

## UNUSUAL ELECTRON MICROSCOPY FINDINGS IN LIVER BIOPSIES FROM PATIENTS WITH VIRAL HEPATITIS B

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*Summary.* — Electron microscopy of 57 needle biopsies from livers of 55 patients with chronic hepatitis, mostly of type B, revealed, in addition to typical hepatitis B virus nucleocapsids in 34 cases, also atypical intranuclear inclusions in 9 biopsies. These inclusions consisted of electron-dense particles similar in size to hepatitis B virus nucleocapsids. The possibility of an involvement of a hepatitis non-A non-B virus is discussed.

*Key words:* hepatitis B; hepatitis non-A non-B; electron microscopy

From 1975 to 1979 we carried out a complex investigation of 57 liver biopsies and sera from fifty-five patients mainly suffering from a chronic form of hepatitis B (HB). All patients were subjected to detailed anamnestic and physical examination of their livers, clinical biochemical examinations, classical histology with eventual orcein staining. Most patients were examined scintigraphically and laparoscopy was done in some of them. In parallel, HB surface antigen (HBsAg) was assayed by counter-immunoelectrophoresis (CIEP). The sera were examined once by immune electron microscopy (IEM) for circulating HB virus (HBV) particles, HBsAg and their immune complexes. Most sera were also examined for antibody against HBV core antigen (anti-HBc) with a CORAB (RIA) kit (Abbott, U.S.A.). The results of these examinations will be published elsewhere.

We are reporting now about a finding in the course of electron microscope examination of liver biopsies.

Portions of liver biopsies taken by a Menghini needle were fixed in 2.5% glutaraldehyde and 1% OsO<sub>4</sub> and embedded into Durcupan ACM (Fluka). Ultrathin sections cut on an LKB III ultramicrotome were contrasted with uranyl acetate and lead citrate and examined in a JEM 7A electron microscope at 80 kV.

In 34 of 57 (approx. 60%) biopsies examined we found typical HBV nucleocapsids (Fig. 1). They occurred either singly or in small groups as round structures limited by an outer shell, with an electron-translucent

**Table 1. Findings in 9 patients whose liver biopsies revealed the presence of electron-dense particles in the nuclei of hepatocytes**

Biopsy No.	Patient, year of birth (males)	Final diagnosis	Electron microscopy				RIA		CIEP
			HBV NC <sup>1)</sup>	Dane particles	HBsAg density	IC <sup>2)</sup>	Anti-HBc <sup>3)</sup>	HBsAg	HBsAg
10	M. Š., 1948	CPH	+	+	2+ - 4+	0	10.8	ND	3+
11	J. K., 1915	CAH	+	+	+	0	11.9	ND	3+
17	J. Š., 1957	Chronic carrier	0	+	+	0	ND	ND	3+
36	D. H., 1958	Chronic carrier	0	0	+ - 2+	0	10.7	ND	2+
37	S. Ž., 1928	Steatofibrosis	0	0	0	0	0	0	0
42	M. K., 1926	Cirrhosis	++	+	2+ - 3+	+	8.1	ND	3+
45	L. B., 1923	Cirrhosis	++	+	2+ - 3+	+	11.0	ND	3+
49	J. M., 1940	Steatofibrosis	0	+	+	+	7.4	ND	3+
53	F. Ž., 1929	CPH	+	+	+ - 2+	0	11.8	ND	3+

<sup>1)</sup> HBV nucleocapsids in hepatocytes.

<sup>2)</sup> Circulating immune complexes.

<sup>3)</sup> Positivity indices.

CPH — Chronic persistent hepatitis; CAH — chronic active hepatitis.

ND — not done.

0 = finding negative; + - 4+ = positive findings evaluated semiquantitatively.

central part and measuring 20–23 nm. These nucleocapsids occurred only in the nuclei of hepatocytes.

In 9 (approx. 15%) of the 57 biopsies examined we detected, again in the nuclei of hepatocytes, inclusions of various sizes, composed of electron-dense particles of a size similar to that of HBV nucleocapsids (Fig. 2). Since these particles had no inner shell we considered them to represent "virus-unlike" particles, most probably intranuclear glycogen particles, and the whole formations to represent glycogen pseudoinclusions. But based on the reports by Hantz *et al.* (1979), Shimizu *et al.* (1979), Bradley *et al.* (1979) and Trepo *et al.* (1980) we are now inclined to accept the hypothesis that a potential agent of non-A non-B hepatitis (HNANB) could be involved. Our particles approach most closely those of the second HNANB virus in experimentally infected chimpanzees, namely the strain H (Shimizu *et al.*, 1979). Of course, this is only a working hypothesis. Serological identification of the structures observed is still open.

Irritating in our findings was the fact that in 5 of 9 cases a mixture with typical HBV nucleocapsids was involved (Fig. 3). Further data are evident from Table 1, summarizing the final clinically-pathological diagnoses and markers related to HBV replication rate in the patients. Should our working hypothesis be confirmed, actual infection with two species of hepatitis virus, HB and HNANB, would have taken place in 8 of the 9 patients. HB was excluded by radioimmunoassay in patient S. Ž. (biopsy No. 37).

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*Explanation of Electron Micrographs (Plates VI and VII):*

*Fig. 1.* Single nucleocapsids of HBV in the nucleus of a hepatocyte.  $\times 100\ 000$ .

*Fig. 2.* Part of an inclusion in the nucleus of a hepatocyte, composed only of electron-dense particles.  $\times 87\ 000$ .

*Fig. 3.* Detail of an inclusion containing both HBV nucleocapsids and electron-dense particles.  $\times 130\ 450$ .