Cholesterol depletion and replenishment of hepatitis B virions reversibly alter their ultrastructure and infectivity

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Hepatitis B virus (HBV) infection of primary hepatocyte cultures is dependent on the presence of viral cholesterol. Extraction of cholesterol from highly purified HBV from plasma of HBV-infected patients with methyl-beta-cyclodextrin (MβCD) leads to a strongly reduced level of infection but addition of exogenous cholesterol restores infectivity of HBV [1]. We analyzed ultrastructural changes of HBV that parallel these effects in untreated HBV (group 1), cholesterol-depleted HBV (group 2) and cholesterol-depleted HBV after cholesterol-replenishment (group 3).

Conventional negative stain preparations were inspected in an EM912 AB (ZEISS) TEM at 120 kV under zero loss conditions. Images were recorded using a slow scan-ccd camera (Proscan) and the SIS software package (Olympus) at a magnification of 50.000 x (pixel size = 0.27 nm).

In all three groups the proportion of disrupted particles was below 5%. Particles were isomorphic regardless of previous treatment indicating that neither the MβCD-treatment nor the cholesterol-replenishment destroyed the integrity of the virions. HBV from group 1 appeared as mainly negatively stained spherical particles with a diameter of 48.4 ± 4.2 nm. The nucleocapsid of these virions displayed only a relatively weak positive contrast. MβCD-treated viral particles from group 2 (which lost their infectivity) had a clearly smaller diameter of 38.7 ± 4.9 nm and and showed an overall positive contrast. Viral particles replenished with cholesterol (group 3) with regained infectivity appeared as negatively stained again and nearly but not fully reached wild-type diameters ranging at 46.5 ± 4.0 nm (compare Fig. 1).

In order to study the viral morphology in more detail 3d models from virions of all three groups were calculated and inspected using EMAN and CHIMERA. The resolution (FSC) of these models was rather poor (~ 35 Å), due to the limited resolution of negative staining and to the relatively low number of particles included in the modeling (n~40 to 50 particles per model). The models reflect the size differences between the virions of the three groups revealed by the statistical analysis of the single images. Within the capsid of all three models it was possible to detect twofold, threefold and fivefold symmetry axes corresponding to an icosahedral symmetry (T=4 organisation). The innermost structures however show features pointing towards an octahedral packing of the internal DNA. The envelope of the infectious HBV (group 1and group 3) appears to be closed with no clear symmetry whereas in the cholesterol-depleted HBV (group 2) it appears as a very porous meshwork of structures (compare Fig. 2).

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These results suggest that cholesterol-depletion of the HBV particles and replenishment strongly affect the viral envelope whereas the nucleocapsids probably remain unaffected.

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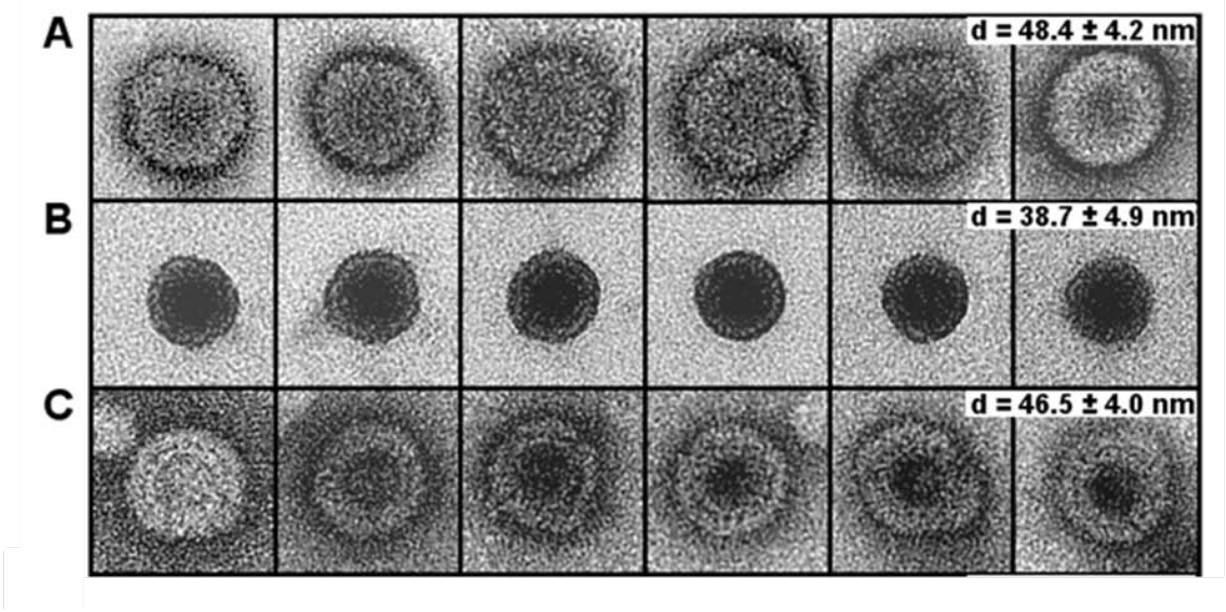

Figure 1. Representative images of negatively stained HBV particles (A: group 1 - untreated; B: group 2 - after cholesterol-depletion with MβCD; C: group 3 - cholesterol replenished particles).

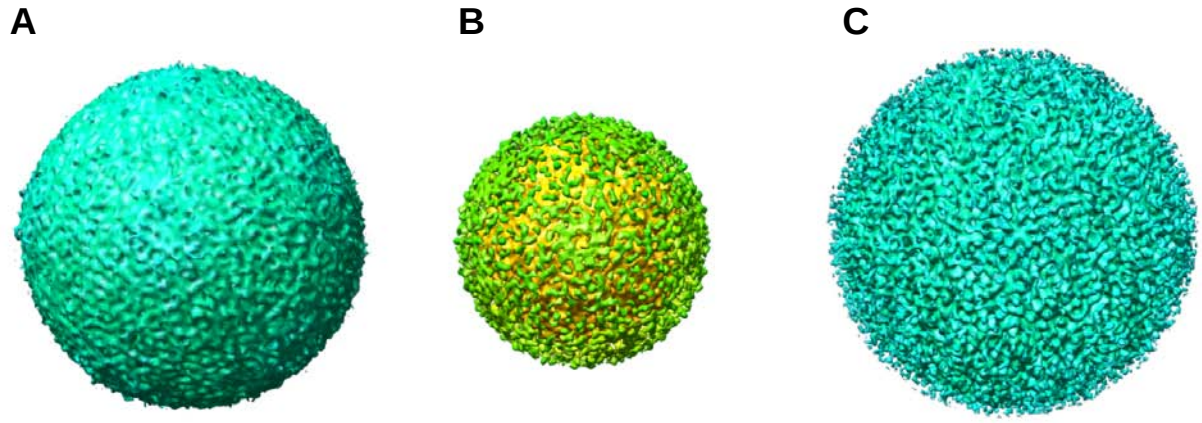

Figure 2. 3d models of untreated HBV particles (A), cholesterol-depleted HBV particles (B), cholesterol replenished HBV particles (C).

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