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A VIRAL INFECTION CAUSING CYTOMEGALIC INCLUSION DISEASE IN THE RENAL EPITHELIUM OF THE PLATYPUS (*ORNITHORHYNCHUS ANATINUS*)

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ABSTRACT: Cytomegaly and intranuclear inclusion bodies were observed in the renal collecting duct epithelium in three of four wild caught platypuses (*Ornithorhynchus anatinus*) from New South Wales using light and electron microscopy during routine pathological studies. Non-enveloped, spherical virions measuring about 80 nm in diameter were present in the nucleus and cytoplasm of affected cells as well as in the lumen of the renal tubule. A single enveloped virion measuring about 150 nm in diameter was found. There was no serological evidence of infection with cytomegalovirus (AD169 antigen) or adenovirus (mammalian and avian group antigens) in any of the platypuses. Although the identity of the virus was not confirmed, it was probably an adenovirus based on morphological grounds. The infection appeared to have little effect on the host.

Key words: Platypus, *Ornithorhynchus anatinus*, monotreme, viral infection, cytomegaly, renal collecting ducts, natural infections, serology, pathology.

INTRODUCTION

Cytomegalic inclusion diseases, which affect a variety of tissues, are characterised histologically by dramatic cell enlargement and the presence of intranuclear and intracytoplasmic inclusion bodies. They occur in eutherian mammals and marsupials and are caused by infection with one of at least two kinds of virus: herpesvirus and adenovirus (Lussier, 1975; Hoover and Thacker, 1979a, b; Barker et al., 1981; Munday and Obendorf, 1983; Durham et al., 1988). The herpesviruses responsible for cytomegalic inclusion disease are referred to as cytomegaloviruses (CMV) and have been well studied in man, mice, guinea pigs and swine (Lussier, 1975; Jones and Hunt, 1983). CMV cause acute, chronic and latent infections typical of the herpesviruses but are generally not pathogenic unless the host is immunologically immature or immunodeficient (Robbins and Cotran, 1979) or unless the virus has been subjected to passage in the laboratory (Jones and Hunt, 1983). The pathogenesis of known adenovirus infections is similar except that reactivation of latent infection is not known (Straus, 1984).

This report describes the light and electron microscopic findings in a viral infection of the renal tubular epithelium of platypuses, and is the first report of viral infection in a monotreme. Although it was not possible to identify the virus with certainty, the evidence suggests that it was an adenovirus.

MATERIALS AND METHODS

Four platypuses that drowned in fishing nets in rivers in southeastern New South Wales (Australia) were packed in ice and transported to the laboratory. Animal details are: Animal One, 27 March 1988, Abercrombie River, 5- to 6-mo-old, male, length 42.5 cm bill tip to tail tip, 1,100 g; Animal Two, 16 January 1986, Murrumbidgee River, >24-mo-old, male, 53.0 cm, 2,200 g; Animal Three, 16 January 1986, Murrumbidgee River, >24-mo-old, male, 52.0 cm, 1,700 g; Animal Four, 29 May 1986, Queenbeyan River, >18-mo-old, female, 44.0 cm, 1,070 g. Routine necropsy was performed within 24 hr of the estimated time of death. A blood sample was obtained from the heart of each animal and samples of kidney, liver, spleen, pancreas, gonad, gastrointestinal tract, tongue, salivary

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gland, lung, thymus, heart and skin were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 5 μm and stained with haematoxylin and eosin (H&E). In addition, kidney sections from Animal One also were stained with Warthin-Starry, Ziehl-Neelsen, modified Ziehl-Neelsen, periodic acid Schiff, Gomori's methenamine silver, and Brown and Hopps techniques (Luna, 1968). Measurements of cell size were performed with a calibrated eyepiece micrometer.

Formalin-fixed samples of kidney from Animal One were postfixed in 1% osmium tetroxide, followed by 0.5% uranyl acetate, and embedded in Spurr's resin (Spurr, 1969). Ultrathin sections were then cut, mounted on copper grids and stained with 2% uranyl acetate and 4.3% lead citrate. They were examined ultrastructurally using a Philips EM 300 transmission electron microscope (Philips Electronics Instruments, Inc., Mahwah, New Jersey 07430, USA).

Sera from Animals One to Four and from 110 platypuses captured in the Shoalhaven River (New South Wales, Australia) between 1983 and 1987 were examined in a complement fixation test (CFT) for the presence of antibody against CMV using the LBCF micro method (Casey, 1965). Human CMV strain AD169 (Cytomegalovirus antigen, Behringwerke AG, Marburg, Federal Republic of Germany) was used as antigen in test plates while CMV free tissue culture medium control (Cytomegalovirus-Varicella/Zoster-/Control antigen, neg., Behringwerke AG, Marburg, Federal Republic of Germany) was used in anticomplementary control plates run in parallel on each serum. All sera were tested in doubling dilutions from 1:8 to 1:1,024. Sera from the three platypuses in which virus was demonstrated histopathologically were tested also in a passive latex agglutination test employing CMV AD169 as antigen (CMV Scan, Becton Dickinson, Cockeysville, Maryland 21030, USA).

Sera from Animals One to Four and from three other platypuses captured on the Shoalhaven River as previously described, were examined in agar gel precipitin (AGP) tests for the presence of antibody against adenovirus (Beard, 1980). Four Australian adenovirus isolates, representing the mammalian and three avian antigenic groups (Bagust, 1982) were used as antigens. Specific positive antisera and a known negative serum were included as controls in each test.

Representative histologic sections from CMV lesions are deposited in the Registry of Veterinary Pathology (Taronga Park Zoological Gardens, Mosman, New South Wales 2088, Australia; Accession Number B0014).

RESULTS

Animals One to Four appeared to be in good physical condition. Except for marked pulmonary congestion consistent with drowning in each animal, there were no significant gross lesions observed.

Sections of kidneys examined by light microscopy had two abnormalities. Megalocytes with prominent intranuclear inclusion bodies were present in the epithelium of the collecting ducts of Animals One, Two and Three, while multifocal, nonsuppurative, interstitial nephritis occurred in Animals Two, Three and Four. The latter lesion was associated with the presence of leptospires in tubular lumens and will be described in a separate report.

Intranuclear inclusion bodies occurred segmentally in the epithelium of the collecting ducts and their presence was associated with cytomegaly (Fig. 1). Affected cells were readily apparent at low magnification. The infection was not uniform throughout the kidney, being present in about one in three sections of renal medulla from infected animals. There appeared to be a progression of changes in some segments of collecting duct. Initially, one or occasionally two dense, round, homogenous inclusions measuring 2 to 3 μm in diameter were present centrally in the dispersed chromatin of the nucleus. Cells so affected had increased from the normal diameter of approximately 6 to 10 μm to a diameter of 15 to 20 μm. Their nuclei were correspondingly enlarged from the normal diameter of 5 to 8 μm to a size of 8 to 11 μm. As cells enlarged further, margination of chromatin created a clear halo around inclusions, which then enlarged, became less dense and more granular, and occasionally had a geometric internal structure resembling honeycomb. The cytoplasm of these cells was abundant and consisted of granular substance interspersed with hyaline droplets and clear vacuoles. Cells in which the entire nucleus measuring up to 20 μm in diameter consisted of granular material were enlarged

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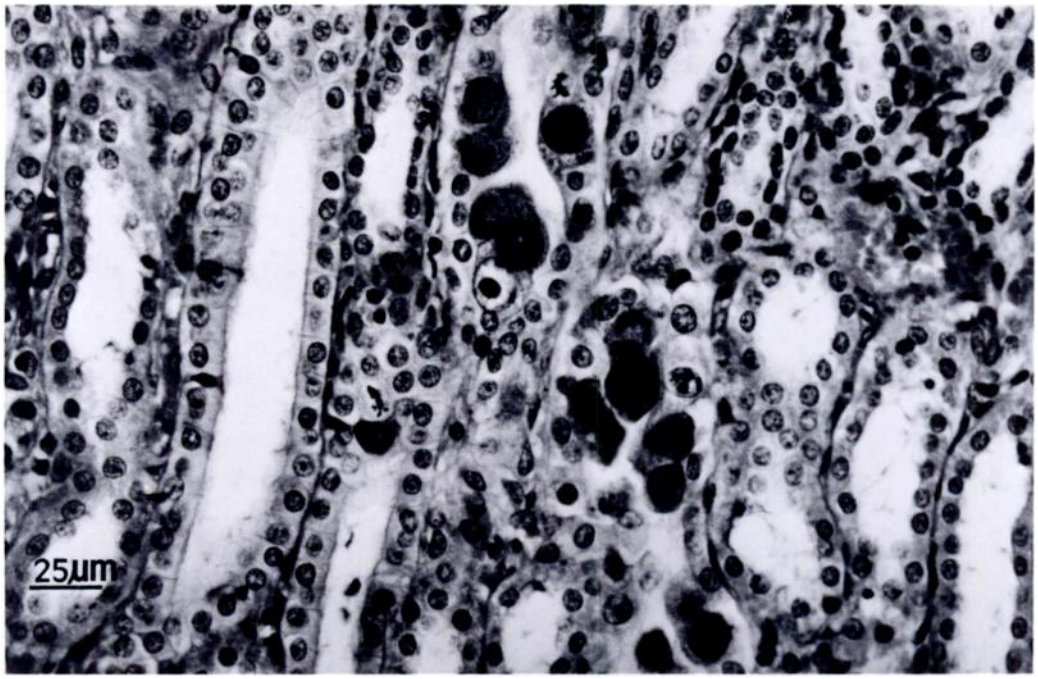

FIGURE 1. Cytomegaly and intranuclear inclusions in the renal collecting duct epithelium of a platypus. H&E.

up to a diameter of 35 μm. Prior to being sloughed into the lumen, the cytoplasm condensed, became deeply eosinophilic to amphophilic leaving enlarged, distorted nuclei protruding into the lumen. In a few cells, enlarged nuclei were empty except for numerous small, intensely basophilic granules. There was no inflammatory reaction in the medulla adjacent to these epithelial lesions. In Animal One, the inclusions had variable staining characteristics with H&E, the smaller, denser bodies appearing basophilic but the larger, more granular inclusions were eosinophilic. The inclusions did not stain preferentially with any of the special stains used. In Animal Three, all inclusions were eosinophilic with H&E. There was no evidence of cytomegaly in mandibular and lingual salivary glands nor in other tissues of any animal.

On electron microscopic examination of kidney, a range of lesions was evident. Some epithelial cells had slightly swollen, rounded nuclei which had peripheral accumulations of electron-dense material, and small numbers of scattered or clustered virions. Other cells had markedly swollen nuclei which distended into the lumen of the renal tubule (Fig. 2). Accumulations of finely granular material and small, spherical electron-dense bodies were scattered throughout some nuclei, together with moderate numbers of virions. Small crystalline arrays of virus were observed in the nucleus of some cells. Cell cytoplasm was compressed and contained many small vesicles but few organelles. Areas of the cytoplasm were densely packed with virions and contained a few large, irregular dense bodies (Fig. 2). The nuclear membrane was indistinct adjacent to these cytoplasmic viral inclusions. Virions were also observed in the lumen along the remnants of the microvillous cell border (Fig. 3). Entire and empty viral nucleocapsids were found in the nucleus and cytoplasm of infected cells (Fig. 4). Virions were spherical in shape and measured approximately 80

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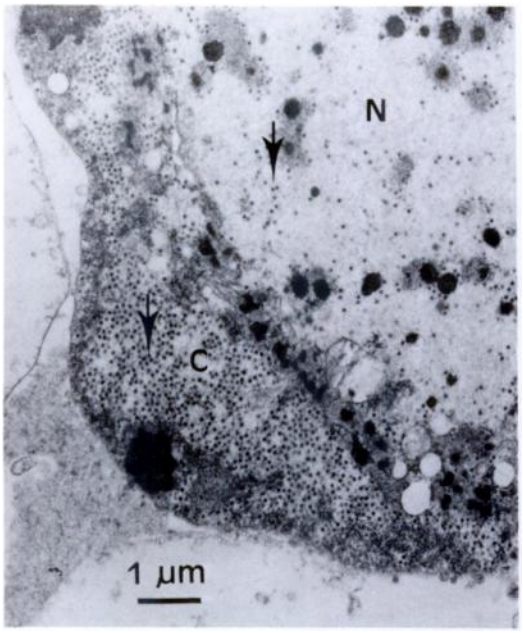

FIGURE 2. Renal tubular epithelial cell of a platypus with markedly swollen nucleus (N) displacing the cytoplasm (C). Note loss of chromatin, intranuclear and intracytoplasmic viral particles (arrows) and electron dense material.

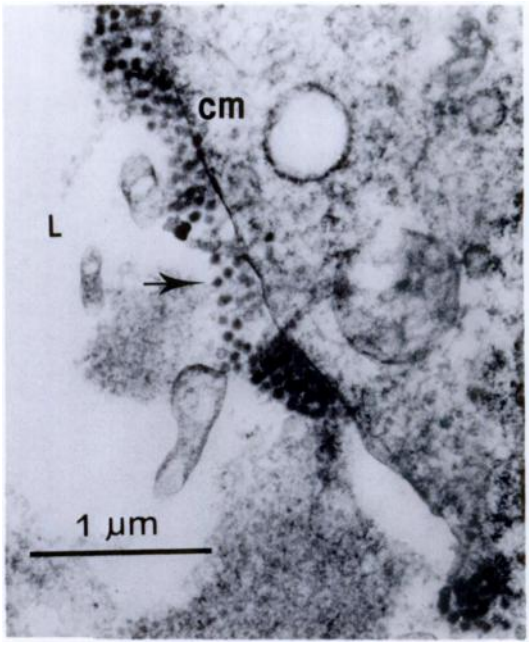

FIGURE 3. Viral particles (arrow) accumulating in the tubular lumen (L) adjacent to the cell membrane (CM) in a platypus kidney.

nm in diameter. Most consisted of a single outer membrane surrounding an electron dense core of about 60 nm diameter, however some particles lacked this core and appeared empty (Fig. 4). Apart from a single enveloped virion, approximately 150 nm in diameter found adjacent to the nuclear membrane (Fig. 5), enveloped virions were not observed.

Eighteen of 114 sera tested by complement fixation for the presence of antibody against CMV had titres of <1:8. The remaining sera, including sera from Animals One and Three, were anticomplementary at titres of between 1:8 and 1:512. There was no instance where the titre obtained using antigen exceeded and anticomplementary titre. Sera from the three platypuses with demonstrable cytomegalic inclusion disease gave negative results in the latex agglutination test for antibody against CMV.

No lines of precipitation between platypus sera and mammalian and avian adenovirus group antigens were observed in AGP tests.

DISCUSSION

This study confirmed that cytomegalic inclusion disease affecting the renal collecting duct epithelium of three platypuses was due to a viral infection. The light microscopic findings were consistent with those considered by some authors to be pathognomonic for CMV infection (Lussier, 1975), but electron microscopy on one platypus cast doubt on the identity of the infecting virus being CMV. In the last decade the identification of viruses causing cytomegalic inclusion disease has been advanced by the realization (Hoover and Thacker, 1979a, b) that adenoviruses in addition to herpesviruses may cause the "characteristic" light microscopic lesion.

Herpesviruses and adenoviruses both contain double stranded DNA, have icosahedral symmetry, are assembled in the cell nucleus and may induce the formation

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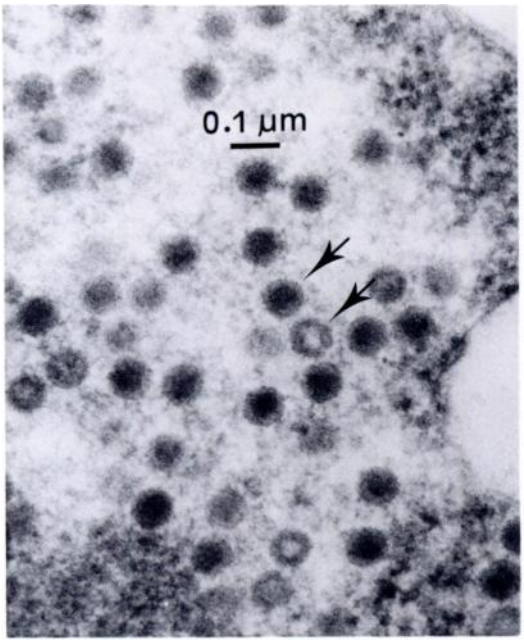

FIGURE 4. Entire and empty viral particles (arrow) in the nucleus of a renal collecting duct epithelial cell of a platypus.

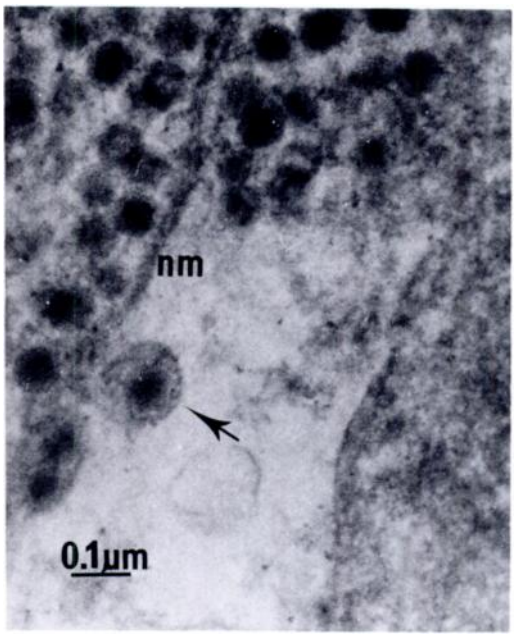

FIGURE 5. Enveloped virion (arrow) adjacent to nuclear membrane (NM) of renal collecting duct epithelial cell of a platypus.

of intranuclear inclusion bodies visible with the light microscope (Fenner et al., 1974). Herpesviruses acquire a lipid envelope by budding through the nuclear membrane and are gradually released from the cell membrane or by exocytosis from cytoplasmic vesicles. In contrast, adenoviruses are released by rupture or partitioning of the nuclear membrane and remain unenveloped. Adenoviruses commonly form crystalline arrays in the nucleus while herpesviruses do this only when large numbers of virions are formed rapidly and synchronously. Enveloped and non-enveloped herpesviruses typically measure 150 nm and 100 nm in diameter, respectively, while adenoviruses are in the range 70 to 80 nm.

The virions observed in the kidney of the platypus were almost invariably non-enveloped regardless of their location in the nucleus (free or arrayed), cytoplasm or in the lumen of the renal tubule. This suggests that they were not a herpesvirus. In addition, the virions were approximately 80 nm in diameter, which is consistent with the size of adenoviruses (Fenner et al., 1974). The finding of a single enveloped virion which appeared to have budded from the nuclear membrane clouded the identification of the virus, but the apparent rarity of this event suggested that it was aberrant or that there was coinfection with an unrelated virus. Unfortunately, the resolution obtained in the electron micrographs was limited by tissue preservation and little information could be obtained about the development and morphology of the virions other than to note their size and spherical shape.

An attempt was made to demonstrate antibody against CMV and adenovirus in the platypus. This was felt to be worthwhile because although CMV are antigenically diverse, serological cross reactions have been reported between some strains in complement fixation tests (Black et al., 1963) and the adenoviruses have group specific antigens (Fenner et al., 1974). It was our impression that all apparent titres

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in the complement fixation test for CMV were due to the anticomplementary activity of platypus serum and CMV latex agglutination test results in virus positive platypuses were negative. Similarly, attempts to demonstrate antibody against adenovirus group specific antigens were unsuccessful. These results do not rule out the presence of CMV or adenovirus infection because the antigens used in the tests may have been inappropriate (i.e., putative CMV or adenovirus infecting the platypus may be antigenically distinct from the test antigens).

The available evidence, principally size and morphology of the virions, suggested that the virus was more likely to be an adenovirus than a cytomegalovirus, but further work is required to confirm this. Cytomegalic inclusion disease of renal epithelium caused by adenovirus has been reported in the bettong *Bettongia gaimardi* (Munday and Obendorf, 1983) and the ground squirrel *Spermophilus franklini* (Durham et al., 1988). The agents responsible for cytomegalic inclusion disease in renal epithelium of brushtailed possums (*Trichosurus velpecula*; Hurst et al., 1943), sheep (Hartley and Done, 1963) and antechinus (*Antechinus stuartii*; Barker et al., 1981) have not been investigated but may also be due to adenovirus infection. Nuclear enlargement with intranuclear inclusions due to adenovirus infection were recently found in the renal epithelium of psittacine birds (Mori et al., 1989).

There was no inflammatory reaction associated with this viral infection of platypuses, a finding consistent with it being chronic and persistent. As virions were seen grouped in the tubular lumens and in sloughed epithelial cells, the urine is likely to be an important means of shedding virus. Urine and/or reproductive tract secretions are probably the major source of CMV infection in marsupials (Barker et al., 1981; Munday and Obendorf, 1983). Although platypuses may come into close contact in burrows, especially when females are suckling offspring, water-borne virus may be a more important means of transmission. Kalter and Millstein (1976) suggested that water supplies contaminated with human urine containing high concentrations of CMV may be a source of human CMV infection. This may also be possible with adenoviruses which do not lose infectivity immediately after excretion in urine (Ishibashi and Yasue, 1984).

The prevalence of this viral infection in platypuses, while uncertain, is probably high. The discovery of infection in these animals was by chance during a routine pathological survey, the animals being in effect randomly selected from wild populations. In addition, histopathology does not detect latent viral infection (Lussier, 1975). Thus, the prevalence of infection in platypuses may be greater than the 75% indicated here. This is consistent with both CMV and adenovirus infections in other mammals where prevalence may reach 100% (Gold and Nankervis, 1978; Straus, 1984).

Cytomegalic inclusion disease of renal collecting duct epithelium is not restricted to wild platypuses as sections of kidney from some captive animals contained the same lesions (R. J. Whittington, unpubl. obs.). It is of some interest that similar inclusions occur in the collecting duct epithelium of the closely related monotreme, the echidna (*Tachyglossus aculeatus*; Whittington, 1988). Although electron microscopy will be required to confirm the presence of a virus in the echidna, there is little doubt that both monotremes are subject to infection with a similar virus.

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