Glutathione transferases (GSTs) are a multigene family of phase II drug-metabolizing enzymes that catalyze the conjugation of glutathione with a range of α-haloacids, including dichloroacetic acid (DCA), and the penultimate step in the tyrosine degradation pathway. DCA is a rodenticide and a common drinking water contaminant. DCA also causes multiorgan toxicity in rodents and dogs. The objective of this study was to determine the expression and activities of GSTZ1–1 in rat tissues with maleylacetone and chlorofluoroacetic acid as substrates. GSTZ1–1 protein was detected in most tissues by immunoblot analysis after immunoprecipitation of GSTZ1–1 and by immunohistochemical analysis; intense staining was observed in the liver, testis, and prostate; moderate staining was observed in the brain, heart, pancreatic islets, adrenal medulla, and the epithelial lining of the gastrointestinal tract, airways, and bladder; and sparse staining was observed in the renal juxtaglomerular regions, skeletal muscle, and peripheral nerve tissue. These patterns of expression corresponded to GSTZ1–1 activities in the different tissues with maleylacetone and chlorofluoroacetic acid as substrates. Specific activities ranged from 0.09 ± 0.01 (kidney) nmol/min/mg of protein with maleylacetone as substrate and from 4.6 ± 0.89 (liver) to 17 (liver) to 1.1 ± 0.4 (muscle) nmol/min/mg of protein with maleylacetone as substrate and from 4.6 ± 0.89 (liver) to 0.09 ± 0.01 (kidney) nmol/min/mg of protein with chlorofluoroacetic acid as substrate. Rats given DCA had reduced amounts of immunoreactive GSTZ1–1 protein and activities of GSTZ1–1 in most tissues, especially in the liver. These findings indicate that the DCA-induced inactivation of GSTZ1–1 in different tissues may result in multiorgan disorders that may be associated with perturbed tyrosine metabolism.

This research was supported in part by the University of Rochester Wilmot Cancer Research Fellowship Program (H.B.M.L.) and by National Institute of Environmental Health Sciences Grant ES03127 (M.W.A.).

1 Abbreviations used are: GST, glutathione transferases; GSTZ, glutathione transferase zeta; DCA, dichloroacetic acid; CFA, chlorofluoroacetic acid; MA, maleylacetone; GSTA, glutathione transferase alpha; GSTP, glutathione transferase mu; GSTT, glutathione transferase theta; GSTO, glutathione transferase omega; GA, fumarylacetone; CHAPS, 3-[3-cholamidopropyl(dimethylammonio)-propanesulfonate.

Address correspondence to: M. W. Anders, Department of Pharmacology and Physiology, University of Rochester Medical Center, 601 Elmwood Avenue, Box 711, Rochester, NY 14642. E-mail: mw_anders@urmc.rochester.edu
Humans are exposed to DCA via environmental and medical sources. DCA is a by-product of the chlorination of drinking water, and humans may consume 0.1 to 3 μg of DCA/kg/day (Uden and Miller, 1983; Weisel et al., 1998). DCA is also a metabolite of trichloroethylene and chloral hydrate; trichloroethylene is found in industrial solvents and degreasing agents to which humans are exposed, and chloral hydrate is a sedative (Henderson et al., 1997; Merdink et al., 1998). DCA is also used in the clinical management of congenital lactic acidosis (Stacpoole et al., 1983, 1998).

DCA is teratogenic in rats and in mouse embryos (Smith et al., 1992; Hunter et al., 1996). DCA-induced toxicities, including peripheral neuropathies, testicular atrophy (Katz et al., 1981; Toth et al., 1992; Linder et al., 1994), and hepatocellular carcinomas (Bull et al., 1990; Nelson et al., 1990; DeAngelo et al., 1991; Carter et al., 1995), are observed in rats, mice, and dogs. Rats are more susceptible than mice to DCA-induced hepatocellular carcinomas, and male rats are more susceptible than female rats (Richmond et al., 1995; DeAngelo et al., 1996; Pereira, 1996).

The mechanism by which DCA exerts its toxicity has not been elucidated. Studies on the biotransformation of DCA by GSTZ1–1 show that DCA is a mechanism-based inactivator of GSTZ1–1 (Tzeng et al., 2000). Moreover, GSTZ1–1 activities and immunoreactive GSTZ1–1 protein concentrations are reduced in hepatic cytosolic fractions from rats given DCA for 5 days (Anderson et al., 1999). The DCA-induced inactivation of GSTZ1–1 perturbs tyrosine metabolism in rats (Cornett et al., 1999), and these perturbations may be associated with the multiorgan toxicity of DCA. The multiorgan toxicity of DCA also indicates that GSTZ1–1 may be expressed in different organs. Northern blot analyses of mouse and human tissue samples show that GSTZ1–1 is expressed in multiple tissues (Board et al., 1997; Fernández-Cañón et al., 1999). Protein expression and activities of GSTZ1–1 in rat tissues have not been determined.

The objective of this study was to determine the subcellular localization of GSTZ1–1 by immunohistochemistry and to quantify the activities of GSTZ1–1 in different rat tissues with MA and CFA as substrates. The patterns of expression of GSTZ1–1 were also compared with those of other GSTs.

Materials and Methods

Immunohistochemistry. Male, Fischer 344 rats (175–200 g; Charles River Laboratories, Inc., Wilmington, MA) were anesthetized with ether and then sacrificed. Organs were removed, fixed in 10% (v/v) neutralized formalin (J. T. Baker, Phillipsburg, NJ), and embedded in paraffin. Two 5-μm serial sections were cut for each tissue and mounted on Superfrost Plus slides (VWR Scientific Products, West Chester, PA) to provide a negative control adjacent to each antibody-treated section.

Slides were stained by the avidin/biotinylated enzyme complex method with standard methods (Otieno et al., 1997). Briefly, serial paraffin-embedded tissues sections were mounted on slides, deparaffinized, and hydrated with an ethanol gradient (100–30%). The endogenous peroxidase activities in the tissues were then quenched, and antigen retrieval was performed prior to permeabilization and blocking by incubating the tissue with 0.5% Triton X-100 and 20% avidin D in normal goat serum in a humid chamber for 30 min at room temperature. On each slide, one tissue section was incubated with polyclonal anti-GSTZ1–1 antibodies (1:500 dilution) or without primary anti-body and stained with the reagents in the avidin/biotin and Vectastain Elite ABC kits (Vector Laboratories, Burlingame, CA) according to the manufacturer’s instructions. Each slide was counterstained with Mayer hematoxylin stain (VWR Scientific Products) before mounting in an aqueous mounting medium. The slides were coded before interpretation by the pathologist.

GSTZ1–1 Activity Assays and Immunoblotting Studies. Male, Fischer 344 rats (175–200 g; Charles River Laboratories, Inc.) were housed in metabolic cages and provided with double-distilled water and Purina rodent chow ad libitum (Purina, St. Louis, MO). The rats were injected i.p. once daily for 5 days with normal saline or with 1.2 mmol/kg DCA (Aldrich Chemical Co., Milwaukee, WI) or CFA, prepared as described previously (Tong et al., 1999b), dissolved in normal saline, and brought to pH 7.4 with NaOH. Twenty-four hours after giving the last dose of DCA or CFA, the rats were anesthetized with ether and then sacrificed; organs were removed and placed in ice-cold 20 mM potassium phosphate buffer (pH 7.4) containing 2 mM EDTA, 2 mM DTT, 100 μM phenylmethylsulfonyl fluoride (Sigma-Aldrich, St. Louis, MO), and 1.15% KCl. The tissues were homogenized, and the homogenates were centrifuged at 3,000 rpm (700g) for 15 min. The supernatant fractions were dialyzed overnight in 30 volumes of the homogenization buffer that lacked KCl and were stored at −80°C until used.

Immunoblotting Analyses. Whole tissue supernatants were diluted in phosphate-buffered saline containing 0.5% CHAPS (3-[3-cholamidopropyl]-dimethylammonio)-propanesulfonate; Sigma-Aldrich) buffer to solubilize the membranes. Polyclonal anti-GSTZ1–1 antibodies (1:1,000 dilution), prepared as described previously (Board et al., 1997; Tong et al., 1999a), were incubated with 250 μg of total protein overnight at 4°C on a shaker. Protein A-Sepharose beads (Sigma-Aldrich) were added to the mixture (10% v/v), which was incubated for 2 h at 4°C with shaking. The mixture was centrifuged at 10,000 rpm for 2 min at 4°C. The centrifugates were suspended and centrifuged twice in 200 μl of 0.5% CHAPS buffer to remove unbound protein; 60 μl of Laemmli’s sample buffer (Bio-Rad Laboratories, Hercules CA) containing 2% β-mercaptoethanol were added to the precipitates. The samples were loaded (20–40 μl per lane) on 15% polyacrylamide gels and separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Mini Protein gel apparatus; Bio-Rad Laboratories). Proteins were transferred to 0.2-μm nitrocellulose membranes with a semidry transfer apparatus (Bio-Rad Laboratories), and the membranes were soaked in blocking solution (10% [w/v] nonfat dry milk (Bio-Rad Laboratories) in 20 mM Tris HCl, 150 mM NaCl, and 0.15% [w/v] Tween 20 [pH 7.4]) overnight and then incubated with polyclonal anti-GSTZ1–1 antibodies (diluted 1:5,000) for 10 h. The membranes were rinsed with blocking solution and then incubated with horseradish peroxidase conjugated with mouse anti-rabbit IgG (Bio-Rad Laboratories) diluted 1:5,000 in blocking buffer lacking milk for 2 h at room temperature. The membranes were rinsed again in blocking buffer lacking milk and incubated with the enhanced chemiluminescent substrate for detection of horseradish peroxidase (SuperSignal West Pico Chemiluminescent substrate kit; Pierce Chemical Co., Rockford, IL) following manufacturer’s instructions. Kodak X-OMAT films (Eastman Kodak Co., Rochester, NY) were exposed to the membranes and developed by standard procedures.

Activities with Maleylacetone as Substrate. The isomerase activities in homogenates of the different tissues were determined by measuring the rate of formation of FA from MA (Fowler and Seltzer, 1970). Reaction mixtures contained protein (10–80 μg), MA (1 mM), and glutathione (1 mM) in a final volume of 0.5 ml of 0.01 M potassium phosphate buffer (pH 7.4). The reaction mixtures were incubated for 5 min at 37°C; the reactions were initiated by addition of MA and were quenched after 1 to 5 min by addition of 50 μl of concentrated HCl. Fifty microliters of a salicylic acid solution (1.37 mg in 1 ml) was added to each sample as an internal standard. Samples (50 μl) were analyzed on a Hewlett Packard 1090 liquid chromatograph (Hewlett Packard, Mississauga, ON) equipped with a μBondapak C18 column (3.9 mm × 300 mm, 10-μm particle size; Waters Corp., Milford, MA). The column was eluted with a 0 to 30% methanol gradient at a flow rate of 0.75 ml/min over 30 min; solvent A contained 0.075% acetic acid in water, and solvent B contained 0.075% acetic acid and 60% methanol in water. The absorbances of MA and FA in the eluate were monitored with a diode-array detector at 312 nm. Concentrations of FA in the reaction mixtures were quantified with a calibration curve prepared with known concentrations of FA. The retention times of MA and FA were tR = 9.6 min and tR = 22.3 min, respectively. The rate of nonenzymatic conversion of MA to FA (0.027 mmol/min/mg of protein) was determined by analysis of a solution containing MA (1 mM), glutathione (1 mM), and heat-inactivated homogenates in 0.01 M potassium phosphate buffer (pH 7.4) and was subtracted from each sample.

Activities with Chlorofluoroacetic Acid as Substrate. The formation of glyoxylate from CFA was measured spectrophotometrically, as previously described (Tong et al., 1999b). Reaction mixtures contained 150 to 500 μM of homogenate protein, CFA (1 mM), and 1 mM glutathione in a final volume of 1 ml of 0.1 M potassium phosphate buffer (pH 7.4) and were incubated at 37°C.
Statistical Analysis. The activity data (Tables 2 and 3) were analyzed by one-way analysis of variance with Bonferroni’s post-test. A level of $p < 0.05$ was chosen for acceptance or rejection of the null hypothesis. Activities with MA and CFA as substrate were compared with the two-tailed Pearson correlation analysis.

Results and Discussion

Analysis of GSTZ1–1 Expression in Rat Tissues. Polyclonal rabbit anti-hGSTZ1–1 antibodies raised against hGSTZ1–1 recognize the rat ortholog (Board et al., 1997; Tong et al., 1998a). Immunoblot analyses of purified GSTs and 100 $\mu$g whole-liver homogenates showed that these polyclonal anti-GSTZ1–1 antibodies do not cross-react with other GSTs (Board et al., 1997; Tong et al., 1998a). Immunoblot analyses of 100 $\mu$g of total protein of whole homogenates from rat tissues revealed a faint band in the lane loaded with liver homogenate, and no bands were visible in lanes that contained homogenates of heart, testis, brain, kidney, and skeletal muscle tissue (data not shown). To increase the sensitivity of the analysis, GSTZ1–1 was immunoprecipitated from homogenates from liver, heart, testis, brain, kidney, and skeletal muscle to concentrate the antigen and determine the expression of GSTZ1–1 in about 250 $\mu$g of total protein from these tissues. This analysis also permitted the examination of the cross reactivity of polyclonal anti-GSTZ1–1 antibodies with other proteins in extrahepatic tissues.

An immunoreactive protein with an apparent molecular mass of about 25 kDa was readily detected in the liver homogenates, and the protein comigrated with purified hGSTZ1c-1c subunits (Fig. 1). Faint bands were also identified in the lanes loaded with heart, brain, and testis homogenates after prolonged exposure of the film (data not shown). These bands were readily detectable in homogenates of rats given CFA (Fig. 2), but were not detected in homogenates of rats given DCA (data not shown), indicating that these bands corresponded to GSTZ1–1 protein. Two other bands, with apparent molecular masses greater than 25 kDa, were also seen in lanes loaded with heart, testis, and brain homogenates of rats treated with CFA. The antigens that give rise to these bands in these tissues have not been identified. Nonetheless, these blots indicate that the polyclonal anti-GSTZ1–1 antibody is specific for GSTZ1–1 antigens in different tissues. The 50-kDa band seen in all lanes of the immunoblot is the large fragment of anti-GSTZ1–1 antibody IgG, which is expected to be seen in gels of immunoprecipitated proteins blotted with the same antibody.

Immunostaining of GSTZ1–1 in Different Tissues. Immunohistochemical analyses of 16 tissues showed that GSTZ1–1 protein was detected in several rat tissues, but with differing intensities.

Liver. Hepatocytes were intensely stained; the staining was condensed and appeared as intracytoplasmic masses that were localized mostly around the perinuclear membrane (Fig. 3). The central, midzonal, and periportal regions of the hepatic lobules were uniformly stained. The bile duct epithelium and the vascular endothelium were not stained. Nonparenchymal cells that were visible in some fields were also not stained. This pattern of hepatic staining indicates that GSTZ1–1 is expressed only in hepatocytes and is not expressed in other cells present in liver lobules. The intense staining of GSTZ1–1 in the liver reflects the high activities seen in the liver (see below).

Hepatocytes are rich in $\alpha$, $\mu$, and microsomal classes of GSTs, and hepatocytes within the centrilobular region of the liver are more intensely stained for those proteins than are hepatocytes from the midzonal and periportal regions of the lobule (Redick et al., 1982; Sundberg et al., 1993; Otieno et al., 1997; Rowe et al., 1997). GSTP is expressed only in biliary epithelium (Redick et al., 1982). GSTO is expressed in the cytoplasm and nuclei of hepatocytes and cells of the bile duct epithelium (Yin et al., 2001). The pattern of expression of GSTZ1–1 in the liver differed from that of other cytosolic GSTs (Table 1).

Testis. The germ cells in the seminiferous tubules showed intense staining (Fig. 4). The staining appeared to increase in intensity from the basal layers toward the superficial layers, indicating an increased expression of GSTZ1–1 associated with maturation and differentiation of germ cells into spermatozoa. The absence of staining between the germ cells and in the interstitial areas indicated that Sertoli cells and Leydig cells, respectively, did not express GSTZ1–1.

Testes are one of the richest sources of GSTs (Table 1). GSTM isozymes are expressed abundantly in most cellular components of the testis. The expression of GSTA and GSTP and of microsomal GST1 is low in the different cellular components of the testis (Hayes and Mantle, 1986; Terrer et al., 1990; Campbell et al., 1991; Hussey and Hayes, 1993; Sundberg et al., 1993; Gandy et al., 1996; Otieno et al., 1997).
GSTO expression in the testis is weak and limited to Leydig cells (Yin et al., 2001). GSTs in the testis have been postulated to protect germ cells from reactive chemicals and products of oxidative stress (Listowsky et al., 1998). GSTM also has steroid isomerase activities, which are important in the synthesis of testosterone and progesterone (Johansson and Mannervik, 2001).

Cerebrum and cerebellum. A dotted pattern of staining was observed in cross sections of the cerebral and cerebellar cortices (Fig. 5). This staining was associated with the pyramidal cells in the cerebral cortex and the Purkinje cells in the cerebellar cortex, based on their localization and sizes. Little or no staining was observed in other tissues, such as glial cells and the cells lining the choroid plexus and ventricles. GSTZ1–1 was expressed only in some neurons of the central nervous system. This pattern of expression is unique to GSTZ1–1; GSTA and GSTM are expressed mostly in the choroid plexus, ventricular lining, and vascular epithelia; GSTP and GSTO are expressed only in astrocytes or glial cells; and microsomal GST1 is expressed in most regions in the brain (Abramovitz et al., 1988; Carder et al., 1990; Terrier et al., 1990; Campbell et al., 1991; Johnson et al., 1993; Juronen et al., 1996; Otieno et al., 1997; Sherratt et al., 1997; Yin et al., 2001) (Table 1). No staining was observed in a peripheral nerve embedded in skeletal muscle, indicating that GSTZ1–1 may not be expressed in peripheral nerves (Fig. 8B). Only GSTP has been described in Schwann cells of peripheral nerves (Terrier et al., 1990).

Gastrointestinal tract. The epithelial lining of the esophagus, stomach, small intestine, and colon was stained intensely, more so in the superficial layers than in the basal layers (Fig. 6). In the stomach, the parietal cells showed more staining than the chief cells. In the intestines, the cuboidal cells of the villi were stained; sloughed-off cells in the lumen were also stained, whereas mucous-secreting cells were not stained. The interstitial tissues underlying the epithelia were not stained. GSTZ1–1 was, therefore, uniformly expressed in the epithelial lining of the gastrointestinal tract.

GSTP is the most abundantly expressed GST in the epithelial lining of the gastrointestinal tract; GSTA, GSTO, and microsomal GST1 expressions are much lower; and GSTM is absent in the gastrointestinal tract lining (Hayes and Mantle, 1986; Terrier et al., 1990; Campbell et al., 1991; Sundberg et al., 1993; Juronen et al., 1996; Otieno et al., 1997; Sherratt et al., 1997; Yin et al., 2001).

Kidney. Staining was weak throughout the kidney (Fig. 7A). The juxtaglomerular regions of the renal cortex showed the most intense staining. Moderate staining of a subset of proximal tubule epithelial cells was also observed. The staining was more intense in the apical side of the cells (Fig. 7B). Staining was also observed in the lumen of the tubules but was absent in glomerular cells and in the epithelia of the distal convoluted tubules and collecting ducts. No staining was observed in the medulla and renal pelvis. GSTZ1–1 was, therefore, discretely expressed in the proximal tubular epithelial cells.

Other GSTs show distinct patterns of expression in the kidney (Sundberg et al., 1994a,b): proximal tubular cells are rich in GSTA and cells of the distal tubule, loop of Henle, and collecting ducts are rich in GSTP. The expression of GSTM and microsomal GST1 is low and is localized in the cells of the distal tubules and collecting ducts (Hayes and Mantle, 1986; Terrier et al., 1990; Campbell et al., 1991; Rozell et al., 1993; Sundberg et al., 1993; Juronen et al., 1996; Otieno et al., 1997; Sherratt et al., 1997). GSTO is expressed in tubular epithelial cells (Yin et al., 2001). Although the abundant and distinct localization of GSTA and GSTP support their use as biomarkers for determining regioselective renal damage (Sundberg et al., 1994a,b), the low expression of GSTZ1–1 precludes its use as a biomarker for renal damage.

Heart. Staining in the heart was moderate and uniformly distributed in the sarcoplasm of all cardiomyocytes (Fig. 8A). Nuclear staining was not observed. GSTP is highly expressed in both human and rat heart tissue, whereas expression of GSTA and GSTM is low (Hayes and Mantle, 1986; Terrier et al., 1990; Sundberg et al., 1993; Juronen et al., 1996; Sherratt et al., 1997). GSTO is expressed in human cardiac myocytes (Yin et al., 2001). GSTZ1–1, GSTO, and GSTP appear to be the most abundant GSTs in the heart.

Skeletal muscle and vascular smooth muscle. The staining in the skeletal muscle was weak and uniformly distributed in the sarcoplasm (Fig. 8B). Smooth muscle tissue in blood vessels and the intestinal muscularis were intensely stained. GSTZ1–1 was, therefore, expressed in muscle tissue, more so in smooth muscle than skeletal muscle. GSTP, GSTM, and GSTT have been identified in skeletal muscle, and GSTM is the only other GST expressed in smooth muscle (Terrier et al., 1990; Hussey and Hayes, 1993; Sundberg et al., 1993; Juronen et al., 1996; Sherratt et al., 1997).

Adrenal gland. Staining in the adrenal gland was diffuse (Fig. 9, left panel), but was most prominent in the adrenal medulla. Staining of the zona glomerulosa, zona fasciculata, and zona reticularis of the adrenal cortex was weak. In rat adrenals, microsomal GST1 has been detected in the medulla (Otieno et al., 1997). In human adrenals, moderate amounts of GSTA, GSTM, and GSTP are expressed in the cortex, and only GSTP is expressed in the medulla (Terrier et al., 1990; Campbell et al., 1991; Sundberg et al., 1993). The adrenal medulla is the body’s major source of epinephrine, which is a metabolite of tyrosine. A high expression of GSTZ1–1 in the adrenal medulla indicates that tyrosine metabolism to catecholamines and tyrosine degradation to fumaryl-
expression is very low (Campbell et al., 1991; Sundberg et al., 1993; Sherratt et al., 1997). GSTO is expressed in acinar, islet, and ductal cells of the pancreas (Yin et al., 2001).

**Lung.** Staining in the lung tissue was weak and limited to the cuboidal epithelial lining of bronchi and bronchioli (Fig. 10A). Sparse, spotty staining was observed in the alveoli. Other GSTs are also expressed predominantly in the bronchial cuboidal epithelium, and staining decreases progressively and in a distal direction (Terrier et al., 1990; Anttila et al., 1993; Sundberg et al., 1993; Juronen et al., 1996; Otieno et al., 1997; Sherratt et al., 1997; Yin et al., 2001). Little staining is observed in the alveolar Clara cells, and GSTs are not expressed in mucus-secreting goblet cells (Hussey and Hayes, 1986; Anttila et al., 1993). Alveolar macrophages also show weak staining for GSTM and GSTO (Sundberg et al., 1993; Yin et al., 2001).

Cetoacetate are competing pathways for the biotransformation of tyrosine within the adrenals; hence, inactivation of GSTZ1-1 by DCA may affect catecholamine homeostasis.

**Pancreas.** The islets of Langerhans showed strong staining, and the staining appeared to be punctate within the cytoplasm and intense in the perinuclear region of cells (Fig. 9, right panel). In contrast, pancreatic acinar cells were weakly stained, and staining was most prominent in the basolateral region. The epithelial linings of the pancreatic ducts were not stained. The pattern of GSTZ1-1 expression in the pancreas differs from that of other GSTs: GSTP is expressed only in the ductal epithelium, GSTA and microsomal GST1 show moderate staining in acinar and ductal epithelial cells, and GSTM expression is very low (Campbell et al., 1991; Sundberg et al., 1993; Sherratt et al., 1997).
**Bladder.** The transitional epithelium was intensely stained, more so in the superficial layers than in the basal layers (Fig. 10B). Staining in the subepithelial tissue was faint. GSTP is the only other GST that has been identified in the bladder epithelium, and its expression is weak (Terrier et al., 1990; Sundberg et al., 1993).

**Prostate.** The glandular epithelium of the prostate was weakly stained, although intense staining was observed in secretory regions of the prostate gland (Fig. 10C). GSTM, GSTP, and GSTO have also been identified in the prostate, and their expression is observed in the basal layer of the epithelium (Terrier et al., 1990; Sundberg et al., 1993; Sherratt et al., 1997). GSTZ1–1, unlike other GSTs, appears to be secreted by the prostate gland and concentrated in prostatic fluid; hence, its expression in prostatic fluid may be unique among the GSTs.

These immunohistochemical analyses showed that GSTZ1–1 is variably expressed in different tissues. The multiorgan expression of GSTZ1–1 indicates that the degradation of tyrosine and biotransformation of its substrates occurs in a range of tissues other than in the liver and kidney (Knox and Edwards, 1955b; Mitchell et al., 1995).

**Effect of DCA Treatment on GSTZ1–1 Expression.** To determine the effects of DCA treatment on the expression of GSTZ1–1 in different tissues, rats were given DCA (1.2 mmol/kg i.p. for 5 days prior to sacrifice) or CFA (1.2 mmol/kg i.p. for 5 days prior to sacrifice), and tissue sections were examined by immunohistochemistry. Sections from different tissues, notably liver, brain, and testis, of rats given DCA showed sparse staining for GSTZ1–1, whereas the staining on sections from rats given CFA was similar to that of untreated rats (data not shown). These data indicate that DCA reduced the amount of immunoreactive GSTZ1–1 in hepatic and extra-hepatic tissues and corroborate the observations showing that DCA-inactivated GSTZ1–1 is degraded (Anderson et al., 1999) and that CFA is a poor inactivator of GSTZ1–1 (Tzeng et al., 2000).

**GSTZ1–1 Activities with MA and CFA as Substrates in Rat Tissues.** GSTZ1–1 activities with MA and CFA as substrates were determined in whole homogenates of six different tissues. Activities with MA as substrate were much higher than with CFA as substrate, and with both substrates, GSTZ1–1 activities in the liver were much higher than activities in extrahepatic tissues (Tables 2 and 3). Two-tailed Pearson correlation analysis showed a significant correlation between the activities in the different tissues with MA as the substrate compared to those with CFA as the substrate (Pearson r = 0.9931; p = 0.0007). The tissue-dependent differences in activities with both substrates reflected the pattern of expression of GSTZ1–1 observed by immunohistochemistry.

The activities of GSTZ1–1 in different tissues with MA and CFA as substrates were also determined in rats given 1.2 mmol/kg DCA for 5 days. The effects of DCA on GSTZ1–1 activities in different tissues were significant only in the liver compared with extrahepatic tissues: hepatic MA isomerase activity was reduced by 99.1%, and the rate of CFA biotransformation was reduced by 95.3%. Extrahepatic tissues from DCA-treated rats showed differing amounts of residual activity with MA and CFA as substrates (Table 2 and 3).

The activity assays confirm that catalytically active GSTZ1–1 is expressed in different tissues. The hepatic activity accounted for the majority of GSTZ1–1 activity in the whole organism. Other tissues showed low specific activities, which may indicate limited expression of GSTZ1–1. DCA (1.2 mmol/kg/day) reduced GSTZ1–1 activity in most tissues, but the reduction was significant only in the liver.
Lipscomb et al. previously showed that DCA is metabolized by whole-tissue homogenates from different tissues with activities in the order: liver > lung > kidney > intestine > muscle (Lipscomb et al., 1995). The limited effects of DCA on GSTZ1–1 activities in extrahepatic tissue may be associated with the much lower turnover of DCA in these tissues compared with the rates in the liver where inactivation is rapid (Anderson et al., 1999; Tzeng et al., 2000). Given that MA turnover in extrahepatic tissues was not significantly different from in DCA-treated rats compared with control rats, it is possible that other GSTs also catalyze the isomerization of MA to FA, but DCA is not a substrate for GSTA, GSTM, GSTP, and GSTT (W. B. Anderson and M. W. Anders, unpublished observations). The limited effects of DCA in extrahepatic tissues are unlikely to be associated with differences in tissue distribution of DCA (Lin et al., 1993).

Tissue-Dependent Differences in Expression and Activities of GSTZ1–1. Several mechanisms have been proposed to explain the tissue-dependent expression of GSTs. GSTZ1–1 may be regulated by its substrates in different tissues. High hepatic expression and activities of GSTZ1–1 is consistent with its role in tyrosine degradation. The abundant expression of GSTZ1–1 in the gastrointestinal epithelium indicates that GSTZ1–1 may affect the bioavailability of tyrosine and α-haloacids. No evidence for induction of GSTZ1–1 expression has been reported. Hormones (Hatayama et al., 1986), such as testosterone that regulates the expression of some GSTs (Catania et al., 2000), may also regulate the expression of GSTZ1–1 in different tissues. This is consistent with the observation that many endocrine tissues, such as the pancreas, adrenals, and testis expressed high amount of GSTZ1–1.

Effects of cis-acting elements in the promoter regions of GST genes in different tissues, such as the octamer DNA binding motif implicated in the tissue specific transcription of Yb3 subclass of GSTM (Abramovitz et al., 1995), have been elucidated. Tissue-specific transcription factors, such as the octomer-binding protein, are associated with tissue-dependent expression of different gene products of the same GST class (Abramovitz et al., 1995). Other signaling proteins may also regulate the tissue-dependent expression of GSTs; the GSTP1 sequence has a ras-response element similar to that of multidrug resistance protein 1 and P-glycoprotein and is activated when ras

Fig. 6. Immunohistochemical detection of GSTZ1–1 protein in the digestive tract.

Sections of (panel A) the esophagus (100×), (panel B) stomach (400×), (panel C) duodenum (100×), and (panel D) colon (100×) were incubated with polyclonal anti-GSTZ1–1 antibodies and stained by the avidin-biotin complex procedure, as described under Materials and Methods. The epithelial lining (arrowheads) of the digestive tract was stained intensely, more so in the superficial layers than in the basal layers. The mucous lining of the lumen (L) containing sloughed cells that also stained strongly. Moderately intense staining was also observed with the smooth muscle layers (*) underlying the epithelia. The parietal cells (P) of the stomach lining stained more intensely than the chief cells (C). The interstitial regions (I) in all segments were negative for GSTZ1–1.

Fig. 7. Immunohistochemical detection of GSTZ1–1 in rat kidney.

Rat kidney sections were incubated with (panel A) or without (panel B) polyclonal anti-GSTZ1–1 antibodies and stained by the avidin-biotin complex procedure, as described under Materials and Methods. Panel A, tubular regions of the cortex (C), particularly the juxtamedullary region (JM), were weakly stained. The medullar (M) and pelvic (P) regions were not stained. Original magnification was 20×. Panel B, the apical side of the proximal convoluted tubules (P) was sparsely stained; perinuclear staining is visible in some cells. The glomerular cells (G) were not stained. Original magnification was 1000×.
FIG. 8. Immunohistochemical detection of GSTZ1–1 in rat heart and other muscle tissue.

Sections of tissue from (panel A) heart and (panel B) skeletal muscle were incubated with polyclonal anti-GSTZ1–1 antibodies and stained by the avidin-biotin complex procedure, as described under Materials and Methods. Panel A, myocardial fibers were homogenously and moderately stained regardless of their location throughout the heart (400×). Panel B, myocytes of skeletal muscle (M) were weakly stained. In the cross-section, the staining of smooth muscle myocytes (S) of the major artery and nearby arterioles were strongly stained. The vascular endothelial lining of the artery was not stained. Embedded in the interstitial region around the artery is a peripheral nerve fiber (N) that was not stained (400×).

FIG. 9. Immunohistochemical detection of GSTZ1–1 in rat adrenal gland and pancreas.

Sections of rat adrenal (left panel) and pancreatic (right panel) tissues were incubated with polyclonal anti-GSTZ1–1 antibodies and stained by the avidin-biotin complex procedure, as described under Materials and Methods. Left panel, the adrenal medulla (M) was moderately intensely stained. The staining in the adrenal cortex was sparse and more intense in the zona glomerulosa (G) than in the zona fasciculata (F) and zona reticularis (R). Original magnification was 200×. Right panel, pancreatic islets (I) showed strong staining, and the acinar cells (A) showed sparse staining that was confined to the basal side of the cells (400×).

FIG. 10. Immunohistochemical detection of GSTZ1–1 in rat lung, bladder, and prostate gland.

Sections of rat lung (panel A), bladder (panel B), and prostate (panel C) tissues were incubated with polyclonal anti-GSTZ1–1 antibodies and stained by the avidin-biotin complex procedure as described under Materials and Methods. Panel A, the cuboidal epithelial cells lining the bronchi (B) and bronchioles (O) showed sparsely intense staining. The epithelial cells lining the alveoli (A) showed little or no staining. The original magnification was 400×. Panel B, the superficial transitional epithelial cells of the urinary bladder showed intense staining (<r>); staining was less intense in the deeper (>) layers than in the superficial layers (400×). Panel C, secretions in the prostate gland were intensely stained. The staining of the glandular epithelial lining (>) was faint (100×).
is induced (Okuda et al., 1987). Stress-response elements also regulate expression of GSTP in bile epithelium, as described in alcoholic liver disease (Harrison et al., 1990b). Investigation of the promoter region of GSTZ1-1 may provide insights into the regulation of the tissue-dependent expression of GSTZ1-1.

Tissue-specific differences in the post-translational modifications, such as glycosylation, phosphorylation, and carbamylation have been associated with tissue-dependent activities of some GSTs (Johnson et al., 1992), but studies done with electrospray mass spectrometry identified N-terminal acetylation of GSTs as the only natural modification of a variety of GSTs (Rowe et al., 1997). The amino acid sequence of GSTZ1-1 contains putative glycosylation and phosphorylation sites, but no evidence for post-translational modification of hepatic GSTZ1-1 has been presented (Tong et al., 1998a). GSTZ1-1 of rat liver is N-terminal blocked (Tong et al., 1998a), but this has not been investigated in extrahepatic tissues. The immunoreactive proteins observed in Fig. 2 may indicate that GSTZ1-1 undergoes pre- or post-translational modifications in a tissue dependent manner.

Tissue-dependent differences in specific activities may be associated with differences in protein stability during tissue processing. For example, rat GSTM5 purified from the testis is labile under oxidative conditions (Rowe et al., 1998). This phenomenon has been observed in other studies (Terrier et al., 1990; Juronen et al., 1996).

**Relationship between DCA-induced Toxicity and GSTZ1-1 Expression.** As indicated in the Introduction, DCA-induced toxicity is observed in several organs and tissues in rats and dogs given DCA. These effects range from cardiac malformations in rats (Smith et al., 1992) to hepatocellular carcinomas in rats and mice (Bhat et al., 1991; DeAngelo et al., 1991, 1996; Daniel et al., 1992). In addition, DCA is spermatotoxic in rats (Bhat et al., 1991; Linder et al., 1997); causes central and peripheral nervous system disorders in humans (Stacpoole et al., 1998), dogs (Katz et al., 1981; Cicmanec et al., 1991), and rats (Moser et al., 1999); causes diffuse degeneration of the tubular epithelium and glomerular cells in rats (Mather et al., 1990); and causes bronchial toxicity and prostatic glandular atrophy in dogs (Cicmanec et al., 1991). A time- and dose-dependent decrease in plasma insulin concentrations are observed in B6C3F1 mice given DCA, and this effect has been associated with DCA-induced inactivation of GSTZ1-1 (Lingohr et al., 2001).

Some of the toxic effects associated with chronic exposure to DCA occur in tissues and subcellular regions that express GSTZ1-1, as shown by the data presented herein. The toxic effects of DCA in the pancreas, which are associated with DCA-induced inactivation of GSTZ1-1 (Lingohr et al., 2001), may indicate that the multiorgan toxicity of DCA may be associated with the decreased expression and activities of GSTZ1-1 in the affected tissues. Studies that explore in detail the relationship between expression, inactivation, or both, of GSTZ1-1 are warranted to understand better DCA-induced toxicity. This study shows that GSTZ1-1 is expressed in many rat tissues and that its pattern of expression differs from that of other GSTs. Expression was observed by immunoblotting, immunohistochemical analyses, and activity determinations in many of the organs in which DCA-induced toxicity is observed. These findings indicate that the DCA-induced toxicity that is observed after prolonged treatment of rats with DCA may be associated with DCA-induced inactivation of GSTZ1-1 in the different target tissues. Tissues and cells that are most affected by DCA appear to be those that expressed high amounts of GSTZ1-1. Other mechanisms of DCA-induced toxicity, such as DCA-induced thiamine deficiency (Stacpoole et al., 1990), may also play a role. The present data point to other organs that may be affected by DCA, such as the adrenals, in addition to the liver, heart, testis, nervous system, pancreas, lung, and prostate.

**References**


**TABLE 2**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Specific Activity</th>
<th>Saline-Treated</th>
<th>DCA-Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>nmol/min/mg protein</td>
<td>258 ± 17</td>
<td>2.4 ± 0.1*</td>
</tr>
<tr>
<td>Heart</td>
<td>4.9 ± 1.6</td>
<td>4.9 ± 1.1</td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>3.9 ± 1.5</td>
<td>3.1 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>Testis</td>
<td>1.1 ± 0.2</td>
<td>1.7 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>Muscle</td>
<td>1.1 ± 0.4</td>
<td>1.2 ± 0.1</td>
<td></td>
</tr>
</tbody>
</table>

* Significantly different from liver tissue from saline-treated rats.

**TABLE 3**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Specific Activity</th>
<th>Saline-Treated</th>
<th>DCA-Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>nmol/min/mg protein</td>
<td>4.61 ± 0.89</td>
<td>0.22 ± 0.01*</td>
</tr>
<tr>
<td>Heart</td>
<td>0.75 ± 0.11</td>
<td>0.40 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>0.82 ± 0.07</td>
<td>0.22 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>Testis</td>
<td>0.28 ± 0.01</td>
<td>0.20 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>Muscle</td>
<td>0.34 ± 0.04</td>
<td>0.21 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>0.09 ± 0.01</td>
<td>0.14 ± 0.01</td>
<td></td>
</tr>
</tbody>
</table>

* Significantly different from liver tissue from saline-treated rats.

DeAngelio AB, Daniel FB, Most BM, and Olson GR (1996) The carcinogenicity of dichloroacetic acid in the male Fischer 344 rat. Toxicology 114:207–221.


