Biochemical Pharmacology, Vol. 30, pp. 589–594.

0006-2952/81/060589-06 $02.00/0

EFFECTS OF FASTING, BODY WEIGHT, METHYLCELLULOSE, AND CARBOXYMETHYLCELLULOSE ON HEPATIC GLUTATHIONE LEVELS IN MICE AND HAMSTERS

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(*Received* 17 *May* 1980; *accepted* 27 *August* 1980)

Abstract—Male mice fasted overnight had only about half the hepatic reduced glutathione (GSH) concentration of fed mice. In contrast, fasted male hamsters had 68 per cent higher hepatic GSH levels than controls. No strong correlation of hepatic GSH concentration with body weight was found in either species. Single doses of 0.5% (w/v) methylcellulose (10 ml/kg, i.p.) decreased the hepatic GSH concentration 18–36 per cent in mice and hamsters. Multiple oral doses of 0.5% (w/v) methylcellulose (10 ml/kg once per day for 9 days) caused an initial doubling of the GSH level after two doses in hamsters, but after four doses the GSH level returned to normal. Chronic (60-day) oral administration of 0.5% (w/v) sodium carboxymethylcellulose (10 ml/kg twice daily) to hamsters caused a time-dependent depression of hepatic GSH concentration and a 4-fold increase in hepatic aniline hydroxylase activity after 45 days. No changes in the activities of aminopyrine *N*-demethylase and hexobarbital oxidase were found. It was concluded that hepatic glutathione levels in mice and hamsters are volatile and respond to relatively innocuous physiological (fasting, time of day) and pharmacological (methylcellulose, carboxymethylcellulose) stimuli.

The role of glutathione in protecting the liver from toxicity induced by acetaminophen has been studied extensively in several species [1–4]. Acetaminophen in high doses has been shown to cause 70–80 per cent depletion of GSH† from liver prior to inducing cellular necrosis. It has been suggested that a reactive metabolic intermediate of acetaminophen combines with liver GSH. When the GSH concentration is sufficiently reduced, the intermediate binds covalently to liver macromolecules and causes cell death. A similar mechanism is involved in liver toxicity due to other compounds, including bromobenzene [5], phenacetin [6, 7], trichloroethylene [8], and 1,1dichloroethylene [9].

In the course of experiments on the effects of various substances on hepatic GSH levels in mice and hamsters, the influence of fasting, body weight, and dosing with two suspending agents commonly used in pharmacological studies has been determined. Methylcellulose was administered intraperitoneally in acute studies in both species and orally in a subacute (9-day) study in hamsters. Sodium carboxymethylcellulose was administered orally in a chronic (60-day) study in hamsters. Results from these experiments show differences between mice and hamsters, with respect to effects of fasting on hepatic GSH concentrations, and demonstrate moderate changes in hepatic GSH levels under certain conditions of administration of methylcellulose and carboxymethylcellulose.

MATERIALS AND METHODS

Chemicals. Reduced glutathione was obtained from the Nutritional Biochemicals Corp. (Cleveland, OH), *ortho*-phthalicdicarboxaldehyde (*ortho*-phthalaldehyde) from the Aldrich Chemical Co. (Milwaukee, WI), spectral grade methanol from Matheson, Coleman & Bell (Norwood, OH), methylcellulose (Methocel 4000 cps) from the Dow Chemical Co. (Williamsville Center, NY), and sodium carboxymethylcellulose, Type 78MSF, from Hercules Powder (Wilmington, DE). Other chemicals were of reagent grade.

Animals. Outbred male mice [TAC: (SW)Fbr] were purchased from Taconic Farms, Germantown, NY, and maintained in wire-mesh cages (36 × 19 × 17.5 cm high) with free access to food (Purina Formulab Chow, Ralston-Purina Co., Richmond, IN) and water. Outbred male Golden Syrian hamsters [Lak: LVG(Syr)], 90–100 g, were purchased from Charles River Lakeview, Wilmington, MA. For acute and subacute (9-day) experiments, the animals were housed four per cage in open plastic boxes (48 × 26 × 16 cm deep) with free access to food and water as above. For the chronic (60-day) study, hamsters were housed individually in wire-mesh cages as described for mice. All animals were kept at 21° under a lights-on schedule from 6:00 a.m. to 6:00 p.m.

Treatment of animals. Unless otherwise specified, the animals were killed and the liver segments were

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† Abbreviation: GSH, reduced glutathione.

removed between 8:00 a.m. and 10:00 a.m. In acute experiments, animals were either not treated or received 0.5% (w/v) methylcellulose, 10 ml/kg, i.p., 90 min prior to being killed by cervical dislocation (mice) or by stunning and decapitation (hamsters). In the subacute experiment, hamsters received methylcellulose (10 ml/kg) by gavage via a soft rubber stomach tube once daily in the morning for 9 days. Groups of seven animals each were killed 24 hr after doses 2, 4, 7 and 9. In the chronic (60-day) study, hamsters were dosed orally each day at 7:00 a.m. and 3:00 p.m. with 0.5% (w/v) sodium carboxymethylcellulose (10 ml/kg per dose). No dosing was done on the day an animal was killed. Groups of seven animals were killed on days 15, 30 and 45. On day 60, eleven remaining animals were killed.

Glutathione assay. GSH was assayed in liver homogenates by the procedure of Hissin and Hilf [10]. GSH forms a fluorescent adduct with *ortho*-phthalaldehyde at pH 8.0. Assays were generally performed on fresh samples immediately after the animals were killed, but it was found that storage for at least 5 days at −20° did not decrease GSH levels. Liver segments weighing approximately 250 mg were minced with a scalpel. For every 250 mg, 3.75 ml of 0.1 mM sodium phosphate–5 mM EDTA buffer (pH 8.0 at 22°) and 1 ml of 25% (w/v) metaphosphoric acid were added. The tissue was homogenized with three 3-sec applications of a Polytron homogenizer, using a PT 10 ST probe generator (Brinkman Instruments, New York, NY). Entire homogenates were transferred to chilled, 8.8-ml polycarbonate centrifuge bottles (catalog No. 338574, Beckman Instruments, Wakefield, MA) and centrifuged for 30 min at 100,000 *g* (40,000 rpm, Beckman Type 50Ti rotor). Supernatant fluids were diluted 1 ml to 10 ml with buffer, and 0.1 ml of each of the resulting dilutions was transferred to another glass tube. To each tube 1.8 ml of buffer was added, followed by 0.1 ml of *ortho*-phthalaldehyde (1 mg/ml in methanol). Fluorescence was read 18 min later at 420 nm with excitation at 350 nm using a model A8106 spectrophotofluorometer (Aminco–Bowman, Silver Spring, MD). For each assay a standard curve with authentic GSH (0.4 to 2 μg) was constructed.

Assays of drug-metabolizing enzymes (chronic study). Six livers, at each interval during the 60-day study, were combined into three samples of two livers each, washed with ice-cold 1.5% (w/v) KCl–20 mM Tris–HCl buffer (pH 7.4 at 22°), and minced with iris scissors. Each minced liver sample was homogenized with 4 vol. (ml/g) of buffer in a glass Potter–Elvehjem homogenizer for 30 sec and then centrifuged at 9000 *g* for 20 min (8600 rpm, Sorvall SS-34 rotor). One ml of the supernatant fluid, equivalent to 200 mg of liver (wet weight), was used in each incubation flask for the determination of aniline hydroxylase, aminopyrine *N*-demethylase, or hexobarbital oxidase activity according to the procedures described by Mazel [11] with minor modifications. The cofactor mixture consisted of NADP (0.65 μmole), glucose-6-phosphate (10 μmoles), nicotinamide (50 μmoles), and $MgCl_2$ (25 μmoles) in 3.0 ml of 0.1 M potassium phosphate buffer (pH 7.4 at 22°). Two ml of the cofactor mixture was used in each incubation mixture for the assay of aniline hydroxylase and 3 ml was used for assay of the other two enzymes. The cofactor mixture used for the determination of aminopyrine *N*-demethylase contained, in addition, semicarbazide (45 μmoles/3 ml). In the hexobarbital oxidase determination, the hexobarbital concentration was 0.75 mg/ml.

Treatment of data. Standard curves for the GSH assay and for the enzyme assays were fitted with straight lines by the method of least squares. GSH concentrations in liver samples, expressed as μg GSH/g wet weight of tissue, and enzyme activity, expressed as shown in Table 4, were calculated from the equations of the standard curves. Group means were compared for differences by a two-tailed *t*-test. Plots of hepatic GSH concentration versus body weight were fitted with straight lines by the method of least squares, and correlations were tested for significance according to Snedecor and Cochran [12].

RESULTS

Effect of fasting and time of killing. Table 1 shows the effects of a 17-hr overnight fast on hepatic GSH levels in male mice and hamsters. Fasted mice had a concentration of GSH 51 per cent lower than unfasted controls (1308 vs 2658 μg/g). In contrast, fasted hamsters had a 68 per cent higher GSH concentration than unfasted animals (3399 vs 2023 μg/g). These differences due to fasting were highly significant. Similar results were found when mice and hamsters were dosed intraperitoneally with 10 ml/kg of 0.5% (w/v) methylcellulose 90 min before being killed. Fasted mice and hamsters, both dosed with methylcellulose, had −45 and +32 per cent changes, respectively, in GSH concentration compared to their unfasted, dosed controls. The influence of time of day at which the animal was killed on hepatic GSH concentration in fasted and unfasted mice was further studied. Both groups of mice showed a significant decrease between 8:00 a.m. and 2:00 p.m. (17–23 hr of fasting), but fed mice exhibited the more dramatic decrease. At 11:00 a.m. and 2:00 p.m. fed mice showed −41 and −36 per cent changes, respectively, from the 8:00 a.m. values. Fasted mice, which started out at 8:00 a.m. with markedly less GSH, exhibited only a small (<15 per cent) further loss between 8:00 a.m. and 2:00 p.m.

To determine whether a stress in addition to brief fasting (4.5 hr) could further deplete hepatic GSH in mice, animals were subjected to cold stress (4–6°) for 2 hr. In the experiment of Table 2, half of each group received 0.5% (w/v) methylcellulose (10 ml/kg, i.p.) at the start of the cold stress period. Rather than decreasing hepatic GSH concentration, cold stress significantly increased GSH in the undosed mice. The methylcellulose-treated animals did not show significant differences in hepatic GSH levels as a result of cold stress.

Effects of methylcellulose and sodium carboxymethylcellulose. The data of Table 1 indicated that animals dosed i.p. with methylcellulose had lower hepatic GSH concentrations, regardless of the fasting state. In fasted and fed mice, methylcellulose had decreased GSH 18 and 26 per cent respectively. The latter value represents a significant ($P < 0.001$) decrease. In fasted and fed hamsters, GSH concen-

Table 1. Effects of fasting, time of day, and methylcellulose on hepatic GSH concentrations of mice and hamsters*

Species	Dose of 0.5% (w/v) methylcellulose, i.p. (ml/kg)	Time of day	Fasted		Not fasted		Treatment effect on GSH (% change)	
			Body wt (g)	GSH (μg/g)	Body wt (g)	GSH (μg/g)	Fasting	Dosing
Mouse	0	8:00 a.m.	19 ± 0.2	1308 ± 64.2 (7)	22 ± 0.5	2658 ± 169 (7)	−51†	−18 (fasted)
		11:00 a.m.	18 ± 0.5	1285 ± 53.2 (7)	23 ± 0.5	1565 ± 160‡ (7)	−18	NA§
		2:00 p.m.	18 ± 0.3	1135 ± 45.5‡ (7)	22 ± 0.5	1697 ± 165‡ (7)	−33‖	NA§
	10	8:00 a.m.	21 ± 0.5	1071 ± 31.2 (126)	30 ± 0.8	1963 ± 63.9 (22)	−45†	−26† (fed)
Hamster	0	8:00 a.m.	116 ± 2.6	3399 ± 127 (5)	136 ± 2.6	2023 ± 47.8 (39)	+68†	−36† (fasted)
	10	8:00 a.m.	110 ± 1.6	2164 ± 47.0 (77)	134 ± 3.0	1644 ± 52.7 (36)	+32†	−19† (fed)

* Fasted males were without food (water *ad lib.*) from 3:00 p.m. of the day before the experiment. Dosed animals received methylcellulose, i.p., 90 min before being killed. Values are means ± S.E. for the number of animals shown in parentheses. Treatment effect is expressed as per cent change from the value in the not fasted or not dosed group. Probability levels were determined by Students' two tailed *t*-test.

† Significant, $P < 0.001$.

‡ Significantly lower than the corresponding 8:00 a.m. value, $P < 0.05$.

§ Not applicable.

‖ Significant, $P < 0.01$.

trations in methylcellulose-treated animals were 36 and 19 per cent lower, respectively, than the corresponding control values. Both these decreases were significant ($P < 0.001$). In the cold-stress experiment (Table 2) in which all the mice, whether dosed with methylcellulose or not, had relatively low GSH levels, there was no significant lowering attributable to methylcellulose.

In another experiment, hamsters were dosed with 0.5% (w/v) methylcellulose (10 ml/kg) orally once per day for 9 days. Groups of seven hamsters each were killed 24 hr after doses 2, 4, 7 and 9. In contrast to the decrease seen after a single i.p. dose (Table 1, fed hamsters), a significant ($P < 0.001$) increase was observed in hepatic GSH concentration after two doses (Table 3). In this group the mean GSH level was approximately double the usual value of 2023 μg/g for fed hamsters (Table 1). Measurements after the other doses showed that hepatic GSH levels had returned to normal. Although no undosed control group was available in this study (because the methylcellulose-dosed hamsters were themselves the control group for animals given a compound suspended in the vehicle), the size of the GSH increase seen after two doses supports the idea that this was an effect of methylcellulose.

Carboxymethylcellulose is another frequently used suspending agent for oral administration of compounds to animals. In light of the above effects of methylcellulose on hepatic GSH levels, data on hamsters dosed with carboxymethylcellulose were assembled from an experiment in which these animals were the control group. Hamsters were treated twice daily with 0.5% (w/v) carboxymethylcellulose, 10 ml/kg, p.o., for 60 days. Groups of seven animals were killed on days 15, 30 and 45, and a group of 11 was killed on day 60. The hepatic GSH concentrations for the 15-, 30-, 45- and 60-day treatment groups were, respectively, 1530, 1111, 1395 and 1199 μg/g (Table 4). The 15-day group had significantly higher hepatic GSH concentration than animals dosed longer, but even this GSH level (1530 μg/g) was significantly ($P < 0.001$) depressed from the usual figure of 2023 μg/g (Table 1) seen with fed hamsters. Although these results suggest some depression of hepatic GSH levels, this conclusion must be held tentatively since no group of undosed animals handled in exactly the same manner was available in this study.

In this same experiment, activities of three drug-metabolizing enzymes of liver were measured. Aminopyrine *N*-demethylase and hexobarbital oxidase did not change systematically over the 60 days. Aniline hydroxylase was significantly ($P < 0.01$) elevated by a factor of 4 at days 45 and 60 as compared to day 15 (Table 4). Whether this was due to carboxymethylcellulose is uncertain in the absence of an undosed group of hamsters.

Effects of body weight. Since weights of mice and hamsters used in these experiments varied somewhat (see Tables 1–4), the possible effect of this variable on hepatic GSH concentration was assessed by fitting least squares linear regression lines to plots of GSH versus body weight. For mice segregated according to fasting state, time of being killed, and methylcellulose treatment, only one of eight regressions

Table 2. Effects of cold stress and methylcellulose on hepatic GSH concentration in mice*

Dose of 0.5% (w/v) methylcellulose, i.p. (ml/kg)	Stressed		Not stressed		Treatment effect on GSH (% change)	
	Body wt (g)	GSH (μg/g)	Body wt (g)	GSH (μg/g)	Stress	Dosing
0	19 ± 0.4	1642 ± 155	17 ± 0.7	1102 ± 137	+49†	−25 (stressed)
10	19 ± 0.2	1238 ± 104	19 ± 0.8	1319 ± 55.6	−6	+20 (not stressed)

* Twenty-eight male mice were fasted from 8:30 a.m. At 1:00 p.m. fourteen mice received 0.5% (w/v) methylcellulose, 10 ml/kg, i.p. Half of each group was placed at 4–6° for 2 hr. At 3:00 p.m. all mice were killed. Body weights were determined at 8:30 a.m. Values are means ± S.E. (seven mice/group).
† Significant, $P < 0.05$.

was significant ($P < 0.01$). Results for this group, which was fasted, dosed, and killed in the morning, showed an increase of 23 μg/g in hepatic GSH concentration with a gram increase in body weight between 15 and 45 g (Fig. 1). For hamsters, only one of four groups exhibited a significant ($P < 0.01$) cor-

Table 3. Effect of subacute oral administration of methylcellulose on hepatic GSH concentration in hamsters*

Number of daily doses	GSH (μg/g)
2	4333 ± 187†
4	2216 ± 109
7	1898 ± 109
9	1916 ± 172

* Male hamsters (99 ± 0.86 g) were given daily oral doses, 10 ml/kg, of 0.5% (w/v) methylcellulose. Groups of seven were killed between 8:00 and 9:00 a.m. 24 hr after the indicated number of doses. Values are means ± S.E.
† Significantly higher than other values, $P < 0.001$.

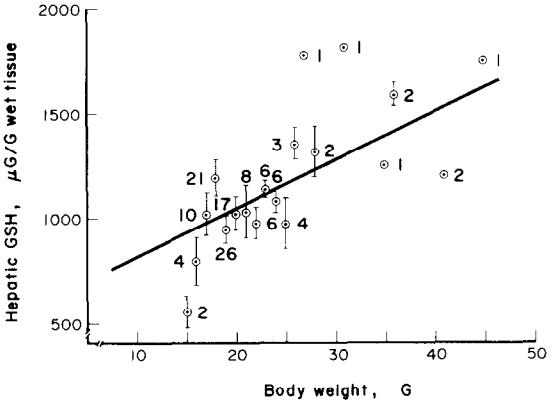

Fig. 1. Correlation of body weight and hepatic GSH in male mice. Mice were fasted 14–16 hr before administration of 10 ml/kg, i.p., of 0.5% (w/v) methylcellulose between 8:00 and 10:00 a.m. Upon killing the animals 90 min after dosing, livers were removed for GSH determination as described in Materials and Methods. Data are means with S.E. bars for the number of mice shown next to the points (126 total). The least squares regression line, GSH = 22.8 × body weight + 586 ($r = 0.356$), is drawn.

Table 4. Effects of chronic oral administration of sodium carboxymethylcellulose to hamsters on GSH concentration and drug-metabolizing enzyme activities of liver*

Days treated	Number of animals	Mean body wt (g)	Mean liver wt (g)	GSH (μg/g)	Enzyme activity		
					Aminopyrine N-demethylase	Aniline hydroxylase	Hexobarbital oxidase
15	7	107 ± 2.46	4.60 ± 0.281	1530 ± 78.6	2.66 ± 0.08	76.4 ± 12.2	ND†
30	7	118 ± 2.34	4.93 ± 0.237	1111 ± 25.1‡	2.98 ± 0.18	25.6 ± 41.6	229 ± 20.8
45	7	123 ± 3.10	4.93 ± 0.208	1395 ± 86.9	2.00 ± 0.21	411 ± 45.1§	118 ± 75.0
60	11	138 ± 3.22	5.43 ± 0.271	1199 ± 54.0‡	2.73 ± 0.24	373 ± 10.2§	175 ± 67.1

* Male hamsters (initial mean body weight = 99 g) were given oral doses of 0.5% (w/v) sodium carboxymethylcellulose (10 ml/kg per dose) twice daily at 7:00 a.m. and 2:00 p.m. for 60 days or until killed. Hepatic GSH and enzyme activities were determined as described in Materials and Methods. Values are means ± S.E. for either the number of animals shown (GSH) or for determinations on three homogenates of two livers each (enzyme activities) as described in Materials and Methods. Enzyme activities are expressed as follows: aminopyrine N-demethylase = μmoles HCHO/g per 30 min; aniline hydroxylase = nmoles p-aminophenol/g per 20 min; and hexobarbital oxidase = nmoles hexobarbital/g per 30 min.
† Not determined.
‡ Significantly different from day 15 value, $P < 0.001$.
§ Significantly different from day 15 value, $P < 0.01$.

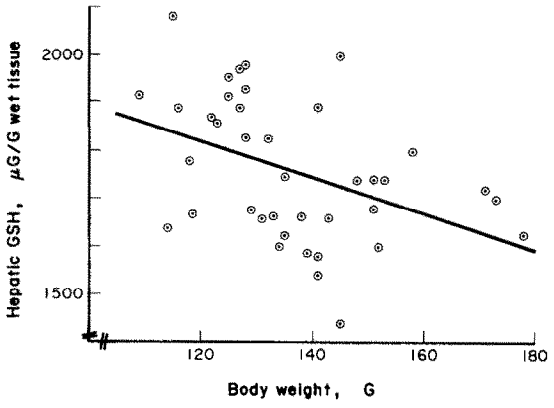

Fig. 2. Correlation of body weight and hepatic GSH concentration in male hamsters. Hamsters were neither fasted nor dosed with methylcellulose before being killed (between 8:00 and 10:00 a.m.). Livers were removed for GSH determination as described in Materials and Methods. Results from thirty-nine individual hamsters are shown. The least squares regression line, GSH = $-7.67 \times$ body weight + 3070 ($r = -0.417$), is shown.

relation. These hamsters were neither fasted nor dosed and, in contrast to the results with mice (Fig. 1), showed a slight 7.67 μg/g decrease in hepatic GSH level with a gram increase in body weight between 99 and 178 g (Fig. 2).

DISCUSSION

It is known that hepatic GSH concentration undergoes diurnal variation in rats [4, 9] and mice [13]. Jaeger *et al.* [9] found that fed rats show a maximum hepatic GSH level between 7:00 a.m. and 1:00 p.m., with a minimum between 7:00 p.m. and 1:00 a.m., and a similar pattern for mice was reported by Beck *et al.* [13]. Fasted rats exhibited a diurnal variation with decreased magnitude. Maruyama *et al.* [14] have shown that fasting causes significant reduction in hepatic GSH of rats. Results presented here support these findings on diurnal variation of hepatic GSH for mice.

The effect of fasting on hamsters was to increase morning levels of hepatic GSH. This is the opposite of what is seen in mice (this study) and rats [14]. This difference may be related to the differences in level and timing of nocturnal activity, including feeding, in hamsters as compared to rats and mice.

Beck and Linkenheimer [15] reported that exposure of male mice to cold (5 hr at 5–10°) decreased hepatic GSH to about half the control value. A later report [13] questioned whether the observed decrease was an effect of cold stress or reflected diurnal variation, since control mice were not killed at the same time as stressed mice. Results in Table 2 show no significant decrease in hepatic GSH of mice stressed for 2 hr at 4–6° when compared with control mice killed at the same time of day. In fact, in mice not treated with methylcellulose a brief cold stress increased hepatic GSH concentration by 49 per cent. The mechanism for this increase and its suppression by methylcellulose is unknown.

Davis *et al.* [2] have reported that hamsters are more susceptible than mice to the hepatotoxic effects of intraperitoneally administered acetaminophen. Cellular necrosis was correlated with depletion of GSH from liver. In view of the different relative morning levels of GSH—low in fed hamsters, high in fed mice—it may be that acetaminophen sensitivity was less a reflection of intrinsic species differences than of time of sampling.

Effects of methylcellulose and sodium carboxymethylcellulose on man and laboratory animals after acute administration have been reviewed [16]. Virtually no changes attributable to these agents have been identified, and no liver toxicity has been found. More recently some evidence of biochemical effects in rats has been reported [17, 18]. When female rats were given methylcellulose ($2 \times 20\,mg \cdot kg^{-1} \cdot day^{-1}$) or carboxymethylcellulose ($2 \times 10\,mg \cdot kg^{-1} \cdot day^{-1}$) orally for 4 weeks, 5 days per week, a moderate degree of uncoupling of oxidative phosphorylation in heart and liver mitochronria was observed. Carboxymethylcellulose, but not methylcellulose, caused a moderate and transient inhibition of mixed function oxidase activity in 9000 *g* supernatant fluids of liver. It was concluded that these common suspending agents could affect certain liver enzymes. These findings are consistent with the effects of methylcellulose on hepatic GSH and of carboxymethylcellulose on GSH and on aniline hydroxylase activity reported here.

It must be emphasized, however, that these results do not in any way indicate that methylcellulose and carboxymethylcellulose are toxic. The magnitude of the changes in GSH levels caused by these agents is small. Indeed, simple fasting and diurnal variation produce equal or greater changes. But the fact that these two agents can mildly decrease hepatic GSH levels under certain circumstances should be recognized, since these effects could aid interpretation of studies of hepatotrophic chemicals. Thus, a mild (<70 per cent) depleter of hepatic GSH may cause no hepatotoxicity by itself, but it could do so if administered in a methylcellulose or carboxymethylcellulose vehicle due to an additive effect of chemical and vehicle. It would be incorrect to classify such a mild depleter as a hepatotoxin working via GSH depletion. It might have no significant liver toxicity at all, just as methylcellulose and carboxymethylcellulose have no liver toxicity [16].

Neither mice nor hamsters showed a marked dependence of hepatic GSH on body weight. This is consistent with the work of Lambert and Thorgeirsson [19] showing that hepatic GSH concentration in mice increases from birth until day 10 and then levels off. The slight correlations observed in the present study were in the opposite directions for the two species. It may be concluded that body weight differences do not explain the changes in hepatic GSH levels attributed to fasting, time of killing, or dosing with methylcellulose and carboxymethylcellulose.

Acknowledgements—We thank Mr. Gordon Rose and Ms. Cheryl Warren for assistance in the GSH experiments, and Ms. Christine Tinkham for the determination of liver enzyme activities. Drs. Marvin M. Goldenberg and Alan F. Moore kindly reviewed the manuscript.

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