Ultrastructural Characterization and Hepatic Pathogenesis of Duck Plague Virus

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Summary
Six-week-old white Pekin ducks were inoculated intravenously with duck plague virus (DPV) isolated from wild waterfowl. The virus replicated in hepatic macrophages, hepatocytes, and bile duct epithelium. In ultrathin sections, herpes-like nucleocapsids and virions were found respectively in the nucleus and cytoplasm of infected cells. Typical herpesviral capsids and virions were seen in negatively-stained preparations of duck embryo fibroblasts. Antibodies against Holland-attenuated strain of DPV reacted with virions of this isolate.

Introduction
A major epornitic of duck plague (duck virus enteritis) occurred in free-flying waterfowl at Lake Andes, South Dakota, United States of America, in January, 1973. An estimated 42,000 mallard ducks and 270 Canada geese died during the epornitic.

At necropsy, multiple foci of hemorrhage and necrosis were reported in the livers of ducks, geese, and swans infected with DPV. Histologically, multiple foci of nonzonal necrosis, hemorrhage, and sinusoidal congestion were present throughout hepatic parenchyma. Intranuclear inclusion bodies were present in hepatocytes around foci of hepatic necrosis (4, 6, 8, 11—13).

Enzymatic studies and the morphology of viral particles in ultrathin sections indicated DPV was a herpesvirus (2). Enzymatic studies demonstrated DPV was a DNA virus. Cytoplasmic virions in ultrathin sections of fibroblasts measured 181 nm and contained 75 nm nucleocapsids. Nucleocapsids in the karyoplasm of fibroblasts had a diameter of 91 nm.

The pathogenesis of hepatic cytopathology of duck plague has not been studied ultrastructurally, and the capsid morphology has not been described. The purpose of this study was: 1. to determine sites of viral replication in the liver of infected...
ducks, and 2. to examine the virions and viral related products of DPV isolated
during the 1973 epornitic at Lake Andes, South Dakota.

Materials and Methods

Virus

Virus Preparation

The DPV used was isolated from a mallard duck that died during the epornitic at Lake Andes, South Dakota in 1973. Duck embryo fibroblasts were inoculated with a 10 per cent liver suspension, and the stock virus was titered in duck embryos as previously described by Erickson et al. (5). A dilution that contained $10^4$ duck embryo median lethal doses (DELD$_{50}$) per ml was used to inoculate ducks.

Negative Staining

Duck embryo fibroblasts were harvested when cytopathic effects were observed in 50 per cent of the cells. Cultures were processed through one freeze-thaw cycle and clarified by centrifugation at 5000 rpm (3000×g) for 15 minutes. The supernatant was removed and centrifuged at 15,000 rpm (23,000×g) for 35 minutes in a SW-25.1 Spinco rotor. Pelleted particles were resuspended in water and negatively stained with neutralized phosphotungstic acid (PTA) as previously described by Ritchie and Fernelius (18). A drop of virus suspension was added to a spotplate well containing 2 to 4 drops of 4 per cent PTA, 15 to 20 drops of distilled water, and 1 drop of a freshly prepared aqueous 1.0 per cent solution of bovine serum albumin (Cohn’s fraction V). After gentle mixing, the suspension was sprayed on carbon-coated, collodion-filmed grids with an all-glass nebulizer. Grids were examined immediately in a Philips 200 electron microscope operated at 60 KV with double condenser illumination and a 25 micrometer gold foil objective aperture.

Direct Immuno-Electron Microscopy

A portion of the above pelleted viral particles was resuspended in water and reacted overnight at 4°C with an excess of attenuated-Holland duck plague virus antisera. The mixture was centrifuged at 15,000 rpm (23,000×g) for 35 minutes to separate the unreacted serum components, and the pelleted material was then negatively stained with PTA as above.

Laboratory Animals

Inoculation and Tissue Collection

Thirty, 6-week-old white Pekin ducks were placed in a 4.6 × 5.5 m isolation room. Each was inoculated intravenously with 0.5 ml of viral suspension containing $10^4$DELD$_{50}$ per ml. Twenty control ducks were placed in a separate isolation room. Two control and 2 inoculated ducks were killed every 12 hours. Ducks that died were discarded. One mm thick sections of liver were removed and placed in 2.5 per cent glutaraldehyde in pH 7.4 sodium cacodylate buffer. Another small section of liver was placed in 2 per cent methylcellulose and frozen at 30°C.

Ultrathin Sections

After fixation for 2 hours in 2.5 per cent glutaraldehyde, the liver tissues were washed in cacodylate buffer pH 7.4, postfixed in 1 per cent osmium tetroxide, dehydrated in graded alcohols, embedded in Epon (14), and ultrathin sections were cut with an ultramicrotome. Sections were stained with lead citrate and uranyl acetate (21) and examined using an electron microscope operated at 60 KV.

Fluorescent Antibody Stained Tissue Sections

Antiserum against attenuated-Holland duck plague virus was prepared in mature ewes and conjugated with fluorescein isothiocyanate as previously described (5). Two frozen sections, each 10 μm thick, were cut in a cryostat from each frozen specimen.