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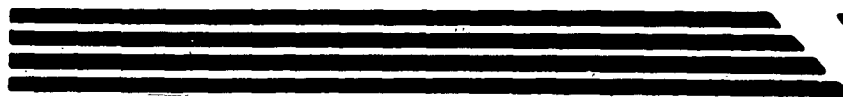
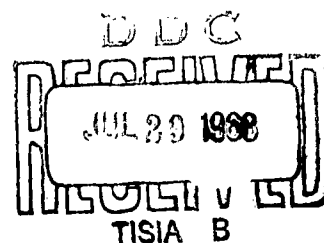
US ARMY MEDICAL RESEARCH LABORATORY

FORT KNOX, KENTUCKY

REPORT NO. 556

ELECTRON MICROSCOPIC OBSERVATIONS ON
TRANSITIONAL EPITHELIUM OF DOGS INFECTED
WITH CANINE DISTEMPER

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MEDICAL RESEARCH AND DEVELOPMENT COMMAND

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WITH CANINE DISTEMPER**

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Biochemistry
Task 03
Basic Research in Life Sciences
USAMRL Project No. 6X99-26-001

Report No. 556
USAMRL Project No. 6X99-26-001-03

ABSTRACT

ELECTRON MICROSCOPIC OBSERVATIONS ON
TRANSITIONAL EPITHELIUM OF DOGS INFECTED
WITH CANINE DISTEMPER

OBJECT

To provide information on the structure and intracellular development of canine distemper virus and the intracellular changes it produces.

RESULTS

The urinary bladder epithelium of four dogs with natural infections of canine distemper was observed with the electron microscope. Numerous abnormal formations were observed including a finely stippled body and a virus containing inclusion. The viral elementary bodies found in this inclusion were measured and found to range in size from 900 to 1200 Å in closely packed inclusions and 1100 to 1400 Å in degenerating inclusions.

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ELECTRON MICROSCOPIC OBSERVATIONS ON TRANSITIONAL EPITHELIUM OF DOGS INFECTED WITH CANINE DISTEMPER

I. INTRODUCTION

Inclusion bodies were first described in dogs dying of canine distemper in 1909 (5). Today the presence of these inclusions is routinely used as diagnostic evidence of the disease. The causative agent, a virus, was originally described in 1905 (1). Recently, studies using fluorescent-labelled antibodies suggested that the inclusions contain antigenic material (8). However, little is known of the exact nature of these inclusions and their relationship to the causative agent of the disease.

Because the electron microscope has greater resolving power than the light microscope, making it possible to observe intracellular detail, it was decided to utilize this instrument to observe transitional epithelial cells of dogs infected with canine distemper and to describe changes occurring in these cells. Transitional epithelium of the urinary bladder was chosen for this study because large numbers of cytoplasmic inclusions appear here in infected animals and because the bladder is easily accessible for the rapid removal and fixation which is necessary for high resolution electron microscopy. It was hoped that typical inclusions could be located and their structure observed.

II. MATERIALS AND METHODS

This study was made on six dogs with classical symptoms and lesions of canine distemper and three normal dogs. Four of the animals were in the acute respiratory stage of the disease and were moribund at the time tissues were taken for electron microscopy. They were selected because of the typical clinical course and the severity of the disease in the hope that they would have abundant cytoplasmic inclusions in the transitional epithelium.

The other two infected animals had passed through a mild respiratory stage and were suffering from nervous symptoms of several months duration. These animals were selected to see if any cellular evidence of the disease was present in the transitional epithelium at this late stage. All animals with distemper were from our animal colony and were naturally occurring cases of the disease.

Post-mortem histological examination of the four acute cases revealed the presence of typical inclusion bodies in the transitional epithelium of the urinary bladder, in the bronchial epithelium, and in the gastric epithelium. There were also typical lesions of spongiosis and gliosis, with intranuclear inclusions of the glial cells present in the white tracts of the cerebellum. The two animals with central nervous symptoms also had lesions of the cerebellum with typical intranuclear inclusions but no inclusions were observed in the urinary bladder, stomach, or bronchi.

Tissues for electron microscopy were surgically removed from the urinary bladder with the animal under pentobarbital Na anesthesia. The animal was then sacrificed for post mortem examination and collection of tissues for light microscopy. Bladder tissue for electron microscopy was fixed within 15 seconds in a pool of fixative maintained at ice bath temperatures. The tissue was sliced into 0.5 to 1.0 mm square fragments while in the fixative and then transferred to fresh fixative for 15 to 20 minutes in an ice bath. The fixative was osmium tetroxide buffered to pH 7.4 with 0.015 Gm of sucrose added per ml (2, 9). Dehydration was carried out rapidly in 50, 70, 95, and 100 per cent ethanol.

Tissues from four animals were embedded in methacrylate and two were embedded in Epon 812 (6). Embedment in methacrylate was carried out by two changes in a methacrylate mixture of nine parts n-butyl and one part methyl methacrylate. A final change of methacrylate plus catalyst (benzoyl peroxide) was made prior to placing the tissues in gelatin capsules containing methacrylate which was prepolymerized for 50 minutes at 60°C. Polymerization of these capsules was completed by holding them in an oven at 60°C overnight. Ultrathin sections were either cut on a Porter Blum or a Leitz-Moran ultramicrotome and then mounted on grids coated with formvar and stabilized with carbon. Some sections of Epon embedded tissues were mounted on uncoated grids. All grids were then examined with an RCA EMU-3 electron microscope. Thick sections (2 - 4 μ) were cut from the methacrylate blocks and stained with hematoxylin and eosin or examined by phase microscopy.

III. RESULTS

Light microscopy of paraffin sections of the four acute respiratory cases revealed the presence of typical cytoplasmic inclusion bodies in the urinary bladder epithelium. These inclusions were moderately numerous with one or two being present in the average high power field.

Inclusion bodies were also present in the bronchial epithelium and gastric epithelium but they were not numerous. It required considerable searching to locate these structures.

Electron microscopic examination of transitional epithelium from the same cases revealed many fine order changes within the epithelial cells even though adjacent thick sections displayed no abnormalities when viewed with the light microscope. Two abnormal intracellular structures were observed with the electron microscope: a finely stippled body and a membrane limited inclusion of viral elementary particles. These two structures were associated with varying degrees of cellular degeneration and were most common in the superficial epithelial cell layer, although occasionally occurring in deeper cells.

The most common finding was the presence of a finely stippled body of variable size often as large as the nucleus (Figs. 1, 2, and 3). One or several of different sizes occurred in many of the cells. In all four of the animals examined at least one third or more of the superficial cells and a few deeper cells contained stipple bodies. They were located centrally in the cytoplasm closer to the nucleus than to the cell wall. The surrounding cytoplasm was either normal or slightly vacuolated and mitochondria were mechanically displaced to the cell periphery when large stipple bodies were present (Fig. 2).

This structure was irregular in outline and had a distinct border without a definite limiting membrane although a poorly developed membrane was observed in a few examples. It was composed of finely stippled material which was evenly scattered and of uniform moderate density. At high magnification the stippled material was not composed of discrete particles or distinct filaments but consisted of irregular dense material which tended to be arranged in rows, producing the effect of coarse but indefinite filaments. Often a few vesicles or other cytoplasmic components were trapped within the stipple body. There was no other specific association with any cytoplasmic structure. A few of the stipple bodies from methacrylate embedded material were filled with fine particles ranging from 50 Å to 100 Å in size. These particles were not observed in Epon embedded materials and their significance as artefact or actual structure is unknown.

The second abnormal structure observed in the bladder epithelium was an inclusion of closely packed viral elementary bodies (Fig. 4). This inclusion ranged in size from larger than a nucleus down to less than a micron in diameter. Inclusions of various sizes were often present in a single cell and they were smallest in size when they were most

numerous. They were surrounded by a double membrane and were filled with elementary bodies (Fig. 5). Some inclusions were partially broken down with rupture of the limiting membrane and scattering of the elementary bodies. An elaborate membrane system was often present in the inclusion with elementary bodies wrapped in membranes or located in channels between membranes (Fig. 6).

The viral elementary bodies varied in size depending on the structure and appearance of the inclusion in which they were contained. They were smallest in compact inclusions with distinct internal structure and largest in those inclusions which were disintegrating. This may indicate that the particles increase in size as they mature because the disintegrating inclusions are probably older and in a more advanced stage of virus formation than the densely packed inclusions with internal membranes. It is possible that the membranes enclosing the virus particles either add an outer coat or become an outer coat of the virus. Elementary bodies ranged in size from 900 to 1200 Å in the well packed inclusions and 1100 to 1400 Å in those which showed signs of breaking up. In one case, 1800 Å particles were observed in a severely degenerating inclusion.

Structure of the elementary bodies also varied as did size. The smallest particles were dense masses with no internal structure visible. Some contained one or two concentric rings of dense material or a dense central body surrounded by a clear zone and then by a dense outer ring. The larger particles were usually less dense and often consisted of a dense outer ring and a clear central space. In the inclusion containing 1800 Å particles mentioned above, the elementary bodies consisted of a dense outer ring enclosing a clear space containing one or two dense 100 Å polar bodies (Figs. 7 and 8).

Cytoplasmic damage varied from slight or non-existent in those cells with small stipple bodies to extensive or complete in those with large viral inclusions. There was mild vacuolization of cytoplasm in areas near numerous or large stipple bodies. Degeneration was more extensive in those cells with viral inclusions especially those with large inclusions. It consisted of dissolution of many cytoplasmic structures, swelling or rupture of mitochondria, and shrinking of the nucleus. A few cells were observed which were no more than membrane limited sacks of cytoplasmic debris. Such a cell is illustrated in Figure 7 and contains a shrunken nucleus, various forms of debris, and a disintegrating viral inclusion. It was this inclusion which contained the 1800 Å elementary bodies described earlier.

Some severely injured cells contained one or more masses of dense osmophilic material, 0.5 to 3.0 microns in size. Close examination revealed that a few scattered viral particles were contained in the dense masses which probably represent old viral inclusions undergoing degenerative changes.

A few nuclei of cells with stipple bodies contained an enlarged nucleolar-like structure. The largest of these were often surrounded by a clear ring almost devoid of nuclear material. Usually there were numerous small particles, 50 to 100 Å in diameter packed in the nucleolar structures but not in the rest of the nucleus.

An attempt was made to correlate the structures observed in this study with the typical canine distemper inclusion body of light microscopy. Thin sections (less than 1000 Å) were cut for electron microscopy and adjacent thick sections (2μ) were cut for staining and study by light microscopy. It was not possible to demonstrate stained inclusion bodies in osmium fixed methacrylate embedded tissues examined with the light microscope. Numerous staining techniques were tried but none stained the inclusions. Therefore, no direct correlation could be made with the structures observed with the electron microscope. However, the stipple bodies were much more numerous than stainable inclusion bodies found in formalin fixed tissues from the same bladder. Stipple bodies were found in one third or more of the superficial cells while only one or two stained inclusion bodies were found per high power light microscope field.

The viral inclusion was observed with the electron microscope in about the same frequency as the stainable inclusion was seen with the light microscope and both of these structures were about the same size and shape. Because of this it is very probable that the viral inclusion described here stains as an inclusion body for light microscopy. It is also possible that some stipple bodies and various forms of cytoplasmic debris may also stain as inclusion bodies but further studies are needed to definitely establish the exact relationship of the stainable inclusion body of light microscopy and the several structures observed with the electron microscope.

The two animals with central nervous symptoms had lesions of the cerebellum including intranuclear inclusions. Electron microscopic examination was not conducted on these brain lesions. Electron microscopy did not reveal any significant changes in the urinary bladders of these animals.

IV. DISCUSSION

From the various abnormalities found in infected cells it is obvious that a series of morphologic changes occur in a cell following entry of the canine distemper virus. It is not possible to reconstruct a series of events from the limited observations of four clinical cases. Changes which are common in these animals may not be typical in others. All of these animals were in late stages of the disease and early changes may not appear as seen here. Because electron microscopy does not allow the study of large areas or large numbers of cells, there is always a danger in generalizing from limited observations.

However, the changes and structures observed in these four animals were found in many cells and were quite distinctive. There is a hint of time sequence in relation to cell damage. The cells with stipple bodies never showed as severe a degeneration as those with viral inclusions. Therefore, they could be an early stage of infection. There were no observations of a transition from stipple bodies to viral inclusions so there can be no definite conclusion that stipple bodies form or help to form viral inclusions. However, vaccinia virus has been shown to develop in areas of fibrillar material similar in appearance to these stipple bodies (3). These observations were made by a series of examinations of tissue culture cells infected with vaccinia virus.

It is difficult to prove that the particles referred to as viral elementary bodies are actually virus and that they are the virus of canine distemper. They probably are virus because of their size and their internal structure which is similar to that of some known viruses (4, 7). The significance of the variation in structure is not known. Some of the forms described are undoubtedly steps in the formation and maturation of the virus. Others may possibly be abnormal virus or degenerating virus. Observations on the development of this virus in tissue culture should help to establish a more definite sequence of events in the formation of infective virus and the associated cellular changes.

Previously reported electron microscopic measurements of purified canine distemper virus indicate that it is 2000 to 2200 Å in diameter (10). This is larger than the 900 to 1400 Å particles observed here but one inclusion did have particles 1800 Å in size. The larger forms may represent the mature infective particle while the smaller forms may be a slightly earlier stage of viral development. There may also be a size difference as a result of differences in specimen preparation for tissues and purified virus suspensions.

V. SUMMARY

The urinary bladder epithelium of four dogs with natural infections of canine distemper was observed with the electron microscope. Numerous abnormal formations were observed including a finely stippled body and a virus containing inclusion. The viral elementary bodies found in this inclusion were measured and found to range in size from 900 to 1200 Å in closely packed inclusions and 1100 to 1400 Å in degenerating inclusions.

VI. REFERENCES

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Fig. 1. Transitional epithelial cell containing several fine stipple bodies (SB). The nucleus contains a large nucleolar-like structure (No). Nucleus (N) and mitochondria (M) are indicated. X7,500.



Fig. 2. Another view of the fine stipple bodies (SB) illustrating their irregular outline. Note the vacuolated cytoplasm (V) adjacent to the bodies and the movement of mitochondria (M) to the cell periphery. Nuclei are indicated by N and bladder lumen by L. X3,700.

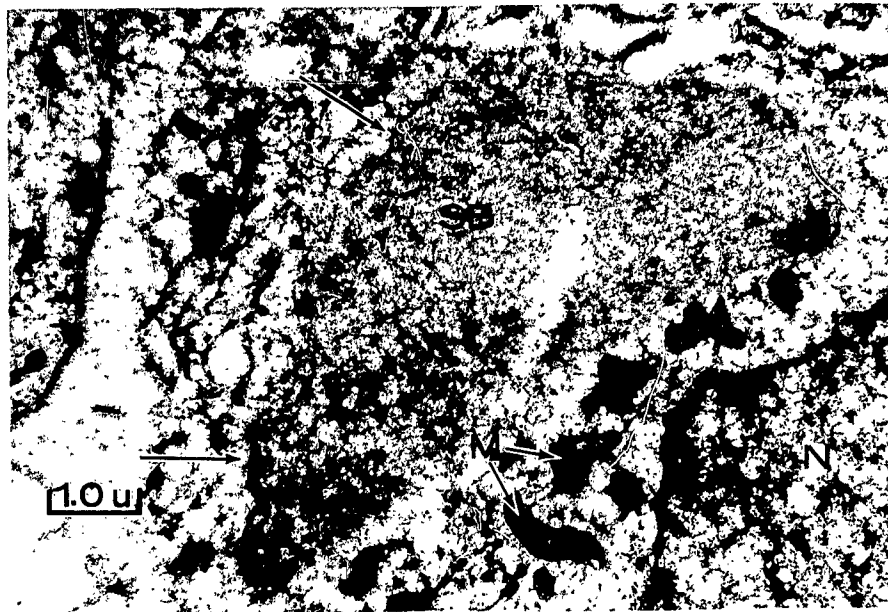


Fig. 3. Higher magnification of a fine stipple body (SB) indicating filamentous internal structure and possible membrane formation (arrows). Nucleus (N) and mitochondria (M) are indicated. X12,500.

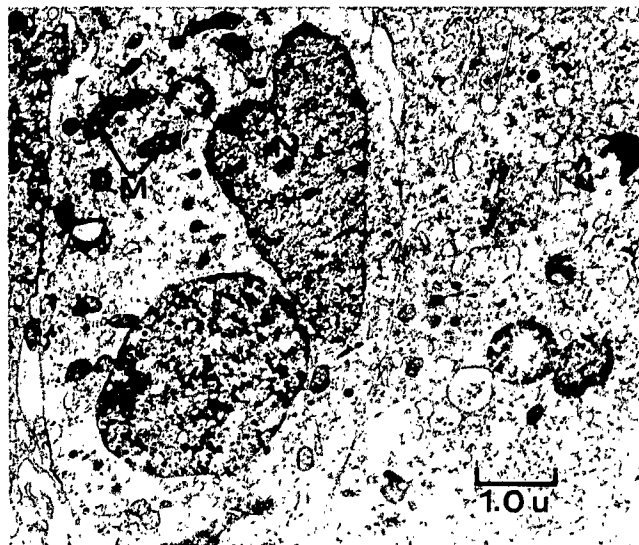


Fig. 4. A transitional epithelial cell containing a large viral inclusion (VI). Note the many small virus particles in the inclusion and its surrounding membrane. The nucleus (N) is shrunken and the mitochondria (M) are enlarged. X12,000.

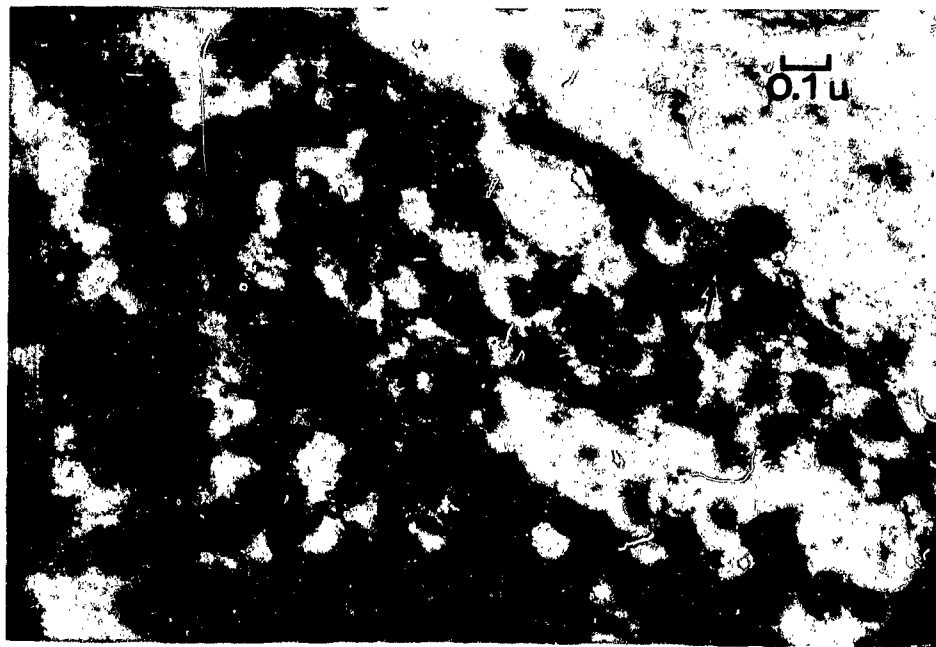


Fig. 5. A higher magnification of the viral inclusion seen in Figure 4. Note the internal structure of one of the virus particles (VP). X110,000.



Fig. 6. A viral inclusion (VI) with the viral particles separated by an elaborate membrane system. Nucleus (N) is at the upper left. X43,000.

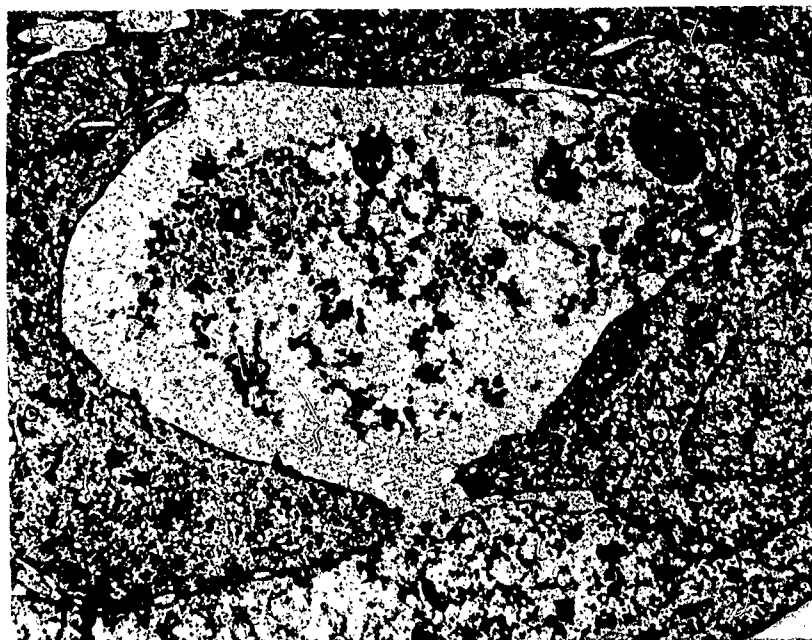


Fig. 7. A transitional epithelial cell which has undergone severe degeneration. The nucleus (N) is very small and no cytoplasmic structure is identifiable. A disintegrating viral inclusion (VI) is present. X6,000.

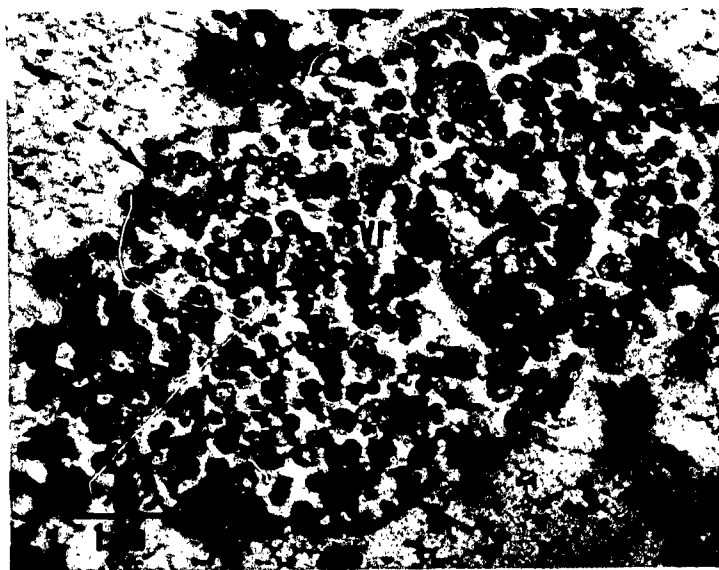


Fig. 8. A higher magnification of the viral inclusion (VI) of Figure 7. Note the disintegrating membrane (arrows) and the viral particles. Many have one or two visible polar bodies. X20,000.