Alteration of Liver Glutathione *S*-Transferase and Protease Activities by Cobalt Chloride Treatment of Rats

Ai Daido[1] and Yoko Aniya[1,2,*]

[1]*Laboratory of Physiology and Pharmacology, School of Health Sciences,* [2]*Research Center of Comprehensive Medicine, Faculty of Medicine, University of the Ryukyus, 207 Uehara, Nishihara, Okinawa 903-01, Japan*

Received April 18, 1994 Accepted August 16, 1994

ABSTRACT—Effects of cobalt chloride on liver glutathione *S*-transferase and protease activities were studied. When cobalt chloride (60 mg/kg) was given to rats, liver microsomal glutathione *S*-transferase and protease activities were significantly increased 24 hr after the injection, whereas glutathione peroxidase activity in microsomes was decreased. The increase in glutathione *S*-transferase activity caused by cobalt chloride treatment was not reversed by dithiothreitol, and the activation of the transferase by *N*-ethylmaleimide was similar to that of the control, indicating that the increase in the transferase activity by cobalt chloride is not due to a modification of the sulfhydryl group of the enzyme. Immunochemical analysis of the liver microsomes did not detect any proteolytic product of microsomal glutathione *S*-transferase. In puromycin- or actinomycin D-treated rats, an increase in the transferase activity caused by cobalt chloride treatment was depressed. Thus it was suggested that liver microsomal glutathione *S*-transferase is induced by cobalt chloride treatment, but not activated by limited proteolysis via microsomal protease.

Keywords: Cobalt chloride, Glutathione *S*-transferase, Liver microsome, Enzyme induction, Protease

Rat liver microsomal glutathione (GSH) *S*-transferase (EC 2. 5. 1. 18) is a multifunctional protein that detoxifies toxic metabolites by GSH conjugation and also acts as a GSH peroxidase and binding protein (1, 2). In contrast to the cytosolic GSH transferases, microsomal GSH *S*-transferase is activated by reactive oxygen species or oxidative stress via formation of the dimeric protein and mixed disulfide bond formation (3-7). Since microsomal GSH *S*-transferase is not induced by drug metabolizing enzyme inducers such as phenobarbital and 3-methylchoranthrene (8-11), it was considered that the oxidative activation of the enzyme is a quick adaptation to detoxify toxic metabolites under oxidative stress and is important as a regulation system of the enzyme in vivo. However, it has been reported that microsomal GSH *S*-transferase is induced by an antioxidant (12) and the possibility of enzyme induction still remains. During a series of studies on the transferase, we observed an increase in microsomal GSH *S*-transferase activity by heavy metal ions. In the present study, the mechanism of the increase in microsomal GSH *S*-transferase activity by cobalt chloride ($CoCl_2$) was investigated and the induction of the transferase by $CoCl_2$ was suggested.

* To whom correspondence should be addressed[(1)].

MATERIALS AND METHODS

Chemicals

Reagents used were as follows: Reduced glutathione (GSH), cumene hydroperoxide, glutathione reductase and actinomycin D were from Sigma Chemicals (St. Louis, MO, USA). Sodium phenobarbital, puromycin, thiobarbituric acid, 1-chloro-2,4-dinitrobenzene and 2,4-dinitrofluorobenzene were from Wako Pure Chemicals (Osaka). The Immuno-Blot Assay kits were from Bio-Rad Laboratories (Richmond, CA, USA). All other reagents used were of analytical grade.

Animal treatment

Male Sprague Dawley rats (140-330 g) were used. Rats were given subcutaneously $CoCl_2$ (30 mg/kg) once a day for 3 days or a single injection of 60 mg/kg. When given in combination with phenobarbital, $CoCl_2$ (30 mg/kg, s.c.) was given after phenobarbital (75 mg/kg, intraperitoneally) administration once a day for 3 days. Actinomycin D (0.25 mg/kg) or puromycin (20 mg/kg) was given to rats intraperitoneally at the indicated times after

$CoCl_2$ (60 mg/kg, s.c.) administration. Control rats were given 0.9% sodium chloride solution instead of $CoCl_2$. All rats were killed by decapitation after overnight starvation, and liver cytosol and microsomes were prepared as described previously (5).

Assay

GSH *S*-transferase and GSH peroxidase activities were measured by the methods of Habig et al. (13) with 1-chloro-2,4-dinitrobenzene and Reddy et al. (14) with cumene hydroperoxide, respectively. The content of reduced and oxidized glutathione was measured by the method of Reed et al. (15) using high performance liquid chromatography. Protease activity with casein was determined (16). Lipid peroxidation was measured by the method of Buege and Aust (17) using a reaction of thiobarbituric acid with malondialdehyde and represented as thiobarbituric-acid-reactive substance (TBARS). Protein concentration was measured by the method of Lowry et al. (18).

Statistical analysis

Data are expressed as the mean±S.D. The significance of the difference was calculated by Student's *t*-test, and P values <0.05 were taken as significant.

RESULTS

Alteration of liver GSH S-transferase, GSH peroxidase and protease activities after $CoCl_2$ treatment of rats

As shown in Table 1, liver microsomal GSH *S*-transferase activity was increased to 1.4-fold by phenobarbital, to 2.2-fold by combination with $CoCl_2$, and to 1.3-fold by $CoCl_2$ alone. Hydrogen peroxide formation in microsomes was increased to 3.2-fold by phenobarbital, but was decreased to 76% of the control by $CoCl_2$. Figure 1 shows time-dependent changes of enzyme activity in the liver of $CoCl_2$-treated rats. Microsomal GSH *S*-transferase activity was increased to 120% of the control at 14 hr after $CoCl_2$ administration and to 141% at 24 hr, whereas the microsomal GSH peroxidase activity decreased to 68% at 14 hr and to 54% at 24 hr. A slight increase in cytosolic GSH *S*-transferase activity was observed at 24 hr after $CoCl_2$ treatment but GSH peroxidase activity was not changed.

As shown in Table 2, when microsomal GSH *S*-transferase activity was increased to 126% of the control by $CoCl_2$ (30 mg/kg, 3 days, s.c.) or to 141% by a dose of 60 mg/kg, microsomal protease activity was also increased to 129% in both cases, although GSH peroxidase activity in the microsomes was decreased significantly.

Table 1. Effect of phenobarbital and cobalt chloride ($CoCl_2$) on activities of GSH *S*-transferase and protease and hydrogen peroxide formation in liver microsomes of rats

Treatment	GSTm (μmol/mg/min)	H_2O_2 (nmol/mg/10 min)
Control	0.098 (100)	53.2 (100)
PB	0.134 (137)**	172.9 (325)**
PB+$CoCl_2$	0.217 (221)**	87.5 (164)*
Control	0.092 (100)	118.2 (100)
$CoCl_2$	0.124 (135)**	89.5 (76)*

Phenobarbital (PB, 75 mg/kg, intraperitoneally) and $CoCl_2$ (30 mg/kg, subcutaneously) were given to rats once a day for 3 days, and the rats were killed 24 hr after the last injections. GSH *S*-transferase (GSTm) activity and hydrogen peroxide (H_2O_2) formation (in the presence of a NADPH generating system, 37℃, 10 or 30 min) in liver microsomes were determined (ref. 19). Values represent means for 2 to 4 rats. *P<0.05, **P<0.01, vs control.

Electrophoresis and immunoblotting

When the liver microsomal proteins prepared from $CoCl_2$-treated rats were analyzed by Western blotting after SDS-gel electrophoresis, neither the dimeric protein nor a fragment formed by limited proteolysis of microsomal GSH *S*-transferase, both are activated forms

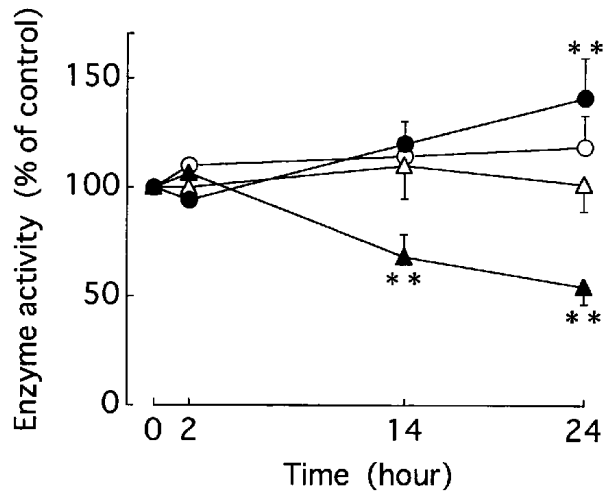

Fig. 1. Time dependent change of enzyme activity in the liver of cobalt chloride ($CoCl_2$)-treated rats. Rats were given $CoCl_2$ (60 mg/kg, s.c.) and killed at the indicated time. Control rats received saline. The liver was removed after perfusion with 1.15% potassium chloride solution. GSH *S*-transferase (GST) and GSH peroxidase (GSH-Px) activities were measured as described in Materials and Methods. Results are expressed as a percent of the control, and each point shows the mean±S.D. for 2 to 6 rats. The activity of GST in the control group (μmol/mg protein/min) was as follows: cytosolic GST (○): 1.452±0.278−1.694±0.301; microsomal GST (●): 0.070±0.011−0.074±0.017. The activity of GSH-Px in the control group (μmol/mg protein/min) was follows: cytosolic GSH-Px (△): 0.364±0.018−0.592±0.072; microsomal GSH-Px (▲): 0.084±0.005−0.085±0.012. **P<0.01 vs control.

Table 2. Effect of cobalt chloride ($CoCl_2$) on liver enzyme activities of rats in vivo

Treatment	n	Microsomes		
		GST (μmol/mg/min)	GSH-Px (μmol/mg/min)	Protease (OD_{280}/mg)
Control	5	0.092±0.011 (100)	0.111±0.016 (100)	0.284±0.035 (100)
$CoCl_2$ (30 mg/kg)	6	0.116±0.019* (126)	0.081±0.012** (73)	0.366±0.043* (129)
Control	6	0.074±0.017 (100)	0.085±0.012 (100)	0.189±0.044 (100)
$CoCl_2$ (60 mg/kg)	6	0.104±0.013** (141)	0.046±0.007** (54)	0.243±0.027* (129)

Rats were given $CoCl_2$ (30 mg/kg, s.c.) once a day for 3 days and then were killed 24 hr after the last injection or were given $CoCl_2$ (60 mg/kg, s.c.) followed by killing 24 hr later. The activities of GSH S-transferase (GST), GSH peroxidase (GSH-Px) and protease were measured as described in Materials and Methods. Values are presented as means±S.D. *$P<0.05$, **$P<0.01$, vs control.

of the transferase, was detected (Fig. 2). The immunoblot analysis did not clearly indicate whether there was in increase in microsomal GSH S-transferase protein.

Effect of puromycin and actinomycin D on liver enzyme activity of $CoCl_2$-treated rats

As shown in Table 3, an increase (121−132% of the control) in liver microsomal GSH S-transferase activity after $CoCl_2$ treatment was depressed by administration of inhibitors of protein synthesis, actinomycin D and puromycin. Although microsomal protease activity in the liver was also increased by $CoCl_2$ treatment, the increase was not prevented by the protein synthesis inhibitors. Cytosolic GSH S-transferase activity was slightly increased by $CoCl_2$, but not altered by treatment with the inhibitors. Lipid peroxidation in liver microsomes of rats was depressed significantly by administration of $CoCl_2$ either with or without the inhibitors.

Effect of agents on liver microsomal GSH S-transferase activity of $CoCl_2$-treated rats

As shown in Table 4, GSH S-transferase activity in microsomes was increased to 142% of the control by $CoCl_2$ treatment, and the increase was not reversed by dithiothreitol. When an SH-alkylating agent, N-ethylmaleimide, was incubated with microsomes from $CoCl_2$-treated rats, the microsomal GSH S-transferase activity was increased 4.7-fold, while it also increased the activity (by 4.1-fold) in the control as well. The liver microsomal GSH S-transferase from $CoCl_2$-treated rats was activated 3-fold by only a high concentration of trypsin (1.87 μg/ml), as was the transferase in the control. These results indicate that the microsomal GSH S-transferase is not activated by modulation of the sulfhydryl group of the enzyme.

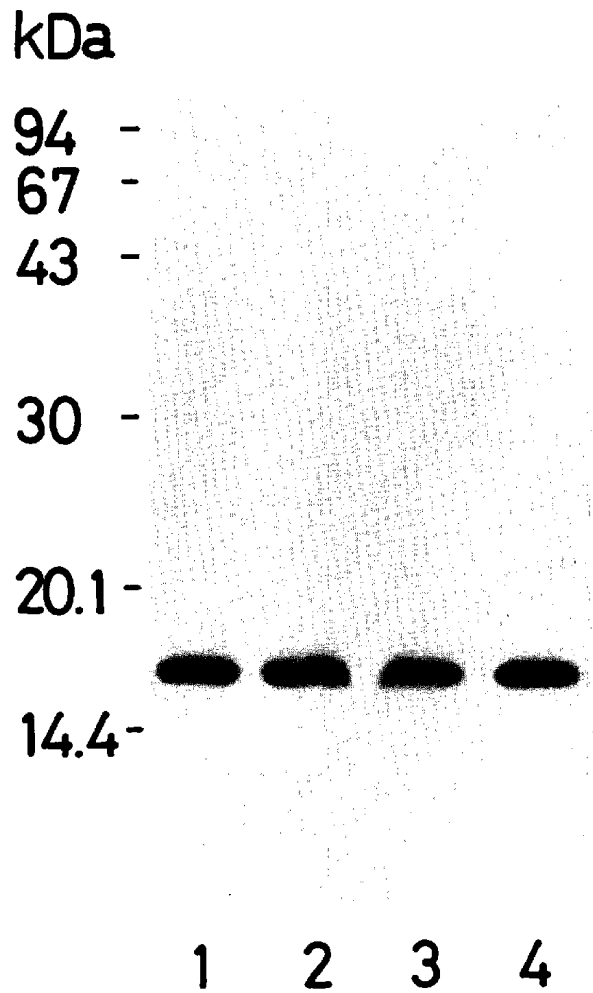

Fig. 2. Gel electrophoresis and immunoblotting of liver proteins prepared from control or cobalt chloride ($CoCl_2$)-treated rats. Rats were given $CoCl_2$ (30 mg/kg) once a day for 3 days and then were killed 24 hr after the last injection of $CoCl_2$. The liver homogenate was centrifuged at 9,000×g, and the microsomal pellet (50 μg) was applied to each lane of 15% SDS-polyacrylamide gels. Electrophoresis was performed according to the method of Laemmli (ref. 26) under non-reducing conditions at 6℃. Immunoblotting was carried out by transferring proteins from the polyacrylamide gels to nitrocellulose paper as described previously (ref. 5). Lanes 1 and 2: $CoCl_2$-treated microsomes, Lanes 3 and 4: control microsomes.

DISCUSSION

Our previous report showed that liver microsomal GSH S-transferase is activated by oxidative modification of the enzyme by oxygen radicals which are increased following the induction of cytochrome P-450 with phenobarbital (19). Since $CoCl_2$ can induce heme oxygenase followed by heme degradation, resulting in a decrease in cytochrome

Table 3. Effect of pretreatment of actinomycin D (Act-D) or puromycin (PM) on enzyme activities and lipid peroxidation of the liver in cobalt chloride ($CoCl_2$)-treated rats

Treatment	Microsomes			Cytosol
	GST (μmol/mg/min)	Protease (OD_{280}/mg)	TBARS (nmol/mg)	GST (μmol/mg/min)
Control	0.081±0.016 (100)	0.217±0.015 (100)	0.167±0.017 (100)	1.460±0.358 (100)
$CoCl_2$	0.107±0.011* (132)	0.258±0.013* (119)	0.105±0.028** (63)	1.705±0.114 (117)
Act-D+$CoCl_2$	0.095±0.014 (117)	0.238±0.019 (110)	0.102±0.026** (61)	1.767±0.383 (121)
Control	0.070±0.006 (100)	0.202±0.040 (100)	0.202±0.039 (100)	1.310±0.257 (100)
$CoCl_2$	0.085±0.008* (121)	0.251±0.058* (124)	0.148±0.033** (73)	1.367±0.202 (112)
PM+$CoCl_2$	0.078±0.008 (111)	0.275±0.081* (136)	0.133±0.026** (66)	1.395±0.125 (114)

Rats were given Act-D (0.25 mg/kg, i.p.) 1 hr prior to and 7 hr after or were given PM (20 mg/kg, i.p.) 1 hr prior to and 5 hr and 10 hr after the $CoCl_2$ (60 mg/kg, s.c.) injection. Control animals received saline alone. Rats were killed 24 hr after $CoCl_2$ injection. The activities of GSH *S*-transferase (GST) and protease and lipid peroxidation (TBARS) in the liver were measured as described in Materials and Methods. Values are presented as means±S.D. for 4 to 6 rats. *$P<0.05$, **$P<0.01$, vs control. TBARS: Thiobarbituric-acid-reactive substances.

P-450 content, administration of $CoCl_2$ together with phenobarbital was expected to depress the activation of microsomal GSH *S*-transferase induced by phenobarbital. However, as shown in Table 1, regardless of a decrease in hydrogen peroxide formation after $CoCl_2$ treatment, microsomal GSH *S*-transferase activity was increased additively by the combination of phenobarbital with $CoCl_2$ administration. Thus, it was suggested that $CoCl_2$ can increase liver microsomal GSH *S*-transferase activity by a different mechanism from oxidative modification of the enzyme.

Table 4. Effect of dithiothreitol (DTT), *N*-ethylmaleimide (NEM) or trypsin on liver microsomal GSH *S*-transferase activity of cobalt chloride ($CoCl_2$)-treated rats

Treatment	Addition	GSH *S*-transferase		
		(μmol/mg/min)	(%)	
Control	None	0.072±0.012	100	
	DTT (10 mM)	0.062±0.013	86	
$CoCl_2$	None	0.102±0.011	142	100
	DTT (10 mM)	0.086±0.011	119	84
Control	None	0.071±0.003	100	
	NEM (1 mM)	0.291±0.003	410	
$CoCl_2$	None	0.093±0.004	131	100
	NEM (1 mM)	0.436±0.002	614	469
Control	None	0.071±0.002	100	
	Trypsin (1.87 μg/ml)	0.152±0.006	214	
	(0.12 μg/ml)	0.072±0.000	101	
$CoCl_2$	None	0.083±0.000	117	100
	Trypsin (1.87 μg/ml)	0.224±0.004	315	270
	(0.12 μg/ml)	0.090±0.001	127	108

Rats were killed 24 hr after administration of $CoCl_2$ (60 mg/kg, s.c.), and then the liver microsomes were prepared. The microsomes were incubated with DTT for 10 min, with NEM for 2 min or with trypsin for 30 min, at room temperature. GSH *S*-transferase activity was measured as described in Materials and Methods. Values are presented as means±S.D. for triplicate determinations.

Liver microsomal GSH *S*-transferase was increased by single or repeated injections of $CoCl_2$ to rats (Fig. 1 and Table 2). In $CoCl_2$-treated liver, small increases (110–120% of the control) in liver weight and protein contents in the cytosol and microsomes were observed. Furthermore, the total activity of GSH *S*-transferase in microsomes was also increased, corresponding to the increase in the specific activity of microsomal GSH *S*-transferase (data not shown). This means that a net increase in microsomal GSH *S*-transferase was caused by $CoCl_2$ administration, but not an apparent increase due to such a loss of microsomal protein.

Microsomal protease activity was also increased after $CoCl_2$-treatment (Table 2). Considering that microsomal GSH *S*-transferase is activated by limited proteolysis (20), it was suggested that the increased protease activity after $CoCl_2$ treatment may contribute to proteolytic activation of the enzyme. However, as judged from the immunoblot analysis, neither the proteolytic product seen in immunoblots of trypsin-treated microsomes (20) nor the dimeric protein, which is an oxidative activated form of the microsomal GSH *S*-transferase (5), was detected. These results indicate that the increase in GSH *S*-transferase

activity in microsomes caused by $CoCl_2$ administration was not due to proteolytic activation or to oxidative activation. These findings were supported in the in vitro experiments. If the sulfhydryl group of microsomal GSH *S*-transferase is modified oxidatively via disulfide bond formation by $CoCl_2$ treatment, the increased activity may be reversed by dithiothreitol, and the activation of the enzyme by *N*-ethylmaleimide may decrease. However, the effects of these agents on the transferase were similar to those on the control. Furthermore, although the transferase with a modified sulfhydryl group becomes sensitive to proteolytic attack (Y. Aniya, unpublished data), the trypsin activation of GSH *S*-transferase obtained from $CoCl_2$-treated rats was the same as that of the control. All these data confirmed that the increase in microsomal GSH *S*-transferase activity caused by $CoCl_2$ treatment is not due to a modification of the sulfhydryl group of the enzyme. In addition, microsomal GSH *S*-transferase was not activated in vitro by incubation of microsomes with $CoCl_2$ and the increase in the transferase activity was not observed at early times after $CoCl_2$ administration, showing that $CoCl_2$ does not directly activate the microsomal GSH *S*-transferase. It is, therefore, suggested that the increase in microsomal GSH *S*-transferase activity seen after $CoCl_2$ administration may be due to an induction or stabilization of the enzyme.

Thus, the possibility of an induction of microsomal GSH *S*-transferase by $CoCl_2$ was examined. When a protein synthesis inhibitor, actinomycin D or puromycin, was given to rats, the increase in microsomal GSH *S*-transferase activity by $CoCl_2$ treatment was depressed (Table 3), suggesting an induction of the transferase by $CoCl_2$. Although $CoCl_2$ can induce heme oxygenase and cytosolic GSH *S*-transferase (21–23), it has not been reported in microsomal GSH *S*-transferase induction by $CoCl_2$. Microsomal GSH *S*-transferase is not induced by a drug metabolizing enzyme inducer such as phenobarbital (11), but a possibility of transferase induction was shown by the antioxidant, 2(3)-*tert*-butyl-4-hydroxyanisole (12). It is, therefore, suggested that liver microsomal GSH *S*-transferase may be induced by a different mechanism from the enzyme induction by phenobarbital. Although the protein content is expected to be increased in the case of enzyme induction, immunoblot analysis showed no clear increase in the content of microsomal GSH *S*-transferase compared to the observed 20–40% increase in the transferase activity. Further studies on the possible induction mechanism of the transferase are needed.

Judging from the marked decrease in microsomal lipid peroxidation of $CoCl_2$-treated rats, $CoCl_2$ seems to act as an antioxidant. Since heavy metal ions can induce metalthioneins in the liver (24, 25), the thioneins may play an antioxidative role against lipid peroxidation. Although cadmium chloride, which is a metalthionein inducer, also increases microsomal GSH *S*-transferase (data not shown), it is unknown whether or not thioneins contribute to an increase in the transferase activity.

Although microsomal GSH *S*-transferase has a GSH peroxidase activity, in the $CoCl_2$-treated rats, the peroxidase activity was decreased in spite of an increase in the transferase activity. This dissociation of the transferase activity and peroxidase activity in the enzyme seems to reflect a different active or regulation site of the enzyme, but this has not yet been proven.

In summary, GSH *S*-transferase in liver microsomes is increased by $CoCl_2$ administration, suggesting an induction of the transferase.

REFERENCES

1 Habig WH: Glutathione *S*-transferase: Versatile enzymes of detoxication. *In* Radioprotectors and Anticarcinogens, Edited by Nygaard OF and Simic MG, pp 169–190, Academic Press, New York (1983)

2 Morgenstern R and DePierre JW: Microsomal glutathione *S*-transferase. Rev Biochem Toxicol **7**, 67–104 (1985)

3 Aniya Y and Anders MW: Regulation of rat liver microsomal glutathione *S*-transferase activity by thiol/disulfide exchange. Arch Biochem Biophys **270**, 330–334 (1989)

4 Aniya Y and Anders MW: Activation of rat liver microsomal glutathione *S*-transferase by reduced oxygen species. J Biol Chem **264**, 1998–2002 (1989)

5 Aniya Y and Anders MW: Activation of rat liver microsomal glutathione *S*-transferase by hydrogen peroxide: Role for protein-dimer formation. Arch Biochem Biophys **296**, 611–616 (1992)

6 Aniya Y and Anders MW: Activation of hepatic microsomal glutathione *S*-transferase by hydrogen peroxide and diamide. *In* Oxygen Radicals, Edited by Yagi K, Kondo M, Niki E and Yoshikawa T, pp 737–740, Elsevier, Amsterdam (1992)

7 Aniya Y and Naito A: Oxidative stress-induced activation of microsomal glutathione *S*-transferase in isolated rat liver. Biochem Pharmacol **45**, 37–42 (1993)

8 Vandenberghe Y, Tee L, Morel F, Rogiers V, Guillouzo A and Yeoh G: Regulation of glutathione *S*-transferase gene expression by phenobarbital in cultured adult rat hepatocytes. FEBS Lett **284**, 103–108 (1991)

9 Pinkus R, Bergelson S and Daniel V: Phenobarbital induction of AP-1 binding activity mediates activation of glutathione *S*-transferase and quinone reductase gene expression. Biochem J **290**, 637–640 (1993)

10 Conney AH: Pharmacological implications of microsomal enzyme induction. Pharmacol Rev **19**, 317–366 (1967)

11 DeJong JL, Morgenstern R, Jornvall H, DePierre JW and Tu C-PD: Gene expression of rat and human microsomal glutathione *S*-transferases. J Biol Chem **263**, 8430–8436 (1988)

12 Morgenstern R and Dock L: A comparison of induction of microsomal glutathione *S*-transferase activity in the liver of the mouse and rat by dietary 2(3)-*tert*-butyl-4-hydroxyanisole (BHA). Acta Chem Scand **B36**, 255–279 (1982)

13 Habig WH, Pabst MJ and Jakoby WB: Glutathione *S*-trans-

ferase. J Biol Chem **249**, 7130–7139 (1974)
14 Reddy CC, Tu C-PD, Burgess JR, Ho C-Y, Scholz RW and Massaro EJ: Evidence for the occurrence of selenium-independent glutathione peroxidase activity in rat liver microsomes. Biochem Biophys Res Commun **101**, 970–978 (1981)
15 Reed DJ, Babson JR, Beatty PW, Brodie AE, Ellis WW and Potter DW: High-performance liquid chromatography analysis of nanomole levels of glutathione, glutathione disulfide, and related thiols and disulfides. Anal Biochem **106**, 55–62 (1980)
16 Laskowski M: Trypsinogen and trypsin. Methods Enzymol **2**, 26–36 (1955)
17 Buege JA and Aust SD: Microsomal lipid peroxidation. Methods Enzymol **52**, 302–310 (1978)
18 Lowry OH, Rosebrough NJ, Farr AL and Randall RJ: Protein measurement with the Folin phenol reagent. J Biol Chem **193**, 265–275 (1951)
19 Aniya Y, Shimoji M and Naito A: Increase in liver microsomal glutathione *S*-transferase activity by phenobarbital treatment of rats. Possible involvement of oxidative activation via cytochrome P-450. Biochem Pharmacol **46**, 1741–1747 (1993)
20 Morgenstern R, Lundquist G, Jörnvall H and DePierre JW: Activation of rat liver microsomal glutathione transferase by limited proteolysis. Biochem J **260**, 577–582 (1989)
21 Tomaro ML, Frydman J and Frydman RB: Heme oxygenase induction by $CoCl_2$, Co-protoporphyrin IX, phenylhydrazine, and diamide: Evidence for oxidative stress involvement. Arch Biochem Biophys **286**, 610–617 (1991)
22 Francis VNK, Dwarki VIJ and Padmanaban G: A comparative study of the regulation of cytochrome P-450 and glutathione transferase gene expression in rat liver. Nucleic Acids Res **14**, 2497–2510 (1986)
23 Yoshida T, Numazawa S and Kuroiwa Y: Induction of hepatic and renal ornithine decarboxylase by cobalt and other metal ions in rats. Biochem J **233**, 577–581 (1986)
24 Hamer DH: Metallothionein. Annu Rev Biochem **55**, 913–951 (1986)
25 Arizono K, Okanari E, Ueno K and Ariyoshi T: Heme oxygenase activity and cytochrome P-450 content associated with induced metallothionein in the liver of rats treated with various metals. J Environ Sci Health **A26**, 941–951 (1991)
26 Laemmli UK: Cleavage of structural proteins during the assembly of the head of Bacteriophage T4. Nature **227**, 680–685 (1970)