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Hepatocyte Localization of Hepatitis B Core and Surface Antigens in Renal Transplant Recipients

An Ultrastructural Prospective Study

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Summary. A prospective series of 45 liver biopsies taken from 22 renal transplant patients was investigated for the presence of hepatitis B antigen core (HBc) and surface (HBs) components by electron microscopy. At the time of each biopsy serum HBs Ag was sought by radioimmunoassay. Sections were taken for the detection of HBs Ag by immunofluorescence.

In seropositive patients, intravesicular tubular structures resembling HBs Ag were found in 61% of biopsies while the intranuclear core HBc was present in 69%. No correlation could be made between the ultrastructural pattern of the viral components and the intensity of the histological liver damage. During the follow up, there was an accumulation of both HBs and HBc Ag even in a period as short as 1 year. The 9 liver specimens examined after three years of transplantation showed a marked accumulation of both antigens. Thus the expression of HB Ag at the hepatocellular level seems to correlate better with the duration of antigenaemia than with the histological pattern.

Lastly, on matched semithin and ultrathin sections, the ground glass appearance of cytoplasm appeared to correlate with smooth endoplasmic reticulum distorsion, irrespective of the simultaneous presence or absence of intravesicular tubular structures. The sanded nuclei expressed a rare massive accumulation of core antigen.

Key words: Liver biopsy – Renal transplant recipients – HBs and HBc Ag – Electron microscopy

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Introduction

At present, the two main components of the hepatitis B virus (HBV) have been well defined by both immunological and ultrastructural criteria. In the hepatocyte, the lipoprotein surface antigen (HBs Ag) corresponds to 20–22 nm diameter tubular structures of variable length located within smooth endoplasmic reticulum (SER) vesicles, while the DNA associated core antigen (HBc Ag) appears electronmicroscopically as 22–25 nm diameter spherical particles located in the nucleus (Ahmed et al. 1971; Huang 1971; Scotto et al. 1971; Stein et al. 1972; Huang and Groh 1973; Sun et al. 1974; Gerber et al. 1974–1975; Gudat et al. 1975; Yamada and Kosaka 1975; Yamada and Nakane 1977; Thery et al. 1977; Huang and Neurath 1979).

The true significance of the presence of these viral particles within the hepatocytes is not clear mainly because sequential studies are lacking. In this report we communicate the first information concerning a prospective liver biopsy study in 22 renal transplant recipients coming from a single medical center. The high prevalence of HBV infection in such patients is well known (Sopko and Anvras 1978; Degos et al. 1980). The ultrastructural findings of serial liver biopsies taken over a 3 year period with particular emphasis on the presence of viral particles are presented. This study was planned to investigate 1. the relationship between the presence of HBs and HBc antigens independently of each other and of the intensity of the liver damage, 2. the evolution in time of the two viral components, 3. the correlation between the electron microscopic and light microscopic appearance of both antigens at the hepatocellular level.

Material and Methods

Fourty-five liver biopsies from twenty-two renal transplant recipients were studied. All the twenty-two patients had a biopsy on the day of transplantation; among them, fourteen had a second biopsy one year later, and nine had a third biopsy after three years. All the patients received prednisone (0.25 mg/kg/day) and azathioprine (3 mg/kg/day). At the time of each biopsy serum HBs Ag was sought by solid phase radioimmunoassay (Austria II, Abbott, North Chicago, Illinois, USA).

The 45 liver specimens were then divided into two groups. Group I included 36 specimens from serum HBs Ag carriers, while group II was made up of 9 specimens taken from sero-negative patients at the time the biopsy was performed. It must be noted that in this series no sergo-negative patient became positive for HBs in the course of the follow-up. More details concerning these patients are given in a previous paper (Degos et al 1980).

Each liver specimen was examined both by light and electron microscopy. For light microscopy a part of the specimen was fixed in Bouin's fluid and embedded in paraffin. Five micron thick sections were stained with haematoxylin-eosin-safran, Masson's trichrome, Perls and the Gordon-sweet method for reticulin. Histological diagnosis was performed, as well as a search for ground glass appearance and sanded nuclei in the hepatocytes. Paraffin sections were also taken for the detection of HBs Ag, using indirect immunofluorescence, as we have already reported (Camilleri et al. 1977). For electron microscopy another part of the specimen was cut into 1 mm^3 fragments and fixed for 90 min in 2.5% glutaraldehyde with a 0.15 M cacodylate buffer pH 7.4. After post-fixing in 1% osmium tetroxide, and embedding in Epon 812,1 µm semi-thin sections were cut from 10 to 15 blocks of each specimen and stained with toluidine blue. Ultrathin sections of selected areas were stained with uranyl acetate and lead citrate and examined with a Siemens Elmiskop 101 electron microscope.

Results

Electron microscopic findings are summarized in Table 1.

Intravesicular filamentous tubular structures corresponding to HBs Ag were found in the hepatocytes of 22 biopsies (61%) from HBs seropositive patients (Group I). They were 20 nm diameter and of variable length. The cytoplasms containing such intravesicular structures showed scattered glycogen and randomly distributed organelles, especially mitochondria. In these areas SER vesicles were numerous and dilated. The accumulation of tubular structures gave the cytoplasm a particular reticular appearance (Fig. 1). Elsewhere, tubular struc-

Table 1. Electron microscopic findings

	HBs seropositive[a] patients: Group I	HBs seronegative[a] patients: Group II
Intravesicular tubular structures	22 (61%)	0
Intranuclear cores	25 (69%)	0
Absence of intravesicular structures and intranuclear cores	8 (22%)	0
Smooth endoplasmic reticulum changes	28 (77%)	0
Number of cases	36	9

[a] Serum HBs Ag was sought by radioimmunoassay

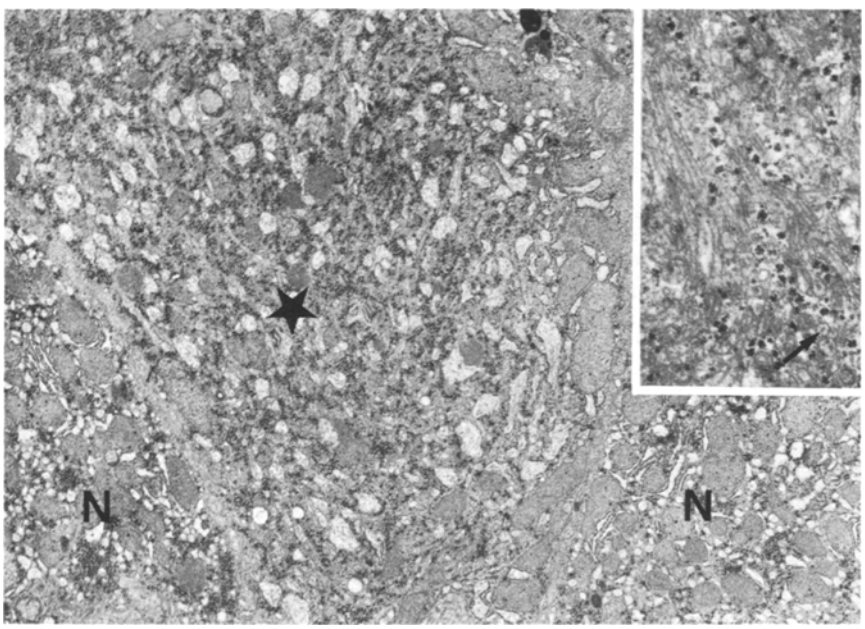

Fig. 1. Electron micrograph showing one HBs Ag containing hepatocyte with marked distorsion of smooth endoplasmic reiculum vesicles (star). *N*, normal hepatocytes; ×8,500. *Inset:* dilated vesicles of smooth endoplasmic reticulum containing filamentous tubular structures with several cross-sections *(arrow)*. ×30,000

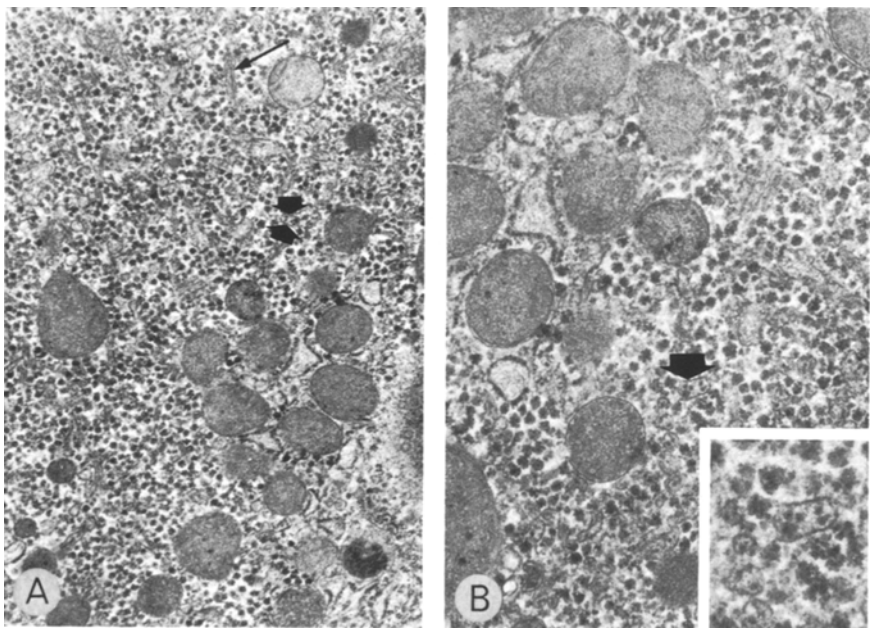

Fig. 2. A, B. Normal looking hepatocytes showing no evidence of cytoplasmic changes. **A** Scarce tubular structures can be seen on cross-sections *(thick arrow)* authenticated by the presence of longitudinal sections *(thin arrow)*. ×20,000. **B** Identification of one vesicle containing five cross-sectioned tubular structures. ×30,000. *Inset:* High power view of the same vesicle. ×100,000

tures were far less numerous and found only by systematic examination of the cytoplasm at high magnification. They were difficult to identify in cross-section becouse they appeared as electron-dense structures strongly contrasted by osmium tetroxide. When a SER vesicle contained a single cross-section, it looked like a rosette and could be confused with the entire virion described by Dane et al. (1970). These difficulties were elucidated by observing a few longitudinally cut tubular structures (Figs. 2 and 3).

Hepatocytes containing intravesicular tubular structures appeared to have no distinct localization within the liver lobules. They mostly occured in small clumps of 2 or 3 cells.

Intravesicular tubular structures were only observed in biopsies of HBs Ag seropositive patients. However, they were not a constant finding in these patients: only two thirds of the liver specimens from Group I contained them. This percentage was in contrast with the occurence of ground-glass hepatocytes as noticed on light microscopy (88%) and the high incidence of HBs positivity using indirect immunofluorescence (IF)-positive cells corresponded in distribution to ground-glass hepatocytes. They mostly showed a spotty expression as an isolated cell or as small groups. By alternate semi and ultrathin sectioning of Epon-embedded material, hepatocytes with ground-glass appearance could be matched with cells having characteristic intravesicular tubular structures. On electron microscopy, ground glass hepatocytes selected from semithin sec-

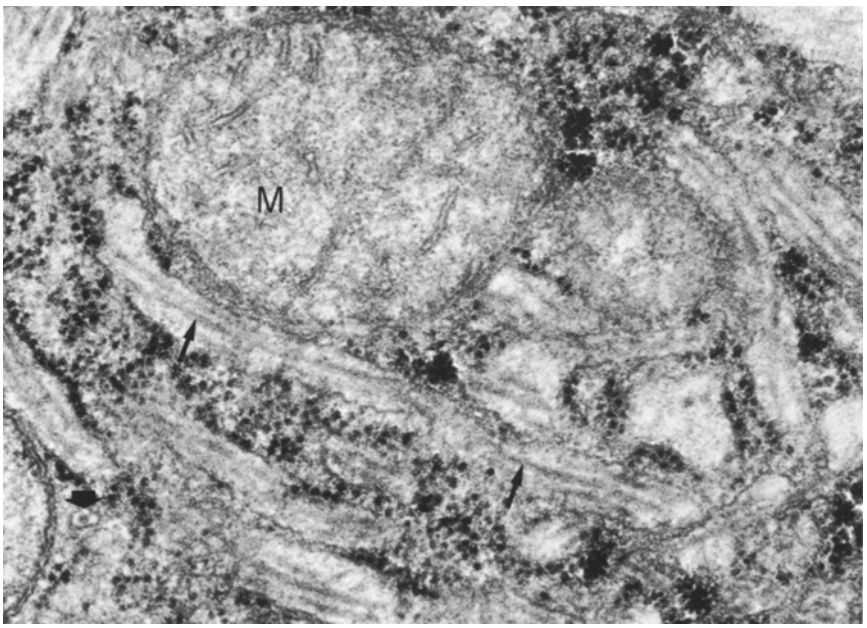

Fig. 3. Electron micrograph showing longitudinal sections of tubular structures located within dilated smooth endoplasmic reticulum vesicles *(thin arrow)*. Note cross sections *(thick arrow)*. *M*, mitochondrion. ×84,000

tions always showed distorsion of the SER. Some of them did not contain tubular structures but showed large areas of numerous gathered, dilated vesicles displacing other cell organelles towards the cell periphery. Most of these dilated vesicules contained an amorphous flocculent material (Fig. 4). These changes in SER were found in 28 biopsies taken from Group I (77%), seven did not contain tubular structures. They were never found in biopsies taken from seronegative patients (Group II).

Twenty-five biopsies (69%) from Group I showed intranuclear cores resembling HBc Ag. They were regularly shaped round particles 22–25 nm in diameter; at high magnification they showed an electron-lucent center and an hexagonal periphery (Fig. 5). These particles were seen everywhere in the nuclear area but most often towards the nuclear membrane. In the series reported, a heavy gathering of cores showing polycyclic electron-lucent nuclear inclusions was observed in one case (Fig. 6). The nuclei containing cores were numerous and appeared to have no particular location within the lobule. Such nuclei were characterized by an unusual dispersal of chromatin giving them an electron-lucent aspect (Fig. 7). Neither nuclear bodies nor nucleolar changes were noted. In 3 biopsies round particles of the same diameter were found in the cytoplasm near the nuclear pores; in these cases there were intranuclear HBc cores.

In matched semithin and ultrathin sections there was no clearcut light microscopic aspect of nuclei containing HBc cores. The chromatin dispersal most

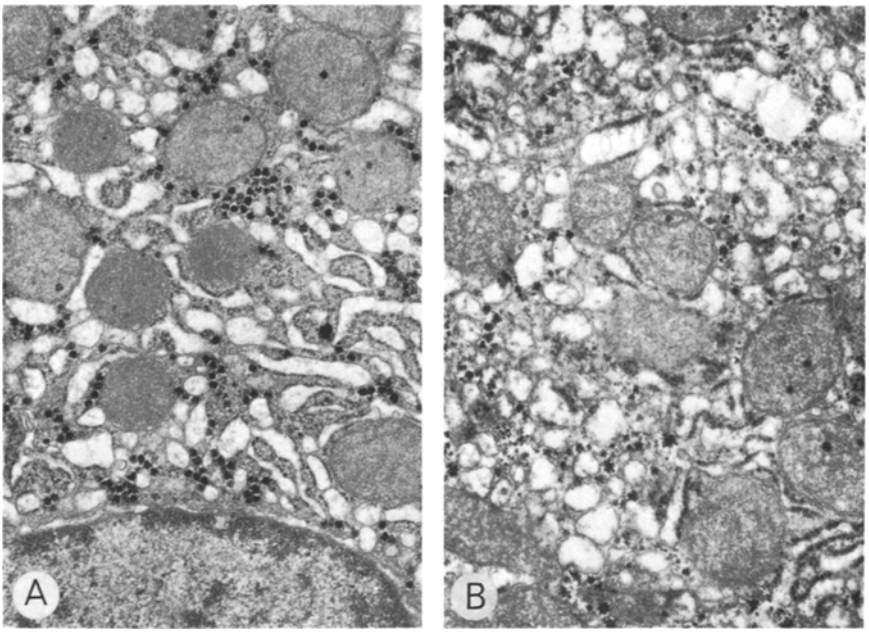

Fig. 4. A Electron micrograph showing numerous dilated smooth Endoplasmic reticulum vesicles without tubular structures in a HBs seropositive patient. **B** Another hepatocytes from the same patient showing flocculent material in the dilated vesicles. × 89,000

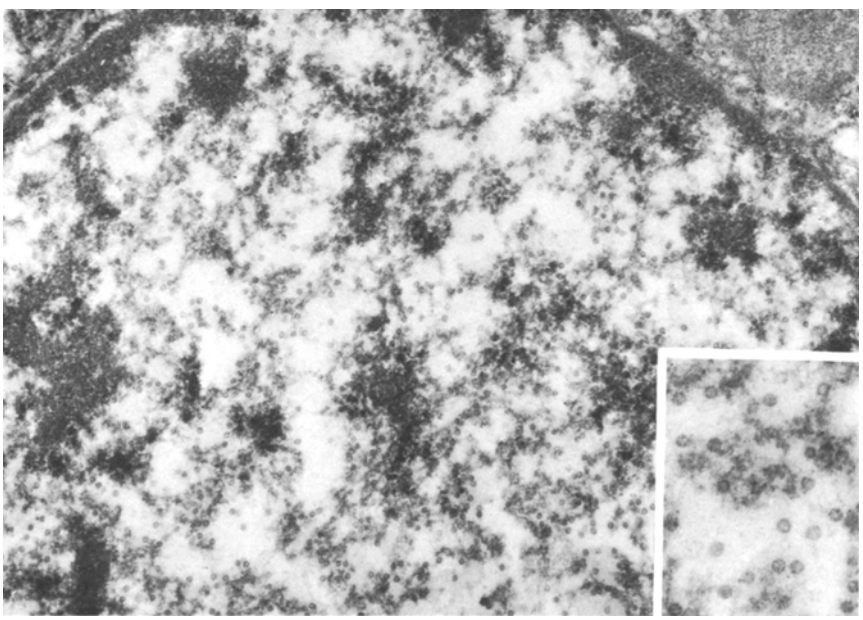

Fig. 5. Scattered cores in a hepatocyte nucleus; some of them are gathered in small clumps. × 40,000. *Inset:* high power view of cores. × 105,000

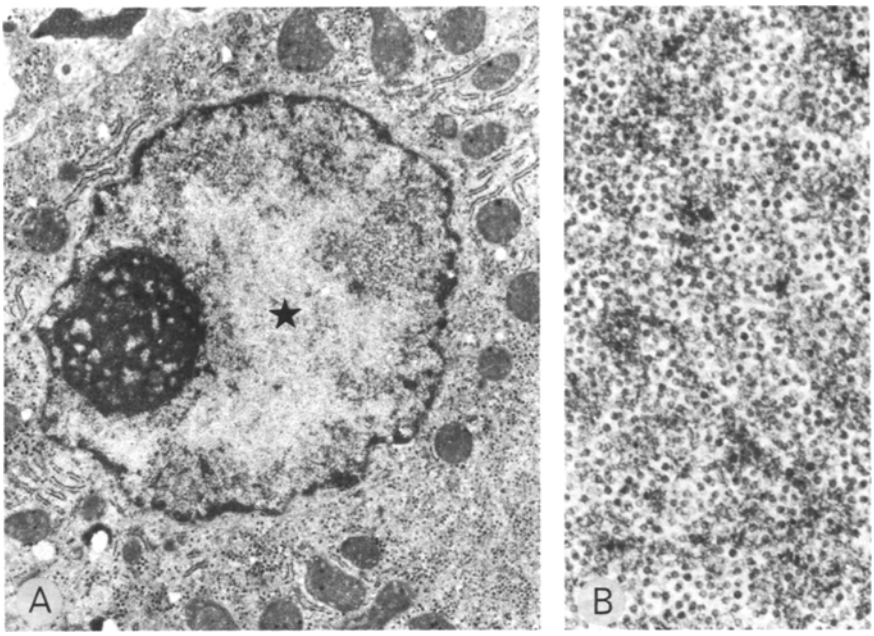

Fig. 6 A, B. Electron micrograph showing massive intranuclear gathering of core particles. **A** Electron lucent polycyclic inclusions *(star)*. × 12,000. **B** Higher magnification of the same nucleus showing close packing of core particles. × 60,000

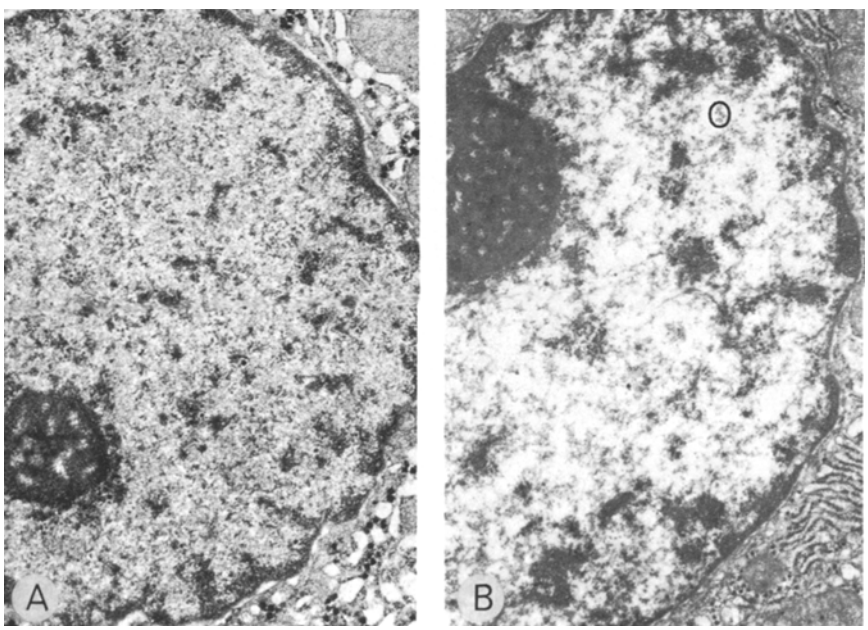

Fig. 7 A, B. Electron micrograph showing two different nuclei at the same magnification. **A** Normal looking nucleus. **B** Nucleus with core particles (circle). Note the dispersal of chromatin. × 16,000

Table 2. Distribution of intravesicular tubular structures and intranuclear cores in biopsies from HBs Ag carriers (Group I)

Intravesicular tubular structures only	3
Intranuclear cores only	6
Intravesicular tubular structures associated with intranuclear cores	19
Absence of intravesicular tubular structures and intranuclear cores	8
Number of cases	36

Table 3. Electron microscopic findings in serial biopsies over 3 years (9 patients)

	Intravesicular tubular structures	Intranuclear cores
At the time of transplantation	5 (55%)	5 (55%)
1 year after transplantation	6 (66%)	7 (77%)
3 years after transplantation	9 (100%)	9 (100%)

Table 4. Correlations between pathological changes and electron microscopy findings in biopsies from serum HBs Ag carriers (Group II)

	Histopathological changes			
	CAH	CPH	ALH	NL
Intravesicular tubular structures only	3	0	0	0
Intranuclear cores only	1	3	0	2
Intravesicular tubular structures associated with intranuclear cores	6	13	0	0
Absence of intravesicular tubular structures and intranuclear cores	1	6	1	0
Number of cases	11	22	1	2

CAH = chronic active/aggressive hepatitis; CPH = chronic persistant hepatitis; ALH = acute lobular hepatitis; NL = normal liver

often gave the nucleus a non-specific faintly stained appearance without increase in size. A fine granular sandy appearance was only observed in the case where numerous intranuclear cores were seen on electron microscopy.

Intravesicular tubular structures and intranuclear cores were found in 19 specimens from Group I, either in the same cell or in different cells. A single antigenic component was observed in 9 cases (HBc 6 times, HBs 3 times) (Table 2). Intracellular entire virions were never found.

The study of serial biopsies taken from these patients showed a progressive increase in the number of positive cases for the two main antigenic components (HBs and c Ag), (Table 3). In nine patients biopsies were performed on the day of transplantation and repeated one year and three years later. Even in as short a period as a year an increased storage of viral antigens was observed. After 3 years of transplantation, all nine patients showed large amounts of both HBs and HBc Ag.

Lastly, there was no relationship between the HB Ag expression at the cellular level and the pathological diapnosis (normal liver NL, acute lobular hepatitis ALH, chronic persistent hepatitis CPH, chronic agressive/active hepatitis CAH) (Table 4).

Discussion

The ultrastructural findings presented are in agreement with the previously published description of the two main antigens of the hepatitis B virus. The data suggest that the HB viral antigens are not at the same location within the hepatocyte. The core antigen replicates in the nucleus and acquires a protein coat supporting the surface antigen which is probably synthetized in the SER vesicles. The precise site of entire virus assembly remains a matter of debate (Yamada and Nakane 1977; Huang and Neurath 1979).

While the hepatitis B virus has been well defined on immunological and ultrastructural grounds, several questions remain concerning what happens in long term follow-up studies. This 3 year prospective study of an homogeneous group of patients known to be more susceptible to HBV infection, might provide new insight in this field. Firstly, a relationship between the expression of core and surface antigens in liver tissue and the different forms of histopathologic pictures was suggested by Gudat et al. (1975). According to these authors, prominent HBs expression is found in non aggressive type of liver damage (CPH), while chronic aggressive/active hepatitis is usually associated with spotty HBs and HBc equivalence pattern. In our series, we failed to find any difference in viral antigenic expression between the biopsies with CPH (22 cases) or normal liver (2 cases) and the biopsies with CAH (11 cases). HBs and c Ag equivalence was mostly observed; it was found in 6/11 CAH and 13/22 CPH. Our data are in agreement with Huang and Neurath's findings (1979). Secondly, serial biopsies performed in nine seropositive patients demonstrated a progressive storage of both HBs and c Ag. All patients from this group showed large amounts of both antigens in their hepatocytes after three years of transplantation. These data confirm the results we have already obtained for HBs Ag, using an immunofluorescence technique (Camilleri et al. 1977). The quantitative expression of the two main viral antigens seems to correlate better with the duration of antigenaemia than with the histological pattern. This evolution might be related either to the patients who are known to be immunodepressed and/or the immunosuppressive therapy (Degos et al. 1980).

The last point of this study deals with the correlation between the ultrastructural aspect of the two main antigens and the light microscopic changes in the infected hepatocytes. The nuclei containing round particles of core antigen show an unusual dispersal of chromatin on electron microscopy. On semithin sections this change corresponds to a faintly stained appearance. However the sanded nuclei described by Bianchi and Gudat (1976) expressed a heavy gathering of cores which was found only in one patient in this series. The ground glass appearance of hepatocytes is usually related to the accumulation of tubular structures within the vesicles of SER. In our series, these structures linked to HBs Ag were noticed in hepatocytes in 61% of the biopsies taken from

seropositive patients. In contrast, HBs immunofluorescence positive cells were found in 91% cases. This discrepancy might be partly explained by the small number of hepatocytes studied on electron microscopy and the spotty pattern of the HBs containing cells. However, on matched semithin and ultrathin sections it appears that cells with the ground-glass appearance do not always show intravesicular structures. In such cases SER changes seem almost constant (28 out of 36 specimes in Group I and never in Group II). Thus it appears likely that there is a relationship between SER changes and ground-glass appearance of the cytoplasm and HBs antigenaemia, irrespective of a simultaneous presence or absence of intravesicular tubular structures (Winckler et al. 1976). Such changes of SER in HBs Ag seropositive patients have been reported previously (Gerber et al. 1975; Gudat et al, 1975; Thery et al. 1977). It is probable that HB viral antigens are not always linked to structures recognizable on electron microscopy; a recent immunoperoxidase study showed that immunoreactive HBs Ag could be present in the vesicles of the SER in a nontubular form (Yamada and Nakane 1977; Huang and Neurath 1979). However, similar increase in vesicular SER has been said to occur in human liver following drug or alcohol administration (Jezequel et al. 1971–1974; Thomsen et al. 1976).

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