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 $\beta$-glucuronidase and arylsulfatase, were measured in liver samples from six 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, and UDP-glucuronosyltransferase, $\beta$-glucuronidase, sulfotransferase, and sulfatase 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, and UDP-glucuronosyltransferase, $\beta$-glucuronidase, sulfotransferase, and sulfatase 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, arylsulfatase, and glutathione S-transferase, and sulfatase) or spectrophotometrically (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 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-acetylamino-7-fluorene 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-acetylamino-7-fluorene (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, arylsulfatase 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 cardiopulmonary supports after death until the organs were reperfused with perfusate. Organ donors were placed on cardiopulmonary supports after death until the organs were reperfused with perfusate.

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.

**Table 1** Biochemical changes associated with experimental hepatocarcinogenesis

<table>
<thead>
<tr>
<th>Chemical mutants</th>
<th>Peroxisomal proliferators</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixed-function oxygenases</td>
<td>↑ (2, 3, 33, 49) ↓</td>
</tr>
<tr>
<td>Epoxide hydrolase</td>
<td>↑ (3, 13) ↓</td>
</tr>
<tr>
<td>Glutathione S-transferases</td>
<td>↑ (2, 3, 11) ↓</td>
</tr>
<tr>
<td>γ-Glutamyltranspeptidase</td>
<td>↑ (2, 11, 51) ↓</td>
</tr>
<tr>
<td>UDP-Glucuronosyltransferase</td>
<td>↑ (2, 3) ↓</td>
</tr>
<tr>
<td>Mutagenicity and DNA damage</td>
<td>↑↑ (50) ↓</td>
</tr>
</tbody>
</table>

*↑*, increased; *↓*, decreased; *→*, unchanged.

**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>nr</sub> and P-450<sub>ap</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, horseradish peroxidase:antiperoxidase rabbit antiperoxidase complex, and a mixture of 4-chloro-1-naphthol and H<sub>2</sub>O<sub>2</sub> (17). Each nitrocellulose sheet contained a series of standards (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 concentration 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>nr</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>nr</sub> and P-450<sub>ap</sub>) are two of the major forms of cytochrome P-450 found in human liver and, along with the antibodies, have been described 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. l-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 homogenate 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 umbelliferone was extracted and the formed glucuronide was hydrolyzed.

**Table 2** Patient characteristics

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Age (yr) and sex</th>
<th>% of tumor-free parenchyma&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Abnormal liver function tests&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (immediate autopsy) (N = 6)</td>
<td>16–36 (5 M, 1 F)</td>
<td>100</td>
<td>ALP (471–1998)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>One alcoholic</td>
</tr>
<tr>
<td>Colon cancer (N = 5)</td>
<td>60–84 (2 M, 3 F)</td>
<td>100</td>
<td>LDH (267–791)</td>
<td>One smoker</td>
</tr>
<tr>
<td>Colon cancer with liver metastasis (N = 14)</td>
<td>33–67 (9 M, 5 F)</td>
<td>66 (25–95)</td>
<td>SGOT and SGPT (86–255)</td>
<td>One alcoholic</td>
</tr>
<tr>
<td>Hepatoma (N = 8)</td>
<td>36–66 (6 M, 2 F)</td>
<td>33 (20–70)</td>
<td>ALP (393–1530)</td>
<td>One smoker and alcoholic</td>
</tr>
</tbody>
</table>

<sup>a</sup>Based on evaluation of computer-assisted tomography scan and surgical estimate.

<sup>b</sup>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).

<sup>c</sup>Numbers in parentheses, range.

<sup>d</sup>Numbers in brackets, number of cases with abnormal liver function tests.
by adding β-glucuronidase. The liberated umbelliferone was measured fluorometrically (excitation, 340 nm; emission, 420 nm) (24).

Glutathione S-transferase Activity. This was assayed by using an adaptation of the technique described by Habig et al. (25). Formation of the 1-chloro-2,4-dinitrobenzene-glutathione conjugate by liver homogenate equivalent to 10 µg wet weight was measured continuously in a spectrophotometer at 340 nm using an extinction coefficient of 9.6 mM−1·cm−1 (25).

β-Glucuronidase Activity. This was determined by incubating liver homogenates, equivalent to 5 µg wet weight, in 10 µl of reagent composed of 75 mM sodium acetate buffer (pH 4.7), 0.02% bovine serum albumin, and 0.5 mM methylumbelliferone glucuronide. After 30 min at 37°C, the released methylumbelliferone was measured fluorometrically as described above.

Sulfatase Activity. This was measured in liver homogenates incubated at 37°C in the presence of 200 µM 4-methylumbelliferone sulfate. After a 30-min incubation the liberated methylumbelliferone was measured fluorometrically as described above.

Sublobular Distribution of Enzymes. The distribution of UDP-glucuronosyltransferase, β-glucuronidase, and glutathione S-transferase across the liver lobule was determined by using quantitative microhistochemical sampling techniques as described previously (15, 26).

Induction of Hyperplastic Nodules in Rat Livers

Nodules were induced in male Fischer 344 rats weighing 150–200 g (Charles River Breeding Laboratories, Inc., Wilmington, MA) by giving diethylnitrosamine (40 ppm) in their drinking water for 4 months. Control rats were kept under similar conditions. Hyperplastic nodules and the adjacent liver tissue were sampled 3 weeks after diethylnitrosamine was removed from the animals' drinking water (27). Hyperplastic nodules stained positively for γ-glutamyltranspeptidase. These specimens and liver samples from control rats were frozen in liquid nitrogen and stored at −80°C until assayed for arylsulfatase activity in homogenates as described above.

Materials

Substrates (umbelliferone, 4-methylumbelliferone sulfate and glucuronide, reduced glutathione, 1-chloro-2,4-dinitrobenzene, and d-saccharic acid-1,4-lactone) and enzymes (β-glucuronidase and sulfatase) used in this study were purchased from Sigma Chemical Co. (St. Louis, MO). All other chemicals used were of the highest purity obtained from standard commercial sources.

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 P-450 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
there were no significant differences in activities of the trans-
ferases 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 associ-
ated 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 ex-
ceeded that of sulfotransferase.

Measurement of arylsulfatase in postmitochondrial super-
natant fractions of homogenates from normal liver and hepa-
tocellular 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 concen-
trated 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/h, re-
spectively. The same activities measured in the 9000 x g super-
natant fractions of these homogenates were 718 and 514 nmol/
mg protein/h.

Because of the marked decrease of sulfatase activity in hepa-
tomas and the striking difference in its activity between the
two types of primary hepatic malignancies, the possibility of
the presence of different forms of the enzyme was considered.
Sulfatase activity was at least 10-fold lower in some samples of
hepatocellular carcinoma than that in cholangiocarcinoma (data
not shown). The difference in sulfatase activity in hepa-
tocellular and cholangiocarcinoma could be a significant differ-
ence between these two types of primary tumor, because activ-
ities 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 $K_m$ values for the enzyme; however, the $V_{max}$ in
the hepatocellular carcinoma appeared to be at least 10 times lower
than that in the cholangiocarcinoma sample. Given the large
variation in $V_{max}$ 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.

Arylsulphatase 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 hetero-
geneous in their distribution and the hydrolase was evenly
distributed across the liver lobule. Glucuronosyltransferase and
glutathione S-transferase were significantly higher in pericen-
tral than peripherotubular regions of liver specimens from metastatic

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

<table>
<thead>
<tr>
<th>Group</th>
<th>$V_{max}$ nmol/mg protein/hr</th>
<th>$K_m$ (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (N = 6)</td>
<td>164 ± 18</td>
<td>406 ± 20</td>
</tr>
<tr>
<td>Colon cancer (N = 5)</td>
<td>159 ± 39</td>
<td>132 ± 28</td>
</tr>
<tr>
<td>Nodular region</td>
<td>160 ± 18</td>
<td>4.7 ± 0.2</td>
</tr>
<tr>
<td>Distant region</td>
<td>139 ± 11</td>
<td>4.7 ± 0.1</td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
<td>277 ± 35</td>
<td>192 ± 14</td>
</tr>
<tr>
<td>Cholangiocarcinoma</td>
<td>137 ± 14</td>
<td>33 ± 1.5</td>
</tr>
</tbody>
</table>

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 $V_{max}$ and $K_m$
in each of the three groups.
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, $\beta$-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 about one-third of the liver. Therefore, changes in drug-metabolizing enzymes in primary hepatomas may have significant effects on the pharmacokinetics of specific agents in vivo and may contribute to alterations in the endocrine status of these patients. The marked decreases in arylsulfatase in primary hepatomas suggest that the metabolism of sulfate conjugates may be particularly affected in patients with primary hepatocellular carcinoma.

Although it is well known that the transferases and hydrolases studied exist in multiple forms (28, 29), only those isozymes that utilize methylumbelliferyl conjugates or umbelliferone as substrates were measured in the present study. The possibility that other isozymes of transferases and hydrolases exhibit different response in primary and secondary hepatic tumors cannot be ruled out. Activities of glutathione $S$-transferase assayed in this study can be compared with data from most other studies which also use l-chloro-2,4-dinitrobenzene (2, 3, 11, 30, 31). The placental form of glutathione $S$-transferase, which is significantly increased in chemical mutagen-induced hepatocellular carcinoma, was proposed as a possible marker for hepatocarcinogenesis (11, 12, 37). It is not known whether this form of glutathione $S$-transferase is also induced or depressed in non-DNA-damaging hepatocarcinogenesis (peroxisomal proliferators). Antibodies raised against the placental form of glutathione $S$-transferase from rats were the only form expressed in non-DNA-damaging hepatocarcinogenesis. Antibodies raised against the human form indicates that the (human) placental form of glutathione $S$-transferase is present in human colonic carcinomas and adenomas (38). This placental form in human hepatic tumors and non-tumor tissues obtained by autopsy has been assayed by immunodiffusion and found to be increased in tumor tissue when compared with non-tumor tissue (39). In view of the marked changes noted above in glutathione $S$-transferases, 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-
acetylaminofluorene, 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 transferases 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 hepatic tumors. 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 hepatocellular foci induced by hypolipidemic peroxisomal proliferators (47). Mixed-function oxidases are decreased in liver after prolonged administration of hypolipidemic peroxisome proliferators; however, administration of clofibrate for shorter periods of time (14 days) has been associated with induction of cytochrome P-450 (48). Taken together, data above indicate that changes in drug-metabolizing enzymes in primary hepatic tumors in humans more closely resemble those that occur in non-DNA-damaging, peroxisome proliferator-induced hepatocellular carcinomas than those carcinomas induced by classic genotoxic agents.

ACKNOWLEDGMENTS

The authors are grateful to Dr. Ronald Thurman, Department of Pharmacology, School of Medicine, University of North Carolina at Chapel Hill, for graciously providing liver specimens from dibutylnitrosamine-treated rats, and to Roxanne Evans for her technical assistance in assembling the manuscript.

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OXIDATIVE AND CONJUGATIVE ENZYMES IN HEPATIC TUMORS


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*Cancer Res* 1987;47:460-466.

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