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## Electron microscopic observation of hepatitis B virus budding from hepatocytes into bile canaliculi.

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# Electron microscopic observation of hepatitis B virus budding from hepatocytes into bile canaliculi.\*

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## Abstract

In electron microscopic observation of a liver biopsy obtained from a hepatitis B surface antigen-positive patient, noncoated core particles were occasionally seen budding into the hepatocytic cisterni and many Dane particles were found in the pericanalicular vesicles of hepatocytes. Noncoated core particles were also localized in clusters within the bleb of microvilli. There were some core particles being protruded from microvilli into the lumen of bile canaliculi by budding. These findings suggest hepatitis B virus being released from the hepatocyte to the bile canaliculi by two different modes; through vesicle by reversed phagocytosis and from the microvilli by budding.

**KEYWORDS:** type B hepatitis, hepatitis B virus, Dane particle, bile canaliculi.

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— BRIEF NOTE —

**ELECTRON MICROSCOPIC OBSERVATION OF HEPATITIS  
B VIRUS BUDDING FROM HEPATOCYTES  
INTO BILE CANALICULI**

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*Abstract.* In electron microscopic observation of a liver biopsy obtained from a hepatitis B surface antigen-positive patient, noncoated core particles were occasionally seen budding into the hepatocytic cisterni and many Dane particles were found in the pericanalicular vesicles of hepatocytes. Noncoated core particles were also localized in clusters within the bleb of microvilli. There were some core particles being protruded from microvilli into the lumen of bile canaliculi by budding. These findings suggest hepatitis B virus being released from the hepatocyte to the bile canaliculi by two different modes; through vesicles by reversed phagocytosis and from the microvilli by budding.

*Key words:* type B hepatitis, hepatitis B virus, Dane particle, bile canaliculi.

Many studies have been reported concerning the replicative cycle of hepatitis B virus (HBV) from ultrastructural and immune electron microscopic studies (1-3), and from animal experiments (4). Nevertheless, it has not yet been clarified how hepatitis B surface antigen (HBsAg)-positive particles including Dane particles are released from hepatocytes into the bile duct. Recently we have demonstrated HBV and associated particles in bile canaliculi by electron microscopy (5). To learn more about them being released from hepatocytes into bile canaliculi, a detailed ultrastructure has been studied in the hepatocytic cytoplasm surrounding the lumen of bile canaliculi.

*Materials and Methods.* The liver biopsy specimen was obtained from a case diagnosed as chronic hepatitis by biochemical and histological findings. HBsAg in sera was always positive by reversed passive hemagglutination tests. Under the light microscope, the liver showed chronic aggressive hepatitis of a severe form. Part of the liver tissue was fixed in a PLP fixative (1) and prepared for detection of HBsAg and hepatitis B core antigen (HBcAg) using a peroxidase

label antibody method (1). The rest was cut less than 1 mm in size, fixed in 3% glutaraldehyde and 1% osmium tetroxide, dehydrated in graded ethanols, and embedded in Epon. Sections were stained with uranyl acetate and lead citrate and examined under the electron microscope (Hitachi H-700H) at magnifications from 6,000 to 50,000.

*Results.* In light microscopic observation of the liver specimen, HBcAg was predominantly stained in the cytoplasm of almost all hepatocytes and HBsAg was present both in the cytoplasm and on the cell membrane of liver cells by peroxidase labeled antibody method. Routine electron microscopy revealed many uncoated core particles in the cytoplasm and a lot of Dane particles in the cisterni of hepatocytes. Some of these Dane particles had electron-dense core particles suggestive of complete HBV (Fig. 1). A few core particles were budding into the cisterni (Fig. 2, inset). Some Dane particles were also observed in vesicles of the cytoplasm surrounding the lumen of bile canaliculi (Fig. 1 & 2). Uncoated core particles occasionally appeared in clusters within the bleb of microvillus of the bile canaliculus (Fig. 3) and in a broadened dense zone of cytoplasm surrounding the lumen. A few Dane particles and tubular forms were demonstrated in the bile canaliculi. The outer membrane of some Dane particles

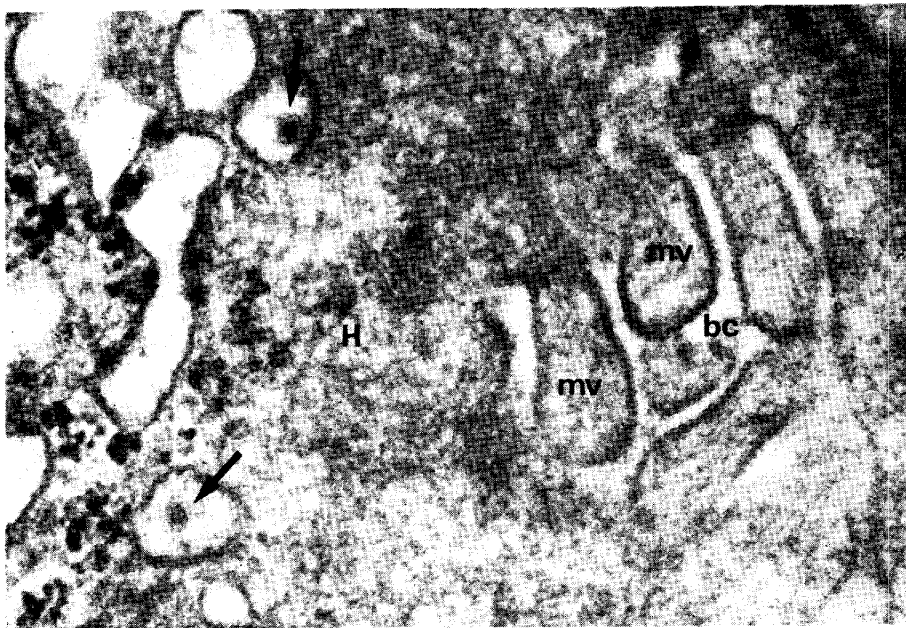


Fig. 1. Dane particles (arrow) are observed in the pericanalicular vesicles. One of two Dane particles has an electron-dense core particle. The dense zone surrounding the lumen of bile canaliculus is broadened. H: cytoplasm of hepatocyte, bc: the lumen of bile canaliculus, mv: microvillus of bile canaliculus.  $\times 90,000$

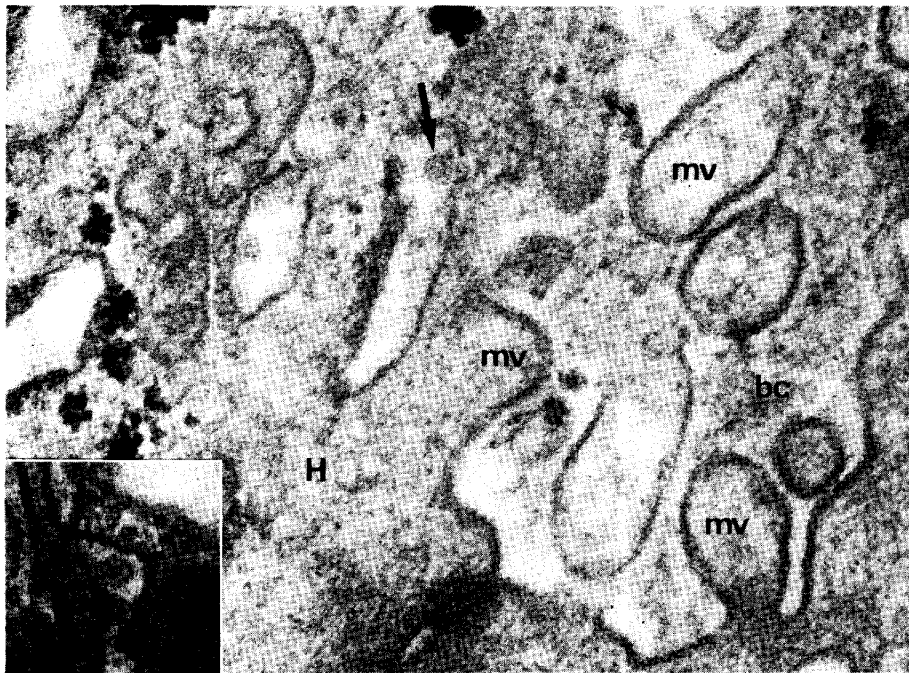


Fig. 2. A Dane particle (arrow) is demonstrated in the vesicle adjacent to the bile canaliculus. Inset: A core particle is budding into the cistern. H: cytoplasm of a hepatocyte, bc: the lumen of bile canaliculus, mv: microvillus of bile canaliculus.  $\times 90,000$

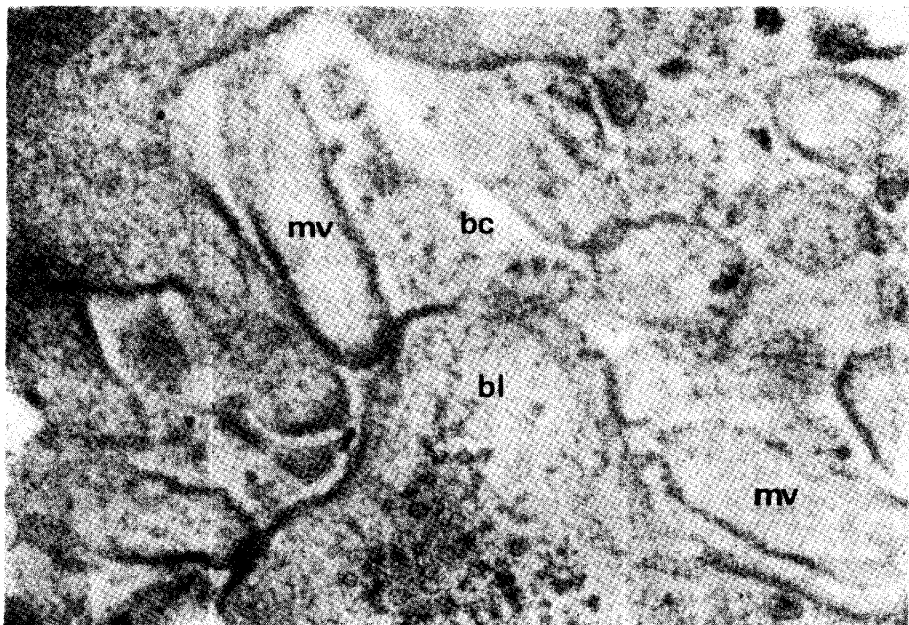


Fig. 3. Uncoated core particles appear in clusters in the bleb of microvillus. bc: the lumen of bile canaliculus, bl: the bleb of microvillus, mv: microvillus of bile canaliculus.  $\times 90,000$

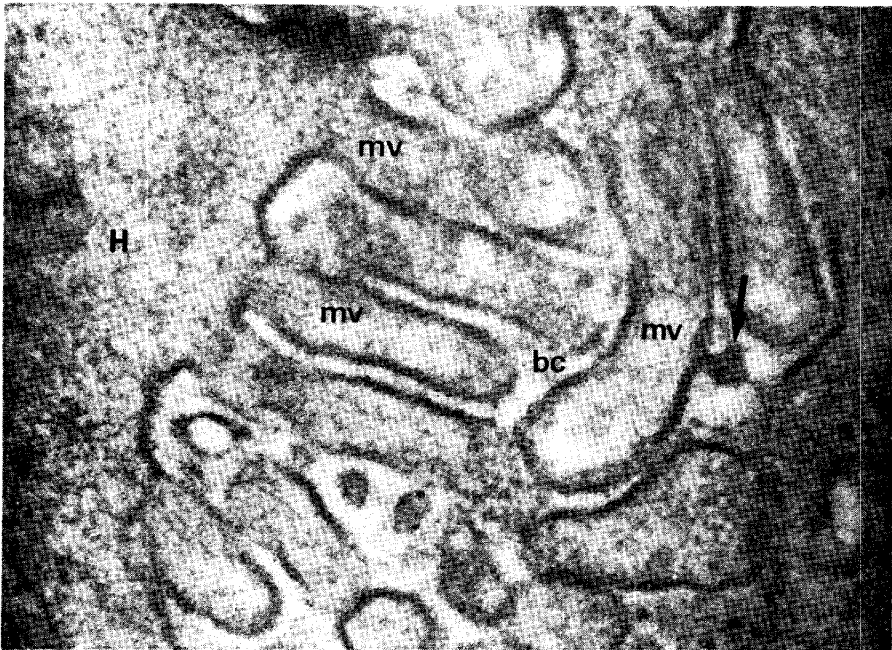


Fig. 4. The outer membrane of a Dane particles (arrow) in the lumen of bile canaliculus is fused with the membrane of microvillus. H: cytoplasm of a hepatocyte, bc: the lumen of the bile canaliculus, mv: microvillus of the canaliculus.  $\times 90,000$

was fused with the membrane of the microvillus (Fig. 4).

*Discussion.* Both the structural and nonstructural forms of HBsAg and HBcAg with respect to their probable intracellular sites of synthesis and distribution have been studied employing routine and immune electron microscopy (1-3, 6). These studies suggest possible modes of HBV being released from the hepatocyte to the presinusoidal space. However, there is little information concerning passage of HBV from liver to intestine via biliary excretion.

In our observation noncoated core particles are budding into the hepatocytic cisterni, and Dane particles are present simultaneously in the pericanalicular vesicles and in bile canaliculi. These findings strongly suggest them being released through vesicles by reversed phagocytosis from hepatocytes to bile canaliculi. However, the demonstration of noncoated core particles in the bleb suggests that HBV may be assembled and released by budding from the surface of the lining hepatocytes into the canalicular lumen. This is also supported by core particles being protruded from microvilli into the lumen, as shown in Fig. 4. Further observations relating to bile canaliculi will lead to better understanding of the replicative cycle of HBV, and to insight into the pathogenesis of intrahepatic cholestasis caused by HBV infection.

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