White Mountain, California. Therefore the $\delta^{18}O$ of the cellulose does not serve as a sensitive or reliable indicator of temperature between regions or species.

Two models have been proposed to account for the $\delta^{18}O$ of the cellulose from terrestrial plants. In model A it is simply assumed that the difference between the $\delta^{18}O$-δD relationship of aquatic plants and that of terrestrial plants is due to the effect of evaporative transpiration on $\delta^{18}O$ and δD of the plant leaf water in terrestrial plants. In model B it is assumed that some of the $\delta^{18}O$ increase in the terrestrial cellulose is due to the use by terrestrial plants of atmospheric CO_2, whose $\delta^{18}O$ of 41 per mil is not altered isotopically during its fixation. Model B seems to be more compatible with the data. Nevertheless, these are tentative models which must be analyzed more rigorously by laboratory experiments.

The δD of cellulose by itself most closely reflects the isotopic composition of meteoric waters and thus climatic temperature. Together, δD and $\delta^{18}O$ can be used to evaluate the effect of the humidity of the growth area during the time when the plant grew. The oxygen and hydrogen isotope data for cellulose and other components of plants are potentially powerful tools for evaluating the relationship between plant growth and the use of water by the plants.

References and Notes

1. S. Epstein, C. J. Yapp, J. H. Hall, *Earth Planet. Sci. Lett.* **30**, 241 (1976).
2. S. Epstein and C. J. Yapp, *ibid.*, p. 252.
3. C. J. Yapp and S. Epstein, *ibid.* **34**, 333 (1977).
4. The δ values are defined as

$$\delta = \left(\frac{R_{sample}}{R_{standard}} - 1 \right) \times 1000$$

where for $\delta^{18}O$, $R = {}^{18}O/{}^{16}O$; and for δD, R = D/H. The standard is standard mean ocean water (SMOW).
5. H. Craig, *Science* **133**, 1702 (1961).
6. M. Cohn and H. C. Urey, *J. Am. Chem. Soc.* **60**, 679 (1938).
7. Y. Bottinga and H. Craig, *Earth Planet. Sci. Lett.* **5**, 285 (1969).
8. H. Craig, L. I. Gordon, Y. Horibe, *J. Geophys. Res.* **68**, 5079 (1963).
9. J. Bricout, *J. Assoc. Off. Anal. Chem.* **56**, 739 (1953).
10. S. Epstein and T. Mayeda, *Geochim. Cosmochim. Acta* **4**, 213 (1953).
11. I. Friedman, *ibid.*, p. 89.
12. L. E. Wise, *Wood Chemistry* (Reinhold, New York, 1944).
13. For example, see H. G. Hardcastle and I. Friedman, *Geophys. Lett.* **1** (No. 4) (1974).
14. D. Rittenberg and L. Ponticorvo, *Int. J. Appl. Radiat. Isot.* **1**, 208 (1956).
15. J. C. Vogel, P. M. Groates, W. G. Mark, *Z. Phys.* **230**, 225 (1970).
16. The reaction for the CO_2 fixation in C_3-type plants is

ribulose 1,5-diphosphate + CO_2 + H_2O → 2 (3-phosphoglycerate)
17. It has been assumed that virtually no oxygen isotopic fractionation occurs during the conversion of CO_2 and H_2O into cellulose. This assumption may not be correct. If oxygen isotopic fractionation does occur, then for model A

$$\delta^{18}O_{cellulose} = 2/3\,[(\delta^{18}O_{H_2O} + 41) + a] + 1/3\,[\delta^{18}O_{H_2O} + b]$$

where $\delta^{18}O_{H_2O} + 41 = \delta^{18}O_{CO_2aq}$. For model B

$$\delta^{18}O_{cellulose} = 2/3[41 + a] + 1/3[\delta^{18}O_{H_2O} + b]$$

where a is the $\delta^{18}O$ change due to fractionation during CO_2 incorporation and b is the $\delta^{18}O$ change due to fractionation during H_2O incorporation. Models A and B could be considered special cases where $b = -2a$; that is, incorporation of H_2O into the cellulose involves an oxygen isotope fractionation twice as large as and in the opposite direction to the oxygen isotopic fractionation associated with CO_2 incorporation into cellulose.
18. L. M. Libby, L. J. Pandolfi, P. H. Payton, J. Marshall III, B. Becker, V. Giertz-Sienhenlist, *Nature (London)* **261**, 284 (1976).
19. S. Epstein and C. J. Yapp, *ibid.* **266**, 478 (1977).
20. J. Gray and P. Thompson, *ibid.* **262**, 481 (1976).
21. H. P. Taylor, Jr., *Econ. Geol.* **69**, 843 (1974).

22. We wish to thank R. Potter, C. Kendall, M. Johnson, and J. Way for making many of the analyses. M. DeNiro helped collect samples, provided scientific advice, helped in many of the operations carried out, and critically read the manuscript. H.-W. Yeh provided both technical and scientific help throughout the project. Discussion with J. F. Bonner was most welcome throughout this research. Samples were provided by C. Emiliani of the University of Miami, H. D. Bruhn of the University of Wisconsin, and H. P. Taylor of the California Institute of Technology. The work was supported by the National Science Foundation, Office of Climate Dynamics, grant number ATM76-03972. This is contribution 2897 from the Division of Geological and Planetary Sciences, California Institute of Technology, Pasadena 91125.

Metals as Regulators of Heme Metabolism

Mahin D. Maines and Attallah Kappas

Heme is a red pigment comprised of four subunits called pyrroles; these subunits are joined together as a single large tetrapyrrole (porphyrin) ring structure. At the center of this porphyrin a metal atom is chelated. In higher organisms the chelated metal is usually iron and the porphyrin is protoporphyrin IX; however, in more primitive species other metalloporphyrin complexes are also formed, for example copper-uroporphyrin is found in the feathers of the Turaco bird (*1*) and cobalt-coproporphyrin is formed in *Propionibacterium arabinosum* (*2*). In order that the metalloporphyrin be of metabolic significance, the central metal ion must be a transition element and capable of undergoing reversible changes in oxidation state (for example, $Fe^{2+} \rightleftarrows Fe^{3+}$, $Cu^{1+} \rightleftarrows Cu^{2+}$, $Co^{2+} \rightleftarrows Co^{3+}$).

In physiological systems heme is bound to certain proteins, and these heme proteins bind oxygen at the site of the metal atom or they function as components of membrane-bound electron transport chains. The ability of heme proteins to carry out these functions is a property of the chelated central metal ion. The porphyrin ring enhances the catalytic activity of the metal, and this activity is further augmented by the complexing of the porphyrin with its apoprotein moiety. The extent of enhancement of the catalytic properties of metals in heme-protein complexes may be of the order of 10^5 to 10^{10}, as compared with unchelated elements (*3*). The porphyrin ring may also serve as an inter- and intracellular carrier of metal ions to regulatory sites for the rate-limiting enzymes of heme synthesis and heme degradation. Porphyrins without a central metal iron are incapable of carrying out those metabolic functions attributed to heme; however, various forms of free porphyrins may have other biological purposes, such as serving as the pigments of mollusc shells and the eggs and feathers of certain birds.

Heme proteins may be soluble—for example, hemoglobin, catalase, tryptophan pyrrolase—or they may be bound to cellular membranes, in which case they are termed "cytochromes." Cytochromes function as terminal oxidases—for example, mitochondrial cytochromes a, endoplasmic reticulum bound cytochromes b_5 and P-450; or as intermediates in electron transfer chains such as mitochondrial cytochromes b and c. Cellular respiration, energy generation, and chemical oxidations are dependent

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on these heme proteins, and their concentrations are, for the most part, a function of the rates of enzymatic synthesis and degradation of heme.

In this article we review certain newly discovered regulatory actions of trace metals on the enzymes of heme synthesis and degradation with emphasis on the consequences of these actions for the heme protein, cytochrome P-450. This cytochrome has proved to be of critical importance as the terminal oxidase (*4*) in the metabolic disposition of a variety of endogenous and exogenous chemicals, including hormones, drugs, insecticides, carcinogens, and various environmental pollutants.

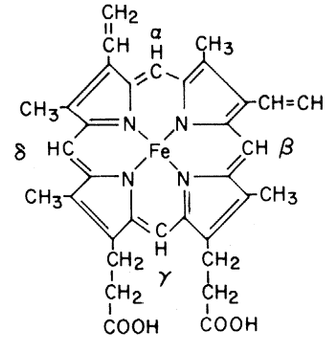
Summary. Heme is essential for cell respiration, energy generation, and oxidative biotransformations. The latter function is exemplified by the oxidative metabolism of various endogenous and exogenous chemicals catalyzed by the heme protein cytochrome P-450. Recent studies have establishd that metal ions directly regulate cellular content of heme, and thus of heme proteins by controlling production of δ-aminolevulinate synthetase and heme oxygenase, the rate-limiting enzymes for heme synthesis and degradation, respectively. Metal ions also alter cellular content of glutathione. In excess amounts, metal ions greatly accelerate the turnover and degradation of heme and substantially impair the oxidative functions of cells—particularly those dependent on cytochrome P-450. As a result, the biological impact of chemicals which are detoxified or metabolically transformed by the P-450 system is greatly altered.

Heme Biosynthesis and Degradation

Heme (Fe-protoporphyrin-IX) is an essentially planar porphyrin structure in which the four pyrrole rings are joined by unsaturated carbon bridges (referred to as methene bridges) with replacement of the eight hydrogen atoms in the β position to the pyrrole nitrogens by various substituents (Fig. 1). Heme is formed through displacement by a divalent ion (for example, Fe^{2+}, Co^{2+}, Mg^{2+}, Mn^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, and Sn^{2+}) of two protons from two pyrrole rings. Metals may be inserted into the porphyrin enzymatically (for example, Fe, Cu, and Co) or chemically (for example, Ni, Sn). Depending on the spatial positions of the different side chains in positions 1 through 8 of the tetrapyrrole nucleus, various position isomers are possible. Protoporphyrin IX, which is the immediate precursor of heme (type b) has three different types of β side chains. The biosynthesis of chlorophyll follows the same pathway and diverges from heme synthesis after the formation of protoporphyrin IX. The biosynthetic pathway of heme (Fig. 2) is a sequence of enzymatic steps in which eight molecules each of pyridoxyl-activated glycine and coenzyme A–activated succinate are ultimately condensed into a heme molecule. The reaction whereby the α carbon of glycine combines with the carbonyl carbon of succinate to form δ-aminolevulinic acid (ALA) is catalyzed by the mitochondrial enzyme ALA synthetase. This enzyme is believed to control the rate-limiting reaction in the pathway (*5*). One of the major, and newly defined, actions of trace metals is to regulate—principally by repression—the synthesis of this rate-limiting enzyme in heme formation (*6–11*).

Through a series of enzymatic reactions taking place in the cytosol a tetracarboxylic acid porphyrin intermediate (coproporphyrinogen III) is formed from ALA. This porphyrin then enters mitochondria where it is decarboxylated and oxidized to form protoporphyrin IX. Heme is synthesized after the incorporation of iron into the protoporphyrin IX ring by the mitochondrial enzyme ferrochelatase.

In mammals and other vertebrates, heme is oxidatively degraded (Fig. 2) to form the open chain tetrapyrrole biliverdin in the course of which three molecules of oxygen are utilized (*12*). One mole of carbon monoxide is generated in this reaction, and the central metal ion of heme is released. The enzyme that catalyzes the oxidation of heme is an endoplasmic reticulum (microsomal)-bound enzyme referred to as "heme oxygenase" (*12–14*). Heme oxygenase has been found in all mammalian tissues studied to date, with the highest rate of enzyme activity being present in the spleen, liver, and kidney. The biliverdin formed from heme oxidation is of the IX α-isomer type—that is, the oxidation of the heme molecule takes place at the α-methene carbon bridge. In mammals biliverdin is reduced to bilirubin by the action of biliverdin reductase—a cytosol enzyme that may utilize NADPH or NADH (reduced forms of nicotinamide adenine dinucleotide phosphate or nicotinamide adenine dinucleotide) (*15*). The reductase is an isomer-specific enzyme that is reactive almost exclusively toward the biliverdin IX α-isomer (*16*). The evolutionary significance of the capacity of mammals to convert biliverdin to bilirubin may lie in the differential lipid solubility of these bile pigments, which facilitates clearance of the latter compound from the fetus.



Heme

Fig. 1. The structure of heme (type b). α, β, γ, and δ refer to the methene bridge.

Heme Regulation of Heme Synthesis and Degradation

The ability of the heme molecule itself to regulate its own biosynthesis and degradation has been well described. Heme regulation of its own formation is reflected in the feedback inhibition of ALA synthetase in erythroid precursors, which was first reported by Karibian and London (*18*), and in the feedback repression of the synthesis and induction of the enzyme in hepatic cells, which was first demonstrated by Granick (*5, 19*).

With respect to heme degradation Sjöstrand's observations (*20*) concerning patients with hemolysis suggested the possibility that carbon monoxide in blood might be derived from released hemoglobin and permitted the inference

that the rate of heme breakdown is elevated in the presence of erythrocyte destruction—that is, endogenous heme might induce an enzyme system that catalyzes its own degradation. Later studies by Gray *et al.* (*21*) proved that the carbon monoxide produced from the breakdown in vivo of hemoglobin originates exclusively from the α-methene bridge of heme; subsequently it was demonstrated (*22*) that, in rats subjected to experimental hemolytic anemia or treated with hemoglobin or methemalbumin, the enzymatic rate of heme degradation in cells was in fact increased. Heme control of its own synthesis and degradation has been shown to require an intact nucleic acid and protein-synthesizing apparatus in the cell.

Although the heme molecule is comprised of two distinct chemical species, the porphyrin ring and the central metal ion, interpretation of its regulatory actions on its own metabolism has always been made in terms of the metalloporphyrin chelate acting as a single molecular unit. The study of the proximate mechanism of heme regulation indicates, however, that metal ions alone can control both the production and the degradation of heme by regulating synthesis of the rate-limiting enzymes that catalyze these processes in the cell in the same manner as does the heme molecule itself (*6–11, 23*).

Metal Control of Heme Synthesis

The catalytic activities of several enzymes of heme metabolism are thought to be SH dependent to various degrees—such as heme oxygenase, ALA dehydratase, uroporphyrinogen I synthetase (UROS), and ferrochelatase—and it is clear that direct metal interactions with these enzymes can inhibit their activities. ALA synthetase (an enzyme that is not SH dependent) is also inhibited by metal ions, but this inhibition is not expressed directly in vitro but rather is expressed in vivo. The mechanism of this action involves metal repression of the rate of formation of ALA synthetase rather than an inhibition of the preformed enzyme (*8, 9*). Metals also inhibit the induction of ALA synthetase by otherwise potent organic chemical inducers of this enzyme (*8, 24*). In these respects metal ions mimic the known regulatory actions of heme on ALA-synthetase formation. The inhibition of ALA-synthetase production by metals is not limited to those elements that are capable of forming heme in vivo, but is a property shared by other elements such as nickel and platinum (*10, 11*) which are not substrates for ferrochelatase (*25*). Therefore metal regulation of ALA-synthetase production does not necessarily involve a porphyrin chelate form of the metal. Tissue culture studies (*9*) support the idea that the metal action on ALA-synthetase production is a direct one. Moreover, of the enzymes of heme metabolism, ALA synthetase appears to be particularly sensitive to metal ions; for example, in cultured liver cells (*9*) a 3-μM concentration of cobalt causes a 60 to 90 percent inhibition of the induction of ALA synthetase by potent inducers of the enzyme such as dicarbethoxydihydrocollidine (DDC) and allylisopropylacetamide (AIA)—agents that otherwise can induce hepatic ALA synthetase to levels as much as 50 times normal. The similarity in cellular responses to metal ions and to heme extends to the secondary, or

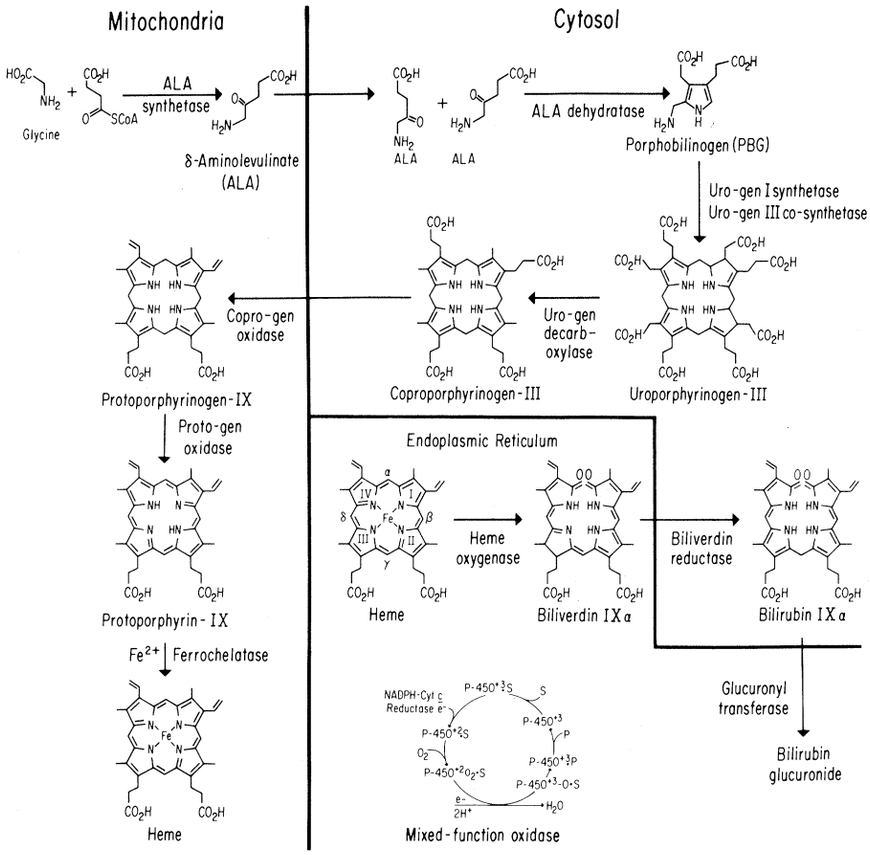


Fig. 2. The pathways of heme biosynthesis and degradation and of heme-dependent mixed-function oxidase activity. The scheme of the mixed-function oxidase system is based on that of Gillette *et al.* (*62*).

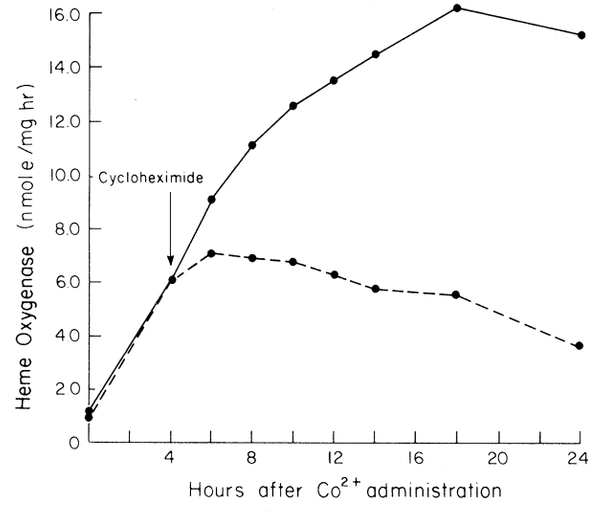


Fig. 3. Cycloheximide inhibition of heme oxygenase induction by cobalt in rat liver. Rats were injected (subcutaneously) with $CoCl_2 \cdot 6H_2O$ (25 μmole/100 g). Cycloheximide (0.18 mg/100 g) was injected (subcutaneously) into one group and the second group received the control solvent •—•, Co^{2+}; •---•, Co^{2+} + cycloheximide.

Table 1. Metal ion effects on heme oxygenase and P-450 dependent metabolism of chemicals in liver. Male rats were injected (subcutaneously) with cobalt chloride (250 μmole/kg), or iron dextran (50 μmole/kg). Twenty-four hours later the rats were killed and various assays were carried out. The *N*-demethylation of ethylmorphine was measured as formaldehyde liberated (*58*). Aniline hydroxylase was measured as *p*-aminophenol formed (*59*). Benzopyrene (a cytochrome P-448 substrate) hydroxylase was measured as hydroxybenzopyrene (OHBP) (*60*). Heme oxygenase activity was measured as bilirubin formed (*6*), and the cytochrome P-450 content was determined by the method of Omura and Sato (*61*).

Treatment	Ethylmorphine *N*-demethylase as HCHO (nmole/mg hr)	Aniline hydroxylase as *p*-aminophenol (nmole/mg hr)	Benzopyrene hydroxylase as OHBP (nmole/mg hr)	Heme oxygenase as bilirubin (nmole/mg hr)	Cytochrome P-450 (nmole/mg)
Control	292.9	18.3	2.93	1.6	0.72
Co^{2+}	88.3	6.4	1.33	14.7	0.33
Fe^{2+}	234.7	15.6	2.50*	7.8	0.64

*The only parameter which was not significantly different from the control.

"rebound" increases in ALA synthetase (*6, 26*), which are produced by these agents in intact animals. This biphasic response of ALA synthetase to heme and to metals such as cobalt has been shown to be a protein synthesis dependent phenomenon.

The activities of heme pathway enzymes subsequent to ALA synthetase are altered differentially by various metals. For example, not all metals that have a high affinity for SH groups have an inhibitory effect on ALA dehydratase in vivo, and not all such metals will inhibit ferrochelatase or UROS in the intact animal. Moreover, there is a degree of tissue and metal specificity in the inhibition response of these enzymes. For example, erythrocyte ALA dehydratase is highly sensitive to lead in vivo, while renal ALA dehydratase is particularly inhibited by platinum (*11*).

Metal Control of Heme Degradation

Metal ions enhance the rate of cellular heme degradation—an action that reflects metal induction of the de novo synthesis of heme oxygenase. For example, a single injection of cobalt to rats causes in 16 hours a nearly 15-fold increase in the oxidative degradation of heme in liver (Fig. 3). The onset of this enzyme induction is very rapid and within 2 hours a significant increase in heme oxygenase activity can be detected (*7*). This inducing action of metals is clearly exerted at the regulatory site for the synthesis of the enzyme, since metal ions in vitro inhibit the preformed enzyme [heme oxygenase is an SH dependent enzyme (*14*)]. The increase of heme oxygenase is maintained up to 72 to 96 hours after a single injection of metal. Metal induction of heme oxygenase is not limited to the liver but extends to other organs such as kidney, heart, lung, intestinal mucosa, and skin (*19*) and is mediated by other transition elements and certain heavy metals as well, for example, Cr, Mn, Fe, Ni, Cu, Zn, Cd, Sn, Pt, Au, Hg, Pb, and Se. The effects of certain of these metal ions on different organs are shown in Fig. 4. The fact that heme oxygenase can also be induced in tissue culture cells grown in synthetically defined medium (*9*) emphasizes that this induction phenomenon reflects a direct action of metals on the enzyme regulatory site and does not involve intracellular intermediary factors such as hormones. The potency of heme oxygenase induction by metals varies from one tissue to another; for example, when administered in equimolar amounts, cobalt is the most potent (7 to 15 times) inducer of heme oxygenase activity in liver, while Sn and Ni are considerably more potent inducers of the enzyme in kidney (10 to 30 times). On the other hand, the ability of mercury to induce this enzyme activity in heart exceeds that of other metal ions. This difference in tissue responsiveness to metal induction of heme oxygenase may reflect differences in binding affinities, cellular contents of functional groups which complex and block metal actions, and the like. The ability of metals to induce the de novo formation of heme oxygenase proved to be not only of biochemical interest but of major pharmacological and toxicological significance when it was shown that, as a result of this enzyme inducing action, there was a resultant depletion of content of cellular heme proteins such as the mitochondrial respiratory cytochromes (*27, 28*), the heme proteins of microsomal origin [such as cytochromes P-450 (*27, 29*), P-448 (*30*), and b_5], and cellular "free heme" (*27, 29*).

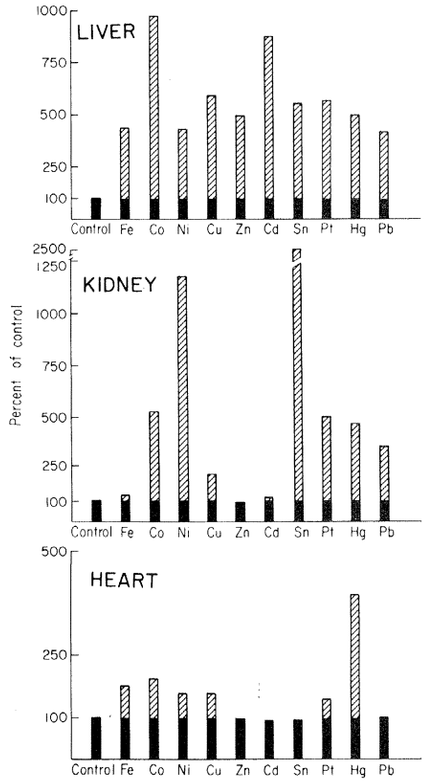


Fig. 4. Effect of various metal ions on heme oxygenase activity of liver, kidney, and heart. The animals were treated (subcutaneously) with 25 μmole/100 g of the metal ions, except in the case of copper and chromium (12.5 μmole/100 g) and cadmium (2.5 μmole/100 g). Microsomal heme oxygenase activity was measured as described (*6*).

Metals and Cytochrome P-450 Dependent Chemical Metabolism

The microsomal drug-metabolizing electron transport chain, that is, the mixed-function oxidase system, is composed of two major enzyme components—a heme protein, cytochrome P-450, and a flavoprotein reductase, NADPH-cytochrome P-450 reductase (Fig. 2). Metal ions cause impairment in the oxidative activity of this system by diminishing cellular heme, an effect that is reflected directly by diminished microsomal content of cytochrome P-450, and by decreased P-450 dependent drug metabolism (Fig. 5). Metals do not significantly affect the activity of the P-450 reductase (*6*).

The liver is the main organ of detoxification. Its capacity to carry out various oxidative transformations is largely due to its high cellular content and multiple species of P-450 (*31*). Although the specific oxidative activity of liver cells by far surpasses that of any organ in the body, other cell types may contribute significantly to overall detoxifying ability. Organs such as the lung and skin, as well as the intestinal mucosa, do not have high specific drug oxidative activities; however, the sheer size of these organs can compensate for this. Moreover,

these organs are the primary portals of entry for exogenous chemicals, and this fact emphasizes the potential importance of their detoxification capabilities (*32*).

In the majority of instances, increased levels of microsomal oxidative activity and cytochrome P-450 content are desirable since, for most drugs and some carcinogens (*33, 34*), the terms "oxidation" and "detoxification" may be used interchangeably. In contrast, for a small group of chemicals to which certain carcinogens belong oxidation and activation are synonymous (*35*). For example, in cultured liver cells benzopyrene is more toxic to cells with high oxidative activity than to cells with low activity (*34, 36*), and there are other lines of evidence indicating that the metabolism of certain carcinogenic polycyclic hydrocarbons is a prerequisite for their carcinogenicity—that is, the metabolites of these chemicals rather than the parent compounds are the ultimate carcinogens (*37*). Similarly certain drugs achieve therapeutic efficacy subsequent to their oxidative metabolism by microsomes (*38*).

During the 1960's—the period in which many properties of the then novel cytochrome P-450 system were being discovered—the function of this cytochrome as the terminal oxidase for various biological oxidations of endogenous and exogenous substrates was established. However, this assignment of function was not accurate in all cases. One such example was the oxidative degradation of heme itself, which was originally thought to be dependent upon cytochrome P-450 (*39*). Later studies with liver (*6, 13, 14, 17*) and spleen (*40*) conclusively disassociated P-450 from the enzyme that catalyzed the degradation of heme. It was observed in the initial studies with cobalt in liver that there was a reciprocal relation between heme oxygenase activity and cellular P-450 content (*6, 17*) (Fig. 5). This suggested the likelihood of the catabolism of microsomal heme by heme oxygenase. The degradation of P-450 heme by heme oxygenase was substantiated by experiments in which the oxidation of microsomal heme labeled with [^{14}C]ALA was measured in rats with bile fistulas and correlated with the rate of metal induction of heme oxygenase. These studies showed a direct correlation between the elevation of heme oxygenase, the increase in biliary excretion of ^{14}C-labeled bilirubin, and the decrease in microsomal content of P-450 (*27, 29, 41*). Reciprocal relations between heme oxygenase activity and the depression of hepatic content of cytochrome P-450 were produced by a number of other metals including Cr, Mn, Fe, Ni, Cu, Zn, Cd, Sn, Pt, Au, Hg, Pb, and by Se (*42*).

However, studies in vitro showed that the heme moiety of intact cytochrome P-450 was in fact not degraded by heme oxygenase; rather it was the heme of cytochrome P-420, the aberrant form of P-450, which was the substrate for the enzyme (*29*). P-420 is produced by denaturation of P-450, and unlike the latter, P-420 cannot catalyze biological oxidations. However, its heme moiety is considerably more labile than that of P-450 (*43*). Accordingly, in order for metal ions to decrease cytochrome P-450 content they must not only increase the rate of degradation of heme, but also accelerate the rate of conversion of P-450 to P-420. This could be accomplished either by increasing the cellular content of a native "denaturent" of P-450 (*29, 41*) or by modifying the affinity of apoprotein P-450 for heme. Recent evidence indicates that the heme moiety of cytochrome P-448 is also catalyzed by metal-induced heme oxygenase (*30*). P-448 is a species of microsomal heme protein which is induced by polycyclic hydrocarbon carcinogens (*44*) and polychlorinated biphenyls (*45*) and serves as the terminal oxidase for many of these compounds.

The decreases in the cellular content of cytochromes P-450 and P-448 produced by trace metals are directly reflected in the ability of cells to carry out drug and carcinogen oxidations that depend on these heme proteins. This metal-mediated depression of drug metabolism extends across the spectrum of chemicals that are substrates for the mixed-function oxidase system and that bind directly to the heme or vicinal to it; these include such compounds as aniline and ethylmorphine, as well as P-448 substrates such as benzopyrene (Table 1). The decrease in drug-metabolizing activity of cells that follows trace metal administration persists for prolonged periods paralleling the enhanced heme oxygenase activity. The decreases observed in drug metabolism in vitro are not limited to the liver but are noted in other organs such as kidney and skin. Several studies have provided in vivo data demonstrating that metal ions diminish the ability of animals to oxidize drugs as judged by criteria such as the prolongation of barbiturate sleeping time (*46*). Others have also reported decreases in P-450 dependent drug-metabolizing ability in humans after long- or short-term exposure to certain metals. For example, studies on chronic lead toxicity in children have shown that these children may display a diminished ability to oxidatively metabolize drugs (*47*). These findings in humans are in keeping with the observation that lead toxicity in animals decreases cytochrome P-450 and heme

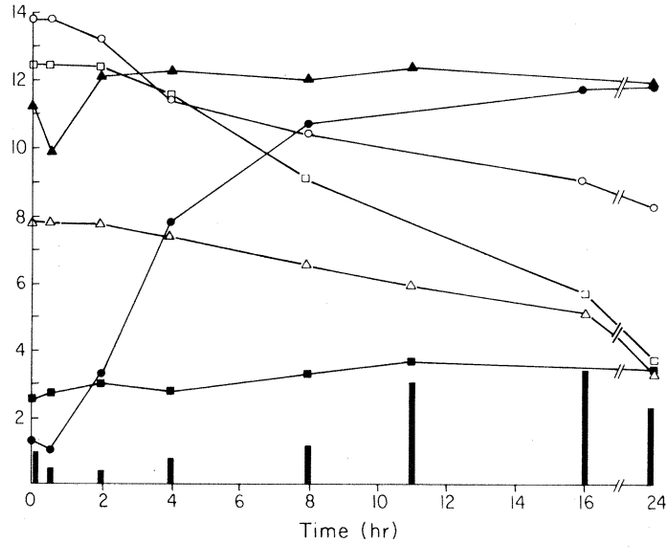


Fig. 5. Effects of cobalt on hepatic heme metabolism. Rats were injected with cobalt (25 μmole/100 g, subcutaneously) as $CoCl_2$. The individual animals were killed at the intervals indicated. Various cellular fractions were prepared (*6, 8*) and assayed for the indicated parameters. Heme oxygenase was measured in the microsomal fractions (*6*), ALA synthetase was measured by the method of Marver *et al.* (*63*), total porphyrin was measured by the method of Granick *et al.* (*64*), cytochrome P-450 was measured by the method of Omura and Sato (*61*), and total microsomal heme was measured by the pyridine hemochromogen spectrum (*65*). Symbols: ●—●, Heme oxygenase (nmole/mg hr); ○—○, microsomal heme (10^{-1} nmole/mg); △—△, cytochrome P-450 (10^{-1} nmole/mg); □—□, *N*-demethylase (20 nmole/mg hr); ▲—▲, ferrochelatase (nmole/mg hr); ■—■, porphyrin (pmole/mg); and ▮, ALA synthetase (10^2 pmole/mg hr).

contents (*48*). In other studies copper, when administered to rats in drinking water for 15 days, has been shown to reduce aniline hydroxylation activity by 75 percent (*49*). Copper is a potent inducer of heme oxygenase (*23*). Trace metals also have synergistic effects on cellular heme metabolism, which further emphasizes the potential of metal ions to act as enzyme regulators. For example, when rats were treated simultaneously with small doses of iron (as iron dextran) and cobalt ($CoCl_2$), which individually caused only a moderate (two- to threefold) increase of heme oxygenase activity in liver and a moderate reduction in cytochrome P-450 content (20 percent), a strong synergistic effect was observed when both metals were given simultaneously. In this instance heme oxygenase increased the control level nearly tenfold, and the cytochrome P-450 concentration decreased to a fraction of that of the control (*50*). Similar synergistic effects are seen in animals treated for a long period with one metal, such as iron, and then given a single treatment with another metal such as gold. Both iron and gold are used therapeutically in humans.

The depletion of cellular heme proteins by metals should not always be considered a means by which the toxicity of a compound will be augmented; as was mentioned earlier for some carcinogens, there is evidence indicating that the products of their oxidative metabolism are the biologically active species. Thus, with such compounds the metal-mediated decreases in cytochrome P-448 content of cells may be viewed as a means of preventing formation of toxic metabolites (*33-36*).

Metals as Physiological Regulators of Heme Metabolism

All experimental findings to date indicate that metal ions regulate ALA synthetase and heme oxygenase in a manner entirely analogous to the control exerted on these enzymes by heme itself. Although several of the metals that share these regulatory properties are capable of enzymatically forming heme complexes—that is, Co, Fe, and Cu; other metals such as Ni and Pt have not been found as heme complexes in nature nor have they been shown to be capable of forming tetrapyrrole chelates enzymatically (*25*). Nevertheless Ni and Pt mimic exactly the regulatory actions of heme on ALA synthetase and heme oxygenase (*10, 11*). These facts provide conclusive evidence that metal ions alone act as proximate regulators of ALA synthetase and heme oxygenase and they permit the conclusion that it is the metal moiety of heme itself, namely iron, which mediates the enzyme regulatory properties attributed to the entire metalloporphyrin complex.

The reciprocal relation between increased rates of heme oxygenase activity and diminished ALA synthetase together with impaired P-450 dependent oxidations of drugs is not only observed after experimental exposure to metal ions, but also may occur naturally (*51*). For example, in the newborn rat (*52, 53*) or the newborn human the liver content of cytochrome P-450 and drug-metabolizing ability are greatly diminished as is ALA-synthetase activity, but the heme oxygenase activity is substantially higher than that of adults. Moreover hepatic ALA-synthetase activity, at least in the newborn rat, is completely unresponsive to induction by chemicals which in the adult induce this enzyme (*54*). The high activity of heme oxygenase and the low levels of ALA-synthetase activity may be significant causative factors for the low content of P-450 in the newborn; the high heme oxygenase activity may also contribute substantially to the overproduction of the toxic heme metabolite bilirubin, which in severely jaundiced infants deposits in central nervous system cells, causing the neurological syndrome referred to as "Kernicterus" (*52*). The high activity of heme oxygenase in the newborn of some species could reflect the enzyme-inducing action of heme or of iron (or both) derived from the breakdown of fetal erythrocytes, and these agents could also act to repress ALA-synthetase formation (*51*). The fact that chelation therapy in the newborn restores hepatic ALA-synthetase activity to normal levels and to normal responsiveness to induction by chemicals such as AIA indicates that repression of this enzyme is due to the iron released from heme through heme oxygenase activity (*51*). Neonatal control of ALA synthetase by metal ions represents one major area in which physiological expression of the enzyme regulatory action of metals is clearly demonstrable, as well as offering a potential situation in which manipulation of heme synthesis and heme degradation by agents that alter metal metabolism may have therapeutic value.

Cellular SH Content and Metal Regulation of Heme

All of the metals that control heme metabolism have by virtue of their atomic structures substantial affinity for electronegative nucleophilic species. A relationship between metal ions and nucleophilic binding sites (for example, SH) for regulation of enzyme synthesis can thus be predicted and has been examined experimentally. On the basis of various studies, some of which are discussed here, the following conclusions have been drawn: (i) metals must be in the ionic form and capable of covalently binding at cellular regulatory sites in order to mediate their effects on the enzymes of heme metabolism (*10, 23*); and (ii) there is a direct relation between the cellular content of SH groups, for example, glutathione (GSH), and the ability of metals to affect the synthesis of the enzymes. When metals having a high affinity for SH groups are complexed with cysteine or GSH, their ability to alter heme metabolism is totally blocked (*10, 23*). The complexing of the metals also blocks their inhibitory effects in vitro on the activity of heme oxygenase.

The antagonistic effect of SH agents on the regulatory actions of metals on cellular heme can also be demonstrated in vivo (*10*); for example, if the endogenous content of SH groups is increased by the oral administration of agents such as cysteine before the injection of metals the actions of metals on heme pathway enzymes are blocked. Conversely, the effects of metals on various aspects of heme metabolism can be magnified by depleting the cellular content of SH groups prior to metal administration (*23*). A number of carbonyl compounds are capable of depleting SH groups mainly by diminishing GSH. One of the most effective agents of this type is diethyl maleate (DEM). It is known that within 30 minutes of one injection of DEM (3 mmole/kg), GSH levels in rat liver are decreased to about 10 percent of the normal value (*55*). Prior treatment of rats with DEM as described above more than doubles the production of heme oxygenase by a number of trace metals such as Co, Ni, and Fe (*9, 10, 23, 50*). This augmentation effect is not limited to the intact animal liver but can also be demonstrated in cultured hepatocytes (*9*).

Metal ions themselves have been found to alter the cellular content of GSH significantly (*1*). For example, Co and Ni cause an initial decrease of cellular GSH content (in 6 hours) which is subsequently followed by an increase 200 to 300 percent above normal (*10, 56*). This biphasic pattern of cellular GSH content resembles the responses that metal ions (as well as heme) elicit in the activities of heme oxygenase and ALA synthetase in liver cells and indicates that metal ions may also regulate GSH possibly by increasing its rate of synthesis.

The concomitant increases in GSH and heme oxygenase could have adaptive significance since GSH would provide the organism with an added protective mechanism for detoxification reactions. It is known that a number of compounds are biologically inactivated as a result of their conjugation with GSH (*57*). Since metal ions, by increasing the rate of heme degradation, diminish the ability of cells to detoxify chemicals, the increase in cellular GSH would thus partially compensate for the metal impairment of the P-450 dependent detoxification process. In contrast since heme oxygenase is an SH dependent enzyme another function of GSH could be to protect the thiol moiety of the protein and thus prevent its inactivation. In this instance, the increased GSH levels produced by metals could in a sense be considered to further potentiate the impairment of detoxification capacity produced by metal induction of this enzyme. Therefore the relation between increased GSH and heme oxygenase is a complex phenomenon although, on balance, the metal effect on GSH is probably beneficial in terms of the overall detoxifying ability of cells.

Conclusions

Trace metals have been found to be unique regulators of cellular heme and thus of heme proteins in that they control both the synthesis and the degradation of this metalloporphyrin. This control is exerted through initial repression of ALA synthetase, the rate-limiting enzyme in heme synthesis, and induction of heme oxygenase, the rate-limiting enzyme in heme degradation. Metal ions mimic in all respects the regulatory effects of heme itself on the enzymes of heme metabolism, including the biphasic response of ALA synthetase (initial repression followed by rebound induction). Considerable experimental evidence supports the idea that the rate of both heme biosynthesis and degradation may primarily be a function of metal ion concentrations at appropriate regulatory sites in the cell. According to this view it is the metal moiety of heme (Fe-protoporphyrin IX) which is in fact the proximately active species in controlling heme metabolism. The tetrapyrrole may be primarily an efficient inter- and intracellular carrier of the metal to the sites for regulation of enzyme synthesis.

The consequences of the metal actions on heme synthesis and degradation are to deplete cellular contents of heme and heme proteins and to impair oxidative activities dependent on these compounds. Many of the metal ions that have the biological properties described above gain entrance to the body in numerous and diverse ways. Moreover, a number of these metals tend to accumulate in various tissues throughout life. Since heme—apart from its role in cellular respiration—is essential for the oxidative detoxification of a wide spectrum of endogenous and exogenous chemicals the potent ability of metals to deplete cellular heme content emphasizes the considerable biomedical significance of these elements.

References and Notes

1. C. Rimington, *Proc. R. Soc. London Ser. B* **127**, 106 (1939).
2. N. Sone, *J. Biochem. (Tokyo)* **69**, 753 (1971).
3. M. Calvin, *Chemical Evolution* (Oxford Univ. Press, New York, 1969), p. 9.
4. H. S. Mason, W. B. Fowlks, E. W. Peterson, *J. Am. Chem. Soc.* **77**, 2914 (1955); O. Hayaishi, M. Katageri, S. Rothberg, *ibid.*, p. 5450.
5. S. Granick, *J. Biol. Chem.* **241**, 1359 (1966).
6. M. D. Maines and A. Kappas, *ibid.* **250**, 4171 (1975).
7. _____, in *Porphyrins in Human Diseases*, M. Doss, Ed. (Karger, Basel, 1976), pp. 43–52.
8. M. D. Maines, V. Janoušek, J. M. Tomio, A. Kappas, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 1499 (1976).
9. M. D. Maines and P. Sinclair, *J. Biol. Chem.* **252**, 219 (1977).
10. M. D. Maines and A. Kappas, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 1875 (1977).
11. _____, *J. Exp. Med.* **146**, 1286 (1977).
12. R. Tenhunen, H. S. Marver, R. Schmid, *J. Biol. Chem.* **244**, 6388 (1969).
13. _____, *Proc. Natl. Acad. Sci. U.S.A.* **61**, 748 (1968).
14. M. D. Maines, I. Ibrahim, A. Kappas, *J. Biol. Chem.* **252**, 5900 (1977).
15. J. W. Singleton and L. Laster, *ibid.* **240**, 4780 (1965).
16. P. O'Carra and E. Colleran, *FEBS Lett.* **15**, 295 (1969).
17. M. D. Maines and J. Cohn, *J. Exp. Med.* **145**, 1054 (1977); M. D. Maines and A. Kappas, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 4293 (1974).
18. D. Karibian and I. M. London, *Biochem. Biophys. Res. Commun.* **18**, 243 (1965).
19. G. Urata and S. Granick, *J. Biol. Chem.* **238**, 821 (1963).
20. T. Sjöstrand, *Scand. J. Clin. Lab. Invest.* **1**, 201 (1949).
21. C. H. Gray, D. C. Nicholson, R. A. Nicolaus, *Nature (London)* **181**, 181 (1958).
22. N. R. Pimstone, P. Engel, R. Tenhunen, P. T. Seitz, H. S. Marver, R. Schmid, *J. Clin. Invest.* **50**, 2042 (1971); R. Tenhunen, H. S. Marver, R. Schmid, *J. Lab. Clin. Med.* **75**, 410 (1970).
23. M. D. Maines and A. Kappas, *Biochem. J.* **154**, 125 (1976).
24. M. D. Maines, J. M. Tomio, V. Janoušek, *J. Clin. Chem. Clin. Biochem.*, in press.
25. R. F. Labbee and N. Hubbard, *Biochim. Biophys. Acta* **41**, 185 (1960).
26. D. P. Tschudy, A. Waxman, A. Collins, *Proc. Natl. Acad. Sci. U.S.A.* **58**, 1944 (1967).
27. M. D. Maines and A. Kappas, *Ann. Clin. Res.* **8**, 39 (1976).
28. A. Kappas and M. D. Maines, *Science* **192**, 60 (1976).
29. M. D. Maines, in *Microsomes and Drug Oxidations*, V. Ullrich, A. G. Hilderbrandt, I. Roots, R. W. Estabrook, A. Conney, Eds. (Pergamon, New York, 1977), p. 543.
30. _____, *J. Exp. Med.* **144**, 1509 (1976).
31. P. E. Thomas, A. Y. H. Lu, D. Ryan, S. B. West, K. Kawalek, W. Levin, *J. Biol. Chem.* **251**, 1385 (1976); N. E. Sladek and G. J. Mannering, *Biochem. Biophys. Res. Commun.* **24**, 668 (1966); A. P. Alvares, G. Schilling, W. Levin, R. Kuntzman, *ibid.* **29**, 152 (1967); D. A. Haugen, T. A. Van der Hoenen, M. J. Coon, *J. Biol. Chem.* **250**, 3567 (1975).
32. A. H. Conney, E. J. Pantuck, K.-C. Hsiao, R. Kuntzman, A. P. Alvares, A. Kappas, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **36**, 1647 (1977); A. P. Alvares, K. Anderson, A. H. Conney, A. Kappas, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 2501 (1976).
33. H. L. Richardson, A. R. Stier, E. Borsos-Nachtnebel, *Cancer Res.* **12**, 356 (1952); A. H. Conney, E. C. Miller, J. A. Miller, *ibid.* **16**, 450 (1956); A. H. Conney, *Pharmacol. Rev.* **19**, 317 (1967).
34. J. A. Miller and E. C. Miller, *J. Natl. Cancer Inst.* **47**, 5 (1971).
35. H. V. Gelboin and F. J. Wiebel. *Ann. N.Y. Acad. Sci.* **179**, 529 (1971); H. V. Gelboin, E. Huberman, L. Sachs, *Proc. Natl. Acad. Sci. U.S.A.* **64**, 1188 (1969).
36. J. Kapitulnik, W. Levin, A. H. Conney, *Nature (London)* **266**, 378 (1977); P. Sims, P. L. Grover, A. Swaisland, K. Pal, A. Hewer, *ibid.* **252**, 326 (1974); A. Borgen, H. Darney, N. Castagnoli, T. T. Crocker, R. E. Rasmussen, I. Y. Wang, *J. Med. Chem.* **16**, 502 (1973); L. W. Wattenberg and J. L. Leong, *Cancer Res.* **30**, 1922 (1970); P. L. Grover, P. Sims, E. Huberman, H. Marquardt, T. Kuroko, C. Heidelberger, *Biochem. Biophys. Res. Commun.* **43**, 1010 (1969).
37. H. V. Gelboin, *Cancer Res.* **29**, 1272 (1969); W. Levin, A. W. Wood, H. Yagi, D. M. Jerina, A. H. Conney, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 386 (1976).
38. A. E. Takemari and G. J. Mannering, *J. Pharmacol. Exp. Ther.* **123**, 171 (1958).
39. R. Tenhunen, H. S. Marver, R. Schmid, *Trans. Assoc. Am. Physicians* **82**, 363 (1969); R. Schmid, *N. Engl. J. Med.* **287**, 703 (1972).
40. T. Yoshida and G. Kikuchi, *J. Biochem. (Tokyo)* **81**, 265 (1977).
41. M. D. Maines, in *The Year in Hematology*, A. S. Gordon, R. Silber, J. LoBue, Eds. (Plenum, New York, 1977), vol. 1, chap. 1.
42. M. D. Maines and A. Kappas, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 4428 (1976).
43. M. D. Maines and M. W. Anders, *Mol. Pharmacol.* **9**, 219 (1973); _____, U. Muller-Eberhard, *ibid.* **10**, 204 (1974).
44. J. H. Mennear and A. P. Rudzik, *Life Sci.* **7**, 213 (1968); D. W. Nebert, J. Winker, H. V. Gelboin, *Cancer Res.* **29**, 1763 (1969); D. W. Nebert and H. V. Gelboin, *Arch. Biochem. Biophys.* **134**, 76 (1969); N. E. Sladek and G. J. Mannering, *Biochem. Biophys. Res. Commun.* **24**, 668 (1966).
45. A. P. Alvares, D. R. Bickers, A. Kappas, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 1321 (1973); J. A. Cohn, A. P. Alvares, A. Kappas, *J. Exp. Med.* **145**, 1607 (1977).
46. R. E. Johnston, T. S. Miga, R. C. Schnell, *Biochem. Pharmacol.* **24**, 877 (1975); D. J. Holbrook, M. E. Washington, H. B. Leake, P. E. Brubaker, *J. Tox. Env. Health*, **1**, 1067 (1976).
47. K. E. Anderson, A. Fischbein, D. Kestenbaum, S. Sassa, A. P. Alvares, A. Kappas, *Am. J. Med.* **63**, 306 (1977).
48. P. Scoppa, M. Roumengous, W. Penning, *Experientia* **29**, 970 (1973); A. P. Alvares, A. Fischbein, S. Sassa, K. E. Anderson, A. Kappas, *Clin. Pharmacol. Ther.* **19**, 183 (1976).
49. E. Moffitta, Jr., and S. D. Murphy, *Biochem. Pharmacol.* **22**, 1463 (1973).
50. M. D. Maines and A. Kappas, *Clin. Pharmacol. Ther.* **22**, 780 (1977).
51. _____, *J. Biol. Chem.*, in press.
52. _____, *J. Exp. Med.* **141**, 1400 (1975).
53. M. M. Thaler, D. L. Gemsa, A. F. Bakken, *Ped. Res.* **6**, 1971 (1972).
54. C. S. Song, H. C. Moses, A. S. Rosenthal, N. A. Gelb, A. Kappas, *J. Exp. Med.* **134**, 1349 (1971); J. S. Woods and R. Dixon, *Biochem. Pharmacol.* **19**, 1951 (1970).
55. E. Boyland and L. F. Chasseaud, *Biochem. Pharmacol.* **19**, 1526 (1970).
56. M. D. Maines and A. Kappas, in *Clinical Chemistry and Clinical Toxicology of Metals*, S. S. Brown, Ed. (Elsevier, New York, 1977); H. Remmer, R. W. Estrabrook, J. Shenkman, H. Greim, *Arch. Pharmacol. Exp. Pathol.* **259**, 98 (1968).
57. J. R. Mitchell, D. J. Jollow, W. Z. Potter, J. R. Gillette, B. B. Brodie, *J. Pharmacol. Exp. Ther.* **187**, 211 (1973); E. Boyland and L. F. Chasseaud, *Adv. Enzymol.* **32**, 173 (1969); L. F. Chasseaud, in *Glutathione: Metabolism and Function*, I. M. Arias and W. B. Jakoby, Eds. (Raven, New York, 1976), p. 77.
58. T. Nash, *Biochem. J.* **55**, 416 (1953).
59. Y. Imai, A. Ito, R. Sato, *J. Biochem. (Tokyo)* **60**, 417 (1966).
60. D. W. Nebert and H. V. Gelboin, *J. Biol. Chem.* **243**, 6242 (1968).
61. T. Omura and R. Sato, *ibid.* **239**, 2379 (1964).
62. J. Gillette, H. Sasame, B. Stripp, *Drug Metab. Disp.* **1**, 164 (1973).
63. H. S. Marver, D. P. Tschudy, M. G. Perlroth, A. Collins, *J. Biol. Chem.* **241**, 2803 (1966).
64. S. Granick, P. Sinclair, S. Sassa, G. Grieninger, *ibid.* **250**, 9215 (1975).
65. K. G. Paul, H. Theorell, A. Akeson, *Acta Med. Scand.* **7**, 1284 (1953).