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Hepatitis B Antigen: Nature and Distribution of Cytoplasmic Antigen in Hepatocytes of Carriers (37912)

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Two patterns of hepatitis B antigen (HB Ag, Australia antigen, Hepatitis associated antigen) in the liver have been demonstrated by fluorescence microscopy. One, in liver disease, particularly chronic hepatitis, is characterized by nuclear HB Ag in many hepatocytes and cytoplasmic HB Ag in scattered hepatocytes and Kupffer cells (1–4). This picture is accentuated by immunosuppression and is associated with spherical particles in the hepatocytic nuclei under the electron microscope (5–11). These particles have HB antigenic determinants as shown by immunoelectron microscopy (9, 12) and were interpreted as virus core (13, 14). The second pattern, found predominantly in healthy carriers, consists of abundant cytoplasmic HB Ag in many hepatocytes without localization in hepatocytic nuclei or Kupffer cells (3, 15, 16). The cytoplasm of these hepatocytes has a peculiar ground-glass appearance under the light microscope (17). Since the nuclear and cytoplasmic antigens seem to have different specificities (18, 19), the question arose as to the ultrastructural nature of the cytoplasmic antigen and the possible presence of particles.

Material and Methods. Nineteen liver biopsy specimens and five autopsy specimens from 21 HB Ag sero-positive persons and 3 control patients were examined by immunofluorescence and electron microscopy and in three cases also by immunoelectron microscopy. Immunofluorescence studies were performed on all specimens as described previously (3) and revealed the two patterns described above after staining with fluoresceinated antisera to HB Ag (Table I). Electron microscopy was performed on specimens which had been fixed in 2.5% glutaraldehyde or in 10% formaldehyde, postfixed in 1% osmium tetroxide, dehydrated, and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a Hitachi HS7S or HS8 electron microscope. In addition, liver biopsy specimens from one HB Ag carrier, from one patient with HB Ag sero-positive chronic hepatitis, and from one HB Ag sero-negative patient were subjected to immunoelectron microscopy. The method employed was the indirect ferritin-labeled antibody technique described previously (9). Briefly, 4–6-μm-thick frozen sections were fixed for 5–30 min in 2% paraformaldehyde, incubated with human antisera to HB Ag, followed by ferritin-labeled goat antihuman IgG, postfixed in 1% osmium tetroxide, dehydrated, and embedded in Epon 812 using inverted Beem capsules.

Results. The hepatocytic cytoplasm in 12 of 14 asymptomatic carriers contained numerous circular and filamentous structures measuring 20–30 nm in diameter (Fig. 1). Some of the circular structures appeared to be cross-sections of the filamentous forms and some had an electron-dense center. These structures were single or in clusters of 3 or more and always within the cisternae of the endoplasmic reticulum

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TABLE I. Correlation of Immunofluorescence and Electron Microscopic Results.

Diagnosis	No. of cases	Serum HB Ag	Hepatocytic HB Ag fluorescence in			Particles on electron microscopy in hepatocytes	
			Nuclei	Cytoplasm of		Nuclei	Cytoplasm
				>20% of cells	<20% of cells		
Carrier	14	+	2[a]/14	11/14	3[a]/14	2[a]/14	12/14
Chronic hepatitis	6[b]	+	5/6	1/6	5/6	5/6	2/6
Acute hepatitis	1	+	—	—	—	—	—
Controls	3	−	—	—	—	—	—

[a] One of these carriers was treated with immunosuppressive therapy and two had elevated serum transaminase activity.

[b] One of the patients with chronic hepatitis was treated with immunosuppressive therapy.

(ER) which appeared dilated and distorted. The surrounding membrane of ER was smooth but occasionally a few ribosomes were attached. "Budding" from the membrane was not seen. In many hepatocytes the ER was increased in amount and the particles were numerous. The number of particle-containing hepatocytes corresponded

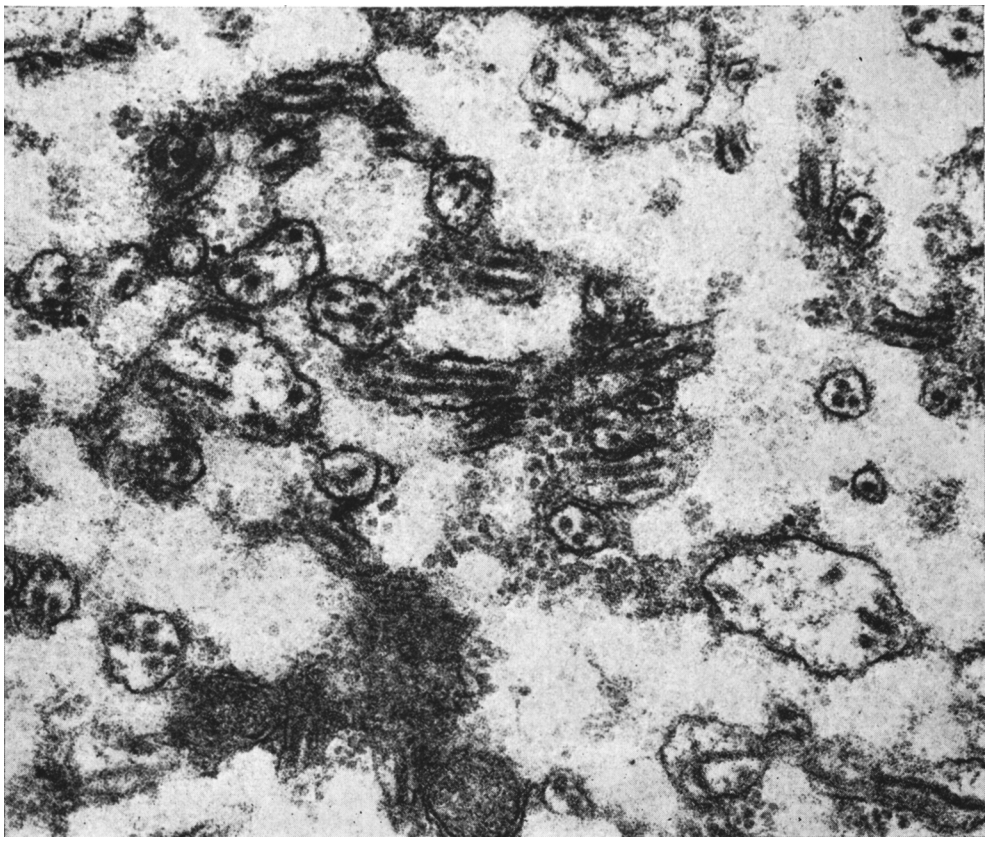

FIG. 1. Circular and filamentous structures in the endoplasmic reticulum of a hepatocyte from a carrier (uranyl acetate and lead citrate, ×54,000).

to the number of hepatocytes with cytoplasmic HB Ag-specific fluorescence. The livers of the two carriers in which no particles were detected showed only a few scattered hepatocytes with HB Ag-specific fluorescence in the cytoplasm. No particles were seen in the nuclei of the carriers with normal transaminase activity. The hepatocytic nuclei of 5 patients with chronic hepatitis and HB Ag-specific nuclear fluorescence contained 20–25-nm spherical particles with or without an electron-dense center. In a few hepatocytes in two of these patients, cytoplasmic structures resembling those described in the carriers were found. Two "carriers" with elevated transaminase activity also had nuclear particles in hepatocytes and one had cytoplasmic particles as well. Occasionally, nuclear and cytoplasmic particles were observed within the same hepatocyte. Neither HB Ag-specific fluorescence nor particles were seen in the livers of 3 HB Ag sero-negative patients.

On immunoelectron microscopy, ferritin granules, indicating the binding of antibody to HB Ag, were associated with the membranes of ER and with the enclosed circular and filamentous structures (Fig. 2). The distribution of ferritin along these structures and in different sections was discontinuous and irregular. The nuclear particles in the liver specimen of the patient (Fig. 3) with chronic hepatitis were aggregated within a moderately electron-dense material and surrounded by ferritin. Only scattered ferritin granules were seen along other cell organelles or in cells free of the circular or filamentous structures. Incubation of liver sections of the carrier with normal human plasma and of the control specimen with antiserum to HB Ag followed by ferritin-labeled antihuman IgG did not show specific binding of ferritin-labeled antibody.

Discussion. The electron microscopic examinations clarify the nature of the hepatocytes with "ground-glass"-appearing cyto-

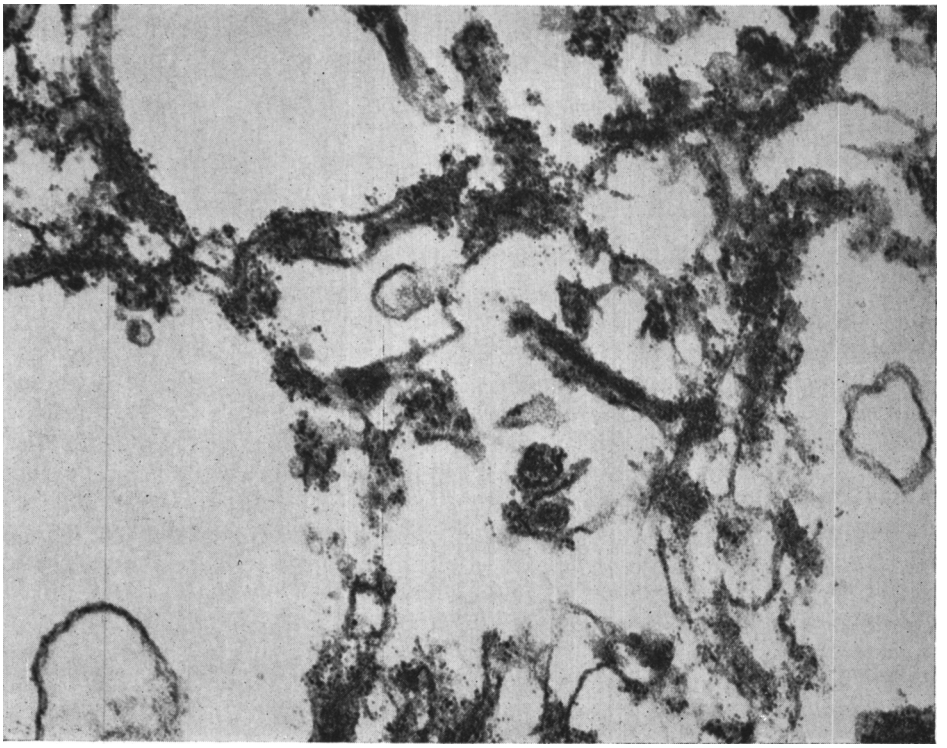

FIG. 2. Ferritin granules along the endoplasmic reticulum and the enclosed circular and filamentous structures in a hepatocyte from a carrier after incubation with antibody to HB Ag followed by ferritin-labeled anti-human IgG (uranyl acetate and lead citrate, ×54,000).

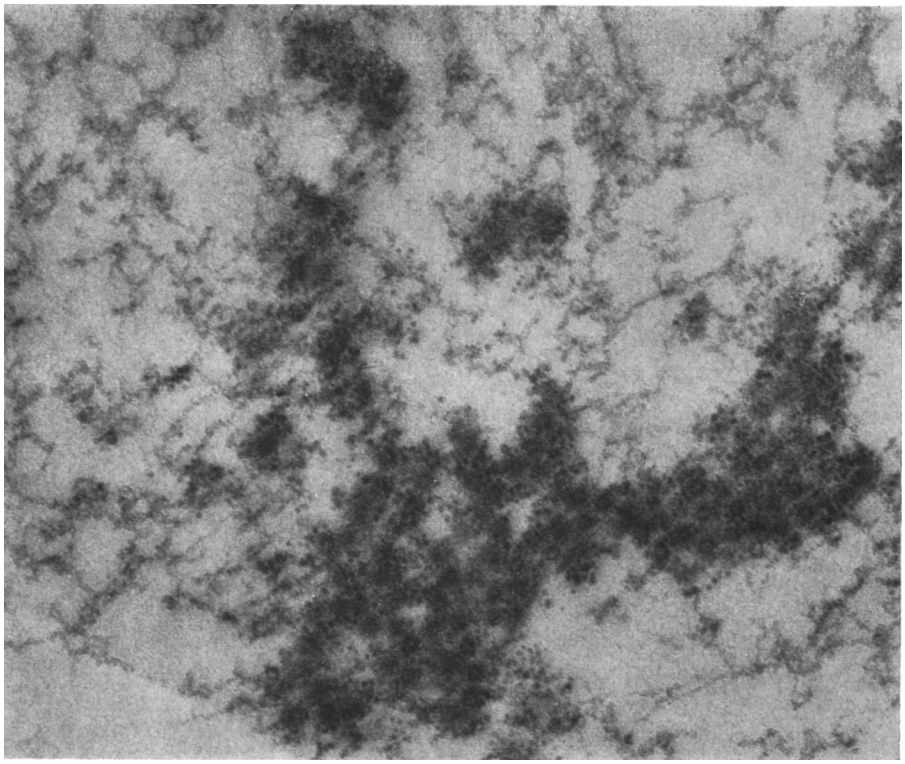

FIG. 3. Ferritin granules around 20-nm particles in the hepatocytic nucleus of a patient with chronic hepatitis after incubation with antibody to HB Ag followed by ferritin-labeled anti-human IgG (uranyl acetate and lead citrate, ×90,000).

plasm in asymptomatic HB Ag carriers which on immunofluorescence contain HB Ag. These hepatocytes resemble those seen in man after treatment with drugs inducing the microsomal biotransformation system and increasing the smooth ER (20). In carriers the "ground-glass"-appearing hepatocytes contain filamentous and circular structures in the cisternae of abundant ER. Similar particles had been noted by Stein *et al.* in the hepatocytic cytoplasm of HB Ag carriers (21). The immunoelectron microscopic findings indicate that the ultrastructural correlates of the cytoplasmic HB Ag in our material, the circular and filamentous structures with a surrounding membrane of ER, contain HB antigenic determinants.

These cytoplasmic structures could be hepatitis B virus, virus core being coated in the ER, surplus virus coat, or host material containing HB Ag. We favor the assumption that the intranuclear spherical particles are the internal core of the hepatitis B virus (HBV) which replicates in the hepatocytic nucleus and that the cytoplasmic circular and filamentous structures represent virally coded proteins, probably surplus coat material which is packaged in the ER. The difference in distribution of abundant cytoplasmic and little or no nuclear HB Ag in carriers, in contrast to much nuclear and little cytoplasmic HB Ag in chronic hepatitis and under immunosuppression, suggests that in carriers the HBV infection results in little virus replication in the nucleus, but abundant formation of virally coded proteins in the cytoplasm of hepatocytes. In chronic hepatitis and under immunosuppression, the intranuclear virus-like particles become prominent with a concomitant decrease of the virally coded material in the hepatocytic cytoplasm and uptake of HB Ag by Kupffer cells. The

epidemiologic implications of this behavior require further study.

Summary. Abundant ER with 20–30-nm circular and filamentous structures was found by electron microscopy in hepatocytes of asymptomatic carriers with "ground-glass"-appearing cytoplasm which by immunofluorescence had been shown to contain HB Ag. On immunoelectron microscopy, the circular and filamentous structures as well as the surrounding ER reacted with antibody to HB Ag. In HB Ag-positive chronic hepatitis, cytoplasmic antigen was less prominent, but HB Ag was localized in many hepatocytic nuclei by immunofluorescence, electron microscopy, and immunoelectron microscopy. This difference in intracellular localization of HB Ag between asymptomatic carriers and patients with chronic hepatitis may have epidemiologic implications.

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