Glutathione transferases (E.C. 2.5.1.18) catalyze the reaction of glutathione with a range of xenobiotic and endogenous electrophiles (1–3). Multiple GSTs exist, and at least 20 GST genes have been identified in the rat (4). Demonstration of the tissue-selective expression and subcellular localization of cytosolic GSTs has improved the understanding of their role in xenobiotic disposition and has provided strategies for assessing tissue-selective cell damage (5, 6).

Both soluble and membrane-bound GSTs have been identified. Cytosolic GST isoenzymes are a family of homo- and heterodimeric proteins that are grouped into five classes (α, μ, π, θ, and σ) based on primary structure, substrate selectivity, sensitivity to inhibitors, and immunological properties (2, 7). Soluble GSTs are present in mitochondria, and a unique θ-class (GST 13-13, now called GST T3*-3*) has been isolated from mitochondrial matrices (8–10). The role of the GSTs in the detoxication of electrophilic chemicals and metabolites is well characterized (1). In addition, the glutathione-dependent bioactivation of a range of xenobiotics—including vicinal dihaloalkanes (11), isocyanates and isothiocyanates (12), quinones (13), and haloalkanes (14)—is catalyzed by GSTs.

Membrane-bound mGST is a homotrimeric protein that is structurally and immunologically distinct from the cytosolic transferases and is classified separately (2, 7). mGST differs from the cytosolic GSTs in that it can be activated several-fold by sulfhydryl reagents, such as N-ethylmaleimide; this property has been exploited to obtain purified mGST that is not contaminated with cytosolic GSTs (15). Leukotriene C₄ synthesis is catalyzed by a membrane-bound GST that is distinct from the N-ethylmaleimide-activatable mGST, as well as from the α-, μ-, π-, σ-, and θ-classes of cytosolic GSTs (16). Human and rat liver microsomal glutathione transferases have been cloned, and the cDNA sequences share 77% identity in the coding region (17). Ontogeny of mGST and the structural organization of human mGST gene have recently been reported (18, 19). Antibodies raised against the human and rat mGSTs cross-react (20). mGST catalyzes the reaction of glutathione with a range of electrophilic compounds, and its activity as a selenium-independent peroxidase may be an important cytoprotective function (21). Oxidants and oxidative stress activate the mGST (22), which may also contribute to its cytoprotective actions. In addition to its cytoprotective action, mGST catalyzes the first step in the glutathione- and cysteine conjugate B-lyase–dependent bioactivation of nephrotoxic haloalkanes (14). mGST activity is present in various rat organs, and the highest activity is present in the liver and testes (23). Although activity in whole-organ homogenates may be relatively low, mGST activity may be high in some cell populations and may, thereby, contribute to xenobiotic detoxication or bioactivation. The objective of this study was to examine the cellular localization of mGST in various rat organs by immunohistochemistry.

**Methods**

**Tissue Preparations.** Fischer 344 rats (200–250 g; Charles River Breeding Laboratories, Inc., Wilmington, MA) were used. Rats were anesthetized with sodium pentobarbital:chloral hydrate (50 mg/kg:250 mg/kg, ip) and perfused through the left ventricle with PBS, followed by 10% neutral-buffered formalin. Organs were removed, fixed in 10% buffered formalin, and embedded in paraffin. Sections (5 μm) were prepared and mounted on poly-L-lysine-coated slides.

**Western Blotting.** mGST was purified from the microsomal fraction of human liver by a combination of hydroxyapatite, gel filtration, and CM-cellulose chromatography (20). Female New Zealand white rabbits were each initially immunized with 200 μg of purified mGST in incomplete Freund’s adjuvant, which was followed 6 weeks later by another inoculation with 200 μg of mGST in incomplete Freund’s adjuvant (24). Animals were bled 2 weeks after the second inoculation, and serum was prepared. After the addition of sodium azide to a final concentration of 0.1%, serum was stored at −70°C until

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**ABSTRACT:**

Distribution of microsomal glutathione transferase (mGST) protein in rat tissues was investigated by immunohistochemistry. Studies on the localization of mGST are of interest because of its involvement in the detoxication and bioactivation of xenobiotics. mGST antigen was detected in the cytoplasm of some hepatocytes and in bile ducts. In kidney, focal staining of mGST was observed in distal tubules and collecting ducts. Cerebral cortical and cerebellar Purkinje neurons showed good immunoreactivity, and nuclear staining was observed in the choroid plexus. The antigen was detected in epithelial cells of respiratory bronchioles and in the crypt cells of the duodenum. Exocrine cells of the pancreas stained for mGST. Nuclear immunostaining for this protein was observed in primary spermatocytes. mGST antigen was detected in the cytoplasm of the adrenal medulla as a granular stain. Leydig and Sertoli cells in testsis also stained for the antigen. Distribution of mGST protein differs from that observed with cytosolic transferases and may be important in determining cell-selective susceptibility to xenobiotics.
Samples of liver (lanes 1 and 2, 2 μg protein), lung (lanes 3 and 4, 10 μg protein), kidney (lanes 5 and 6, 10 μg protein), and testes (lanes 7 and 8, 10 μg protein) were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting, as described in Materials and Methods. Mobility of the molecular weight marker (lysozyme, 17.9 kDa) is also shown.

### TABLE 1

Summary of the distribution of cytosolic and mGST antigen in various rat and human tissues

<table>
<thead>
<tr>
<th>Organ</th>
<th>mGST</th>
<th>α</th>
<th>π</th>
<th>μ</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>27, 28, 35, 44, 46</td>
</tr>
<tr>
<td>Hepatocytes</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>27, 28, 35</td>
</tr>
<tr>
<td>Bile duct epithelum</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>5, 27, 28, 35–37, 42</td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distal convoluted tubules</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>27, 28, 35–37</td>
</tr>
<tr>
<td>Proximal convoluted tubules</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>5, 27, 28, 35–37, 42</td>
</tr>
<tr>
<td>Glomeruli</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Collecting ducts, cortex, and medulla</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Thick Loop of Henle</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td></td>
</tr>
<tr>
<td>Thin Loop of Henle</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Choroid plexus</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>28, 35, 44–46</td>
</tr>
<tr>
<td>Cerebral cortex</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Cerebellum, Purkinje neurons</td>
<td>+</td>
<td>±</td>
<td>−</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Glia</td>
<td>ND</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bronchioles</td>
<td>+</td>
<td>±</td>
<td>+</td>
<td>−</td>
<td>28, 35, 54, 55</td>
</tr>
<tr>
<td>Alveoli</td>
<td>−</td>
<td>±</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Intestine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duodenum</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>27, 28, 35</td>
</tr>
<tr>
<td>Pancreas</td>
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<tr>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>27, 28, 35, 58</td>
</tr>
<tr>
<td>Islets of Langerhans</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>±</td>
<td>27, 28, 35, 58</td>
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<td>Adrenal gland</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortex</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>28, 35</td>
</tr>
<tr>
<td>Medulla</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Testes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leydig cells</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>27, 28, 60, 61</td>
</tr>
<tr>
<td>Sertoli cells</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Spermatocytes</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td></td>
</tr>
</tbody>
</table>

+ , Antigen clearly present; − , antigen not detected; ± , both presence and absence of antigen reported; ND, not determined.
with diaminobenzidine were counterstained with hematoxylin, dehydrated, and mounted in an organic mounting medium (Permunt; Fisher Scientific, Fair Lawn, NJ); slides treated with 3-amino-9-ethylcarbazole were also counterstained with hematoxylin, but were mounted in an aqueous mount (Aqua-polymount; Polysciences, Inc., Warrington, PA).

Results and Discussion

This study represents the first immunohistochemical description of the comparative distribution of mGST protein in rat tissues. mGST antigen was detected in differing amounts in the organs studied by immunoblotting and immunohistochemistry.

Immunoblotting studies with human anti-mGST antibodies showed the presence of a single protein band of identical mobility in microsomal fractions from rat liver, testis, kidney, and lung (fig. 1). The immunoreactive band displayed a mobility consistent with its identity as mGST and was estimated to have a molecular mass of \(-17\) kDa; the calculated molecular mass of rat liver mGST is \(17,430\) Da (17). Furthermore, antibodies yielded a single band in the immunoblots, indicating selectivity for mGST. Antibodies raised against the mGST do not cross-react with cytosolic GSTs (15). (Because the amount of mGST protein in the tissues studied differed, the amount of protein applied to the gel varied with the tissue being studied; fig. 1 cannot be used to compare the relative amounts of mGST in various tissues.)

Cellular localization of mGST protein in various rat organs was studied by immunohistochemistry. A comparison of the localization of mGST found in this study and that reported for other rat and human cytosolic GSTs is summarized in table 1.

In the liver, mGST antigen was demonstrable as a faintly granular cytoplasmic material in most, but not all, hepatocytes (fig. 2A, C, and E) or with preimmune serum (B, D, and E) and stained by the avidin-biotin complex procedure with a final chromogen of 3-amino-9-ethylcarbazole, as described in Materials and Methods. (A) Note the occasional, poorly stained hepatocytes (arrow). T, Portal triad; c, central vein. Original magnification 100×; bar = 100 \(\mu\)m. (C) Higher magnification 200×; bar = 50 \(\mu\)m of centrilobular area. Note the intensely stained cell (arrow). T, Portal triad; c, Central vein. (E) Higher magnification 200×; bar = 50 \(\mu\)m of portal triad. Note that the bile duct epithelial cells show both cytoplasmic and nuclear staining. b, bile duct.

Liver sections were incubated with polyclonal anti-mGST antibodies (A, C, and E) or with preimmune serum (B, D, and E) and stained by the avidin-biotin complex procedure with a final chromogen of 3-amino-9-ethylcarbazole, as described in Materials and Methods. (A) Note the occasional, poorly stained hepatocytes (arrow). T, Portal triad; c, central vein. Original magnification 100×; bar = 100 \(\mu\)m. (C) Higher magnification 200×; bar = 50 \(\mu\)m of centrilobular area. Note the intensely stained cell (arrow). T, Portal triad; c, Central vein. (E) Higher magnification 200×; bar = 50 \(\mu\)m of portal triad. Note that the bile duct epithelial cells show both cytoplasmic and nuclear staining. b, bile duct.

![Fig. 2. Immunohistochemical detection of mGST protein in rat liver.](https://example.com/fig2.jpg)
reported (27, 28). In the portal triad, cytoplasmic components of the bile duct epithelium were well stained (fig. 2C). GSTs have the capacity to bind a wide range of lipophilic compounds, including bile acids and bilirubin (29, 30). mGST in the biliary epithelium may serve as a carrier protein for the transport of such molecules, as well as function to detoxify xenobiotics. Nonspecific staining was not observed in sections incubated with preimmune serum (fig. 2, B, D, and F).

Immunohistochemical observation of mGST protein in hepatocytes is consistent with the observation that hepatic mGST activity exerts a cytoprotective function by providing glutathione-dependent detoxication against lipid peroxidation (21, 31). Hepatic mGST also catalyzes the first step in the glutathione- and β-lyase–dependent bioactivation of nephrotoxic haloalkenes. Also, in rat hepatocytes, 85% of glutathione-conjugate formation with chlorotrifluoroethene as the substrate is catalyzed by mGST (32). Hexafluoropropene is converted to 5-(1,1,2,3,3,3-hexafluoropropyl)glutathione in rat hepatic cytosolic fractions and to S-(1,2,3,3,3-pentafluoropropenyl)glutathione in rat hepatic microsomal fractions, but total conjugate formation is greater in microsomes than in cytosol (33).

Other immunohistochemical studies show the presence of α-, μ-, and θ-class GSTs in rat liver hepatocytes. Like mGSTs, staining was more intense in the centrilobular region than in the midzonal or periportal regions (34). GST-π is not seen in adult hepatocytes, but, like μ- and θ-class GSTs, it is expressed in biliary epithelium (27, 28, 35). GST-α is not present in bile ducts (28).

In the renal cortex, the most intense staining for mGST antigen was observed in the distal convoluted tubules (fig. 3). Faint staining was observed in some glomerular cells, and proximal convoluted tubules demonstrated very light apical staining; no staining was observed in the brush border. Original magnification 200×; bar = 50 μm. (C) Collecting ducts (CD) in the cortex and medulla were well stained. Original magnification 200×; bar = 50 μm. (E) The thick portions of the loop of Henle (L) stained intensely. Original magnification 100×; bar = 100 μm.
mGST antigen in the distal tubules and collecting ducts and of GST-α in the proximal tubules demonstrates the ubiquitous distribution of GSTs throughout the nephron.

The presence of GSTs in the kidney is well established (38). Renal glutathione-conjugate formation has been detected in isolated rabbit renal proximal tubules with chlorotrifluoroethene as the substrate, although the enzyme that catalyzes the reaction was not identified (39). Renal cytosolic fractions, but not renal microsomal fractions, catalyze the conversion of hexafluoropropene to \( S-(1,1,2,3,3,3\text{-hexafluoropropyl})\)glutathione, but \( S-(1,2,3,3,3\text{-pentafluoropropenyl})\)-glutathione formation was not detected in kidney cytosol or microsomes.

The release of GST-α from its proximal tubular location and its detection in urine has previously been used as a diagnostic measure of renal tubular damage in cyclosporin-induced nephrotoxicity (40, 41). Use of GST-π in urine, as a marker of renal tubular injury, has also been reported (42, 43). \( S-(1,2\text{-Dichlorovinyl})\)-l-cysteine is selectively toxic to the S3 segment of the proximal tubule and is an example of renal toxins that target specific segments of the renal tubules, leaving other segments unharmed. Selective markers for renal tubular injury are useful diagnostic tools for such toxins. As has been demonstrated for GST-α and -π, focal localization of mGST makes it a potentially suitable candidate as a marker for distal tubular injury; in addition the lack of immunoreactivity of anti-mGST antibodies with cytosolic GSTs (15) is a property that could be exploited to design sensitive assays that would distinguish it from other GSTs also used as markers.

mGST protein was also detected in the brain (fig. 4). Choroid epithelial cells were intensely stained (fig. 4A, arrow), and abundant antigen was detected in both the nucleus and cytoplasm. Neuronal nuclei in the cerebral cortex were well stained (fig. 4B), as were cerebellar Purkinje neurons and the molecular-layer neurophil (fig. 4E). Nonspecific staining was not observed in sections incubated with preimmune serum (fig. 4, B, D, and F).

In human brains, GST-α and -π have not been detected in neurons, but weak staining is observed with GST-π in glial cells (27, 28, 44). However, studies with rat brain show nuclear localization of GST-α in Purkinje cells and neurons in the brainstem, hippocampus, and cerebral cortex (45). Immunohistochemical studies with rat \( \mu \)-class GSTs show that the protein is localized in the glial cells lining the ventricles and in astrocyte endfeet and processes (46).
GST activity is much higher in cultured chick astrocytes than in neurons or forebrain (47). Whole-brain mGST activity is much lower (~6%) than liver mGST activity (23).

Glutathione-dependent conjugation in brain cytosol has been demonstrated with the neurotoxin acrylamide (48). There is also evidence that brain GSTs can bind hormones and neurotransmitters (46). The role of mGST in the nucleus is to be determined. Glutathione peroxidase activity of GSTs has been postulated to play a role in the detoxication of peroxidized DNA, and mGST has been shown to protect against lipid peroxidation. mGST may play a significant role in detoxifying peroxides in the nucleus, because selenium-dependent glutathione peroxidase activity is low in this organelle (49, 50). Bennett et al. (51) have also reported that the DNA-binding protein, protein BA that colocalizes with U-snRNPs in the cell nucleus is a glutathione transferase.

In lung tissue, there was scanty, focal presence of antigen in the epithelial cells of the terminal respiratory bronchioles, most notably in the apical portions, and alveolar epithelial cells showed little detectable cytoplasmic antigen (fig. 5A). Nonspecific staining was not observed in sections incubated with preimmune serum (fig. 5B).
mGST activity in lung is 12% of that found in liver (23). Lung mGST may play a role in the bioactivation of xenobiotics: Patel et al. (52) found that, of the extrahepatic tissues studied, lung microsomes had the highest activity toward dichloroacetylene, a neurotoxin, nephrotoxin, and nephrocarcinogen. Activity was not observed in the lung cytosol.

Proteins corresponding to \( \alpha \)- and \( \mu \)-class GSTs have been purified from rat lung (53). GST-\( \alpha \) protein was not detected in the epithelial cells of the lung or bronchi (28). In contrast, Anttila et al. (54) reported localization of all GST classes in the bronchial epithelium. Others have detected GST-\( \pi \) in ciliated epithelial cells and GST-\( \alpha \), -\( \mu \), and -\( \pi \) in Clara cells (55).

In the intestinal tract, duodenal tissue was also examined for the presence of mGST antigen. Staining was most intense in the apical portions (brush border) of the enteric epithelial cells, particularly in the cryptal areas (fig. 6A). Nonspecific staining was not observed in sections incubated with preimmune serum (fig. 6B). Specific activity of mGST in rat intestine is 48% of that present in the liver (23).

In the rat intestine, GST activity is highest in the duodenum and jejunum (56, 57). GST-\( \pi \) is expressed at significant levels in the duodenum (28).

In pancreatic tissue, the Islets of Langerhans lacked antigen (fig. 7A). Exocrine epithelial cells contained some antigen, most notably in the apical portions of epithelial cells. Nonspecific staining was not observed in sections incubated with preimmune serum (fig. 7B).

GST-\( \alpha \) and GST-\( \pi \) antigens have not been detected in the Islets of Langerhans (27, 35). GST-\( \alpha \) has been detected in the cytoplasm of acinar cells, whereas GST-\( \pi \) is present in both the cytoplasm and some nuclei of these cells in rat pancreas (58).

Cells of the adrenal cortex showed little staining, but medullary cells contained variable amounts of faintly staining granular material in the cytoplasm (fig. 8A). Occasional cells contained abundant antigen, but adjacent cells showed scanty staining material. Nonspecific staining was not observed in sections incubated with preimmune serum (fig. 8B). Specific activity of mGST in rat adrenal is 41% of that found in the liver (23).

GST-\( \alpha \) protein is found in the zona reticularis of the adrenal cortex, but not in the outer layers of the cortex or medulla (28). GST-\( \pi \) was detected faintly in both layers (27).

In the testes, the interstitial cells showed modest cytoplasmic and nuclear staining (fig. 9A). All stages of sperm-cell maturation were stained in the seminiferous tubules, and staining was pronounced in the nuclei of primary spermatocytes. Nonspecific staining was not observed in sections incubated with preimmune serum (fig. 9B). Activity studies show that the specific activity of mGST in rat testis is similar to that in the liver (23), but a physiological role for mGST in the testis has not been established. Peroxidative DNA damage has been observed in proliferating cells (3) and, because mGST shows peroxidase activity (59), it is possible to speculate that testicular...
mGST may function to reduce damage to DNA caused by oxidative stress.

Immunohistochemical studies with cytosolic GSTs show the presence of all GST classes in Leydig cells in the testis; GST-α and GST-μ were detected in Sertoli cells, whereas all stages of sperm-cell maturation were unreactive to antibodies against α-, π-, or μ-class GSTs (27, 28, 60, 61). Distribution of GSTs along the rat epididymis seems to be region-specific; recent evidence shows selective expression of GST-π in basal cells of the epididymal epithelium (62). Basal cells have been proposed to play an active role in detoxication in the epididymis, thus further demonstrating the cytoprotective role that GSTs may play in the testes. Finally, the presence of μ- and ω-class GSTs in testicular microsomes has recently been reported (63).

Present studies show that the distribution of mGST protein differs from that of cytosolic GSTs that have been studied. Both mGST and cytosolic GSTs play important roles in xenobiotic detoxication and bioactivation. As described previously, peroxidative activity of mGST may exert a detoxification function in testes. mGST is important in the bioactivation of nephrotoxic haloalkanes. Several haloalkanes are selective nephrotoxins and undergo glutathione and cysteine conjugation (14, 64, 65). Biotransformation of haloalkanes to glutathione conjugates is preferentially catalyzed by hepatic mGST (32). Present findings show the presence of small amounts of mGST antigen in renal proximal tubules, which is consistent with the observation that mGST activity is much lower in the kidney than in the liver (23). As described previously, studies with chlorotrifluoroethene show that ~85% of hepatic glutathione conjugate formation is catalyzed by mGST (32).

Immunohistochemical studies reported herein demonstrate unique patterns of distribution of mGST protein across organs and show that the distribution pattern seen with mGST differs from the patterns seen with most cytosolic transferases, although there are some similarities between the distribution of mGST and GST-α. Distribution of GST activities may play a role in determining the susceptibility of a tissue or cell type to different xenobiotics, because mGST and cytosolic GSTs have different, but overlapping, substrate selectivities.

References


