Biochemical Pharmacology, Vol. 34, No. 24, pp. 4239–4244, 1985.
Printed in Great Britain.

0006-2952/85 $3.00 + 0.00

ALTERATION OF HEPATIC GLUTATHIONE *S*-TRANSFERASES AND RELEASE INTO SERUM AFTER TREATMENT WITH BROMOBENZENE, CARBON TETRACHLORIDE, OR *N*-NITROSODIMETHYLAMINE

YOKO ANIYA* and M. W. ANDERS†
Department of Pharmacology, University of Rochester, Rochester, NY 14642, U.S.A.

(*Received* 30 *August* 1984; *accepted* 3 *May* 1985)

Abstract—The effects of bromobenzene, carbon tetrachloride, and *N*-nitrosodimethylamine (DMN) on hepatic glutathione *S*-transferase activity were studied in untreated and in phenobarbital- or ethanol-treated rats. In phenobarbital-treated rats, the isozymic composition of the hepatic cytosolic glutathione *S*-transferases was changed after giving hepatotoxic chemicals; glutathione *S*-transferases 2-2(AA), 3-3(A), 1-2(B), 3-4(C), and 4-4 + 5-5(D+E) were present in cytosol from control rats, but only glutathione *S*-transferases cochromatographing with transferases 4-4 + 5-5(D+E) were detected in rats given carbon tetrachloride or bromobenzene. A marked decrease in hepatic and an increase in serum glutathione *S*-transferase activity were also observed after carbon tetrachloride or bromobenzene treatment, but little change was seen after giving DMN. On the contrary, in untreated or ethanol-treated rats, DMN administration decreased hepatic glutathione *S*-transferase activity and caused an elevation in serum glutathione *S*-transferase activity. The isozymic composition of the hepatic cytosolic glutathione *S*-transferases after giving DMN to untreated rats was also altered, but the alteration was much less than that observed after giving carbon tetrachloride or bromobenzene to phenobarbital-treated rats. The elevation in serum glutathione *S*-transferase activity was accompanied by an increase in both serum glutamate-pyruvate transaminase activity and serum bilirubin concentrations. Thus, hepatic glutathione *S*-transferase activity was altered and released into serum after giving hepatotoxic chemicals, and the alteration in glutathione *S*-transferase activity was dependent on treatment with phenobarbital or ethanol.

The glutathione *S*-transferases (EC 2.5.1.18) are multifunctional proteins, which serve as binding, scavenger, or transfer proteins and as catalysts for the reaction of electrophilic compounds with glutathione [1–3]. Active metabolites, which are generated from toxic chemicals by hepatic microsomal cytochrome P-450-dependent mixed-function oxidases, are conjugated with glutathione by hepatic glutathione *S*-transferases [4].

It has been demonstrated that chloroform, which causes hepatotoxicity after bioactivation by microsomal enzymes, alters the isozymic composition of the hepatic cytosolic glutathione *S*-transferases and releases the transferases into serum [5]. This study suggested that both an active metabolite of chloroform and bilirubin play a role in the alteration of the glutathione *S*-transferases. The hepatotoxicity of carbon tetrachloride, bromobenzene, and *N*-nitrosodimethylamine (DMN‡) is also associated with active metabolite formation by the microsomal mixed-function oxidases [6–11]. The present study investigated whether alterations of the glutathione *S*-transferases, such as those observed after chloroform treatment, can be generalized to other compounds. Accordingly, the effects of giving carbon tetrachloride, bromobenzene, or DMN on glutathione *S*-transferases were studied. The effects of giving phenobarbital or ethanol on the hepatotoxic action of carbon tetrachloride, bromobenzene, or DMN were also investigated.

MATERIALS AND METHODS

Carbon tetrachloride, bromobenze, and *N*-nitrosodimethylamine were purchased from the Aldrich Chemical Co. (Milwaukee, WI). Sephadex G-100 (super fine) was purchased from Pharmacia Fine Chemicals (Piscataway, NJ). DE52 and CM52 were obtained from Whatman, Inc. (Clifton, NJ), and CDNB was purchased from the Eastman Chemical Co. (Rochester, NY).

Treatment of animals. Male Long–Evans rats (170–250 g) were used. Rats were given phenobarbital sodium (75 mg/kg) intraperitoneally once daily for 3 days. Hepatotoxic chemicals were administered intraperitoneally 24 hr after the last dose of phenobarbital as follows: carbon tetrachloride (0.2 ml/kg) and bromobenzene (0.2 ml/kg) were given as corn oil solutions, and *N*-nitrosodimethylamine (60 mg/kg) was given as a 0.9% sodium chloride solution. Ethanol-treated rats were given 15% ethanol in the

* On leave from the University of the Ryukyus, Okinawa, Japan.
† Address all correspondence to: Prof. M. W. Anders, Department of Pharmacology, University of Rochester, 601 Elmwood Ave., Rochester, NY 14642, U.S.A.
‡ Abbreviations: DMN, *N*-nitrosodimethylamine; CDNB, 1-chloro-2,4-dinitrobenzene; DE52, diethylaminoethyl-cellulose; CM52, carboxymethyl-cellulose.

drinking water for 3.5 days and were allowed free access to food. The same doses of hepatotoxic chemicals were given intraperitoneally to ethanol-treated rats 16 hr after withholding ethanol. Control rats were given corn oil (0.3 ml/100 g) intraperitoneally. Rats were allowed free access to food and water in all experiments except ethanol-treated rats. Rats were killed by decapitation, and the blood was collected from the stump.

Assays. Hepatic cytosolic fractions were prepared as previously reported [5]. Samples of the cytosol were used for the measurement of glutathione *S*-transferase activity on the same day, and the remainder was used for column chromatography, either on the same day or within 2 weeks after storage at −20°. Glutathione *S*-transferase activity in hepatic cytosol and in serum was assayed by the method of Habig *et al.* [12] with CDNB as substrate. Serum bilirubin concentrations and serum glutamate-pyruvate transaminase (GPT) activities were determined with Sigma Kit No. 605 (Sigma Chemical Co., St. Louis, MO) and Beckman Enzymatic ALT reagent (Beckman, Carlsbad, CA) respectively. Protein concentrations were measured by the method of Lowry *et al.* [13].

Glutathione *S*-transferases in hepatic cytosol and in serum were isolated as previously reported [5].

RESULTS

Alteration of hepatic cytosolic glutathione S-transferases after giving hepatotoxins to phenobarbital-treated rats. Hepatic cytosolic glutathione *S*-transferases were isolated 5 hr after administration of hepatotoxic chemicals to phenobarbital-treated rats. Cytosol (12 ml, 150–190 mg protein) was chromatographed on a DE52 column followed by Sephadex G-100 gel filtration chromatography. Protein recovery of Sephadex G-100 fractions with glutathione *S*-transferase activity was about 8% of that present in cytosol in each case. Figure 1 shows chromatographic profiles of the Sephadex G-100 fractions on a CM52 column. Glutathione *S*-transferases 2-2(AA), 3-3(A), 1-2(B), 3-4(C), and 4-4 + 5-5(D+E) were observed in control and in DMN-treated rats, although glutathione *S*-transferases 1-2(B) and 3-4(C) were decreased slightly in DMN-treated rats. After giving carbon tetrachloride or bromobenzene, however, the basic glutathione *S*-transferases 2-2(AA), 3-3(A), 1-2(B), and 3-4(C) were lost, and

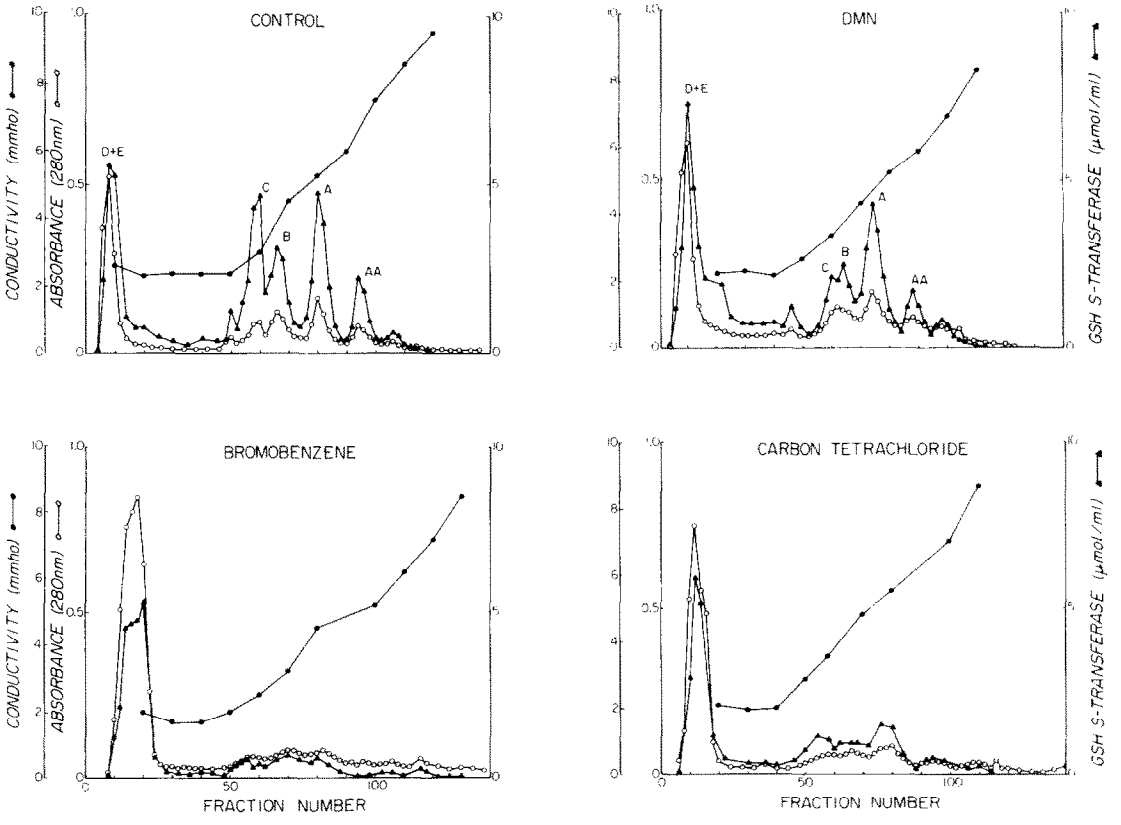

Fig. 1. Column chromatography of hepatic cytosolic glutathione *S*-transferases on CM52 after treatment with hepatotoxic chemicals. Bromobenzene (0.2 ml/kg), carbon tetrachloride (0.2 ml/kg), or DMN (60 mg/kg) was injected intraperitoneally 24 hr after the last dose of sodium phenobarbital (75 mg/kg), which was given daily for 3 days. Rats were killed 5 hr after giving the toxins, and the hepatic cytosolic glutathione *S*-transferases were isolated as described in Materials and Methods. The Sephadex G-100 fractions with glutathione *S*-transferase activity were applied to the CM52 column (0.9 × 20 cm) and were eluted with a linear gradient of potassium chloride (0–100 mM) in 0.1 M phosphate buffer (pH 6.7); 1.2-ml fractions were collected. The amounts of protein applied to the column were 14, 18, 25, or 16 mg from control, DMN-, bromobenzene-, or carbon tetrachloride-treated rats respectively.

only glutathione S-transferases cochromatographing with transferases 4-4 + 5-5(D+E) were detected. The total recovery of glutathione S-transferase activity from CM52 columns was 41% of that present in cytosol in control and 34% in DMN-treated rats. Glutathione S-transferase activity in the first peak (4-4 + 5-5) from the column in control, DMN-, carbon tetrachloride-, and bromobenzene-treated rats was 9.4, 11.2, 31.7, and 37.4% respectively. Recovery of protein, chromatographing as glutathione S-transferases 4-4 + 5-5(D+E), calculated from the same amount (16 mg) of protein applied to the column, from control, DMN-, carbon tetrachloride-, or bromobenzene-treated rats was 5.7, 6.7, 8.3, and 8.7 mg respectively. The specific activity of the peak chromatographing as transferases 4-4 + 5-5(D+E) was 8.62, 6.45, 5.97, and 10.20 μmoles/mg protein with CDNB as substrate respectively. In repeated experiments with bromobenzene treatment, an increase in the specific activity of the glutathione S-transferase 4-4 + 5-5(D+E)-like fraction was observed (10.2 ± 3.3 μmoles/mg, $N = 5$). These results suggest that the more basic glutathione S-transferases 2-2(AA), 3-3(A), 1-2(B), and 3-4(C) were partially recovered as a glutathione S-transferase 4-4 + 5-5(D+E)-like fraction after treatment with carbon tetrachloride or bromobenzene and that these basic transferases are altered, but retained their catalytic activities after bromobenzene treatment but not after carbon tetrachloride treatment.

Figure 2 shows the relationship among hepatic cytosolic and serum glutathione S-transferase activities, serum bilirubin concentrations, and serum GPT activities after giving hepatotoxins. Hepatic cytosolic glutathione S-transferase activity was decreased by the treatment with hepatotoxic chemicals: 5 hr after treatment, the transferase activity was 74% of the control after giving carbon tetrachloride, and little decrease was observed after giving bromobenzene or DMN. Elevated serum glutathione S-transferase activities were detected 5 hr after the administration of carbon tetrachloride or bromobenzene, but no increased activity was seen after giving DMN. Twenty-four hours after the administration of carbon tetrachloride, bromobenzene, or DMN, the hepatic cytosolic glutathione S-transferase activity was decreased to 77, 56, and 89% of control respectively. A marked elevation of the serum glutathione S-transferase activity was observed 24 hr after giving carbon tetrachloride or bromobenzene, and DMN moderately increased the serum transferase activity. Glutathione S-transferase activity in serum was increased to 9-fold at 5 hr and 32-fold at 24 hr after carbon tetrachloride treatment, and the increase after bromobenzene treatment was 3-fold at 5 hr and 51-fold at 24 hr. Both bilirubin concentrations and GPT activities in serum were elevated in parallel with the increase in serum glutathione S-transferase activity after treatment with the hepatotoxins.

Effects of hepatotoxins in untreated and phenobarbital- or ethanol-treated rats. Table 1 shows serum glutathione S-transferase activity 5 hr after giving hepatotoxins to untreated and phenobarbital- or ethanol-treated rats. Carbon tetrachloride administration caused a marked and a moderate elevation of transferase activity in phenobarbital- and ethanol-treated rats respectively; in the case of bromobenzene, an elevation was observed only in phenobarbital-treated rats. On the contrary, DMN increased the serum transferase activity in untreated

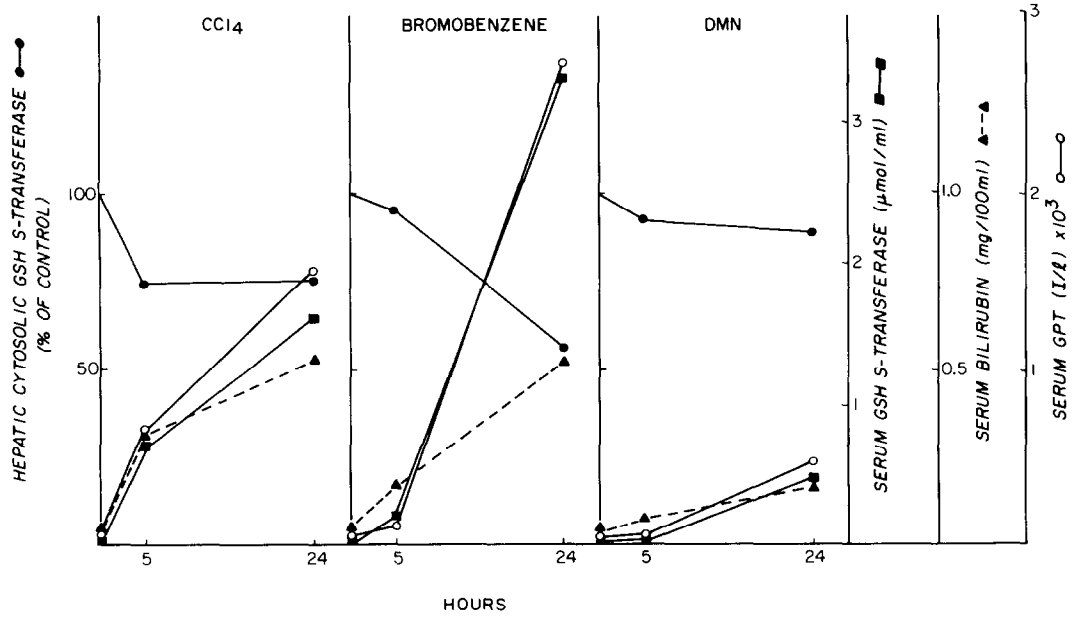

Fig. 2. Effects of hepatoxic chemicals on serum and hepatic glutathione S-transferase activities, serum GPT activity, and serum bilirubin concentrations. Bromobenzene, carbon tetrachloride, or DMN was given to phenobarbital-treated rats as described in the legend of Fig. 1, and the rats were killed 5 or 24 hr later. Hepatic cytosol and serum were prepared and activities were measured as described in Materials and Methods. Blood was collected from the stump after decapitation. Values are shown as the mean of four or five rats.

Table 1. Serum glutathione *S*-transferase activity 5 hr after giving carbon tetrachloride, bromobenzene, or DMN

Treatment	Serum glutathione *S*-transferase (μmoles/ml)			
	Control	CCl_4	Brz	DMN
None	0.04 ± 0.01	0.05 ± 0.03	0.02 ± 0.01	0.09 ± 0.03*
Ethanol	0.04 ± 0.01	0.09 ± 0.01*	0.04 ± 0.02	0.09 ± 0.01*
Phenobarbital	0.05 ± 0.03	0.73 ± 0.17*	0.20 ± 0.14	0.05 ± 0.02

Rats were given carbon tetrachloride (CCl_4, 0.2 ml/kg), bromobenzene (Brz, 0.2 ml/kg), or DMN (60 mg/kg) intraperitoneally after treatment with phenobarbital or ethanol, as described in Materials and Methods. Blood was taken from the tail vein 5 hr after the administration of the toxins. Values are shown as mean ± S.D.; N = 4.
* Analysis of variance: control vs treated, $P < 0.05$.

and ethanol-treated rats. As shown in Table 2, in untreated rats DMN decreased the hepatic glutathione *S*-transferase activity to 61% of the control and increased serum transferase activities, serum bilirubin concentrations, and GPT activities after 24 hr. Ethanol treatment potentiated the elevation of the serum transferase or GPT activity caused by DMN. Carbon tetrachloride and bromobenzene administration caused a small decrease in hepatic cytosolic and a small increase in serum glutathione *S*-transferase activity in untreated rats, and this alteration was enhanced by ethanol treatment.

To clarify the alteration of hepatic cytosolic glutathione *S*-transferases after giving DMN, the transferases were isolated from untreated rats by the same methods employed with phenobarbital-treated rats. Figure 3 shows CM52 column chromatographic profiles 5 and 24 hr after giving DMN. In comparison with phenobarbital-treated rats, the activity of glutathione *S*-transferases A and AA of control was low, and transferase B and C activities were not well separated (Fig. 3A). Glutathione *S*-transferases B and C were diminished by DMN treatment, and the transferase activities decreased to 60–70% of control. Protein recovery of glutathione *S*-transferases as glutathione *S*-transferases 4-4 + 5-5(D+E) fraction of control and 5 and 24 hr after giving DMN was 2.5 mg (19%), 4.1 mg (32%), and 3.9 mg (30%) respectively. Moreover, the specific activity of transferases chromatographing as transferases 4-4 + 5-5(D+E) decreased from 16.5 in control to 14.6 at 5 hr and 11.9 μmoles product/mg protein 24 hr after DMN treatment.

DISCUSSION

Rat hepatic cytosol contains several glutathione *S*-transferases, which are designated 2-2(AA), 3-3(A), 1-2(B), 3-4(C), and 4-4 + 5-5(D+E) in reverse order of elution from a cation exchange column [12]. Previous reports [5] showed that the more basic glutathione *S*-transferases, such as 2-2, 3-3, 1-2, and 3-4, are decreased markedly and glutathione *S*-transferases chromatographing with transferases 4-4 + 5-5(D+E) are detected in the serum after chloroform treatment. In the present study, a marked alteration of hepatic cytosolic glutathione *S*-transferases was also observed after giving hepatotoxic chemicals. Glutathione *S*-transferases 2-2(AA), 3-3(A), 1-2(B),

Table 2. Relationship among hepatic and serum glutathione *S*-transferase activities, serum GPT activities, and serum bilirubin concentrations 24 hr after the administration of hepatotoxins

Treatment	Glutathione *S*-transferase		Serum bilirubin (mg/100 ml)	Serum GPT (I.U./l)
	Cytosol (μmoles/mg)	Serum (μmoles/ml)		
Untreated				
Control (N = 4)	1.83 ± 0.36	0.03 ± 0.02	0.09 ± 0.08	45 ± 11
CCl_4 (N = 4)	1.47 ± 0.16	0.09 ± 0.03	0.11 ± 0.04	127 ± 54
Brz (N = 4)	1.76 ± 0.12	0.05 ± 0.02	0.18 ± 0.15	55 ± 7
DMN (N = 4)	1.11 ± 0.21*	0.98 ± 0.32*	1.51 ± 0.42*	877 ± 487*
Ethanol-treated				
Control (N = 3)	1.63 ± 0.07	0.05 ± 0.01	0.09 ± 0.01	40 ± 5
CCl_4 (N = 3)	1.26 ± 0.05†	0.23 ± 0.08†	0.09 ± 0.01	205 ± 57
Brz (N = 3)	1.56 ± 0.09†	0.39 ± 0.27†	0.15 ± 0.05	306 ± 37†
DMN (N = 4)	1.07 ± 0.07*	1.53 ± 0.57*	0.86 ± 0.46*	2615 ± 981*†

Rats were treated with ethanol as described in Materials and Methods. Carbon tetrachloride (CCl_4, 0.2 ml/kg), bromobenzene (Brz, 0.2 ml/kg), or DMN (60 mg/kg) was given 16 hr after withholding ethanol, and rats were killed 24 hr later. Values are presented as mean ± S.D.
*,† Analysis of variance: * control vs treated, $P < 0.05$; † untreated vs ethanol-treated, $P < 0.05$.

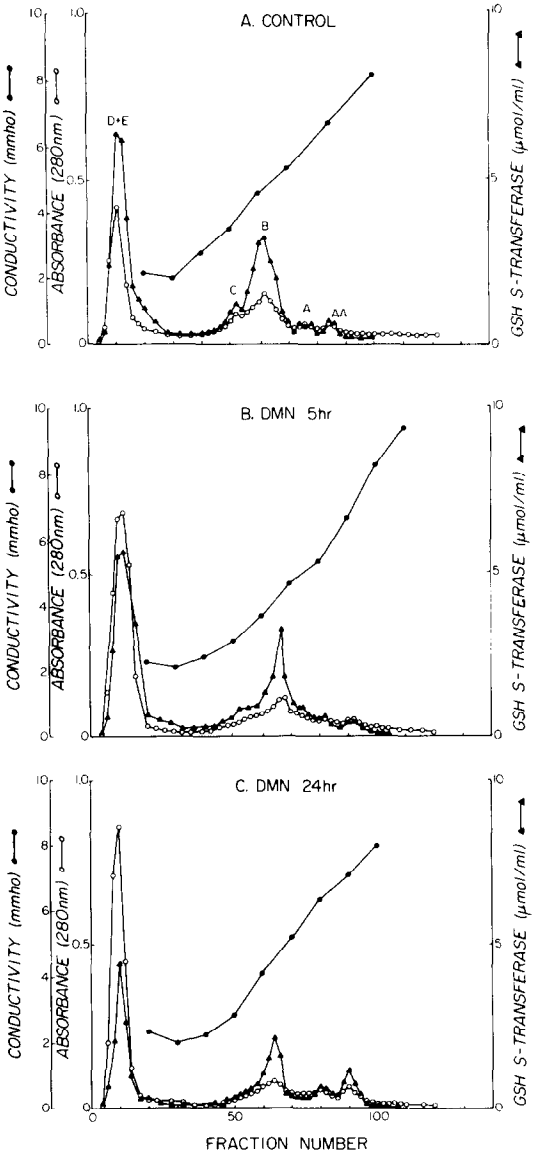

Fig. 3. CM52 column chromatography of hepatic cytosolic glutathione S-transferases after giving DMN to untreated rats. The Sephadex G-100 fractions (A and C 13 mg, B 19 mg protein) with glutathione S-transferase activity obtained from giving DMN to untreated rats were applied to the CM52 column (0.9 × 20 cm) and eluted with the same conditions as shown in Fig. 1. (A) control, (B) 5 hr after giving DMN, and (C) 24 hr after giving DMN.

and 3-4(C) were lost, and only transferases co-chromatographing with glutathione S-transferases 4-4 + 5-5(D+E) were detected in hepatic cytosol 5 hr after giving bromobenzene or carbon tetrachloride to phenobarbital-treated rats. DMN administration caused a small decrease in glutathione S-transferase 1-2(B) and 3-4(C) (Fig. 1). Protein recovery in the glutathione S-transferase 4-4 + 5-5(D+E)-like fraction was increased after treatment with hepatotoxins. Moreover, the transferase activity was almost completely recovered in a glutathione S-transferase 4-4 + 5-5(D+E) fraction from a CM52 column after administration of carbon tetrachloride or bromobenzene. These results suggested that more basic glutathione S-transferases were altered and partially recovered in the glutathione S-transferase 4-4 + 5-5(D+E) fraction, as occurred after chloroform administration [5].

Since serum glutathione S-transferase activity was elevated markedly in the case of carbon tetrachloride, it was suggested that cytosolic glutathione S-transferases are altered and released into serum. Serum glutathione S-transferase activity was increased only modestly after giving bromobenzene, although marked alterations of the chromatographic behavior and increases in the specific activity of the glutathione S-transferase 4-4 + 5-5(D+E)-like fraction were observed. Furthermore, the eluate from the CM52 column showed that the CM52-1 fraction separated as two peaks, and that the second peak, which was accompanied by an increase in conductivity, possessed high transferase activity (data not shown). The increase of the specific activity in the glutathione S-transferase 4-4 + 5-5(D+E)-like fraction and the decrease in the basic transferases, such as 1-2 or 3-4, were also observed in *in vitro* experiments with liver homogenates (unpublished data). Since glutathione S-transferases 1-2, 3-3, and 3-4 show a higher activity with CDNB as substrate than does transferase 5-5 [12], it is suggested that more basic glutathione S-transferases are altered, but retain their catalytic activities, and are recovered in a glutathione S-transferase 4-4 + 5-5(D+E)-like fraction after administration of bromobenzene. It is not known why the alteration of the hepatic glutathione S-transferases is accompanied by an increase in conductivity. The isozymic composition of the hepatic cytosolic glutathione S-transferases after giving DMN to untreated rats was also altered, but the alteration was much less than that observed after giving carbon tetrachloride or bromobenzene to phenobarbital-treated rats; nevertheless, the magnitudes of the decrease in hepatic cytosolic transferases and of the increase in serum transferase activity were similar. These results suggest that the action of DMN on the glutathione S-transferases is different from that of carbon tetrachloride or bromobenzene.

A marked decrease in liver cytosolic and an increase in serum glutathione S-transferase activity were observed 24 hr after administration of carbon tetrachloride or bromobenzene to phenobarbital-treated rats (Fig. 2). The serum GPT activity and serum bilirubin concentrations were also increased in parallel to the elevation in glutathione S-transferase activity in serum after giving carbon tetrachloride or bromobenzene. DMN treatment produced little change in liver cytosolic and serum glutathione S-transferase activities 24 hr after administration.

It is well known that the toxicity induced by carbon tetrachloride, bromobenzene, and DMN is associated with bioactivation to toxic metabolites by hepatic microsomal cytochrome P-450-dependent mixed-function oxidases [6–11]. It was, therefore, of interest to study the effect of cytochrome P-450 inducers on the alterations of the glutathione S-transferases after administration of hepatotoxins. Ethanol treatment potentiated the DMN-induced hepatotoxicity compared with untreated rats. Car-

bon tetrachloride and bromobenzene slightly changed the glutathione *S*-transferase activity and serum parameters measured in untreated rats, and ethanol treatment increased these effects. Our results agree with previous reports that ethanol treatment enhanced carbon tetrachloride-, bromobenzene-, and DMN-induced toxicity [14–17]. On the other hand, it has been reported that hepatotoxicity induced by carbon tetrachloride or bromobenzene is potentiated by phenobarbital treatment [18–20], but that DMN metabolism is decreased rather than increased [21, 22]. Our results confirmed the above observations and showed that the alterations of liver cytosolic and serum glutathione *S*-transferases after administration of the hepatotoxins were dependent on the treatment of animals with cytochrome P-450 inducers. It is, therefore, suggested that reactive metabolites, which are produced by cytochrome P-450-dependent monooxygenases from carbon tetrachloride, bromobenzene, and DMN, contribute to the alterations of the glutathione *S*-transferases.

We showed previously [5] that bilirubin preferentially alters glutathione *S*-tranferases 1-2(B) and 3-4(C) to a transferase 4-4 + 5-5(D+E)-like fraction. Because all toxins used in our studies increase bilirubin concentrations, it is possible that bilirubin contributes to the alteration of the cytosolic glutathione *S*-transferases after treatment with hepatotoxins.

The elevation of glutathione *S*-transferase activity in serum paralleled the increase of both serum GPT activity and serum bilirubin concentrations. Thus, serum glutathione *S*-transferase measurements, which have been observed by others [23–26], may be useful for the evaluation of liver dysfunction.

Acknowledgement—This research was supported by National Institutes of Health Grant ES 03126.

REFERENCES

1. W. B. Jakoby, *Adv. Enzymol.* **46**, 383 (1978).
2. A. W. Wolkoff, R. A. Weisiger and W. B. Jakoby, *Prog. Liver Dis.* **6**, 213 (1979).
3. W. B. Jakoby and W. H. Habig, in *Enzymatic Basis of Detoxication* (Ed. W. B. Jakoby), Vol. 2, p. 63. Academic Press, New York (1980).
4. B. Ketterer, B. Coles and D. J. Meyer, *Environ. Hlth Perspect.* **49**, 59 (1983).
5. Y. Aniya and M. W. Anders, *Biochem. Pharmac.* **34**, 249 (1985).
6. D. J. Jollow, J. R. Mitchell, N. Zampaglione and J. R. Gillette, *Pharmacology* **11**, 151 (1974).
7. D. F. Heath, *Biochem. J.* **85**, 72 (1962).
8. P. N. Magee and J. M. Barnes, *Adv. Cancer Res.* **10**, 163 (1967).
9. B. B. Brodie, W. D. Reid, A. K. Cho, G. Sipes, G. Krishna and J. R. Gillette, *Proc. natn. Acad. Sci. U.S.A.* **68**, 160 (1971).
10. M. C. Villarruel, E. G. D. Toranzo and J. A. Castro, *Toxic. appl. Pharmac.* **41**, 337 (1977).
11. T. F. Slater, in *Biochemical Mechanisms of Liver Injury* (Ed. T. F. Slater), p. 1. Academic Press, London (1978).
12. W. H. Habig, M. J. Pabst and W. B. Jakoby, *J. biol. Chem.* **249**, 7130 (1974).
13. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
14. H. M. Maling, B. Stripp, I. G. Sipes, B. Highman, W. Saul and M. A. Williams, *Toxic. appl. Pharmac.* **33**, 291 (1975).
15. O. Strubelt, F. Obermeier and C. P. Siegers, *Acta pharmac. tox.* **43**, 211 (1978).
16. A. J. Garro, H. K. Seitz and C. S. Lieber, *Cancer Res.* **41**, 120 (1981).
17. K. W. Miller and C. S. Yang, *Archs Biochem. Biophys.* **229**, 483 (1984).
18. W. D. Reid and G. Krishna, *Expl molec. Path.* **18**, 80 (1973).
19. N. Zampaglione, D. J. Jollow, J. R. Mitchell, B. Stripp, M. Hamrick and J. R. Gillette, *J. Pharmac. exp. Ther.* **187**, 218 (1973).
20. P. Pani, M. V. Torrielli, L. Gabriel and E. Gravela, *Expl molec. Path.* **19**, 15 (1973).
21. R. Kato, H. Shoji and A. Takanaka, *Gann* **58**, 467 (1967).
22. J. C. Arcos, G. M. Bryant, N. Venkatesan and M. F. Argus, *Biochem. Pharmac.* **24**, 1544 (1975).
23. M. Sherman, J. A. H. Campbell, S. A. Titmuss, M. C. Kew and R. E. Kirsch, *Hepatology* **3**, 170 (1983).
24. N. Ohmi and I. M. Arias, *Hepatology* **4**, 316 (1981).
25. N. M. Bass, R. E. Kirsch, S. A. Tuff and S. J. Saunders, *Gastroenterology* **75**, 589 (1978).
26. P. Di Simplicio and B. Mannervik, *Toxic. Lett.* **18**, 285 (1983).