Biochemical Pharmacology, Vol. 33, No. 4, pp. 571–576, 1984.
Printed in Great Britain.

0006-2952/84 $3.00 + 0.00
Pergamon Press Ltd.

STUDIES ON THE MECHANISMS OF THALLIUM-MEDIATED INHIBITION OF HEPATIC MIXED FUNCTION OXIDASE ACTIVITY

CORRELATION WITH INHIBITION OF NADPH–CYTOCHROME *c* (P-450) REDUCTASE

JAMES S. WOODS* and BRUCE A. FOWLER

Laboratory of Environmental Toxicology, National Institute of Environmental Health Sciences, National Institutes of Health, Research Trinagle Park, NC 27709, U.S.A.

and

DAVID L. EATON

Department of Environmental Health, University of Washington, Seattle, WA 98195, U.S.A.

(*Received* 4 *April* 1983; *accepted* 15 *August* 1983)

Abstract—Thallium ($TlCl_3$) administration to rats produced a dose-dependent loss of hepatic NADPH–cytochrome *c* (P-450) reductase and microsomal mixed function oxidase activities within 2–4 hr following treatment. These changes occurred independently of apparent effects on microsomal heme or cytochrome P-450 content, both of which remained unchanged with respect to control levels despite transient inhibition of δ-aminolevulinic acid (ALA) synthetase and induction of heme oxygenase. These results are consistent with the recognized properties of thallium as both a flavoprotein antagonist and sulfhydryl inhibitor and differ uniquely from the action of other metals which impair mixed function oxidase activity through compromise of heme biosynthesis and heme depletion. The potential utility of thallium compounds in further evaluating the functional characteristics of NADPH-cytochrome *c* (P-450) reductase and its role in microsomal oxidative processes is suggested from these observations.

Previous studies from these and other laboratories have demonstrated the development of a deficiency in microsomal mixed function oxidase (MFO) activities in livers of animals following treatment with various trace metals and metal-containing compounds [1–10]. Although the precise mechanisms by which this phenomenon occurs have not been elucidated, these events appear to be closely related to reduction of hepatocellular heme levels resulting from inhibition of δ-aminolevulinic acid (ALA) synthetase and/or induction of heme oxygenase, the rate-limiting enzymes in heme biosynthesis [11] and degradation [12] respectively. Cellular heme depletion subsequently leads to reduction in cytochrome P-450 content with concomitant compromise of P-450-dependent oxygenation reactions. Impairment of the mixed function oxidase system resulting from inhibition of intermediate enzymes of the heme biosynthetic pathway by various metals has also been demonstrated [1, 2, 4, 7, 13].

Typically, alterations of heme metabolism and consequent effects on heme-dependent MFO activity are not precisely correlated in time, the former event usually preceding the latter by several hours or days following metal treatment. Thus, a latency period of 24 hr or more is often required for depletion of cellular heme to an extent sufficient to affect P-450-dependent enzyme activities. Within the context of these observations, therefore, it was interesting to observe in recent studies from these laboratories [5, 14–16] on the mechanisms of action of group III trace metals, which includes aluminum, indium and thallium, that several elements of this series produce a rapid depression of MFO enzyme activities within a time course following treatment which is substantially less than that anticipated on the basis of the usual latent period for this effect observed with other metals. Moreover, this effect occurred at dose levels which do not produce generalized inhibition of protein synthesis. Thallium is especially effective in this regard, producing a pronounced depression of microsomal aniline hydroxylase within 2–4 hr after intraperitoneal administration, with no apparent significant effect on cytochrome P-450 or microsomal heme levels. This observation is particularly intriguing inasmuch as it suggests that thallium might contribute to impairment of MFO activities through mechanisms other than those involving direct effects on heme metabolism and heme-dependent components of the mixed function oxidase system. Moreover, the known properties of thallium as a flavoprotein antagonist [17, 18] and as a potent sulfhdryl group inhibitor [19, 20] suggest that interference with microsomal oxidative metabolism might be mediated via inhibition of the flavoprotein component of that system, NADPH–cytochrome *c* (P-450) reductase (EC 1.6.2.4).

* Present address, to which galley proofs and all correspondence regarding this manuscript should be sent: James S. Woods, Ph.D., Battelle Seattle Research Center, 4000 N.E. 41st St., Seattle, WA 98105.

The present studies were undertaken to investigate the mechanisms through which thallium mediates inhibition of microsomal MFO activities in rat liver. The effects of thallium on heme biosynthetic and degradative enzymes were evaluated relative to the impact on microsomal heme and cytochrome P-450 levels. The dose-related effects of thallium on cytochrome *c* (P-450) reductase activity and the temporal relationship of these effects on microsomal MFO function were also investigated. The results suggest that thallium-mediated inhibition of hepatic MFO activity is closely correlated with inhibition of NADPH–cytochrome *c* (P-450) reductase *in vivo*. This action occurs independently of metal-induced effects on cellular heme metabolism and may represent an important component of metal-related effects on hepatocellular mixed function oxidative capacity.

MATERIALS AND METHODS

Treatment of animals. Male Sprague–Dawley rats (CD strain) (150–200 g) were obtained from Charles River Laboratories, Boston, MA, and were maintained in individual wire-bottom cages in barrier isolation rooms with free access to laboratory chow and water. Prior to being killed, animals were divided into five groups of five animals each and were treated by intraperitoneal injection with aqueous solutions of thallium chloride ($TlCl_3 \cdot 4H_2O$) (Alfa Products, Danvers, MA) at the doses indicated. Controls received vehicle only. All animals were fasted for the duration of exposure to the experimental agent.

Preparation of tissues. Animals were killed by decapitation. Livers were rapidly excised, washed, weighed, and homogenized in 9 vol. of 0.25 M sucrose containing 0.05 M Tris–HCl buffer, pH 7.5, in a Potter–Elvehjem homogenizer fitted with a Teflon pestle. Mitochondrial and microsomal fractions were prepared as previously described [21]. All assays were performed on fresh tissues immediately following sacrifice.

Assay of enzyme activities. ALA synthetase activity in hepatic mitochondria was measured by a modification of the method of Scholnick *et al.* [22], as previously described [21]. Reaction mixtures contained approximately 4 mg of mitochondrial protein/ml. ALA dehydratase activity was measured in 9000 *g* supernatant fractions of liver homogenates by a modification of the method of Gibson *et al.* [23], as described by Baron and Tephly [24]. Uroporphyrinogen I synthetase activity in liver was measured in the 9000 *g* supernatant fractions after heating at 65° for 15 min, by the method of Levin and Coleman [25], as previously described [1]. Mitochondrial heme synthetase activity was measured by a modification of the method of Porra [26] as previously described [2]. Microsomal heme oxygenase activity was measured by a modification of the method of Correia and Schmid [27], as previously described [2]. Microsomal hemes were quantitated by the pyridine hemochromogen method as described by Falk [28].

Cytochrome P-450 in rat liver microsomes was determined by difference spectrophotometry using the method of Omura and Sato [29]. Microsomal aminopyrine demethylase was measured by a modification of the method of Orrenius [30], as described by Lucier *et al.* [31]. Microsomal aniline hydroxylase activity was measured by the method described by Mazel [32]. NADPH–cytochrome *c* (P-450) reductase was measured in microsomes by following the reduction of cytochrome *c* at 550 nm [33]. In studies *in vitro*, enzyme activity was measured in previously frozen microsomes prepared from pooled livers of three rats pretreated for 3 days prior to sacrifice with phenobarbital (80 mg/kg/day).

Protein determinations were performed by the

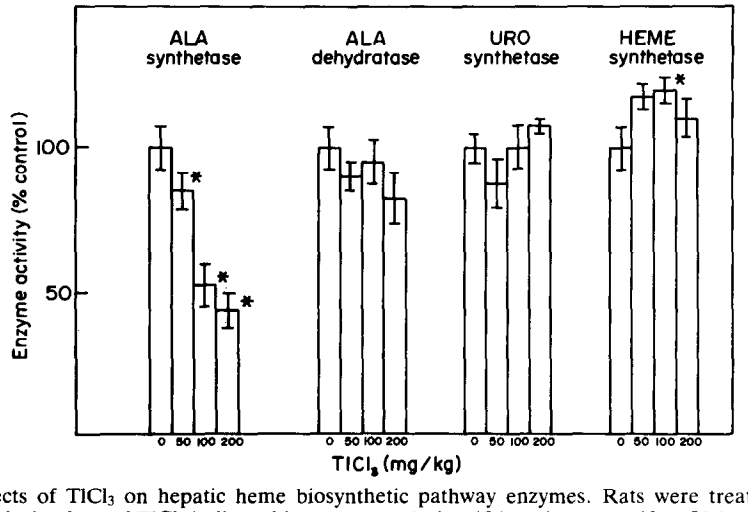

Fig. 1. Effects of $TlCl_3$ on hepatic heme biosynthetic pathway enzymes. Rats were treated by i.p. injection with the dose of $TlCl_3$ indicated in aqueous solution 12 hr prior to sacrifice. Values represent means ± S.E. expressed as a percentage of control enzyme activities as follows: ALA synthetase, 0.64 ± 0.06 nmole ALA/mg protein/hr; ALA dehydratase, 5.26 ± 0.42 nmoles PBG/mg protein/hr; uroporphyrinogen I synthetase, 6.08 ± 0.13 nmoles PBG/mg protein/hr; and heme synthetase, 2.43 ± 0.28 nmoles mesohme/mg protein/hr. An asterisk (*) indicates significantly different from the control value at $P < 0.05$.

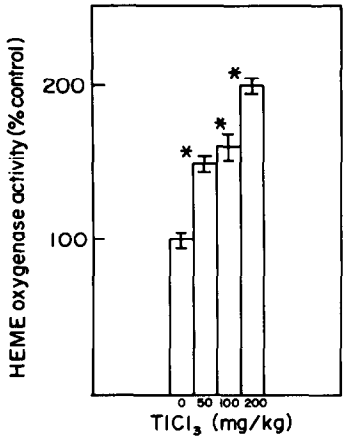

Fig. 2. Effect of $TlCl_3$ on hepatic microsomal heme oxygenase. Rats were treated by i.p. injection with the dose of $TlCl_3$ indicated 12 hr prior to sacrifice. Values represent mean ± S.E. expressed as a percentage of control enzyme activity, 2.50 ± 0.28 pmoles bilirubin/mg protein/min. An asterisk (*) indicates significantly different from the control value at $P < 0.05$.

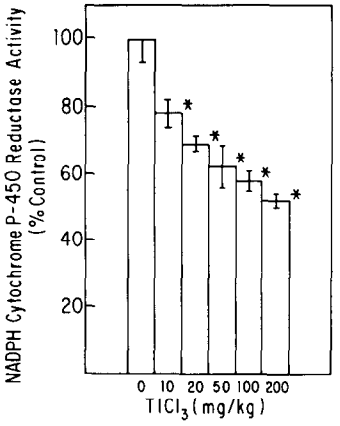

Fig. 3. Effect of $TlCl_3$ on hepatic microsomal NADPH–cytochrome c (P-450) reductase. Rats were treated by i.p. injection with the dose of $TlCl_3$ indicated 12 hr prior to sacrifice. Values represent mean ± S.E. as a percentage of control enzyme activity, 140.6 ± 9.9 nmoles/mg protein/min. An asterisk (*) indicates significantly different from the control value at $P < 0.05$.

method of Lowry *et al.* [34], using bovine serum albumin (Fraction V) as a standard.

Statistical analyses. Analyses of significance of differences between groups were performed by means of Student's *t*-test. The level of significance was chosen as $P < 0.05$.

RESULTS

The action of thallium on heme biosynthetic capability in the liver was assessed by measuring the effects of $TlCl_3$ on several enzymes of the heme biosynthetic pathway including ALA synthetase, the rate-limiting enzyme in this process. As indicated in Fig. 1, ALA synthetase was highly susceptible to inhibition by thallium *in vivo*, a significant decrease in activity occurring at all dose levels 12 hr following treatment. A decline in enzyme activity to 85% of control values occurred at 50 mg/kg, whereas a decrease to 43% of control values was seen at 200 mg/kg. ALA synthetase was also inhibited by thallium *in vitro*, with concentrations in the reaction mixture of 0.05, 0.1 and 0.2 mg/ml inhibiting the enzyme to 63, 47 and 22% of control values respectively.

Interestingly, thallium had no significant effects *in vivo* on the activities of several intermediate heme biosynthetic pathway enzymes (Fig. 1), except for a slight increase in the activity of heme synthetase (ferrochelatase), the last enzyme of the heme biosynthetic process. Neither ALA dehydratase, a sulfhydryl enzyme which catalyzes porphobilinogen (PBG) formation from ALA, nor uroporphyrinogen I synthetase, which catalyzes the synthesis of uroporphyrinogen from PBG, was altered significantly following thallium treatment at any dose level. ALA dehydratase and heme synthetase, but not uro synthetase, were, however, inhibited significantly by thallium *in vitro* at the same concentrations sited for ALA synthetase.

The action of thallium on heme degradation in the liver was assessed by measuring the activity of microsomal heme oxygenase in thallium-treated rats. The effects of enzyme activity are shown in Fig. 2. As indicated, thallium treatment produced a significant increase in heme oxygenase activity at each dose level. A mean maximal 2-fold increase in enzyme activity was observed at 12 hr following treatment.

The results of thallium-mediated alteration of heme biosynthesis and degradation on hepatocellular

Table 1. Effects of $TlCl_3$ treatment on cytochrome P-450, microsomal heme and aniline hydroxylase in rat liver *in vivo**

$TlCl_3$ (mg/kg)	Cytochrome P-450 (nmoles/mg protein)	Microsomal heme (nmoles/mg protein)	Aniline hydroxylase (nmoles/mg protein/min)
0	0.89 ± 0.15	1.99 ± 0.26	43.94 ± 1.95
50	0.79 ± 0.16	1.63 ± 0.28	38.91 ± 1.04†
100	0.83 ± 0.07	1.80 ± 0.42	34.28 ± 0.94†
200	0.84 ± 0.11	1.73 ± 0.44	23.73 ± 1.62†

* Values represent the mean ± S.E. of five experiments. Livers from five rats from each treatment group were pooled for each experimental value. Assays were performed as described under Materials and Methods.

† Significantly different from tho control value at $P < 0.05$.

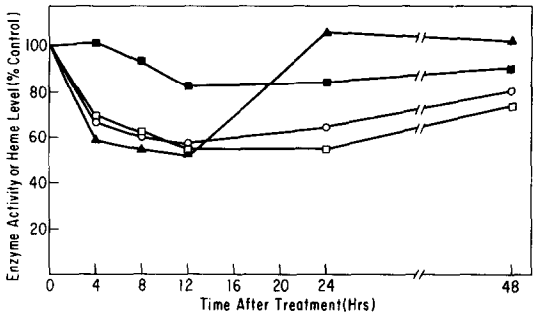

Fig. 4. Time course of $TlCl_3$ effects on ALA synthetase (▲), microsomal heme (■), aniline hydroxylase (○) and NADPH–cytochrome *c* (P-450) reductase (□). Rats were treated by i.p. injection with 100 mg/kg $TlCl_3$ and killed at the times indicated. Control (zero time) levels for parameters measured are indicated in other figures and tables.

heme levels and heme-dependent mixed function oxidase activity were assessed by measuring microsomal heme and cytochrome P-450 content as well as aniline hydroxylase activity in rat liver at 12–16 hr following thallium treatment. As indicated in Table 1, neither cytochrome P-450 nor microsomal heme content was altered significantly by thallium treatment at any dose level. In contrast, aniline hydroxylase activity was decreased significantly at all dose levels. A similar though less dramatic reduction in activity was observed with respect to aminopyrine demethylase (6.22 ± 0.3 vs 4.14 ± 0.4 nmoles HCOH/mg protein/min at 200 mg/kg).

The observed reduction in mixed function oxidase activity described in Table 1 was accompanied by a pronounced depression of NADPH–cytochrome *c* (P-450) reductase activity. As indicated in Fig. 3, thallium significantly inhibited cytochrome *c* (P-450) reductase at all dose levels tested. Moreover, significant inhibition was observed at doses as low as 10 mg/kg, a dose substantially less than those at which effects on heme enzymes or other measured parameters were observed.

Thallium was also an effective inhibitor of NADPH–cytochrome *c* (P-450) reductase *in vitro*. As shown in Table 2, thallium produced a dose-related inhibition of the reductase, with a complete loss of activity observed at a concentration of 0.2 mg/ml (0.1 mM Tl^{3+}) in the reaction mixture. Interestingly, inhibition was prevented completely by addition of the sulfhydryl reagent dithiothreitol (DTT) (3 mM) to the sample cuvette 5 min prior to the addition of thallium (0.1 mM). However, enzyme activity was only partially restored when DTT was introduced 5 min subsequent to the addition of thallium at either 0.1 or 0.2 mg/ml. In other studies *in vitro*, NADPH–cytochrome *c* (P-450) reductase was inhibited to the same extent by thallium regardless of whether thallium was preincubated with the enzyme prior to initiation of the reaction with cytochrome *c* or added concomitantly with cytochrome *c* to the reaction mixture. Moreover, the inhibition of reductase by thallium was not reversed or prevented by either excess cytochrome *c* or NADPH added either subsequently or prior to the addition of the thallium salt.

To more fully characterize the relationship between heme synthesis, NADPH–cytochrome *c* (P-450) reductase, and other components of the mixed function oxidase system, time–course studies *in vivo* were performed wherein the levels of ALA synthetase, microsomal heme, cytochrome *c* (P-450) reductase and aniline hydroxylase were measured over a 48-hr period following thallium treatment (100 mg/kg). The results of these studies are shown in Fig. 4. A decline in ALA synthetase activity occurred within the first 4–8 hr following thallium administration, but activity returned to control levels within the subsequent 8- to 12-hr period. The decline in ALA synthetase activity was followed by a slight but statistically non-significant decrease in microsomal heme content, which remained slightly below control levels throughout the remainder of the observation period. Cytochrome P-450 levels were not altered during the course of observation, values ranging from 0.88 ± 0.15 nmole/mg protein at zero time to 0.79 ± 0.16 nmole/mg protein at 48 hr after treatment. In contrast, a significant decrease ($P < 0.05$) in the activities of both NADPH–cytochrome *c* (P-450) reductase and aniline hydroxylase occurred

Table 2. Effects of $TlCl_3$ on NADPH–cytochrome *c* (P-450) reductase activity *in vitro**

$TlCl_3$ (mg/kg)	NADPH–cytochrome *c* (P-450) reductase (nmoles/mg protein/min)	Inhibition (% of control)
0	69.11 ± 4.53	100
0.05	42.47 ± 2.23†	61
0.1	18.84 ± 1.56†	27
0.2	0†	0
0.1 after DTT	71.10 ± 2.76	103
0.1 followed by DTT	31.71 ± 4.06†	46
0.2 followed by DTT	31.71 ± 3.76†	46

* Values represent the mean ± S.E. of three experiments. Activity was measured in previously frozen microsomes prepared from pooled livers of three rats pretreated for 3 days prior to sacrifice with phenobarbital (80 mg/kg/day). Assays were performed as described under Materials and Methods. Dithiothreitol (DTT) (3 mM) was added either 5 min before or after $TlCl_3$, as indicated.

† Significantly different from the control value at $P < 0.05$.

within the first 2–4 hr following thallium treatment, and parallel changes in the enzyme activities were observed throughout the remainder of the time course.

DISCUSSION

The results of the present studies demonstrate a newly defined property of thallium to significantly impair the microsomal mixed function oxidase system in rat liver *in vivo*. This effect appears to be mediated primarily through inhibition of NADPH–cytochrome *c* (P-450) reductase, an integral component of the microsomal electron transfer system. Although compromise of MFO activity by specific inhibitors of cytochrome *c* (P-450) reductase has been described previously [35–37], we believe this study to be the first report of impairment of hepatic MFO activity through inhibition of the reductase by a specific metal. This effect is unique inasmuch as compromise of MFO function heretofore reported in association with metal exposure has been largely attributed to alteration of heme biosynthetic capacity and depletion of cellular heme levels.

The precise mechanisms by which thallium inhibits cytochrome *c* (P-450) reductase have not as yet been determined, but such activity is consistent with the known properties of thallium as both a flavoprotein antagonist [18] as well as a sulfhydryl group inhibitor [19, 20]. While little is known of the former mechanism, support for the latter process is derived from recent studies on the structural properties of cytochrome *c* (P-450) reductase [38, 39], which provide evidence that the catalytic activity of the enzyme is largely dependent on functional cysteinyl residue(s) [35, 40]. As a "soft" Lewis acid [41] with a d^{10} electronic configuration, thallium is particularly amenable to coordinate covalent bond formation with sulfhydryl groups, themselves among the most chemically reactive functional groups found in cells [42]. This fact, together with the observation that all cysteine residues of the reductase are present as free sulfhydryl forms, i.e. with no disulfite bridges [39], supports the exceptional sensitivity of this enzyme to inhibition resulting from metal-mercaptide formation with thallium.

The interesting observation from the present studies *in vitro*, that DTT only partially reversed thallium-inhibited cytochrome *c* (P-450) reductase, further supports the idea that several mechanisms may be involved in the interaction of thallium with this enzyme. Thus, DTT treatment restored reductase activity to approximately 50% of control levels regardless of the initial extent of inhibition in the presence of thallium alone. This observation may represent the extent to which metal-mercaptide formation is responsible for enzyme inhibition by thallium or, alternatively, may reflect the inability of the sulfhydryl reagent to overcome the metal-mercaptide bond formation which has already formed between thallium and sulfhydryl groups of the enzyme. On the other hand, an entirely different interaction between thallium and the enzyme, such as metal binding to the flavin portion of the protein, as previously suggested, may account for the sustained inhibition of reductase activity which is observed in the presence of DTT.

The failure of thallium to have a significantly pronounced effect on microsomal heme content or P-450 levels is curious in light of the substantial inhibition of ALA synthetase and induction of heme oxygenase observed following thallium treatment. One explanation for this observation is the transitory nature of the effects of thallium on ALA synthetase as observed in the time–course studies. Inasmuch as ALA synthetase activity returned to control levels within less than 24 hr after thallium administration, it is possible that impairment of heme biosynthesis was insufficient to have a significant impact on overall microsomal heme content. Moreover, an increase in heme oxygenase activity of only 2-fold was observed following thallium treatment. This effect is substantially less than that produced by many metals whose induction of heme oxygenase is associated with subsequent reduction of hepatocellular heme and P-450 levels. Alternatively, numerous forms of cytochrome P-450 which mediate specific MFO reactions are considered to exist in the liver, and it is possible that differential inhibition of one or more of those forms by thallium may have gone undetected in these studies where overall P-450 content was assessed. This prospect seems unlikely in light of the observed inhibition of aniline hydroxylase, an enzyme associated with the major phenobarbital-inducible form of P-450 in the liver. Nevertheless, further studies are required to evaluate this possibility.

That thallium did not appear to inhibit *in vivo* enzymes of the heme biosynthetic pathway other than ALA synthetase is an interesting observation in light of the properties of thallium as a potent sulfhydryl group inhibitor. One possible explanation for this phenomenon, applicable especially to the soluble enzymes such as ALA dehydratase, is that thallium concentrations were diluted below levels sufficient for enzyme inhibition during cell fractionation in preparation for enzyme assays. This possibility is unlikely, however, in light of the strength of the coordinate covalent bond which characterizes thallium-mercaptide formation. A more likely explanation of the lack of effects of thallium on the soluble enzymes evaluated may be found in the results from previous studies from these laboratories [14, 16] which demonstrate the property of group III and other metals to be preferentially localized within specific compartments of the cell, wherein their effects are predominantly expressed. Thus, thallium is known to be preferentially sequestered by mitochondria and other membranous sub-cellular organelles [19, 43], suggesting that effects of thallium *in vivo* are restricted predominantly to membrane bound enzyme systems associated with those organelles. In this respect, the sensitivity of ALA synthetase to thallium may be explained on the basis of metal-mercaptide formation which disrupts the juxtaposition of the loosely bound enzyme to the mitochondrial inner membrane [44], as is required for optimal activity. The tight or integral binding of heme synthetase to the inner membrane [45], on the other hand, may account for the lack of sensitivity of this enzyme to inhibition by thallium *in vivo*, as observed in these studies. Inasmuch as the function of NADPH–cytochrome *c* (P-450) reductase in elec-

tron transfer between NADPH and cytochrome P-450 is highly dependent on the integrity of its binding domain for attachment to the microsomal membrane [46, 47], metal-mercaptide formation at that site could most likely preclude appropriate orientation for optimal electron transfer and, in effect, account for the exquisite sensitivity of this enzyme to thallium inhibition.

Several investigators have proposed that various site-specific inhibitors of NADPH–cytochrome *c* (P-450) reductase might serve as appropriate tools for further investigation of the nature of the enzyme and its role in cellular oxidative processes [35–37]. The results of the present study suggest that thallium may be of particular utility in this respect, owing to the sensitivity of the interaction between this metal and the reductase as observed here. Such utility might include further elucidation of the nature of the interaction between the reductase and NADPH with respect to the sulfhydryl residue at the binding site [39], or investigation of the requirement for sulfhydryl binding in the interaction between the reductase and cytochrome P-450 in the microsomal membrane. Additional studies *in vitro* involving purified or reconstituted preparations of the enzyme would be of particular interest in further defining the nature of the interaction of thallium with both flavin and sulfhydryl components of the enzyme and for assessing the overall contribution of these components to enzyme function.

REFERENCES

1. J. S. Woods, *Biochem. Pharmac.* **25**, 2147 (1976).
2. J. S. Woods and G. T. Carver, *Drug Metab. Dispos.* **5**, 487 (1977).
3. J. S. Woods and B. A. Fowler, *J. Lab. clin. Med.* **90**, 266 (1977).
4. J. S. Woods and B. A. Fowler, *Toxic. appl. Pharmac.* **43**, 361 (1978).
5. J. S. Woods, G. T. Carver and B. A. Fowler, *Toxic. appl. Pharmac.* **49**, 455 (1979).
6. D. W. Rosenberg, G. S. Drummond, H. C. Cornish and A. Kappas, *Biochem. J.* **190**, 465 (1980).
7. J. L. Eiseman and A. P. Alvares, *J. Pharmac. exp. Ther.* **214**, 250 (1980).
8. M. D. Maines and A. Kappas, *Science* **198**, 1215 (1977).
9. M. D. Maines and A. Kappas, in *Clinical Chemistry and Clinical Toxicology of Metals* (Ed. S. S. Brown), pp. 75–87. Elsevier/North-Holland, Amsterdam (1977).
10. T. R. Tephly, C. Webb, P. Trussler, E. Kniffin, E. Hasegawa and W. N. Piper, *Drug Metab. Dispos.* **1**, 259 (1973).
11. S. Granick and G. Urata, *J. biol. Chem.* **238**, 821 (1963).
12. R. Tenhunen, H. S. Marver and R. Schmidt, *J. biol. Chem.* **244**, 6388 (1969).
13. A. Alvarez and A. Kappas, *Drug Metab. Rev.* **10**, 91 (1979).
14. J. S. Woods and B. A. Fowler, *Exp. molec. Path.* **36**, 306 (1982).
15. J. S. Woods, G. T. Carver and B. A. Fowler, *Toxicologist* **1**, 117 (1981).
16. B. A. Fowler, R. M. Kardish and J. S. Woods, *Lab. Invest.* **48**, 471 (1983).
17. A. Pentschew and F. Garro, *J. Neuropath. exp. Neurol.* **28**, 163 (1969).
18. R. Kuhn, H. Rudy and T. Wagner-Jauregg, *Ber. dt. chem. Ges.* **66**, 1950 (1933).
19. M. M. Herman and K. G. Bensch, *Toxic. appl. Pharmac.* **10**, 199 (1967).
20. A. Saddique and C. D. Peterson, *Vet. hum. Toxic.* **25**, 16 (1983).
21. J. S. Woods, *Molec. Pharmac.* **10**, 389 (1974).
22. P. L. Scholnick, L. E. Hammaker and H. S. Marver, *J. biol. Chem.* **247**, 4126 (1972).
23. K. D. Gibson, A. Neuberger and J. J. Scott, *Biochem. J.* **61**, 618 (1955).
24. J. Baron and T. R. Tephly, *Molec. Pharmac.* **5**, 10 (1969).
25. E. Y. Levin and D. L. Coleman, *J. biol. Chem.* **242**, 4248 (1967).
26. R. J. Porra, *Analyt. Biochem.* **68**, 289 (1975).
27. M. A. Correia and R. Schmid, *Biochem. biophys. Res. Commun.* **65**, 1378 (1975).
28. J. E. Falk, *Porphyrins and Metalloporphyrins*, p. 188. Elsevier, Amsterdam (1964).
29. T. Omura and R. Sato, *J. biol. Chem.* **239**, 2370 (1964).
30. S. Orrienius, *J. Cell Biol.* **26**, 713 (1965).
31. G. W. Lucier, O. S. McDaniel, R. Klein and P. E. Brubaker, *Chem. Biol. Interact.* **4**, 265 (1972).
32. P. Mazel, in *Fundamentals of Drug Metabolism and Disposition* (Eds. B. N. La Du, H. G. Mandel and E. L. Way), pp. 546–82. Williams & Wilkins, Baltimore, MD (1971).
33. P. Mazel, in *Fundamentals of Drug Metabolism and Disposition* (Eds. B. N. La Du, H. G. Mandel and E. L. Way), pp. 575–7. Williams & Wilkins, Baltimore, MD (1971).
34. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
35. A. J. Marinello, M. J. Berrigan, R. F. Struck, F. P. Guengerich and H. L. Gurtoo, *Biochem. biophys. Res. Commun.* **99**, 399 (1981).
36. R. E. Ebel, *Archs Biochem. Biophys.* **211**, 227 (1981).
37. T. M. Guenthner, G. F. Kahl and D. W. Nebert, *Biochem. Pharmac.* **29**, 89 (1980).
38. B. M. Anderson, S. T. Kohler and C. D. Anderson, *Archs Biochem. Biophys.* **188**, 214 (1978).
39. T. Lazar, H. Ehrig and L. Lumper, *Eur. J. Biochem.* **76**, 365 (1977).
40. H. Nishibayashi-Yamashita and R. Sato, *J. Biochem., Tokyo* **67**, 199 (1970).
41. R. G. Pearson, *J. chem. Educ.* **45**, 581 (1968).
42. P. D. Boyer, in *The Enzymes* (Eds. P. D. Boyer, H. Lardy and K. Myrback), Vol. 1, pp. 511–88. Academic Press, New York (1959).
43. A. M. Potts and L. M. Gonasun, in *Toxicology—The Basic Science of Poisons* (Eds. J. Doull, C. D. Klaassen and M. O. Amdur), Chap. 13, pp. 275–310. Macmillan, New York (1980).
44. G. M. Patton and D. S. Beattie, *J. biol. Chem.* **248**, 4467 (1973).
45. R. J. Porra, K. S. Vitols, R. F. Labbe and N. A. Newton, *Biochem. J.* **104**, 321 (1967).
46. H. W. Strobel, J. D. Dignam and J. R. Gum, *Pharmac. Ther.* **8**, 525 (1980).
47. S. D. Black and M. J. Coon, *J. biol. Chem.* **257**, 5929 (1982).