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CYTOMEGALOVIRUS INFECTION OF THE PROSTATE IN THE DASYURID MARSUPIALS, *Phascogale tapoatafa* AND *Antechinus stuartii*

I.K. BARKER,[1] P.L. CARBONELL[2] and A.J. BRADLEY[3]

Abstract: Infection by a herpesvirus producing cytomegalic disease in the prostate was demonstrated in the dasyurid marsupials, *Phascogale tapoatafa* and *Antechinus stuartii*. The prevalence of lesions among the latter was highest in mature animals during breeding, when the animals are known to be under stress, and in animals treated daily with high levels of exogenous corticosteroid. Occasional cytomegaly was observed in the kidneys of some *A. stuartii* and may represent a site of latent infection. Virus particles of herpes type were demonstrated by electron microscopy in the nucleus and cytoplasm of infected prostatic cells. It is suggested that the infection may be venereal.

INTRODUCTION

Cytomegalovirus (CMV) infection occurs in a number of mammals[9,14] but apparently has yet to be confirmed from marsupials. Typically, these herpes viruses cause inapparent or latent infection characterized by the presence of enlarged cells with intranuclear inclusions in one or more organs, particularly salivary gland and kidney. However, systemic infection, which may not be clinically apparent, presumably occurs prior to the development of latent infection.

During the course of investigations of mortality among small dasyurid mrsupials,[1,4] we have encountered cytomegalovirus infection of the prostate in two species, *Phascogale tapoatafa* and *Antechinus stuartii*.

MATERIALS AND METHODS

Tissues (brain, liver, lung, spleen, kidney, prostate) from 1 male and 2 female *P. tapoatafa* which had been held in the laboratory, and died of systemic infection with *Erysipelothrix rhusiopathiae*, were fixed in 10% neutral buffered formalin, embedded in paraffin and processed, sectioned and stained routinely with haematoxylin and eosin.

Prostates from 13 groups of male *A. stuartii* totalling 73 individuals trapped within a 60 km radius of Melbourne, Victoria, were examined for infection with cytomegalovirus. Age, breeding status and treatment of these groups are summarized in Table 1. The prostatic urethra of immature *A. stuartii* (Groups 1, 2) weighed less than 6 mg, while that of maturing animals (Group 3) in which primordial prostatic cells were proliferating, weighed 8 to 36 mg. Within a short period, as animals come into breeding condition, the prostate hypertrophies so that in mature breeding males (Groups 4, 5) it usually weighed 200 to 300 mg, a size maintained

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throughout the breeding season (unpubl.).

In addition to material from animals examined immediately after capture, or after dying during the breeding period, prostates were available from animals treated with corticosteroids or sham injected during the breeding season (Groups 8 to 13).[4] Formalin-fixed prostates from immature animals were embedded entire, while a complete cross section of larger organs was embedded in paraffin, sectioned and stained with haematoxylin and eosin, and in some cases with a Gram stain. The criterion for infection was the presence of enlarged epithelial cells containing intranuclear inclusions on a single cross section of the gland.

Brain, liver, lung, spleen, heart, gastrointestinal tissue, skeletal muscle, tongue, kidney and, in some cases, pancreas and parapancreatic lymph node, were examined microscopically from the 22 animals in Groups 4, 6 and 7 and from 17 females trapped and killed at the same time.[1]

Infected formalin-fixed prostatic tissue from the male *P. tapoatafa* and from several *A. stuartii* was cut into 1 mm cubes, post-fixed in osmium tetroxide, embedded in Epon 812 resin and ultra thin sections were cut and stained with lead citrate and uranyl acetate for examination in a Philips EM 300 electron microscope.

RESULTS

Prevalence of Infection. The male phascogale and 15 of 73 male *A. stuartii* had evidence of virus infection in the prostate (Table 1). Among the *A. stuartii* the prevalence of infection was 0/13 in immature animals (Groups 1 to 3) and 7/26 in untreated sexually mature animals (Groups 4 to 7). High dose corticosteroid treatment of mature animals seemed to increase the prevalence of infection among Groups 8 to 13 (pooled values-control, 1/11; 1 mg/kg/d, 2/11; 10 mg/kg/d, 5/12), but the response was variable, and Chi-squared analysis indicated the difference in prevalence

TABLE 1. Age, breeding status, treatment of male *A. stuartii* and prevalence of prostatic cytomegalovirus infection.

Group	Age and Breeding Status	Treatment	Prevalence of CMV
1	5 mo. (1974) immature	Killed after capture	0/4
2	7 mo. (1974) immature	Killed after capture	0/4
3	10 mo. (1974) maturing	Killed after capture	0/5
4	10.5 mo. (1974) mature	Held until death	0/6
5	11 mo. (1973) breeding	Killed after capture	1/4
6	11.5 mo. (1974) breeding	Killed after capture	3/6
7	11.5 mo. (1974) breeding	Held until death	3/10
8	10.5-11 mo. (1973) mature	Injected with vehicle daily for 1 mo.	1/6
9	10.5-11 mo (1974) mature	Injected with vehicle daily for 1 mo.	0/5
10	10.5-11 mo. (1973) mature	1 mg/kg/d hydrocortisone acetate for 1 mo.	0/5
11	10.5-11 mo. (1974) mature	1 mg/kg/d hydrocortisone acetate for 1 mo.	2/6
12	10.5-11 mo. (1973) mature	10 mg/kg/d hydrocortisone acetate for 1 mo.	2/6
13	10.5-11 mo. (1974) mature	10 mg/kg/d hydrocortisone acetate for 1 mo.	3/6

between untreated and 10 mg/kg/d corticosteroid injected animals could not be considered significant ($p > 0.10$).

Microscopic Findings. Virus-infected prostatic tissue was similar (Figs. 1 to 3) in both species. Normal prostatic epithelial cell nuclei were about 5 μm in diameter. The nucleus of infected cells was enlarged to up to 20 μm in diameter, chromatin was peripheralized and a central spherical eosinophilic inclusion appeared in the nucleus (Figs. 2, 3). At this stage, the cytoplasm of infected cells tended to be increased in volume, vacuolated and more basophilic than normal, and the cells were irregular and distorted. The inclusions increased in size to occupy virtually the entire nucleus, at the same time becoming more basophilic and occasionally adopting a fine, stippled, vacuolate appearance. Cells with this type of inclusion sloughed into the lumina of prostatic glands where they appeared to disintegrate. Some infected glands contained masses of granular or homogeneous, basophilic material in place of their normal eosinophilic secretion (Fig. 2). In some animals only a few infected cells were detected, often near the periphery of the organ, while in other cases, either a large proportion of the length of a gland or a major segment of the cross-section of the organ (Fig. 1) was involved. No inflammatory response was observed.

In two *A. stuartii* treated in separate years with the high dose of hydrocortisone acetate, a severe prostatitis was observed involving acute necrosis of a large portion of the organ and a heavy polymorphonuclear infiltrate, associated with the presence of large numbers of Gram positive bacilli, possibly *Listeria*, which often is cultured from moribund

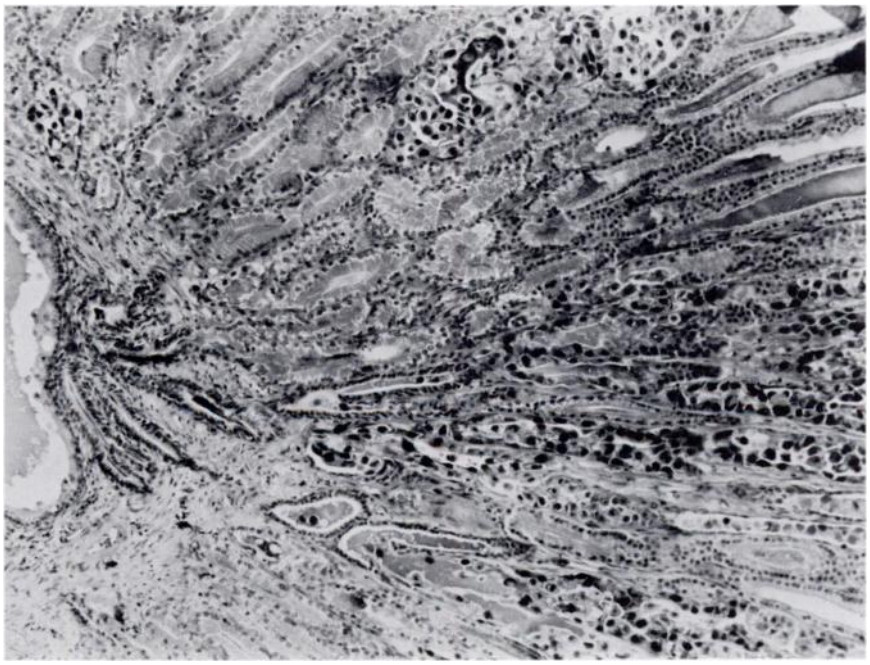

FIGURE 1. Cytomegalovirus infection of glands in a segment of sectioned prostate of *Antechinus stuartii*. The urethral lumen is to the left. HE × 95.

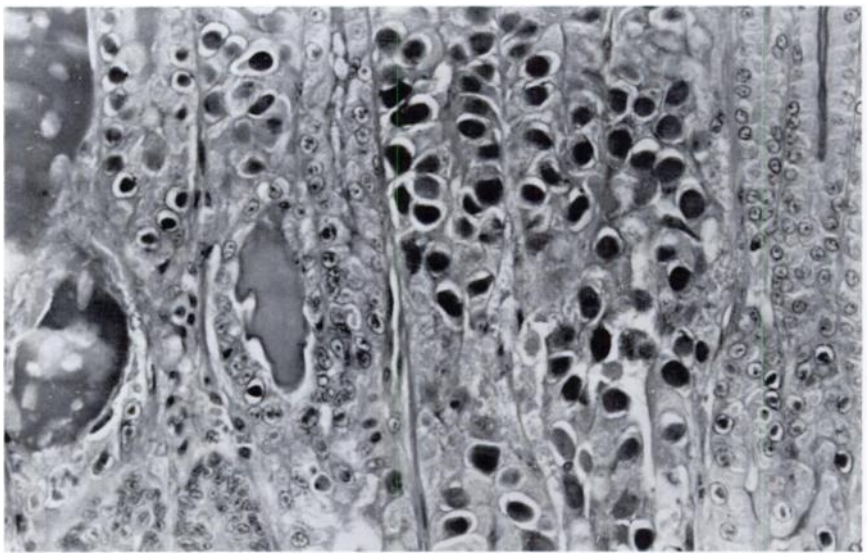

FIGURE 2. CMV infection in prostatic glands in *Phascogale tapoatafa*. Normal gland epithelium is present on the right, and dilated glands containing homogenous basophilic material are seen on the left. HE × 240.

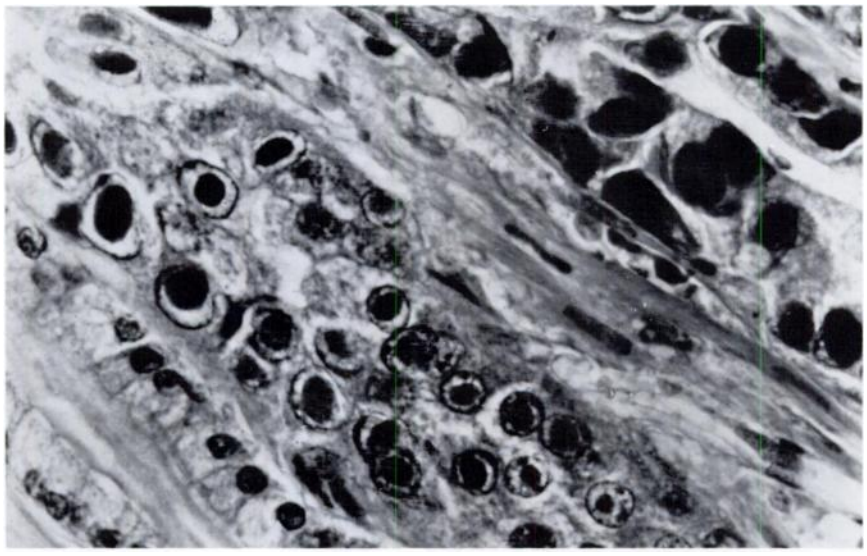

FIGURE 3. The evolution of the development of CMV infection in cells of the prostate of *A. stuartii* is evident, with normal epithelium on the lower left, cells with eosinophilic intranuclear inclusions in the center, and large basophilic inclusions in degenerate cells on the upper right. HE × 600.

male *A. stuartii*.[1] In one of these animals the prostate weighed 511 mg and the process had advanced to local peritonitis with adhesions on the surface of the prostate. In this animal there was evidence of cytomegalovirus infection, but not in the other.

In eight of the male *A. stuartii* in Groups 4, 6 and 7, occasional hypertrophied epithelial cells with a basophilic cytoplasm and a nucleus enlarged up to 25×13 μm were seen at all levels of the nephron in the cortex and outer medulla of the kidney. In most of these cells, the chromatin was condensed around the periphery of the nucleus and ill-defined irregular eosinophilic bodies filled the bulk of the nucleus, while in other instances, the chromatin was evenly distributed, basophilic and stippled, resembling a small protozoan cyst. These cells were not found in any of the males with cytomegalovirus infection of the prostate nor in kidneys of any of the females killed at the same time.

In two males, rare small rhomboid eosinophilic intranuclear inclusions were seen in normal-sized hepatocytes.

Ultrastructural Findings. In general, nuclear structure including the nuclear membrane appeared well preserved; however, cytoplasmic organelles and the plasma membrane seemed poorly preserved. Intranuclear inclusions appeared as patchy aggregations of finely granular electron dense material, which occupied the central part of the nucleus and were separated from the nuclear membrane by an electron-lucent zone. In both species of marsupial, viral particles were numerous within the inclusions, where they occurred as evenly distributed single particles and as small groups (Fig. 4). Within

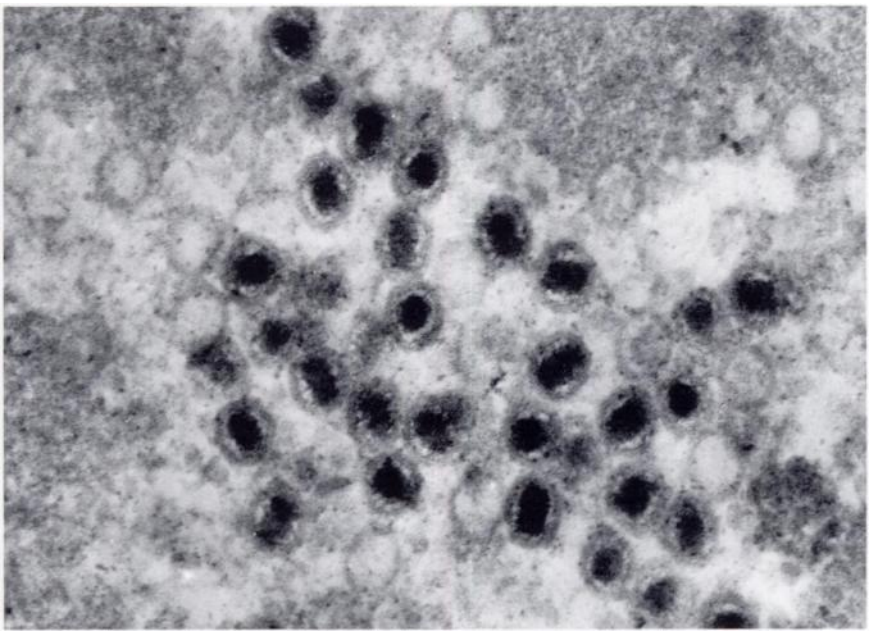

FIGURE 4. Virus particles in an intranuclear inclusion in the prostatic epithelium of *P. tapoatafa*. Some particles have a core and a single membrane, while empty membranes are also present. $\times$ 66,000.

nuclei, viral particles were always limited by a single thin, well-defined, membrane which surrounded the central highly electron dense core. Probably as a result of plane of section the shape of this core varied in different particles, in some cases appearing circular, in others, rhomboid. Many particles also appeared to lack a core (Fig. 4).

The electron-lucent zone between the inclusions and the nuclear membrane was usually free of virus particles, but in a few cells large numbers of coreless particles were seen in this region. The nuclear membrane was apparent in most infected cells, but in some, it appeared discontinuous. Dense granular material, corresponding to the marginated chromatin seen by light microscopy, was present adjacent to the inner aspect of the nuclear membrane. Near the nuclear membrane in some cells, aggregates of viral particles were seen in closely packed groups. In many cases these aggregates were enclosed within a unit membrane similar to that of the nucleus, although it could not be established with certainty that it was continuous with the nuclear membrane.

The cytoplasm of infected cells contained few recognizable organelles, although in some small amounts of rough surfaced endoplasmic reticulum were found. Infected cells almost invariably failed to contain the dense membrane-found granules seen in the apical cytoplasm of uninfected cells in adjacent tubules. The majority of infected cells appeared to be desquamated, with a circular or oval, rather than rectangular outline.

Viral particles were frequent in the cytoplasm, and occurred in two forms. The majority of cytoplasmic particles had a single membrane like those in the nucleus and were distributed randomly throughout the cytoplasm. In some cells, groups of such particles were seen surrounding small cytoplasmic vesicles (Fig. 5). Particles with a second outer membrane, broader and less distinct than the inner, were less frequent, and occurred as scattered small aggregations or as large densely-packed masses in the cytoplasm. In many sections, large portions of the contents of prostatic tubules consisted of similar particles (Fig. 6). These appeared to correspond to areas of intense basophilia of tubular contents seen in sections examined by light microscopy.

DISCUSSION

The morphologic characteristics of infected cells in the prostate and the presence of virus morophologically identical to herpes viruses[11] in ultra-thin sections of nuclei indicate that this is a cytomegalovirus infection.

An opportunity to attempt virus isolation has not arisen since all male *A. stuartii* die after breeding and infection is overt for only a short period. Apart from displacement of normal glandular tissue, infection with this agent does not appear to be detrimental to the host and incites no inflammatory response. The bacterial prostatitis observed in one virus-infected *A. stuartii* was probably only fortuitously associated.

The data on prevalence of infection indicate that lesions in the prostate are manifest only in breeding animals under normal circumstances. At this time, males have elevated plasma corticosteroid concentrations[2,4] and show a syndrome apparently related to stress, with involution of lymphoid tissue, gastrointestinal hemorrhage and initiation or recrudescence of listerial and babesial infection.[1,4] It seems possible that CMV infection, which in mice and man is known to occur more commonly in immunosuppressed individuals,[5,6,10] was acquired or activated as immune processes were suppressed by stress. The tendency for increased prevalence of infection in the *A. stuartii* treated with the higher level of hydrocortisone acetate,

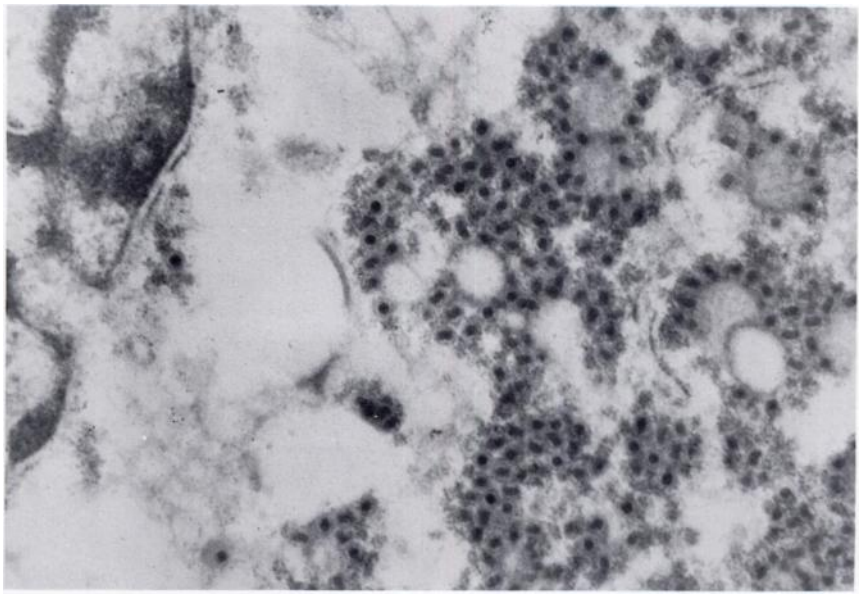

FIGURE 5. Virions aggregating around vesicles in the cytoplasm of an infected cell. *P. tapoatafa*. × 30,000.

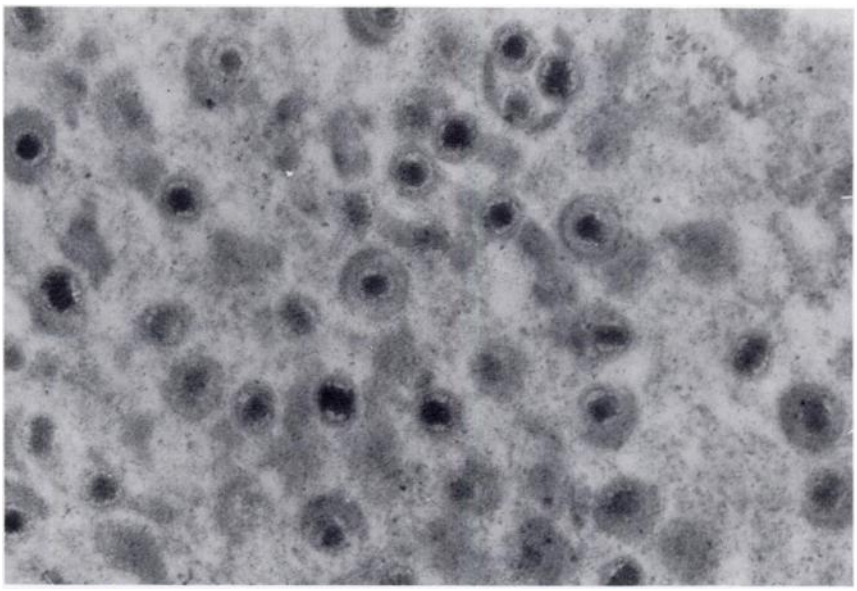

FIGURE 6. Dense aggregates of virus particles with an outer envelope, free in the lumen of a prostatic gland. *P. tapoatafa*. × 60,000.

which produced circulating levels of corticosteroid similar to those in naturally stressed animals[3] may support this contention. Cytomegalovirus infection of one *P. tapoatafa* suffering *Erysipelothrix* bacteraemia is consistent with infection occurring in a stressed animal.

The hypertrophied cells seen in renal epithelium may indicate that latent infection with cytomegalovirus occurs, although their relationship with prostatic infection is unproven. Similar cells have been reported in renal epithelium in brush-tailed phalangers *(Trichosurus vulpecula)*[7] and if they represent a site of latent infection, could indicate that cytomegalovirus occurs in other marsupials. Lesions suggestive of CMV infection were not found in other tissues, including lingual glands, but only limited observations have been made on other salivary glands.

CMV has not been detected in females, although they have not been investigated in detail when stressed and their reproductive organs have not been examined systematically. However, if, as it currently appears, the main target organ is the prostate in males, the epizootiology may be complex. Populations of *A. stuartii* are devoid of other than neonatal male animals for several months following the total postbreeding mortality of males,[12,13] eliminating the possibility of horizontal transmission between generations of males, and implying that infection must be transmitted vertically by females. The possibility of venereal transmission of CMV exists since the virus is undoubtedly in high titre in the semen of some breeding males; venereal transmission of CMV may also occur in humans where it has been demonstrated in seminal plasma.[8]

Thus, CMV infection in dasyurids, particularly in *A. stuartii*, presents a number of intriguing virological, immunological and epizootiological problems which await further investigation.

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