Immunohistochemical Localization of Glutathione S-Transferases in Livers of Untreated Rats*

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Sheep antibodies raised against three isoenzymes of glutathione S-transferase (EC 2.5.1.18), transferases B, C, and E, which were isolated and purified to apparent homogeneity from rat liver, have been employed to localize these enzymes at the light microscopic level within livers of untreated rats. Using these antibodies in an unlabeled antibody peroxidase-antiperoxidase staining technique, each glutathione S-transferase was detected immunohistochemically within parenchymal cells throughout the liver lobule. In addition, immunohistochemical staining for transferases C and E, but not for transferase B, was observed within bile duct epithelium. While all parenchymal cells were stained with each glutathione S-transferase antibody, the patterns of immunohistochemical staining intensity observed across the liver lobule with the three anti-transferases were not uniform: parenchymal cells within the centrilobular region were more intensely stained for each isoenzyme than were those within the midzonal and periportal regions of the lobule. The results of this immunohistochemical study thus demonstrate that glutathione S-transferases are not distributed uniformly throughout the liver lobule and that each transferase is present in the greatest concentration within the centrilobular region of the lobule.

The glutathione S-transferases are a family of multifunctional enzymes that play significant roles in the detoxication of both endogenous and exogenous substances (1, 2). They are not only capable of catalyzing the conjugation of the sulphydryl group of reduced glutathione with a wide variety of electrophilic compounds, among which is the first step leading to the formation of water-soluble mercapturic acid derivatives (1, 2), but they also have the capacity for binding a large number of potentially toxic, hydrophobic nonsubstrates such as bilirubin (2-6). Given the capacity of the glutathione S-transferases to inactivate many potentially toxic substances, knowledge of the precise cellular localizations of these enzymes within a tissue such as the liver would contribute to an understanding of the basis for the relatively selective cellular nature of many chemically induced toxicities. The presence of the glutathione S-transferases has been demonstrated biochemically within the liver and other tissues in many species (2, 7-12), and glutathione S-transferase B (ligandin) has been localized within parenchymal cells in human (13, 14), rat (13, 15), and hamster (13) liver. However, little is known regarding the distributions and cellular localizations of different glutathione S-transferases within the liver lobule. To provide this information, sheep antibodies raised against three isoenzymes of hepatic glutathione S-transferase, transferases B, C, and E, have been utilized in an unlabeled antibody peroxidase-antiperoxidase staining technique in the present study to determine the cellular localizations of each of these enzymes within livers of untreated rats.

EXPERIMENTAL PROCEDURES

Materials—Parabenzoquinone was obtained from Fisher Chemical Co., 3,3'-diaminobenzidine tetrahydrochloride was from Hach Chemical Co., and normal (nonimmune) sheep serum was from Cappel Laboratories. The soluble goat peroxidase-antiperoxidase complex and rabbit antiserum to sheep IgG were produced by DAKO (Dakopatts of Denmark) and purchased through Accurate Chemical and Scientific Corp. All other chemicals employed were of the highest purity available.

Methods—The glutathione S-transferases were isolated and purified to apparent homogeneity from livers of Sprague-Dawley rats as described by Habig et al. (16) for transferases B and C and by Fjellstad et al. (17) for transferase E. It should be noted that antibodies directed against transferase C cross-react with transferase A and that antibodies directed against transferase A cross-react with transferase C (16). This is not surprising since it is now known that these two transferases share a common subunit (18, 19). Thus, references to the results obtained with antibody to transferase C should be interpreted as indicating the presence of either transferase C or transferase A. The antibody to transferase B does not cross-react with transferases A, AA, C, or E, and the antibody to transferase E is similarly specific for this enzyme (2). Sheep antisera against the three transferases were obtained, and the IgG fractions were prepared from both normal and immune sheep sera as described previously (16, 20). Male albino Holtzman rats weighing 200-250 g were used in this study and were fasted for 24 h prior to killing by decapitation. Blocks of liver, approximately 5 x 3 x 1 mm, were obtained from the median lobe and were fixed at 4 °C for a total period of 4 h by immersion in several changes of a solution containing 0.35% (w/v) paraformaldehyde, 0.02 M CaCl2 in 0.2 M sodium cacodylate buffer, pH 7.4. Following fixation, the liver blocks were dehydrated, cleared, and embedded in paraffin, and serial sections 7 μm in thickness were prepared. The glutathione S-transferases were localized at the light microscopic level employing minor modifications of the unlabeled antibody peroxidase-antiperoxidase technique (20-25). In control experiments, sections were exposed to normal sheep IgG rather than to sheep anti-glutathione S-transferase IgG.

RESULTS AND DISCUSSION

When sections of livers from untreated male rats were exposed to IgG prepared from sheep antisera to glutathione S-transferases B, C, and E in the unlabeled antibody peroxidase-antiperoxidase staining technique, the presence of immunohistochemical staining for each isoenzyme was evident within parenchymal cells throughout the liver lobule (Figs. 1-
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**FIG. 1. Immunohistochemical localization of glutathione S-transferase B within the liver of an untreated male rat.** The photomicrographs in A and E show the same areas in serial sections. A, section exposed to sheep anti-glutathione S-transferase B IgG (diluted to a final concentration of 0.1 mg of protein/ml); E, section exposed to normal sheep IgG (0.1 mg of protein/ml). B and F, C and G, and D and H show higher magnification photomicrographs of the centrilobular, midzonal, and periportal regions, respectively, in the two serial sections. B–D, exposed to sheep anti-glutathione S-transferase B IgG; F–H, exposed to normal sheep IgG. The photomicrographs in I–K show serial sections of a portal triad. I, exposed to sheep anti-glutathione S-transferase B IgG; J, stained with hematoxylin and eosin; K, exposed to normal sheep IgG. A central vein (v), portal triad (p), and bile duct (d) are indicated.

**FIG. 2. Immunohistochemical localization of glutathione S-transferase C within the liver of an untreated male rat.** The photomicrographs in A and E show the same areas in serial sections. A, section exposed to sheep anti-glutathione S-transferase C IgG (diluted to a final concentration of 0.07 mg of protein/ml); E, section exposed to normal sheep IgG (0.07 mg of protein/ml). B and F, C and G, and D and H show higher magnification photomicrographs of the centrilobular, midzonal, and periportal regions, respectively, in the two serial sections. B–D, exposed to sheep anti-glutathione S-transferase C IgG; F–H, exposed to normal sheep IgG. The photomicrographs in I–K show serial sections of a portal triad. I, exposed to sheep anti-glutathione S-transferase C IgG; J, stained with hematoxylin and eosin; K, exposed to normal sheep IgG. A central vein (v), portal triad (p), and bile duct (d) are indicated.
Immunohistochemical staining was not demonstrable within these cells when sections were exposed to normal sheep IgG (Figs. 1–3, E–H). Although parenchymal cells throughout the liver lobule were stained for the three glutathione S-transferases, it is readily apparent from the photomicrographs in Figs. 1–3, A, that immunohistochemical staining produced with each antibody was not of uniform intensity across the liver lobule: parenchymal cells lying within the centrilobular regions exhibited the greatest intensity of staining whereas those found within the perinuclear regions of the lobule exhibited the least. Similar findings for the distribution of immunohistochemical staining for ligandin within the liver have previously demonstrated similar differences in the intralobular distributions of cytochrome P-450 isoenzymes (23,26,27), NADPH-cytochrome c (P-450) reductase (20, 26, 28), cytochrome b₅, (28), and epoxide hydrolase (24,26,29) within livers of untreated rats. These findings, especially for glutathione S-transferase, suggest that detoxication may occur within bile ducts.

Examination of the higher magnification photomicrographs in Figs. 1–3, B–D, reveals that nuclei were not appreciably stained by antibodies against transferases B and C, whereas some parenchymal cell nuclei were intensely stained for transferase E. On the other hand, antibodies against transferases B and C frequently produced rings of stain around nuclei, indicating association of the transferases with the nuclear envelope. These findings, especially for glutathione S-transferase B, are in disagreement with those of others who have reported intense immunohistochemical staining of parenchymal cell nuclei with antibodies directed against ligandin (13–15). The reasons for this discrepancy are unknown, although they do not appear to be a result of tissue processing, since findings similar to those shown in Figs. 1–3 have been made by us using a number of different fixatives as well as unfixed, cryostat liver sections (data not shown). It is apparent, however, that in addition to differing in their specific cellular localizations within the liver, glutathione S-transferase isoenzymes exhibit differences in their localizations within hepatic parenchymal cells.

REFERENCES
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