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EFFECT OF DIETHYLMALEATE AND OTHER GLUTATHIONE DEPLETORS ON PROTEIN SYNTHESIS*

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Abstract—The α,β-unsaturated carbonyl compound diethylmaleate (DEM) depletes glutathione (GSH) from liver and other tissues, and for this reason it is often used in toxicological research to study the GSH-mediated metabolism of xenobiotics. In addition to GSH depletion, however, DEM has been shown to have other nonspecific effects, such as alteration of monooxygenase activities or glycogen metabolism. In this study we found that DEM (1 ml/kg) inhibited protein synthesis in brain and liver, following *in vivo* administration to mice. Protein synthesis was measured as the incorporation of [^{3}H]valine into trichloroacetic acid-precipitable material. Administration of DEM also decreased body temperature by 2–3°. By increasing the environmental temperature from 22° to 35° the hypothermic effect of DEM was prevented, without affecting its ability to deplete GSH from brain and liver. Furthermore, when mice were maintained at 35°, DEM still caused a significant decrease in protein synthesis, suggesting that this effect was only partially due to hypothermia. To test whether inhibition of protein synthesis was related to GSH depletion, groups of animals were dosed with the α,β-unsaturated carbonyl phorone (diisopropylidenacetone) or the specific inhibitor of GSH synthesis, buthionine sulfoximine (BSO). Phorone decreased GSH in liver and brain; however, it had no effect on protein synthesis. BSO decreased GSH levels in liver and kidney, but not in brain, and did not have any effect on protein synthesis in any of these tissues, nor did it cause any hypothermia. Furthermore, when hepatic GSH content was decreased by *in vivo* administration of DEM or BSO, there was no inhibition of protein synthesis measured *in vitro*. These results indicate that, at the dose normally used to deplete GSH from various tissues, DEM also exerts an inhibitory effect on protein synthesis, which appears to be only partially due to its hypothermic effect, and is independent from GSH depletion. BSO, which, in our experimental conditions, lacks this and other nonspecific effects, might be a good alternative for studies aimed at characterizing the role of GSH in the metabolism and toxicity of chemicals.

Diethylmaleate (DEM) is an α,β-unsaturated carbonyl compound which depletes glutathione from the liver of rats, mice and hamsters [1–3]. The reaction between DEM and glutathione is both spontaneous and catalyzed by a cytosolic glutathione transferase [1, 3]. Glutathione in erythrocytes, kidney, lung and brain is also depleted by DEM although to a lesser extent than in the liver [2]. For this reason DEM has been widely used in toxicological studies in order to investigate the possible role of glutathione-mediated reactions in the metabolism of various drugs and chemicals either *in vivo* or in isolated hepatocytes [4–7].

The effects of DEM, however, are not restricted to depletion of glutathione. For example, DEM has been shown to affect hepatic microsomal monooxygenase activities [8–11], to stimulate bile flow [12], and to impair glycogen metabolism [13]. Other effects of DEM, such as lipid peroxidation [7, 14] or acute ulcerogenesis [15], may be a consequence of glutathione depletion rather than a separate effect [2].

Buthionine sulfoximine (BSO), a specific inhibitor of gamma-glutamylcysteine synthetase, a key enzyme in glutathione synthesis [16], has been shown to decrease hepatic and renal glutathione without affecting other enzymes involved in xenobiotic biotransformation [17, 18]. For this reason it has been suggested as a preferable glutathione depletor for use in the investigation of mechanisms of xenobiotic-induced toxicities [17, 18].

Two studies [19, 20] reported that DEM is capable of inhibiting protein synthesis in isolated hepatocytes. This effect occurred at concentrations of DEM higher than those required to decrease glutathione levels and was thought to be a separate effect of DEM rather than a consequence of glutathione depletion [19, 20]. In the present study we report that DEM inhibited protein synthesis in mouse brain and liver, following *in vivo* administration. This effect was not related to DEM-induced hypothermia and did not appear to be a consequence of glutathione depletion. On the other hand, BSO was capable of depleting GSH without having any effect on protein synthesis or body temperature.

MATERIALS AND METHODS

Animals and treatments. Male Swiss–Webster mice (25–35 g; Tyler Laboratories, Bellevue, WA) were housed five per cage under standard laboratory conditions and had free access to food (Purina Lab Chow) and water. DEM was injected i.p. at a dose of 1 ml/kg. BSO was dissolved in distilled water

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alkalinized to pH 8.5 with 0.1 N NaOH and injected i.p. at a dose of 4 or 8 mmoles/kg. Phorone (diisopropylidenacetone) was dissolved in corn oil and administered i.p. at a 250 mg/kg dose. [^{3}H]Valine was administered 1 hr after DEM or phorone, or 2 hr after BSO. Animals were kept either at room temperature (22 ± 1°) or at 35 ± 1°.

Protein synthesis. For estimation of protein synthesis, we measured the incorporation of [^{3}H]valine into trichloroacetic acid (TCA)-precipitable material, following the methods of Dunlop *et al.* [21] and Reith *et al.* [22] with slight modifications. Mice were injected i.p. with a 500 mM solution of L-valine containing 10 μCi/ml of [^{3}H]valine. Injection volume was 20 ml/kg. Preliminary experiments indicated that incorporation of [^{3}H]valine was linear for 120 min (data not shown), and a 1-hr time-point was selected for all experiments. One hour following administration of valine, mice were decapitated, and their brain and liver were rapidly removed on ice and weighed. Tissues were homogenized in 9 vol. of 10% TCA with a Teflon–glass homogenizer. An aliquot of the homogenate was transferred to centrifuge tubes and centrifuged at 20,000 *g* for 10 min. An aliquot of the supernatant fraction containing free valine and its metabolites was counted for radioactivity. No significant differences between control and treated animals were found (data not shown). The pellet was washed three times by resuspension and centrifugation with 3 ml of 10% TCA. Three milliliters of 0.6 N NaOH was then added and the tubes were incubated for 3 hr at 60° under gentle agitation to dissolve the pellet. A 0.5-ml aliquot (corresponding to 50 mg of original tissue) was then counted in 10 ml of Liquiscint in a Packard Tricarb Scintillation Spectrometer at an efficiency of 57%.

In initial experiments we also compared two washing procedures. In one, samples were washed three times with 10% TCA, then once each with methanol, chloroform–methanol (1:1, v/v) and ether, before dissolving the pellet in 0.6 N NaOH. No significant differences were found between this method and the one described previously in the amount of [^{3}H]valine incorporated into protein (data not shown). Protein synthesis is usually expressed as dpm of [^{3}H]valine incorporated per mg of tissue. Similar results were obtained when data were expressed as dpm per mg of protein (measured by the method of Lowry *et al.* [23]).

In a few experiments, protein synthesis was measured *in vitro*, according to the method of Deutsch *et al.* [24]. Liver and brain of control or treated mice were homogenized in 9 vol. of Jones and McIlwain medium [25] adjusted at pH 7.4 with 95% O_2–5% CO_2, with a Teflon–glass homogenizer. Fifty microliters of homogenate, equivalent to 0.7 to 0.9 mg of protein, was added to 1 ml of incubation medium [25] containing 5 μCi of [^{3}H]valine. Prior to incubation the tubes were flushed with 95% O_2–5% CO_2 and sealed. After 60 min at 37°, the incubation was terminated by placing the tubes on ice and adding 15 μl of bovine serum albumin (10 μg/ml), 50 μl of L-valine (50 mg/ml) and 7 μl of beta-mercaptoethanol. After standing 15 min at 0°, each sample was treated with 0.5 ml of an ice-cold mixture of valine and TCA (30% TCA–0.3 M L-valine). Following centrifugation at 20,000 *g* for 10 min, the resulting pellet was washed three times with 10% TCA and dissolved in 1.5 ml of 0.6 N NaOH before counting in 10 ml Liquiscint. In these experiments, a blank group was used, in which the homogenate was boiled for 15 min before incubation. The counts present in this boiled tissue blank, presumably representing nonspecific binding of [^{3}H]valine, were subtracted from the control values. Cycloheximide was also used as a control. At a concentration of 10^{-5} M, it inhibited protein synthesis by 60–70%.

Tissue non-protein sulfhydryls (NPSH). Tissue NPSH were determined by a modification of the method of Ellman [26] as described by Benke *et al.* [27]. Twenty percent tissue homogenates, prepared in 5% trichloroacetic acid in 10^{-3} M NaEDTA, were centrifuged at 2000 *g* for 10 min (liver) or 20 min (brain and kidney).

A 0.2-ml aliquot of each supernatant fraction was transferred to another tube containing 4.75 ml of 0.1 M sodium phosphate buffer (pH 8.0), and 0.05 ml of 10^{-2} M 5,5-dithiobis-2-nitrobenzoic acid was added. The absorbances were read at 412 nm, and NPSH concentrations were calculated from a standard curve of reduced glutathione.

Colonic temperature. Taken as an index of body temperature, colonic temperature was measured by a thermistor mounted in a rectal probe connected to a Telehermometer (Yellow Springs Instrument Co., Yellow Springs, OH), as previously described [28]. The flexible thermistor probe was inserted 25 mm deep into the rectum. The average of two measurements taken during an interval of 30 min before treatment was considered as the initial temperature at 0 time. During temperature measurements, mice were kept in a plastic restrainer and the probe was retained in the rectum until a constant temperature reading was obtained.

Analysis of data. Data were analyzed for statistical significance by Student's *t*-test.

Materials. Diethylmaleate (practical grade) was purchased from the Eastman Kodak Co. (Rochester, NY). L-Buthionine-*S*,*R*-sulfoximine (BSO) was obtained from the Chemical Dynamics Corp. (South Plainfield, NJ) and phorone (diisopropylidenacetone) was purchased from the Aldrich Chemical Co. (Milwaukee, WI). [^{3}H]L-Valine (54.5 Ci/mmole) was purchased from New England Nuclear (Boston, MA). Liquiscint was purchased from National Diagnostic (Somerville, NJ). All other chemicals were from the Sigma Chemical Co. (St. Louis, MO).

RESULTS

Administration of DEM to mice at a dose of 1 ml/kg decreased NPSH levels in brain and liver by 40 and 81% respectively (Table 1 and Fig. 1). The same dose of DEM also caused a significant decrease of protein synthesis in both tissues (Table 1). Since a decrease in body temperature is known to cause alterations in protein synthesis [29], we controlled for a possible hypothermic effect of DEM. As shown in Table 2, when animals were kept at normal laboratory temperature (22°), colonic temperature was decreased 2.8° following administration of DEM. On the other hand, when mice were kept at 35° for the

Table 1. Effect of DEM on protein synthesis and NPSH levels

Treatment	Protein synthesis (dpm/mg tissue)		NPSH levels (μmoles/g tissue)	
	Brain	Liver	Brain	Liver
Control	11.3 ± 1.17	45.6 ± 3.80	2.82 ± 0.06	9.12 ± 0.32
DEM	6.6 ± 0.56*	29.5 ± 4.99*	1.69 ± 0.07†	1.73 ± 0.09†
% of Control	(58.4)	(64.7)	(60.0)	(18.9)

Mice were injected i.p. with distilled water or DEM (1 ml/kg). [^{3}H]Valine was injected 1 hr after DEM or water, and animals were killed 1 hr after valine administration. NPSH levels were measured in two groups of similarly treated mice, 1 hr after administration of water or DEM. Results are the mean ± S.E.M. of six mice.

* Significantly different from control, $P < 0.05$.

† Significantly different from control, $P < 0.01$.

Table 2. Effect of environmental temperature on DEM-induced hypothermia and NPSH depletion

Treatment	NPSH levels (μmoles/g tissue)				Colonic temperature (°C)	
	22°		35°			
	Brain	Liver	Brain	Liver	22°	35°
Control	2.46 ± 0.07	8.82 ± 0.19	3.11 ± 0.25	9.12 ± 0.13	37.3 ± 0.2	37.3 ± 0.1
DEM	1.48 ± 0.25*	0.79 ± 0.04*	1.95 ± 0.25*	0.63 ± 0.10*	34.5 ± 0.3*	36.9 ± 0.1
% of Control	(60.2)	(9.0)	(62.7)	(6.9)		

Mice were kept at 22° or at 35° for at least 2 hr to allow temperature to stabilize. Colonic temperature was measured before the administration of distilled water or DEM (1 ml/kg, i.p.). After the injection animals were kept for 1 hr at 22° or at 35°. After a second temperature measurement mice were killed for NPSH assays. Results are the mean ± S.E.M. of six mice.

* Significantly different from controls, $P < 0.01$.

whole duration of the experiments, the hypothermic effect of DEM was prevented (Table 2). Since glutathione is related to the biosynthesis of prostaglandins, which are thought to be involved in temperature control [30], and it appears to be involved in the response of cells to thermal stimulation [31], we first investigated whether there was a correlation between hypothermia and NPSH depletion. However, as shown in Table 2, DEM decreased NPSH content to the same extent at both environmental temperatures, suggesting that these two (i.e. hypothermia and glutathione depletion) effects are not related.

We then conducted experiments to determine whether DEM would inhibit protein synthesis when animals were kept at 35°. Table 3 shows that even at this higher environmental temperature, DEM significantly decreased protein synthesis in brain and liver, although to a less extent than at 22°. The difference between inhibition of protein synthesis by DEM at 22° and 35° represents the effect due to its hypothermic action; the decreased body temperature induced by DEM caused a decrease of protein synthesis of about 6% per °C of hypothermia, close to the 8% decrease in amino acid incorporation per each °C of hypothermia previously reported [32].

Table 3. Effect of DEM on protein synthesis at different environmental temperatures

Treatment	Protein synthesis (dpm/mg tissue)			
	Brain		Liver	
	22°	35°	22°	35°
Control	9.9 ± 0.36	11.2 ± 0.28	38.1 ± 0.85	36.5 ± 0.63
DEM	6.2 ± 0.40*	8.5 ± 0.10*	24.4 ± 1.40*	28.0 ± 2.35*
% of Control	(62.6)	(75.9)	(64.0)	(76.7)

Mice were administered distilled water or DEM (1 ml/kg). [^{3}H]Valine was injected 1 hr after DEM, and animals were killed 1 hr after valine administration. Mice were kept at 22° or 35° for at least 2 hr before injection of DEM or water, and for the whole duration of the experiment. Colonic temperature decreased by 2.5 ± 0.2° ($P < 0.05$) and by 0.2 ± 0.05° in mice kept at 22° and 35° respectively. Results are the mean ± S.E.M. of six mice.

* Significantly different from control, $P < 0.05$.

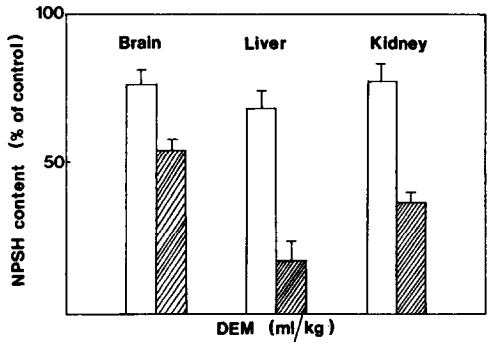

Fig. 1. Effect of DEM on NPSH levels in brain, liver and kidney. DEM was administered by i.p. injection at a dose of 0.25 ml/kg (white bars) or 1 ml/kg (hatched bars), and mice were killed 1 hr after administration. NPSH levels in control tissues were (μmoles/g tissue): brain, 2.22 ± 0.06; liver, 9.12 ± 0.32; and kidney, 5.16 ± 0.31. Results are the mean (± S.E.M.) of five mice. All the values (raw data) were significantly different from control, $P < 0.05$.

Since these results suggested that DEM is capable of decreasing protein synthesis and NPSH content independently from the environmental temperature, we designed a series of experiments aimed at determining whether inhibition of protein synthesis might be a consequence of NPSH depletion. For this purpose we investigated the effects of two other known glutathione depletors, phorone and buthionine sulfoximine (BSO). Phorone is an α,β-unsaturated carbonyl compound similar to DEM [3], while BSO decreases GSH levels by inhibiting gamma-glutamylcysteine, a key enzyme in the synthesis and maintenance of cellular glutathione levels [16].

Administration of phorone (250 mg/kg, i.p.) caused a 2.5 ± 0.6° ($N = 5$, $P < 0.01$) decrease in body temperature, which could be prevented by keeping the animals at 35°. At this environmental temperature, phorone decreased NPSH levels in liver by 65 ± 7% and in brain by 18 ± 4% ($N = 5$, $P < 0.01$). However, protein synthesis was not affected by phorone. Incorporation of [^{3}H]valine (in dpm/mg tissue) was 35.3 ± 1.72 versus 32.7 ± 0.67 in liver and 7.6 ± 0.57 versus 7.2 ± 0.27 in brain of control and phorone-treated mice respectively ($N = 5$).

A second series of experiments investigated the effects of BSO on protein synthesis, NPSH depletion, and colonic temperature. The results of these studies are shown in Table 4. Administration of 4 mmoles/kg BSO decreased NPSH levels in liver and kidney, but not in brain, similarly to what was previously reported [33]. BSO did not have any effect on either colonic temperature or protein synthesis in brain and liver. A higher dose of BSO (8 mmoles/kg) caused a further decrease of NPSH content in liver and kidney, but had no effect on protein synthesis in these two organs (Table 4).

In an additional experiment we examined whether pretreatment of mice with either DEM (1 ml/kg) or BSO (8 mmoles/kg) would alter hepatic protein synthesis *in vitro*. Table 5 shows that both compounds depleted NPSH in liver by 95 and 76% respectively; however *in vitro* protein synthesis was

Table 4. Effect of BSO on NPSH content, colonic temperature and protein synthesis after *in vivo* administration

	NPSH content (μmoles/g tissue)			Colonic temperature (°C)	Protein synthesis (dpm/mg tissue)		
	Brain	Liver	Kidney		Brain	Liver	Kidney
Control	2.11 ± 0.21	8.66 ± 0.27	4.00 ± 0.19	36.7 ± 0.2	9.8 ± 0.50	41.3 ± 2.24	
BSO (4 mmoles/kg)	2.00 ± 0.04	3.42 ± 0.27*	1.04 ± 0.08*	36.8 ± 0.2	8.8 ± 0.29	37.1 ± 2.07	
% of Control	(94.8)	(39.5)	(26.0)		(89.8)	(89.8)	
Control	2.10 ± 0.06	6.45 ± 0.42	3.03 ± 0.21	36.9 ± 0.3		37.9 ± 4.21	34.1 ± 4.32
BSO (8 mmoles/kg)	2.13 ± 0.12	1.66 ± 0.77*	0.47 ± 0.04*	36.6 ± 0.3		35.3 ± 6.50	32.9 ± 5.92
% of Control	(102.7)	(25.7)	(15.5)			(93.1)	(96.5)

BSO was dissolved in distilled water and administered i.p. at a dose of 4 or 8 mmoles/kg. Control mice were injected with distilled water (10 ml/kg). Colonic temperature and NPSH content were measured 2 hr after BSO. [^{3}H]Valine was administered 2 hr after BSO, and animals were killed 1 hr after valine. Results are the mean ± S.E.M. of six to ten mice.

* Significantly different from control, $P < 0.01$.

Table 5. Effects of DEM and BSO on hepatic NPSH levels and *in vitro* protein synthesis

	NPSH content (% of control)	P	*In vitro* protein synthesis (% of control)	P
DEM	4.9 ± 0.4	<0.01	93.9 ± 3.3	>0.1
BSO	24.1 ± 4.3	<0.01	100.0 ± 7.3	>0.1

Mice were injected i.p. with 1 ml/kg DEM or 8 mmoles/kg BSO and killed 1 or 2 hr later respectively. Control animals were injected with corn oil (for the DEM experiment) or with H_2O brought to pH 8.5 with 0.01 N NaOH (the vehicle for BSO). Animals were kept at 35° until they were killed. Neither compound altered colonic temperature under these conditions. Results are expressed as percent of control and are the mean ± S.E.M. of five mice. The probabilities that the values would occur by chance were calculated using the raw data, for which 100% NPSH = 6.30 ± 0.33 μmoles/g tissue and 100% protein synthesis = 2166 ± 129 dpm/mg tissue.

not affected in either case. DEM also decreased NPSH in brain by 54% without any effect on *in vitro* protein synthesis (data not shown).

DISCUSSION

The present study shows that DEM inhibited protein synthesis after *in vivo* administration, and presents evidence suggesting that this effect is not related to hypothermia or to glutathione depletion.

When mice were kept at 22°, DEM caused a large decrease of NPSH content in various tissues, significantly lowered colonic temperature, and inhibited brain and liver protein synthesis by 35–42%. The hypothermic effect of DEM could be prevented by increasing the environmental temperature to 35°, and this change had no effect on the ability of DEM to decrease NPSH levels, suggesting that hypothermia is not related to glutathione depletion.

This hypothermic effect, however, could be responsible for the inhibition of protein synthesis observed following administration of DEM. Several chemicals that alter protein synthesis rates have been shown to do so by changing body temperature [29]. For example, the inhibitory effect of chlorpromazine on brain protein synthesis is prevented by increasing the environmental temperature [34], and part of the reduction in protein synthesis observed after exposure to cigarette smoke is due to its hypothermic effect [32]. When mice were kept at 35°, the inhibitory effect of DEM on protein synthesis was reduced but was still significant (Table 3). A recent study found an 8% decrease in amino acid incorporation per each °C of reduction of body temperature [32]. Similarly, in our experiments, we calculated a temperature dependence of protein synthesis of about 6% per °C. Inhibition of protein synthesis by DEM, therefore, does not appear to be only a consequence of its hypothermic effect, since the latter could be prevented without abolishing the effect on protein synthesis.

One question arising from these studies is whether inhibition of protein synthesis is a result of glutathione depletion. Kosower and Kosower [35] reported that adequate glutathione levels are necessary for efficient protein synthesis in a variety of cells and cell-free systems, and that depletion of glutathione and/or addition of GSSG leads to the inhibition of protein synthesis. In particular, GSSG, which can be formed by oxidizing GSH with diamide, is capable of inhibiting initiation and elongation, the major stages of protein synthesis [35]. Recently, Fischer *et al.* [36] reported that acetaminophen inhibition of hepatic protein synthesis correlates well with depletion of glutathione. Other investigators, however, found that, in isolated hepatocytes, glutathione was strongly depleted by concentrations of DEM which did not affect protein synthesis [19, 20], a finding which led them to conclude that there is no link [19] or only a possible indirect link [20] between glutathione depletion and inhibition of protein synthesis.

Our results seem to support the hypothesis that the effect of DEM on protein synthesis is not related to its depletion of glutathione. This conclusion is based primarily on the findings that NPSH could be depleted in other experimental conditions without an alteration of protein synthesis. First, the α,β-carbonyl compound phorone, which is similar to DEM [3], decreased NPSH in both liver and brain and had no effect on protein synthesis. Second, BSO, an inhibitor of glutathione synthesis, decreased NPSH levels in liver and kidney without any effect on protein synthesis. Third, hepatic NPSH could be depleted *in vivo* by either DEM or BSO without altering protein synthesis *in vitro*. Fourth, a lower dose of DEM decreased NPSH levels by 20–30% without affecting protein synthesis (see below and Fig. 1).

On the basis of these observations, our tentative conclusion is that DEM exerts its inhibitory effect on protein synthesis independently from its interaction with glutathione. The molecular mechanism of such effect on protein synthesis, however, remains to be determined.

In addition to inhibition of protein synthesis and hypothermia, reported in this study, DEM has also been shown to impair glycogen metabolism [13] and to alter the activity of P-450-dependent enzymes [8–11]. This suggests that the use of DEM as a specific and selective GSH depletor in toxicological studies should be reconsidered. In *in vitro* experiments, using isolated hepatocytes or other cells, one can use concentrations of DEM which deplete glutathione by about 80%, without any other apparent effect [19, 20, 37]. Goethals *et al.* [19] showed that 0.2 mM

DEM induces a strong GSH depletion, while a more than 4-fold higher concentration (0.9 mM) is needed to affect protein synthesis. *In vivo*, however, administration of doses of DEM less than the commonly used 1 ml/kg results in much less depletion of glutathione. Figure 1 shows that at a dose of 0.25 ml/kg (4-fold less than the dose which inhibited protein synthesis), DEM caused only a 20–30% decrease in NPSH content in brain, kidney and liver. This dose of DEM had no effect on colonic temperature (controls 37.6 ± 0.3° vs DEM-treated 36.8 ± 0.3°, at 22°), nor on protein synthesis in brain (controls 11.4 ± 0.23 vs 9.5 ± 0.41 dpm/mg tissue) or in liver (controls 43.8 ± 1.22 vs DEM-treated 39.5 ± 1.54 dpm/mg tissue; for all data N = 4). To achieve *in vivo* larger decreases in glutathione content, it seems preferable, therefore, to use compounds lacking all or most of the other nonspecific effects of DEM. In particular, the specific inhibitor of GSH synthesis, BSO, appears to be a better candidate in studies aimed at demonstrating a role of GSH in xenobiotic metabolism and toxicity. BSO depletes GSH by 70% or more in liver and kidney, but not in brain [this study; and Refs. 17 and 33], and does not have any effect on body temperature, protein synthesis (this study), cytochrome P-450 levels and a range of cytochrome P-450-dependent and -independent enzyme activities [17].

In conclusion, DEM, at a dose normally used in toxicological studies to deplete glutathione from various tissues, caused a significant inhibition of protein synthesis in brain and liver. This effect was due only in part to hypothermia and did not seem to be a result of glutathione depletion. In our experimental conditions, BSO did not cause hypothermia nor did it inhibit protein synthesis; since it also lacks various other nonspecific effects of DEM [17], it might be a better choice over other glutathione depletors.

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