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Control of Heme and Cytochrome P-450 Metabolism by Inorganic Metals, Organometals and Synthetic Metalloporphyrins

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The heme–cytochrome P-450 complexes represent sensitive metabolic systems for examining the biological impact of metals on important cellular functions. Many metals, both in the inorganic form and bound to organic moieties, potently induce heme oxygenase, the rate limiting enzyme of heme degradation. The resulting increase in the rate of heme breakdown is reflected in a marked depression of cellular cytochrome P-450 content and impairment of the oxidative metabolism of natural and foreign chemicals dependent on this hemeprotein. Organometal complexes do not mimic in all their aspects the actions of the inorganic elements which they contain. For example, organotins, in contrast to inorganic tin, produce a prolonged induction response of heme oxygenase in the liver but not in the kidney. Co-protoporphyrin is a much more potent inducer of heme oxygenase in liver than is inorganic cobalt; and Sn-protoporphyrin inhibits heme oxygenase activity nearly completely, whereas inorganic tin is a powerful inducer of the renal enzyme. Contrasting effects on heme metabolism exist as well within the metalloporphyrin species as demonstrated by the effects *in vivo* of Co-protoporphyrin and Sn-protoporphyrin on heme oxygenase activity; the former induces the enzyme whereas the latter potently inhibits it. *In vitro*, however, both compounds competitively inhibit heme oxidation activity. These differences, among others which characterize metal actions *in vivo* and *in vitro* attest to the importance of pharmacokinetic, adaptive and other host factors in defining the responses of the heme–cytochrome P-450 systems to the impact of metals in the whole animal.

Introduction

Environmental chemicals can produce detrimental effects on cellular processes by perturbing the physiologic control mechanisms for important metabolic systems. The heme-cytochrome P-450 complexes, which play a central role in the oxidative metabolism or detoxification of many foreign chemicals (*1*), are representative of such systems. The effects of many environmental chemicals on specific aspects of heme metabolism are moreover readily definable; for example, both δ-aminolevulinate (ALA) synthase and heme oxygenase, the rate-limiting enzymes of heme synthesis (*2*) and degradation (*3,4*), respectively, are highly inducible enzymes, and the resultant alterations in their activities produced by many chemicals can elicit marked changes in cellular functions dependent on heme. Metals, both inorganic (*5*) as well as complexed to organic moieties (*6*), represent a major category of environmental chemicals which can produce major perturbations of heme and cytochrome P-450 metabolism. Some examples of these metal actions are summarized in this report.

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Methods

Male (175–225 g) and 15-day synchronized pregnant rats were purchased from Taconic Farms (Germantown, NY) and Holtzman (Madison, WI), respectively. The animals were maintained on standard rat chow and drinking water *ad libitum* and were housed in the Rockefeller University Laboratory Animal Research Facility. Metalloporphyrins were purchased from Porphyrin Products (Logan, UT); diethyltin dichloride and tricyclohexyltin were generous gifts of the M&T Chemical Company (Rahway, NJ). All other chemicals were of the highest grade obtainable from either Fisher or Sigma. Animals were treated as indicated in the figures and table; microsomes were prepared and assays performed as previously described (*7,8*).

Results

Effects of Inorganic Metals on Heme Metabolism

Cobalt and other trace elements, as well as certain heavy metals, are potent inducers of heme oxygenase in liver (*5,9*). The administration of cobalt to an animal

produces a rapid rise in heme oxygenase activity which reaches a maximum at 16 hr (Fig. 1) before gradually declining to initial levels by 72 hr (*5*). This action appears to reflect metal induction of *de novo* synthesis of heme oxygenase (*10*). Concurrent perturbations of ALA-synthase activity and a substantial depression of cytochrome P-450 content also occur (Fig. 1). The perturbations in ALA-synthase are characterized by an early depression of enzyme activity followed by a "rebound" phenomenon (*10*). The decline in cytochrome P-450 content is accompanied by parallel decreases in chemical biotransformations mediated by this hemeprotein. In addition to cobalt, inorganic elements such as nickel, platinum, cadmium, tin and chromium, among others, induce these marked but short-term alterations in heme metabolism (*5*). The heme oxygenase inducing action of metals has been studied extensively in liver but also extends to other tissues (Fig. 2). Administration of inorganic tin results in an especially marked increase of heme oxygenase in kidney (Fig. 2).

The heme oxygenase inducing action of some inorganic metals can be blocked by other inorganic elements. For example, manganese when administered simultaneously with tin prevents the increase in heme oxygenase induced by the latter metal (Fig. 2); zinc also has the same blocking effect (*11*). Manganese and zinc administration also prevents the decline in cytochrome P-450 content and the changes in ALA-synthase activity associated with tin administration. The blocking effect of these metals on heme oxygenase induction is lost when they are administered 10 min or more after the inducer element (Fig. 2) indicating that the enzyme synthetic process triggered by tin, and other metals, is initiated rapidly after these inducers are administered.

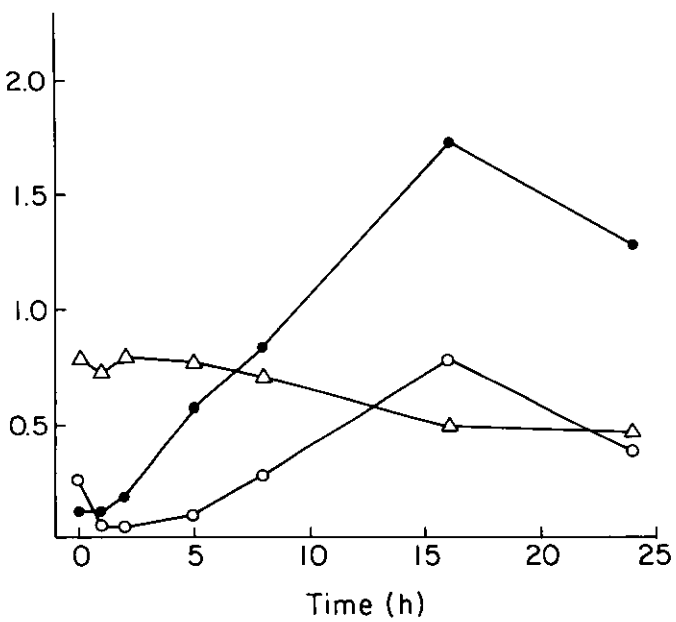

FIGURE 1. Effect of a single dose of cobalt chloride (250 μmole/kg body weight) on heme metabolism in liver. The metal was administered subcutaneously in the nuchal region of adult male rats. Each point represents the average of three animals killed at the times indicated. (Δ) Cytochrome P-450 (nmole/mg); (○) ALA-synthase (nmole ALA/mg/hr); (●) heme oxygenase (nmole bilirubin/mg per hr) × 10^{-1}.

Effects of Organometals on Heme Metabolism

Organometals are compounds in which a central metal atom is directly bound to a carbon atom of an organic moiety; such complexes may have potent and prolonged effects on heme metabolism in the liver and other organs. For example, organometals containing tin, such as tricyclohexyltin and diethyltin dichloride (*6*), produce substantial increases in hepatic heme oxygenase activity in contrast to inorganic tin which does not under the same experimental conditions, produce a significant increase in the activity of this enzyme in liver (Table 1). The effect of a single dose of tricyclohexyltin on hepatic heme oxygenase is shown in Figure 3. Heme oxygenase activity was significantly increased, reaching a maximum 48 hr after organometal administration; associated with the increase in enzyme activity was a concurrent decrease in cytochrome P-450 content and characteristic perturbations in ALA-synthase activity. These alterations in heme metabolism

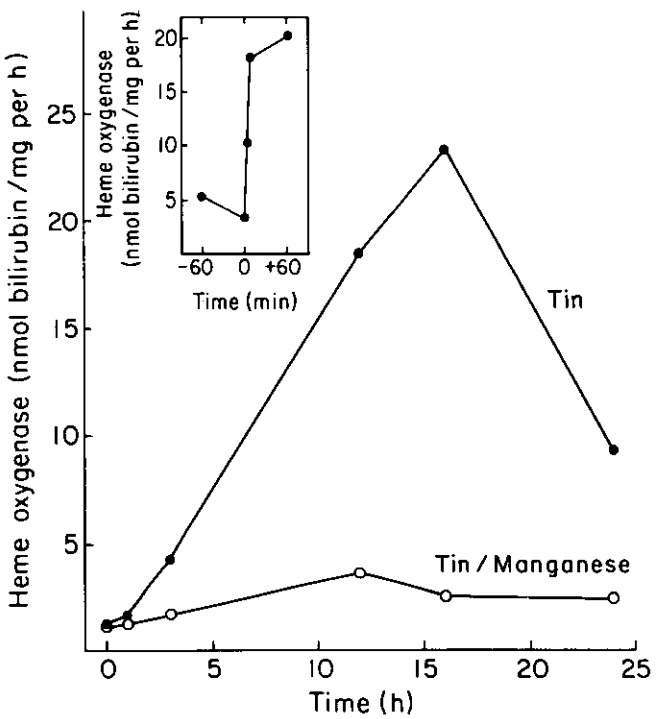

FIGURE 2. Effect of the simultaneous administration of stannous chloride (250 μmole/kg body weight) and manganese chloride (250 μmole/kg body weight) on heme oxygenase activity in kidney. Metals were administered at separate sites in the nuchal region of adult male rats. Each point represents the average of three animals killed at the times indicated. Inset: manganese chloride was administered at the designated times, which ranged from 60 min before (-) to 60 min after (+) stannous chloride, which was administered at zero time.

extend for one week or more after administration of this organometal, in marked contrast to inorganic metals which produce perturbations in heme metabolism which are generally completed by 72 hr (*5*). In addition, while inorganic tin is a potent inducer of heme oxygenase in kidney (Fig. 2) but not in liver (Table 1), the reverse is true for tricyclohexyltin (Table 1), indicating that organification can change the tissue site of action of a metal.

Effects of Synthetic Metalloporphyrins on Heme Metabolism

A third group of metals which can perturb cellular regulatory mechanisms for heme and cytochrome P-450 metabolism are synthetic metalloporphyrins; i.e., metals, other than iron, which are complexed with the protoporphyrin IX moiety. The duration and intensity of the inducing effect on liver heme oxygenase produced by a single dose (125 μmole/kg body weight) of cobalt protoporphyrin (Co-protoporphyrin) are shown in Figure 4. The enzyme inducing effect is striking in terms of the marked elevation of heme oxygenase produced and the prolonged period of time—at least 21 days—during which the enzyme activity remains elevated above normal levels. A concomitant pronounced and sustained depression of cytochrome P-450 is also produced following administration of a single dose of Co-protoporphyrin; the effect shown in Figure 4 lasted for at least 3 weeks. The activities of cytochrome P-450 dependent enzymes paralleled the depression of cellular cytochrome P-450 content. In addition, the activity of ALA-synthase was markedly diminished throughout this time period. One metabolic consequence of Co-protoporphyrin administration in the rat is a rapid and profound suppression of peripheral endocrine secretions (data not shown).

Polyacrylamide gel electrophoresis of microsomal proteins from Co-protoporphyrin treated animals revealed a rapid loss of major bands in the region of M_r 50,000 to 55,000 compared with microsomes isolated from control animals (Fig. 5). The loss of these bands in the region associated with various cytochrome P-450 species parallels the loss in cytochrome P-450 content. The relation of enhanced heme oxygenase activity and profoundly diminished levels of cytochrome P-450, to other expressions of cellular toxicity is not clear. Thus one metal inducer of heme oxygenase, indium, has been shown to produce significant morphological damage in liver (*12*); while Co-protoporphyrin, which produces more pronounced and sustained changes in heme oxygenase and cytochrome P-450, elicits no structural alterations in this organ (*13*).

The ability of another metalloporphyrin tin protoporphyrin (Sn-protoporphyrin) to alter heme metabolic processes stands in marked contrast to the effects of Co-protoporphyrin. Sn-protoporphyrin has the property *in vivo* of potently inhibiting heme oxygenase activity in liver, kidney and spleen (Fig. 6) by acting as a competitive (nonmetabolized) substrate for the enzyme (*14,15*). This effect is long-lasting in all tissues studied

Table 1. Effects of tin and organotins on heme oxygenase activity and cytochrome P-450 content in liver.[a]

Treatment	Dose, mg/kg body wt	Heme oxygenase, nmole bilirubin/mg per hr	Cytochrome P-450, nmole/mg
Saline	—	2.50 ± 0.14	0.74 ± 0.03
Inorganic tin	30	3.16 ± 0.19	0.69 ± 0.06
Diethyltin dichloride	15	7.51 ± 0.31*	0.47 ± 0.01*
Tricyclohexyltin	15	4.75 ± 0.09*	0.49 ± 0.01*

[a]Animals were killed 16 hr after metal administration. Compounds were administered subcutaneously in the nuchal region.
*$p < 0.05$ when compared to control (saline).

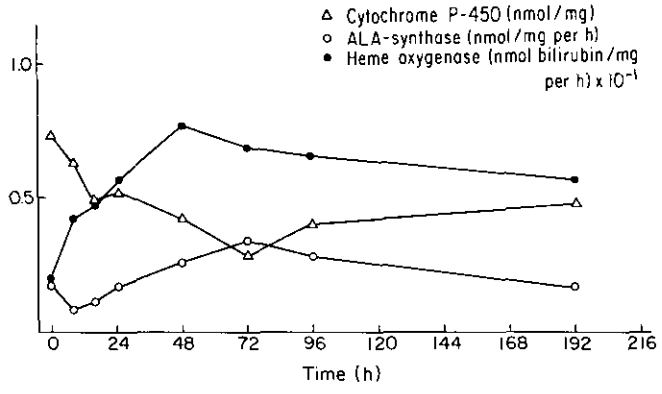

FIGURE 3. Effect of a single dose of tricyclohexyltin (40 μmole/kg bw) on heme metabolism in liver. Tricyclohexyltin was dissolved in 95% EtOH and administered subcutaneously in the nuchal region of adult male rats. Each point represents the average of three animals killed at the times indicated.

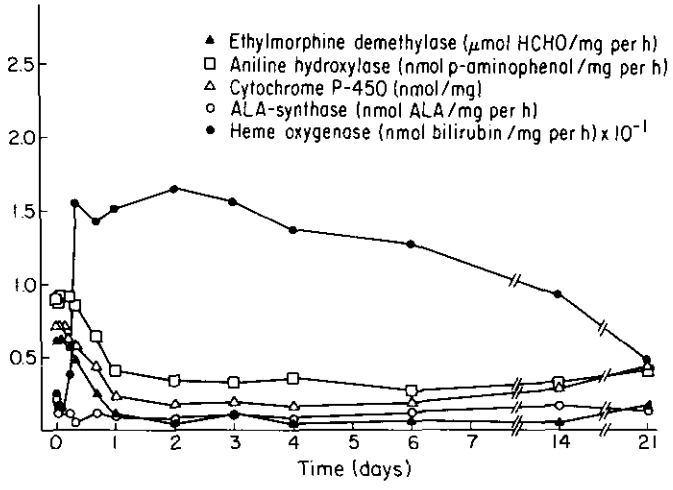

FIGURE 4. Effect of a single dose of Co-protoporphyrin (125 μmole/kg body weight) on heme metabolism in liver. Co-protoporphyrin was dissolved in 0.5N NaOH (0.2 mL/mL final volume), the pH adjusted with 1N HCl to pH 7.4 and made up to final volume with saline. The solution was then injected subcutaneously in the nuchal region within 10 min of preparation. Each point represents the average of three animals killed at the times indicated.

(Fig. 6). The ability of Sn-protoporphyrin to inhibit heme oxygenase *in vivo* for considerable periods of time has been explored in the neonate to determine whether this metalloporphyrin could prevent the developmental changes in heme degradation which characterize the postnatal period (*16*) and whether the metalloporphyrin could concomitantly suppress the hyperbilirubinemia which occurs at this time. As shown in Figure 7, Sn-protoporphyrin administration in a single dose at birth produced a prompt decrease in hepatic heme oxygenase activity; low levels of enzyme activity were maintained throughout the 14-day study period; similar results were noted in kidney. Sn-protoporphyrin also prevented the normal postnatal increase of splenic heme oxygenase (Fig. 7) and produced a prompt (within 24 hr) decline in serum bilirubin to levels comparable to those in normal adult animals. Thus Sn-protoporphyrin proved to be highly effective in suppressing the hyperbilirubinemia that occurs in the immediate postnatal period in this species. Chromium protoporphyrin

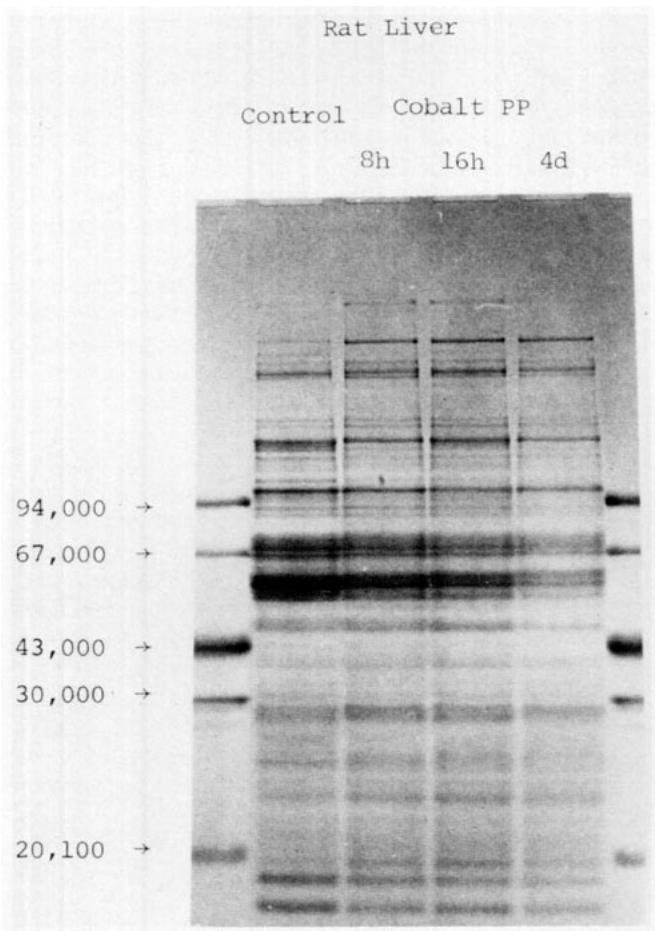

FIGURE 5. $NaDodSO_4$/polyacrylamide gel electrophoresis of hepatic microsomal proteins isolated from control and Co-protoporphyrin (cobalt PP, 125 μmole/kg body weight)-treated rats at the times indicated. The gels were run on the basis of equal amounts of microsomal protein and were stained with Coomassie Blue. The stacking and separating gels contained 5% and 7.5% acrylamide, respectively.

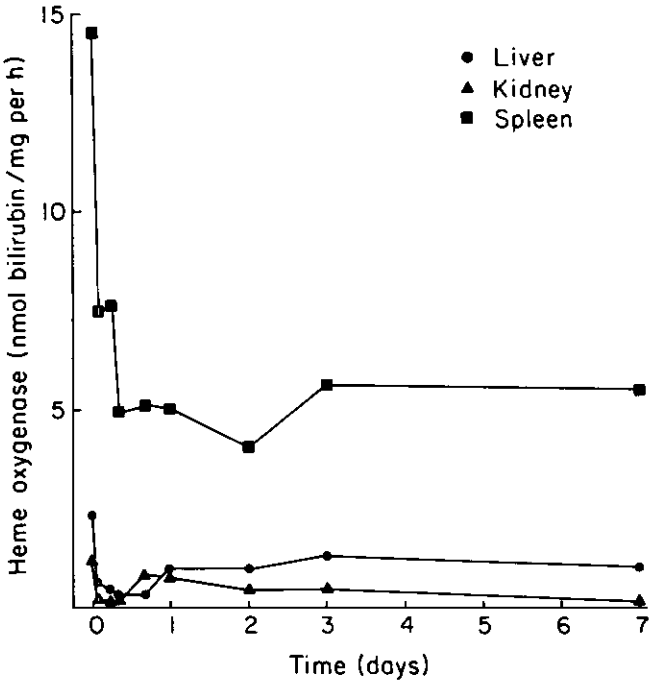

FIGURE 6. Inhibition *in vivo* of heme oxygenase in (●) liver, (▲) kidney and (■) spleen in adult rats after a single administration of Sn-protoporphyrin (5 μmole/kg body weight). Sn-protoporphyrin was prepared in an identical manner to Co-protoporphyrin (Fig. 4) and administered by subcutaneous injection in the nuchal region. Each time point represents the average of at least three animals killed at the times indicated.

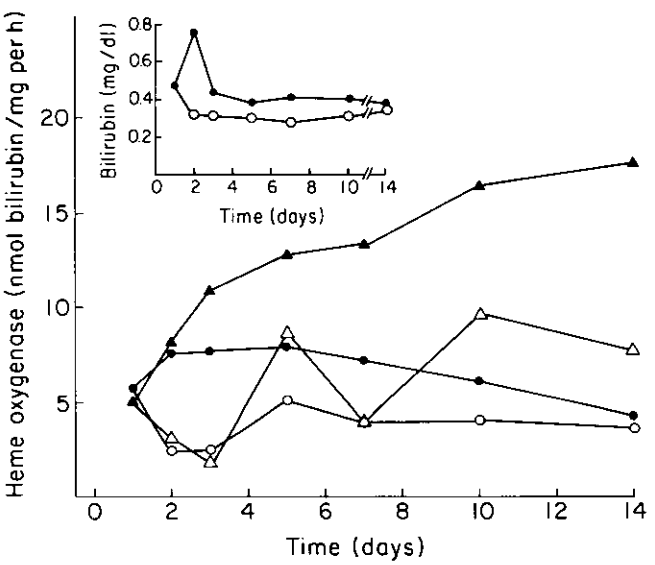

FIGURE 7. Inhibition *in vivo* of heme oxygenase in the liver and spleen of rat neonates after a single administration of Sn-protoporphyrin (10 μmole/kg body weight) at birth: (●) liver heme oxygenase control; (○) treated; (▲) spleen heme oxygenase control; (Δ) treated. The compound was prepared in an identical manner to Co-protoporphyrin (Fig. 4). Each time point represents the average of 12 (later points) to 30 (earlier points) neonates. The total bilirubin levels (inset) were determined fluorometrically on pooled serum samples of (●) control and (○) treated neonates as described earlier (*14*).

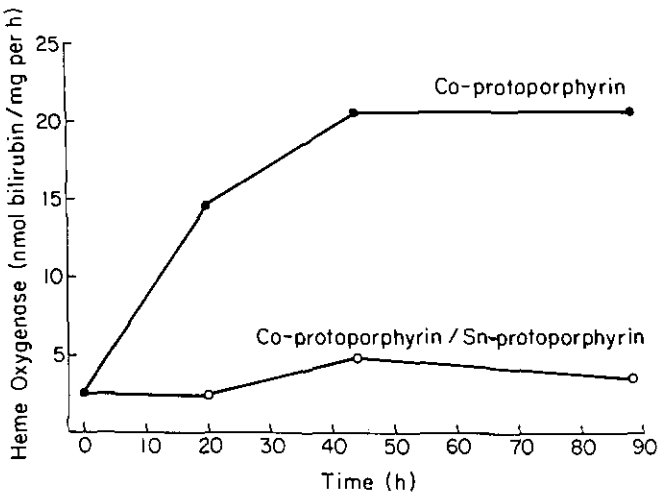

FIGURE 8. Effect of Sn-protoporphyrin administration on Co-protoporphyrin induction of heme oxygenase in the liver of adult male rats. Both metalloporphyrins were administered in a dose of 50 μmole/kg body weight. Sn-protoporphyrin was administered 30 min after Co-protoporphyrin. The route of administration was by subcutaneous injection at separate sites in the nuchal region. Each point represents the average of at least three animals killed at the times indicated.

(Cr-protoporphyrin) exerts a similar suppressive effect on neonatal jaundice (*8*), but other metalloporphyrins studied, such as Mn-protoporphyrin and Zn-protoporphyrins do not, in extremely large amounts, show this property in the rat (*14,17*), even though they can inhibit heme oxygenase activity *in vitro* (*14,15,18*). Co-protoporphyrin also inhibits heme oxygenase activity *in vitro* (*15*), but, as noted, this metalloporphyrin has proved to be *in vivo* an exceptionally potent inducer of the enzyme (*7*). Thus, the ability of a synthetic metalloporphyrin to competitively inhibit heme oxygenase *in vitro* does not assure that the compound can also suppress hyperbilirubinemia in the whole animal.

Sn-protoporphyrin can inhibit heme oxygenase activity, not only in normal animals and in neonates, but also in animals in which the enzyme has been previously induced by other metals or by Co-protoporphyrin (Fig. 8). Sn-protoporphyrin also suppresses the hyperbilirubinemia associated with severe genetic hemolytic anemias in mice (*19*) and that produced by bile duct ligation or by administration of large amounts of heme or the heme precursor, ALA in rats (*20,21*).

Discussion

The heme–cytochrome P-450 complexes provide sensitive metabolic systems for studying the impact of metals on important cellular functions. Inorganic metals (Figs. 1 and 2), organometals (Table 1 and Fig. 3) and synthetic metalloporphyrins (Fig. 4) can all potently induce the rate-limiting enzyme of heme degradation, heme oxygenase, resulting in an enhanced rate of heme degradation. The associated depression of cellular cytochrome P-450 content and impairment of the oxidative metabolism of xenobiotics reflect this increased heme breakdown. The net effect of these changes is to diminish the capacity of the liver to carry out cytochrome P-450-dependent inactivation of chemical substances for which oxidative metabolism results in biological inactivation.

Inorganic elements such as cobalt or tin produce short-term though pronounced alterations in heme metabolism (Figs. 1 and 2). Organification of the metal may lead to a marked prolongation of the induced perturbations in heme metabolism (Figs. 3 and 4); these effects occur with considerably lower doses of the organometals compared with those of the inorganic elements which they contain. In addition, studies with organotins reveal that organification of a metal can also lead to a shift in the organ site of its action.

Complexed metals may not mimic in all respects the effects of their inorganic elements on heme metabolism. The striking difference in potency of heme oxygenase induction by inorganic cobalt (Fig. 1) and Co-protoporphyrin (Fig. 4) attests to this fact; as does the inability of Sn-protoporphyrin to induce heme oxygenase activity in kidney (Fig. 6) whereas inorganic tin is a powerful inducer of the enzyme in this organ (*5,11*). Whether the potent inhibitory effect of Sn-protoporphyrin on heme oxygenase activity obscures an underlying ability of the metalloporphyrin to also induce *de novo* synthesis of the enzyme protein remains to be determined by appropriate immunological means. In the case of Co-protoporphyrin, both actions can be manifest—an inhibitory effect of the compound *in vitro* (*15*) and an enzyme inducing effect *in vivo* (*7*). In addition, markedly contrasting effects on heme metabolism exist within the metalloporphyrin species as demonstrated by the *in vitro* and *in vivo* studies with Co-protoporphyrin and Sn-protoporphyrin (*7,14*).

Metals, in the inorganic form or as complexes, can also exert useful experimental, and possibly clinical, effects on heme metabolism. Simultaneous administration of manganese or zinc with tin for example (Fig. 2) prevents the occurrence of the characteristic, and presumably deleterious perturbations of heme metabolism associated with the administration of tin alone. Sn-protoporphyrin is a potent inhibitor *in vivo* of heme oxygenase activity in normal animals and in animals in which the enzyme has been previously induced by another agent, e.g., Co-protoporphyrin (Fig. 8) or in animals, i.e., neonates, in which the enzyme activity has been induced by the heme of hemoglobin derived from the postnatal lysis of fetal red cells. Sn-protoporphyrin when administered immediately after birth has been successfully employed (*14,17*) in the latter circumstance to suppress or prevent the hyperbilirubinemia that occurs in the neonate (Fig. 7). This action of Sn-protoporphyrin, which also extends to Cr-protoporphyrin (*8*), affirms the suggestion made from this laboratory in 1975 (*16*) that it may be possible to approach, therapeutically, the problem of neonatal jaundice by considering means for suppressing the

enhanced rates of tissue heme oxidation activity which characterize the newborn and which contribute to the hyperbilirubinemia which occurs in the neonatal period. Our initial clinical studies with Sn-protoporphyrin confirm that the ability of this compound to suppress excessive hyperbilirubinemia in animals also extends to humans (*20*).

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