Virchows Archiv A Pathol Anat (1991) 419:409–415

Virchows Archiv A
Pathological Anatomy and Histopathology

The histopathology of the liver in older patients with hepatitis B virus surface antigenaemia

Haruhiko Sugimura[1,2], Toshio Fukazawa[3], Megumu Saitoh[3], Ken-ichi Nakamura[1], Hiroyuki Shimada[4], and Kohichiroh Ohtsubo[1]

[1] Department of Clinical Pathology, Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan
[2] Department of Pathology, Faculty of Medicine, University of Tokyo, Tokyo, Japan
Departments of [3] Medicine and [4] Pathology, Tokyo Metropolitan Geriatric Hospital, Tokyo, Japan

Received March 10, 1991 / Received after revision July 2, 1991 / Accepted July 11, 1991

Summary. The histopathology of the liver and the detectability of intrahepatic hepatitis B virus (HBV) markers were studied in 34 autopsy cases in elderly patients (mean age 73.9 years, range 60–91 years) who had had a history of positive HBV surface antigenaemia prior to death. Seven of 14 persistent HBV carrier cases (group A) in which long-lasting HBV surface antigen (HBsAg) carriage in the sera had been confirmed by sequential assays, and 5 out of 15 HBV-infected people (group C, single assay) showed significant primary liver damages including chronic hepatitis, toxic hepatitis, liver cirrhosis and hepatocellular carcinoma. In 5 cases (group B), one of which was type B liver cirrhosis, HBsAg became negative and HBsAb appeared during the follow-up period (up to 33 months). Among confirmed HBV carriers, HBsAg and HBV core antigen were most frequently found in the liver of cirrhotic cases with and without hepatocellular carcinoma (5 of 6), whereas these were rarely detected in those with non-specific changes or slight hepatitic activity (1 of 7). All 5 cases in group B were negative for histological HBV-related antigens and the findings in group C were variously interpreted. Post-mortem cases of the aged HBV carriers who survived their mean life expectancy represent an important population in which to study the natural history of HBV carriers.

Key words: Hepatitis B virus – Hepatitis B virus carrier – Liver – Pathology

Introduction

A considerable number of reports have been published on the histopathology of liver disease associated with hepatitis B virus (HBV) and so-called asymptomatic HBV carriers, but almost all the previous reports have dealt mainly with young, healthy blood donors and middle-aged people with and without hepatic symptoms (Reinicke et al. 1972; Simon and Patel 1974; Vittal et al. 1974; Sakurai and Nakaso, 1982; Suzuki et al., 1985). Little information is available about HBV infection in elderly people with or without liver disease, whether it is a chronic or transient infection. The elderly HBV carriers may be of interest in observing the sequelae of the life-long carriage of HBV, since most Japanese HBV carriers are assumed to be infected by perinatal transmission or horizontal infection in the very early stages of life (Hoofnagel et al. 1987).

In this study, histological changes in the liver are related to the detectability of intrahepatic HBV markers. Postmortem examinations performed at the Department of Pathology, Tokyo Metropolitan Geriatric Hospital during the last 10 years provided the material. Only the livers of elderly people whose serum had been shown to be positive for HBV surface antigen (HBsAg) at least once during the follow-up period prior to autopsy were examined. The purpose of this investigation was to identify those histological features of HBV-infected liver in aged patients who had survived for approximately the mean life expectancy of the contemporary Japanese (male 74.95 years, female 80.75 years) (Vital Statistics, Japan, 1986).

Materials and methods

From autopsy files and attached clinical protocols, cases with a history of HBs antigenaemia were extracted, and in each case two or three liver blocks were examined for histological detection of HBV-related antigens in addition to usual routine pathological studies. In 5 cases numerous blocks including a whole frontal slice of the liver were investigated in order to estimate the quantity of HBsAg and to look for possible trace amounts of HBsAg. Dako's anti-HBsAg and anti-HBcAg kit were used for histopathological detection of HBsAg and HBV core antigen (HBcAg) respectively. Peroxidase-antiperoxidase staining was carried out in accordance with the company's instructions and positive controls were provided with the kits. On each batch unambiguous staining for HBsAg and HBcAg was confirmed for the positive controls. Non-immune serum was used as the primary antibody for the negative

Offprint requests to: H. Sugimura, First Department of Pathology, Hamamatsu University School of Medicine, 3600, Handa-cho, Hamamatsu, Shizuoka, Japan 431-31

control and non-specific staining was excluded. Shikata's orcein (Shikata et al. 1974) and Victoria blue stain (Tanaka et al. 1981) were also applied for HBsAg. Human anti-delta antibody and positive control specimen were gifts from Dr. Suzuki and Dr. Uchida at Nihon University. Indirect immunohistochemistry was performed for the detection of delta antigen in the liver.

Clinical history, including transaminase values, history of blood transfusion, habitual alcohol intakes and the immediate cause of death were recorded. Serum HBsAg was assayed by reversed passive haemagglutination assay, anti-HBsAg antibody (HBsAb) by passive haemagglutination, and anti-HBcAg antibody (HBcAb) by radioimmunoassay. According to the available profiles of the serum HBV markers, all the cases were categorized into three groups: (A) HBs antigenaemia lasting more than 6 months confirmed by serial assays; (B) cases in which HBsAg became negative during the follow-up period; (C) HBs antigenaemia proved by only a single assay.

Results

From 2875 autopsy cases (male 1495, female 1374) during the last 10 years (1976–1985) 34 cases (male 23, female 11; 1.5%) were positive for HBsAg at least once before their death. The average age of the subjects was 73.9 years; male 74.9 years, female 71.9 years. Considering that the mode of age distribution of all autopsy cases in this institution was around 80 years in both sexes and the male to female ratio was around 1.0, the mean age of HBV carriers was slightly lower, with an increased incidence in males. Of the cases, 14, 5 and 15 were categorized as groups A, B and C as described above.

The interval of the last date of serum HBV marker assay and death ranged from 0 to 24 months in group A, from 0 to 4 months in group B, and from 0.5 to 22 months in group C (column b in Table 1).

Clinical diagnoses were made on the grounds of clinical symptoms, laboratory findings including transaminase, image diagnosis such as liver scintigraphy, echography and CT. Laparoscopy and liver biopsy were also done in some cases. In 12 cases of the 34, no serious liver disease had ever been diagnosed. However, 15 cases manifested primary liver damage. These were liver cirrhosis (6 cases), liver cirrhosis with hepatocellular carcinoma (HCC) (4 cases), chronic hepatitis (2 cases), acute hepatitis (1 case) and drug-induced hepatitis (2 cases). Furthermore, metastatic liver cancer (2 from the stomach and 1 from the lung), 2 infiltrating bile duct carcinomas and 2 cases of sepsis had been among the cases of clinically apparent liver damage.

A past history of transfusion was noticed in 7 cases (20%) while that of post-transfusional hepatitis was recorded only in 1 case. Data on the titre of HBsAg and HBcAb were limited. The clinical profiles of the cases studied are summarized in Table 1.

Autopsied livers weighed from 350 to 2020 g, and the spleens from 10 to 330 g. No significant differences were noticed among the three groups.

In the group of persistent HBV carriers (group A), 6 of 14 cases had liver cirrhosis with or without HCC. All the cirrhosis cases except 1 showed thin fibrotic septa and regenerative nodules with a diameter of more than 5 mm and were compatible with post-hepatitic cirrhosis.

Table 1. Clinical profiles of the cases studied

A. Cases with positive HBsAg in the sera by serial assays

Case no.	Age (years)	Sex	a	b	c	d	e
1.	68	M	8	3	−	−	+
2.	73	M	25	4	++	Biliary LC	−
3.	62	M	3	2	−	−	−
4.	61	F	6	6	(+)	Drug-induced	−
5.	67	M	1	0.5	+	LC+HCC	−
6.	66	M	31	4	++	LC	++
7.	73	M	16	10	+	LC+HCC	−
8.	60	M	14	1	++	Ch hepatitis	+
9.	75	F	7	3	−	Meta	−
10.	80	M	7	2	−	−	−
11.	77	F	10	9	−	−	−
12.	80	M	4	0.1	−	−	ND
13.	72	M	7	2	ND	LC+HCC	−
14.	70	M	14	24	+++	LC	ND

B. The cases in which HBsAg disappeared during the follow-up

Case no.	Age (years)	Sex	a	b	c	d	e
1.	80	M	21	4	+	−	−
2.	80	F	1	2	+	Sepsis	−
3.	65	F	3	0	+	Ac hepatitis	−
4.	78	M	3	3	+	Ca infiltration	+
5.	79	M	32	4	+	LC	−

C. The cases in which only a single assay was performed

Case no.	Age (years)	Sex	b	c	d	e
1.	80	F	12	−	−	−
2.	81	M	22	−	−	−
3.	72	F	2	+++	LC	+
4.	89	M	1	−	−	−
5.	85	M	0.5	−	Sepsis	−
6.	80	F	3	++	INH	−
7.	60	F	1.5	+++	Meta	−
8.	70	M	2	+++	Meta	+
9.	74	F	0.5	(+)	−	+
10.	84	M	1.0	+	−	−
11.	67	F	10	+++	LC	−
12.	91	M	0.5	(+)	−	−
13.	79	M	11	+	Ch hepatitis	−
14.	64	M	4	−	Meta	−
15.	72	M	14	+	LC+HCC	−

Column/abbreviation
a. The interval of the serial assays (months)
b. The lag time between the last date of the assay and the autopsy (months)
c. The abnormality of transaminase. −, +, ++, +++ indicate no, slight (less than 50 IU), moderate (more than 50 IU), marked (more than 100) abnormality, respectively
d. Symptoms associated with liver damage. −, asymptomatic or not particular
e. Past history of transfusion. ++, post-transfusional hepatitis

Ch, Chronic; Ac, acute; Meta, metastatic carcinoma; Ca, cancer; INH = isonicotine hydrazide; LC, liver cirrhosis; HCC, hepatocellular carcinoma; ND, not determined

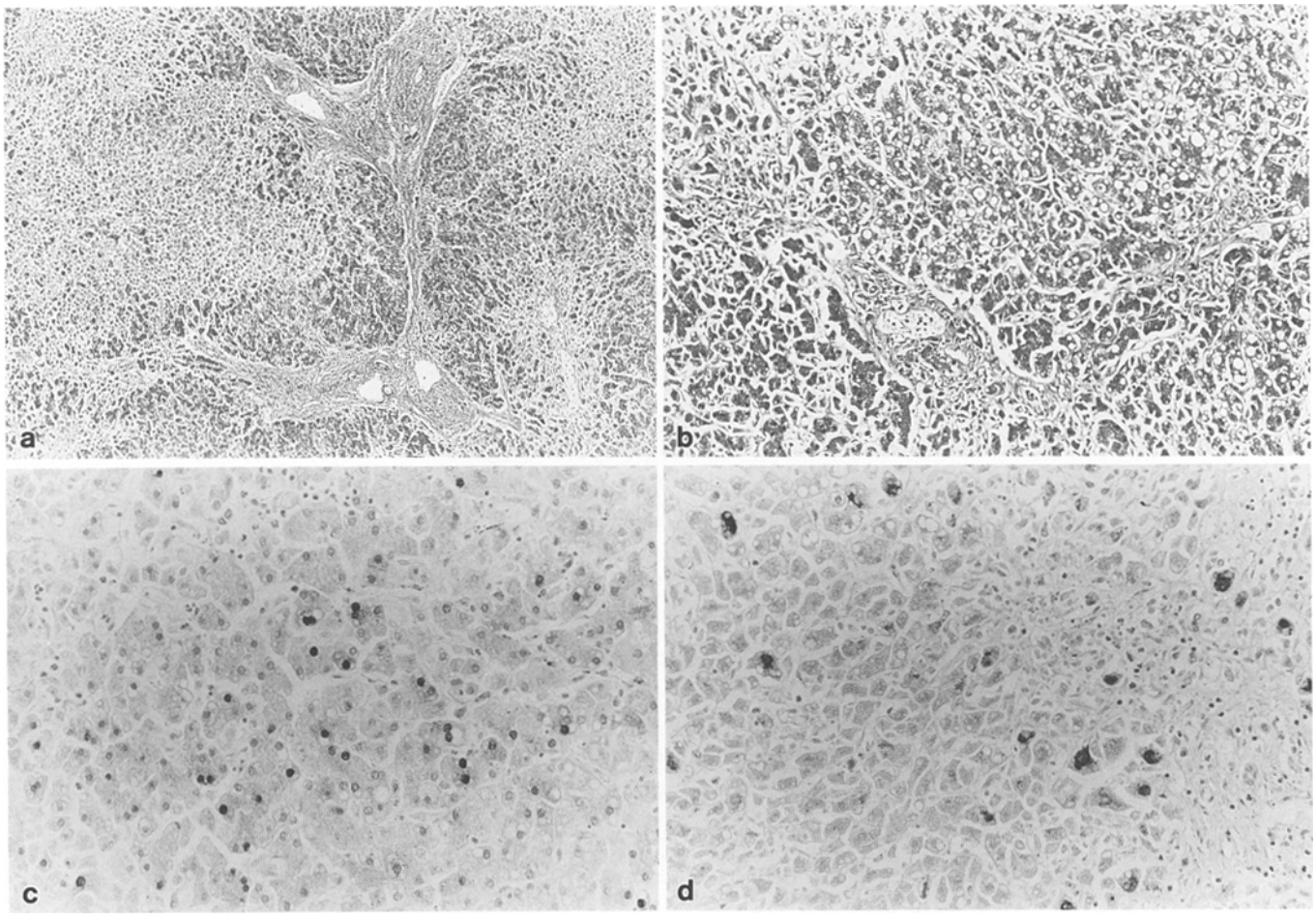

Fig. 1. a Wide areas of the parenchyma of the liver are devastated. Fibrosis and marked fatty changes were seen in case 8 in group A. ×40. **b** Higher magnification. Marked fatty metamorphosis and fibrosis are noted in this case. H&E, ×100. **c** HBcAg is shown in a clustered hepatocytic nucleus. Anti-HBcAg PAP, ×100. **d** HBsAg is shown in the cytoplasm of hepatocytes in a case of liver cirrhosis. Victoria blue stain, ×100

One of the other cases, a heavy drinker, had alcoholic cirrhosis, without cancer. A case of persistent hepatitis was found in this group. Five of these 6 cirrhotic cases contained HBsAg and 1 of them also had HBcAg. The HBsAg was cytoplasmic in all the positive cases. Another case (case 8 in group A) presented with so-called acute-on-chronic liver damage clinically and clinical presentation after operation was for a pituitary tumour. The histological findings in the liver showed hypoxic necrosis with fibrosis (substantial loss of hepatocytes and remarkable fatty metamorphosis) (Fig. 1a, b). In this case, HBcAg was demonstrated in the liver nuclei (Fig. 1c), and HBsAg was also detected (Fig. 1d). In 6 cases in this group, non-specific changes, such as slight portal fibrosis and centrilobular necrosis due to circulatory failure, were found. HBsAg was found only in 1 among these pathologically unremarkable livers. This positive case (case 12, group A) showed faintly stained HBsAg with dilatation of the hepatic sinusoids. A total of 65 blocks covering a whole frontal slice of this liver were investigated to determine the quantity of HBsAg which was found to be diffuse in distribution in this case. The same procedure failed to detect the presumably localized HBsAg in the other asymptomatic cases.

In group B, type B liver cirrhosis was demonstrated in 1 case, acute hepatitis and septic change in another, but the other 3 cases had no significant change suggesting primary liver disease. Neither HBsAg nor HBcAg was detected in the liver in any case in group B.

Histological changes in the liver in group C were various; mild chronic hepatitis was recognized in 3 cases, liver cirrhosis with or without HCC in 3 cases, non-specific changes such as centrilobular necrosis, fatty change and senile atrophy in 7 cases. In 1 aged patient, HBsAg was found in the area of marked liver cell atrophy and sinusoidal dilatation (case 12 in group C). HBcAg was also detected both in the nuclei and cytoplasm in this case (Fig. 2a, b). A case of acute hepatitis accompanied by bile duct carcinoma and toxic hepatitis was also found. The latter case (toxic hepatitis) was suspected to be drug-induced, because severe symptoms appeared just after anti-tuberculosis therapy had been started in a patient with mild transaminase abnormality. Clinically, so-called subacute hepatitis was also suspected.

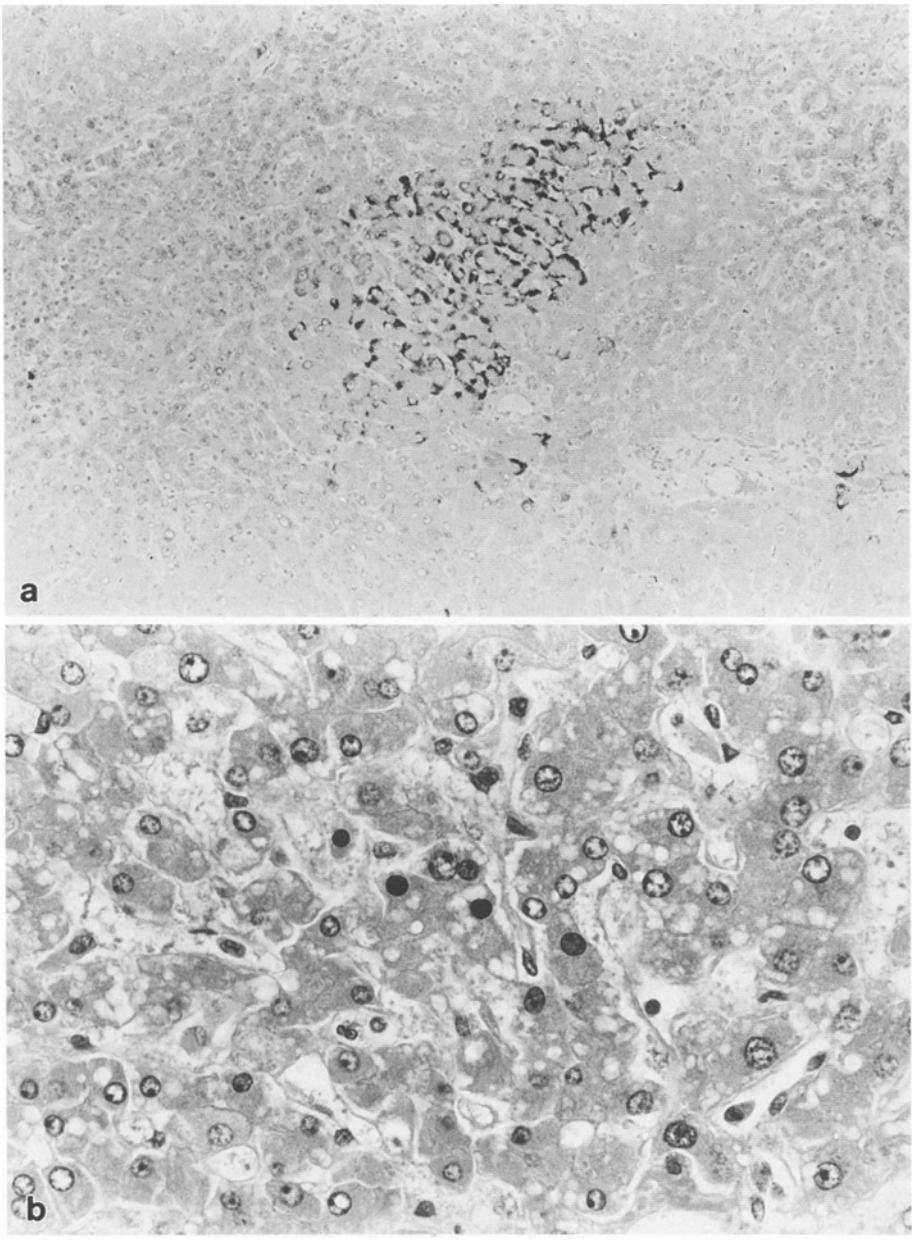

Fig. 2. a Clusters of HBsAg-containing hepatocytes are present in the area where liver cell atrophy and sinusoidal dilatation are marked. Victoria blue stain, ×40. **b** HBcAg in the same area as **a**. Nuclear localization of HBcAg is clear in this case. Anti-HBcAg PAP stain, ×200

The histological picture in this case showed active hepatitis with pseudocholangiolar proliferation and marked lymphoid cell aggregation (Fig. 3a–c). Membranous HBsAg was remarkable and HBcAg was also found in this case.

HBsAg was detected in 3 cases of mild and non-specific hepatitis and non-specific change, 2 cases of liver cirrhosis, and 1 case of toxic hepatitis. HBcAg was found in a case of acute hepatitis, a case of toxic hepatitis, and 2 cases of livers with non-specific changes and a case of liver cirrhosis with HCC.

Histological findings and detectability of HBV markers in the liver are summarized in Table 2.

In total, 12 of 34 cases (35%) were HBsAg positive histologically; 42% in group A and 40% in group C. Except for 2 cases, significant hepatitic activity or terminal stage of chronic liver disease was identified in these HBsAg-containing livers. HBcAg was found in 7 out of 25 informative cases, and only in 1 case with histologically mild change. Delta antigen was not found histologically in the 25 cases examined.

Discussion

A significant proportion of HBsAg carriers, whether symptomatic or not at the time of diagnosis, may develop chronic liver disease, liver cirrhosis and finally HCC in their life span (Beasley et al. 1981; Feinman et al. 1982; Sakuma et al. 1982; Iijima et al. 1984; James and Nuttall 1984; Tamura et al. 1986; Wu et al. 1987). However, the existence of HBsAg carriers without any symptoms both clinically and pathologically is well known. Recently follow-up studies of blood donors revealed that

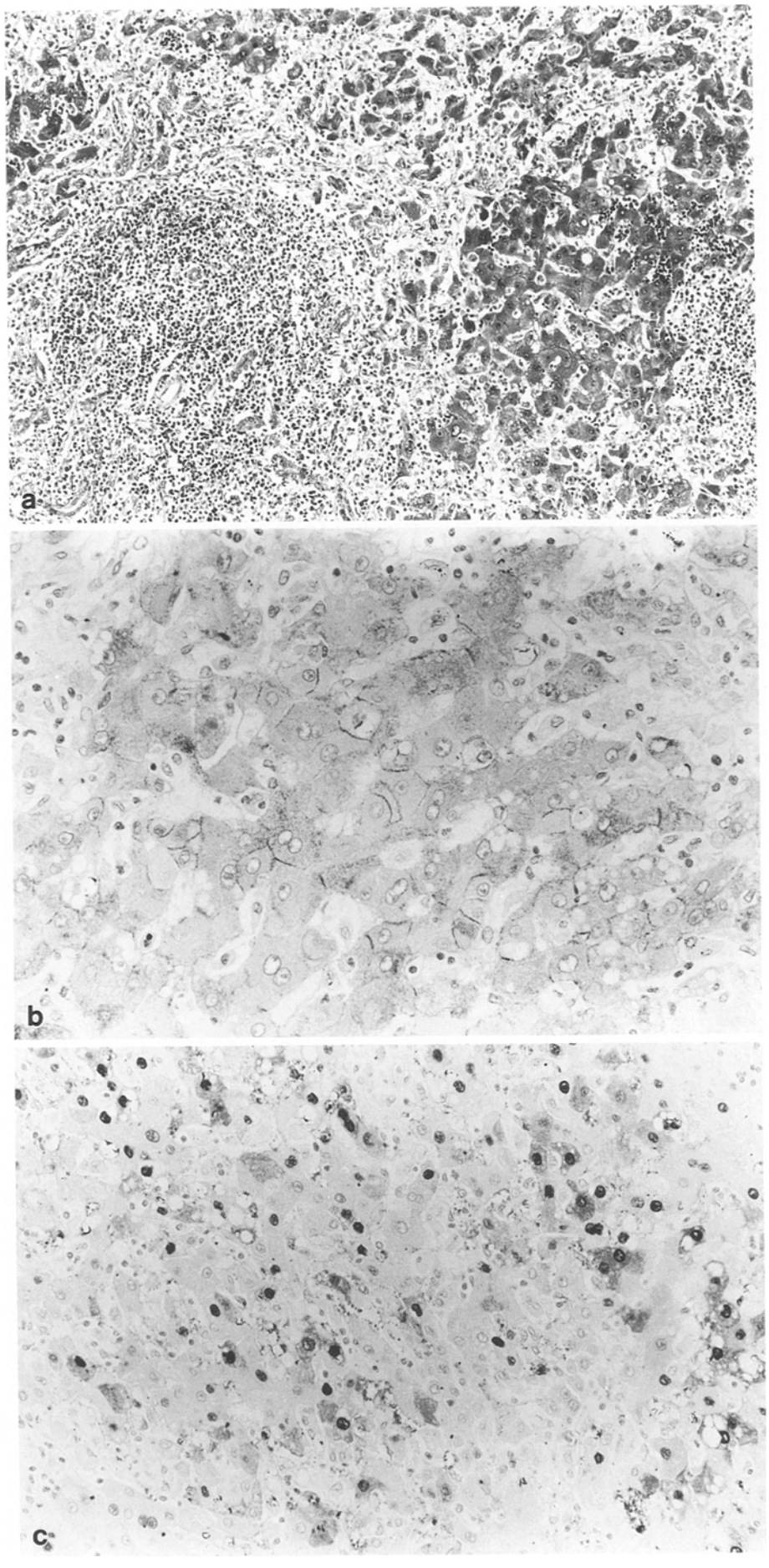

Fig. 3. **a** Hepatocellular necrosis and marked small round cell infiltration mainly in the portal area in case 6 in group C. H&E, ×40. **b** Membranous localization of HBsAg (Ray et al. 1976) which is suggestive of the presence of HBsAg in the liver cell membrane is of note in this case. Anti-HBsAg, PAP stain, ×100. **c** Prominent HBcAg is found in the cytoplasm as well as in the nuclei of case 6 in group C. Anti-HBcAg PAP, ×100

Table 2. Pathological findings of the liver

Case no.	Pathological diagnosis	HBsAg[a]	HBcAg
Group A			
1.	Non-specific change	−	ND
2.	Type A, post-necrotic liver cirrhosis	+	ND
3.	Non-specific change	−	ND
4.	Chronic persistent hepatitis	−	ND
5.	HCC with LC	−	ND
6.	LC	+	ND
7.	HCC with LC	+	ND
8.	Submassive hepatic necrosis with marked fatty change	+	++
9.	Metastatic liver cancer	−	−
10.	Non-specific change	−	−
11.	Fibrosis	−	−
12.	Non-specific change	+	−
13.	HCC with LC	+	−
14.	LC	++	+
Group B			
1.	Non-specific change	−	−
2.	Non-specific change	−	−
3.	Non-specific change	−	−
4.	Metastatic liver cancer	−	−
5.	LC	−	−
Group C			
1.	Periportal fibrosis, fatty change	−	ND
2.	Chronic persistent hepatitis	++	−
3.	Type A liver cirrhosis	−	−
4.	Marked fatty metamorphosis	−	−
5.	Non-specific change	−	−
6.	Chronic toxic hepatitis	+	++
7.	Metastatic liver cancer	−	−
8.	Acute cholestatic hepatitis Bile duct cancer	−	+
9.	Non-specific change	−	−
10.	Non-specific change	−	−
11.	LC	+	++
12.	Senile atrophy of the liver	+	++
13.	Chronic persistent hepatitis	+	−
14.	Metastatic liver cancer	−	−
15.	HCC with LC	+	+

Non-specific changes include slight centrilobular necrosis, fatty metamorphosis, slight inflammatory cell infiltration. HCC, Hepatocellular carcinoma; LC, liver cirrhosis; ND, not determined

[a] The data about the detectability of HBsAg are based on immunohistochemistry and Victoria blue or orcein stain. Those three were consistent in our study except that membranous HBsAg was only detected by immunohistochemistry. ++, Presence of larger numbers of positive cells than +

HBsAg in the serum of HBsAG carriers could disappear (Iijima et al. 1984; James and Nuttall 1984). In this context, it is interesting to study the pathological sequence of HBV carriers, especially elderly and healthy ones. As far as our autopsy cases are concerned, half of the patients, who had been clinically diagnosed as HBV carriers and survived almost their mean life expectancy to die of various diseases, showed no remarkable histology in their livers. HBV markers were only detectable in 1 of 7 those cases.

Histological detectablility of HBsAg in our total series was 35% and 8 out of 11 (72%) in chronic liver disease, and it is higher in groups A and C than in group B. This is compatible with a previous report of seropositive liver cirrhosis and liver cirrhosis with HCC (23 out of 30 sero-positive autopsy cases) (Mori et al. 1980). There is little information about how many asymptomatic carriers have detectable HBsAg in their histologically normal liver. Interestingly in our series, histological detectability of HBV markers correlated with the presence of histological changes of chronic liver disease. In the cases with histologically undetectable HBV, it is possible that HBV had been cleared during the follow-up after the last serum assay. We assumed that trace amounts of HBsAg might be detectable by studying extensive areas of the liver, but found no evidence of the presence of HBsAg.

Alternately, there is a possibility that the method we adopted is not sensitive enough or is inappropriate to detect small amounts of HBsAg. However, it is difficult to argue about the sensitivity, because our knowledge about the turn-over of HBsAg in the liver is still limited and "positive" staining does not always mean the presence of antigen. We concluded, at reasonable sensitivity, that our histological results support the possibility of HBV clearance during the life of HBV carriers.

Our study is not a prospective one and there are therefore several reservations about our conclusions. Although it is accepted that the majority of HBV carriers acquire their positive state during infancy (Hoofnagel et al. 1987), the actual duration of HBsAg carriage of each case could not be determined; serum samples of our subjects from 50 to 70 years ago were clearly not obtainable. We studied the birth places of our patients and found that they came from an ethnic and socioeconomical background similar to those known to be infected with HBV in early life.

Long-standing histological retention of HBsAg may be the cause of the liver damage or the result of affected liver function, or both. Host factors may also influence the sequelae. Histopathological studies on liver from autopsy cases of elderly HBV carriers are instructive from the standpoint of the natural history of this state.

Acknowledgement. This work was supported by Grant in Aid for Cancer Research from the Ministry of Education in Japan. The authors thank Miss Yamada for typing the manuscript and Prof. Machinami for useful comments.

References

Beasley RP, Hwang LY, Lin CH-CH, Chien C-S (1981) Hepatocellular carcinoma and hepatitis B virus. A prospective study of 22707 men in Taiwan. Lancet II:1129–1133

Feinman SV, Berris B, Cooter N, Sinclair JC, Wrobel DM (1982) The results of a long term prospective study of hepatitis B surface antigen (HBsAg) carrier state. Hepatogastroenterology 29:58–61

Hoofnagel JH, Shafritz DA, Popper H (1987) Chronic type B hepatitis and healthy HBsAg carrier state. Hepatology 7:758–763

Iijima T, Saitoh N, Nobutomo K, Nambu M, Sakuma K (1984) A prospective cohort study of hepatitis B surface antigen carrier state. Hepatogastroenterology 29:58–61

James V, Nuttall PA (1984) Reduction in numbers of HBsAg positive donors. BMJ 289:1424

Mori W, Machinami R, Tanaka K (1980) Pathology of hepatocellular carcinoma. Pathol Res Pract 169:4–20

Ray MB, Desmet VJ, Fevery J, De Groote J, Bradburne AF, Desmyter J (1976) Distribution pattern of hepatitis B surface antigen (HBsAg) in the liver of hepatitis patients. J Clin Pathol 29:94–100

Reinicke V, Dybkiaen E, Poulson H, Banke O, Lylloff K, Nordenfelt E (1972) A study of Australia antigen positive blood donors and their recipients, with special reference to liver histology. N Engl J Med 286:867–870

Sakuma K, Takahara T, Tsuda F, Mayumi M (1982) Prognosis of hepatitis B virus surface antigen carriers in relation to the routine liver function tests: a prospective study. Gastroenterology 83:114–117

Sakurai M, Nakaso S (1982) Distribution of HBs antigen in livers revealed by orcein staining. Hepatogastroenterology 29:9–12

Shikata T, Uzawa T, Yoshikawa N, Akatsuka T, Yamazaki S (1974) Staining methods of Australian antigen in paraffin sections. Jpn J Exp Med 44:25–36

Simon JB, Patel S (1974) Liver disease in asymptomatic carriers of hepatitis B antigen. Gastroenterology 66:1020–1028

Statistics and Information Department, Minister's Secretariat, Ministry of Health and Welfare (1986) Vital Statistics of Japan

Suzuki K, Uchida T, Horiuchi R, Shikata T (1985) Localization of hepatitis B surface and core antigens in human hepatocellular carcinoma by immunoperoxidase methods. Replication of complete virus in carcinoma cells. Cancer 56:321–327

Tamura I, Kurimura O, Inaba Y (1986) A follow up study of hepatitis B virus carriers at hospital. Jpn J Cancer Res 77:992–997

Tanaka K, Mori W, Suwa K (1981) Victoria blue – nuclear fast red stain for HBs antigen in paraffin section. Acta Pathol Jpn 31:93–98

Vittal SB, Dourdourekas D, Shobassy N, Gerber M, Telischi M, Szanto PB, Steogmann F, Clowdus BF (1974) Asymptomatic hepatic disease in blood donors with hepatitis B antigenemia. Am J Clin Pathol 62:649–654

Wu T-S, Hsu H-C, Chen D-S, Sheu J-C, Su I-J, Chen S-L, Chuang S-M (1987) Clearance of hepatitis B surface antigen (HBsAg) after surgical resection of hepatocellular carcinoma. J Hepatol 4:45–51