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EFFECTS OF BUTHIONINE SULFOXIMINE AND DIETHYL MALEATE ON GLUTATHIONE TURNOVER IN THE CHANNEL CATFISH*

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Abstract—Despite the growing use of fish in toxicological studies, little is known regarding glutathione (GSH) metabolism and turnover in these aquatic species. Therefore, we examined GSH metabolism in the liver and gills of channel catfish (*Ictalurus punctatus*), a commonly employed aquatic toxicological model. Treatment of channel catfish with L-buthionine-*S*,*R*-sulfoximine (BSO, 400 or 1000 mg/kg, i.p.), an inhibitor of GSH biosynthesis, did not deplete hepatic GSH in channel catfish. In addition, hepatic GSH concentrations did not fluctuate in catfish starved for 3 days, indicating relatively slow turnover of hepatic GSH. However, hepatic GSH concentrations were reduced significantly ($P < 0.05$) after 7 days of starvation. Administration of the thiol alkylating agent diethyl maleate (DEM, 0.6 mL/kg, i.p.) resulted in depletion of 85% of hepatic GSH at 6 hr post-DEM, with complete GSH recovery observed at 24 hr post-DEM. Co-administration of BSO and DEM (1000 mg/kg, 0.6 mL/kg, respectively) substantially depleted gill GSH and eliminated detectable liver GSH. Following BSO/DEM, GSH recovery in hepatic mitochondria occurred more rapidly than did liver cytosolic GSH. γ-Glutamylcysteine synthetase (GCS) activities were comparable in the 10,000 *g* supernatants of catfish liver and gills (204 ± 21 and 268 ± 20 nmol/min/mg protein, respectively) whereas γ-glutamyltranspeptidase (GGT) activity was not detected in the 600 *g* post-nuclear fraction of either liver or gills. In conclusion, i.p. administration of DEM was an effective means for achieving short-term hepatic GSH depletion in channel catfish, whereas co-administration of BSO and DEM elicited prolonged and extensive hepatic GSH depletion in this species. Like rodents, channel catfish maintained physiologically distinct hepatic mitochondrial and cytosolic GSH pools, and also regulated hepatic GSH levels by *in situ* hepatic GSH biosynthesis. However, unlike rodents, there was no evidence for a labile hepatic cytosolic GSH pool in channel catfish. These similarities and differences need to be considered when designing toxicological studies involving the GSH pathway in channel catfish and possibly other fish species.

Glutathione (GSH¶) is a major nonprotein thiol associated with a number of critical cellular regulatory functions [1, 2]. GSH protects the cell against both electrophilic agents and free radical metabolites by serving as a cofactor for glutathione *S*-transferases (GSTs) [2] and as a reductant for glutathione peroxidase [2, 3]. Two distinct pools of GSH have been identified in rodent hepatocytes, a labile cytosolic pool ($T_{1/2} = 1.7$ hr) comprising approximately 85% of total cellular GSH, and a stabile mitochondrial pool ($T_{1/2} = 28$ hr) [4, 5]. In addition to detoxification, the cytosolic GSH pool functions in microtubule assembly and as a cysteine reservoir during protein synthesis [2, 6]. Meredith and Reed [4] have suggested that depletion of the mitochondrial GSH pool is a more sensitive indicator of cellular toxicity than cytosolic GSH loss. Accordingly, it has been postulated that the GSH-depleting agent diethyl maleate (DEM) is not cytotoxic because it does not deplete mitochondrial GSH [7]. In rats, the mitochondrial GSH pool is metabolically distinct from the cytosolic pool [8] and is involved in the control of hydrogen peroxide formation through the activity of the mitochondrial GSH peroxidase/reductase pathway. Accordingly, depletion of mitochondrial GSH may compromise the ability of the mitochondria to prevent oxidative damage.

Chemically induced GSH depletion can be an effective means of studying compounds whose toxic effects are attenuated by GSH. For example, the toxicities of formaldehyde in rat hepatocytes [5], of aflatoxin B_1 in rats [9], and of methyl parathion and fenitrothion in mice [10] are enhanced after GSH depletion by DEM pretreatment. Treatment of rainbow trout (*Oncorhynchus mykiss*) with DEM increases the hepatic covalent binding of monochlorobenzene [11] and hepatotoxicity of allyl formate [12]. L-Buthionine-*S*,*R*-sulfoximine (BSO) effectively lowers hepatic and renal GSH in rodents by specifically inhibiting γ-glutamylcysteine synthetase (GCS), the rate-limiting enzyme for GSH synthesis [13]. Like DEM, BSO has been used to study the toxicological role of GSH both *in vitro* [14, 15] and *in vivo* [16]. Monroe and Eaton [17] showed that the extent of hepatic GSH depletion in mice could be enhanced when BSO (600 mg/kg,

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¶ Abbreviations: GSH, glutathione; BSO, L-buthionine-*S*,*R*-sulfoximine; DEM, diethyl maleate; GCS, γ-glutamylcysteine synthetase; GGT, γ-glutamyltranspeptidase; and GSTs, glutathione *S*-transferases.

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i.p.) and DEM (0.75 mL/kg, i.p.) were co-administered. However, care should be exercised when employing these compounds in toxicological studies, as both agents may exert physiological and biochemical effects unrelated to GSH depletion [18, 19].

In addition to providing information on the mechanisms of chemical toxicity, the use of GSH-depleting agents can also shed light on key aspects of GSH metabolism and turnover. For example, the understanding of hepatic cytosolic GSH turnover is a direct result of studies involving BSO [13, 20]. The objectives of the present study were to evaluate BSO and DEM as GSH-depleting agents for studies of xenobiotic metabolism in channel catfish. We also were interested in examining possible differences in hepatic GSH turnover among catfish and rodents. Despite the involvement of GSH in a number of critical cellular processes, little is known regarding its metabolism and turnover in aquatic animals. Since the use of fish in studies of biochemical toxicology and carcinogenesis is increasing, we must extend our basic understanding of detoxification systems in these organisms. While the primary focus of this work was on the liver, certain key experiments examined comparative effects on GSH metabolism in the gills, the initial site of exposure to waterborne pollutants. We report for the first time significant differences in GSH metabolism among an aquatic species and traditional rodent models and discuss the possible mechanisms for, and implications of, these differences.

MATERIALS AND METHODS

Chemicals. Maleic acid, diethyl ester (DEM), GSH, and all other reagents were obtained from the Sigma Chemical Co. (St. Louis, MO). All chemicals used in this study were of reagent or analytical grade.

Animals. Yearling channel catfish (*Ictalurus punctatus*) (50–75 g) were obtained from Aquaculture Advisory Service (Raleigh, NC). The catfish were maintained at 20° (12 hr light/12 hr dark photoperiod) in a 1200 L stainless steel tank with flow-through dechlorinated city water. Fish were fed to satiation every other day with Westco floating catfish food (32% protein, Charlotte, NC). An acclimation period of at least 1 week preceded all experiments. After 7 days, fish were transferred to 50 L flow-through aquaria, four to six fish per tank. Average water quality characteristics in the aquaria were as follows: water temperature 20°; pH 7.0 to 7.2; dissolved oxygen 5.0 to 6.0 mg/L; total hardness 30 mg/L; total alkalinity 37 mg/L; ammonia < 0.1 mg/L; nitrite < 0.1 mg/L. Except for the starvation experiments, feeding was continued over the course of the studies. However, fish typically ate poorly after i.p. injections.

Effects of BSO, DEM, and a BSO/DEM combination on GSH depletion. To determine the effect of BSO on hepatic GSH, BSO was dissolved in saline (1.15% KCl) by alternate heating under hot tap water and vortexing. After cooling to room temperature, BSO (400 or 1000 mg/kg) was injected i.p. and the catfish were placed in the flow-through aquaria. In a second experiment, catfish received DEM (0.6 mL/kg in corn oil, i.p.). For the BSO/DEM co-treatment experiments, catfish received DEM (0.6 mL/kg in corn oil) immediately followed by BSO (1000 mg/kg, i.p. in saline). Control animals in all experiments received equivalent volumes (150 μL) of saline or corn oil, as appropriate. Catfish were killed at the specified time points following chemical treatments by severing the spinal cord. The livers were quickly excised, washed in cold (0–4°) saline, blotted dry and weighed. The liver was then divided into two sections. Once section was placed in 10% buffered formalin (pH 7.0), and the remaining section homogenized in 10% sulfosalicylic acid (1:5, w/v). The fixed liver sections were trimmed and then sent to Experimental Pathology Laboratories (Research Triangle Park, NC) for paraffin embedding and staining with hematoxylin and eosin. To determine the effect of co-treatment of DEM/BSO on gill GSH, the gills were excised 24 and 48 hr following treatment. The gill filaments were carefully trimmed from the gill arches. The gill filaments were then pooled and weighed. Four passes of the tissues through a Brinkmann (Westbury, NY) polytron were used to homogenize both liver and gill tissues. All homogenates were centrifuged at 5000 *g* for 10 min and the acidified, protein free supernatants stored at −70° for subsequent GSH determinations by the method of Tietze [21] as modified by Griffith [22]. The results are expressed as GSH equivalents and include oxidized (GSSG) and reduced (GSH) glutathione.

Cytosolic and mitochondrial GSH depletion and GSH recovery experiments. To determine the subcellular extent of GSH depletion, catfish were injected with DEM/BSO as before and placed in flow-through aquaria. Control animals received injections of equivalent volumes of corn oil and saline. After 24 hr, the catfish were killed and the livers and gills were processed as above. Tissues were weighed and subsequently homogenized in 2 vol. of buffer (2 mM HEPES, 222 mM mannitol, 70 mM sucrose, 1 mM EDTA, pH 7.4) with a teflon homogenizer for 10–15 sec and centrifuged (0–4°) at 660 *g* for 10 min. Following centrifugation, the large particle fraction was discarded. The 660 *g* supernatant was recentrifuged at 8000 *g* for 15 min for isolation of the mitochondrial pellets. The mitochondrial pellets were resuspended in 1 mL of buffer following two washings. The cytosolic fractions were isolated from the 8000 *g* supernatant by centrifugation at 105,000 *g* for 60 min. Purity of subcellular fractions was verified in preliminary experiments by assessing lactate dehydrogenase and cytochrome oxidase activities. Following isolation, the mitochondrial and cytosolic fractions were deproteinized by addition of 10% sulfosalicylic acid (1:1, 1:4, respectively) and centrifuged at 5000 *g* for 10 min. GSH was determined on the isolated fractions as described above. To determine the rate of GSH recovery in hepatic mitochondrial and cytosolic pools following GSH depletion, catfish were treated with BSO/DEM or carriers as described above. Ten fish (five treated, five controls) were killed at 1, 2, 4 and 6 days post-treatment and hepatic mitochondrial and cytosolic pools were isolated and analyzed for GSH content as previously described.

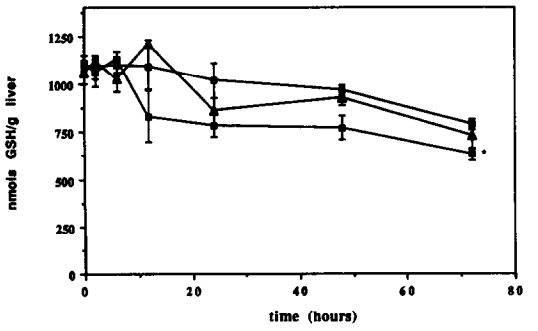

Fig. 1. Time course of hepatic glutathione levels in channel catfish after i.p. injection of 400 mg/kg BSO (▲), 1000 mg/kg BSO (■) or 150 μL saline (□). Catfish were killed at the specified time points and the livers were excised and homogenized in 10% sulfosalicylic acid (1:5, w/v). All homogenates were centrifuged at 5000 rpm for 10 min and the acidified, deproteinized supernatants stored at −70° for subsequent GSH determinations by the method of Griffith [22]. Values are means ± SEM of three animals. The asterisk denotes a value significantly different from control at the respective time point ($P < 0.05$, two-tailed Student's *t*-test).

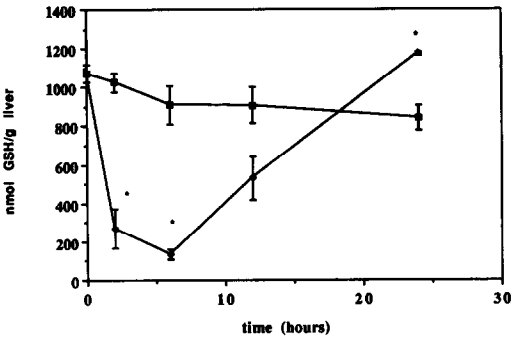

Fig. 2. Time course of hepatic glutathione levels in channel catfish after i.p. injection of 0.6 mL/kg diethyl maleate (◆) or 150 μL corn coil (□). Tissue preparations and assay conditions are described in Materials and Methods. Values are means ± SEM of four animals. Asterisks denote means significantly different from controls at respective time points ($P < 0.05$, two-tailed Student's *t*-test).

Effect of starvation on hepatic GSH. To determine the effect of starvation on hepatic GSH, catfish were placed in flow-through aquaria with continued feeding for 7 days. After 7 days food was withheld. Three or four fish were sampled at 0 hr, 24 hr, 3 days, 7 days, and 14 days after the last feeding, and GSH was determined on liver homogenates as described above.

GCS and γ-glutamyltranspeptidase (GGT) activities and protein determination. Liver and gill tissues from control catfish were homogenized in 0.1 M Tris/HCl containing 5 mM EDTA (pH 7.4). GGT activity was assayed on the 600 *g* post-nuclear fractions by the method of Tate and Meister [23] at pH 8.0. GCS activity was measured in the 10,000 *g* post-mitochondrial supernatant by the method of Seelig and Meister [24] at apparent pH optima for channel catfish liver and gills (pH = 7.75). Substrate saturation and protein linearity were established prior to quantitation of GCS activities. These conditions could not be established for GGT activity in catfish liver and gills due to negligible GGT activity in those tissues. Protein content was measured by the method of Lowry *et al.* [25] using bovine serum albumin as a standard. GCS activities were calculated as specific activity (per mg protein) and total organ activity.

Statistical analysis. Significant differences among control and treatment groups were tested by one-way analysis of variance (ANOVA). Where appropriate, comparisons were made using the two-tailed Student's *t*-test for unpaired sample means. Dunnett's *t*-test was used to assess the significance of starvation effects on hepatic GSH. The chosen level of significance was $P \leq 0.05$.

RESULTS

Effect of depleting agents on hepatic GSH. The results of administration of 400 or 1000 mg/kg BSO i.p. are illustrated in Fig. 1. Hepatic GSH concentrations in BSO-treated catfish were not significantly different than controls for 48 hr post-treatment. At 72 hr, there was a 21% decrease in hepatic GSH concentrations in catfish treated with 1000 mg/kg BSO which was statistically significant ($P < 0.05$). BSO administered by i.p. injection at 1600 mg/kg or by gavage did not increase the extent of hepatic GSH loss (data not shown). Our findings contrast with those of rodent studies which have shown extensive hepatic GSH depletion within 24 hr of *in vivo* administration of BSO [19, 20]. In contrast, treatment of channel catfish with DEM (0.6 mL/kg, i.p.) resulted in depletion of hepatic GSH to 27% and 15% of control levels at 2 and 6 hr, respectively (Fig. 2). Partial recovery of hepatic GSH was observed at 12 hr, and by 24 hr liver GSH concentrations in the DEM-treated catfish exceeded control values by 40% (Fig. 2).

Since the DEM experiment proved the channel catfish were susceptible to short-term GSH depletion, we examined the possibility of using BSO to suppress GSH resynthesis after DEM-induced GSH depletion. As seen in Fig. 3, co-administration of BSO/DEM (1000 mg/kg, 0.6 mL/kg, i.p.) resulted in extensive and sustained hepatic GSH depletion (<4% of control GSH levels), indicating an inhibition of hepatic GSH resynthesis by BSO following DEM-induced GSH depletion. None of the GSH-depleting regimens caused observable toxicity, and examination of livers by light microscopy showed no histological differences among control, DEM, or BSO/DEM-treated catfish.

Recovery of hepatic mitochondrial and cytosolic GSH pools after depletion. Partial recovery of GSH in hepatic mitochondrial fractions (to 14% of control levels) occurred 4 days following BSO/DEM-induced hepatic GSH depletion (Fig. 4). In contrast, partial recovery of the hepatic cytosolic GSH pool was not observed until 6 days post-treatment. By day 6, hepatic cytosolic and mitochondrial pools

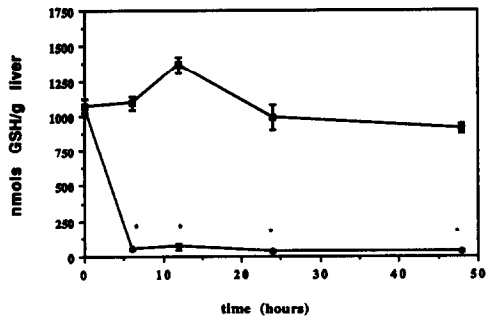

Fig. 3. Time course of hepatic glutathione levels in channel catfish after i.p. co-treatment with 1000 mg/kg BSO and 0.6 mL/kg DEM (◆) or 150 μL corn oil and 150 μL saline (□). Tissue preparations and assay conditions are described in Materials and Methods. Values are means ± SEM of four animals. Asterisks denote means significantly different from controls at respective time points ($P < 0.05$, two-tailed Student's t-test).

Table 1. Effect of BSO/DEM on gill GSH concentrations in channel catfish*

Treatment group	Time (hr)	GSH	
		nmol/g tissue	% Control
Control	24	248 ± 15	(100)
BSO/DEM	24	15 ± 12	6 ± 5
Control	48	220 ± 11	(100)
BSO/DEM	48	146 ± 47	66 ± 21

* Channel catfish (50–75 g) were injected i.p. with DEM (0.6 mL/kg in corn oil) immediately followed by BSO (1000 mg/kg, i.p., in saline). Controls received equivalent volumes of saline and corn oil carriers (150 μL). Catfish were killed 24 and 48 hr post-injection and the gills were excised immediately. Following isolation, the gill filaments were pooled, weighed and homogenized in 10% sulfosalicylic acid. The homogenates were centrifuged at 5000 g for 10 min. GSH was determined on the acidified, deproteinized supernatants by the method of Griffith [22]. Results are means ± SEM of four catfish preparations.

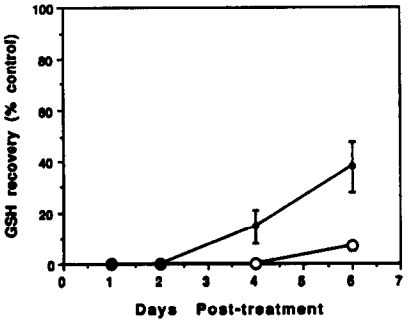

Fig. 4. Time course of GSH recovery measured in hepatic cytosolic (○) and mitochondrial fractions (●) following i.p. injection of BSO and DEM (1000 mg/kg and 0.6 mL/kg, i.p., respectively). Values are expressed as per cent GSH observed in treated groups as compared to controls sampled simultaneously. Absolute control values for mitochondrial and cytosolic GSH were 3.11 ± 0.54 and 19.10 ± 2.75 μmol/g protein, respectively (mean ± SEM). Subcellular isolations and assay conditions are described in Materials and Methods. Zero per cent recovery indicates GSH concentrations below the practical limit of assay detection for the subcellular fractions (80 nmol/mg mitochondrial protein, 300 nmol/mg cytosolic protein). Values are means ± SEM of five animals.

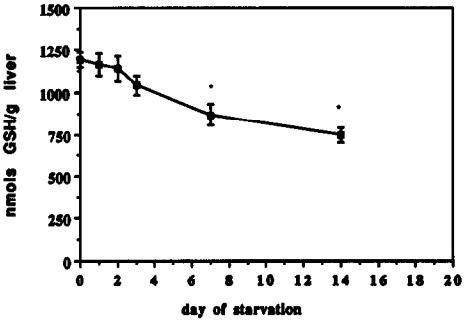

Fig. 5. Time course of hepatic glutathione levels in channel catfish after food restriction. Catfish were killed at the specified time points following food restriction (0 hr). Tissue preparations and assay conditions are described in Materials and Methods. Values are means ± SEM of four animals. Asterisks denote means significantly different from 0 hr controls ($P < 0.05$, Dunnett's t-test).

were restored to 7 and 38% of their respective control GSH concentrations (Fig. 4).

Effect of BSO/DEM on gill GSH. Gill GSH concentrations were reduced to 6% of control levels at 24 hr following BSO/DEM treatment (Table 1). By 48 hr, gill GSH concentrations recovered to 66% of respective control levels (Table 1).

Effect of starvation on hepatic GSH. To further explore possible differences in GSH turnover, hepatic GSH concentrations were assayed in catfish starved for 14 days. As seen in Fig. 5, there was no significant reduction in hepatic GSH concentrations during the first 3 days of starvation. However, hepatic GSH concentrations declined significantly ($P < 0.05$) by day 7 of starvation. At 7 and 14 days of starvation hepatic GSH concentrations declined by 23 and 36%, respectively (Fig. 5).

Liver and gill GGT and GCS activities. GGT activity was not detected (detection limit of 0.6 nmol/min/mg protein) in either liver or gills, indicating the inability of those organs to utilize GSH synthesized in other tissues. In contrast, GCS activities were present in both liver and gills, indicating the presence of the GSH biosynthetic pathway in those tissues. Specific GCS activities were somewhat higher in gills (268 ± 20 nmol/min/mg protein) than in liver (204 ± 21 nmol/min/mg protein) (Table 2). However, total GCS activities (based on tissue weight) were higher in the

Table 2. γ-Glutamylcysteine synthetase (GCS) and γ-glutamyltranspeptidase (GGT) activities in channel catfish liver and gills

Tissue	GCS* Specific activity (nmol/min/mg protein)	GCS* Total activity (μmol/min/organ)	GGT†
Liver	204 ± 21	16.9 ± 1.9	ND
Gills	268 ± 20	12.6 ± 1.5	ND

* GCS activities were determined in 10,000 *g* post-mitochondrial supernatants by the method of Seelig and Meister [24]. Values are the means ± SEM of five catfish.

† GGT activities were determined in 600 *g* post-nuclear fractions by the method of Tate and Meister [23]. GGT activity was not detected (ND, detection limit of 0.6 nmol/min/mg protein) in either tissue. As a positive control, GGT was detected in catfish posterior kidneys (990 ± 21 nmol/min/mg protein, mean ± SEM of five catfish).

liver (16.9 ± 1.9 μmol/min/liver) than in the gills (12.6 ± 1.5 μmol/min/gills).

DISCUSSION

Tissue GSH concentrations are the result of a complex equilibrium between biosynthesis, utilization, and export into plasma and bile [26]. The results of the present study confirm functional differences in the maintenance of tissue GSH levels in catfish and rodents. Administration of BSO (400 or 1000 mg/kg, i.p.) to channel catfish did not result in extensive hepatic GSH depletion, while treatment with DEM (0.6 mL/kg) caused a rapid, albeit reversible depletion of hepatic GSH. Since co-administration of BSO and DEM potentiated the extent and duration of hepatic GSH depletion, it can be inferred that BSO reaches the liver and blocks GSH biosynthesis in channel catfish. Assuming that GSH biosynthesis is inhibited completely and that utilization continues at its normal rate, changes in hepatic GSH concentrations will reflect the rate of GSH turnover into plasma and biliary compartments [13]. Thus, the relative stability of hepatic GSH concentrations in channel catfish following BSO administration in the absence of DEM strongly suggests a slow turnover of catfish hepatic GSH.

This postulation of slow turnover is further supported by the lack of hepatic GSH loss in channel catfish during short-term starvation. Similarly, Thomas and Wofford [27] did not observe fluctuations in hepatic acid-soluble thiol, cysteine or GSH levels in mullet (*Mugil cephalus* L.) starved over 48 hr. Therefore, the stability of hepatic GSH observed in starved channel catfish may be a phenomenon common to other fish species. Rodents starved for 24 hr undergo hepatic GSH depletion due to continued export of cytosolic GSH into plasma, followed by failure of GSH biosynthesis due to a lack of amino acid substrates [6]. On refeeding, the rate of GSH resynthesis is directly proportional to the dietary cysteine concentration [6]. Although the mechanisms of GSH regulation have not been studied in aquatic animals, fish, like rodents, regulate tissue cysteine concentrations through dietary intake, methionine transsulfuration and by reduction of cystine [28]. Since fish are poikilotherms, maintenance energy requirements are relatively low as compared to mammals. Therefore, except in the case of growing animals, the energy requirements of most fish species are relatively small. Thus, it is possible that channel catfish do not possess a highly developed transport system for rapid export of hepatic GSH because there is little need to exploit liver GSH as a cysteine source for either protein synthesis or energy demands.

GSH is found in the major tissues of many species of fishes, albeit at concentrations well below those of rodents [27, 29, 30]. In addition, GSH-cycle enzymes such as GSTs, GSSG-reductase, and GSH-peroxidase typically exhibit lower specific activities and are kinetically less efficient in fish than in mammals [30–32]. Collectively, these reports suggest that fish may be disproportionally sensitive to the toxicity of environmental chemicals which are detoxified by GSH. However, it is possible that fish may overcome this deficit by increasing hepatic GSH concentrations during exposure to environmental pollutants. For example, there are reports of elevated hepatic GSH concentrations in fish exposed to pollutants in the laboratory [27, 33] and also in feral fish inhabiting polluted waterways [34]. In addition, we have observed sustained elevations in hepatic GSH in channel catfish chronically exposed to polycyclic aromatic hydrocarbon-enriched sediments [35]. Thus, it is possible that slow hepatic GSH export, concomitant with the ability for GSH biosynthesis, allows for increased hepatic GSH levels during exposure to environmental pollutants.

Co-administration of BSO and DEM substantially depleted gill GSH. In addition to serving as the key respiratory organ for fish, the gills are the dominant organ for maintaining ionic balance and for the excretion of nitrogenous wastes [36]. As the initial site of exposure to waterborne pollutants, the gills are often a critical target of many environmental pollutants, including organochlorine insecticides [37], organophosphates [26, 37] and cadmium [38]. We have also observed increases in gill GSH in channel catfish exposed to the fungicide

chlorothalonil [39]. In the present study, the gills were sensitive to GSH depletion by BSO/DEM, although recovery occurred more rapidly in the gills than in the liver. The rapid GSH recovery in the gills may reflect an increased capacity for GSH biosynthesis, a lesser amount of BSO/DEM reaching the gills, or faster elimination of BSO/DEM from the gills than liver. The presence of substantial GCS activities in the gills and liver indicates GSH biosynthetic capabilities in those tissues. In contrast, GGT activity, which is present in tissues that have the ability to utilize translocated GSH [13, 23], was not detected in either liver or gills of catfish (GGT activity was easily detected in the posterior kidney as a positive control; data not shown). Thus, it appears that GSH replenishment in liver and gills following chemically induced GSH depletion is a result of *in situ* GSH biosynthesis in those tissues as opposed to GSH import from other physiological sources.

In the present study, catfish hepatic GSH concentrations were depleted by 85% when compared to vehicle-treated animals 6 hr after 0.6 mL/kg DEM. This level of DEM-induced hepatic GSH depletion is similar to that exhibited by rainbow trout (82%) 6 hr after the same dose of DEM [11]. To our knowledge, the present report is the first to combine BSO and DEM to enhance hepatic GSH depletion in fish. When administered alone, BSO does not cross mitochondrial membranes of hepatocytes or deplete mitochondrial GSH in rats [40]. However, as observed in our experiments, co-treatment of channel catfish with BSO and DEM results in a substantial depletion of mitochondrial GSH pools in both liver and gills. As in rodents, the presence of two physiologically distinct subcellular GSH pools is evidenced by differential rates of recovery in hepatic mitochondrial and cytosolic GSH pools following GSH depletion (Fig. 4). The preferential replacement of mitochondrial GSH observed in catfish liver may reflect the need to protect mitochondrial membranes from oxidative damage due to localized hydrogen peroxide production. Such a scenario would especially hold true if catfish hepatocytes are similar to those of rodents in that they do not contain mitochondrial catalase activity.

Consistent with rodent studies [41–43], we found no evidence of hepatotoxicity to channel catfish by DEM or BSO/DEM. However, we have not investigated the effects of BSO or DEM on other physiological functions or drug-metabolizing enzymes in channel catfish. Both DEM [18] and BSO [19, 44] may affect the activities of hepatic drug-metabolizing enzymes in rodents. Therefore, until the potential for physiological effects other than GSH depletion by DEM and BSO has been fully investigated in fish, caution should be applied when employing these agents in studies of aquatic xenobiotic metabolism.

In summary, DEM, and DEM used in combination with BSO, can be effectively used to deplete hepatic GSH for studies of xenobiotic metabolism in channel catfish. However, BSO administered alone does not deplete hepatic GSH in this species. Co-administration of BSO and DEM will extensively deplete catfish hepatic cytosolic and mitochondrial pools, and will also affect peripheral tissues such as the gills. Unlike those involving rodent models, our studies indicate that the hepatic cytosolic GSH pool in channel catfish does not undergo rapid turnover. Although the basis for differences in GSH metabolism between catfish and rodents has not been investigated, the presence of a stable cytosolic GSH pool may provide catfish with a means to maintain adequate GSH levels during exposure to waterborne xenobiotics. Investigators must exercise caution when drawing on the mammalian toxicology literature in designing studies addressing the mechanisms of GSH regulation and detoxification of aquatic contaminants in fish.

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