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Abstract

The liver biopsy specimen from a patient with hepatitis B surface antigen was observed by electron microscopy. Dane particles, uncoated core particles and tubular forms were demonstrated in hepatocytes. Dane particles and tubular forms, approximately 25nm in diameter, were also found in the bile canaliculi. These findings suggest that hepatitis B virus and associated particles are released from hepatocytes into the bile duct.

KEYWORDS: hepatitis B virus, bile canaliculus.

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— BRIEF NOTE —

**HEPATITIS B VIRUS ASSOCIATED PARTICLES
IN THE BILE CANALICULUS**

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Abstract. The liver biopsy specimen from a patient with hepatitis B surface antigen was observed by electron microscopy. Dane particles, uncoated core particles and tubular forms were demonstrated in hepatocytes. Dane particles and tubular forms, approximately 25 nm in diameter, were also found in the bile canaliculi. These findings suggest that hepatitis B virus and associated particles are released from hepatocytes into the bile duct.

Key words: hepatitis B virus, bile canaliculus.

The results of immunological detection of hepatitis B surface antigen (HBsAg) in bile obtained from patients with hepatitis B antigenemia suggested the presence of hepatitis B virus (HBV) in bile (1, 2). Much is known about the formation of HBV and associated particles in hepatocytes, but ultrastructural studies concerning these virus particles in the biliary system remain poorly clarified (3, 4). We have recently found HBV and associated particles in bile canaliculi by electron microscopy.

Materials and methods. The liver biopsy specimen was obtained from a chronic HBsAg carrier. The tissue was fixed in 3% glutaraldehyde in 0.1 M phosphate buffer for 2 h, rinsed in buffer overnight, postfixed in 1% buffered OsO₄ for 2 h, dehydrated through an ethanol-propylene oxide series, and embedded in Epon. Ultrathin sections were stained with 2% uranyl acetate followed by lead citrate and examined with a Hitachi H-700H electron microscope. HBV associated particles isolated from the serum using ultracentrifugation were also observed by electron microscopy after negative staining (5).

Results. Dane particles and tubular forms were often observed in the cisterni of hepatocytes. Uncoated core particles were localized in clusters in the cytosol of the hepatocytic cytoplasm. A few Dane particles were demonstrated in the bile canaliculi. Many tubular forms and spherical particles appeared in

the intracytoplasmic diverticulum and in the bile canaliculus surrounded by three liver cells similar to ductular epithelial cells (Figs. 1, 2). Tubular forms were

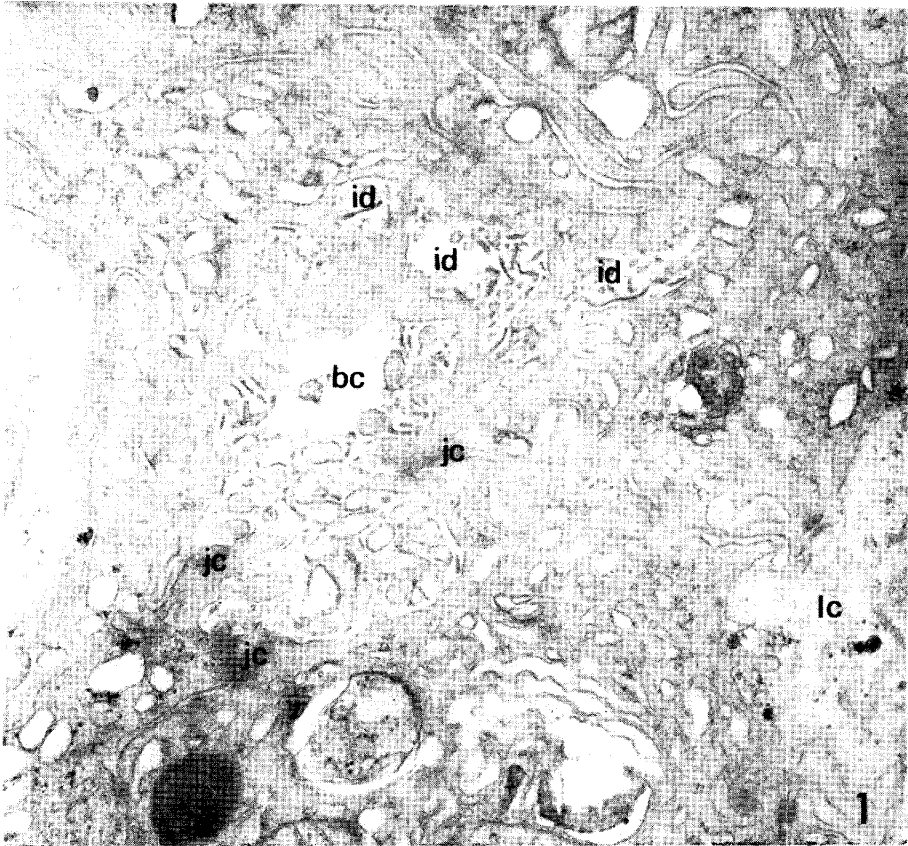


Fig. 1. Many tubular forms and spherical particles are present in the intracytoplasmic diverticulum and in the bile canaliculus surrounded by three hepatocytes similar to ductular epithelial cells. id: intracytoplasmic diverticulum, bc: bile canaliculus, jc: junctional complex, lc: lymphocyte, $\times 33,000$.

approximately 25 nm in diameter and the longest one was about 600 nm in length. The end of some tubular forms were swollen just like tadpoles. The serum of this case contained similar HBV associated particles after negative staining, as shown in Fig. 3.

Discussion. Using electron microscopy, we have confirmed the result reported by Akdamar *et al.* that HBsAg is present in bile (1, 2). HBV and associated particles in bile canaliculi were morphologically identical with HBV associated particles demonstrated in the serum of this case. Furthermore, the

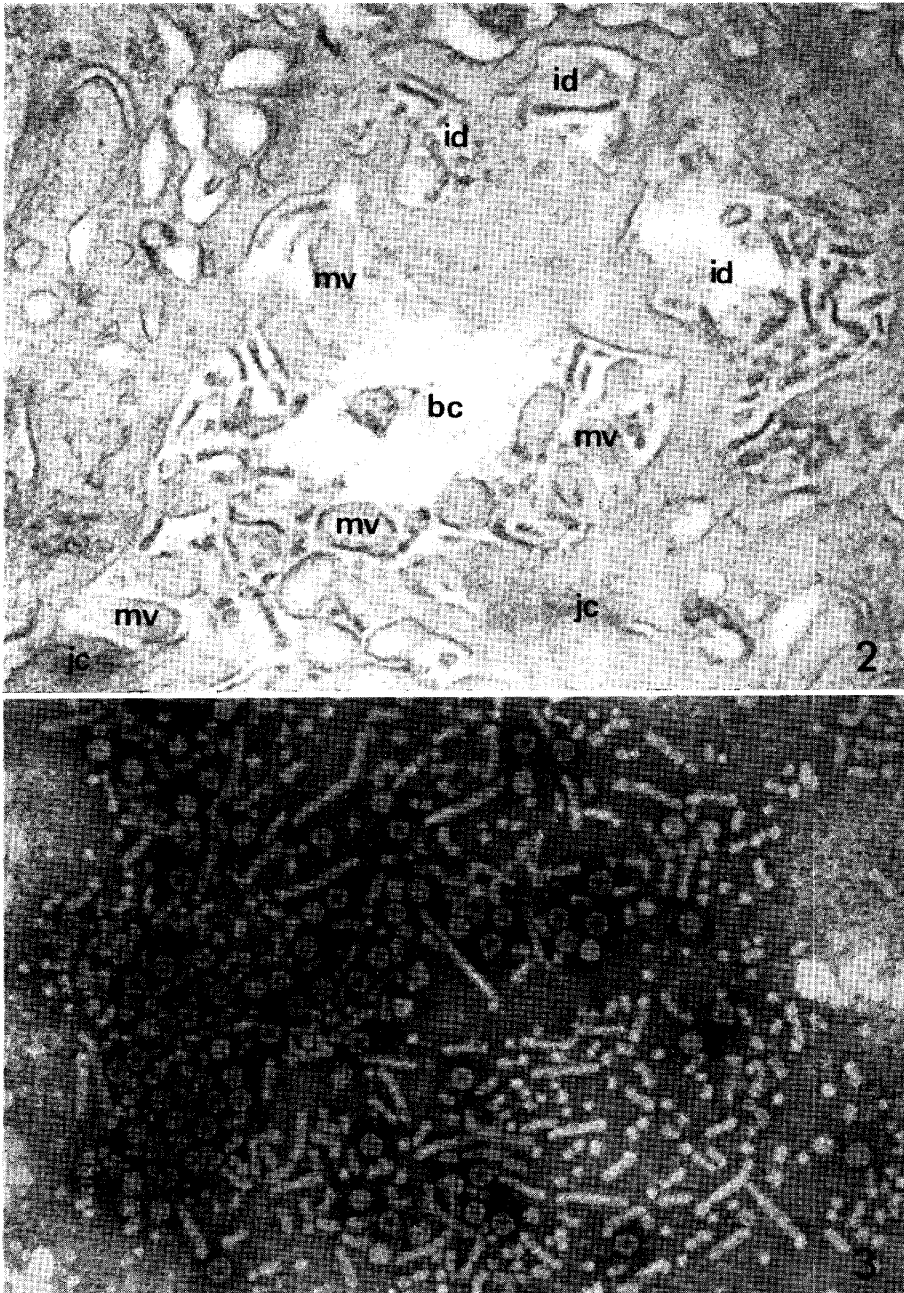


Fig. 2. Enlargement of Fig. 1. Many tubular forms, approximately 25 nm in diameter are demonstrated. The end of a tubular form is swollen like a tadpole. id: intracytoplasmic diverticulum, bc: bile canaliculus, jc: junctional complex. mc: microvilli, $\times 60,000$.

Fig. 3 Hepatitis B virus and associated particles in the serum of this case. Dane particles, tubular forms and small particles are shown. Stained with phosphotungstate. $\times 60,000$.

presence of many of these particles in hepatocytes suggests that HBV is released from hepatocytes into bile canaliculi, as well as being released from hepatocytes to the sinusoid (6, 7). Further work is necessary to clarify how HBV is released from hepatocytes into the bile duct.

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