Proc. Natl. Acad. Sci. USA
Vol. 79, pp. 2384–2388, April 1982
Medical Sciences

The cytochrome *P*-450-depleted animal: An experimental model for *in vivo* studies in chemical biology

[heme oxidase (decycling)/metalloporphyrin/δ-aminolevulinate synthase]

GEORGE S. DRUMMOND AND ATTALLAH KAPPAS

The Rockefeller University Hospital, New York, NY 10021

Communicated by Karl H. Beyer, November 5, 1981

ABSTRACT **An experimental method is described to deplete markedly *in vivo* the cytochrome *P*-450 content of liver for prolonged periods of time. The method uses the synthetic metalloporphyrin cobalt-heme (cobalt protoporphyrin IX), which possesses the dual biological properties of repressing δ-aminolevulinate synthase, the rate-limiting enzyme of heme biosynthesis, and of potently inducing microsomal heme oxygenase, the rate-limiting enzyme of heme catabolism. A single dose of cobalt-heme (125 μmol/kg of body weight) decreased within 48 hr hepatic cytochrome *P*-450 to ≈20% of normal, at which level it remained for 10 days; normal levels were not achieved by 36 days. Periodic administration (total, six injections) of a smaller dose of cobalt-heme (50 μmol/kg of body weight) maintained the cytochrome *P*-450 content at levels ≈15% of normal for >90 days with concurrent profound impairment of mono-oxygenase reactions catalyzed by this heme protein. The ability of cobalt-heme to produce profound and prolonged depletion of cytochrome *P*-450 *in vivo* provides a valuable model for examining the role of cytochrome *P*-450-dependent metabolism in the biology of endogenous and exogenous chemicals.**

It would be useful to have an experimental model in which information, somewhat analogous to that provided by microbiological assays (1), relating to the role of cytochrome *P*-450 in determining the nature of the proximately active chemical species that exert biological actions *in vivo* could be obtained in the whole animal. Such a model would require a method for producing long-sustained depletion of cytochrome *P*-450, especially in the liver, since this organ contains the highest concentration of this heme protein. Comparisons could then be made between the biological actions of chemicals in normal and cytochrome *P*-450-depleted animals or even in the same animal in the normal and depleted state or in the induced and uninduced state. Such studies should provide new insight into the relationship between the cytochrome *P*-450-inducing action of chemicals such as phenobarbital and 3-methylcholanthrene and their metabolic transformation by the cytochrome.

We report here that cobalt protoporphyrin IX (Co-heme) profoundly depresses the level of cytochrome *P*-450 in rat liver. A single injection of the metalloporphyrin decreases hepatic cytochrome *P*-450 content to ≈20% of normal within 48 hours. Recovery to normal levels of the heme protein requires at least 5 weeks. During this period, a marked prolonged induction of heme oxygenase and a sustained depression of δ-aminolevulinate (AmLev) synthase activity occur. Periodic injections of Co-heme depress hepatic cytochrome *P*-450 content markedly; extremely low (≈15% of normal) levels of the heme protein can be sustained for as long as the Co-heme is administered.

The ability of Co-heme to profoundly deplete liver cells of cytochrome *P*-450 for prolonged periods of time is a consequence of its dual biological properties—i.e., its potent capacity to induce microsomal heme oxygenase (2, 3), the rate-limiting enzyme of heme catabolism (4, 5), and its ability to repress the activity of AmLev synthase (2, 6), the rate-limiting enzyme of heme biosynthesis (7). These unique properties of Co-heme provide a valuable experimental means to assess *in vivo* the role of cytochrome *P*-450-dependent metabolic activation of chemicals in determining their biological actions.

MATERIALS AND METHODS

Materials. Male Sprague–Dawley rats (175–200 g) purchased from Holtzman, (Madison, WI) were used throughout this study. Rats were injected subcutaneously with single doses of Co-heme, up to 125 μmol/kg of body weight unless otherwise indicated. The animals were starved for 16 hr but permitted access to water prior to sacrifice. Solutions of Co-heme were prepared by dissolving the compound in a small volume (0.1 ml/ml of Co-heme solution) of 0.1 M sodium hydroxide, adjusting the pH to 7.4 with 1 M HCl, and making up to final volume with 0.9% NaCl. These Co-heme solutions were administered (0.2–0.4 ml/100 g of body weight) within 10 min of preparation. Control animals received an equivalent volume of 0.9% NaCl. In the chronic-condition experiment, animals received six doses of Co-heme (50 μmol/kg of body weight, each dose), the first on day 0 of the study and the subsequent ones on days 7, 14, 32, 60, and 88.

Co-heme was supplied by Porphyrin Products (Logan, UT). All other chemicals used were of the highest grade obtainable and were supplied by either Sigma or Fisher.

Tissue Preparation. Livers were perfused *in situ* with ice-cold 0.9% NaCl, removed, and homogenized, as were other tissues, in 3 vol of 0.1 M potassium phosphate, pH 7.4/0.25 M sucrose. The homogenate was centrifuged at 9000 × *g* for 20 min. The precipitate was washed twice with 0.1 M potassium phosphate, pH 7.4, prior to assaying for AmLev synthase activity. The 9000 × *g* supernatant was centrifuged at 100,000 × *g* for 60 min; the cytosol from untreated liver was used as a source of biliverdin reductase and the microsomal pellet was washed once with and then resuspended in 0.1 M potassium phosphate, pH 7.4, at a protein concentration of 15–20 mg/ml.

Enzyme Assays. The activities of AmLev synthase, aniline hydroxylase, ethylmorphine demethylase, aryl hydrocarbon hydroxylase, and heme oxygenase, respectively, were assayed with modifications as described (8–10). The formaldehyde produced in the ethylmorphine demethylase assay was measured by the method of Nash (11). The *p*-aminophenol produced in the aniline hydroxylase assay was measured by the method of

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: Co-heme, cobalt heme (cobalt protoporphyrin IX); AmLev, δ-aminolevulinate.

Imai *et al.* (12). Bilirubin produced in the heme oxygenase assay was calculated by using an extinction coefficient of 40 $mM^{-1}\cdot cm^{-1}$ between 464 and 530 nm.

Spectral Studies. Cytochrome *P*-450 content was measured from the CO difference spectrum using sodium dithionite as the reducing agent and an extinction coefficient of 91 $mM^{-1}\cdot cm^{-1}$ between 450 and 490 nm (13). Cytochrome b_5 content was measured from the NADH difference spectrum using an extinction coefficient of 185 $mM^{-1}\cdot cm^{-1}$ between 422 and 408 nm (13). Microsomal heme was determined by the reduced-minus-oxidized difference spectrum between 557 and 540 nm using an extinction coefficient of 20.7 $mM^{-1}\cdot cm^{-1}$ (14). These studies were performed on an Aminco-Chance DW2A spectrophotometer in the split beam mode.

Protein concentration was determined by the method of Lowry *et al.* using crystalline bovine serum albumin as standard (15). All experiments were conducted a minimum of three times and the results are expressed as mean ± SEM or as the average of three experiments. The data were analyzed by using the standard Student's *t* test and differences were considered significant if $P < 0.05$.

RESULTS

Effect of Co-heme on Cytochrome *P*-450 Content and Activities of Heme Oxygenase and AmLev Synthase in Liver. The effects of various doses of Co-heme on cytochrome *P*-450 levels *in vivo* 16 hr after administration are shown in Table 1. Co-heme reduced, in a dose-dependent manner, cytochrome *P*-450 content; the level of this heme protein was reduced to 56% of control by administration of the highest dose (125 μmol/kg of body weight) of Co-heme. Because of solubility problems, higher doses of Co-heme could not be administered. The activities of heme oxygenase and AmLev synthase were also determined (Table 1). Heme oxygenase activity increased in a dose-dependent manner, an ≈6-fold increase in enzyme activity being obtained with the highest dose used (125 μmol/kg of body weight). AmLev synthase activity was inhibited to a similar extent (≈50%) by all three concentrations of Co-heme examined. Thus, the ability of Co-heme to induce heme oxygenase with simultaneous reduction of AmLev synthase activity probably accounts for its potent ability to decrease cytochrome *P*-450 content in the liver. This ability of Co-heme to depress cytochrome *P*-450 and to perturb the rate-limiting enzymes of heme synthesis and degradation was further investigated with respect to time.

Time Course of Effects of a Single Administration of Co-heme on Heme Metabolism in Liver. Perturbations of heme metabolism induced by inorganic metal treatment are usually complete within 72 hr (16, 17). The effects on heme metabolism

Table 1. Dose response of cytochrome *P*-450 content and activities of heme oxygenase and AmLev synthase after Co-heme administration in liver

Co-heme, μmol/kg body weight	Cytochrome *P*-450, nmol/mg	Heme oxygenase, nmol of bilirubin·$mg^{-1}\cdot hr^{-1}$	AmLev synthase, nmol of AmLev·$mg^{-1}\cdot hr^{-1}$
0 (Control)	0.79 ± 0.03	2.61 ± 0.14	0.207 ± 0.01
25*	0.54 ± 0.03	7.89 ± 0.93	0.110 ± 0.01
50*	0.46 ± 0.03	10.92 ± 0.75	0.116 ± 0.01
125*	0.44 ± 0.06	13.76 ± 1.23	0.118 ± 0.02

Co-heme was administered subcutaneously as indicated to animals, which were killed 16 hr later. Three to six animals were used for each determination and the assays were performed in duplicate. Data are mean ± SEM.
* $P < 0.05$, compared with control (saline treated).

72 hr after a single dose of Co-heme (125 μmol/kg of body weight) are shown in Table 2. Heme oxygenase activity was still elevated 6-fold while AmLev synthase activity was depressed to ≈40% of control. Cytochrome *P*-450 content decreased to ≈20% of control. Cytochrome *P*-450-dependent drug-metabolizing activities (ethylmorphine demethylase and aniline hydroxylase) were also significantly decreased (11% and 37% of control values, respectively) as were cytochrome b_5 and microsomal heme contents. Co-heme administration also markedly lowered the level of cytochrome *P*-450 in kidney, although this decrease was slower in onset, reaching its lowest level at days 6–10 (results not shown).

The ability of a single dose of Co-heme to depress cytochrome *P*-450 content in liver was both protracted and profound (Fig. 1). The level of heme protein decreased to ≈20% of control by day 2 after administration of metalloporphyrin and remained there until about day 12 when it gradually began to increase (Fig. 1). Cytochrome *P*-450 content was, however, still 40% below initial levels at day 21 and initial levels had not been attained by the end of the 5-week study. Activities of ethylmorphine demethylase and aniline hydroxylase paralleled the decline in cytochrome *P*-450 (results not shown). Heme oxygenase activity increased 6- to 7-fold by day 2 and remained elevated through day 15 (4-fold increase); normal activity of this enzyme was achieved by day 36. AmLev synthase activity declined to ≈40% of initial levels within 2 hr (results not shown) and remained depressed throughout the 36-day study. Cytochrome *P*-450 content had begun to increase at a time when AmLev synthase activity was still depressed although, at this time, heme oxygenase activity had begun to return to normal.

Time Course Study with Multiple Administrations of Co-heme; Effects on Heme Metabolism in Liver. The ability of a series of Co-heme doses (50 μmol/kg each) to depress and main-

Table 2. Effect of a single dose of Co-heme on activities of heme oxygenase and AmLev synthase, cellular contents of heme and heme proteins, and activities of cytochrome *P*-450 drug-metabolizing enzymes in liver

	Saline	Co-heme
Heme oxygenase, nmol of bilirubin·$mg^{-1}\cdot hr$	2.65 ± 0.11	15.62 ± 0.90
AmLev synthase, nmol of AmLev·$mg^{-1}\cdot hr^{-1}$	0.201 ± 0.05	0.082 ± 0.01
Cytochrome *P*-450, nmol/mg	0.80 ± 0.04	0.19 ± 0.03
Cytochrome b_5, nmol/mg	0.35 ± 0.01	0.23 ± 0.01
Microsomal heme, nmol/mg	1.85 ± 0.04	0.89 ± 0.05
Ethylmorphine demethylase, μmol of HCHO·$mg^{-1}\cdot hr^{-1}$	0.555 ± 0.06	0.062 ± 0.02
Aniline hydroxylase, nmol of *p*-aminophenol·$mg^{-1}\cdot hr^{-1}$	89.42 ± 6.90	32.75 ± 3.01

A single dose of Co-heme (125 μmol/kg of body weight) was administered subcutaneously to each of six animals. The animals were permitted food and water for 56 hr; then, the food was removed, and they were permitted water only until they were killed 16 hr later. Each assay was performed in duplicate. Data are mean ± SEM.

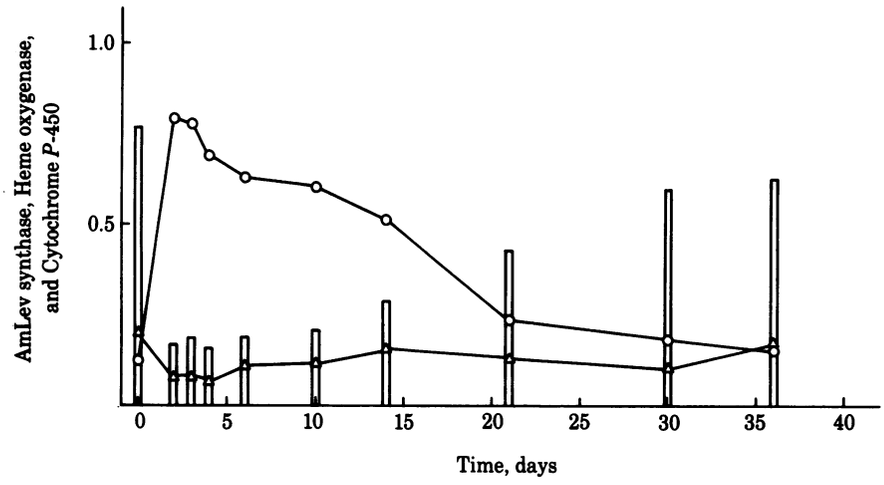

FIG. 1. Effect of a single dose of Co-heme (125 μmol/kg of body weight) on cytochrome *P*-450 content (nmol/mg; ▯) and heme oxygenase (nmol × 0.5 of bilirubin·mg^{-1}·hr^{-1}; ○) and AmLev synthase (nmol of AmLev·mg^{-1}·hr^{-1}; △) activities in liver. Animals were treated subcutaneously with Co-heme and allowed food and water until 16 hr prior to sacrifice, when food was removed. Each point represents the average of three to six animals. Assays were performed in duplicate.

tain at very low levels cytochrome *P*-450 content *in vivo* for extended periods of time was studied in liver (Fig. 2). Co-heme produced an immediate rapid decrease in hepatic cytochrome *P*-450 (≈30% of initial levels by day 2; Fig. 2). The heme protein was then maintained at an average of 15% of control level for the entire 13-week study period by repeated Co-heme administration (total, six doses). In addition, activities of the cytochrome *P*-450-dependent enzymes ethylmorphine demethylase, aniline hydroxylase, and aryl hydrocarbon hydroxylase decreased immediately after Co-heme administration and paralleled the very low cytochrome *P*-450 content throughout the entire study period (Table 3). Similar sustained low levels of cytochrome *P*-450 were noted in kidney (results not shown).

Heme oxygenase activity increased ≈7-fold by day 2 after the initial dose of Co-heme. Successive Co-heme doses served to maintain elevated enzyme activity for >13 weeks (Fig 2). AmLev synthase activity declined markedly (≈50% of normal by day 2) and remained at this or lower levels throughout the entire study period.

DISCUSSION

Our results show that the cytochrome *P*-450 content and the activities of cytochrome *P*-450-dependent drug- and carcinogen-metabolizing enzymes can be markedly decreased *in vivo* for extended periods by single or multiple doses of Co-heme. Within 48 hr of a single dose of Co-heme, cytochrome *P*-450 content decreased to 20% of normal. This decrease persisted for ≈12 days (Fig. 1) before reverting toward normal; however, control cytochrome *P*-450 levels had still not been regained at the end of the 5-week study. A series of smaller doses of Co-heme depressed cytochrome *P*-450 content to an average of 15% of that of control animals for >13 weeks (Fig. 2 and Table 3), at which time the experiment was terminated. Co-heme thus provides a chemical means for producing short-term (i.e., 1 to 2 weeks, Fig. 1) as well as long-term (i.e., 3 months or more, Fig. 2) depletion of hepatic cytochrome *P*-450 in the whole animal.

Co-heme administration also produced marked prolonged changes in the activities of heme oxygenase and AmLev synthase, the rate-limiting enzymes of heme catabolism and heme synthesis, respectively (4, 5, 7). Heme oxygenase was increased in a dose-dependent manner by the metalloporphyrin. A single injection (Fig. 1) of the highest dose examined (125 μmol/kg of body weight) produced a 6- to 7-fold elevation of this enzyme activity by day 2. Activity remained elevated (4-fold) at day 14 but returned to normal by day 36 (Fig. 1). A direct consequence of this protracted increase in heme oxygenase activity is an enhanced rate of heme degradation (18, 19), leading to a substantial decrease in cellular heme. Concomitantly AmLev synthase activity was substantially depressed by Co-heme. Either a single or multiple doses of the metalloporphyrin maintained this activity at very low levels for extended time periods (Figs. 1 and 2). Thus, a high rate of heme catabolism combined with a low rate of heme synthesis produced by Co-heme results in a metabolic situation in which only negligible amounts of heme become available to serve as the prosthetic group for cellular heme

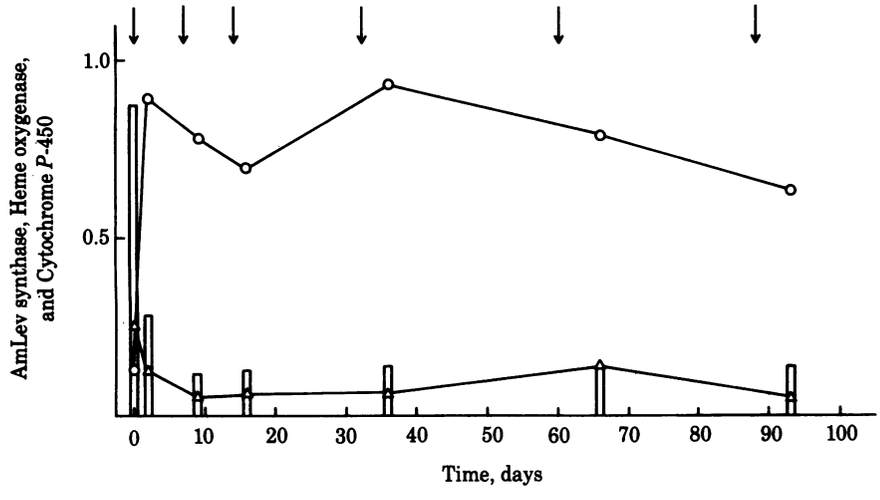

FIG. 2. Effect of multiple doses of Co-heme (50 μmol/kg of body weight) on cytochrome *P*-450 content (nmol/mg; ▯) and heme oxygenase (nmol × 0.5 of bilirubin·mg^{-1}·hr^{-1}; ○) and AmLev synthase (nmol of AmLev·mg^{-1}·hr^{-1}; △) activities in liver. ↓, Times at which animals were treated. Animals were allowed food and water until 16 hr prior to sacrifice when food was removed. Each point represents the average of three to six animals. Assays were performed in duplicate.

Table 3. Effects of multiple doses of Co-heme on cytochrome *P*-450 content and various drug-metabolizing activities in liver

Time, days	Cytochrome *P*-450, nmol/mg	Ethylmorphine demethylase, μmol of $HCHO \cdot mg^{-1} \cdot hr^{-1}$	Aniline hydroxylase, nmol of *p*-aminophenol$\cdot mg^{-1} \cdot hr^{-1}$	Aryl hydrocarbon hydroxylase, nmol of benzo[*a*]pyrene$\cdot mg^{-1} \cdot hr$
0	0.87	0.53	105.6	92.4
2	0.28	0.11	34.8	16.7
9	0.12	0.05	15.4	10.8
16	0.13	0.10	18.3	17.9
36	0.14	0.06	34.9	2.8
66	0.13	0.04	16.9	1.9
93	0.14	0.05	14.9	5.4

Doses of Co-heme (50 μmol/kg of body weight) were administered subcutaneously to a group of rats at days 0, 7, 14, 32, 60, and 88. The animals were permitted food and water until 16 hr before they were killed when the food was removed. Each assay was performed in duplicate. Each point represents the average of pooled microsomes from three to six animals.

proteins. A consequence of this metabolic situation is a marked protracted decrease in cytochrome *P*-450 (Figs. 1 and 2) and of oxidative enzymatic activities dependent on this heme protein (Tables 2 and 3). Dual potent effects of Co-heme on rates of heme synthesis and heme degradation could in themselves account for the reduced hepatic cytochrome *P*-450 observed in these studies. However, as Wagner *et al.* (20) reported, cobalt protoporphyrin IX can be inserted into purified apocytochrome *P*-450 from *P. putida*. If such a process occurred *in vivo*, depletion of catalytically active cytochrome *P*-450 could occur in part from formation of a Co-heme cytochrome *P*-450.

The ability of inorganic cobalt to induce heme oxygenase, thereby decreasing cytochrome *P*-450 content in liver, is well documented (16, 21). However, inorganic cobalt produces short-term effects that return to normal within 72 hr. In contrast, Co-heme produced more substantial and protracted effects on cytochrome *P*-450 and on heme oxygenase and AmLev synthase (Figs. 1 and 2). These properties of Co-heme are probably related in part to the fact that Co-heme is not oxidized by the microsomal heme oxygenase system (22, 23) although, as we recently demonstrated (2, 3), it can interact with heme oxygenase at its catalytic site and serve as a potent competitive inhibitor of heme oxidation to bile pigment.

Co-heme is also a potent inducer of renal heme oxygenase but, in kidney, maximum induction did not occur until 6–10 days after administration (results not shown), a time considerably later than the response produced in liver (Fig. 1).

Phenobarbital induction of cytochrome *P*-450 over prolonged time periods in experimental animals produces major morphological and biochemical alterations in liver, including tumor formation (24–26). The marked depletion of hepatic cytochrome *P*-450 produced by Co-heme during the 3-month experiment (Fig. 2) also produced significant metabolic alterations apart from those involving hepatic heme metabolism. The most evident of these alterations were a prompt halt in weight gain, a considerable loss of body hair, and later tissue wasting, suggesting major alterations of general cellular metabolism apart from those enzymatic changes determined in the liver. Animals commenced to gain weight shortly after the last dose of Co-heme had been administered, at which time cytochrome *P*-450 was returning to normal levels. These observations suggest that Co-heme administration may provide a means to elucidate the physiological role of cytochrome *P*-450 and of heme in the metabolism of endogenously derived chemicals involved in the maintenance of metabolic homeostasis, in the production of hormones, and in the regulation of growth processes in the whole animal.

Bacterial mutagenesis assays use a crude microsomal fraction (S-9 fraction) containing cytochrome *P*-450 to activate chemicals as an integral part of the test procedure. Thus, the profound depletion of cytochrome *P*-450 produced by Co-heme and the parallel decrease in ability of microsomes to carry out *P*-450-dependent metabolic reactions provides a sensitive means for comparing the role of *P*-450-depleted as well as *P*-450-induced microsomes in determining the mutagenicity of chemicals and their metabolic products. Cytochrome *P*-450-depleted microsomes produced by Co-heme treatment *in vivo* could be used to provide appropriate microsomal fractions for use in such *in vitro* studies.

Co-heme depletion of cytochrome *P*-450 *in vivo* also provides a method for examining cytochrome *P*-450-dependent chemical metabolism at different levels of this heme protein in a single animal. For example, by using an inducer of cytochrome(s) *P*-450 such as phenobarbital or 3-methylcholanthrene, cytochrome *P*-450-dependent biotransformations can be studied in the presence of high levels of specific species of this heme protein. Co-heme administration would then permit similar studies to be carried out when the induced species of cytochrome *P*-450 in the liver had been largely depleted. The experimental animal would thus serve as its own internal control, obviating the problems of genetic variation in chemical metabolism that arise when two or more animals are used.

Finally, certain carcinogens, such as TCDD (27) and dieldrin (28), that are nonmutagenic in microbial test systems are considered to require long-term continuous metabolism to become proximately activate cancer-producing agents. A further potential use of Co-heme depletion of cytochrome *P*-450 could thus involve evaluation of the role of long-sustained oxidative metabolism *in vivo* on the tumoregenic properties of various chemicals.

We thank Ms. Susan Spinner for technical assistance, Ms. Michele Bifano for preparation of this manuscript, and Dr. Shigeru Sassa for his helpful and enlightening discussions. This study was supported by U.S. Public Health Service Grant ES-01055 and by a gift from Mr. Ralph Ablon, The Ogden Corporation.

1. Ames, B. N., Durston, W. E., Yamasaki, E. & Lee, F. E. (1973) *Proc. Natl. Acad. Sci. USA* **70**, 2281–2285.
2. Drummond, G. S. & Kappas, A. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 6466–6470.
3. Drummond, G. S. & Kappas, A. (1981) *Fifth International Symposium on Microsomes and Drug Oxidations*, Japan Scientific Societies Press, Tokyo, in press.

4. Tenhunen, R., Marver, H. S. & Schmid, R. (1968) *Proc. Natl. Acad. Sci. USA* **61,** 748–755.
5. Tenhunen, R., Marver, H. S. & Schmid, R. (1969) *J. Biol. Chem.* **244,** 6388–6394.
6. Drummond, G. S. & Kappas, A. (1980) in *Biochemistry, Biophysics and Regulation of Cytochrome P-450,* eds. Gustafson, J. A., Carlstedt-Duke, J., Mode, A. & Rafter, J. (Elsevier/North Holland, Amsterdam), pp. 203–206.
7. Granick, S. & Urata, G. (1963) *J. Biol. Chem.* **238,** 821–827.
8. Sassa, S., Kappas, A., Bernstein, S. E. & Alvares, A. P. (1979) *J. Biol. Chem.* **254,** 729–735.
9. Anderson, K. E., Drummond, G. S., Freddara, U., Sardana, M. K. & Sassa, S. (1981) *Biochim. Biophys. Acta* **676,** 289–299.
10. Maines, M. D. & Kappas, A. (1978) *J. Biol. Chem.* **253,** 2321–2326.
11. Nash, T. (1953) *Biochem. J.* **55,** 416–421.
12. Imai, Y., Ito, A. & Sato, R. (1966) *J. Biochem. (Tokyo)* **60,** 417–428.
13. Omura, T. & Sato, R. (1964) *J. Biol. Chem.* **239,** 2379–2385.
14. Paul, K. G., Thorell, H. & Akeson, A. (1953) *Acta Chem. Scand.* **7,** 1284–1287.
15. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265–275.
16. Maines, M. D. & Kappas, A. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 4293–4297.
17. Maines, M. D. & Kappas, A. (1977) *Science* **198,** 1215–1221.
18. Maines, M. D. & Kappas, A. (1976) *Ann. Clin. Res.* **8,** Suppl. 17, 39–46.
19. DeMatteis, F. & Unseld, A. (1976) *Biochem. Soc. Trans.* **4,** 205–209.
20. Wagner, G. C., Gunsalus, I. C., Wang, M-Y. R. & Hoffman, B. M. (1981) *J. Biol. Chem.* **256,** 6266–6273.
21. Maines, M. D. & Kappas, A. (1975) *J. Biol. Chem.* **250,** 4171–4177.
22. Yoshida, T. & Kikuchi, G. (1978) *J. Biol. Chem.* **253,** 8479–8482.
23. Sassa, S., Walter, R. & Kappas, A. (1979) *Fed. Proc. Fed. Amer. Soc. Exp. Biol.* **38,** 460.
24. Levin, W., Welch, R. M. & Conney, A. H. (1974) *J. Pharmacol. Exp. Ther.* **188,** 287–292.
25. Sultatos, L. G., Vesell, E. S. & Hepner, G. W. (1979) *Biochem. Pharmacol.* **28,** 849–857.
26. Feldman, D., Swarm, R. L. & Becker, J. (1981) *Cancer Res.* **41,** 2151–2162.
27. Wassom, J. A., Huff, J. E. & Lopsieno, N. (1977/1978) *Mutat. Res.* **47,** 141–160.
28. McCann, J., Choi, E., Yamasaki, E. & Ames, B. N. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 5135–5139.