

diseases including carcinomas. In this paper, we demonstrate that GST- $\pi$  is a useful immunohistochemical marker for differential diagnosis of carcinomas originating from epithelial cells of the bile duct system.

### MATERIALS AND METHODS

**Materials:** The details of cases used in the present study are shown in Table I. The materials taken from all the cases were obtained at autopsy, removed surgically or biopsied at either Nara Medical College Hospital or its associated hospitals since 1982. All materials had been fixed in 10% formalin and embedded in paraffin.

The diagnoses described were based on histological examinations and those of hepatobiliary tumors were defined according to the General Rules for the Clinical and Pathological Studies of Primary Liver Cancer<sup>11)</sup> and to the General Rules for Surgical Studies on Cancer of the Biliary Tract.<sup>12)</sup> For hepatocellular carcinomas, Edmondson's classification<sup>13)</sup> was employed in the definition of the grade differentiation of cancer cells.

**Immunohistochemical staining:** The ABC method was employed for immunohistochemical staining. Anti GST- $\pi$  serum was kindly supplied by Professor Kiyomi Sato, Second Department of Biochemistry, Hirosaki University, School of Medicine, Hirosaki. Paraffin embedded specimens were cut to 5 $\mu$ m in thickness and were dewaxed in xylene and graded ethanol. The sections were stained by the ABC method (Vectastain ABC Kit, Vector Labo., Burlingame, Calif.). The sections were incubated with anti-human GST- $\pi$  rabbit antibody at 1:2000 dilution for 120 minutes at room temperature, and then stained with a solution of 0.05% 3,3' diaminobenzidine tetrahydrochloride (DAB, Nakarai Chemicals Ltd., Kyoto) in Tris-HCl buffer for 5 minutes and rinsed quickly in distilled water. Finally, they were counterstained with Mayer's hematoxylin. As a negative control for testing the specificity of anti-GST- $\pi$  antibody, pre-immune rabbit serum was used instead of the antiserum. In histopathological examinations, hematoxylin and eosin (H & E) staining sections were performed.

Table I. Details of cases examined for GST- $\pi$  activity in hepato-biliary diseases

Source	Sex		Number of cases			Total
	F	M	Age distribution	Autopsied	Removed or biopsied	
Fetal liver	5		18-38 weeks	5	0	5
Without liver disease	10	12	0-89	18	4	22
Chronic hepatitis	1	1	63	0	2	2
Liver cirrhosis	4	9	44-79	4	9	13
Hepatocellular carcinoma	4	22	44-75	24	2	26
Cholangiocellular carcinoma*	1	1	65-71	1	1	2
Cancer of the biliary tract**	5	5	59-77	4	6	10

\*: Based on the General Rules for the Clinical and Pathological Study of Primary Liver Cancer.<sup>11)</sup>

\*\* : Based on the General Rules for Surgical Studies on Cancer of the Biliary Tract.<sup>12)</sup>

## RESULTS

GST- $\pi$  activity in various human hepato-biliary diseases was studied and compared with that in fetal liver and undiseased adult liver (Table II). The GST- $\pi$  activity was clearly positive or strongly positive in the epithelial cells of intra-hepatic bile duct in fetal and undiseased adult liver (Figs. 1, 2) and of extra-hepatic bile duct and gall bladder (Fig. 3). Hepatocytes of fetal liver showed a positive staining but GST- $\pi$  activity was decreased following increase of the gestation period (Fig. 1). Hepatocytes of adult liver showed a negative to positive activity and there was a tendency for the GST- $\pi$  staining to be more intense in the periportal area (Fig. 2). Epithelial cells in the mucosa of undiseased gall bladder showed positive to strongly positive staining. The hepatocytes in cases of chronic viral hepatitis were stained positively throughout the lobule. In posthepatic cirrhosis, hepatocytes in regenerating nodules were positively stained. Proliferated and non-proliferated bile duct epithelial cells showed positive to strongly positive staining.

Hepatocellular carcinomas were histologically defined as 20 cases of trabecular, 1 case of pseudoglandular, and 5 cases of scirrhous carcinoma. By Edmondson's classification, 2 cases were grade I, 11 were grade II and III, respectively, and 2 were grade IV. None of these cases showed strongly positive GST- $\pi$  activity. Among GST- $\pi$  activities of histological types and grades of differentiation of cancer cells, no correlation could be found in hepatocellular carcinomas (Fig. 4). The 2 cases of cholangiocarcinoma (Fig. 5) and 7 out of 10 cases of cancer of the biliary tract showed strongly positive activity. Histologically, cancers of the biliary tract were well-or moderately differentiated adenocarcinomas except for 1 case of undifferentiated carcinoma which showed positive activity (Fig. 6).

Table II. GST- $\pi$  activity in epithelial cells of various human hepato-biliary diseases

Cases	No. of cases	Cells examined	No. of cases showing GST- $\pi$ activity*			
			-	$\pm$	+	++
Fetal liver	5	Hepatocytes	1	1	3	0
		Bile duct	0	0	0	5
Liver without disease	22	Hepatocytes	8	10	4	0
		Bile duct	0	0	5	17
Extrahepatic bile duct without disease	12	Bile duct	0	0	5	7
Gall bladder without disease	3	Gallbladder	0	0	2	1
Chronic hepatitis	2	Hepatocytes	0	0	2	0
		Bile duct	0	0	0	2
Cirrhosis	13	Hepatocytes	0	2	11	0
		Bile duct	0	0	5	8
Hepatocellular carcinoma	26	Cancer cells	15	9	2	0
Cholangiocellular carcinoma	2	Cancer cells	0	0	0	2
Cancer of the biliary tract	10	Cancer cells	0	1	2	7

\* -: Negative

 $\pm$ : Consisting of negative and positive cells

+: Positive

++: Strongly positive

## DISCUSSION

Recently, a group of GSTs were purified and the use of immunohistochemical demonstration of GSTs was evaluated in chemically-induced rat liver carcinogenesis with the result that GSTs were considered to be more sensitive markers of putative preneoplastic hepatocyte foci than  $\gamma$ -glutamyl transpeptidase ( $\gamma$ -GT).<sup>7)</sup> GST- $\pi$  was subsequently purified from human placenta and its physico-chemical and immunological properties were described.<sup>14)</sup> The present study showed that in hepatocytes, the intensity of GST- $\pi$  staining depended upon the extent of fetal aging, a stronger intensity being observed in aged fetal liver of 38 weeks, and thereafter in adult liver, the GST- $\pi$  activities were decreased. These findings are compatible with biochemical results previously reported in rats. Studies on  $\alpha$ -fetoprotein have shown that the serum level of this protein is high in the fetus and decreases after birth, but that it reappears at a high level when hepatocellular carcinoma develops.<sup>1)</sup> Furthermore, these findings obtained on serum  $\alpha$ -fetoprotein levels are compatible with the tissue levels of this protein revealed by immunohistochemical studies.<sup>3)</sup>

No cases of hepatocellular carcinoma showed a strongly positive GST- $\pi$  reaction in the present investigation.

The purification and subunit-structural and immunological characterization of five isozymes of glutathione S-transferases in human liver have been reported<sup>15)</sup>, and the acidic form has been shown to be a hepatic tumor marker.<sup>16)</sup> However, in the latter report, no definite histological description of a hepatic tumor was given, so the possibility exists that the tumor might not have been a hepatocellular carcinoma. In rat hepatocarcinogenesis, it is well known that carcinogens alter the pattern of drug-metabolizing enzymes, leading to partial or total resistance of the putative preneoplastic cells to the chemical toxicity, and then causing phenotypical expression of the genes for various enzymes.<sup>7)</sup> The hepatocellular carcinomas studied in the present investigation were obtained from humans, for which the details of chemical involvement in hepatocarcinogenesis are not well understood.

Hepatocytes in regenerating cirrhotic nodules showed stronger GST- $\pi$  activity than that in adult liver without disease.

Thus, further studies are needed to clarify the significance of the appearance of GST- $\pi$  in hepatocyte regeneration, both biochemically and immunohistochemically.

Intra- and extrahepatic bile duct epithelial cells were clearly stained with GST- $\pi$  and the intensity of staining was preserved on cholangiocarcinomas and cancer of the biliary tract. However, among these carcinomas, the intensity of staining decreased in parallel with the decrease of the degree of cancer cell differentiation, from well- to moderately differentiated, and to poorly differentiated carcinomas. A similar tendency has been observed in human gastric and pancreatic carcinomas.<sup>8)10)</sup> In contrast, in human colonic tumor GST- $\pi$  activity is phenotypically expressed and increases with cancer cell differentiation.<sup>9)</sup> As GST- $\pi$  is observed in organs originated from endodermal (unpublished data), the difference of intensity of the GST- $\pi$  activity observed in the cancer cells arising in the

above organs might be due to the normal distribution of this enzyme, since normal epithelial cells in bile duct, pancreatic duct and stomach mucosa are positively but colonic mucosa are negatively stained. Nevertheless, the present results indicate that GST- $\pi$  is a useful immunohistochemical marker for differential diagnosis of carcinomas originating from epithelial cells of the bile duct system.

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#### Explanation of figures

- Fig. 1. A section of fetal liver stained with anti-GST- $\pi$  antibody. GST- $\pi$  is positive in most of the hepatocytes and strongly positive in bile duct epithelial cells.
- Fig. 2. A section of adult liver stained with anti-GST- $\pi$  antibody. GST- $\pi$  shows a tendency to stain more intensely in the periportal area and strongly positive in bile duct epithelial cells.
- Fig. 3. A section of extrahepatic bile duct epithelial cells stained with anti-GST- $\pi$  antibody. GST- $\pi$  is strongly positive in epithelial cells.
- Fig. 4. A section of hepatocellular carcinoma stained with anti-GST- $\pi$  antibody. GST- $\pi$  is negative in cells of hepatocellular carcinoma and positive in proliferating bile duct epithelial cells.
- Fig. 5. A section of cholangiocarcinoma stained with anti-GST- $\pi$  antibody. GST- $\pi$  is strongly positive in cholangiocarcinoma cells.
- Fig. 6. A section of undifferentiated carcinoma of the gallbladder stained with anti-GST- $\pi$  antibody. Positive cells and negative cells produce a mosaiclike appearance.

