynthesis in the liver, contents of hepatic and renal microsomal cytochrome P-450, or hematologic indices such as red blood cell count, Hb and protoporphyrin concentrations, and hematocrit (17). The findings of that study, however, contrasted those of another investigation that reported on the diminished capacity of the hepatic microsomal fraction of juvenile rats treated with Sn-protoporphyrin (50 μmol/kg/d for 6 d) to carry out mixed-function oxidase activities (40).

The results of the present study clearly show that those hemoprotein species that are present in the steroidogenic organ, the adrenals, are highly susceptible to adverse effects of Sn-protoporphyrin. In this organ, activities of both the microsomal 21α-hydroxylase and the mitochondrial 11β-hydroxylase were severely inhibited (Fig. 1). Insofar that the adrenal cortex is the site of glucocorticoid production, as would be predicted, a significant decrease in serum glucocorticoid, corticosterone, was noted as the result of metalloporphyrin treatment (Fig. 2). In addition, the general ability of the organ to carry on cytochrome P-450-dependent biotransformations, as reflected by the metabolism of the prototype drug, benzo(a)pyrene, was significantly curtailed in both the mitochondrial and the microsomal fractions (Table 1). The present study also finds that Zn-protoporphyrin, by and large, does not affect the assessed parameters, although it effectively inhibits heme oxygenase activity.

The observation with adrenals where the concentration of cytochrome P-450 (Fig. 3) was decreased but the decrease was not reflected in side-chain cleavage activity resembles what we have observed in the past with gonadotropin-releasing hormone and human chorionic gonadotropin effects on testis; both hormones cause significant increases in cholesterol side-chain cleavage activity without a concomitant increase in cytochrome P-450 content. These observations, in turn, are analogous to the reported action of ACTH that causes an increase in cytosolic peptide in the adrenal cortex, which in turn increases the rate of association of cholesterol with cytochrome $P\text{-}450_{SCC}$ and stimulates side-chain cleavage activity without increasing the levels of the cytochrome (23). Accordingly, it is plausible that the presently observed absence of a decrease in side-chain cleavage activity of the adrenal mitochondria, in the face of a significant decrease in cytochrome P-450 content, may also reflect an increased association of substrate with the enzyme. Moreover, the underlying basis for decrease in the hemoprotein level appears not to involve a decrease in synthesis or increased degradation

of heme; rather, it appears to reflect a defect in the availability of the apoprotein moiety and/or formation of holocytochrome. Also, potentially, Sn-protoporphyrin could cause destabilization and denaturation of the hemoprotein.

The mechanism by which Sn-protoporphyrin leads to disruption of steroidogenic activity appears to be independent of its effects on heme oxygenase activity. This suggestion is based on the reasoning that a decrease in heme oxygenase would be expected to cause an increase, rather than a decrease, in hemoprotein-dependent functions (41, 42). A plausible mechanism by which Sn-protoporphyrin exerts its adverse effects is through the known activity of the complex to be a potent photosensitizer and generator of singlet oxygen free radicals (43–45). Indeed, various metal chelates of porphyrins are known to directly degrade proteins and hemoprotein constituents of cellular membranes (46, 47). This, plus the fact that the complex has a rather long duration of tissue retention (48), could provide a highly plausible explanation for the mechanism of action of Sn-protoporphyrin and its prolonged effects on steroidogenesis. This suggestion is supported by the report (43) that a concentration of Sn-protoporphyrin, which is an order of magnitude lower than that found in rat tissues (48) 1 wk after s.c. injection of a dose of Sn-protoporphyrin similar to that used in the present study, promotes severe photodegradation of bilirubin. Moreover, although both Sn- and Zn-protoporphyrins are photosensitizing agents (49), when these complexes were compared for bilirubin photodegrading activity (50), the tin compound was found to be by far more potent, and, when the metalloporphyrins were examined for causing mortality in neonatal rats after exposure to light, only the tin compound was found to cause mortality (13). Accordingly, it may be speculated that the seemingly higher potency of Sn-protoporphyrin to inhibit heme oxygenase could in part reflect photodegradation of bilirubin and destruction of the microsomal membrane constituents (45), leading to the appearance that production of the bile pigment by heme oxygenase enzyme is inhibited.

Based on the present findings, demonstrating that although Sn- and Zn-protoporphyrin are effective in inhibiting heme oxygenase activity, the tin complex also exerts notable adverse effects on the adrenal functions; therefore, it would appear wise to exert extreme caution in the use of metalloporphyrins in human infants. Furthermore, in light of the fact that steriods play a major regulatory role in development and gene expression in higher animals including humans (51), the present finding that a rather moderate regimen of Sn-protoporphyrin treatment causes quite notable perturbations in cytochrome P-450-dependent steroidogenesis should be seriously considered when using the tin complex to treat hyperbilirubinemia in humans, particularly infants.

Acknowledgment. The authors thank L. Schenk for preparation of the manuscript.

REFERENCES

1. Maines MD, Kappas A 1977 Enzymatic oxidation of cobalt-protoporphyrin-IX: observations on the mechanism of heme oxygenase. Biochemistry 16:419–422
2. Maines MD 1981 Zinc-protoporphyrin is a selective inhibitor of heme oxygenase activity in the neonatal rat. Biochim Biophys Acta 673:339–350
3. Drummond GS, Kappas A 1981 Prevention of neonatal hyperbilirubinemia by tin protoporphyrin IX, a potent competitive inhibitor of heme oxidation. Proc Natl Acad Sci USA 78:6466–6470
4. Drummond GS, Kappas A 1982 Chemoprevention of neonatal jaundice: potency of tin-protoporphyrin in an animal model. Science 217:1250–1253
5. Maines MD, Veltman JC 1984 Phenylhydrazine-mediated induction of haem oxygenase activity in rat liver and kidney and development of hyperbilirubinemia: inhibition by zinc-protoporphyrin. Biochem J 217:409–417
6. Kappas A, Drummond GS, Simionatto CS, Anderson KE 1984 Control of heme oxygenase and plasma levels of bilirubin by a synthetic heme analogue, tin-protoporphyrin. Hepatology 4:336–341
7. Kappas A, Drummond GS 1985 Synthetic metalloporphyrins: a class of compounds of pharmacological interest. Bioessays 3:256–259
8. Qato M, Maines MD 1985 Prevention of neonatal hyperbilirubinemia in nonhuman primates by Zn-protoporphyrin. Biochem J 226:51–57
9. Kappas A, Drummond GS, Manola T, Petmezaki S, Valaes T 1988 Sn-protoporphyrin use in management of hyperbilirubinemia in term newborns with direct Coombs-positive ABO incompatibility. Pediatrics 81:485–497
10. Berglund L, Angelin B, Blomstrand R, Drummond GS, Kappas A 1988 Sn-protoporphyrin lowers serum bilirubin levels, decreases biliary bilirubin output, enhances biliary heme excretion and potently inhibits hepatic heme oxygenase activity in normal human subjects. Hepatology 8:625–631
11. Hamori CH, Vreman HK, Rodgers PA, Stevenson DK 1989 Zinc protoporphyrin inhibits CO production in rats. J Pediatr Gastroenterol Nutr 8:110–115
12. Berglund L, Angelin B, Hultcrantz R, Einarsson K, Emtestam L, Drummond GS, Kappas A 1990 Studies with the haem oxygenase inhibitor Sn-protoporphyrin in patients with primary biliary cirrhosis and idiopathic haemochromatosis. Gut 31:899–904
13. Hintz SR, Vreman HJ, Stevenson DK 1990 Mortality of metalloporphyrin-treated neonatal rats after light exposure. Dev Pharmacol Ther 14:187–192
14. Maines MD, Trakshel GM, Kutty RK 1986 Characterization of two constitutive forms of rat liver microsomal heme oxygenase: only one molecular species of the enzyme is inducible. J Biol Chem. 261:411–419
15. Tenhunen R, Marver HS, Schmid R 1969 Microsomal heme oxygenase: characterization of the enzyme. J Biol Chem 244:6388–6394
16. Maines MD, Kappas A 1974 Cobalt induction of hepatic heme oxygenase; with evidence that cytochrome P-450 is not essential for this enzyme activity. Proc Natl Acad Sci USA 71:4293–4297
17. Sassa S, Drummond GS, Bernstein SE, Kappas A 1985 Long-term administration of massive doses of Sn-protoporphyrin in anemic mutant mice (sph^{ha}/sph^{ha}). J Exp Med 162:864–876
18. Drummond GS, Rosenberg DW, Kihlström-Johanson AC, Kappas A 1989 Effects of tin-porphyrin on developmental changes in hepatic cytochrome P450 content, selected P450-dependent drug-metabolizing enzyme activities, and brain glutathione levels in the newborn rat. Pharmacology 39:273–284
19. Estabrook RW, Cooper DY, Rosenthal O 1963 The light reversible carbon monoxide inhibition of the steroid C21-hydroxylase system of the adrenal cortex. Biochem J 338:741–755
20. Mitani F 1979 Cytochrome P-450 in adrenocortical mitochondria. Mol Cell Biochem 24:21–43
21. Nozu R, Matsuura S, Catt KJ, Dufau ML 1981 Modulation of Leydig cell androgen biosynthesis and cytochrome P-450 levels during estrogen treatment and human chronic gonadotropin-induced desensitization. J Biol Chem 256:10012–10017
22. Nakajin S, Shinoda M, Haniu M, Shively JE, Hall PF 1984 C21 steroid side chain cleavage enzyme from porcine adrenal microsomes. Purification and characterization of the 17α-hydroxylase C17,20-lyase cytochrome P-450. J Biol Chem 259:3971–3976
23. Simpson ER, Waterman MR 1988 Regulation of the synthesis of steroidogenic enzymes in adrenal cells by ACTH. Am Rev Physiol 50:427–432
24. Zuber MX, Simpson ER, Hall PF, Waterman MR 1985 Effects of adrenocorticotropin on 17α-hydroxylase activity and cytochrome P-450 17α synthesis in adrenocortical cells. J Biol Chem 260:1842–1848
25. Maines MD, Jollie DR 1984 Dissociation of heme metabolic activities from the microsomal cytochrome P-450 turnover in testis of hypophysectomized rats. J Biol Chem 259:9557–9562
26. Maines MD, Mayer RD 1985 Inhibition of testicular cytochrome P-450-dependent steroid biosynthesis by *cis*-platinum: reversal by human chorionic gonadotropin. J Biol Chem. 260:6063–6068
27. Veltman JC, Maines MD 1986 The regulatory effect of copper on rat adrenal cytochrome P-450 and steroid metabolism. Biochem Pharmacol 35:2903–2909
28. Maines MD, Sluss PM, Iscan M 1990 *cis*-Platinum-mediated depression of LH receptors and cytochrome $P\text{-}450_{SCC}$ in rat Leydig cells: a possible mechanism for decrease in serum testosterone. Endocrinology 126:2398–2406
29. Maines MD 1990 Effects of *cis*-platinum on heme, drug and steroid metabolism pathways: possible involvement in infertility and nephrotoxicity. CRC Crit Rev Toxicol 21:1–20
30. Kutty RK, Daniel RF, Ryan DE, Levin W, Maines MD 1988 Rat liver cytochrome P-450b, P-420b, and P-420c are degraded to biliverdin by heme oxygenase. Arch Biochem Biophys 260:638–644
31. Dehnen W, Tomingas R, Roos J 1973 A modified method for the assay of benzo(a)pyrene hydroxylase. Anal Biochem. 53:373–383
32. Strobel HW, Dignam JD 1978 Purification and properties of NADPH-cytochrome P-450 reductase. Methods Enzymol 52:89–96
33. Menard RH, Martin HF, Stripp B, Gillette JR, Bartter FC 1974 Spironolactone and cytochrome P-450: impairment of steroid hydroxylation in the adrenal cortex. Life Sci 15:1639–1648
34. Gallant S, Bruckheimer SM, Brownie AC 1978 The use of high-pressure liquid chromatography in the simultaneous assay of 11β-hydroxylase and 18-hydroxylase in zona fasciculata-reticularis tissue of the rat adrenal cortex. Anal Biochem 89:196–202
35. Mason JI, Arthur JR, Boyd GS 1978 Control of sterol metabolism in rat adrenal mitochondria. Biochem J 174:1045–1051
36. Abraham GE, Buster JE, Kyle FW, Corrales PC, Teller RC 1973 Radioimmunoassay of plasma pregnenolone. J Clin Endocrin Metab 37:40–45
37. Paul KG, Theorell H, Akeson A 1942 The molar light absorption of pyridine ferroprotoporphyrin. Acta Chem Scand 7:1284–1297
38. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ 1951 Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275
39. Stoks PG, Benraad TJ 1983 Rapid separation of plasmoid steroids by reverse-

phase high performance liquid chromatography with timed collection of fractions. J Chromatogr 276:408–412
40. Stout DK, Becker FF 1988 The effects of tin-protoporphyrin administration on hepatic xenobiotic metabolizing enzymes in the juvenile rat. Drug Metab Dispos 16:23–26
41. Maines MD 1984 New developments in the regulation of heme metabolism and their implications. CRC Crit Rev Toxicol 12:241–314
42. Maines MD 1988 Heme oxygenase: function, multiplicity, regulatory mechanisms, and clinical applications. FASEB J 2:2557–2568
43. McDonagh AF, Palma LA 1985 Tin protoporphyrin: a potent photosensitizer of bilirubin obstruction. Photochem Photobiol 42:261–264
44. McDonagh AF 1988 Purple versus yellow: preventing neonatal jaundice with tin-protoporphyrin. J Pediatr 113:777–781
45. Land EJ, McDonagh AF, McGarvey DJ, Truscott TG 1988 Photophysical structures of tin (IV) protoporphyrin IX dichloride, a novel chemotherapeutic agent for the prevention of neonatal jaundice. Proc Natl Acad Sci USA 85:5249–5253
46. Aft RL, Muller GC 1984 Hemin-mediated degradation of proteins. J Biol Chem 259:301–305
47. Maines MD, Kappas A 1975 The degradative effects of porphyrins and heme compounds on the components of the microsomal mixed-function oxidase. J Biol Chem 250:2363–2369
48. Anderson CE, Simionatto CS, Drummond GS, Kappas A 1986 Disposition of tin-protoporphyrin and suppression of hyperbilirubinemia in humans. Clin Pharmacol Ther 39:510–517
49. Delaney JK, Mauzerall D, Drummond GS, Kappas A 1988 Photophysical properties of Sn-porphyrins: potential clinical implications. Pediatrics 81:498–504
50. Vreman HJ, Stevenson DK 1990 Metalloporphyrin-enhanced photodegradation of bilirubin *in vitro*. Am J Dis Child 144:590–594
51. Fink GR 1986 Translational control of transcription in eukaryotes. Cell 45:155–156.