Hepatic Drug-metabolizing Enzymes in Primary and Secondary Tumors of Human Liver

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ABSTRACT

Significant increases in activities of epoxide hydrolase, UDP-glucuronosyltransferase, and glutathione S-transferase, and marked reductions in cytochrome P-450 mixed-function oxidase systems occur in hyperplastic nodules induced in rat liver by chemical mutagens. In contrast, activities of both oxidative (Phase I) and conjugative (Phase II) enzymes are decreased in hepatocellular carcinomas induced by peroxisome proliferators. The present work compares alterations induced by chemical mutagens or peroxisome proliferators with changes in enzyme activities that occur in primary and secondary hepatic tumors in man. The above activities, along with β-glucuronidase and arylsulfatase, were measured in liver samples from 6 normal livers obtained at immediate autopsy, and liver specimens obtained by surgical biopsy from the following patients: 8 with hepatomas, 5 with nonmetastatic colorectal carcinomas, and 14 with metastatic colorectal carcinomas. Cytochromes P-450, P-4501, and P-4503, in addition to epoxide hydrolase were measured by immunoquantitation. Enzymes involved in conjugation reactions were either assayed fluorometrically (UDP-glucuronosyltransferase, β-glucuronidase, sulfotransferase, and sulfatase) or spectrophotometrically (glutathione S-transferase) using umbelliferyl substrates or 1-chloro-2,4-dinitrobenezene. Secondary hepatic tumors showed no significant change in drug-metabolizing enzymes, in contrast to primary hepatomas, which displayed decreases in all of the measured drug metabolizing enzymes. Arylsulfatase was markedly depressed in primary hepatomas (14% of normal values). Thus, activities of drug-metabolizing enzymes in human primary tumors resemble those associated with altered hepatic foci induced by peroxisome proliferators such as ciproflaxin. The marked decreases in sulfatase that occurred in primary but not in secondary human tumors suggest that sulfation of endogenous compounds and xenobiotics may differ in patients with primary and secondary hepatic tumors.

INTRODUCTION

Carcinoma of the colon and rectum is the second most common cancer in the United States with 138,000 new instances each year (1), and the liver represents the most common site of distant metastases. Primary malignancies of the liver are much less common than the metastatic tumors but still occur in significant numbers in the United States (13,600 cases/year) (1). Because of the high incidence of liver tumors, it is important to study the influence of hepatocarcinogenesis on hepatic drug metabolizing enzymes. Although considerable information is available regarding the influence of chemically induced hyperplastic tumors on drug-metabolizing enzymes in laboratory animals (2, 3), there is little information concerning changes in these enzymes induced in human liver by neoplasia. Determining whether alterations in hepatic drug-metabolizing enzymes occur with the development of primary or secondary tumors is important because of the involvement of these enzymes in the metabolism of therapeutic agents as well as circulating hormones such as catecholamines and steroids. Further, changes in activities of these enzymes may also alter the sensitivity of the liver to chemical injury. For example, a direct correlation has been noted between sulfotransferase activity and the susceptibility of the liver to tumors induced by chemical carcinogens that require conjugation with sulfate before forming adducts with nucleic acids (4, 5).

To date, most studies of enzymes in human hepatic cancer have focused mainly on tumor cell enzyme markers such as γ-glutamyltranspeptidase, which is markedly elevated in hepatocellular carcinoma, and glucose 6-phosphatase and canaliculat ATFase, which are often decreased (6). In addition to these enzymes, α1-antitrypsin, α-fetoprotein, and carcinoembryonic antigen (7) have also been used as markers for liver cancer and are often measured in hepatocellular carcinoma. To our knowledge, a comprehensive study of drug-metabolizing enzymes in primary and secondary tumors of human liver has not been made; however, studies of cytochrome P-450-dependent oxidative enzymes in human liver have been made using a variety of methods, including antipyrine half-life (8), ethylmorphine N-demethylation (9), aminopyrine breath test (10), and benzopyrene hydroxylation (8).

The possibility that changes in drug-metabolizing enzymes occur in human hepatic neoplasia is suggested by data obtained from animal studies. Hyperplastic liver nodules from rats treated with chemical mutagens such as 2-acetylaminofluorene have markedly reduced (5-44% of control values) activities of mixed-function oxidases and 2- to 4-fold increases in enzymes involved in conjugation reactions such as UDP-glucuronosyltransferase and glutathione S-transferase (2, 3). Recent reports have also shown that the placental form of glutathione S-transferase, which is very low in normal liver, is markedly increased in hyperplastic nodules and well-differentiated hepatomas in rat liver (11, 12). In addition, the enzyme epoxide hydrolase is elevated 5- to 7-fold in livers of rats fed 2-acetylaminofluorene (13). Patterns of drug-metabolizing enzymes in livers of rats treated with chemical mutagens differ markedly from those in livers of rats treated with hypolipidemic peroxisome proliferators, which also induce hepatic tumors (14). In general, drug metabolizing enzymes decrease in livers of rats treated with the latter group of compounds. A summary of recent studies of both models of hepatocarcinogenesis in rats is given in Table 1.

The present study surveys hepatic mixed-function oxidases and conjugating enzymes in primary and secondary tumors of human liver and compares these data with measurements of drug-metabolizing enzymes in studies of the two current models of hepatocarcinogenesis in rats. Enzymes were studied in liver specimens obtained from cases of adenocarcinoma of the colon with and without liver metastasis, and from patients with primary malignant tumors of the liver. Because of lack of information regarding sulfatase activities in experimental hepato-
carcinogenesis and its marked reduction in primary hepatomas, aroylsulfatase activity was assayed in livers of rats treated with diethylnitrosamine to induce hyperplastic nodules. Our results indicate that primary and secondary human hepatic tumors differ from each other with respect to drug-metabolizing enzymes. The patterns noted in primary hepatomas in human liver more closely resemble those observed in livers of animals treated with peroxisome proliferators than those seen in hyperplastic nodules induced by chemical mutagens.

MATERIALS AND METHODS

Subjects

Of a total of 33 liver specimens, 27 were obtained from patients at the time of operation and 6 were from the immediate autopsy program at the University of Maryland. Of the 27 patients, 8 had hepatomas, 14 had secondary liver cancer from adenocarcinoma of the colon, and 5 had adenocarcinoma of the colon without liver metastasis. Liver samples weighing between 100 mg and 1 g were obtained during the resection of the hepatic tumor or the primary colon tumor. Specimens from nodular, perinodular, and/or distant regions of liver were obtained in most of the cases. Normal liver specimens used as controls were selected from organ donor cases obtained through the immediate autopsy program at the University of Maryland. Organ donors were placed on cardiorespiratory supports after death until the organs were removed. From 20 specimens obtained at immediate autopsy, only 6 had normal histological appearance and were used in this study. The characteristics of all 33 patients, the subject of this study, are summarized in Table 2.

All liver specimens were immediately frozen in liquid nitrogen and stored at -80°C until analyzed to prevent deterioration of the enzyme activities. The maximal interval between removal of biopsy specimen and freezing did not exceed 5 min.

Analytical Procedures

Liver specimens were homogenized (5% w/v) in 2 mM Tris-HCl (pH 8.1), 230 mM mannitol, and 70 mM sucrose using a Polytron homogenizer. Protein concentrations of homogenates were determined according to Lowry et al. (15) using bovine serum albumin as a standard. Drug-metabolizing enzyme activities were determined as described below.

Immunquantitations

Cytochromes P-450<sub>sw</sub> and P-450<sub>ahp</sub> and epoxide hydrolase were quantitated as described in detail previously (16). Briefly, samples of liver homogenates were solubilized and electrophoresed in individual lanes of a sodium dodecyl sulfate-polyacrylamide gel (7.5%, w/v). After electrophoresis, the resolved proteins were electrophoretically transferred to a sheet of nitrocellulose and that sheet was sequentially developed with primary rabbit antiserum raised against the antigen under consideration, goat anti-rabbit immunoglobulin G, horsedarish peroxi-
dase: rabbit anti-peroxidase complex, and a mixture of 4-chloro-1-naph-
thol and H<sub>2</sub>O<sub>2</sub> (17). Each nitrocellulose sheet contained a series of amounts of 0.5-5 µg of the purified antigen for the preparation of a standard curve. In these particular studies an internal standard antigen and antibody were not utilized (18). The density of each stained band was estimated by reflectance densitometry and the apparent concentra-
tion of the antigen in each liver sample was calculated. We have found that the presence of a crude hepatic protein mixture in a lane does not influence the staining intensity (and, thus, the apparent amount of antigen) of purified antigens, at least in the case of P-450<sub>sw</sub> (19).

In the case of epoxide hydrolase, 10 µg of liver or tumor homogenate protein were electrophoresed in each lane. The antiserum (rabbit anti-
human epoxide hydrolase) was used at a dilution of 1:200. The antigen and antibody have been described elsewhere (20, 21).

With the human liver cytochromes P-450, 50 µg of liver or tumor homogenate protein were electrophoresed in each lane. The antigens (P-450<sub>sw</sub> and P-450<sub>ahp</sub>) are two of the major forms of cytochrome P-450 found in human liver and, along with the antibodies, have been de-
scribed elsewhere (22, 23). The antisera were both used at dilutions of 1:100.

Fluorometric and Spectrophotometric Assays

Enzymes involved in conjugation reactions (Phase II reactions) were assayed using highly fluorescent umbelliferone or its glucuronide and sulfate conjugates as substrates. 1-Chloro-2,4-dinitrobenzene was used as a substrate to measure glutathione S-transferase (24). Under conditions described below, all of the assays were linearly proportional with time and tissue.

UDP-Glucuronosyltransferase Activity. This was measured in liver homogenates which were equivalent to 5 µg wet weight and were added to 10 µl reagent containing 10 mM UDP-glucuronic acid and 100 µM umbelliferone. After a 60-min incubation at 37°C, the unreacted um-
belliferone was extracted and the formed glucuronide was hydrolyzed

Table 1

| Table 1 Biochemical changes associated with experimental hepatocarcinogenesis |
|-----------------------------------------|-----------------------------|
| Chemical mutagens                      | Peroxisomal proliferators  |
| Mixed-function oxygenases              | (2, 3, 33, 49)             |
| Epoxide hydrolase                      | (3, 13)                    |
| Glutathione S-transferases             | (2, 11, 11)                |
| γ-Glutamyltranspeptidase               | (2, 3)                     |
| UDP-Glucuronosyltransferase            | (2, 3)                     |
| Mutagenicity and DNA damage            | (2, 3)                     |

* Numbers in parentheses, reference numbers.

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belliferone was extracted and the formed glucuronide was hydrolyzed

Table 2

| Table 2 Patient characteristics |
|--------------------------------|-----------------------------|
| Diagnosis                      | Age (yr) and sex | % of tumor-free parenchyma* | Abnormal liver function testsb | Remarks |
| Normal (immediate autopsy)    | 16-36 (5 M, 1 F) | 100                          | ALP (471-1998) [5] | One alcoholic |
| Colon cancer (N = 5)          | 60-84 (2 M, 3 F) | 100                          | LDH (267-791) [6] | One smoker |
| Colon cancer with liver metastasis (N = 14) | 33-67 (9 M, 5 F) | 66 (25-95) | SGOT and SGPT (86-255) [3] | One alcoholic |
| Hepatoma (N = 8)              | 36-66 (6 M, 2 F) | 33 (20-70) | ALP (393-1530) [4] | One smoker and alcoholic |

* Based on evaluation of computer-assisted tomography scan and surgical estimate.

b Liver function tests: ALP, alkaline phosphatase (N = 95-300 milliunits/ml); LDH, lactate dehydrogenase (N = 80-180 milliunits/ml); SGOT and SGPT, glutamic-oxaloacetic transaminase and glutamic-pyruvic transaminase (N = 10-30 milliunits/ml).

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OXIDATIVE AND CONJUGATIVE ENZYMES IN HEPATIC TUMORS

RESULTS

Cytochromes P-450 and epoxide hydrolase were measured in the four groups of patients and the results are shown in Fig. 1 and Table 3. No consistent changes in levels of cytochrome P-450 or epoxide hydrolase occurred in samples from patients with adenocarcinoma of the colon, with or without liver metastasis. The hepatic parenchyma surrounding the metastatic nodules had cytochrome P-450 levels comparable to that in distant tissue from the nodules. In contrast to results obtained in patients with adenocarcinoma, both forms of cytochrome P-450 were reduced to 45% of control values in primary hepatomas. Activities of these enzymes were not reduced in regions distant from hepatomas. Epoxide hydrolase in specimens from secondary tumors was increased significantly (P < 0.01) in perinodular but not in distant regions compared to normal values. In primary tumors, epoxide hydrolase tended to be lower in the nodular regions. Only traces of polypeptides other than the antigens were recognized by the antibodies (Fig. 1), and the molecular weights of the protein were unchanged in the tumors. Activities of enzymes associated with conjugation reactions are shown in Table 4. Apart from glutathione S-transferase, which was more active, all other enzymes measured in liver homogenates from nonmetastasizing adenocarcinoma of the colon were the same as those measured in normal livers, and

Table 3 Cytochromes P-450 and epoxide hydrolase in human liver malignancies

<table>
<thead>
<tr>
<th></th>
<th>Cytochrome P-450 (pmol/mg protein)</th>
<th>Epoxide hydrolase (pmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P-450&lt;sub&gt;hE&lt;/sub&gt;</td>
<td>P-450&lt;sub&gt;hF&lt;/sub&gt;</td>
</tr>
<tr>
<td>Control (N = 6)</td>
<td>10.6 ± 1.1&lt;sup&gt;±&lt;/sup&gt;</td>
<td>10.6 ± 0.7</td>
</tr>
<tr>
<td>Colon cancer (N = 4)</td>
<td>15.6 ± 0.4</td>
<td>11.8 ± 1.0</td>
</tr>
<tr>
<td>Secondary from colon cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perinodular regions (N = 9)</td>
<td>11.9 ± 1.1</td>
<td>13.1 ± 1.8</td>
</tr>
<tr>
<td>Distant regions (N = 10)</td>
<td>14.5 ± 1.5</td>
<td>11.2 ± 1.1</td>
</tr>
<tr>
<td>Hepatoma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nodular regions (N = 6)</td>
<td>4.4 ± 0.2</td>
<td>5.3 ± 2.2&lt;sup&gt;±&lt;/sup&gt;</td>
</tr>
<tr>
<td>Distant regions (N = 4)</td>
<td>11.4 ± 3.0</td>
<td>12.6 ± 2.7</td>
</tr>
</tbody>
</table>

<sup>±</sup> Mean ± SE.  
<sup>±</sup> P < 0.01 compared to control.  
<sup>±</sup> P < 0.05 compared to control.
OXIDATIVE AND CONJUGATIVE ENZYMES IN HEPATIC TUMORS

Table 4 Hepatic conjugating enzymes and hydrolases in human liver malignancies

Enzyme activities were measured in liver homogenates as described in "Materials and Methods." Data were analyzed statistically using analysis of variance.

<table>
<thead>
<tr>
<th>Group</th>
<th>UDP-Glucuronosyl-transferase</th>
<th>β-Glucuronidase</th>
<th>Sulfotransferase</th>
<th>Sulfatase</th>
<th>Glutathione S-transferase (x 10^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (N = 6)</td>
<td>9.9 ± 2.0*</td>
<td>570 ± 86</td>
<td>119 ± 15</td>
<td>288 ± 56</td>
<td>11.1 ± 1.6</td>
</tr>
<tr>
<td>Colon cancer (N = 5)</td>
<td>6.9 ± 0.8</td>
<td>433 ± 85</td>
<td>149 ± 15</td>
<td>202 ± 22</td>
<td>18.0 ± 2.0*</td>
</tr>
<tr>
<td>Secondary from colon cancer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perinodular regions (N = 10)</td>
<td>11.1 ± 1.0</td>
<td>779 ± 96</td>
<td>106 ± 12</td>
<td>221 ± 25</td>
<td>7.3 ± 1.4</td>
</tr>
<tr>
<td>Distant regions (N = 10)</td>
<td>12.2 ± 0.9</td>
<td>657 ± 95</td>
<td>118 ± 10</td>
<td>239 ± 29</td>
<td>8.7 ± 1.0</td>
</tr>
<tr>
<td>Hepatoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nodular regions (N = 8)</td>
<td>4.4 ± 1.3c</td>
<td>311 ± 44b</td>
<td>34 ± 8c</td>
<td>40 ± 17d</td>
<td>4.6 ± 0.7f</td>
</tr>
<tr>
<td>Distant regions (N = 5)</td>
<td>5.9 ± 1.4</td>
<td>347 ± 68</td>
<td>108 ± 32</td>
<td>129 ± 56</td>
<td>11.7 ± 2.7</td>
</tr>
</tbody>
</table>

* Mean ± SE.
^ P < 0.05 (compared to control).
* * P < 0.001 (compared to control).
* * * P < 0.01 (compared to control).

Table 5 Kinetic properties of arylsulfatase in normal human liver and primary hepatic tumors

Values are kinetic constants ± SE calculated by the Wilkinson weighted regression analysis (54). Each value is a separate experiment done in duplicate using 4-methylumbelliferyl sulfate concentrations ranging between 5 and 500 µM. Specimens from two patients in each group were used to determine Vₘₐₓ and Kₘ in each of the three groups.

<table>
<thead>
<tr>
<th>Liver</th>
<th>Vₘₐₓ nmol/mg protein/hr</th>
<th>Kₘ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>406 ± 20</td>
<td>164 ± 18</td>
</tr>
<tr>
<td>HL84-5</td>
<td>132 ± 28</td>
<td>159 ± 39</td>
</tr>
<tr>
<td>HL84-1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
<td>4.7 ± 0.2</td>
<td>166 ± 18</td>
</tr>
<tr>
<td>SOL85-6 T</td>
<td>4.7 ± 0.1</td>
<td>139 ± 11</td>
</tr>
<tr>
<td>SOL85-17 T</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholangiocarcinoma</td>
<td>192 ± 14</td>
<td>277 ± 35</td>
</tr>
<tr>
<td>SOL85-12 T</td>
<td>33 ± 1.5</td>
<td>137 ± 14</td>
</tr>
</tbody>
</table>

Table 6 Arylsulfatase activity in diethylnitrosamine-induced hyperplastic nodules in rat liver and human hepatic malignancies

Sulfatase activity was assayed using 4-methylumbelliferyl sulfate as substrate as described in "Materials and Methods."

<table>
<thead>
<tr>
<th>Rat activity (nmol/mg protein/hr)</th>
<th>Human activity (nmol/mg protein/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal liver</td>
<td>153 ± 8f (3)</td>
</tr>
<tr>
<td>Pathological liver</td>
<td></td>
</tr>
<tr>
<td>Nodular region</td>
<td>203 ± 22 (3)</td>
</tr>
<tr>
<td>Distant region</td>
<td>239 ± 15c (8)</td>
</tr>
<tr>
<td>Hepatoma</td>
<td></td>
</tr>
<tr>
<td>Secondary from colon cancer</td>
<td></td>
</tr>
</tbody>
</table>

* Mean ± SE of normal and pathological liver samples.
^ Numbers in parentheses, number of animals or humans.
* * P < 0.01 (compared to normal).
* * Data from perinodular region.

there were no significant differences in activities of the transferases and hydrolases in samples from patients with secondary liver tumors from carcinoma of the colon. Enzyme activities in the perinodular and distant regions of samples from these patients were similar. In contrast, all enzyme activities associated with conjugation were depressed markedly in primary hepatic tumors. Sulfatase was the most affected enzyme (14% of normal) and the reduction in sulfatase activity greatly exceeded that of sulfotransferase.

Measurement of arylsulfatase in postmitochondrial supernatant fractions of homogenates from normal liver and hepatocellular carcinoma indicated that the major fraction of aryl-sulfatase was contained in the 9000 x g particulate fraction of normal liver and about equally distributed between the 9000 x g particulate and supernatant fractions in homogenates of the primary hepatoma. In contrast, β-glucuronidase was concentrated primarily in the postmitochondrial supernatant fraction in homogenates of both normal liver and primary hepatoma. Activities of β-glucuronidase measured in homogenates of a normal liver (HL-84-5T; Table 5) and primary hepatoma (SOL-85-17T; Table 5) were 526 and 399 nmol/mg protein/hr, respectively. The same activities measured in the 9000 x g supernatant fractions of these homogenates were 718 and 514 nmol/mg protein/hr.

Because of the marked decrease of sulfatase activity in hepatomas and the striking difference in its activity between the two types of primary tumor, because activities of the other enzymes involved in drug conjugation were essentially the same in both types of hepatomas. The kinetic properties of sulfatase using 4-methylumbelliferone sulfate as a substrate in normal liver and the two types of primary hepatic tumors appears in Table 5. No significant differences were found in Kₘ values for the enzyme; however, the Vₘₐₓ in the hepatocellular carcinoma appeared to be at least 10 times lower than that in the cholangiocarcinoma sample. Given the large variation in Vₘₐₓ among the various samples, additional data are needed for meaningful statistical comparison. Collectively the data indicate that differences in sulfatase activity among the various samples are due primarily to differences in the amounts of sulfatase rather than to differences in the kinetic properties of the enzyme.

Arylsulfatase activity increased in hyperplastic nodules induced in rat livers by diethylnitrosamine (133% in nodular region and 156% in distant regions) (Table 6). These increases are notably different from the marked reductions in sulfatase activity observed in human livers with primary hepatomas.

Since the sublobular distribution of the drug-metabolizing enzymes may be affected by the hepatic carcinogenesis, this possibility was evaluated in liver specimens from patients with secondary tumors (Table 7). The sublobular distribution of the drug-metabolizing enzymes was studied only in livers with secondary tumors because of the difficulty in distinguishing normal liver structure in samples taken from patients with primary hepatomas. In general, the transferases were heterogeneous in their distribution and the hydrolase was evenly distributed across the liver lobule. Glucuronosyltransferase and glutathione S-transferase were significantly higher in pericentral than peripherally regions of liver specimens from metastatic...
carcinoma of colon (P < 0.01 and 0.001, respectively). This heterogeneous distribution of the two transferases across the liver lobule is similar to that observed in normal livers (24). In contrast to the transferases, β-glucuronidase was evenly distributed across the liver lobule.

**DISCUSSION**

Adenocarcinoma of the colon with or without liver metastasis did not influence the activities of hepatic drug-metabolizing enzymes involved in both Phase I (oxidative) and Phase II (conjugative) reactions. In contrast, all of the drug-metabolizing enzymes were decreased in primary hepatic malignancies. Arylsulfatase was the most markedly depressed in livers of these patients. Apart from UDP-glucuronosyltransferase and sulfatase, decreases in enzyme activities were localized mainly in nodular areas and may represent a general decrease due to a higher density of extracellular protein in these regions. Although most enzyme activities in regions distant from the tumor were within the normal range, these regions account for only one-third of the liver. Therefore, changes in drug-metabolizing enzymes in primary hepatic malignancies may have significant effects on the pharmacokinetics of specific agents in vivo and on the development of hepatocellular carcinoma. This change resembles decreases noted in livers of rats treated with chemical mutagens (2, 3, 11). It is noteworthy that this enzyme is depressed in hepatic tumors induced by peroxisome proliferators, but is quite different from the marked increases in glutathione S-transferases, which is significantly increased in chemical mutagen-induced hepatocellular carcinoma, was proposed as a possible marker for hepatocarcinogenesis (11, 12, 37). Glutathione S-transferase activities has been noted previously in studies of human hepatocarcinogenesis with primary hepatomas studied above had cirrhotic livers. It would seem worthwhile to examine alterations in the placental form of the enzyme in specific hepatic tumors obtained by surgical biopsy.

A strong relation between alcoholic cirrhosis and the development of hepatocellular carcinoma has been reported (40). It is not known whether the changes in hepatic drug-metabolizing enzymes associated with hepatocarcinogenesis are due to cirrhosis; however, this seems unlikely because only 2 of 8 patients with primary hepatomas studied above had cirrhotic livers.

Comparison of experimental hepatocarcinogenesis with liver tumors in humans is very difficult, and extrapolation of data from laboratory animals with chemically induced neoplasias to humans with primary and metastatic hepatic tumors must entail caution. Nevertheless, data presented above suggest that changes in drug-metabolizing enzymes in primary hepatomas of human liver resemble those seen in livers of rats treated with peroxisome proliferators. With chemical mutagens such as 2-

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**Table 7** Sublobular distribution of drug-metabolizing enzymes in human liver with secondary tumors

<table>
<thead>
<tr>
<th>Region</th>
<th>Glucuronosyltransferase</th>
<th>β-Glucuronidase</th>
<th>Glutathione S-transferase (× 10⁻⁴)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Periportal</td>
<td>23 ± 4.3* (41)</td>
<td>365 ± 29 (38)</td>
</tr>
<tr>
<td></td>
<td>Pericentral</td>
<td>34 ± 3.1* (34)</td>
<td>388 ± 32 (30)</td>
</tr>
<tr>
<td>Secondary from Colon Cancer</td>
<td>Periportal</td>
<td>16.1 ± 0.9 (21)</td>
<td>431 ± 19 (21)</td>
</tr>
<tr>
<td>Perinodular region</td>
<td>Pericentral</td>
<td>32.5 ± 2.5* (20)</td>
<td>458 ± 21 (17)</td>
</tr>
<tr>
<td>Distant region</td>
<td>Periportal</td>
<td>37.3 ± 1.7 (8)</td>
<td>405 ± 14 (11)</td>
</tr>
<tr>
<td></td>
<td>Pericentral</td>
<td>56.9 ± 1.5* (11)</td>
<td>425 ± 10 (10)</td>
</tr>
</tbody>
</table>

* Mean ± SE as carried out on 4 normal livers and 2 liver specimens from metastatic colon cancer using microhistochemical techniques described in "Materials and Methods."

* Numbers in parentheses, number of determinations.

* P < 0.01 periportal compared to pericentral zone in the same group.

* P < 0.05 periportal compared to pericentral zone in the same group.

* P < 0.001 periportal compared to pericentral zone in the same group.

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4 M. S. Rao, personal communication.
acetylaminoflourene, all Phase II drug-metabolizing transferases including epoxide hydrolase were increased and the mixed-function oxidases were decreased (2, 3, 11, 13). These alterations associated with chemical mutagens support the “resistant hepatocyte” theory of hepatocarcinogenesis (41, 42). On the other hand, peroxisome proliferator-induced experimental hepatocarcinogenesis is associated with a decrease of all Phase II drug-metabolizing enzymes including epoxide hydrolase (36, 43). The activity of arylsulfatase has not been determined in the peroxisome proliferator model of experimental hepatocarcinogenesis, and it would be interesting to compare its activity in this model with that in human hepatocarcinomas. Another activity that might be compared with changes in human primary hepatomas is γ-glutamyltranspeptidase, which is absent in the peroxisomal proliferator model (44), but increased markedly in livers of rats treated with chemical mutagens (45, 46). A recent study using a specific antibody for γ-glutamyltranspeptidase confirmed the absence of this protein in altered hepatocytic foci induced by hypolipidemic peroxisomal proliferators (47).

Mixed-function oxidases are decreased in liver after prolonged administration of hypolipidemic peroxisome proliferators; however, administration of clobarbrate for shorter periods of time (14 days) has been associated with induction of cytochrome P-450 (48). Taken together, data above indicate that changes associated with chemical mutagens support the “resistant hepatocyte” theory of hepatocarcinogenesis, and it would be interesting to compare its activity in this model with that in human hepatocarcinomas. Another activity that might be compared with changes in human primary hepatomas is γ-glutamyltranspeptidase, which is absent in the peroxisomal proliferator model (44), but increased markedly in livers of rats treated with chemical mutagens (45, 46). A recent study using a specific antibody for γ-glutamyltranspeptidase confirmed the absence of this protein in altered hepatocytic foci induced by hypolipidemic peroxisomal proliferators (47).

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Hepatic Drug-metabolizing Enzymes in Primary and Secondary Tumors of Human Liver


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