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# THE EFFECTS OF BUTHIONINE SULPHOXIMINE (BSO) ON GLUTATHIONE DEPLETION AND XENOBIOTIC BIOTRANSFORMATION

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**Abstract**—Buthionine sulphoximine (BSO) is an inhibitor of γ-glutamylcysteine synthetase (γ-GCS) and consequently lowers tissue glutathione (GSH) concentrations. In fed male C3H mice, liver and kidney GSH levels were depleted by BSO in a dose dependent manner with maximum effect (35% of initial levels) occurring with doses between 0.8 and 1.6 g/kg, i.p. At these doses maximum effects on γ-GCS and GSH were observed 2–4 hr after BSO administration; initial γ-GCS activity and GSH content were restored approximately 16 hr post BSO. BSO, either *in vivo* or *in vitro*, had no effect on hepatic microsomal cytochrome P-450 levels, a range of cytochrome P-450 dependent enzyme activities or *p*-nitrophenol glucuronyl transferase activity. Similarly, BSO had no effect on phenol sulphotransferase and two GSH-transferase activities in the 105,000 *g* supernatant fraction. BSO had no effect on the duration of hexobarbitone induced narcosis in mice. Consistent with specific inhibition of GSH synthesis, BSO pretreatment of mice decreased the proportion of a 50 mg/kg dose of paracetamol excreted in the urine as GSH-derived conjugates but did not affect paracetamol clearance through the glucuronidation or sulphation pathways.

Since BSO does not affect cytochrome P-450 or conjugating enzyme activity, its use as a specific depletor of tissue GSH in the investigation of mechanisms of xenobiotic-induced toxicities is preferable to the standard GSH-depleting agents as these have other enzymic effects.

In addition to being involved with the maintenance of cellular redox states and cell membrane integrity, reduced glutathione (GSH) plays an essential role in the amelioration of many xenobiotic-induced toxicities. Indeed depletion of cellular GSH and subsequent exacerbation of toxicity is an accepted procedure for demonstrating that a reactive electrophilic compound may be responsible for chemical-induced toxicity. Current methods of investigating the involvement of GSH in the expression of toxic responses to chemicals relies on a depletion of cellular GSH by agents (*N*-ethylmaleimide, diethyl maleate, iodoacetate, iodoacetamide) which act as competitive substrates for glutathione-transferases. These agents however, produce other cellular effects, apart from depletion of GSH, by reacting with other reduced thiol groups in addition to that of GSH. In some spatial models of cytochrome P-450, thiol groups play an essential role in cytochrome P-450 mediated mixed function oxidation of xenobiotics by the liver [1, 2]. Thus, *N*-ethylmaleimide, diethylmaleate, iodoacetate and iodoacetamide have all been shown to inhibit the activity of cytochrome P-450 [1–5]. Diethylmaleate, the agent most often used to deplete GSH levels, has both inhibitory and inductive effects on mixed function oxidase activity [4]. It has marked effects on haem oxygenase and hence may affect cytochrome P-450 levels [6]. Since cytochrome P-450 is involved in the production of many electrophilic reactive metabolites, experiments which utilize the above agents to deplete GSH need be interpreted with caution.

γ-Glutamylcysteine synthetase (γ-GCS) has a key role in the synthesis and maintenance of cellular GSH levels [7]. This enzyme catalyses the conjugation of cysteine with glutamate to form γ-glutamylcysteine which then conjugates with glycine to form GSH. With respect to other enzymes of the γ-glutamyl cycle, buthionine sulphoximine (BSO) (Fig. 1) has recently been shown to be a specific inhibitor of γ-GCS in the kidney and to markedly lower renal and hepatic GSH levels [8].

In addition to describing dose and temporal effects of BSO on hepatic and renal γ-GCS activity and GSH levels, this communication reports the effects of BSO on other hepatic enzyme systems which are involved in the expression of xenobiotic-metabolite mediated toxicities and demonstrates that BSO is a

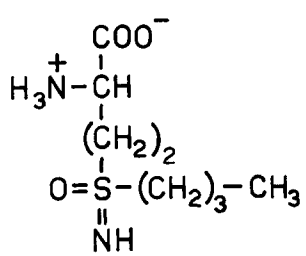

Fig. 1. Structure of buthionine sulphoximine.

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useful, specific agent for indicating the involvement of GSH in xenobiotic induced toxicities.

## MATERIALS AND METHODS

*Chemicals.* BSO was purchased from Chemical Dynamics Corp., South Plainfield, N.J. Biochemical cofactors were obtained from Sigma Chemical Co., St. Louis.

*Animals.* Male C3H mice (20–25 g) were used throughout the study and allowed food and water *ad libitum*. BSO was dissolved in saline with the aid of 0.1 N NaOH (final pH 8.5) and administered intraperitoneally; control animals received an equivalent volume of saline (pH 8.5, 20 ml/kg). At varying times after BSO administration, animals were killed by cervical dislocation and the liver and kidneys quickly removed for enzyme and GSH analysis.

*Tissue preparation and enzyme analysis.* Liver and kidneys were rinsed with cold KCl–Tris buffer (150 mM KCl, 50 mM Tris–HCl, pH 7.4). Subsequent procedures were conducted at 4°. Tissues were homogenized in 4 vol of KCl–Tris buffer using a motor driven Potter–Elvenhjem homogeniser. Aliquots of the homogenate were removed for determination of GSH content and $\gamma$-GCS activity as described below. The remaining portion of the liver homogenate was centrifuged at 10,000 *g* for 20 min and the supernatant removed and further centrifuged at 105,000 *g* for 60 min. The resulting microsomal pellet was resuspended by hand using a Potter–Elvenhjem homogeniser. The protein content of the microsomal suspension and 105,000 *g* supernatant was determined according to Lowry [9] prior to measuring enzyme activities in these fractions.

GSH content of liver and kidney homogenates was determined by a modification of the method of Cohn and Lyle [10]; 0.05 ml of concentrated perchloric acid was added to 0.5 ml of tissue homogenate and centrifuged in the cold at 5000 *g* for 10 min and the reduced GSH in the supernatant determined by conversion to the fluorescent *o*-phthalaldehyde derivative.

Tissue $\gamma$-GCS activity was determined according to Griffith *et al.* [11]. Immediately after tissue homogenisation an aliquot of homogenate was made 1 and 5 mM with respect to $MgCl_2$ and $\beta$-mercaptoethanol. After centrifugation at 5000 *g* for 10 min $\gamma$-GCS activity in the supernatant was determined with L-$\alpha$-amino-*n*-butyric acid, L-glutamate and ATP as cosubstrates. The reaction was stopped with 10% TCA and following centrifugation inorganic phosphate was determined in the supernatant by the method of Fiske and SubbaRow [12].

Cytochrome P-450 content of liver microsomes was determined from the carbon monoxide difference spectrum of dithionite-reduced microsomes assuming a molar extinction coefficient of 91 $cm^{-1}$ $mM^{-1}$ between 450 and 490 nm [13].

Activities of microsomal and soluble enzymes were determined with co-factor and substrate concentrations that were not rate limiting and enzyme activities were linear with respect to incubation time and protein concentration. Aminopyrine *N*-demethylase activity was determined by the method of Cochin and Axelrod [14] utilizing the Nash procedure for detection of formaldehyde production [15]. Aniline hydroxylase was assayed by following the production of p-aminophenol as described by Imai *et al.* [16]. Incubation mixtures for aminopyrine demethylase and aniline hydroxylase activities consisted of an NADPH-generating system (1 mM NADP, 10 mM glucose-6-phosphate, 5 mM $MgCl_2$ and 2 IU of glucose-6-phosphate dehydrogenase), Tris–HCl buffer (100 mM, pH 7.4), microsomal protein (1 mg/ml) and either aminopyrine (25 mM) or aniline (5 mM) in a final volume of 1.5 ml. Biphenyl hydroxylase was assayed by measuring the formation of 4-hydroxy biphenyl as described by Creaven *et al.* [17]. The incubation mixture consisted of 160 mM Tris–HCl buffer (pH 7.4), 0.75 mg microsomal protein, 50 mM biphenyl, 1.2 mM NADP, 12 mM glucose-6-phosphate, 6 mM $MgCl_2$ and 2.4 IU of glucose-6-phosphate dehydrogenase in a final volume of 1.0 ml. 7-Ethoxycoumarin *O*-deethylase activity was measured by following the formation of 7-hydroxycoumarin as described by Greenlee and Poland [18]. The incubation mixture contained 65 mM phosphate buffer (pH 7.4), 0.15 mg protein, 0.5 mM 7-ethoxycoumarin, 1.5 mM NADP, 15 mM glucose-6-phosphate, 7.5 mM $MgCl_2$ and 3 IU of glucose-6-phosphate dehydrogenase in a final volume of 1.0 ml. Aryl hydrocarbon hydroxylase (AHH) activity was determined with benzo(a)pyrene as substrate. The microsomal incubation mixture, containing 40 mM phosphate buffer (pH 7.4), 0.15 mg microsomal protein, 1.88 mM NADP, 18.9 mM glucose-6-phosphate 5mM $MgCl_2$ and 1.2 IU of glucose-6-phosphate dehydrogenase in a final volume of 1.0 ml was preincubated for 3 min at 37° [19]. The reaction was initiated by the addition of benzo(a)pyrene solution in acetone (final incubation concentration 83 $\mu$M) and after a further 5 min the reaction was stopped with 0.2 ml of Triton X-100 in triethylamine 10% v/v [20]. The fluorescence of benzo(a)pyrene metabolites was determined using a fluorimeter standardised against quinine sulphate (0.3 $\mu$g/ml in 0.1 N sulphuric acid). Results are expressed as the relative fluorescence difference ($\Delta F$) between emission wavelengths of 520 and 490 nm, per minute of incubation per mg microsomal of protein using an excitation wavelength of 470 nm [20]. Microsomal UDP-glucuronyl transferase using *p*-nitrophenol (PNP) as substrate was assayed by a modified method of Temple [21]. The assay was conducted aerobically at 37° and the incubation mixture consisted of Tris–HCl buffer (150 mM, pH 7.4), $MgCl_2$ (1.5 mM), UDPGA (5 mM), microsomal protein (1 mg/ml) and PNP (0.5 mM) in a final volume of 1.5 ml.

Hepatic 105,000 *g* supernatant GSH transferases were measured at 37° using 1-(choloro-2, 4-dinitrobenzene (DNCB) and 1,2-epoxy-3-(*p*-nitrophenoxy) propane (ENPP) as substrates [22, 23]. The respective incubation mixtures consisted of phosphate buffer (200 mM, pH 6.5), GSH (2.0 mM), DCNB (2.0 mM) and supernatant protein (0.005 mg); and phosphate buffer (200 mM, pH 6.5), GSH (5 mM), ENPP (0.5 mM) and supernatant protein (1.0 mg) in a final volume of 1.0 ml. Soluble sulphotransferase was measured at 37° using *p*-nitrophenyl sulphate as sulphate donor and phenol as the acceptor substrate [24]. The incubation mixture consisted of Tris–HCl buffer (100 mM, pH 8.0), *p*-

nitrophenyl sulphate (10 mM), phenol (0.5 mM), 3',5'-ADP (0.02 mM) and supernatant protein (0.5 mg) in a final volume of 1.0 ml.

The effect of BSO on *in vivo* drug metabolism was assessed by measuring hexobarbitone sleeping times and the urinary metabolic profile of paracetamol. Hexobarbitone sleeping times were determined according to Fouts [25], with hexobarbitone (80 mg/kg, i.p.) being administered 45 min after BSO (1.6 g/kg, p.o.). Separate groups of mice received BSO (1.6 g/kg, i.p.) 45 min before and 6 hr after a non-toxic dose of paracetamol (50 mg/kg, p.o.). The control group of animals received the paracetamol alone. Urine was collected for 36 hr and immediately assayed by HPLC for unchanged paracetamol and its glucuronide, sulphate and glutathione-derived conjugates as previously described [26].

Results were statistically analysed using the unpaired 2-tailed Student's *t*-test.

## RESULTS

The temporal effect of a single dose of BSO (1.6 g/kg, i.p.) on liver and kidney γ-GCS activity and GSH content is shown in Fig. 2. The time course of inhibition of γ-GCS in both tissues was the same, maximum inhibition occurred 2–4 hr after BSO administration and activity had returned to approximately 85% of control values by 15 hr. In contrast the depletion and recovery of GSH in liver and kidney was different, but was similar to that described by Griffith and Meister [8] following administration of 4 mmole/kg (890 mg/kg) BSO to mice that had been starved for 12 hr. GSH depletion in the liver paralleled changes in γ-GCS activity; maximum depletion of GSH (approx. 35% of control) occurred 4–6 hr after BSO and at 10 and 15 hr GSH content had returned to 62 and 83% of control values. On the other hand maximum depletion of GSH in the kidney (approx. 33% of control) occurred as early as 1 hr after BSO and remained at this low level for 10 hr. At 15 and 24 hr after BSO, kidney GSH levels were 53 and 79% respectively of control values.

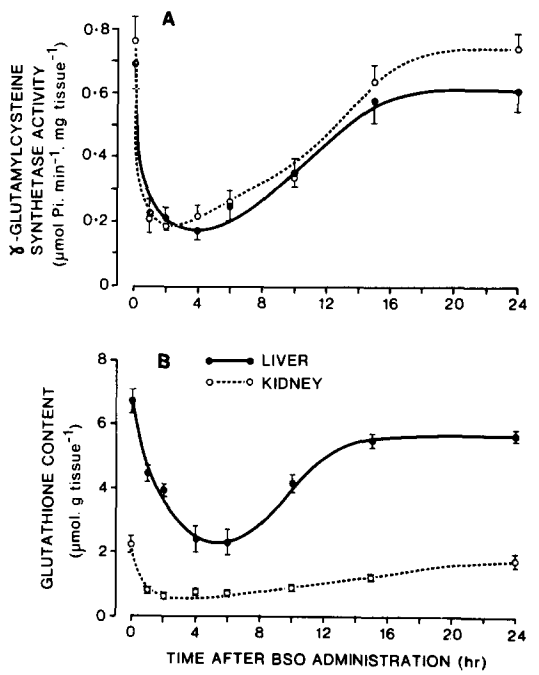


Fig. 2. Time course of inhibition and recovery of γ-glutamyl cysteine synthetase activity (Panel A) and glutathione content (Panel B) in mouse liver and kidney following interperitoneal buthionine sulphoximine (1.6 g/kg). Results are presented as the mean ± S.E.M. of 4 animals at each time point.

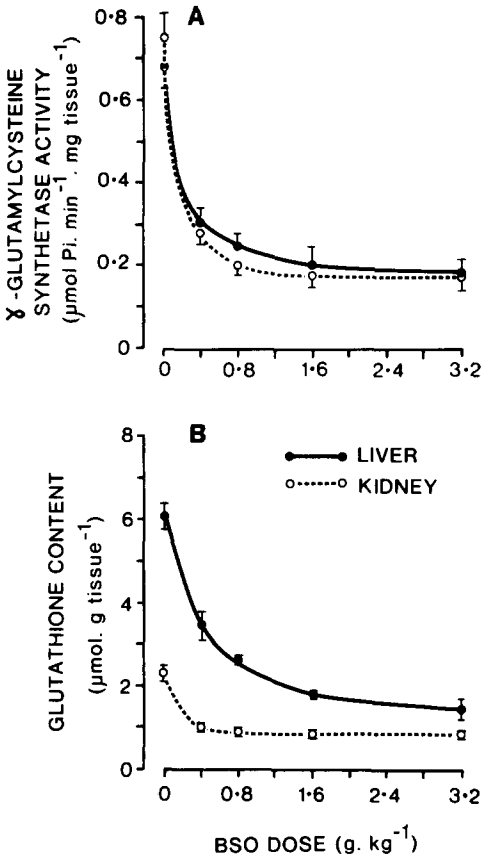


Fig. 3. Effect of different doses of buthionine sulphoximine (BSO) on liver and kidney γ-glutamylcysteine synthetase activity (Panel A) and glutathione content (Panel B). Each point represents the mean ± S.E.M. of 4 animals 4 hr after interperitoneal BSO administration.

The changes in γ-GCS and GSH content in liver and kidney 4 hr after the intraperitoneal administration of different doses of BSO are shown in Fig. 3. In both tissues γ-GCS activity was inhibited to approximately 40% of control activity by a BSO dose of 400 mg/kg, maximum inhibition of 25–30% of control activity was observed with 1600 mg/kg. In the kidney, GSH was reduced to 43% of control content by 400 mg/kg and maximum depletion to approximately 35% of control occurred with 800 mg/kg. Within the dose range employed in these experiments the reduction of hepatic GSH showed a greater dose dependency than did depletion of renal GSH content. Following doses of 400, 800, 1600 and 3200 mg/kg BSO hepatic GSH levels were 57, 43, 30 and 24% respectively of control levels.

Table 1. Effect of BSO on hepatic GSH content and γ-GCS, conjugating and mixed function oxidase activities*

| Activity | Control | Time after BSO administration (hr) 4 | 13 | 24 |
|---|---|---|---|---|
| GSH (μmole/g liver) | 6.46 ± 0.30 | 2.30 ± 0.39† | 4.81 ± 0.34‡ | 5.33 ± 0.30 |
| γ-GCS | 0.54 ± 0.06 | 0.17 ± 0.01† | 0.41 ± 0.04 | 0.49 ± 0.06 |
| GSH-transferase (nmoles product/min/mg protein) | | | | |
| DNCB | 3960 ± 410 | 3910 ± 160 | 3930 ± 280 | 3820 ± 190 |
| ENPP | 78.4 ± 3.5 | 77.9 ± 3.9 | 68.9 ± 3.2 | 84.0 ± 2.6 |
| Phenol sulphotransferase ($\Delta OD_{400} \times 10^2$/min/mg protein) | 2.29 ± 0.10 | 2.14 ± 0.10 | 2.37 ± 0.10 | 2.00 ± 0.08§ |
| *p*-NP glucuronyl transferase (nmoles *p*-NPG/min/mg protein) | 6.26 ± 0.40 | 5.52 ± 0.55 | 6.44 ± 0.12 | 5.79 ± 0.49 |
| Cytochrome P-450 (nmole/mg protein) | 0.64 ± 0.05 | 0.69 ± 0.05 | 0.62 ± 0.03 | 0.63 ± 0.02 |
| Aniline hydroxylase (nmole PNP/min/mg protein) | 9.1 ± 0.4 | 8.6 ± 0.3 | 7.7 ± 0.3§ | 8.1 ± 0.4 |
| Aminopyrine demethylase (nmole HCHO/min/mg protein) | 18.4 ± 0.4 | 19.1 ± 0.3 | 16.6 ± 0.3 | 16.5 ± 1.1 |
| Biphenyl hydroxylase (nmole 4-OH BP/min/mg/protein) | 1.18 ± 0.03 | 1.22 ± 0.03 | 1.16 ± 0.11 | 1.22 ± 0.03 |
| Aryl hydrocarbon hydroxylase ($\Delta F$/min/mg protein) | 239 ± 12 | 224 ± 16 | 236 ± 14 | 233 ± 19 |
| 7-Ethoxycoumarin deethylase (nmole 7-OH coumarin/min/mg protein) | 4.13 ± 0.21 | 4.69 ± 0.46 | 3.94 ± 0.58 | 4.81 ± 0.63 |

* Mice were given BSO intraperitoneally (1600 mg/kg) and at the times indicated groups of 4 were killed and various hepatic parameters measured as described under Methods.

Results are expressed as the mean ± S.E.M.

† Significantly different from controls ($P < 0.005$).

‡ Significantly different from controls ($P < 0.025$).

§ Significantly different from controls ($P < 0.05$).

*Effect on drug metabolising activity* in vitro. The effect of BSO on drug metabolising enzyme activity was studied at various times after an intraperitoneal dose of 1600 mg/kg (Table 1). In this experiment the changes in hepatic γ-GCS and GSH were similar to those reported in Fig. 2. Glutathione transferase activity using DNCB and ENPP as substrates was unaffected as was PNP glucuronyltransferase and phenol sulphotransferase activity. Cytochrome P-450 content was unaltered by BSO and although aniline hydroxylase was slightly decreased 13 hr after BSO the activity of this enzyme was unaltered at other time points. Aminopyrine demethylase, biphenyl hydroxylase, 7-ethoxycoumarin deethylase and aryl hydrocarbon hydroxylase activities were unaffected at all sampling times.

The influence of BSO on enzyme activity was also investigated when the compound was added directly to incubation mixtures. Concentrations up to 500 mM BSO had no affect on the *in vitro* activity of DNCB- or ENPP-glutathione transferase, PNP glucuronyltransferase, phenolsulphotransferase, aniline hydroxylase or aminopyrine demethylase.

*Effect on* in vivo *drug metabolism.* BSO administered 45 min before hexobarbitone had no effect on the induction of loss of righting reflex or on the duration of hexobarbitone induced hypnosis (Table 2). The amount of paracetamol excreted unchanged and as the glucuronide and sulphate was not altered in mice that had received BSO 45 min before and 6 hr after paracetamol administration (Table 2). However, the amount recovered as glutathione-derived metabolites was decreased by 58% following BSO treatment. Total recovery of unchanged paracetamol and its metabolites was significantly lower in the BSO treated animals.

## DISCUSSION

The expression of xenobiotic-metabolite mediated toxicities is determined by a number of interacting factors. The amount of electrophilic reactive metabolite available to react with tissue macromolecules, and presumably initiate a toxic response, is dependent upon the rate and extent of formation and detoxication of the metabolite. The microsomal cytochrome P-450 dependent mixed function oxidase system is usually responsible for the formation of reactive metabolites while conjugation with sulphate, glucuronide and glutathione largely facilitate detoxication and excretion of xenobiotics. In many instances GSH conjugation, and hence the availability of GSH, is qualitatively and quantitatively an important determinant of the final expression of metabolite mediated toxicity. Thus, alteration of toxicity by altering tissue GSH content is an indirect approach to implicate the involvement of electrophilic reactive compounds in the aetiology of xenobiotic-induced toxicities.

Griffith and Meister [8] have shown that in relation to the γ-glutamyl cycle, BSO is a potent and specific inhibitor of γ-GCS and hence of GSH synthesis.

Table 2. Effect of BSO on *in vivo* drug metabolism

| | Control | BSO-treated |
|---|---|---|
| (a) Hexobarbitone sleeping time (min)* | 54.5 ± 2.9 | 48.5 ± 2.5 |
| (b) Paracetamol urinary metabolites† | | |
| glucuronide | 3.63 ± 0.27 | 3.62 ± 0.20 |
| sulphate | 1.04 ± 0.08 | 1.00 ± 0.11 |
| GSH | 1.67 ± 0.13 | 0.70 ± 0.10‡ |
| APAP | 0.86 ± 0.08 | 0.92 ± 0.11 |
| % dose recovered | 94.0 ± 4.2 | 81.9 ± 4.0‡ |

* Mice received BSO (1600 mg/kg, p.o.) 45 min before hexobarbitone (80 mg/kg, i.p.) and the duration of loss of righting reflex determined. Control animals received saline (20 ml/kg, p.o.). Results are the mean ± S.E.M. of groups of 12 animals.

† Urine was collected for 36 hr after paracetamol (50 mg/kg, p.o.). BSO (1600 mg/kg, i.p.) was administered 45 min before and 6 hr after paracetamol. Control animals received saline (20 ml/kg, i.p.) at equivalent times. There were at least 5 animals in each group and results are expressed as mean ± S.E.M. Paracetamol urinary metabolites are expressed as amount (μmole of paracetamol equivalents) excreted in 36 hr.

‡ Significantly different from controls ($P < 0.01$).

Furthermore, these workers demonstrated that BSO did not inhibit glutamine synthetase and produced no abnormal behaviour in mice after intraperitoneal or oral administration. This communication confirms and extends these observations. We have established the time course and dose–response relationship of γ-GCS inhibition and depletion of GSH content in liver and kidneys of fed male mice. The optimal dose for depletion of hepatic GSH was found to be 1.6 g/kg; this dose had no effect on conjugating enzymes or mixed function oxidase activity in microsomes or 105,000 *g* supernatant prepared from the livers of treated mice. Addition of BSO to *in vitro* incubation assays for these enzymes also did not alter enzymic activity. The duration of hexobarbitone narcosis, which is often used as an *in vivo* index of mixed function oxidase activity, was similarly not altered by BSO. It would therefore appear that BSO is able to selectively inhibit the synthesis of GSH and lower tissue GSH levels without influencing enzyme activities that may contribute to the generation and detoxication of reactive metabolites.

The specificity of action of BSO is in sharp contrast to other agents that have been used to alter tissue GSH content. Diethylmaleate, iodoacetate, iodoacetamide and *N*-ethylmaleimide have all been used to deplete tissue GSH levels but have also been shown to affect mixed function oxidase activity [1–6]. *In vitro*, these agents may inhibit or enhance certain mixed function oxidase activities [2, 4, 5, 27, 28]. With regard to the role of GSH in xenobiotic-induced toxicities, depletion of GSH by these agents is subject to different interpretations. For example, Chuang *et al.* [5] hypothesized that depletion of skin GSH by diethylmaleate would increase the dermal tumorigensis of 3-methylcholanthrene by allowing more of the reactive intermediate to combine with DNA. Experimentally however, diethylmaleate markedly decreased 3-methylcholanthrene-induced skin tumorigensis. It was subsequently found that DEM inhibited skin (and liver) aryl hydrocarbon hydroxylase, the enzyme responsible for generating the reactive hydrocarbon metabolite. Chuang *et al.* [5] also demonstrated that cyclohexene sulphide, thought to be a specific inhibitor of glutathione transferase [29], similarly inhibited aryl hydrocarbon hydroxylase activity. It has been argued that GSH does not have a protective role in carbon tetrachloride induced hepatic injury since GSH depletion by diethylmaleate ameliorated $CCl_4$ hepatotoxicity in rats [30]. Since $CCl_4$ must be metabolised to a reactive species, an alternative explanation for diethylmaleate protection of $CCl_4$ hepatoxicity is inhibition of $CCl_4$ bioactivation.

An appreciation of the effects of GSH-depleting agents on enzymes involved in bioactivation/inactivation is important in understanding the changes that these agents cause in xenobiotic urinary metabolite profiles. BSO caused a marked decrease in the formation of GSH-derived metabolites of paracetamol but no change in the excretion of the glucuronide and sulphate conjugates. The decreased excretion of GSH-derived conjugates and the lower recovery of paracetamol-derived products in BSO treated animals reflects the extent of the dose converted to the paracetamol reactive metabolite which would have been conjugated with GSH in the absence of BSO. Further confirmation of the lack of effect of BSO on conjugating enzyme activity is provided by the paracetamol metabolite data. The ratio of metabolite excreted in urine to unchanged paracetamol, commonly called the metabolic ratio, reflects the clearance to that metabolite and thus the activity of the enzyme(s) involved in its formation [26]. Reconsideration of the urinary metabolite data in Table 2 in terms of metabolic ratio confirms that BSO did not affect paracetamol clearance through the glucuronidation and sulphation pathways.

In conclusion, BSO is a specific inhibitor of GSH synthesis and does not affect other enzymes involved in the formation/removal of reactive metabolites. BSO therefore, has distinct advantages over other GSH-depleting agents when used to demonstrate the

role of GSH in xenobiotic-induced toxicities and should find wide application in the elucidation of mechanisms of chemical induced tissue injury.

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## REFERENCES

1. K. J. Netter, *Pharmac. Ther.* **10**, 515 (1980).
2. H. P. A. Illing and K. J. Netter, *Xenobiotica* **5**, 1 (1975).
3. B. Testa and P. Jenner, *Drug Metab. Rev.* **12**, 1 (1981).
4. M. W. Anders, *Biochem. Pharmac.* **27**, 1098 (1978).
5. A. H. L. Chuang, H. Mukhtar and E. Bresnick, *J. natn. Cancer Inst.* **60**, 321 (1978).
6. R. F. Burk and M. A. Correia, *Res. Commun. Chem. Path. Pharmac.* **24**, 205 (1979).
7. A. Meister, in *Functions of Glutathione in Liver and Kidney* (Eds. H. Sies and A. Wendel), p. 43. Springer, Berlin (1978).
8. O. W. Griffith and A. Meister, *Proc. natn. Acad. Sci. U.S.A.* **76**, 5606 (1979).
9. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
10. H. C. Cohn and J. Lyle, *Analyt. Biochem.* **14**, 434 (1966).
11. O. W. Griffith, A. Larsson and A. Meister, *biochem. biophys. Res. Commun.* **79**, 919 (1977).
12. C. H. Fiske and Y. SubbaRow, *J. biol. Chem.* **66**, 35 (1925).
13. T. Omura and R. Sato, *J. biol. Chem.* **239**, 2370 (1964).
14. J. Cochin and J. Axelrod, *J. Pharmac. exp. Ther.* **125**, 105 (1959).
15. T. Nash, *Biochem. J.* **55**, 416 (1953).
16. Y. Imai, A. Ito and R. Sato, *J. Biochem.* **60**, 417 (1966).
17. P. J. Creaven, D. V. Parke and R. T. Williams, *Biochem. J.* **96**, 879 (1965).
18. W. F. Greenlee and A. Poland, *J. Pharmac. exp. Ther.* **205**, 596 (1978).
19. C. S. Young, F. S. Strickhart and L. P. Kicha, *Biochem. Pharmac.* **27**, 2321 (1978).
20. W. Dehnen, R. Tomingas and J. Roos, *Analyt. Biochem.* **53**, 373 (1973).
21. A. R. Temple, A. K. Done and M. S. Clement, *J. Lab. clin. Med.* **77**, 1015 (1971).
22. W. H. Habig, M. J. Pabst and W. B. Jakoby, *J. biol. Chem.* **249**, 7130 (1974).
23. T. A. Fjellstedt, R. H. Allen, B. K. Duncan and W. B. Jakoby, *J. biol. Chem.* **248**, 3702 (1973).
24. G. J. Mulder and E. Scholtens, *Biochem. J.* **165**, 553 (1977).
25. J. R. Fouts, in *Methods in Pharmacology* (Ed. A. Schwaratz), Vol. 1, p. 287. Meredith Corp., NY (1971).
26. J. O. Miners, J. F. Adams and D. J. Birkett, *Clin. exp. Pharmac. Physiol.* **11**, 209 (1984).
27. H. Kampffmeyer and M. Kiese, *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.* **244**, (1963).
28. H. Kampffmeyer and M. Kiese, *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.* **246**, 397 (1964).
29. T. Hayakawa, S. Udenfriend and H. Yogi, *Archs. Biochem. Biophys.* **170**, 438 (1975).
30. K. A. Suarez and P. Bhonsle, *Fedn. Proc.* **36**, 412 (1977).