Virchows Arch A (1987) 412:111–118

Virchows Archiv A

Carcinoembryonic antigen and lectin binding in the bile canalicular structures of hepatocellular carcinoma *

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Summary. Bile canalicular structures of 32 hepatocellular carcinomas and 36 cirrhotic livers were investigated by light microscopy using immunohistochemistry for carcinoembryonic antigen (CEA) and lectin histochemistry. CEA positive bile canalicular structures were found in 17 out of 32(53%) hepatocellular carcinomas and in 33 of 36(92%) cirrhotic livers. Among the 10 lectins examined, about 40% of the CEA positive bile canalicular structures of hepatocellular carcinomas showed positive binding of MPA, DBA, WGA, RCA-I or UEA-I, whereas only MPA or RCA-I bound to the CEA positive bile canalicular structures of cirrhotic liver, in about 20% of cases. It has been shown that the CEA positive bile canalicular structures of hepatocellular carcinomas are heterogeneous and differ from those of cirrhotic liver in lectin histochemistry.

Key words: Hepatocellular carcinoma – Lectin – Carcinoembryonic antigen

Introduction

Immunohistochemical studies have recently shown the presence of carcinoembryonic antigen (CEA) on the surfaces of bile canalicular structures in hepatocellular carcinomas (Hirohashi et al. 1983; Koelma et al. 1986). CEA represents a family of glycoproteins that are found not only in fetal and neoplastic tissues but also in normal and diseased tissues, including bile ducts and bile ductules (Gerber et al. 1983; Koelma et al. 1986). In recent years lectins have been widely used as histochemical markers for various glycocompounds in normal and neoplastic tissues (Ulrich et al. 1985). The lectins bind to sugar residues of cell surface glycoconjugates of various tissues with high affinity (Gulati et al. 1986). Alteration in these cell surface glycoconjugates occurs during embryonic development, differentiation and neoplastic growth (Kellokumpu et al. 1986). The purpose of this study is to compare the lectin binding of CEA positive bile canalicular structures in hepatocellular carcinoma with that in liver cirrhosis and to know the histochemical difference of bile canalicular structures between benign and malignant conditions.

Materials and methods

Tumour tissue from 32 cases of hepatocellular carcinoma including 23 surgically removed cases and 9 autopsy cases, were compared with 25 autopsy cases of liver cirrhosis and the cirrhotic liver obtained from 11 surgically removed cases of hepatocellular carcinoma. The tissues were fixed in 10% formalin and paraffin sections were stained with haematoxylin and eosin, periodic acid-Schiff (PAS) with or without diastase digestion, alcian blue and peroxidase labeled lectins, including WGA, PNA, SBA, DBA, MPA, GS-I, GS-II, UEA-I, RCA-I and Con A. These 10 lectins were purchased from EY Laboratories, Inc, USA and their sugar specificity is shown in Table 1. For staining, all lectins were diluted 1:40(25 μg/ml) or 1:20(50 μg/ml) in phosphate-buffered saline (PBS; pH 7.4). Sections were incubated with the peroxidase-labeled lectins for 60 min in a moist chamber at room temperature. After washing in PBS and Tris-saline (pH 7.4), the peroxidase reaction product was developed by the diaminobenzidine reagent (3 mg diaminobenzidine in 10 ml Tris-saline with 0.1 ml of 1% H_2O_2) for 3 min. After another wash in Tris-saline, the slides were counterstained with hematoxylin, dehydrated and coverslipped with Entellan. The specificity of staining for each lectin was tested by preincubating the lectin with 0.1 M of the appropriate inhibitory sugar before staining of the tissue sections. Replacement of the peroxidase labeled lectins by PBS was also used as control.

Paraffin sections were also examined by immunoperoxidase technique to demonstrate CEA positive bile canalicular structures. Anti-CEA antibody and peroxidase conjugated anti-rabbit IgG were obtained from DAKOPATTS. The sections were

* Presented at the XVI International Congress of International Academy of Pathology (Rikuo Machinami 1986)

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Table 1. Major sugar specificity of lectins

Lectins	Sugar specificity
WGA (wheat germ agglutinin)	N-acetylglucosamine; sialic acid
PNA (peanut agglutinin)	Beta-D-galactose; n-acetylgalactosamine
SBA (soy bean agglutinin)	Alpha-D-galactose; n-acetylgalactosamine
DBA (dolichos biflorus agglutinin)	N-acetylgalactosamine
MPA (maclura pomifera agglutinin)	Alpha-D-galactose
GS-I (griffonia simplicifolia agglutinin I)	Alpha-D-galactose
GS-II (griffonia simplicifolia agglutinin II)	N-acetylglucosamine
UEA-I (ulex europaeus agglutinin I)	Alpha-fucose
RCA-I (ricinus communis agglutinin I)	Beta-D-galactose
Con A (concanavalin agglutinin)	Alpha-D-mannose; alpha-D-glucose

incubated overnight at 4° C with the anti-CEA antibody. After washing with PBS, the sections were incubated with the peroxidase conjugated anti-rabbit IgG, washed again, and developed for 3 min in the diaminobenzidine reagent. Replacement of anti-CEA antibody by normal rabbit serum was used as control.

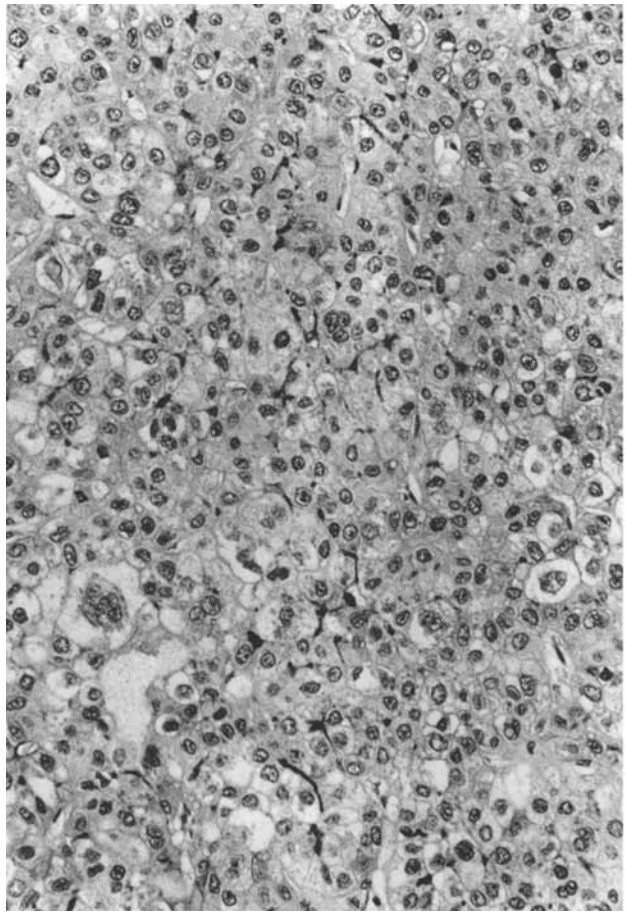
The slides were examined with a light microscope for both the extent and intensity of the staining of CEA and lectins. The extent of the staining was determined according to the percentage of the area with positive bile canalicular staining; slight (+)(<25%), moderate (++)(25–75%) and marked (+++)(>75%). The labeling intensity was graded as either weak (w) or strong (s). When the reaction product could be easily detectable by low magnification, the labelling intensity was graded as strong (s). However, the intensity was graded as weak (w), if the staining was only detectable by higher magnification.

The time between death and post mortem examination was described for autopsy cases in order to assess the effect of post mortem analysis on lectin binding and immunoreactive CEA.

Results

CEA positive bile canalicular structures were found in 17 of 32 (53%) cases of hepatocellular carcinoma; the reaction was slight and weak (+w) in 8 cases, moderate and weak (++w) in 3 cases, marked and weak (+++w) in 1 case, slight and strong (+s) in 1 case, moderate and strong (++s) in 3 cases, and marked and strong (+++s) in 1 case (Fig. 1). The staining reaction of CEA was abolished by replacement of anti-CEA antibody by normal rabbit serum. Among the 10 lectins examined, MPA, DBA, WGA, RCA-I or UEA-I bound to these CEA positive bile canalicular structures of hepatocellular carcinomas. CEA negative cases of hepatocellular carcinoma did not bind any lectins. The staining reactions of these 5 lectins was abolished by replacement of the peroxidase labelled lectins by PBS or coincubation with specific inhibitory sugars such as D-galactose for MPA, N-acetylgalactosamine for DBA, N-acetylglucosamine and sialic acid for WGA, D-galactose for RCA-I, and fucose for UEA-I. The results of CEA activity and lectin binding in these lectin positive cases are shown in Table 2. Seven of 17 (41%) CEA positive cases of hepatocellular carcinoma showed positive lectin binding. Four of the 7 lectin positive cases of hepatocellular carcinoma showed positive binding of MPA (Fig. 2); marked and strong (+++s) staining in 3 cases, and slight and weak (+w) staining in one case. The CEA positive bile canalicular structures were alcian blue positive, showing small acinar structures and containing diastase resistant PAS positive eosinophilic material in one of the 17 CEA positive cases (Fig. 3). Moderate and strong (++s) reaction of CEA, marked and strong (+++s) reaction of MPA and moderate and strong (++s) reaction of UEA-I were found in the acinar struces of this case (Figs. 4, 5, 6). Another two cases with marked and strong (+++s) reaction of MPA also showed moderate and weak (++) reaction of CEA and DBA (Fig. 7), and moderate and weak (++w) reaction of CEA, WGA (Fig. 8) and RCA-I (Fig. 9), respectively. Three of the 7 lectin positive hepatocellular carcinomas were only RCA-I positive; two of them showed moderate and weak (++w) reaction and one showed slight and weak (+w) reaction, respectively.

Although the bile canalicular structures of almost all cases of liver cirrhosis were CEA positive and the percentage of CEA positive cases in liver cirrhosis was significantly higher than that in hepatocellular carcinoma, the percentage of cases with positive lectin binding in CEA positive liver cirrhosis was significantly lower than that in CEA positive hepatocellular carcinoma (Table 3). Thirty-three of 36 (92%) cases of liver cirrhosis were CEA positive in the bile canalicular structures (Fig. 10); the reaction was slight and weak (+w) in 9 cases, moderate and weak (++w) in 6 cases, marked and weak (+++w) in 3 cases, moderate and strong (++s) in 4 cases, and marked and strong (+++s) in 11 cases. Only 7 of these 33 (21%) CEA positive cirrhotic liver tissues showed positive lectin binding in the bile canalicular structures. Five of them were only RCA-I positive (Fig. 11); slight and weak (+w) in 3 cases, moderate and weak (++w) in 1 case, and slight and



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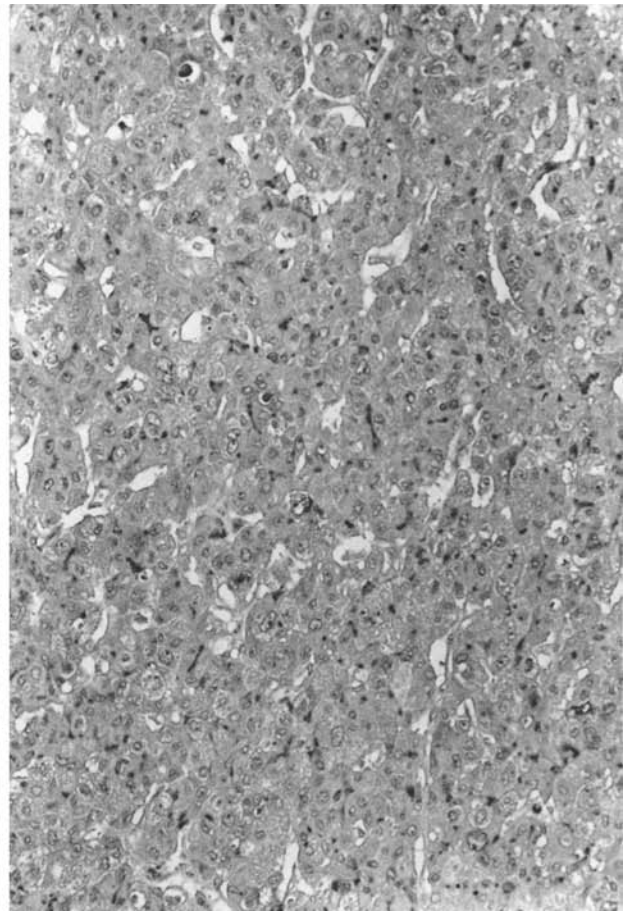

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Fig. 1. Hepatocellular carcinoma with CEA positive bile canalicular structures. The reaction is moderate and strong (++s). (Immunoperoxidase technique, ×75)

Fig. 2. Hepatocellular carcinoma with MPA lectin positive bile canalicular structures showing marked and strong (+++s) staining. (Peroxidase-conjugated MPA, ×60)

Table 2. CEA and lectin binding in the bile canalicular structure of lectin positive hepatocellular carcinoma

Cases	CEA	Lectins
796554*	++s	MPA +++s, UEA-I ++s
791101*	++w	MPA +++s, DBA ++w
78252*	++s	RCA-I +w
6076**	+++s	RCA-I ++w
6065**	++w	MPA +++s, WGA ++w, RCA-I ++w
5753**	++w	RCA-I ++w
2023**	+w	MPA +w

* Surgically removed cases
** Autopsy cases

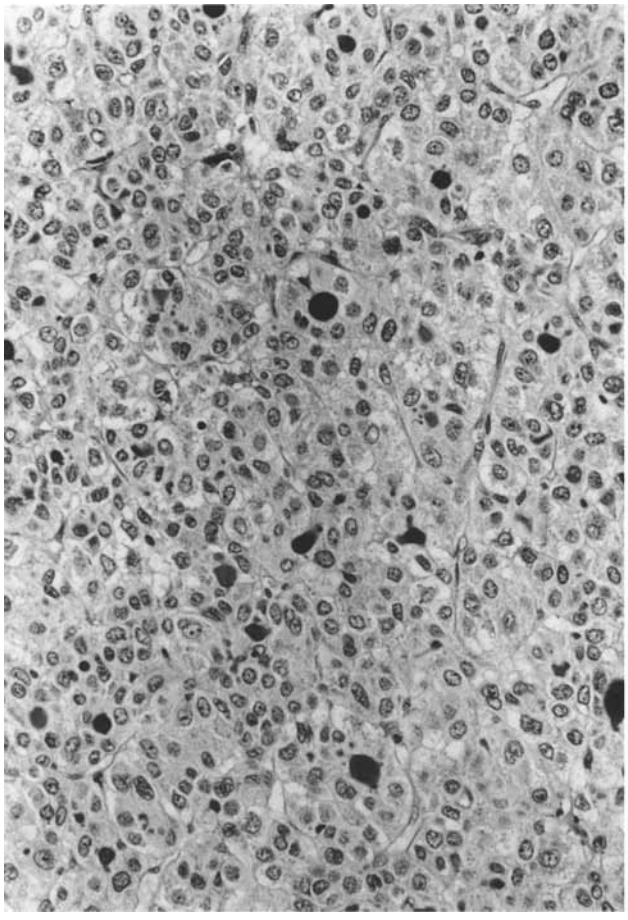
Extent of the staining: slight + (<25%), moderate ++ (25–75%), marked +++ (>75%)

Intensity of the staining: weak (w), strong (s)

Small acinar structures were observed in case 796554

strong (+s) in 1 case. Two of the 7 lectin positive cases were MPA positive (Fig. 12); moderate and weak (++w) reaction in one of them in addition to slight and strong (+s) reaction of RCA-I, and moderate and strong (++s) reaction of MPA in the other case. No lectin binding was found in the CEA negative bile canalicular structures of liver cirrhosis.

The time between death and autopsy for the 9 cases of hepatocellular carcinoma ranged from 45 min to 13 h 30 min and 4 showed positive lectin binding (with the time after death ranging from 45 min to 2 h 40 min). The time between death and post mortem examination ranged from 1 h to 10 h 25 min for the 25 autopsy cases of liver cirrhosis and the time after death for the 5 lectin positive cases ranged from 1 h to 7 h 50 min. The percentage of CEA and lectin positive cases in the



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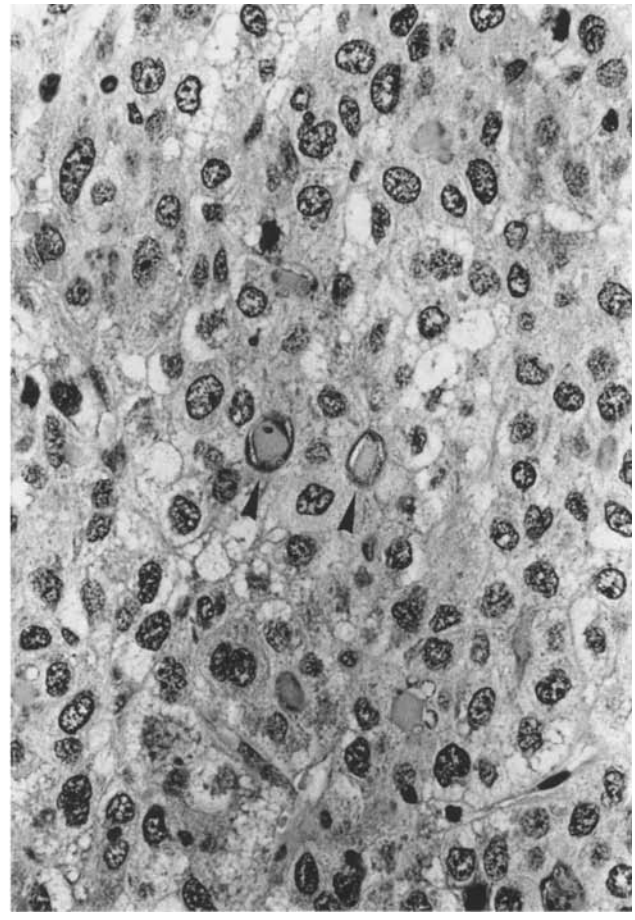

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Fig. 3. Small acinar structures of hepatocellular carcinoma containing diastase resistant PAS positive material. (Diastase-PAS, ×75)

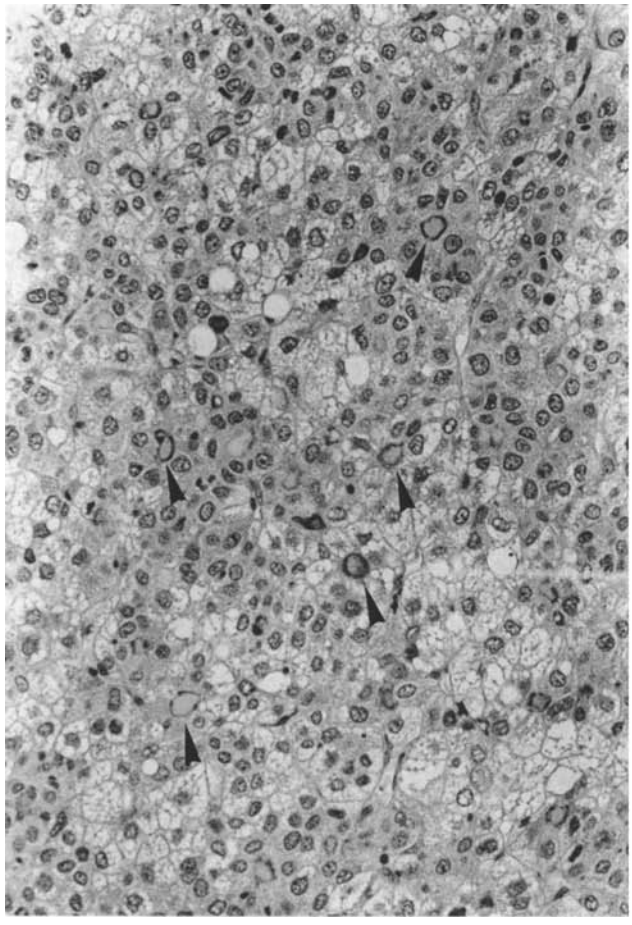
Fig. 4. The small acinar structures shown in Fig. 3 are CEA positive with moderate and strong (++s) reaction (*arrow heads*). (Immunoperoxidase technique, ×150)

surgically removed hepatocellular carcinomas was 52% for CEA and 13% for lectins, while that in autopsy cases was 45% for CEA and 44% for lectins. The bile canalicular structures of cirrhotic liver tissues from surgically removed cases were positive for CEA in 91% and for lectins in 18%, while those from autopsy cases were positive for CEA in 92% and for lectins in 20%.

Discussion

CEA positive bile canalicular structures have been observed in 53% of the cases with hepatocellular carcinoma and 92% of the cases with liver cirrhosis in this study. This confirms the report that the surfaces of bile canaliculi in non-neoplastic liver tissues and the structures resembling bile canaliculi in relatively well-differentiated hepatocellular carcinoma and well-differentiated parts of hepatoblastoma are stained with a rabbit anti-CEA immunoglobulin (Hirohashi et al. 1983). It has also been reported that a murine monoclonal anti-CEA antibody does not stain any neoplastic or non-neoplastic hepatocytes suggesting the presence of a CEA cross-reactive antigen on the surface of bile canaliculi. This antigen corresponds to a CEA cross-reactive antigen detected in bile, termed biliary glycoprotein I (Svenberg et al. 1979). By immunohistochemistry of a polycolonal anti-CEA and of a monoclonal antibody, which both show cross-reactions with CEA and biliary glycoprotein I, a bile canalicular staining pattern has been reported in 80% of cases with hepatocellular carinoma and also in liver cell adenoma, focal nodular hyperplasia, alcoholic hepatitis and secondary biliary cirrhosis (Koelma et al. 1986).

Although the percentage of cases with CEA positive bile canalicular structures was markedly lower in hepatocellular carcinoma than in liver cirrhosis, the lectin binding in these structures was



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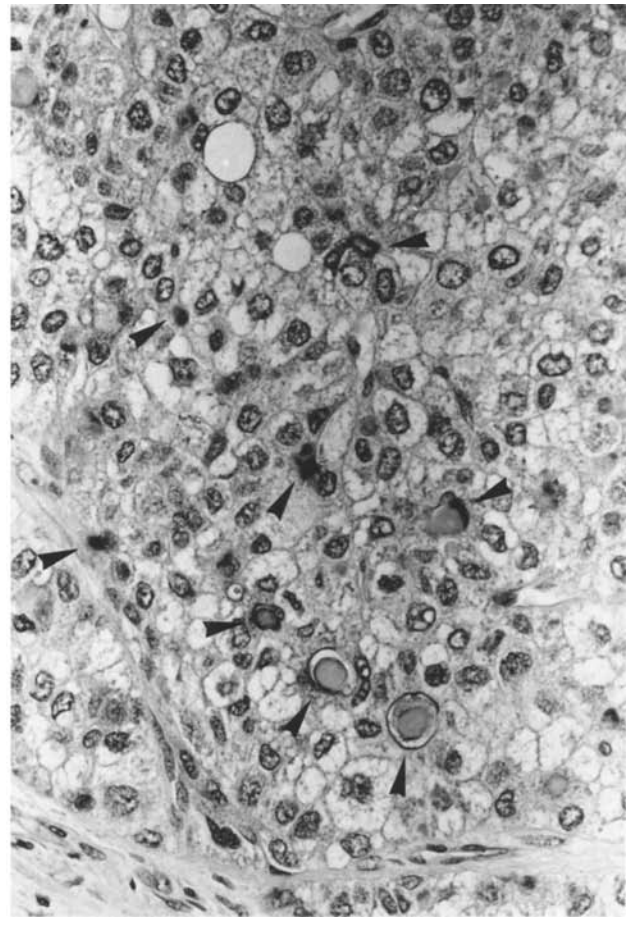

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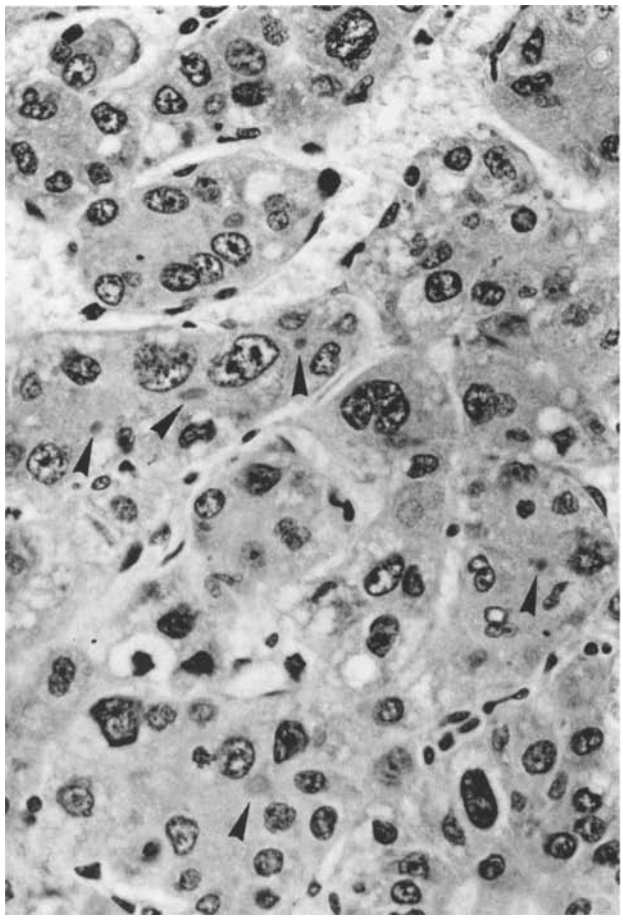
Fig. 5. The small acinar structures shown in Fig. 3 are also MPA lectin positive with marked and strong (+ + +s) binding (*arrow heads*). (Peroxidase-conjugated MPA, × 75)

Fig. 6. The same case of hepatocellular carcinoma shown in Figs. 3, 4 and 5 is positive for UEA-I lectin in the bile canalicular structures with moderate and strong (+ +s) reaction (*arrow heads*). (Peroxidase-conjugated UEA-I, × 130)

significantly more prominent in hepatocellular carcinoma when compared with liver cirrhosis. About 40% of the structures in hepatocellular carcinomas displayed positive binding of several lectins including MPA, DBA, WGA, RCA-I or UEA-I, while only MPA or RCA-I bound to CEA positive liver cirrhosis, in about 20% of cases. Similar differences between neoplastic and non-neoplastic tissues in cell surface associated lectin histochemistry have been shown on several tissues including the appearance of specific sugar residues in breast carcinoma (Louis et al. 1983), heterogeneity of carbohydrate structures of colorectal carcinoma (Kellokumpu et al. 1986), difference in distribution and amounts of normally present sugar rests in renal cell carcinoma (Ulrich et al. 1985) or sialic acid masking of lectin binding sites in gastric carcinoma (Macartney 1986). Among the 5 lectins which showed positive binding, most prominent binding to hepatocellular carcinomas was found in MPS indicating the presence of alpha-D-galactose in the CEA positive bile canalicular structures. Although GS-I and SBA can also bind to alpha-D-galactose, these two lectins did not actually bind to any hepatocellular carcinoma tissue. This seems to suggest the occurrence of masking of lectin binding sites in alpha-D-galactose molecule for GS-I and SBA. The next prominent finding was weak reaction of RCA-I showing the presence of beta-D-galactose in the bile canalicular structures of hepatocellular carcinomas. PNA can also bind to beta-D-galactose but did not bind to any case of hepatocellular carcinoma in this study; this may also be due to masking of the lectin binding sites. Both MPA and RCA-I lectins also bound to some of the cirrhotic liver tissues indicating the presence of the same sugars in the neoplastic and non-neoplastic liver. Three other lectins including UEA-I, DBA and WGA did not bind to any cirrhotic liver tissues but bound to some hepatocellular carcinomas.



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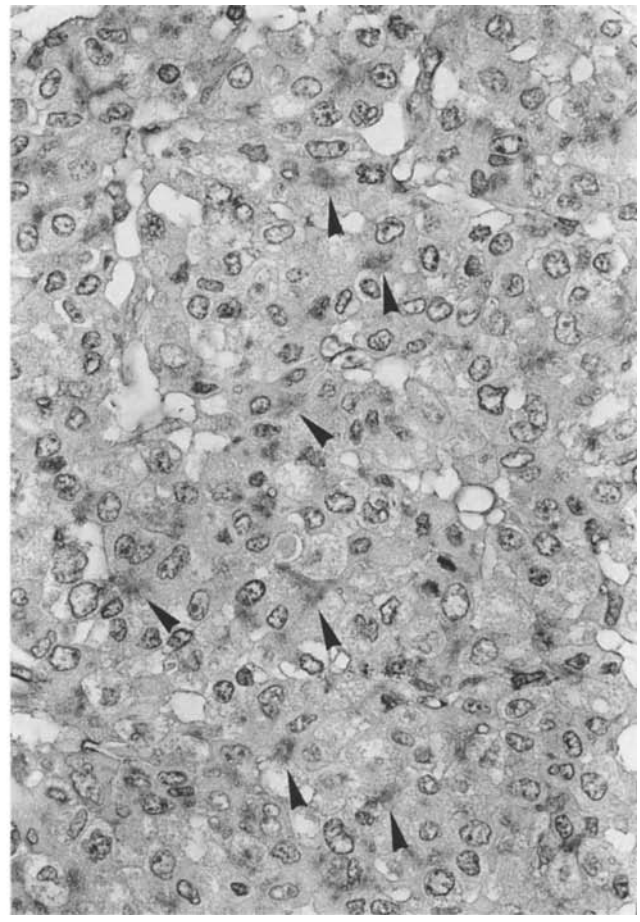

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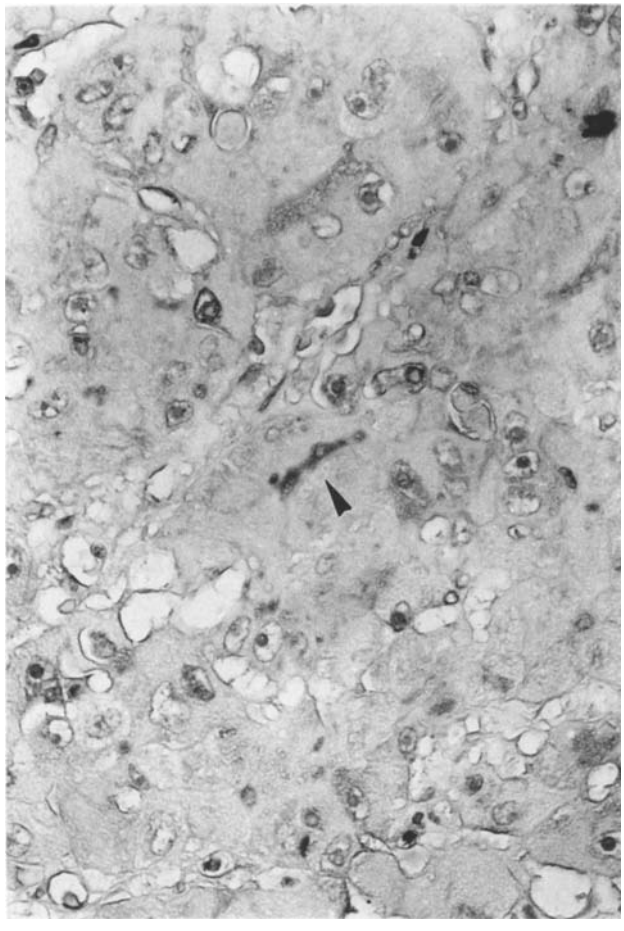

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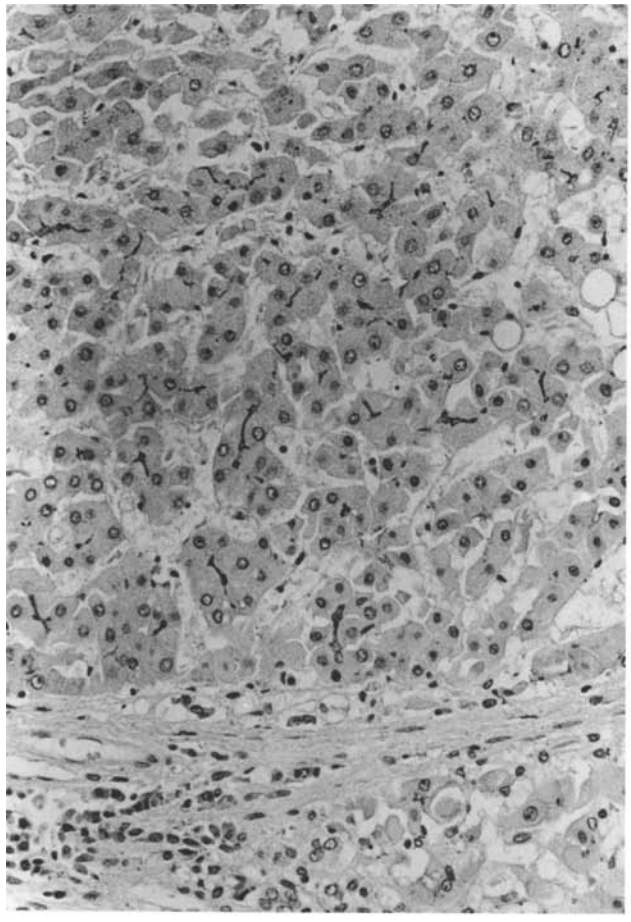
Fig. 7. Hepatocellular carcinoma showing positive binding of DBA lectin with moderate and weak (+ + w) reaction in the bile canalicular structures (*arrow heads*). (Peroxidase-conjugated DBA, × 150)

Fig. 8. Hepatocellular carcinoma showing positive binding of WGA lectin with moderate and weak (+ + w) reaction in the bile canalicular structures (*arrow heads*). (Peroxidase-conjugated WGA, × 150)

Fig. 9. Hepatocellular carcinoma with RCA-I lectin positive bile canalicular structures showing moderate and weak (+ + w) staining (*arrow heads*). (Peroxidase-conjugated RCA-I, × 150)



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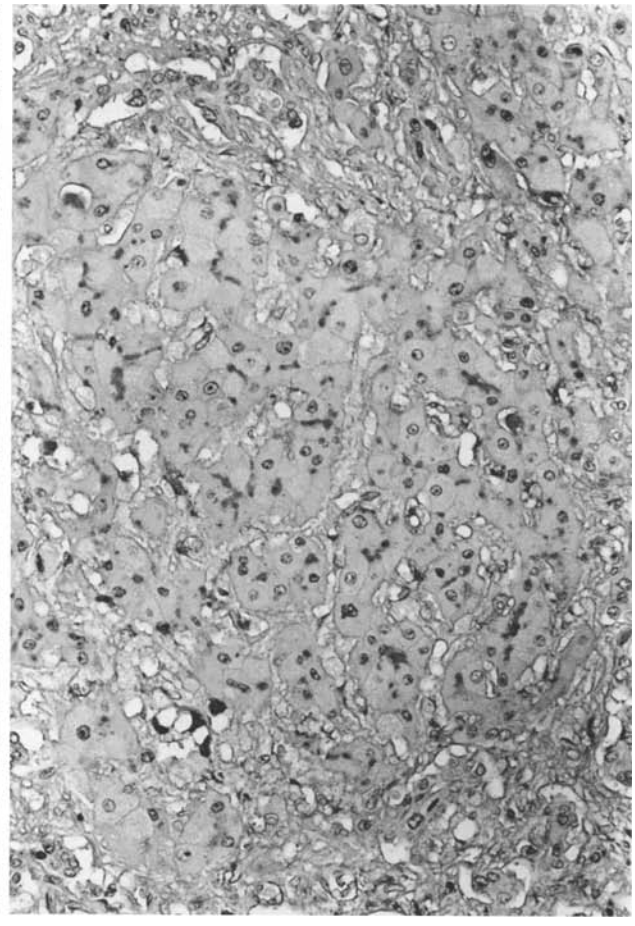

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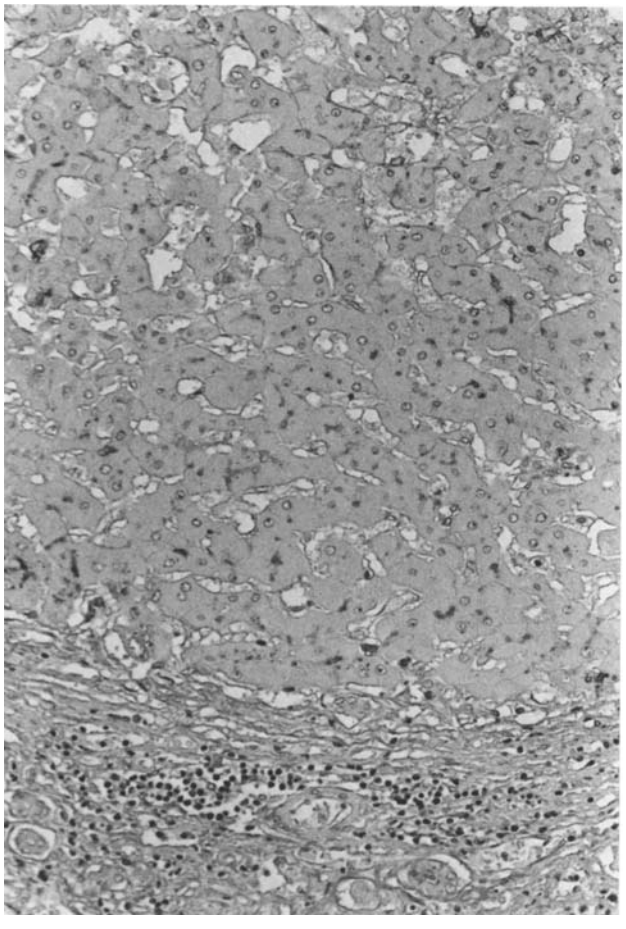

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Fig. 10. Liver cirrhosis showing CEA positive bile canalicular structures with marked and strong (+ + +s) staining. (Immunoperoxidase technique, × 75)

Fig. 11. Liver cirrhosis with RCA-I lectin positive bile canalicular structures with slight and strong (+s) reaction. (Peroxidase-conjugated RCA-I, × 75)

Fig. 12. Liver cirrhosis with MPA lectin positive bile canalicular structures showing moderate and strong (+ +s) staining. (Peroxidase-conjugated MPA, × 60)

Table 3. CEA and lectin binding in the bile canalicular structure of lectin positive liver cirrhosis

Cases	CEA	Lectins
78252*	+++s	RCA-I ++w
70939*	+w	RCA-I +s
6180**	+++s	RCA-I +w
6043**	+++s	RCA-I +w
695**	++w	MPA ++w, RCA-I +s
667**	+++s	MPA ++s
664**	+++s	RCA-I +w

* Surgically removed cases
** Autopsy cases
Extent of the staining: slight + (<25%), moderate ++ (25–75%), marked +++ (>75%)
Intensity of the staining: weak (w), strong (s)

This indicates the presence of alpha-fucose, N-acetylgalactosamine, N-acetylglucosamine, or sialic acid only in the CEA positive bile canalicular structures of hepatocellular carcinomas. Although N-acetylglucosamine and N-acetylgalactosamine can also bind GS-II, and both PNA and SBA, respectively, no binding of these lectins was observed in the present study. This may also be due to the occurrence of masking of binding sites for these lectins. It is suggested that alpha- and beta-D-galactoses are present in both some cases of hepatocellular carcinoma and liver cirrhosis and that alpha-fucose, N-acetylglucosamine, N-acetylgalactosamine and sialic acid are unique to the CEA positive bile canalicular structures of hepatocellular carcinomas.

The percentage of CEA positive autopsy cases of hepatocellular carcinoma did not differ from that of surgically removed cases, while the percentage of lectin positive autopsy cases was significantly higher than that of surgically removed hepatocellular carcinomas. The time between death and autopsy was, however, short in lectin positive hepatocellular carcinomas; it was less than 2 h 40 min. Nevertheless, there was no difference in the percentage of CEA and lectin positive cases of liver cirrhosis between autopsy and surgically removed cases, and lectin binding could be observed even at 7 h 50 min after death. These findings suggest that the effect of post mortem analysis on lectin binding is not serious, though decrease of lectin binding capacity occurs for hepatocellular carcinoma tissues at more than 3 h after death.

Acinar formation has been regarded as a structural alteration characteristic for malignant hepatocytes and a helpful sign for identifying the well differentiated type of small hepatocellular carcinomas (Kondo et al. 1986). CEA positive small acinar structures were found in only one case of hepatocellular carcinoma in this study; they were also positive for MPA and UEA-I lectins. Although the CEA positive bile canalicular structures in other cases of hepatocellular carcinoma were similar to those of liver cirrhosis and seem to reflect differentiation of hepatocellular carcinoma, these structures were heterogeneous and different from those of liver cirrhosis in lectin histochemistry. It has been shown by this study that lectin histochemistry is useful for differentiating the CEA positive bile canalicular structures in some cases of hepatocellular carcinoma from those of liver cirrhosis.

Acknowledgements. The author is grateful to Mrs. Hiroko Obuchi and Miss Tomoko Abe for their technical help in lectin histochemistry and immunohistochemistry, and to Mr. Tatsuro Fukushima for photography.

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Accepted September 11, 1987