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Source: Journal of Wildlife Diseases, 19(2) : 132-135

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-19.2.132

Journal of Wildlife Diseases, 19(2), 1983, pp. 132–135

CYTOMEGALIC LESIONS IN AUSTRALIAN MARSUPIALS

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ABSTRACT: Cytomegalic lesions were detected in nine dusky antechinus (*Antechinus swainsonii*), three bettongs (*Bettongia gaimardi*) and two little pygmy possums (*Cercartetus lepidus*). Virus particles were demonstrated in inclusions in *A. swainsonii* and *B. gaimardi*, but electron microscopy was not performed on tissues from *C. lepidus*.

INTRODUCTION

Cytomegalic lesions can be caused by a number of agents, chief amongst which are viruses and pyrrolizidine alkaloids. Barker et al. (1981) were the first to record the presence of viral-induced cytomegalic lesions in Australian marsupials. They demonstrated lesions due to cytomegalovirus infection in the brown antechinus (*Antechinus stuartii*) and the phascogale (*Phascogale tapoatafa*).

Pyrrolizidine alkaloidosis has rarely been reported in Australian marsupials, probably because only some of the plants containing these alkaloids are palatable to herbivorous marsupials. Munday (1978) quoted a personal communication from W. J. Hartley reporting the occurrence of typical lesions, including hepatic megalocytosis, in red kangaroos (*Macropus rufa*) grazing an area infested with such plants. In contrast, the authors' personal observations have indicated that grey kangaroos (*Macropus giganteus*), Bennett's wallabies (*Macropus rufogriseus*) and pademelons (*Thylogale billardierii*) do not eat ragwort (*Senecio jacobea*), a pyrrolizidine alkaloid-containing plant, even when no other browse is available.

This communication reports cytomegalic lesions in marsupial species other than the brown antechinus and phascogale.

MATERIALS AND METHODS

Sixteen adult, male dusky antechinus were trapped in the Otways State Forest, Victoria, during the month of July in 1978 and 1979. The animals were necropsied as part of an investigation into the natural postmating mortality of male dusky antechinus. A family of three bettongs (1 each, adult male and female, 1 immature female) were collected as road kills in the Blessington area of Tasmania. Also, three adult bettongs (1 male and 2 females) were trapped in the Epping Forest district and examined after being used for pesticide studies. Two female little pygmy possums (*Cercartetus lepidus*) were submitted to the laboratory as part of routine disease surveillance at a Tasmanian wildlife park.

Formalin-fixed tissues, including brain, lung, heart, liver, kidney, and genitalia were embedded in paraffin, sectioned at 5 μm and stained with hematoxylin and eosin for light microscopy. Prostatic tissue from three dusky antechinus and kidney from one bettong were post-fixed in osmium tetroxide, resin-embedded and ultra-thin sections were stained with lead citrate and uranyl acetate for electron microscopy.

RESULTS

Light microscopy revealed cytomegalic lesions in nine of 16 dusky antechinus, three of the five adult bettongs and both pygmy possums. In the dusky antechinus the prostate gland was the only organ in which lesions typical of CMV infection were detected. In all prostates there were focal aggregations of enlarged epithelial cells, each cell containing a single intranuclear inclusion body. It was noted that these aggregations consisted of a number of adjacent tubules in which all the cells were cytomegalic and contained inclusions. Depending upon the stage of the lesions, the proportion of the cell occupied by the cytoplasm and the nucleus varied. In early lesions the nuclear volume was increased with the chromatin marginated around a distinct eosinophilic inclusion. At this stage the cytoplasm was also increased in volume. Later, the cells became degenerate leading to a comparative reduction in the volume of the cytoplasm and a concurrent increase in the size of the nucleus which was completely filled by a basophilic inclusion. These degenerate cells appeared to lose their association with adjoining epithelial cells and some were shed into the lumen of the glands as cellular remnants. Host reaction to this process was minimal, although individual glands did become distended with cellular debris. A few glands with severely eroded epithelium contained plugs of

Received for publication 16 August 1982.

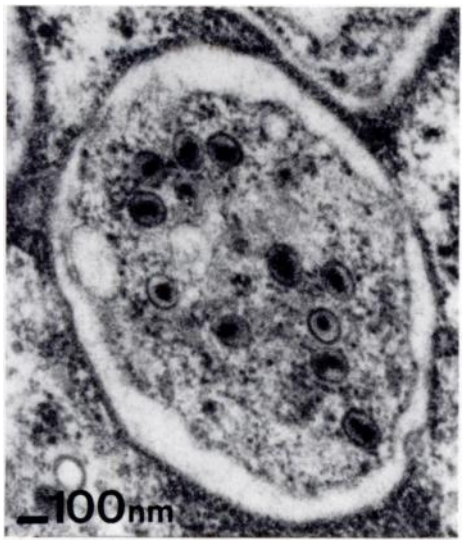

FIGURE 1. Virus particles with single capsid within a vesicle, possibly inside the perinuclear space in a dusky antechinus. ×40,000.

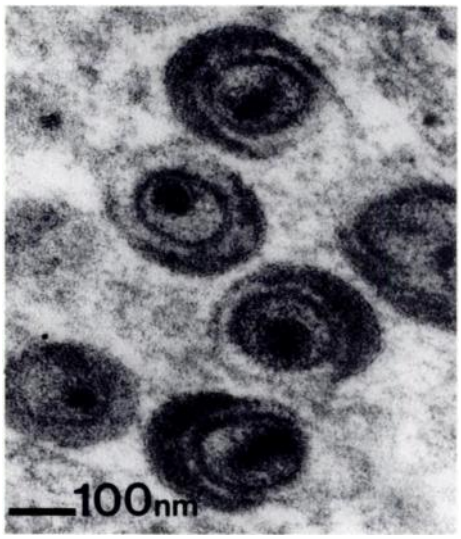

FIGURE 2. Double-enveloped virus particles in cytoplasm in a dusky antechinus. ×90,000.

neutrophils. There was a definite tendency for the pathologic process to be located in the anterior portion of the prostate where the basophilic debris in affected glands contrasted markedly with the eosinophilic secretions of normal glands.

Ultrastructurally, the intranuclear inclusions consisted of a finely granular material containing several intensely electron-dense bodies and many randomly dispersed virus particles. The host cell chromatin was aggregated about the nuclear membrane with a relatively electron-lucent zone separating it from the central inclusion. Virus particles within the inclusion consisted of an electron-dense core surrounded by a single, roughly circular to ovoid membrane or capsid. Several empty capsids were also seen. Clusters of virus particles containing cores were found in close proximity to the nuclear membrane. Occasionally these virus particles were seen within vesicles which appeared to be associated with the inner lining of the nuclear membrane, then passing into the perinuclear space between the two unit membranes of the nuclear lining (Fig. 1). Virus particles were also seen within the rough-surfaced endoplasmic reticulum and in membrane-bound cytoplasmic vesicles. An interesting feature was the presence of electron-dense bodies about which virus particles were clustered. Many virus particles in the cytoplasm were enclosed within a second membrane or envelope (Fig. 2). Toward the lumenal edge of the infected prostatic cell the virus particles were all contained within an outer membrane or envelope. Enveloped virus particles were seen within the short microvillous processes of the secretory edge of the cells and also in the lumen of the gland.

Lesions in the bettongs occurred only in the renal collecting tubules. Not all tubules were involved and not all cells in a tubule were affected. The earliest lesion was cytomegaly, followed by margination of the nuclear chromatin and formation of eosinophilic intranuclear inclusions (Fig. 3). The inclusions enlarged and became more basophilic until they filled the nucleus which finally occupied most of the cell. In some instances, the tubules were virtually occluded by hypertrophied cells. Scattered among the normal and virus-infected tubules were "ghost" tubules which contained only cellular debris.

Electron micrographs of the bettong renal tissue revealed numerous viral particles clustered about the electron-dense material within the nuclear inclusions. Each virus particle was approximately 70 nm in diameter consisting of a roughly hexagonal to spherical envelope or capsid containing a single large electron-dense core (Fig. 4).

Lesions in both pygmy possums were also

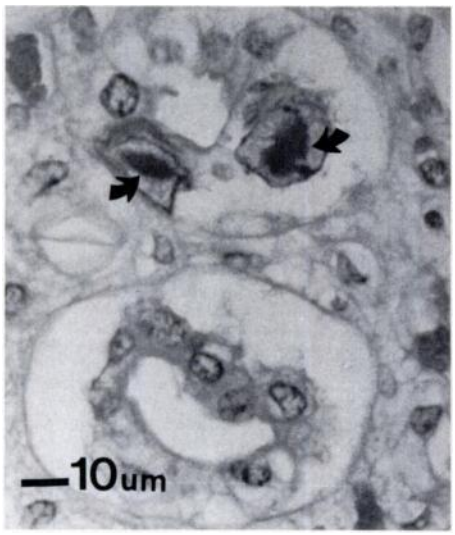

FIGURE 3. Cytomegalic, renal collecting tubules containing eosinophilic, intranuclear inclusion bodies (arrowed) in a bettong. H&E. ×600.

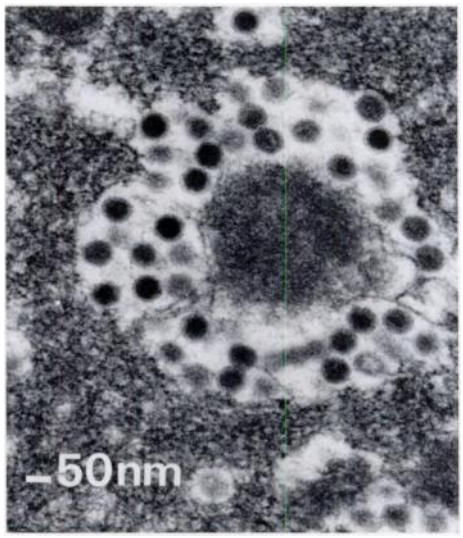

FIGURE 4. Electron-dense body in nucleus with a halo of virus particles in a bettong. ×60,000.

limited to the renal collecting tubules. Only a few tubules were affected and not every cell in the tubules was abnormal. The earliest lesions were those of cytomegaly with the presence of small, eosinophilic, globular inclusions in the nucleus. The nucleus enlarged to practically fill the cell with an amorphous, basophilic inclusion body which sometimes contained eosinophilic globules.

DISCUSSION

The characteristics of the light and electron microscopic lesions and particles in the antechinus were typical of a cytomegalovirus infection. The morphology of the viral particles in the bettong kidney was more typical of an adenovirus than a cytomegalovirus or herpes virus. The enveloped virus particles from the prostates of antechinus measured about 100 nm in diameter with a relatively small electron-dense core. In contrast, the virus particles from the bettong were appreciably smaller with an hexagonal outline and a core which almost filled the capsid. Unfortunately, no cytoplasmic virus particles were present in electron micrographs of the bettong material especially as the presence or absence of enveloped virus particles in this region may have helped identify the virus. As electron microscopy was not performed on the material from the pygmy possums it is not possible to ascertain whether or not a virus was associated with the renal lesions in these animals. As far as could be ascertained they were not exposed to pyrrolizidine alkaloids. It may be relevant, as noted by Hurst et al. (1943), in relation to similar lesions in brush possums (*Trichosurus vulpecula*), that the majority of animals were subjected to stress, such as captivity. Both pygmy possums and three of the six bettongs had been captive animals. *Antechinus* spp., including *A. swainsonii* suffer an abrupt and absolute mortality of males within a 2- or 3-wk period following breeding (Barker et al., 1978; Lee et al., 1982). For *A. swainsonii* in Victoria, the mating season is July, with the male die-off occurring in early August (Lee et al., 1982). Our animals were examined at this time when they showed signs of stress and hyperadrenocorticism such as gastrointestinal ulceration, involution of lymphoid follicles and lymphopenia (D. Obendorf, unpubl. data). This relationship between the post-breeding stress period and occurrence of CMV infection has previously been noted with *A. stuartii* (Barker et al., 1978).

It is possible that CMV and adenoviral infections are quite widespread in Australian marsupials as latent infections which are only recognized when stress, natural or unnatural, lead

to enhanced replication of the viruses and the consequent formation of detectable numbers of viral inclusion bodies.

ACKNOWLEDGMENTS

Assistance with the preparation of ultra-thin sections and electron micrographs were provided by Dr. W. J. Hartley, when he was at the University of Sydney and Miss A. Venstra of the University of Melbourne.

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